Enzyme Induced Carbonate Precipitation (EICP) for Soil Improvement

by

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ABSTRACT

This dissertation presents an investigation of calcium carbonate precipitation via hydrolysis of urea (ureolysis) catalyzed by plant-extracted urease enzyme for soil improvement. In this approach to soil improvement, referred to as enzyme induced carbonate precipitation (EICP), carbonate minerals are precipitated within the soil pores, cementing soil particles together and increasing the dilatancy of the soil. EICP is a bio-inspired solution to improving the properties of cohesionless soil in that no living organisms are engaged in the process, though it uses a biologically-derived material (urease enzyme).

Over the past decade, research has commenced on biologically-mediated solutions like microbially induced carbonate precipitation (MICP) and biologically-inspired solutions like EICP for non-disruptive ground improvement. Both of these approaches rely upon hydrolysis of urea catalyzed by the enzyme urease. Under the right environmental conditions (e.g., pH), the hydrolysis of urea leads to calcium carbonate precipitation in the presence of Ca^{2+}. The rate of carbonate precipitation via hydrolysis of urea can be up to 10^{14} times faster than natural process.

The objective of this research was to ascertain the effectiveness of EICP for soil improvement via hydrolysis of urea (ureolysis) catalyzed by plant-extracted urease enzyme. Elements of this work include: 1) systematic experiments to identify an optimum EICP treatment solution; 2) evaluation of the mechanical properties of EICP-treated soil under different treatment conditions and with varying carbonate contents; 3) investigation of the potential for enhancing the EICP stabilization process by including xanthan gum, natural sisal fiber, and powdered of dried non-fat milk in the EICP treatment solution; and

4) bench-scale studies of the use of EICP to make sub-horizontal columns of cemented soil for soil nailing and vertical columns of cemented soil for foundation support. As part of this research, the effect of three preparation methods (mix-and-compact, percolation, and injection) was also examined as was the influence of the grain size of soil. The results of this study should help make the EICP technique an attractive option for geotechnical engineers for ground improvement and stimulate the development and use of other biogeotechnical techniques for civil engineering purposes.

DEDICATION

This dissertation is dedicated to my parents, Abdulrahman and Asmaa Almajed, who have on all occasions supported me and motivated me to achieve my goals. Also, this dissertation is dedicated to my wife and best friend, Amnah Alsaid, who has supported me and taken care of me and my daughter, and allows me to pursue my degree without facing a lot of obstacles, thank you a lot.

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CHAPTER 1

INTRODUCTION

OBJECTIVE

The objective of this research is to investigate factors that affect the use of enzyme-induced carbonate precipitation (EICP) via hydrolysis of urea (ureolysis) catalyzed by plant-extracted urease enzyme for soil improvement. A key element of this research was the systematic identification of an optimum EICP treatment solution. The strength and ductility of EICP-treated soil was studied under different treatment conditions (sample preparation methods and admixture) and with varying carbonate content. Sample preparation methods include mix-and-compact, percolation, and injection. Admixtures investigated included xanthan gum, sisal fibers, and organic additives. This study also worked towards eliminating, or at least controlling, the drawbacks of the EICP technique to make it a more attractive option for ground improvement, encouraging the use of biological techniques over conventional methods where possible to enhance sustainability in civil engineering.

BACKGROUND

Techniques used in practice to enhance the mechanical properties of granular soil, include stone columns, vibroflotation, removal and re-compaction, dynamic compaction, and chemical grouting. Increasing concerns regarding the sustainability of these conventional soil improvement techniques have driven geotechnical engineers to seek ground improvement solutions that employ biologically-based techniques. These biological-based techniques can be divided into two categories: bio-mediated techniques, which use living organisms for soil improvement; and bio-inspired techniques, which mimic biological processes without actually involving living organisms.

Microbially induced carbonate precipitation (MICP) is a bio-mediated technique that has been investigated for the past decade as an alternative to Portland cement for ground improvement (Dejong et al., 2013). One of the main advantages of MICP over Portland cement for ground improvement is that MICP is non-disruptive. Another appeal of using MICP in place of Portland cement is that doing so certainly reduces cement manufacture-related emissions including carbon dioxide (CO_2) and sulfur dioxide (SO_2). Studies suggest that cementation using MICP can address a variety of significant geotechnical problems in granular soils including the bearing capacity of shallow foundations, excavation stability, erosion and scour, under-seepage of levees, tunneling in running or flowing ground, seismic settlement, and liquefaction (Dejong et al., 2010; Kavazanjian and Karatas, 2008; van Paassen et al., 2010).

MICP relies upon calcium carbonate ($CaCO_3$) precipitation to induce inter-particle binding cementation in sandy soil, limiting their mobility and enhancing the strength and stiffness of the soil (Harkes et al., 2010). $CaCO_3$ precipitation may also fill the voids of the soil, decreasing soil porosity, decreasing the tendency for volume decrease that leads to liquefaction (Andrus and Chung, 1995), and increasing the tendency for volume expansion (dilatancy) during shear (Yang et al. 2016). Additionally, the precipitated $CaCO_3$ is strong and is resistant to dissolution, dissolving slowly on a geological scale under normal conditions (van Paassen et al., 2010b).

$CaCO_3$ precipitation using bacterial cells containing urease enzyme is a bio-mediated technique (Harkes et al., 2010). Several studies have been conducted on using

$CaCO_3$ precipitation facilitated by bacterial urease, sometimes referred to as bio-grouting, as a method to strengthen soil (e.g., van Paassen et al., 2009; Whiffin et al., 2007; Harkes et al., 2010). $CaCO_3$ precipitation using free urease enzyme, referred to herein as Enzyme Induced Carbonate Precipitation (EICP), is a much-less studied technique for soil improvement. EICP may be considered a bio-inspired technique as no living organism is directly involved in the precipitation process. Over the past 6 years, EICP has been investigated as an alternative technique to MICP for soil improvement by Dilrukshi and Kawasaki (2016), Hamdan (2015), Hamdan et al. (2013), Hamdan and Kavazanjian (2016), Kavazanjian and Hamdan (2015), Neupane et al. (2013, 2015a and 2015b), Putra et al. (2016), and Yasuhara et al. (2011 and 2012).

The free urease enzyme used in most EICP studies to date has been isolated from plants, including soybean, jack bean, watermelon seed, and pea plants (Kayastha and Das 1999; Das et al. 2002). The Jack bean, a drought resistant relative of the soybean, is the most common source of free urease. The enzyme is the most expensive component of the EICP solution. It has a size of 12 nm per subunit and is soluble in water, facilitating its transport within the pores of soils (Blakely and Zerner, 1984). As the $CaCO_3$ precipitation reaction through EICP does not employ living organisms, it is not limited by oxygen availability as when using MICP for deep soil treatment. Furthermore, MICP requires nutrients for bacterial activity, is subject to competing effects of other microorganisms, or rely upon cell attachment to soil particles. For these reasons, EICP possesses many practical advantages over MICP. Another advantage of EICP over MICP in some cases is that the reaction process in EICP occurs more rapidly than MICP, making it particularly useful for some applications in arid and semi-arid environments.

POTENTIAL APPLICATIONS OF EICP

Enzyme-induced carbonate precipitation has a wide range of potential applications in geotechnical engineering. This wide range of applications is due in part to the smaller size of the free urease enzyme used in EICP compared to that of the ureolytic bacteria used in MICP. Potential applications of EICP include fugitive dust mitigation, surface water erosion control, the creation of subsurface barriers, excavation stabilization, remediation of cracks in concrete, soil nailing, liquefaction mitigation, and foundation support. While still in early stages of development, EICP application has demonstrated the ability to significantly reduce fugitive dust levels (Knorr 2014; Hamdan 2015; Bang et al.2009; Meyer et al.2011). Both EICP and MICP can be used to mitigate fugitive dust. However, EICP has practical advantages over MICP since the reaction process in EICP occurs more rapidly than in MICP.

POTENTIAL ADVANTAGES OF EICP COMPARED TO MICP

The small size of the solubilized free urease enzyme used in EICP provides it with a unique advantage over microbial urease for engineering implementations that demand penetration into finer grained soils, such as silt. The small urease enzyme sub-unit, on the order of 12 nm in dimension, allows the EICP solution to diffuse into finer soils in which ureolytic microbes, typically sized between 300 and 500 nm, either cannot penetrate or, when combined with $CaCO_3$ precipitation, result in of the bio-plugging (clogging of soil pores), inhibiting transport of nutrients and substrate in to the soil. The water solubility of the free urease enzyme enables it to enter any pore that water could reach, minimizing the risk of bio-plugging.

Another potential advantage of using free urease enzyme is the decrease in the enzymatic activity and functionality over time (Pettit et al., 1976; Marzadori et al., 1998), potentially reducing long term environmental impacts. Ephemeral urease activity would be better in accomplishing short-term engineering goals, after which the enzyme will degrade, avoiding potential long-term biological impacts. Though the free urease enzyme is expected to degrade quickly, the consumed urease sorbed to soil particles can persist for a longer time without any loss of function or proteolytic degradation (Pettit et al., 1976). Using free urease enzyme is also much simpler than using ureolytic microbes, as there is no need to culture or stimulate bacteria.

POTENTIAL DISADVANTAGES OF EICP

There are several potential disadvantages of using free urease enzyme in place of ureolytic microbial urease. Using free enzyme may result in a lack of nucleation points in the soil for calcium carbonate precipitation; MICP microbes typically adhere themselves to the soil particles, providing nucleation points for mineral precipitation. In addition, the rapid precipitation of carbonate in EICP can be disadvantageous because it can result in the formation of vaterite and amorphous $CaCO_3$. These polymorphs of $CaCO_3$ are less structured, and more easily dissolved in water than the calcite phase of $CaCO_3$ (van Paassen, 2009). Another disadvantage of the free enzyme is its relatively high cost. The cost of the free urease enzyme accounts for an estimated 57%–98% of the total cost of applying EICP for practical applications, since lab-grade materials are used in most EICP trials. Another disadvantage of using both EICP and MICP, is that they produce the ammonium chloride (NH_4Cl) a recognized ground water contaminant, as a byproduct of their reactions.

ORGANIZATION

This dissertation is organized into the following sections:

a) Chapter 1 describes the objective of this study and briefly discusses potential applications of EICP and the advantages and disadvantages of EICP;

b) Chapter 2 presents a review of the relevant literature on the use of $CaCO_3$ precipitation for soil improvement through MICP and EICP;

c) Chapter 3 describes initial investigations of the EICP technique for soil improvement, including a systematic evaluation of the optimal concentration of constituents in the EICP solution;

d) Chapter 4 presents the results of studies on the enhancement of EICP for soil improvement using sisal fiber and xanthan gum;

e) Chapter 5 presents the results of studies of beneficially modifying the EICP by using an organic additive;

f) In Chapter 6 the strength of EICP treated specimens using different preparation methods and different gradations of sand is presented; and

g) Chapter 7 provides a summary, conclusions, and suggestions for future research.

CHAPTER 2

LITERATURE REVIEW

INTRODUCTION

In numerous areas of the world, the mechanical properties of soils are inadequate for the desired utilization of the land: roads and railroads require constant maintenance; embankments, dunes, and slopes can easily become unsteady due to weak soil; earthquakes can lead to soil liquefaction, destroying local infrastructure. Conventional ground improvement methods, such as compaction, inserting piles, or mixing the soil with cement or lime, can be used to stabilize the soil and solve some of these problems (Karol, 2003). However, these traditional methods have several limitations. Some of them are expensive and time consuming, and some require specialized machinery. Furthermore, some of these traditional methods may have significant negative environmental impacts.

Diagenesis, including the formation of natural carbonate-cemented sands and carbonate rocks, occurs because of physical, chemical, and biological processes that naturally stabilize soil. Sediments are packed together and bound by naturally precipitated minerals. However, these natural actions that form cemented sand from loose sand are very slow, taking potentially hundreds of thousands of years to transition from a layer of cohesionless earth to a cemented sand.

Directed biological processes can be used to speed up the natural processes that form cemented sand by changing the chemistry of pore water, leading to faster mineral precipitation. Over the past 15 years, geotechnical engineers have investigated a variety of biological processes to induce mineral precipitation in soil. The most common biological technique investigated in these studies is the use of urea hydrolysis to induce carbonate precipitation in soil.

THE CHEMISTRY OF UREA HYDROLYSIS

Urea hydrolysis refers to the chemical reaction where urea is hydrolyzed into ammonia (NH_3) and carbon dioxide (CO_2) in the presence of water. These reactions products speciate into ammonium (NH_4) and carbonate (CO_3^{2-}) ions. These products of ureolysis can induce carbonate precipitation in the presence of suitable divalent cations such as calcium ions. Calcium chloride ($CaCl_2$) is one potential source of the calcium ions. The urease enzyme acts as a catalyst for the hydrolysis reaction. The following is the chemical equation of urea hydrolysis.

$$CO(NH_2)_2 + 2H_2O \xrightarrow{Urease} 2NH_4^+ + CO_3^{2-} \quad \text{-.-.-.-.-.-.-.-.-} \quad (1)$$

The reaction above increases the alkalinity and pH of the solution creating an ideal condition for carbonate precipitation in the presence of divalent cations, e.g., Ca^{2+}. The pH increases as a result of ammonia reacting with water, producing OH^- ions. When a sufficient concentration of the appropriate cation is present, this rise in the pH will result in the development of inorganic carbon leading toward carbonate concentration that then produces carbonate precipitation (Karatas et al. 2008). Jacob (1999) elucidated the connection between pH and carbonate precipitation in water, showing that a higher pH leads to greater production of carbonate CO_3^{2-} as shown below in Figure 1.

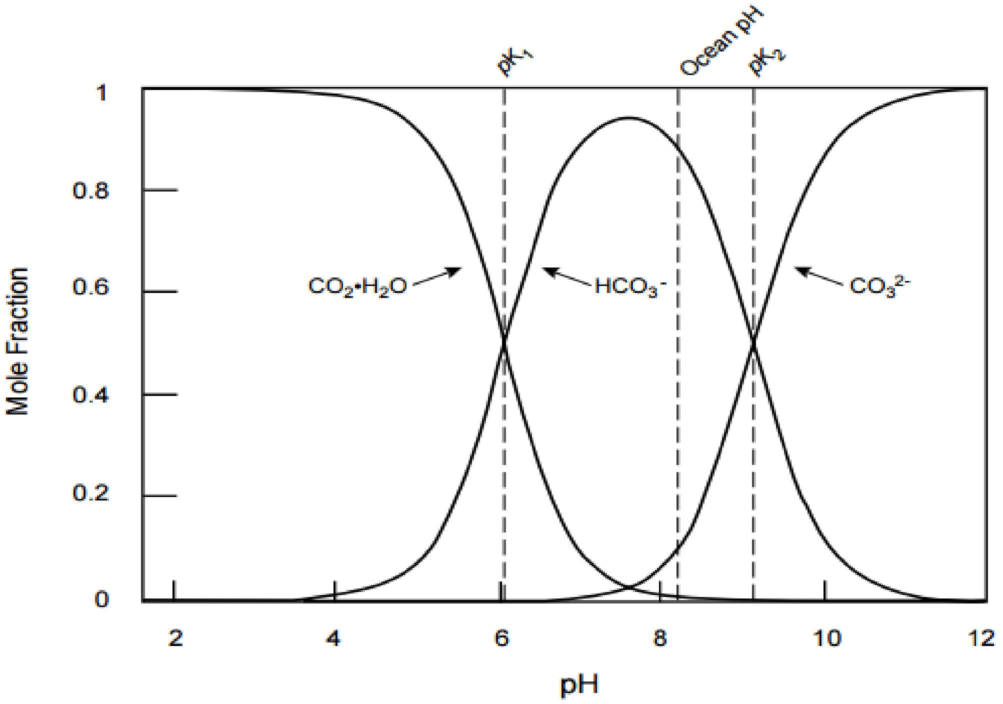


Figure 1 Speciation of Total Carbonate CO_3 in seawater vs. pH (Jacob 1999)

When the reaction in Equation (1) is completed, the reaction shown below as Equation (2) starts. The carbonate ions arising from the reaction precipitate as calcium carbonate ($CaCO_3$) in the presence of calcium ions, as indicated below (van Paassen, 2009).

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

The reaction in Equation (2) is catalyzed by the urease enzyme. There are multiple sources of urease, the most common of which are microorganisms and plants. Urease extracted from plants and microorganisms have both been shown to induce carbonate precipitation (Stocks-Fischer et al., 1999).

If $CaCl_2$ is the source of the calcium ions in Equation (2), this reaction also leads to the production of a potentially undesirable by-product, ammonium chloride:

$$NH_4 + Cl^{-1} \longrightarrow NH_4Cl \quad \text{----------} \quad (3)$$

GROUND IMPROVEMENT VIA UREA HYDROLYSIS

The objective of urea hydrolysis in ground improvement is to induce carbonate precipitation in the soil. Urea hydrolysis is just one of several methods that can induce carbonate precipitation in soil, but it has proven to an efficient method (DeJong et al., 2013). Other processes that can induce carbonate precipitation include sulfate reduction, iron reduction, denitrification, and ankerite precipitation (Karatas et al. 2008 and DeJong et al., 2013). Carbonate precipitation can fill the pore space between two particles of sand and if it occurs at particle content can cement them together.

In 1984, engineers began to use carbonate precipitation for an engineering technique known as "Cleaning in Place," or CIP. In CIP, carbonate precipitation is used to fill the pores in pervious zones in boreholes for oil wells to stop the oil from spreading horizontally and to control the path of the oil's flow (Hitzman, 1984; Mclnerney et al., 1985; Ferris and Stehmeir, 1992; Nemati and Voordouw, 2005). In addition to sealing oil well boreholes, a wide array of applications for calcite precipitation via urea hydrolysis have been proposed. These include the restoration of calcareous stone materials (Tiano et al., 1995; Stocks-Fisher et al., 1999), bio-remediation (Ferris, 2003; Fujita et al., 2000), and water treatment (Hammes et al., 2003).

Most of the studies to date on bio-geotechnical ground improvement are based on carbonate precipitation via urea hydrolysis. Most of the researchers performing these studies rely upon ureolytic bacteria such as *Sporsarcina Pasteurii* as source of the urease enzyme. *Sporsarcina Pasteurii* DSM33 (DSMZ, FRG) is the most popular ureolytic bacteria employed in MICP process for ground improvement. Typically, the ureolytic bacteria used in MICP are grown in the ex-situ under controlled conditions and then inserted into the soil with a solution of urea and calcium chloride (DeJong et al., 2006; Whiffin et al., 2007; van Paassan, 2009; van Paassen, et al. 2010). However, recent studies have explored stimulation of ureolytic bacteria in situ, followed by introduction of a urea and calcium chloride solution (Burbank et al. 2012;Gomez et al. 2016)

Recently, laboratory studies have explored using free urease enzyme extracted from plants to induce carbonate precipitation for soil improvement (Nemati and Voordouw, 2003; Whiffin, 2004; Yasuhara, 2011, 2012; Neupane et al., 2013, 2015; Kavazanjian and Hamdan, 2015; Knorr, 2014; Hamdan and Kavazanjian, 2016). Use of free enzyme has been referred to as enzyme induce carbonate precipitation (EICP). The best known and most studied free urease enzyme is that extracted from the jack bean plant, *Canavaliaensiformis* (Jones and Mobley, 1989; Jabri et al., 1992). There have been limited studies comparing the effects on carbonate precipitation of ureolytic bacteria and plant derived using free urease enzyme. Whiffin (2004) reported higher strength and precipitation in sand cemented via carbonate precipitation using free urease enzyme than sand cemented using ureolytic bacteria. Whiffin (2004) attributed this higher strength and greater precipitation achieved using free urease enzyme to the small size of the free urease enzyme and its lower activity, which resulted in higher precipitation of calcite crystals.

MICP STUDIES ON SOIL STRENGTH

Whiffin (2004) was the first person to conduct a detailed investigation of the impacts of MICP on the physical characteristics of soil. Whiffin (2004) performed triaxial shear strength tests on MICP-treated Koolschijn sand and 90% Koolschijn sand mixed with 10% peat. The results showed that the shear strength for Koolschijn sand was 1800 kPa, whereas Koolschijn sand mixed with 10% peat showed no improvement compared to untreated Koolschijn sand. Whiffin (2004) reported that increasing numbers of cycles of treatment in Koolschijn sand mixed with 10% peat led to improved shear strength. Whiffin inserted varying amounts of urea, $CaCl_2$, and ureolytic bacteria into these soils to catalyze $CaCO_3$ precipitation. Higher concentrations of urea caused an increase in the compressional wave (P-wave) velocity and shear strength of the specimen. Additionally, Whiffin (2004) tested the strength and stiffness of these soils in order to understand the effects of biocementation. Biocementation refers to binding between particles of sand through the precipitation of $CaCO_3$. Whiffin (2004) reported that Koolschijn sand, treated through biocementation, showed an eight-fold increase in the shear strength compared to untreated sand. However, the basis of this statement is not clear since the confining pressure, friction angle, and cohesion were not reported. Furthermore, Whiffin et al. (2007) report that, to achieve a measurable unconfined compressive strength (UCS), the percentage of $CaCO_3$ precipitated into sand should be at least 3.5% (w/w).

Dejong et al. (2006) compared shear strength among MICP-treated and untreated samples of Ottawa 50-70 silica sand in loose and dense conditions (i.e., at 35% and 70% relative density, respectively). The concentration of the solution that was injected into the samples were 0.33 M Urea, 0.95 M calcium chloride, and 2 x 10^6 cells/mL *BacillusPasteurii.* The results showed an increase in shear wave velocity with the number of treatment cycles. After one cycle of treatment, 180 m/s for dense sand, and approximately 155 m/s for loose sand. However, after ten cycles of treatments, the shear wave velocity reached approximately 540 m/s for both conditions. The shear wave velocity of the untreated sand was around 125 m/s. Dejong et al. (2006) noted that there are several factors that must be considered in order to achieve a favorable outcome when treating soil via MICP, including pH of the pore fluid, concentration of microbes, oxygen supply, number of treatment cycles, and availability of ionic calcium.

Van Paassen et al. (2010) performed a large-scale MICP test in a 100 m^3 (8.0 m x 5.6 m x 2.5 m) container that was filled with fine to medium-grain sand under water. Six wells were inserted, three for injection and three for extraction. Within 16 days, around 100 m^3 of a solution containing 1 M urea and calcium chloride, and ureolytic microorganisms was inserted through three injection wells at one end of the box in ten batches of approximately 10 m^3 divided over 16 days. After flushing the solution, 30 m^3 of tap water was needed to rinse out the NH_4^+ (unwanted product from this experiment). After that, the percentage of calcium carbonate and the soil strength was measured by means of the UCS test at various points through the container. The UCS results varied based on the location of the samples from 0.7 MPa at 12.6% $CaCO_3$ w/w to 12.4 MPa at 24.8% $CaCO_3$ w/w. The higher strengths and $CaCO_3$ percentages were located in the vicinity of the injection points.

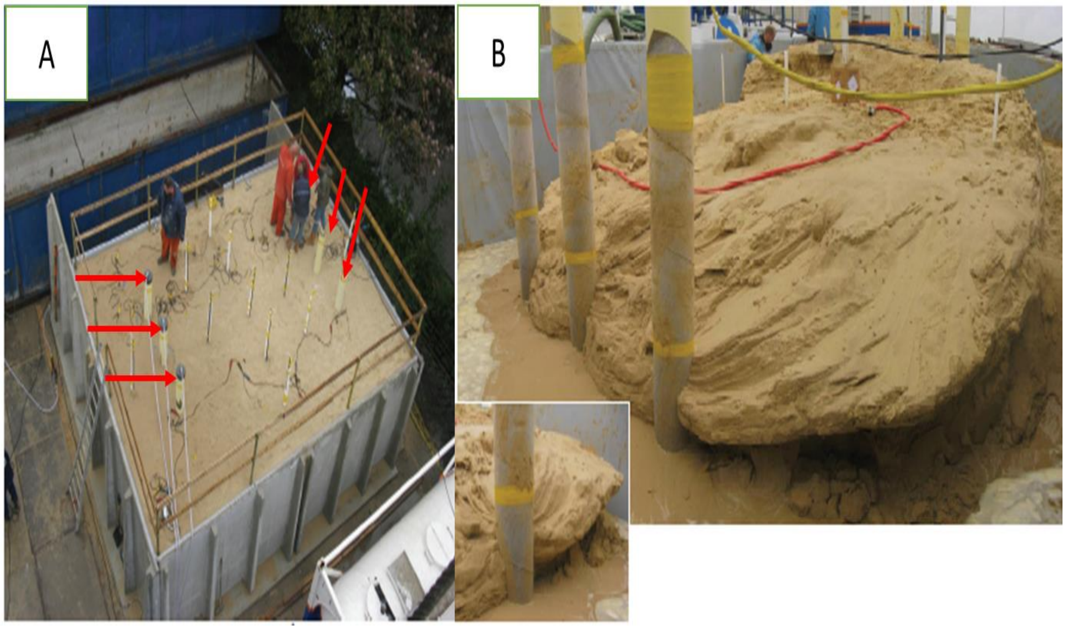


Figure 2. A: Large scale Biogrout experiment set-up (100 m^3). Three injection wells (left) and three extraction wells (right) were used to flush liquids through the sand body; B: The exposed treated sand; C: Close to an extraction well, showing the cementation pattern was clearly related to the flow paths. (Van Paassen et al. (2010))

Bang et al. (2009) showed the ability of both MICP and EICP to mitigate wind erosion. The type of soil that used in these experiments was classified as cohesionless fine sand. This loose sand was poorly graded and sub-rounded. These researchers investigated the effect of three different treatment processes: (1) ureolytic bacteria, (2) urease enzyme only, and (3) a mixture of ureolytic bacteria (*S. pasteurii*) and urease enzyme. They prepared eight boxes with dimensions of 0.61 m x 0.61 m x 0.152 m, and applied 2.39 L/m^2 for each treatment process. Three different concentrations of ureolytic bacteria were used: 1×10^7 cells/ml, 1×10^8 cells/ml, and 1×10^9 cells/ml and one concentration of urease enzyme (0.5 unit / ml). The urease enzyme solution consisted of 333 mM urea, 100 mM $CaCl_2$ and 1% skim milk (concentration). Bang et al. (2009) measured the strength using

Torvane and conducted wind tunnel tests to measure the amount of soil loss. Their results indicated that the bacteria concentrations were not a significant parameter. Also, their results indicated that the urease enzyme treatment gave the highest strength and resulted in the lowest amount of sand detachment (soil loss).

Meyer et al. (2011) investigated the effect of using MICP for fugitive dust mitigation using wind tunnel tests. They looked at the effect of different concentrations of ureolytic bacteria (*S. pasteurii*), temperature and humidity, and effects of soil type (washed and unwashed sand). The type of soil that was used classified as well-graded sand. Different concentrations of bacteria were used (from 1×10^5 cells/ml to 1×10^9 cells/ml) and samples were cured at different temperatures (20, 30, and 45 degrees Celsius) and two relative humidities (20% and 100%). Meyer et al. (2011) found that a higher concentration of ureolytic bacteria led to the lowest percentage of soil loss. Meyer et al. (2011) observed there was a difference between the washed sand and unwashed sand. They conjectured that the taking away of the finer soil by washing led to filling the voids of the specimen with carbonate precipitation. Moreover, they found that low humidity with high temperature led to decrease in soil loss compared to high humidity with low temperature.

Al Qabany et al. (2012) investigated the optimum level and efficiency of *Sporosarcina Pasteurii* in inducing the precipitation of $CaCO_3$ using medium to coarse sand (grain size between 90 μm to 300 μm). Their results showed that the input rates for urea and $CaCl_2$ should be below 0.042 Molar/L/h and that the concentration of the solution of urea and $CaCl_2$ should be less than 1 M to achieve 100% efficiency. Their study showed that lower concentrations will provide more uniform cementation and less clogging than higher concentrations.

Cheng et al. (2013), performed laboratory tests on two types of sands (Cook Industrial, Minerals Pty. Ltd., Western Australia) fine and course sand respectively. Both of these sands were identified as poorly graded sand with a specific gravity of 2.62. Four different degrees of saturation (20%, 40%, 80%, and 100%) were used. Samples were prepared into a PVC column 16 cm in height with a 5.48 cm inner diameter using percolation method. They performed permeability tests, freeze-thaw durability, UCS, and consolidated undrained triaxial tests. Cheng et al. (2013) report that higher strength with less precipitated carbonate was achieved at lower degrees of saturation than the samples with higher degrees of saturation. In the unsaturated samples, carbonate crystals formed primary at the particle contact points, which may explain why they were stronger compared to the more highly-saturated samples. The results of Cheng et al. (2013) suggest that the location of the precipitated $CaCO_3$ in a sample can be more important than the quantity.

Gomez et al. (2014) showed that MICP can be accomplished via bio-stimulations, i.e., by stimulating indigenous ureolytic bacteria in situ. Soil columns 10.2 cm high by 5.1 cm in diameter were used with filters at the bottom to reduce the loss of sand. Eight different types of sand were used and all of them were classified as poorly graded sand (SP). These samples were prepared at target relative densities of approximately 50%. For the first 10 days, these samples were treated with a stimulation solution containing 0.5 M urea, 0.0125 mol/L ammonium chloride, 0.17 mol/L sodium acetate, and 0.1 g/L yeast extract to stimulate native ureolytic microbes. Next, they were treated with a cementation solution containing 0.5 M urea and 0.25 M calcium chloride from day 11 up to day 20. The cementation solution contained the ingredients of the stimulation solution plus calcium chloride dihydrate. The total amounts of solution applied to each specimen were around

300 ml (1.5 pore volume) twice a day. They found that the highest value for unconfined compressive strength was 5.3 MPa, with 13.2% $CaCO_3$ after 10 days of treatment with the cementation solution.

Gomez et al. (2016) examined MICP via stimulation of indigenous microbes on a larger scale to improve the mechanical properties of soil. They used two large circular tanks, 1.7 m in diameter and 0.5 m as height. In each tank, three wells were employed to inject the combined stimulation, augmentation solution, and cementation solution. They used a local sand, classified as poorly graded sand. The sand was placed with target relative density of 45%. Figure 3 demonstrates the set-up of these two tanks. Each well was injected with a different concentration of the solution. For the first five days of treatment, 0.5 pore volume of stimulation solution was injected into each well. Then followed by 0.75 pore volume of cementation solution into each well. Each well received at the end 1.5 pore volume of solution. Cone penetration tests (CPT) and shear wave velocity tests were conducted before and after treatment. The results showed an increased in the soil's shear wave velocity to 961m/s and CPT tip resistance 30.1MPa from initial values of approximately 131 m/s and 5.3 MPa for the untreated soil.

Figure 3. Large diameter tanks used by Gomez et al. (2016)

PREVIOUS STUDIES OF EICP

Yasuhara et al. (2011) investigated the use of free urease enzyme to induce calcium carbonate precipitation in sand. They used test tube experiments to provide basic assessments of the influence of concentrations of urea, $CaCl_2$, and urease on carbonate precipitation. There were two sets of test tube experiments. The first set of test tube experiments was performed to analyze the rate of urea hydrolysis initiated by urease. The second set was conducted to study the effects of the concentrations constituents on calcium carbonate precipitation. Both experiments demonstrated that high concentrations of $CaCl_2$-urea relative to urease enzyme may inhibit urease effectiveness and activity. These tests also illustrated the relatively high speed of the chemical reaction when using free urease and the importance of the urease to $CaCl_2$ and urea solution ratio.

Yasuhara et al. (2012) conducted unconfined compression tests on Toyoura sand samples treated using EICP with two different concentrations of urea to calcium chloride.

The lower concentration was 0.5 M urea, 0.5 M $CaCl_2$, and 1 g/l00 ml of urease enzyme. The higher concentration was 1 M urea, 1 M $CaCl_2$, and 1 g/l of urease enzyme. In these experiments, urease (Kishida Chemical Co., Ltd.: 020-83242) was mixed into the sand as a powder. Note that no information was provided about the urease activity, if it was high or low. The test specimens were 101.6 mm in height and 50.8 mm in diameter. In these experiments, 100 ml of a $CaCl_2$-urea solution (0.80 pore volume) was injected in the samples. After this mixing, a $CaCl_2$-urea solution was injected into the sand. This injection was repeated between four to eight times in half-hour intervals for both concentrations. The UCS of the treated samples with low concentration, (U:C = 0.5 M), was a maximum of 890 kPa after eight cycles of treatment and 6.76% $CaCO_3$. However, a UCS of 1.6 MPa after four cycles of treatment and 5.1% $CaCO_3$ was obtained when the high concentration of urea and calcium chloride was applied.

Neupane et al. (2013) also conducted test tube tests to find the optimum concentration for EICP for improving soil properties. Their results indicate that higher precipitation efficiency was achieved at lower concentrations of urea and calcium chloride. They used high urease activity (Kishida Chemical, 2950U/g) and concentrations of urease from 1 to 4 g/l. Urea and $CaCl_2$ concentrations of 0.5 mol/L and 1.0 mol/L were used in this study. The precipitation ratio, defined as the ratio of the precipitated mass of $CaCO_3$ to the theoretical maximum mass of $CaCO_3$ was observed to decrease after the urease concentration exceeded 3 g/l, as illustrated in Figure 4. Neupane et al. (2013) postulate that the decline in the precipitation ratio after 3 g/l may have occurred because stored urease solution loses some of its activity within a matter of hours. They also decided that the optimum concentration of urease was 2 g/L due to concerns of the cost of the enzyme and relatively little increase in precipitation ratio above this value.

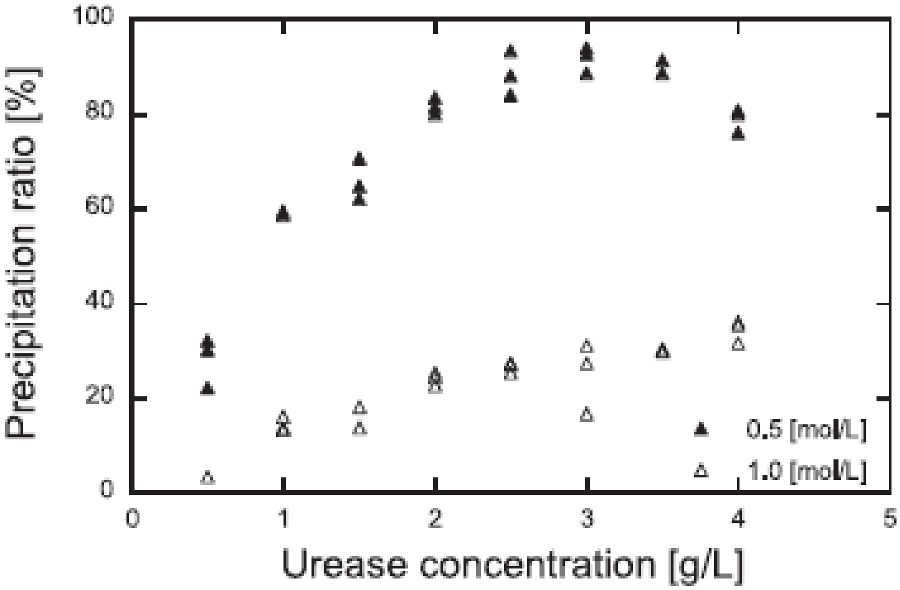


Figure 4: Relationship between urease concentration and CaCO3 precipitation ratio (Neupane et al. (2013))

Neupane et al. (2013) conducted two tests in columns which measured 100 mm in diameter and 200 mm in height using the optimum solution level they postulated from their previous study (0.5 M urea: 0.5 M $CaCl_2$ and 2 g/l of urease enzyme). Toyoura sand was placed into the columns to a height of 100 mm at a target relative density of 60%. Two cycles of treatments were used for each column. The first injection contained 400 ml of the cementation solution, which is equal to 0.75 pore volume based upon the initial 100 mm of sand. After two hours, and after the placement of the additional 70 mm of sand, another 400 ml of the cementation solution was injected. The only difference between the two columns was the addition of 0.1 g/l of green dye to the solution to distinguish the cementation in one column. During the second injection, the cementation solution did not penetrate completely through the soil because of the precipitation that occurred after the first injection. The first injection was under gravity flow. During the second injection, the pressure on the top of the injection tube was increased to 30 kPa to maintain the rate of flow. Following treatment, the columns contained comparable amounts of $CaCO_3$: around 3% at 10 mm beneath the injection tube and 1.6% at 50 mm beneath the injection tube. Subsequently, these investigators treated four drums of soil 850 mm in height and 560 mm in diameter using the same injection technique with 30 L of the solution for each injection. They found that more cycles of treatment resulted in increased precipitation.

Neupane et al. (2015), performed the same experiments described above again and reported that the $CaCO_3$ content was larger nearer to the tube injection point. The maximum value for UCS was found to be 380 kPa after two injections. In these experiments, the main objective was to measure the quantity of carbonate by using the following methods: acid digestion, thermal decomposition, and CO_2 generation. The researchers also sought to determine the effect of ammonium chloride (NH_4Cl) on the carbonate measurement technique. Neupane et al. (2015) found that NH_4Cl affects the results of the acid digestion and thermal decomposition methods, but not the CO_2 evaluation method. They claimed that ammonium chloride does not react with hydrochloric acid. However, this statement may be highly questionable by other researchers as ammonium chloride does react with hydrochloric acid. Neupane et al. (2015) therefore concluded that the CO_2 evaluation method is more accurate than the acid digestion and thermal decomposition methods for measuring the amount of calcium carbonate.

Putra et al. (2016) discuss the addition of magnesium chloride to urea, $CaCl_2$, and urease enzyme to modify the precipitation process. The concentration of magnesium chloride to urea, $CaCl_2$ was fixed 0.50 mol/l, and 1 g/l of urease. Poorly graded sand and high-activity urease enzyme (2950U/g) were used in this experiment. They prepared their samples in PVC columns 50.8 mm in diameter and 101.6 mm in height. They injected 75 ml of the solution (one pore volume) into the samples at slow rates. After curing, the samples achieved an UCS of 0.6 MPa, though no details are available regarding the number of treatment cycles. Putra et al. (2016) report that adding Mg raised the precipitation ratio to 90% of the theoretical maximum precipitation. Moreover, Scanning Electron Microscopy (SEM) showed that larger calcite deposits formed when magnesium was added to the solution.

Knorr (2014) investigated the use of EICP to control fugitive dust through wind tunnel tests. He prepared over 50 pans with three different types of soil and varied concentrations of urea and calcium chloride, from concentrations as low as 0.1 M up to concentrations as high as 2.0 M, but with a fixed concentration of urease enzyme of 0.5 g/l. The pans were 229 mm in diameter and 25 mm deep, and the sands used were native Arizona soil, F-60 silica sand, and mine tailing. In these experiments, 175 ml of EICP solution was sprayed onto the soil filled pans. The pans were then allowed to set for a week before running the wind tunnel tests. Knorr (2014) reports that the detachment of the treated samples took place at a wind velocity greater than 25 m/s (25 m/s was the top speed of the wind tunnel, for safety concerns). On the other hand, the detachment speed for untreated samples was on the order of 8 m/s. His results indicated that the higher concentrations of urea and calcium chloride led to increased wind erosion resistance SEM images showed that there was carbonate precipitation on the surface of the sand.

Kavazanjain and Hamdan (2015) used free urease enzyme to induced carbonate precipitation in two columns containing different types of soil: F-60 silica sand and Ottawa 20-30 sand. They prepared their samples in 152 mm x 51 mm containers by mix-and-compact and injection methods. The solution concentrations that were used was 1.38 M urea, 1.58 M calcium chloride (both reagent grade, Sigma-Aldrich), 0.4 g/L of urease enzyme (low-grade, Jack Bean urease), and 4 g/L of dried non-fat milk. Each column received 100 ml of the EICP solution. The mix-and-compact treatment, resulted in an UCS strength of 529 kPa at 2.82% $CaCO_3$ (w/w) for Ottawa sand and 392 kPa at 4.3% $CaCO_3$ (w/w) for F-60 silica sand.

Kavazanjain and Hamdan (2015) prepared three PVC columns with dimensions of 304.8 mm x 101.6 mm treated by injection method. A 330 mm (13")-long Tygon laboratory tube (R-3603 PVC; 3/8" ID) was perforated with holes 11/64" in diameter, and tube was placed at the center of PVC columns. The tube was wrapped with 250 µm polypropylene mesh (29% open area) to mitigate the potential for clogging of the holes with sand. The solution concentrations that were used was 1.38 M urea, 1.58 M calcium chloride (both reagent grade, Sigma-Aldrich), 0.4 g/L of urease enzyme (low-grade, Jack Bean urease), and 4 g/L of dried non-fat milk. Each column received 700 ml of EICP solution. They reported that they were not able to conduct UCS tests on the 304.8 mm x 101.6 mm columns treated by injecting a cementation solution containing free urease, they had to use a hammer and chisel to extract these samples from the columns. SEM and X-ray powder diffraction (XRD) on extracted samples indicated the presence of calcite in all tests.

Hamdan (2015) and Hamdan et al. (2016) studied the use of xanthan gum, guar gum, and inert polyol-cellulose hydrogels to improve the efficiency of EICP in unsaturated soil via surficial spraying and percolation. They hypothesized that the viscosity of these mixtures would direct the precipitation reaction to the contact points between soil particles. They tested their hypothesis through two experiments. The first one used 50 ml glass beakers to assess the precipitation of EICP without adding soil. The second experiment involved filling paper cups with F-60 silica sand and pouring the EICP solution with and without hydrogel onto the soil. The ratio of Urea to $CaCl_2$ was always 1.5:1 in these experiments, but the initial concentration of Urea was varied from 2 M to 0.4 M. Using both high and low activity of urease enzyme. From the glass beaker experiment, through the observation of gas bubbles, these investigators deduced that hydrogel can reduce gas generation (i.e., ammonia (NH_3) and carbon dioxide (CO_2)) without any effect on carbonate precipitation. The paper cup experiments illustrated that including xanthan gum and guar gum in the cementation solution resulted in less penetration and harder crust thickness than inert polyol-cellulose. Hamdan et al. (2016) report that salts were not removed after rinsing specimens treated with a cementation solution containing a hydrogel because the hydrogel polymer coated the sand particles and was not easily removed.

CHAPTER 3

BASELINE INVESTIGATION ON ENZYME INDUCED CALCIUM CARBONATE PRECIPITATION

INTRODUCTION

This chapter presents a baseline study on enzyme induced calcium carbonate precipitation (EICP), a bio-inspired technique for soil improvement. This study aims to investigate the primary aspects of EICP-treatment of soil. For this purpose, a series of test tube experiments were carried out to determine the optimum recipe for EICP treatment. Next, the effectiveness of two different soil treatment methods (i.e., percolation and mix-and-compact) was evaluated. The mineralogy of the precipitates generated using both methods was examined. The effect of providing calcite seeds in the soil as nucleation sites on mineralogy was also evaluated. Treatment effluent was evaluated to see if it can be used as a source of enzyme and as a source of substrates for further treatment. The effect of water rinsing on treated soil samples (to wash out the soluble substances, e.g., salts) was also assessed.

Sustainability concerns associated with conventional soil improvement techniques have motivated geotechnical engineers to move toward biologically-based solutions. The techniques which have been so far introduced in this regard can be divided into bio-mediated techniques which use living organisms for soil improvement, and bio-inspired techniques which mimic biological processes without any participation of living organisms. Microbially induced carbonate precipitation (MICP), a bio-mediated technique, has received the most attention by researchers studying biological-based-solutions (Chu et al. 2012; DeJong et al. 2006; Montoya and DeJong 2015; van Paassen 2009; Whiffin et al.

2007). In MICP, calcium chloride, urea, ureolytic bacterial cells, and appropriate nutrients for bacterial growth are introduced into granular soil to precipitate calcium carbonate. Calcium carbonate precipitation improves the strength, stiffness, and dilatancy of the soil by pore-filling, particle roughening, and inter-particle binding (Yang et al. 2016). MICP will also decrease the permeability of the soil, though the effect is minimal until relatively larger amount of carbonate is precipitated (Cuthbert et al. 2013).

In MICP, ureolytic bacteria play a catalyzing role through generation of urease enzyme. Bacterial cells also act as nucleation sites through adsorption of calcium ions to their negatively charged surface, creating localized supersaturation (Al-Thawadi 2008). However, relying upon bacteria as the source of urease for MICP creates many unknowns and uncertainties (e.g. physical non-homogeneity, limited oxygen availability, complex pore fluid chemistry, presence of other microorganisms, etc.) that can raise difficulties. These difficulties may include controlling bacterial growth and their enzymatic activity, bacterial cell attachment to soil particles, and interaction between target species and other microorganisms in soil. In addition, MICP via bio-augmentation (i.e., by introducing exogenous bacteria to the soil) in general cannot be applied into soil with pores (or more properly pore throats) smaller than the ureolytic bacteria. Bio-stimulation, in which indigenous ureolytic bacterial cells are activated in-situ, has been developed to overcome some of these difficulties (Burbank et al. 2011 and 2012; Gomez et al. 2014 and 2016).

EICP is expected to eliminate some of the challenges caused by using bacterial cells. In EICP, free urease enzyme is used as the catalyst for hydrolysis of urea. Urease can be isolated from plants and plant product such as jack beans and watermelon seeds. Free urease has a size on the order of 12 nm per subunit (Blakely and Zerner 1984) and is soluble in water, facilitating its transport within the pores of soil. Using EICP, there are no concerns about oxygen availability for deep soil treatment, providing nutrients for bacterial activity, the competing effect of other microorganisms, or cell attachment to soil particles. However, the EICP approach lacks the nucleation sites provided by bacteria, which may cause disorder in precipitation morphology. The rapid rate of precipitation that can be associated with EICP can also adversely affect carbonate morphology. Furthermore, the urease enzymes available in the market are also expensive, as they are of medical grade and are produced in small quantities.

FALCON TUBE TESTS

Optimum EICP solution

Initial tests were carried out without soil in 50-ml Falcon test tubes containing 20 ml of EICP solution. The EICP solutions were prepared by dissolving different concentrations of calcium chloride, urea and enzyme into deionized water. The calcium chloride dihydrate, $CaCl_2.2H_2O$, and urea used is this study were from VWR. The enzyme used throughout the study was low activity jack bean-extracted urease without a specific average activity from Fisher Scientific. However, the results from testing at ASU showed that the average activity of the urease used in this study was around3500 U/g. In order to remove impurities, the enzyme solution was filtered using 0.45 μm syringe filters. Fresh enzyme solution was always used. Various concentrations of $CaCl_2$, urea, and urease enzyme were tested in order to find the optimum EICP solution. Table 1 presents the 37-different concentrations of constituents used in these tests. The electrical conductivity of the calcium chloride and urea in solution, hereinafter called initial electrical conductivity of EICP solution, was measured prior to adding enzyme. The initial electrical conductivity represents the ionic strength of the solution and is a measure of the concentration of urea and calcium chloride in EICP solution.

Table 1. Summary of Falcon Tube Tests

#	[Urea] (M)	[CaCl$_2$] (M)	CaCl$_2$:Urea	EC (mS/cm)	Enzyme (g/l)
1	1.50	1.50	1:1	128.9	5
2	1.50	1.50	1:1	128.9	3
3	1.50	1.50	1:1	128.9	1
4	1.00	1.00	1:1	108.8	6
5	1.00	1.00	1:1	108.8	5
6	1.00	1.00	1:1	108.8	3
7	1.00	1.00	1:1	108.8	1
8	0.50	0.50	1:1	64.73	5
9	0.50	0.50	1:1	64.73	3
10	0.50	0.50	1:1	64.73	1
11	0.25	0.25	1:1	37.44	5
12	0.25	0.25	1:1	37.44	3
13	0.25	0.25	1:1	37.44	1
14	0.25	0.17	1:1.5	26.68	1
15	0.25	0.17	1:1.5	26.68	3
16	0.25	0.17	1:1.5	26.68	5
17	0.61	0.41	1:1.5	55.9	1
18	0.61	0.41	1:1.5	55.9	3
19	0.61	0.41	1:1.5	55.9	5
20	1.00	0.67	1:1.5	80.86	1
21	1.00	0.67	1:1.5	80.86	2
22	1.00	0.67	1:1.5	80.86	3
23	1.00	0.67	1:1.5	80.86	5
24	1.00	0.67	1:1.5	80.86	6
25	0.25	0.14	1:1.75	22.94	1
26	0.25	0.14	1:1.75	22.94	3
27	0.25	0.14	1:1.75	22.94	5
28	0.61	0.35	1:1.75	49.47	1
29	0.61	0.35	1:1.75	49.47	3
30	0.61	0.35	1:1.75	49.47	5
31	1.00	0.57	1:1.75	70.74	1
32	1.00	0.57	1:1.75	70.74	2
33	1.00	0.57	1:1.75	70.74	3
34	1.00	0.57	1:1.75	70.74	5

35	1.00	0.83	1:1.2	94.5	6
36	1.00	0.83	1:1.2	94.5	5
37	1.00	0.83	1:1.2	94.5	3

The test tubes were incubated at 200 rpm and 30 °C for at least 72 hours using an Incubator Shaker. The precipitates in each tube were collected using ashless filter paper, thoroughly rinsed with deionized water, and then dried at 50 °C until a constant mass was achieved. The remaining mass of the precipitates was measured. Precipitation mass versus the theoretical maximum based upon the $CaCl_2$ concentration was defined as the precipitation ratio. Precipitation ratio versus electrical conductivity and enzyme concentration were also evaluated.

Reusability of effluent

The effluent from some of the 50 ml Falcon tube treatment process was tested to determine if it can be used as a source of enzyme and/or substrates for further treatment. To check the availability of active enzyme in effluents, 15 ml of 0.25 M equimolar calcium chloride and urea solution was added into 15 ml fresh filtered effluents from the tests with a precipitation ratio close to 1.0, i.e. the tests with almost no more calcium ions available in the effluent at the end of the experiment (Tests No. 23, 24, 35 and 36). To check reusability of unconsumed substrates in the effluent, the effluent from tests with a precipitation ratio significantly less than 1.0 were employed. For this purpose, 15 ml of 4 g/l and 2 g/l fresh enzyme solution were added into 15 ml of the filtered effluents collected from the tests No. 20 and 21, respectively, with precipitation ratios of 24% and 63%, respectively (i.e., stoichiometrically, effluent of the test No. 20 contained 0.42 M calcium chloride and 0.63 M urea unconsumed, and the effluent of the test No. 21 contained 0.25 M calcium chloride and 0.37 M urea unconsumed).

Effect of Calcite Seeds on Mineralogy of the Precipitates

In order to investigate the effect of calcite seeds on the mineralogy of the precipitates, Test No. 22 was performed with 1% calcite seeds (by mass of the maximum theoretical carbonate precipitation) added into the EICP solution.

Falcon Tube Test Results

Results of the Optimum EICP solution

The Falcon tube tests were carried out to find the optimum recipe for EICP solution for use in further soil treatment. The optimum EICP solution was considered to be the best concentration of ingredients (i.e. calcium chloride, urea and urease enzyme) resulting in both high precipitation mass and precipitation ratio. The objective was to minimum consumption of enzyme for production of the desired quantity of carbonate as the enzyme is the most expensive component of the EICP solution. In this study, considering the price for the laboratory grade chemicals, the enzyme constitutes 57% - 98% of the cost of the EICP solution. Precipitation mass and precipitation ratio for each test concentrations are presents in Table 2.

Changes in precipitation ratio versus enzyme concentration and initial electrical conductivity of EICP solution are illustrated in Figures 5a,5b, and 5c. The initial electrical conductivity of the solution changes as the concentration of calcium chloride and urea is varied as illustrated in Figure 6. As an ionic compound, the increase in conductivity with increasing concentration of calcium chloride is expected. However, it is observed that urea slightly suppresses the ionic strength of the solution at higher concentrations even though it is a covalent compound. For instance, the electrical conductivity of the solution containing 1 M calcium chloride and 1 M urea is 108.8 mS/cm while for the solution with the same concentration of calcium chloride and 1.5 M urea the electrical conductivity is 104.5 mS/cm. The influence of urea on electrical conductivity could be attributed to a reduction in mobility of ions in solutions containing a high concentration of urea.

Table 2. Precipitation mass and precipitation ratio for the Falcon tube tests

#	Precipitation mass (g)	Precipitation ratio (%)
1	0.79	26.34
2	0.60	19.92
3	0.09	3.03
4	1.84	71.24
5	1.22	60.90
6	0.98	48.91
7	0.18	8.89
8	1.00	100
9	1.00	99.61
10	0.57	56.85
11	0.48	95.32
12	0.49	97.92
13	0.49	98.72
14	0.32	95.76
15	0.33	97.82
16	0.33	99.91
17	0.69	84.49
18	0.80	98.32
19	0.83	100
20	0.32	23.7
21	0.84	62.61
22	1.25	93.32
23	1.33	99.31
24	1.38	100
25	0.29	99.56
26	0.28	98.17
27	0.28	97.82
28	0.69	98.91

29	0.70	99.63
30	0.71	100
31	0.39	34.12
32	0.80	69.64
33	1.14	99.30
34	1.14	100
35	1.73	100
36	1.72	100
37	1.25	74.79

Figures 5a demonstrates a meaningful correlation between precipitation ratio and initial electrical conductivity of EICP solution for a given enzyme concentrations. Figure 5b shows that the precipitation ratio is increased by increasing the amount of enzyme per given concentration of calcium chloride and urea. Contrary to the findings of Neupane et al. (2013), a high enzyme content did not lead to a reduction in precipitation ratio (for concentrations up to 6gm/l). However, the rate of increase in precipitation ratio declines as the precipitation ratio approaches 100%. Figure 5a also shows that the precipitation ratio considerably drops in the solutions with an initial electrical conductivity higher than a specific value, hereinafter named the critical electrical conductivity ($EC_{critical}$). The value of ($EC_{critical}$) depends upon enzyme concentration. The $EC_{critical}$ for each enzyme concentration represents the maximum concentration of calcium chloride and urea for which nearly complete precipitation is achieved (for the given ratio of calcium chloride to urea). The higher the value of $EC_{critical}$, the greater mass of precipitation. These results indicate that there is an amount of urease enzyme for an EICP solution with a certain initial electrical conductivity below which the precipitation ratio drastically drops. This observation may be attributed to denaturation and/or precipitation (salting-out) of proteins within a solution containing high concentrations of calcium chloride and urea.

Figure 5a, 5b, 5c and 6, provide a systematic way for estimation of precipitation mass and precipitation ratio for a given concentration and proportion of calcium chloride, urea and urease enzyme. Figure 6 gives the initial electrical conductivity for each concentration of calcium chloride and urea. The required amount of enzyme to obtain a high precipitation ratio can then be estimated using the graph in Figure 5a.

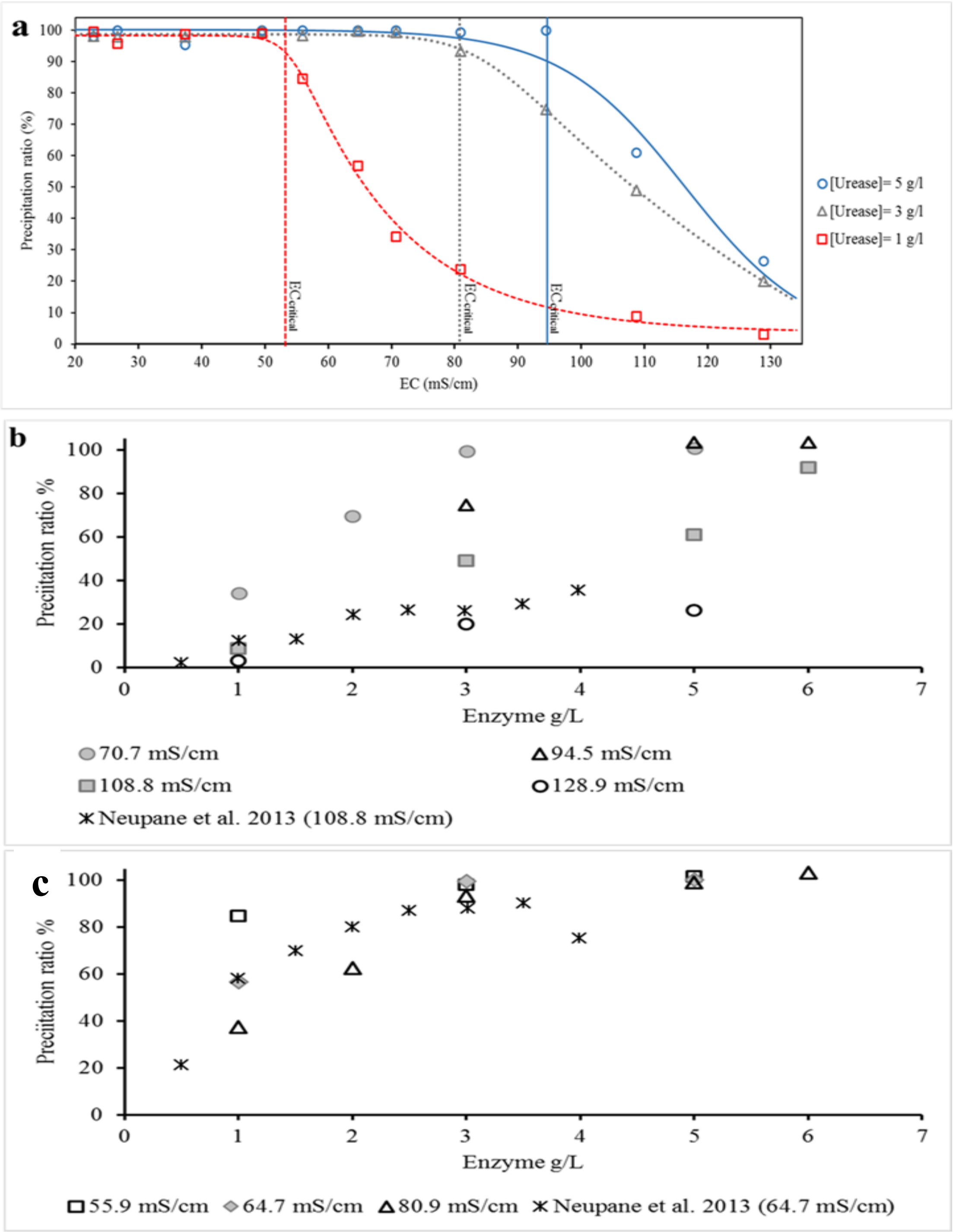


Figure 5 Changes in precipitation ratio versus enzyme concentration and initial electrical conductivity of EICP solution in (a) precipitation ratio and conductivity plane, (b) precipitation ratio and urease concentration plane, and (c) precipitation ratio and

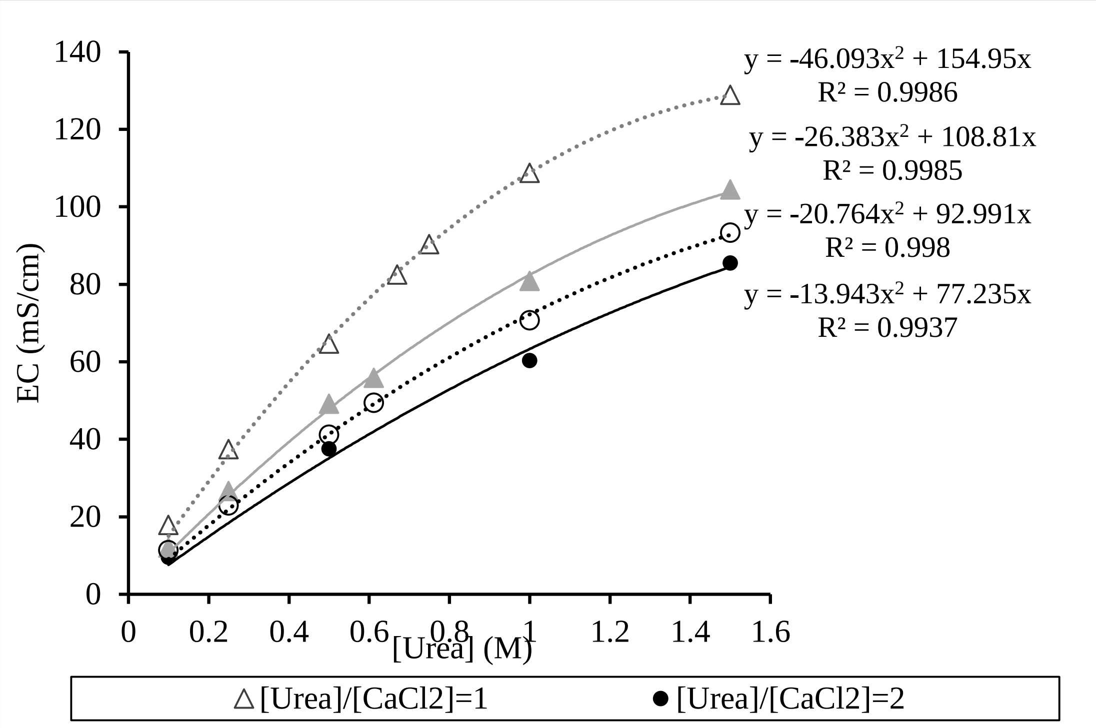


Figure 6. Electrical conductivity versus concentration of calcium chloride and urea.

Figures 5 and 6 were used to find the optimum recipe for an EICP solution in this study. It was determined that although 5 g/l urease provides a higher $EC_{critical}$, it is more cost efficient than to use 3 g/L urease, i.e., 67% more enzyme (5 g/l) only produces a 24% increase in the mass of precipitation. Therefore, on EICP solution with 0.67 M calcium chloride, 1 M urea, and 3 g/l urease enzyme was adopted as the optimum treatment solution for further tests.

Results of Reusability of Effluent

Effluent collected from the initial treatment process was tested for reusability as a source of enzyme and/or substrates. Recycling effluents as a source of urease enzyme for additional usage can substantially reduce the cost of EICP treatment of soil. While enzyme, as a catalyzer, may be expected to remain intact at the end of the reactions, the results of this study yielded no precipitation in any of the tubes containing effluent from previous treatment process. This result suggests that urease enzyme in the previously-used EICP solution is either absent or deactivated (i.e., denatured and/or precipitated (salted-out) during previous precipitation reactions). Excessive increase in ionic strength of a solution can lead to precipitation of proteins. The high chaotropic effect of the ammonium by-product in the solution can cause enzyme denaturation. Chaotropes encourage enzyme extension and denaturation through disrupting the hydrogen bonding network between water molecules and consequently weakening the hydrophobic effect.

To check reusability of effluent as a source of substrates, fresh enzyme was added into the effluent obtained from a residual EICP solution from tests with a precipitation ratio less than 1 (i.e. an EICP solution containing unconsumed calcium chloride and urea). It was observed that adding fresh enzyme solution into the effluents obtained from the tests No. 20 and 21 resulted in 0.49 g and 0.17 g calcium carbonate precipitation, respectively. Subtracting the amount of calcium ions precipitated at previous stage, these amounts of precipitation correspond to a precipitation ratio 79% and 46% for the effluents of tests No. 20 and 21, respectively. Note that a fresh EICP solution with the same concentration of calcium chloride, urea and enzyme gives a precipitation ratio of nearly 100%. The reduced precipitation ratio in the effluent may be attributed to loss of an enzymatic activity due to the presence of the ammonium by product. Furthermore, a chaotropic agent in the effluents and/or to the ionic strength of the effluent. These factors help explained why the effluent obtained from the Test No. 21, which contains a higher ammonium content from the previous precipitation process than the effluent obtained from Test 20, yielded a lower precipitation ratio than Test No. 20.

Additional Falcon tube tests were performed after subsequent testing suggested that the non-fat dried milk used in these experiments had expired and was no longer effective, these tests were conducted in the same manner as described above except that fresh dried non-fat milk was used in the EICP solution. Again, the test results suggest that urease enzyme in the effluent from EICP treatment cannot be reused for further EICP treatment.

Results of pre-existing calcite seeds on mineralogy of the precipitates

Precipitated calcium carbonate can appear as amorphous (i.e. a non-crystalline phase) and five different crystalline phases (polymorphs of calcite, aragonite, vaterite, monohydrocalcite and ikaite, in decreasing order of stability). Rhombohedral calcite is the most desirable polymorph for geotechnical application due to its thermodynamic stability. For calcite crystallization, the solute molecules in a saturated solution must encounter a solid surface (by chance or by intermolecular force) that acts as a nucleation site (De Yoreo and Vekilov 2003). Dust particles, bacterial cells, nascent crystals, and foreign seed crystals are examples of nucleation sites. In the absence of nucleation sites, the solute molecules move freely in the solution in a random flow. The interaction and collision between solute molecules form a basis for the resulting crystal lattice. Placing nucleation sites into solution allows the solute molecules to diffuse across their surface lattice instead of moving randomly around, i.e., provides a surface diffusion mechanism. In the other words, the surface lattice of the nucleation site provides an arrangement pattern for the solute molecules and subsequent a crystal growth. Hence, seeding the precipitation solution with rhombohedral calcite crystals was expected to facilitate increased rhombohedral calcite precipitation (by mass of maximum carbonate theoretical precipitation). Therefore, calcite seeds were added into several Falcon tubes in order to examine the effect of seeding with calcite on nucleation and the mineralogy of the precipitates. SEM images of the precipitates harvested from the seeded solution show densely aggregated rhombohedral calcite crystals whereas the precipitates from the unseeded solution show a disordered morphology as illustrated in Figure 7. This result suggests that it is more likely to get rhombohedral calcite precipitation from an EICP treatment of a soil already containing calcite, although the presence of inhibiting matter such as magnesium ions and organic compounds may disturb calcite crystal formation (Loste et al. 2003).

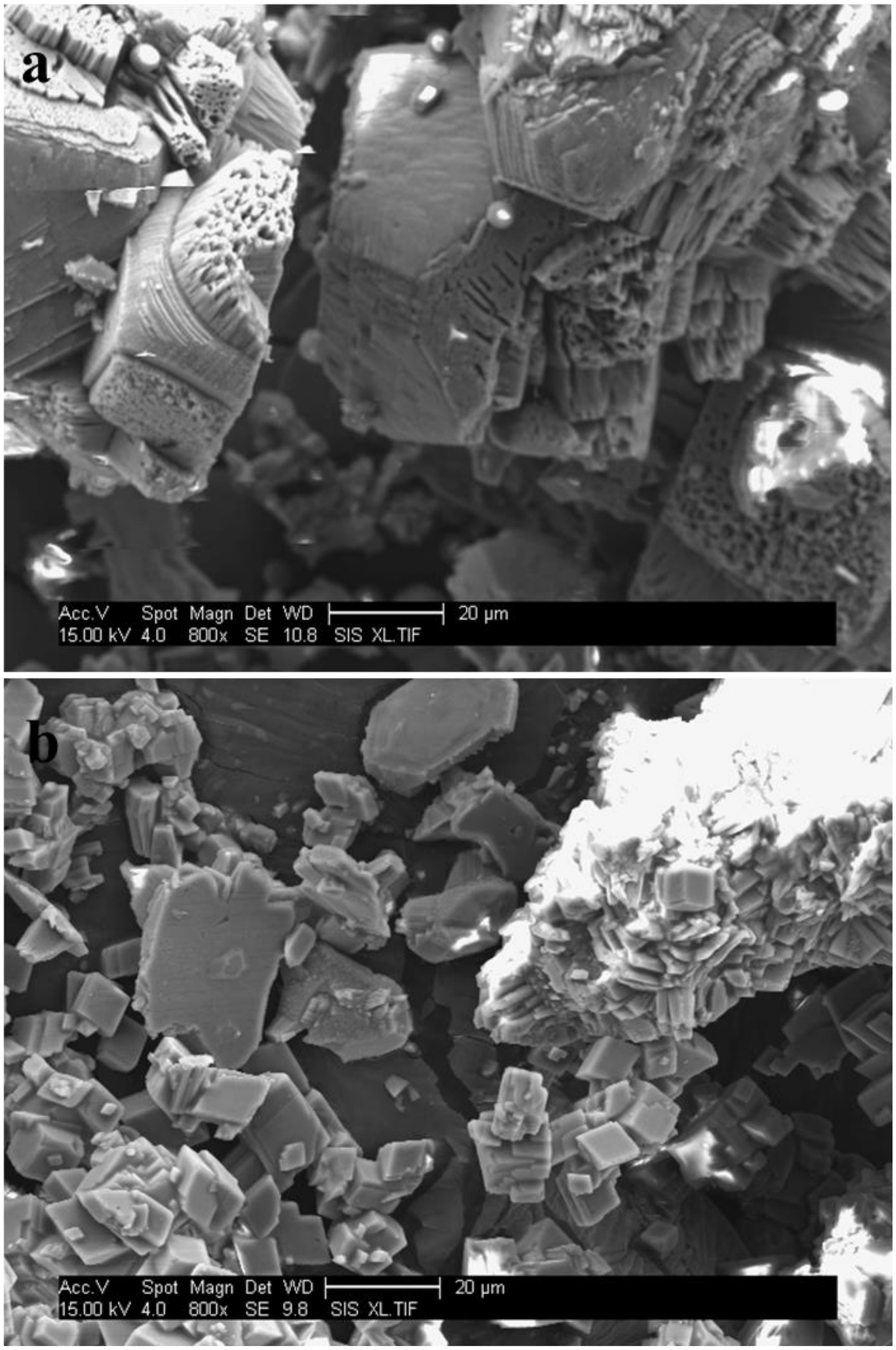


Figure 7 Morphology of the precipitates from (a) unseeded (top), and (b) calcite-seeded EICP solutions (bottom).

SOIL COLUMN TESTS

Soil column treatment

Eight soil columns were treated using the optimum EICP treatment solution (1 M urea, 0.67 M calcium chloride, and 3 g/l enzyme) from the Falcon tube tests. A clear acrylic cylinder 10 cm tall × 5 cm in diameter (4” x 2”) were used for these column tests. Materials used in these experiments, except sand, were cleaned by rinsing them with rubbing alcohol (70% v/v ethanol). As illustrated in Figure 8, a polypropylene liner was placed with the columns to facilitate subsequent extraction and testing of the treated soil. Ottawa 20-30 sand (e_{max}=0.742, e_{min}=0.502, D_{50}=0.72, D_{10}=0.65, G_s=2.65, SiO_2>99%) was used throughout the baseline study. Both percolation and mix-and-compact treatment methods were employed for the first cycle of treatment of the soil columns (4 columns by percolation and 4 columns by mix-and-compact). Percolation was applied for subsequent cycles of treatment in all specimens. In percolation, dry sand was placed into each cylinder at a relative density of around 45% by pluviation. Then, the EICP solution was added from the top of the column with the bottom sealed. For the first treatment cycle in the columns prepared by percolation, 70 ml of cementation solution, corresponding to approximately one pore volume, was introduced to each specimen. To prepare the mix-and-compact column, the amount of dry sand corresponding to a relative density of 45% was quickly mixed with 70 ml of EICP solution prior to placing the mixture into each column. The mixture was placed in three lifts in each column with very gentle compaction to reach a relative density of around 45%. In subsequent cycles of treatment for both the percolation and mix-and-compact columns, the columns were allowed to drain from the bottom by gravity. Four specimens with progressively increasing number of cycles (from 1 to 4) were prepared for each method of sample preparation. 50 ml, 40 ml, and 40 ml of solution was added with the bottom closed for cycles 2, 3, and 4, respectively.

In both methods of sample preparation, the samples were left to cure for 7 days at room temperature after each cycle of treatment. After curing, each column was drained by gravity by perforating the base with a syringe needle and the samples were rinsed with one pore volume (i.e. ~70 ml) of deionized water. After rinsing, specimens were oven-dried at 50 °C until a constant mass was achieved. Unconfined compressive strength (UCS) tests were then conducted to evaluate the strength of the oven dried samples.

After the UCS test, the mass of calcium carbonate precipitated in each sample was determined through acid digestion. In this method, each treated sample was soaked into 4 M Hydrochloric acid (HCl) for 20 minutes to dissolve the carbonate precipitates. The decrease in mass of the specimen before and after acid digestion was taken as the net mass of carbonate precipitation in the soil.

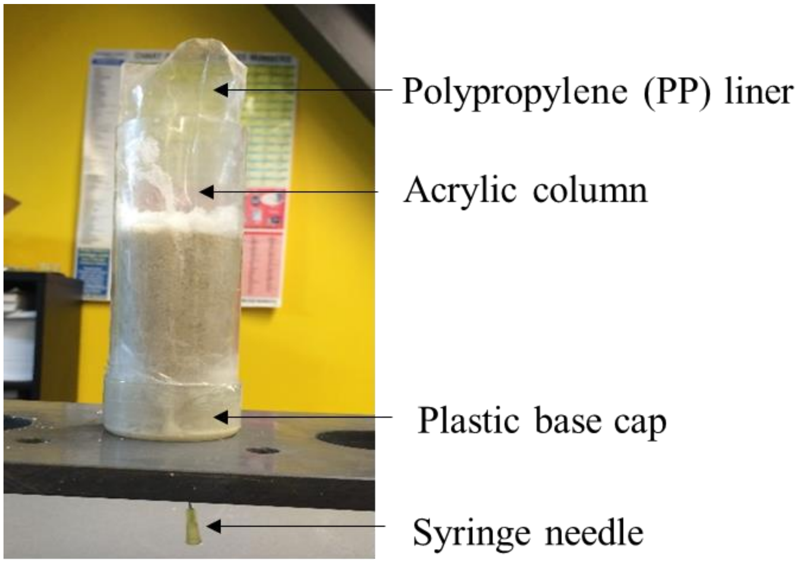


Figure 8. Layout of the soil columns used for treatment

Effect of relative density on strength of the treated samples by using mix-and-compact

The samples treated using mix-and-compact to a relative density of 45% were not strong enough for UCS testing. In order to check if higher relative densities can influence effectiveness of treatment, two columns were treated for one cycle of treatment using mix-and-compact at relative densities of 63% and 92%.

Micro Scale Identification Analysis

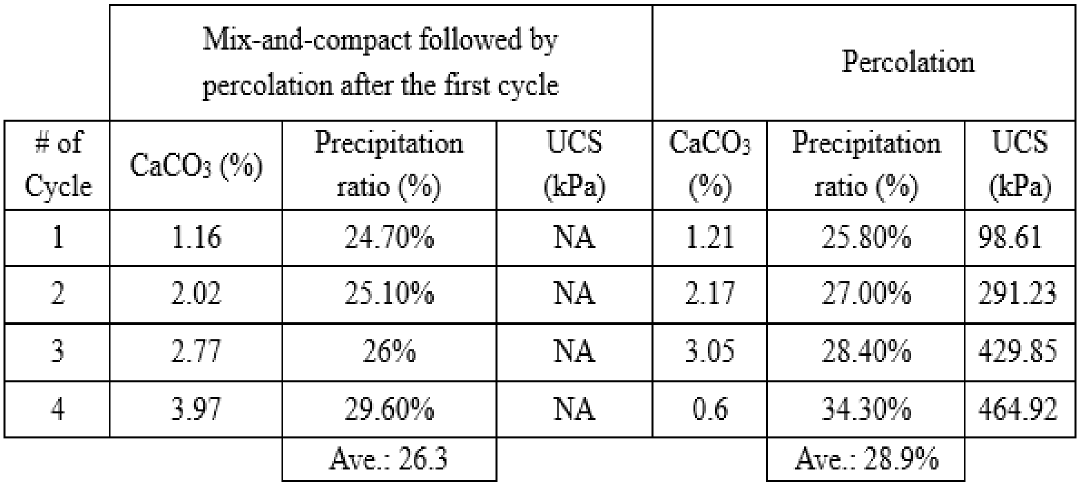
Scanning electron microscopy (SEM) was employed to visualize the precipitates. X-ray diffraction analysis (XRD) was used to identify the crystalline phases in the precipitates. Fourier transform infrared (FTIR) spectroscopy, which identifies the chemical bonds in a molecule, was performed to examine the presence of compounds in the precipitates including amorphous and organic substrates.

Soil Columns Results

Effect of EICP treatment on soil columns

The effect of EICP treatment on carbonate precipitation in the ten Ottawa 20-30 soil columns treated by the mix-and-compact and percolation methods was investigated through UCS testing. The optimum treatment solution established based upon the Falcon tube tests (i.e. 1 M urea, 0.67 M calcium chloride, and 3 g/l enzyme) was applied to all columns. The carbonate content, precipitation ratio, and UCS strength for the columns that prepared with an initial relative density of 45% presented in Table 3.

Table 3. Results of EICP treatment of soil columns with relative density of 45% by using mix-and-compact and percolation.



	Mix-and-compact followed by percolation after the first cycle			Percolation		
# of Cycle	$CaCO_3$ (%)	Precipitation ratio (%)	UCS (kPa)	$CaCO_3$ (%)	Precipitation ratio (%)	UCS (kPa)
1	1.16	24.70%	NA	1.21	25.80%	98.61
2	2.02	25.10%	NA	2.17	27.00%	291.23
3	2.77	26%	NA	3.05	28.40%	429.85
4	3.97	29.60%	NA	0.6	34.30%	464.92
		Ave.: 26.3			Ave.: 28.9%	

As expected, the amount of precipitation increased with increasing the number of treatment cycles for both methods. However, the precipitation ratio was around 25% for all cycles for both types of sample preparation method. Even though the Falcon tube tests indicated that the concentration ratio employed should be able achieve about 100% precipitation ratio, a maximum precipitation ratio of 34% was obtained. This reduced precipitation ratio may be ascribed to insufficient interaction between urease molecules and urea molecules in the soil pores due to the absence of the mechanical mixing (i.e., centrifuge mixing) as employed during the Falcon tube tests. To evaluate this hypothesis, Falcon tube tests with the optimum treatment solution were performed with no centrifuge shaking. Results yield a precipitation ratio of less than 50%, substantiating the hypothesis that shaking significantly influences the precipitation ratio.

As noted preciously, all the columns with an initial relative density of approximately 45% treated via mix-and-compact did not have sufficient cementation for

UCS testing. As illustrated in Figure 9, there were large intact chunks of cemented sand in these columns. However, these columns fell apart after removing the polypropylene (PP) liner. The pattern of behavior illustrated in Figure 9 indicates that calcium carbonate precipitation did not thoroughly bind the sand particles between lifts of soil in mix-and-compact method.

It seems the mix-and-compact method results in more precipitation on the particles surface and in the pores due to disturbance during mixing and placing the sand into the columns whereas percolation provides more precipitation between particles (resulting in more binding of particles together). Samples treated by percolation remained intact upon removal from the acrylic columns, suggesting more continuous cementation than mix-and-compact method. The SEM images in Figure 10 shows aggregation of precipitates along what appears to be a flow path resulting from a sample prepared by percolation. Inter-particle binding leads to an increase in compressive strength. Figure 11 presents the unconfined compressive strength of the treated samples via percolation versus the mass percentage of precipitation (i.e. mass of precipitates to initial mass of dry sand, $CaCO_3$ (%)). This figure suggests a strength increase of around 115 kPa per percentage of carbonate precipitation.

Figure 9. EICP-treated samples by using mix-and-compact (left) and percolation (right) methods.

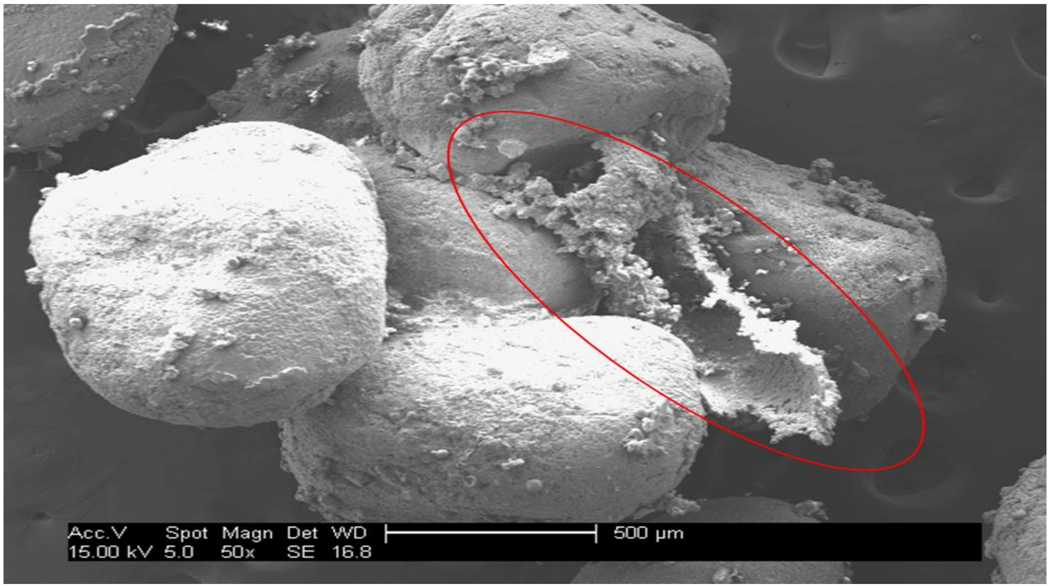


Figure 10 Precipitation between particles along flow path in percolation method.

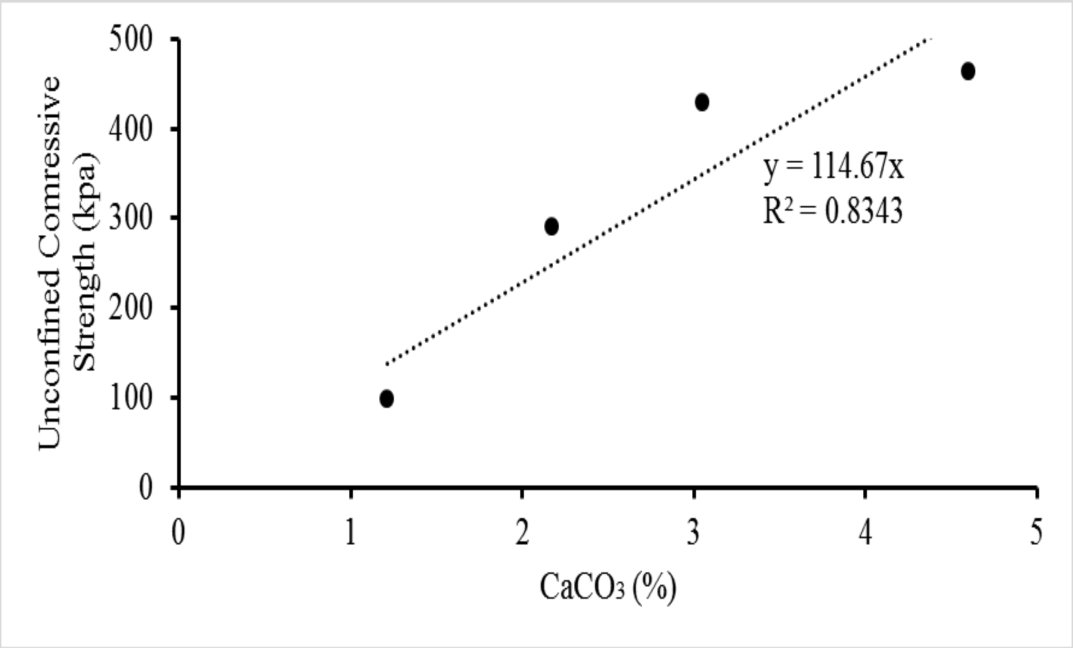


Figure 11. Strength versus percentage of precipitation mass in EICP-treated samples prepared using percolation method.

The efficiencies of the mix-and-compact treatment method at higher initial relative density levels (D_r = 63% and 92%) was evaluated to see if the mix-and-compact method is sufficient to bind the particles together at higher particle densities. Table 4 compares the carbonate content and UCS strength for samples prepared using mix-and-compact at relative density of 63% and 97% subject to a single cycle of treatment to the sample that was prepared at a relative density of 45% using mix-and-compact and subject to 4 cycles of treatment. The soil columns treated at the higher compaction levels were intact enough to perform unconfined compression tests, even at lower carbonate contents. This observation indicates that the mechanism of strength formation in EICP-treated specimens is not necessarily governed entirely by the amount of precipitation, but may also influenced by particle packing (i.e., density).

Table 4. Strength of EICP-treated columns by using mix-and-compact.

# of Cycle	Mix-and-compact		
	D_r	$CaCO_3$ (%)	UCS (kPa)
4	45%	3.97	NA
1	63%	0.8	72.5
1	92%	0.75	88.8

Effect of Water Rinsing on EICP-Treated Sand

Rinsing the treated columns with DI water caused some samples that were prepared at a relative density of 45% using mix-and-compact or percolation to crumble or completely lose their strength. This observation suggests that the precipitate produced by EICP-treatment may contain organic compounds, inorganic salts, and amorphous and/or metastable polymorphs of calcium carbonate which are fully or partially soluble in water. SEM, XRD and FTIR analysis were performed to examine the mineralogy of the precipitates before and after water rinsing. SEM images of the precipitates before rinsing, presented in Figure 12a, manifest an organic crust covering the sand particles and binding them together. In SEM images in Figure 12b of particles after rinsing, the organic crust can no longer be observed. Figure 12b displays rhombohedral calcite crystals cladding the particle surface. XRD spectra of the precipitates, presented in Figure 13a, showed the presence of ammonium chloride, quartz and calcite crystals prior to rinsing. However, in the XRD results after rinsing, presented in Figure 13b, the peaks corresponding to ammonium chloride have disappeared. These results indicate that ammonium chloride salts remained in treated soil after evaporation of EICP solution during drying of the samples. The FTIR spectra of the precipitates presented in Figure 14 are consistent with the spectrum associated with the unrinsed precipitates as it is comprised of major peaks for calcite, quartz and ammonium chloride, whereas the first ammonium peak is absent in the spectrum associated with the rinsed sample. Furthermore, small sharp peaks at 1480-1520 cm^{-1} and a small broad peak at 1660 cm^{-1} in the spectrum of the unrinsed sample may be attributed to the presence of organic compounds, as these bands represent amide bonds in protein structures (Kong and Yu 2007). The FTIR spectrum of the unrinsed sample does not exhibit all the peaks corresponding to the bonds in protein structure, which may be due to overlapping the peaks and very tiny mass of organic compound compared to mass of quartz, ammonium chloride and calcite. Lack of the sharp peak at ~711 cm^{-1} is indicative of a distinction between amorphous $CaCO_3$ and calcite (Muller et al. 2015). However, this was not observed which could be due to the overlap between amorphous $CaCO_3$ and calcite.

Overall, these results indicate that water rinsing may dissolve the inter-particle binding created by precipitation of organic compounds and ammonium chloride, which consequently causes changes in mechanical properties of an EICP-treated soil. Since these water-soluble compounds are likely to be leached from the soil in the field, it is suggested that all EICP and MICP treated soils be thoroughly rinsed with water prior to strength or stiffness testing to remove soluble precipitates which may falsely contribute to the soils.

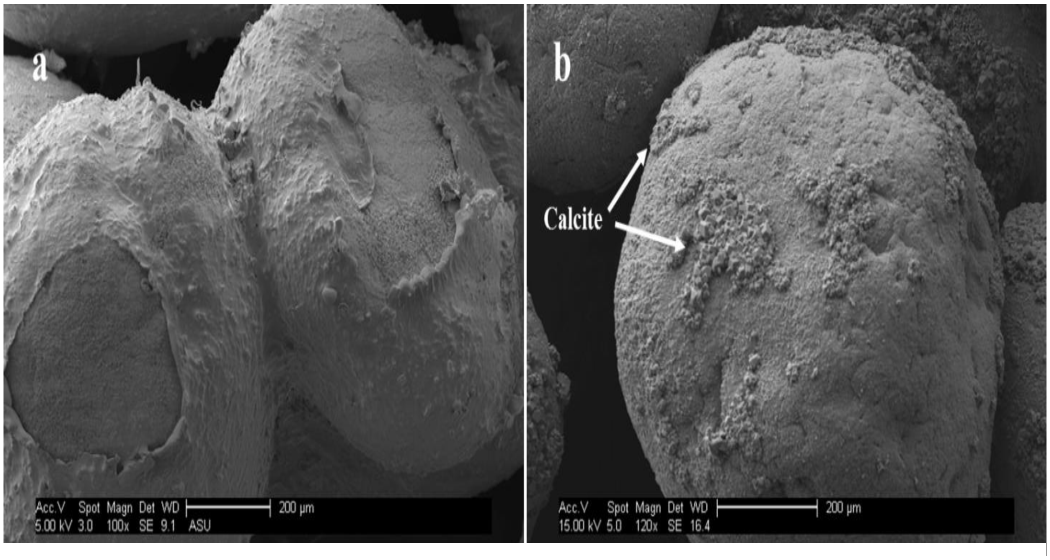


Figure 12 SEM micrographs of EICP-treated soil (a) before and (b) after water rinsing.

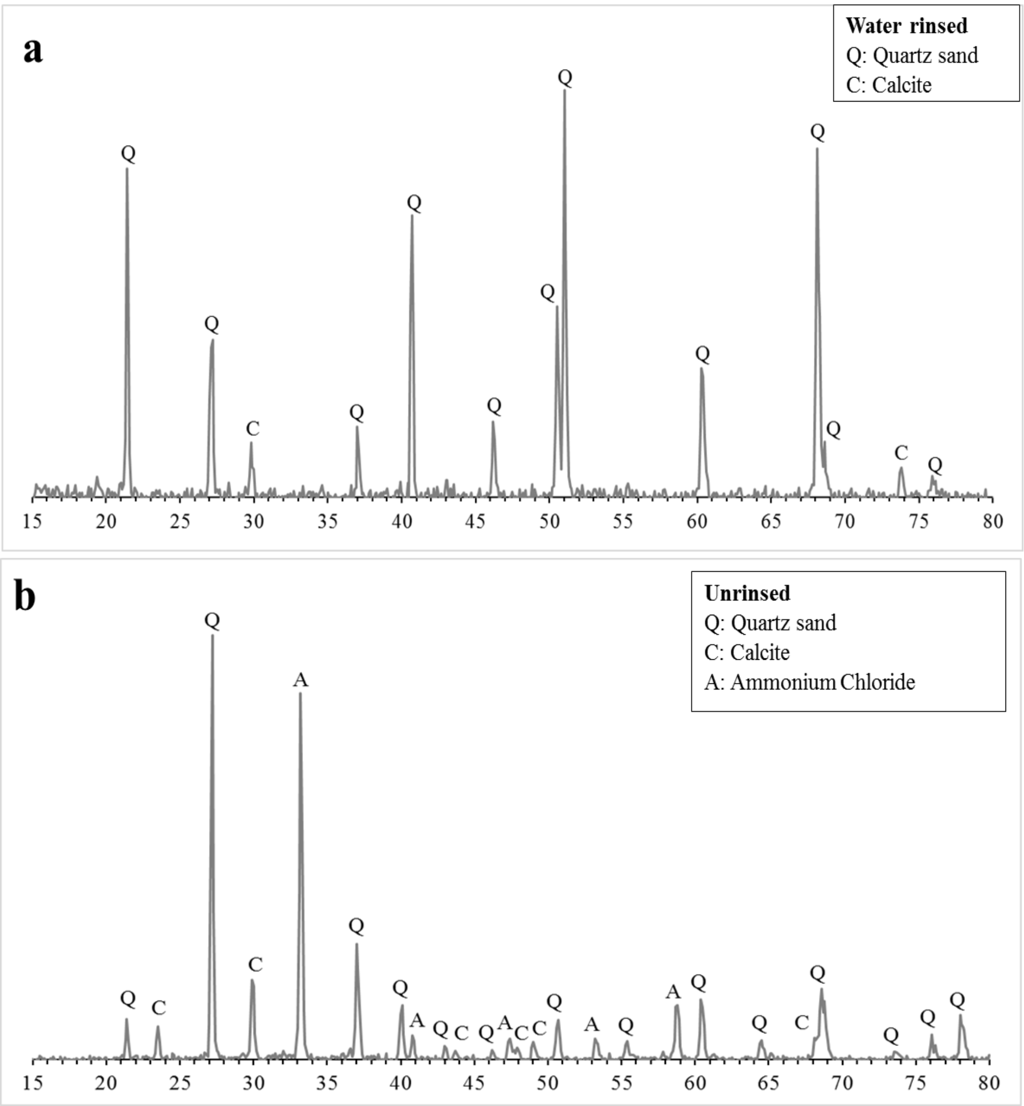


Figure 13. XRD analysis of the treated soil (a) before and (b) after water rinsing.

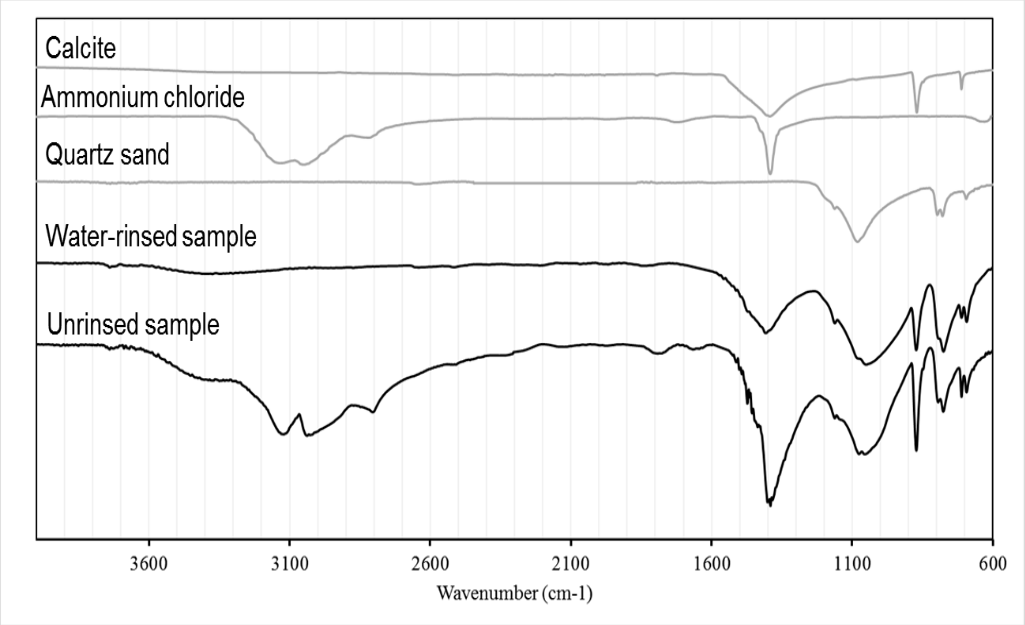


Figure 14. FTIR spectra of the treated soil before and after water rinse.

Effect of Calcite Seeding

A test was also conducted in which the calcite seeds were mixed into the soil before the EICP solution was applied. The UCS of the sample treated in this manner was 1268 kPa after four cycles of treatment, compared to the UCS of 464 kPa obtained after four cycle treatment of soil without the addition of calcite seeds. However, the calcite content of the specimen in which the seeds were mixed with the soil was not measured and precipitation ratio is unknown.

CONCLUSION

A systematic evaluation of precipitation ratio and the mass of precipitation as a function of the concentrations of calcium chloride, urea, and urease enzyme was conducted. Through this systematic evaluation, an optimum recipe for EICP treatment of 1 M urea, 0.67 M $CaCl_2$ and 3 g/l urease enzyme was established. It was also demonstrated that the effluent from prior EICP treatment cannot be used as a source of enzyme for additional treatment. However, unconsumed calcium chloride and urea in the effluent from an incomplete treatment process can be precipitated by adding fresh enzyme, suggesting the potential to reuse the effluent containing unconsumed calcium chloride and urea as a source of these substrates. This is an important conclusion, as the maximum precipitation ratio achieved in treatment of soil columns was only 34%.

SEM images suggests that the presence of calcite seeds in an EICP treatment solution can improve the morphology of the precipitates. An additional test conducted with the calcite sees mixed into the soil before the EICP solution was applied yielded a high UCS (1268 kPa versus 429 kPa). However, the calcite content of the specimen in which the seeds were mixed with the soil was not measured and precipitation ratio is unknown.

It was found that EICP-treatment of soil via the mix-and-compact method may not provide efficient inter-particle binding in soil in a loose condition. The percolation method was observed to be more efficient in inter-particle binding for loose soils. The SEM images suggest that methods which promote more interaction between urease molecules and substrate in the pores during treatment, such as continuous flow, will provide a higher precipitation ratio. Specimens prepared by percolation exhibited a strength increase of around 115 kPa per unit mass percentage of carbonate precipitation. Rinsing the treated soil with DI water caused loss of some precipitates, indicating that EICP treated soil should always be thoroughly rinsed with water prior to measuring mechanical properties (as done in this study).

CHAPTER 4

ENHANCEMENT OF EICP FOR SOIL IMPROVEMENT USING FIBERS AND HYDROGELS

INTRODUCTION

This chapter presents the materials, methods, and results from experiments to enhance Enzyme Induced Carbonate Precipitation (EICP) through the addition of natural fibers (in the form of sisal fibers) and hydrogels to the soil matrix. Hydrogels and sisal fibers can potentially enhance the performance of EICP-treated soil by increasing its strength and ductility, extending its applicability for ground improvement and other purposes.

All soil columns in this chapter were made using the mix-and-compact method with an initial target relative density of 90%. The objectives of the experiments described in this chapter included the following: (1) finding the optimum amount of sisal fibers or xanthan gum hydrogel to mix into the soil to enhance strength and ductility; (2) observing the effects of sisal fibers and hydrogels on binding between two particles using SEM images; and (3) determining how fibers and hydrogels may interfere with EICP. Overall, the question addressed here in this chapter is how can fibers and hydrogels make EICP a more effective process for soil improvement?

EXPERIMENTAL METHODS AND PROCEDURES

Column Preparation

Materials used in these experiments, except sand, were cleaned by rinsing them with rubbing alcohol (70% v/v ethanol). All the soil columns used in these experiments used Ottawa 20-30 silica sand (US Silica Company), a uniform quartz sand with a mean grain size of 0.6 mm. Three different concentrations of Enzyme Induced Carbonate Precipitation (EICP) solution were employed in these experiments.

Columns were prepared in acrylic cylinders 101.6 mm high and 50.8 mm in inner diameter (4” x 2”). While the acrylic columns are clear, a polypropylene (PP) liner was put inside of the cylinders to facilitate extraction of the samples after EICP treatment, i.e. to prevent calcium carbonate $CaCO_3$ precipitation from adhering to the acrylic cylinders. The bottom of the columns was closed with a rubber cap and sealed with silicone adhesive. The top the polypropylene liner was closed between treatments to minimize evaporation of the EICP solution. Figure 15 shows an array of columns undergoing treatment.

Figure 15. Samples were setting on the pedestal after treatment.

For each of three concentrations of solution, two columns were prepared in an identical way to evaluate the reproducibility of the experiments. The EICP solution has two parts: 1) An aqueous solution of calcium chloride dihydrate and urea; 2) an aqueous solution of free urease enzyme and an organic stabilizer (dried non-fat milk). Deionized water was used to make these solutions. Three different concentrations of urea and $CaCl_2$ (both reagent grade, Sigma-Aldrich) were used in these experiments with a fixed concentration of urease enzyme (Fisher Scientific, low-grade Jack Bean Urease, ≈ 1500-2500 U/g activity based on our lab measurement at ASU). The urea: $CaCl_2$ concentrations were 2.65 M:1.51 M, 1.75 M:1 M, and 0.875 M:0.5 M. The cementation solution consisted of 0.85 g/L low activity urease and 4.0 g/L of dried non-fat milk as stabilizer.

In these experiments, every column was prepared using the mix-and-compact method according to ASTM method D2168. First, the cementation solution was poured into a bowl containing the soil, then the soil and solution were thoroughly mixed. Second, the soil was placed into the cylinder in three layers and every layer was compacted approximately 25 times with a small hammer. The hammer weight was 11.56 N (2.6 Ib) and it had a diameter of approximately 10.20 mm. The soil was gently tamped with the hammer. Each column was filled with a mixture of 350 gm (dry mass) Ottawa 20-30 sand and 75 ml (approximately one pore volume) of the cementation solution. After tamping, the top of the first and second layer was scarified prior to adding the next layer in order to increase the interaction between these layers.

After preparing the columns, all column was left on the pedestal (as shown in Figure 15) for three days with the PP liner taped closed with duct tape to minimize evaporation. After four days, the columns were drained by punching holes at the bottom of the rubber caps with a 20-gauge needle. The holes were made with a needle to avoid losing soil from the column (as there was no a screen at the bottom of the columns). The cementation solution that drained from each column was collected and pH of the solution was recorded. Then, all columns were flushed with at least 100 ml (approximately 1.3 per volumes) of 18.2 MΩ deionized (DI) water to flush salt and organic material from the column.

Adding Xanthan Gum to Dry Soil

A biodegradable hydrogel, xanthan gum, was mixed with the dry soil in an attempt to enhance the cementation process. Hamdan (2015) has previously conducted studies on EICP enhanced with xanthan gum to create soil crust for wind erosion control. In that work, the percentage of xanthan gum was about 1.1% by dry weight of soil. Also, Martin et al. (1996) used xanthan gum to enhance the strength and reduce the permeability of a silty soil. They found the optimum amount of xanthan gum was from 1% to 2%. They recommended using around 1% since there was little difference between results for these two percentages.

In the tests conducted herein, 1.1% xanthan gum (by dry weight of sand) was employed. Xanthan gum was added as a powder directly to the soil and mixed with the soil in the bowl before adding the cementation solution. Three different EICP solution formulations were used to create six columns (two with each formulation):

1) 0.875 M of urea; 0.50 M of $CaCl_2$-$2H_2O$; 0.85 g/L urease enzyme and (4.0 g/L stabilizer)
2) 1.75 M of urea; 1.00 M of $CaCl_2$-$2H_2O$; 0.85 g/L urease enzyme and (4.0 g/L stabilizer)
3) 2.65 M of urea; 1.51 M of $CaCl_2$-$2H_2O$; 0.85 g/L urease enzyme and (4.0 g/L stabilizer)

The xanthan gum columns could not be flushed with DI water after compaction due to the xanthan gum clogging the voids. Instead, these columns were soaked in a bowl that contained DI water for 20 minutes. After soaking, columns were put in the oven at 40 C^0 for four days prior to UCS testing.

Adding Xanthan Gum to the EICP Solution

Experiments in which the hydrogel was mixed with the EICP solution employed a similar procedure as that described in the previous Section. The only difference was that the xanthan gum was mixed with urea-$CaCl_2$ solution. Once the hydrogel dissolved in the urea-$CaCl_2$ solution, the urease enzyme and dried non-fat milk were mixed with urea-$CaCl_2$ solution. Then the resulting the EICP solution was added to the soil, mixed in a bowl, and compacted in three lifts into the acrylic cylinders. An additional six columns (two for each EICP mixture) were prepared using xanthan gum mixed with the EICP solution. Similar to the samples created using dry xanthan gum powder, after compaction and curing these columns were soaked in DI water for 20 minutes, dried in the oven at 40 C^o for one month, and then capped for UCS testing.

Adding Sisal Fibers

A set of experiments was conducted to evaluate the benefit of including sisal fibers in the EICP formulation. Sisal fibers are natural fibers with a tensile strength exceeding that of steel. Sisal fibers are derived from the sisal plant, native to Brazil (Gross et al.1979). Before flowering, sisal plants produce between 200 to 250 leaves. Sisal leaves contain 4% fiber, 8% dry matter, 1% cuticle and 87% water (Mukherjee and Satyanaray, 1984). Each leaf contains between 700 to 1400 fiber bundles. These fiber bundles are 0.5 to 1.0 m in length (Oksman et al.2002). Sisal fibers stretch from the base to the tip of the plant and maintain high mechanical strength (Nutman1937). Sisal fiber reinforced cement composites have shown a large amount of ductility (de Andrade Silva et al. 2011).

The EICP solution formulation for the sisal fibers experiments was kept at 0.875 M of urea, 0.50 M of $CaCl_2$-$2H_2O$, 0.85 g/l urease enzyme, and 4.0 g/l of dried non-fat milk stabilizer. Based upon a review of the literature (Hejazi et al. 2012), a fiber content of 0.75% (w/w soil) was chosen. Fiber lengths of 10 mm and 20 mm were initially employed. Unfortunately, the 20 mm fibers did not generate any specimens suitable for UCS testing. An additional column made with 20 mm fibers at 0.85% w/w soil for curiosity, also failed to yield a specimen suitable for strength testing.

Following the initial experiments with a fiber content of 0.75% w/w dry soil, six columns were prepared with 10 mm at lower percentages of fiber per dry mass of dry soil. Duplicate soil columns with fiber content of 0.2, 0.3, and 0.4% w/w soil were prepared.

Unconfined Compressive Strength (UCS) Testing

All columns were extracted from the acrylic cylinders and the polypropylene (PP) liner was removed to prepare the columns for unconfined compressive strength (UCS) testing. The top and the bottom of these columns had to be leveled before running the UCS test. Initially sand paper was used in an attempt to level the top and bottom surface of the columns. However, the sand particles abraded easily and surfaces could not be leveled with this procedure. Next, capping with sulfur at the top and the bottom was attempted. However, heat that generated by the sulfur caps also had a deleterious which effect on the columns, making the top and bottom of the columns disintegrate. Through trial and error, Devcon glue was found to be the best way to make the top and the bottom surfaces flat and smooth without affecting the columns themselves.

The capping procedure using Devcon glue was as follows. First, the top and the bottom platens for the column were wiped with clean cloth until the surface was very clean. Then waxed paper was placed on the surface of the platens to avoid adhesion between the platen and the column. The glue is a two-part composition (a white part and black part).

The white part is mixed with the black part at a ratio of 1:1.7. Then a spoon was used to put the glue on the top of the column and put the column against the top platen perpendicular to the surface and wait for at least 30 minutes for the glue to dry. The procedure was repeated for the bottom of the column and then the column was ready for UCS testing. Two columns were prepared and tested with EICP solution but with zero fiber or xanthan gum but with 0.875 M of urea, 0.50 M of $CaCl_2$-$2H_2O$, 0.85 g/l urease enzyme, and 4.0 g/l of dried non-fat milk as control columns for comparison to earlier test results.

Scanning Electron Microscope (SEM) Imaging

After UCS testing, intact chunks of cemented sand were subject to SEM imaging. The AFEI/Philips XL-30 Field Emission Environmental Scanning Electron Microscope (ESEM) was used to image the morphological features on coated (gold-palladium, 50-50) pieces of the cemented soil.

Acid Digestion

The $CaCO_3$ content of intact pieces of the cemented soil was evaluated by washing the samples with 4 M hydrochloric acid (HCl) solution to dissolve the precipitated of $CaCO_3$ (the columns had previously been flushed with DI water to remove salts and organics). Then the samples were washed with DI water and placed in an oven at 105 Celsius for 24 hours. The difference in weight between the original specimen and the oven-dried specimen was considered to be the weight carbonate that was precipitated in the specimen.

RESULTS AND DISCUSSION

Xanthan Gum Mixed with Dry Soil

After extraction from acrylic cylinder columns, xanthan gum/dry powder columns 1A and 1B, with the highest concentrations of urea-$CaCl_2$ (2.65 M: 1.5 M), were easily deformed in a plastic manner and were not suitable for UCS testing. The other xanthan gum/dry power columns were subject to USC tests. Table 5 summarizes the results of the UCS testing on the columns prepared by mixing powdered xanthan gum into the soil. All column subjected to UCS testing failed along a shear plane at approximately 45 degrees, as illustrated in Figure 16. The percentage of $CaCO_3$ in Table 5 was calculated using the acid digestion procedure described previously in. Figures 17 and 18 shows the stress-strain curves from UCS testing. These figures show relatively good reproducibility of results, despite the somewhat lower strength for sample 2A compared to sample 2B.

Table 5 Results of USC tests at 1.1% (w/w) Xanthan gum mixed with dry soil

Column	Concentration of Urea: $CaCl_2$	Peak Strength (kPa)	ε_f	$CaCO_3$%	Water content after air drying
1A*	2.65:1.5	n/a	n/a	n/a	n/a
1B*	2.65:1.5	n/a	n/a	n/a	n/a
2A	1.75:1.00	1160	1.4	3.93	10%
2B	1.75:1.00	1461	1.8	3.44	9.7%
3A	0.875:0.50	977	2	4.01	8%
3B	0.875:0.50	1010	2.2	4.50	8.2%

*= S*pecimens 1A and 1B were not suitable for UCS testing.*

Figure 16: Shear plane for specimen 3B

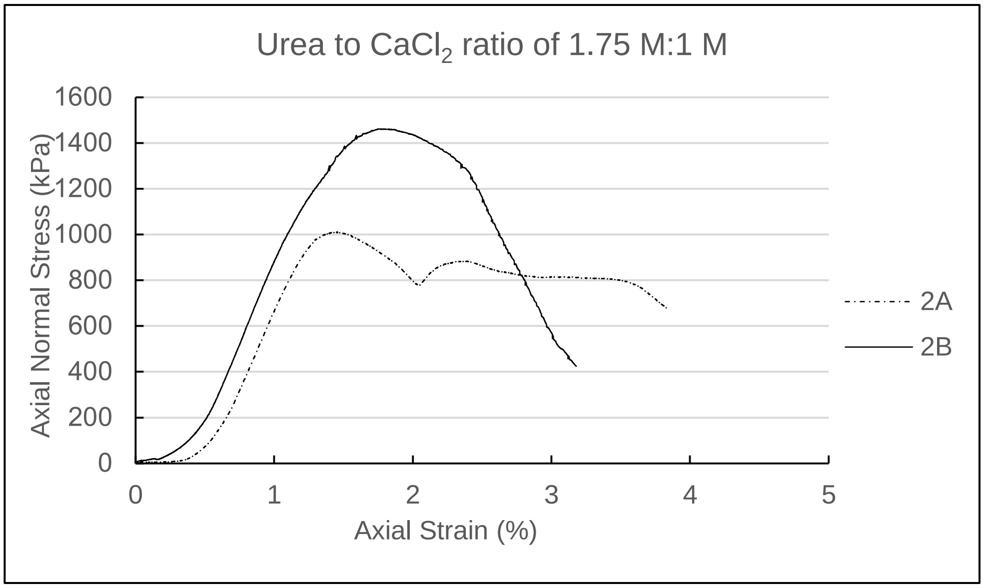


Figure 17. Relationship between Axial Normal Stress and Axial Strain for Samples Prepared by Adding Xanthan Gum as Dry Powder at U:C= 1. 75 M:1 M

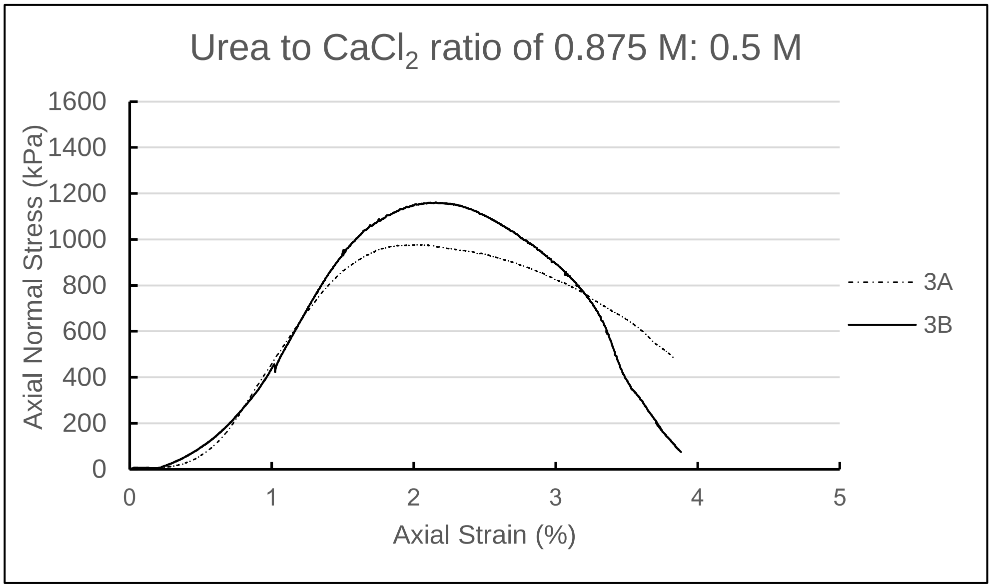


Figure 18. Relationship between Axial Normal Stress and Axial Strain for Samples Prepared by Adding Xanthan Gum as Dry Powder at U:C= 0.875 M:0.5 M

Table 6 shows a comparison of the effects of the EICP solution when xanthan gum powder added to soil and to the samples that treated without the addition of xanthan gum. Two concentrations of urea and $CaCl_2$ are shown in Table 6: 1.75 M: 1.00 M and 0.875 M:

0.50 M. The parameters that are being compared are UCS, $CaCO_3$%, and strain at failure. The percentage of $CaCO_3$ precipitated in these columns and the UCS of the columns were significantly different from the columns without xanthan gum at the same concentrations of urea, $CaCl_2$, and urease.

It is observed that xanthan gum has a significant effect on the UCS of the EICP treated soil, with an increase in strength up to 94% for the specimens that included xanthan gum mixed in as dry powder compared to the case without xanthan gum. It is also observed that acid digestion suggests that the $CaCO_3$% also increases when adding xanthan gum. However, a reason for this could be that the xanthan gum encapsulated ammonium chloride, salts, and organic cementation leading to an inaccurate increase in $CaCO_3$%. Note that the $CaCO_3$% in the case without xanthan gum in general (at different concentrations), is close to the theoretical maximum value. Furthermore, when comparing the strain at failure with and without xanthan gum, it is observed that, in general, adding xanthan gum leads to an increase in the strain at failure (i.e., in ductility). However, it should be noted that due to the non-uniformity of the cementation process, the strain values do not show a clear trend within each case (with or without xanthan gum). Lastly, it is observed that decreasing the molar concentration of urea and $CaCl_2$ does not significantly change the effects observed on UCS and $CaCO_3$%.

Table 6. Compares the UCS strength, strain at failure (ε_f), and carbonate content for specimens prepared with and without the addition of powder xanthan gum

Concentration of M Urea: M $CaCl_2$	w/ Xanthan Gum Powder			w/o Xanthan Gum		
	UCS (kPa)	$CaCO_3$%	ε_f	UCS (kPa)	$CaCO_3$%	ε_f
1.75:1.00	1160	3.93	1.4	71.5	1.74	0.9
1.75:1.00	1461	3.44	1.8	76	2.1	3.4
0.875:0.50	977	4.01	2	73.1	*	1.2
0.875:0.50	1010	4.50	2.2	113	1.23	0.7

*Specimen not tested for $CaCO_3$

After the UCS tests were completed, two intact chunks from the xanthan gum columns were soaked with DI water for 24 hours to see if some of the cementation was due to salt precipitated in the soil. After 24 hours of soaking, the intact chunks did not dissociate, but visual and manual observations indicated that some strength was lost.

A FEI/Philips XL-30 Field Emission Environmental Scanning Electron Microscope (ESEM) was used to investigate morphological features of the precipitate, i.e., to identify the types of crystal that were precipitated. Fragments of the samples prepared by mixing powdered xanthan gum with soil were saved for XL30- SEM analysis after mechanical testing was completed. Images from columns 2A and 3A, presented in Figure 19, illustrate the bonding between the particle and type of crystal. These images, taken at three different magnifications, show (1) shape of the sand particles; (2) a concave $CaCO_3$ face that is interpreted as a separation point where one particle was linked to another particle; (3) the bonding between two particles; and (4) the surface texture of sand particles after precipitation at high magnificent (i.e., magnification =2620X). From these images, we were not able to see clear carbonate crystals due to the biofilm that covering the texture.

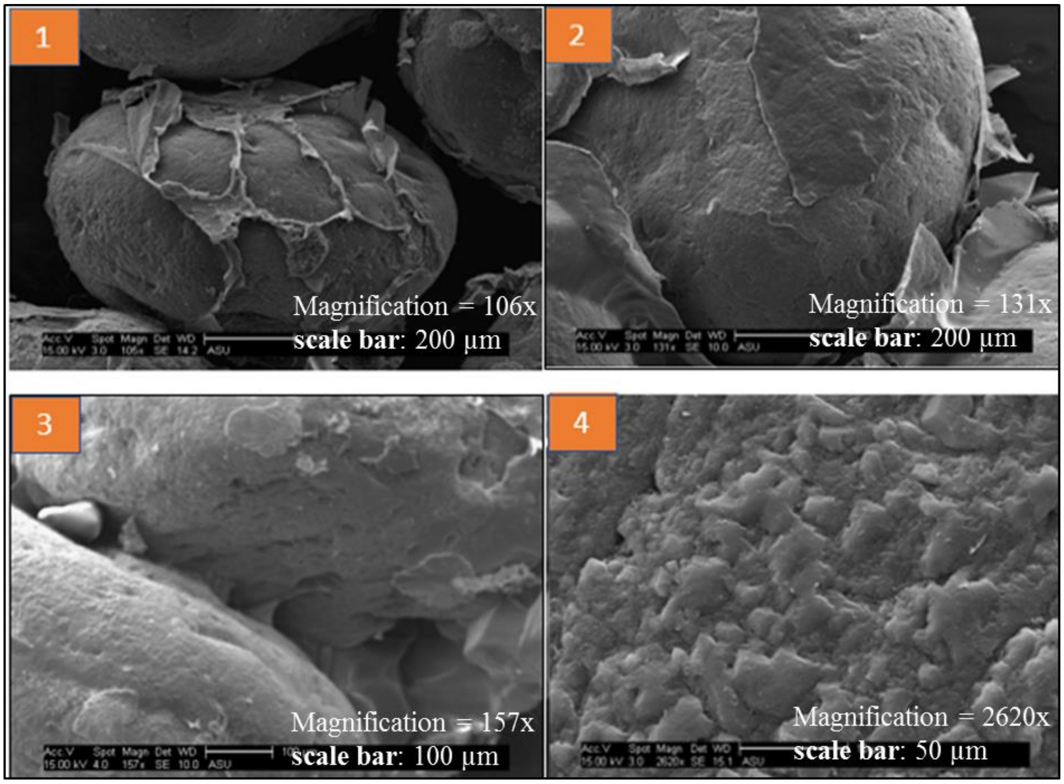


Figure 19. SEM images from sample prepared by mix and compact with xanthan gum mixed into the soil as powder: (1) the shape of the sand particles coated with residual organic material; (2) a concave $CaCO_3$ face that is interpreted as a separation point where this particle was linked to another sand particle; (3) the bonding between two particles; and (4) the surface texture of sand particles after precipitated.

Xanthan Gum as a Part of EICP solution

Similar to the columns prepared by mixing powdered xanthan gum with dry soil, columns 1AA and 1BB, the columns with xanthan gum mixed with the EICP solution with the highest concentrations of urea-$CaCl_2$, did not have enough cementation for UCS testing. The other 4 columns in which xanthan gum was mixed with the EICP solution were soaked in DI water for 20 minutes to flush salts organic matter from the system. These columns seemed by visual observation more shiny and moist than columns prepared using xanthan gum as dry powder. Therefore, these specimens were left in the oven at 40 °C for one month to dry them out. After oven drying, these columns were subjected to UCS testing. After UCS testing, the percentage of $CaCO_3$ was measured by acid digestion. Table 7 summarizes the results and observations from testing of the columns prepared using xanthan gum as part of the EICP solution.

Table 7 Results and observations from using xanthan gum as part of the EICP solution

Column	Concentration of Urea: $CaCl_2$	Peak Strength (kPa)	ε_f	$CaCO_3$%	Water content after air drying
1AA*	2.65:1.5	n/a	n/a	n/a	n/a
1BB*	2.65:1.5	n/a	n/a	n/a	n/a
2AA	1.75:1.00	830	4.1	4.63	12.29%
2BB	1.75:1.00	899	1.95	3.00	10.91%
3AA	0.875:0.50	159	0.6	2.83	10.41%
3BB	0.875:0.50	155	3	3.63	11.01%

*=Specimen 1AA and 1BB were prepared with high concentration of urea: $CaCL_2$ and by that specimen were not suitable for UCS testing (i.e. were easily deformed in a plastic manner due to Xanthan gum).

The failure pattern from the UCS testing of columns prepared by mixing xanthan gum with the EICP solution were different than those of the columns prepared using xanthan gum as powder. The failure pattern for the samples prepared by mixing xanthan gum with the EICP solution was bulging at the bottom of the column. Figure 20 illustrates the pattern failure in UCS testing when xanthan gum was mixed with EICP solution. Figures 21 and 22 shows the stress-strain curves from UCS testing for the specimens treated by dissolving xanthan gum in the EICP solution

Figure 20. Bulging failure when xanthan gum used as part of EICP solution

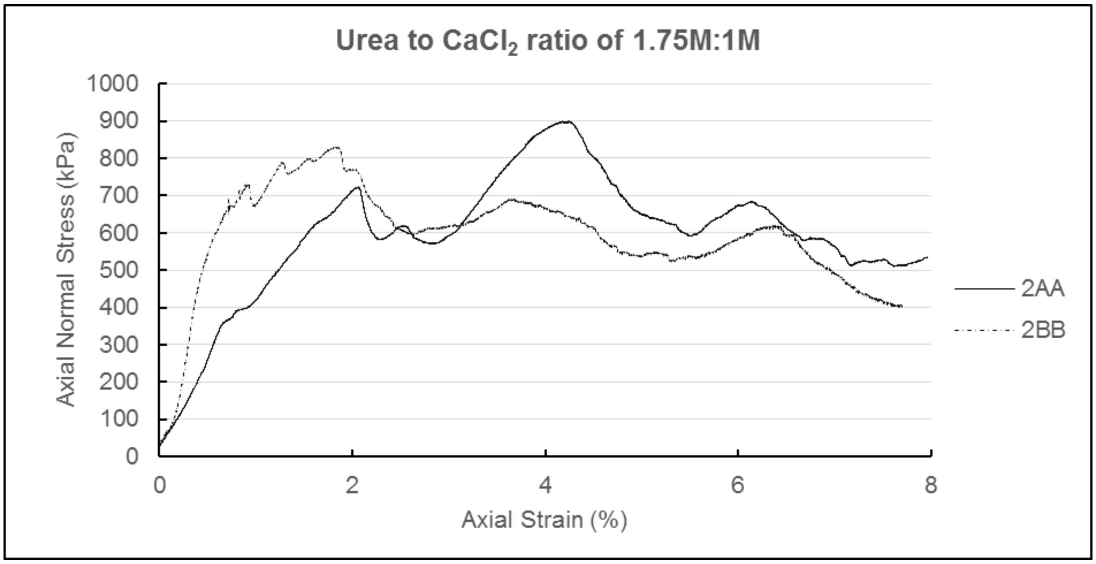


Figure 21. Relationship between Axial Normal Stress and Axial Strain for Samples Prepared by Adding Xanthan Gum to the EICP solution at U:C=1. 75 M:1 M

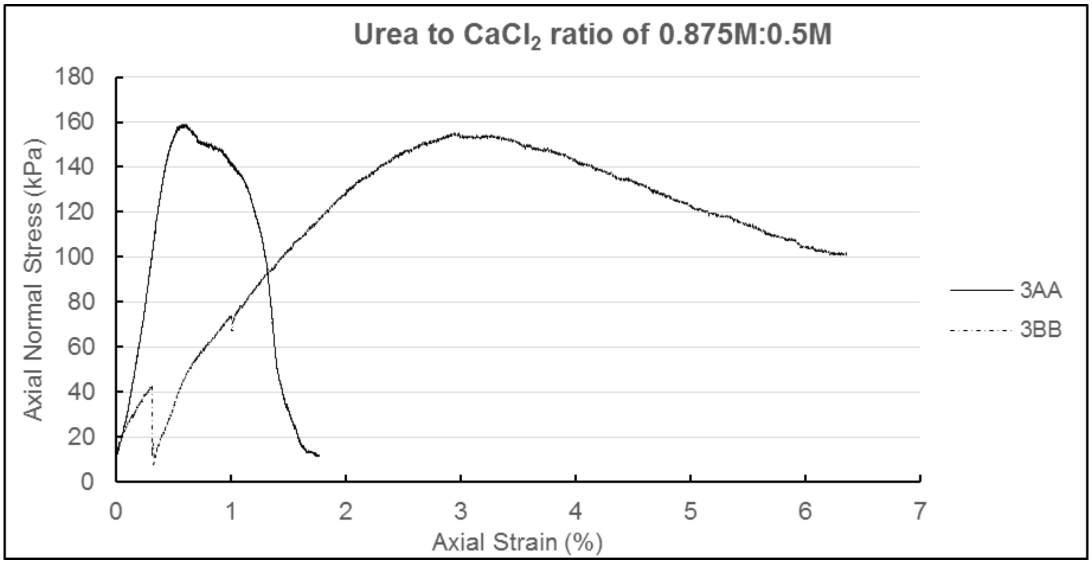


Figure 22. Relationship between Axial Normal Stress and Axial Strain for Samples Prepared by Adding Xanthan Gum to the EICP solution at U:C=0.8 75 M:0.5 M

Table 8 shows a comparison of the effects of mixing xanthan gum as part of the EICP solution versus adding xanthan gum as a powder to the dry soil. It is observed that for the higher and lower molar concentrations of urea and $CaCl_2$, the UCS for the case of adding xanthan gum as part of the EICP solution is lower than that for xanthan gum mixed as powder with soil. For the lower molar concentrations, this difference is significant (by a factor of greater than 6).

Table 8. UCS strength, strain at failure (ε_f), and carbonate content for specimens prepared using different methods to add xanthan gum.

Concentration of M Urea: M $CaCl_2$	Xanthan gum as part of EICP solution			Xanthan gum mixed as powdered with dry soil		
	UCS (kPa)	$CaCO_3$%	ε_f	UCS (kPa)	$CaCO_3$%	ε_f
1.75:1.00	830	4.62	1.95	1160	3.93	1.4
1.75:1.00	899.28	3.0	4.1	1461	3.44	1.8
0.875:0.50	159	2.83	0.6	977	4.01	2
0.875:0.50	155	3.63	3	1010	4.50	2.2

By comparing the two different ways of using xanthan gum, the results show that xanthan gum as a powder gives higher strength and ductility than using it as part of EICP solution. Table 8 showed also that for all cases with xanthan gum, the $CaCO_3$% is higher than the case without xanthan gum due to the additional encapsulated material as previously explained.

Intact chunks from UCS testing were saved for imaging using the AFEI/Philips XL-30 SEM testing. Images taken at three different magnifications are presented in Figure 23. Panel (1) of Figure 23 shows the exterior of a soil particle of soil at low magnification. Panel (2) of Figure 23, using increased magnification appears to show precipitated of

$CaCO_3$ but it is hard to identify the type of calcite crystals. Panel (3) of Figure 23, at an even higher magnification, does show precipitated the calcite crystals. Upon close inspection, panels 2 and 3 in Figure 23 also shows a small amount of salt attached to the sand particles. Therefore, it seems that salts were not entirely removed by soaking in DI water for 20 minutes. Furthermore, all specimens that had been subjected to SEM imaging showed xanthan gum covering the surface of the sand particles.

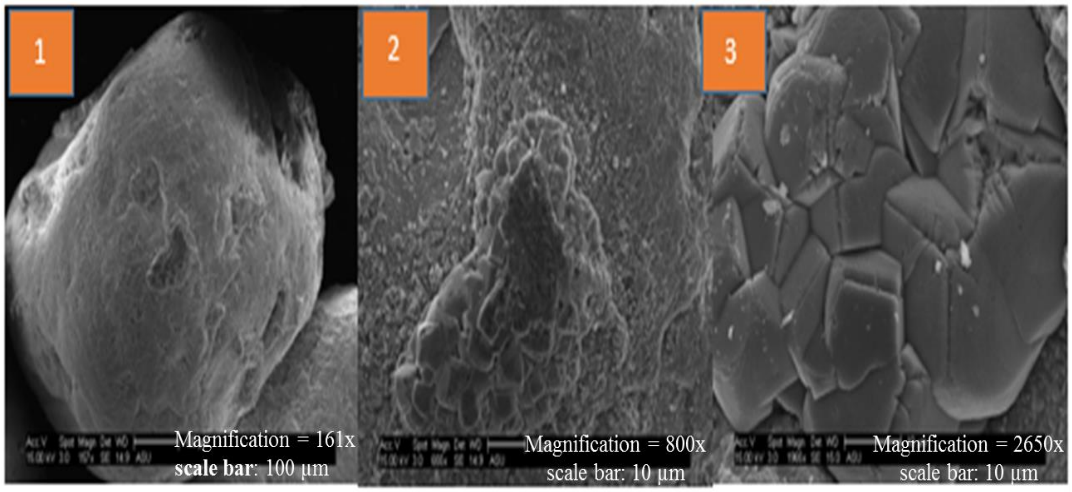


Figure 23 (1) is the exterior of the particle of soil at low magnification, (2) is with increased magnification, and (3) is with even higher magnification.

SISAL FIBER

UCS Testing

Six columns were prepared using mix and compact with the percentage of fiber equal to 0.75 % per dry mass of sand and a length of 20 mm at concentrations of 0.875 M urea, 0.5 M $CaCl_2$,0.85 g/l urease enzyme, and 4.0 g/l of dried non-fat milk as stabilizer. After they were extracted, these samples were unsuitable for UCS testing because the soil fell apart immediately after taking the polypropylene (PP) liner out from the cylinders due to the effect of the fibers, as shown in Figure 24.

Figure 24. Samples with 0.75% Fiber (w/w)

Ten new samples were then prepared with the same cementation solution (0.875 M urea, 0.5 M $CaCl_2$,0.85 g/l urease enzyme, and 4.0 g/l of dried non-fat milk as stabilizer), but with four different percentages of fiber (0.2%, 0.3%, 0.4%, and 0.75%) with a length of about 10 mm to evaluate how sisal fiber can improve the properties of soil. Two tests were conducted for each percentage of sisal fiber with two additional samples without sisal fiber. The columns were extracted and capped in the same way as described above for UCS testing, as shown in Figure 25.

Figure 25: Sisal Fiber Column Prepared for UCS Testing

When the strength of these columns was examined for UCS test, it was seen that the soil strength increased until the fiber content equaled 0.3%. After 0.3% fiber content, the soil strength began to decline as shown in Figure 26. Also, it noted that the strength of the EICP-treated sample with sisal fiber at 0.3% (w/w) was approximately four times higher than samples prepared without addition of sisal fiber. Table 9 presents a summary of all the results of UCS testing and percentage of $CaCO_3$ for the samples prepared using sisal fiber.

Table 9: Summary of the Results of Using Sisal Fiber (10 mm Length)

	Test 1			Test 2		
Percentage by dry weight	Peak Strength (kPa)	ε_f	$CaCO_3$% by weight	Peak Strength (kPa)	ε_f	$CaCO_3$% by weight
0	54	0.8	1.80	65	0.7	1.69
0.2	180	0.35	1.86	220	0.6	1.59
0.3	289	0.50	1.52	296	1.15	1.12
0.4	135	0.55	1.44	150	1.6	1.91
0.75	25	0.20	1.64	N. A	N.A	1.72

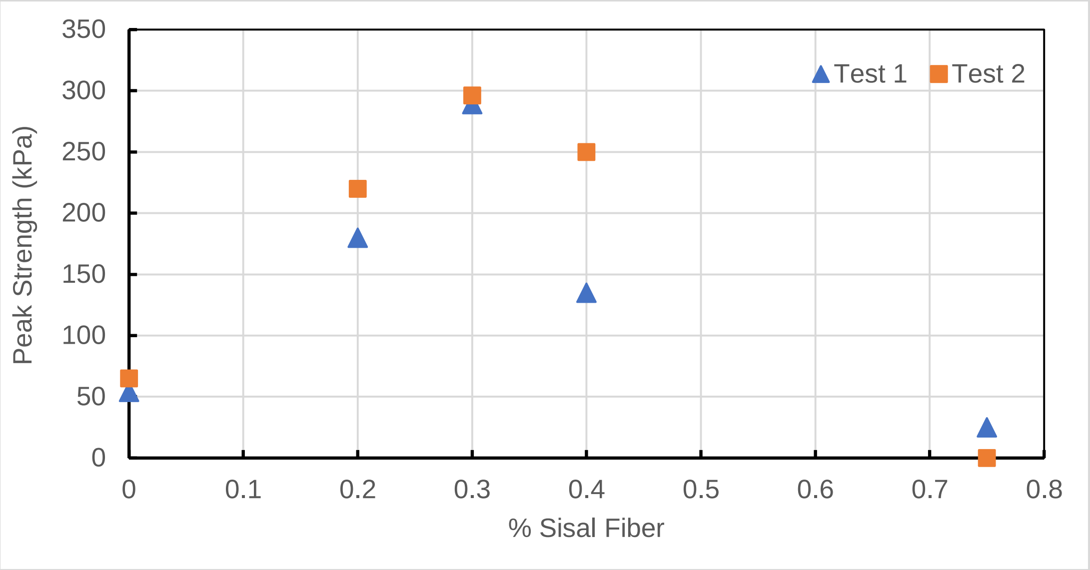


Figure 26 Relationship between the Percentage of 10 mm Sisal Fibers and Peak Strength

As shown above in Figure 26 and Table 9, higher amounts of fibers and longer lengths can have a negative impact on the strength. Li et al (2015) came to a similar conclusion regarding the influence of fiber on the mechanical properties of MICP-treated sand. Li et al. (2015) used a synthetic fiber instead of sisal fibers and found, as illustrated in Figure 27a, the maximum strength was achieved at 0.2 % per mass of dry soil (versus 0.3% for the tests reported herein).

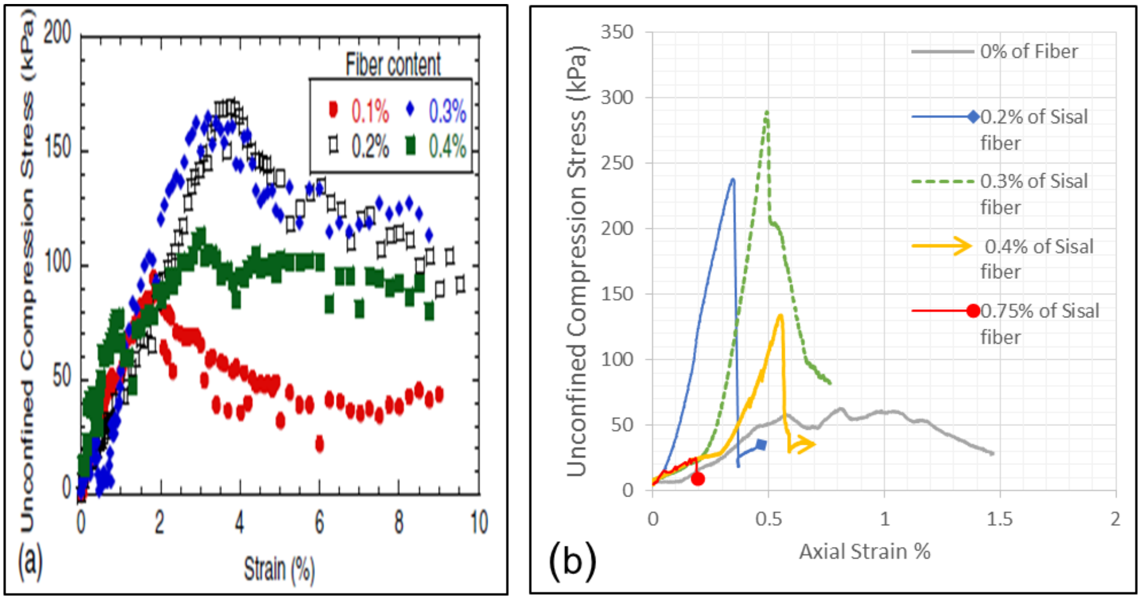


Figure 27: (a) MICP Treated-Sand with Fiber, Li et al (2015), and (b) EICP Treated-Sand with Sisal Fiber (This Study*)*

SEM Imaging of Cemented Sample with Sisal Fiber

Intact chunks of cemented samples were prepared for XL30- SEM imaging after the UCS tests were completed. Images were taken at three different magnifications. These images, some of which are presented in Figure 28, showed relatively little carbonate crystal precipitation. However, it was hard to take clear SEM images of specimens that contained fiber because fibers were charged.

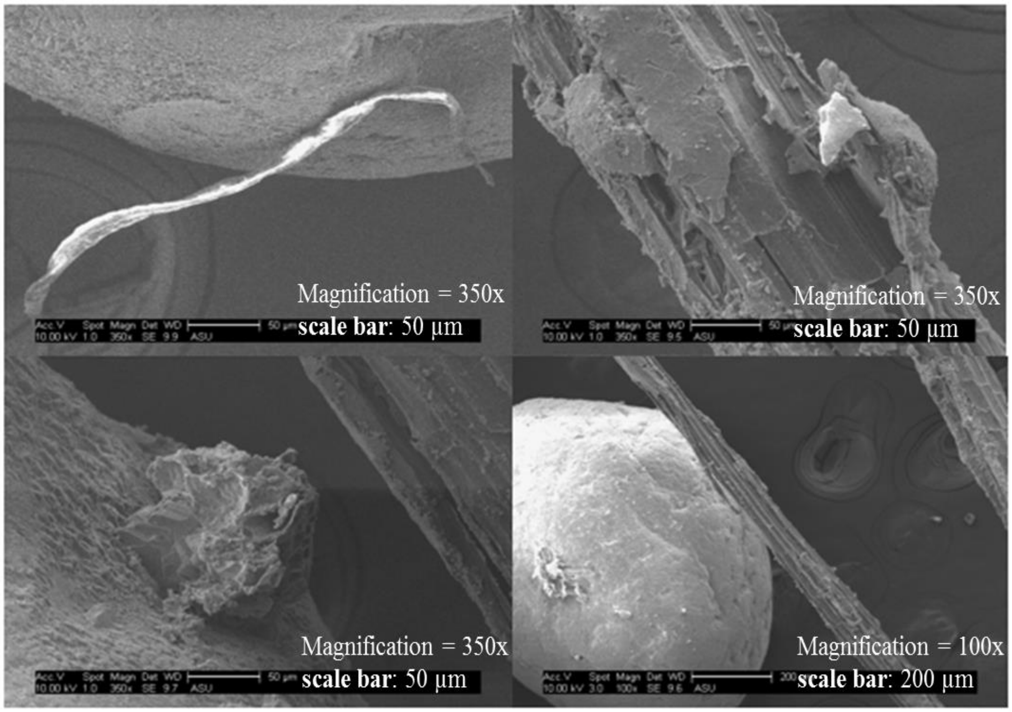


Figure 28 Illustrative SEM images of calcite crystals with fiber at different magnifications and locations

CONCLUSION

The potential for enhancing the strength of EICP improved Ottawa 20-30 by adding two different natural materials, xanthan gum and sisal fiber, was evaluated. The optimum amount of xanthan gum for enhancing unconfined compressive strength was 1.1% (w/w) of the dry weight of the soil. Xanthan gum was applied in two ways: mixed as powder with dry soil and mixed with EICP solution. Mixing the xanthan gum as powder into dry soil showed higher strength and ductility than mixing the xanthan gum into the EICP solution.

For both method of application of xanthan gum, it was very hard to dry the samples, even in an oven at 105 °*C*. Acid digestion of samples prepared using both methods yielded a higher percentage of $CaCO_3$ than the theoretical amount based upon the amount of calcium in the EICP solution. This discrepancy is attributed to salts (including ammonium chloride) and organic materials that were not removed by soaking them in DI water for 20 minutes. SEM imaging showed that xanthan gum covered the particle surface and bridged between particles.

Ten (10) samples were prepared using different percentages of sisal fiber and a fiber length of 10 mm. The results of UCS tests on these samples show that fiber content is an important factor in regard to UCS. The UCS test showed increases in strength and ductility up to 0.3% (w/w) fiber content. After 0.3% (w/w) fiber content, there was a sharp decline in strength. The strength of the EICP-treated sample with sisal fiber at 0.3% (w/w) was approximately four times higher than samples prepared without addition of sisal fiber. It was hard to take clear SEM images of fibers improved samples because of the charge of the fiber.

CHAPTER 5

MODIFYING EICP SOLUTION BY ADDING AN ORGANIC ADDITIVES

INTRODUCTION

This chapter presents results of unconfined compression strength tests on columns of Ottawa 20-30 sand enhanced by adding a “fresh” organic additive (dried non-fat milk) to the EICP solution. The tests described in Chapters 4 and 6 were conducted using old (expired) powdered dried non-fat milk as a stabilizer. In this chapter, fresh (unexpired) powdered dried non-fat milk was used. Unexpectedly, the tests described in this chapter, achieved significantly higher strengths at significantly lower carbonate content than the earlier tests. Tests were also conducting using a different organic additive, green cabbage juice. But the effect of this additive was negligible.

The tests reported in the chapter that used the fresh additive achieved an unconfined compression strength (UCS) as high as 2 MPa at 1% $CaCO_3$ content with only one cycle of treatment. All the experiments were conducted using soil-filled acrylic columns lined with polypropylene sleeves to facilitate extraction of the samples for UCS testing. Initial experiments were repeated several times to confirm the unexpected results. These experiments also showed a better morphology than earlier tests based on scanning electron microscope (SEM) imaging. Furthermore, tensile splitting was observed as the failure mode in the UCS tests, indicating a shear strength in excess of the UCS strength. Therefore, a series of triaxial compression tests at three confining pressures (50 kPa, 100 kPa, and 150 kPa) were conducted to assess the shear strength of specimens prepared using the fresh dried non-fat milk.

METHODS AND MATERIALS

EICP Treatment Solutions

EICP solutions were prepared by dissolving the EICP constituents in deionized water. Solutions were prepared using: (a) green cabbage juice, and (b) dried non-fat milk. Tests employed two different concentrations of the substrate and enzyme: one a relatively low concentration (0.37 M urea, 0.25 M $CaCl_2$, 0.8 g/l urease) and one a relatively high concentration (1 M urea, 0.67 M $CaCl_2$, 3.0 g/l urease). Concentrations of constituents used in the tests described in this chapter were:

- **Solution 1:** 1 M of urea: 0.67 M of $CaCl_2$, and 3 g/l of urease enzyme (no powdered dried non-fat milk or cabbage juice added).
- **Solution 2:** 1 M of urea: 0.67 M of $CaCl_2$, 3 g/l of urease enzyme, and 4 g/l dried non-fat milk.
- **Solution 3:** 1 M of urea: 0.67 M of $CaCl_2$, different amount of green cabbage juice, and 3 g/l of urease enzyme.
- **Solution 4:** 1 M of urea: 0.67 M of $CaCl_2$, different amounts of green cabbage juice, 3 g/l of urease enzyme, and 4 g/l of dried non-fat milk.
- **Solution 5:** 0.37 M of urea: 0.25 M of $CaCl_2$, 0.8 g/l of urease enzyme, and 4 g/l of dried non-fat milk.

EICP Solution 1 listed is the same solution as employed in the baseline tests described in Chapter 3. EICP Solution 2 is the same as used in Chapter 3 except that fresh dried non-fat milk was added. In EICP Solution 3, cabbage juice was used instead of dried non-fat milk. In EICP Solution 4, both cabbage juice and dried non-fat milk were used. In EICP Solution 5, reduced concentrations of urea, calcium chloride, and urease enzyme were used with dried non-fat milk in an attempt to increase the utilization of the calcium in the substrate. $CaCl_2$ in the form of ($CaCl_2$-$2H_2O$) and urea were used in all of these solutions. Both of these reagents were Sigma-Aldrich reagents' grade and had a purity of greater than ≥ 99%. The urease was low grade activity (jack bean urease) from Fisher Scientific.

Dried non-fat milk was added to the ECIP solution because it has been reported to act as a stabilizer for the enzyme (Nemati, M., and Voordouw, G. (2003); Iyer, P.V, and Ananthanarayan, L. (2008)). Cabbage juice was used in the EICP solution in an attempt to slow down the reaction. It was reported in Olech et al (2014) that cabbage juice was able to inhibit the ureolysis reaction for a short time. Juice from garlic and Brussel sprouts were reportedly able to inhibit the reaction completely. As the objective of this organic additive was to slow down, not stop the reaction, cabbage juice was used in these experiments. The objective in slowing down the precipitation rate was to allow the injected solution to penetrate deeper into the pores of the soil before the decrease in permeability accompanying precipitation hinders penetration. A slower precipitation rate may also result in precipitation of greater amounts of calcite compared to other polymorphs of calcium carbonate such aragonite and vaterite and may lead to larger calcite crystals, resulting in a stronger and more durable precipitate.

The cabbage juice was extracted from green cabbages purchased from an organic grocery store. The active ingredient in the cabbage juice that inhibits the ureolysis reaction is reported to be thiosulfinate (Olech et al.2014).

Sample Preparation

Soil columns were prepared in acrylic cylinders were filled with Ottawa 20-30 sand (US Silica Company), a uniform quartz sand with a mean grain size of 0.6 mm, to investigate enzymatic ureolytic $CaCO_3$ precipitation. The acrylic cylinders measured 101.6 mm high and 50.8 mm in inner diameter (4” × 2”). A polypropylene (PP) liner was placed inside of the acrylic columns to ease sample extraction for UCS testing. The bottoms of the columns were closed with rubber caps and sealed with silicone glue to minimize leakage. The PP liner extended above the acrylic cylinder and its top was loosely closed to minimize evaporation of the EICP solution. Each soil column was prepared by first pouring 75 ml of the EICP solution (corresponding to slightly more than one pore volume of the final specimen) into a bowl containing 350 g of 20-30 Ottawa sand and then thoroughly mixing the sand and solution. The sand-ECIP solution mixture was then quickly placed into the acrylic column in three layers. Each layer was compacted using the same compaction procedure described in Chapter 4, i.e., by tamping 25 times with a small hammer to reach a relative density of 90% (based upon the dry weight of soil). The hammer weighed 11.56 N (2.6 lb.). After compaction, the solution was always a few millimeters above the soil, indicating that the soil is almost under a saturated condition.

Sample Curing

The compacted soil and cementation fluid was allowed to stand in the acrylic cylinders for at least 72 hours at room temperature. The residual fluid was then drained out from the bottom of the cylinder. Drainage was accomplished by puncturing the rubber base of the cylinder with a syringe needle. When drainage was completed, 80 ml of DI water (slightly more than one-pore volume) was added slowly to the top of each column to flush EICP solution and soluble salts from the column. After draining and flushing were complete, each treated specimen was extracted from the acrylic cylinder. Then, selected samples were soaked in DI water for at least one half hour (as described subsequently). All samples were placed into an oven at (40 °C) until a constant mass was achieved. Once oven drying was complete, specimens were prepared for the UCS Test.

UCS Testing

UCS testing was conducted in according with ASTM D4918. Prior to performing the UCS test, the top and bottom surface of each sample was leveled using plastic steel putty from ITW Devcon, as described previously (in Chapter 4).

Triaxial Testing

Undrained triaxial tests were conducted on samples prepared using Solution 2 in order to measure the shear strength of the treated soil. In these tests, the Ottawa 20-30 sand was prepared at a relative density of 40%, instead of 90% for comparison to results of specimens compacted to 40% relative density and treated using microbially induced carbonate preparation (MICP). The samples were prepared by pouring the dry soil through a funnel (air pluviation of dry soil). Before applying the confining pressure, each sample was back pressure saturated until Skempton's B parameter was above 0.95. Samples were tested at confining pressures of 50 kPa, 100 kPa, and 150 kPa. Each test was performed at a constant axial strain rate of 0.5% per minute to a final strain of 20%.

Acid Digestion

Following UCS and triaxial testing, intact pieces of samples were dried in an oven at 105 $°C$ for 24 hours, weighed, and then washed with a strong (4 M) hydrochloric acid (HCl) solution to determine the mass of calcium carbonate precipitates. Following treatment with HCl, the samples were rinsed with deionized (DI) water and dried in the oven at 105 °C. The mass difference before and after the acid-wash was considered as the mass of the carbonates precipitated in the specimen.

Micro Scale Identification Analysis

X-Ray Diffraction (XRD) analysis was performed on intact pieces of selected specimens to identify mineral crystal phases existing in each sample. The samples were ground using an agate mortar and pestle and powdered onto a standard glass slide for XRD analysis. Scanning electron microscopy (SEM) imaging was also performed on intact cemented chunks of material using an Agilent 8500 Low-Voltage SEM (LV-SEM). Energy dispersive X-ray (EDX) analysis was also carried out in conjunction with SEM imaging to determine the elemental composition of each sample. The samples were coated with carbon prior to SEM/EDX analysis.

RESULTS

Tests Using EICP Solution 1

Two columns were prepared using EICP Solution 1, i.e., composed of 1 M urea, 0.67 M $CaCl_2$, and 3 g/L of urease enzyme (Solution 1, which does not have powdered dried non-fat milk or cabbage juice into the EICP solution, is the baseline solution tested in Chapter 3). The results of UCS testing and acid digestion on these two specimens are shown in Table 10. These results are similar to the results shown in Table 4 in Chapter 3 for specimens prepared using the same formulation as Solution 1.

Table 10. Results of UCS and Carbonate Precipitation Using Solution 1

Column	$CaCO_3$ (%)	Peak Strength (kPa)
5-1	1.63	133
5-2	0.98	158

SEM images were taken of these samples to provide visual evidence of $CaCO_3$ precipitation. The image in Figure 29 shows that the calcium carbonate precipitate was spread around the surface of the sand particle. A similar precipitation pattern was reported on specimens in described Chapter 3 of this dissertation and in the EICP work by Hamdan (2015) for specimens prepared using the same EICP formulation as Solution 1 but with expired powdered dried non-fat milk.

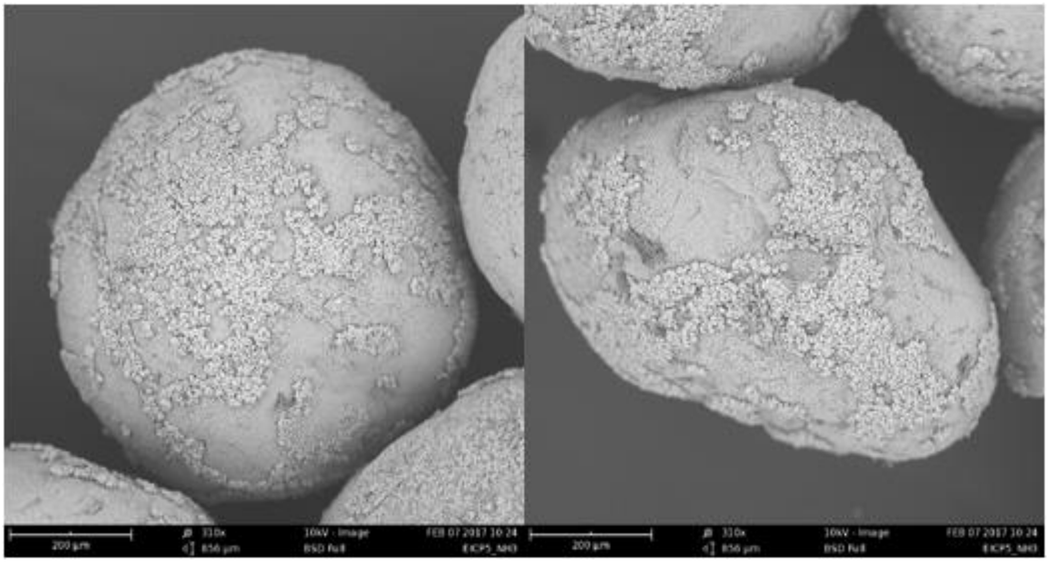


Figure 29. SEM of samples treated using Solution 1 (Baseline Solution)

Tests Using EICP Solution 2

A series of specimens were prepared using EICP Solution 2, a solution containing 1 M of urea: 0.67 M of $CaCl_2$, 3 g/l of urease enzyme, and 4 g/l of fresh powdered dried non-fat milk. This solution is the same as Solution 1 except with the addition of fresh dried non-fat milk. UCS testing yielded a significantly higher strength compared to specimens were prepared with EICP Solution 1 (i.e., by a factor between 6 and 20) at similar carbonate content. The UCS and carbonate content of the tests using Solution 2 are presented in Table 11. It should be noted that, except for the specimen on which the cap failed (Test 5-6), the test with the lowest strength (Test 5-3) was soaked overnight in DI water following rinsing with approximately one pore volume while the other tests were simply rinsed using one pore volume of DI water. However, the strength of the sample soaked overnight in DI water was still significantly greater (by a factor of around 6) than tests that did not use fresh dried non-fat milk. The variability of strength and carbonate content among specimens treated in exactly the same manner is also noteworthy, as it indicated that EICP is a relatively non-uniform process even under the best of conditions.

Table 11. Strength and percentage of precipitation mass for the samples treated with Solution 2

1M Urea, 0.67M $CaCl_2$, 3g/L of urease, 4g/L dried non-fat milk			
Column	Peak Strength (kPa)	$CaCO_3$ (%)	Rinsed or Soaked
5-3	911	1.02	Soaked Overnight
5-4	1817	0.82	Rinsed in Column
5-5	1010	0.78	Rinsed in Column
5-6	463*	0.84	Rinsed in Column
5-7	1654	0.79	Rinsed in Column
5-8	1763	1.17	Rinsed in Column

*: Cap failed, so tested just the bottom of the column

Scanning electron microscope (SEM) images of specimens prepared using dried non-fat milk (Solution 2) are presented in Figure 30. These images exhibit a pattern of precipitation that may be characterized as densely agglomerated calcite crystals at inter-particle contact points. The higher strength obtained using dried non-fat milk in the EICP solution can be attributed to this pattern of precipitation. Figure 31, present the results of EDX analysis focused at the contact point of a specimen treated using Solution 2. The EDX analysis yields a carbonate percentage of around 2%, higher than measured for the bulk sample (as expected), but still significantly lower than reported by others for the same UCS. The results from XRD analysis, presented in Figure 32, demonstrate that the precipitate is primarily calcite.

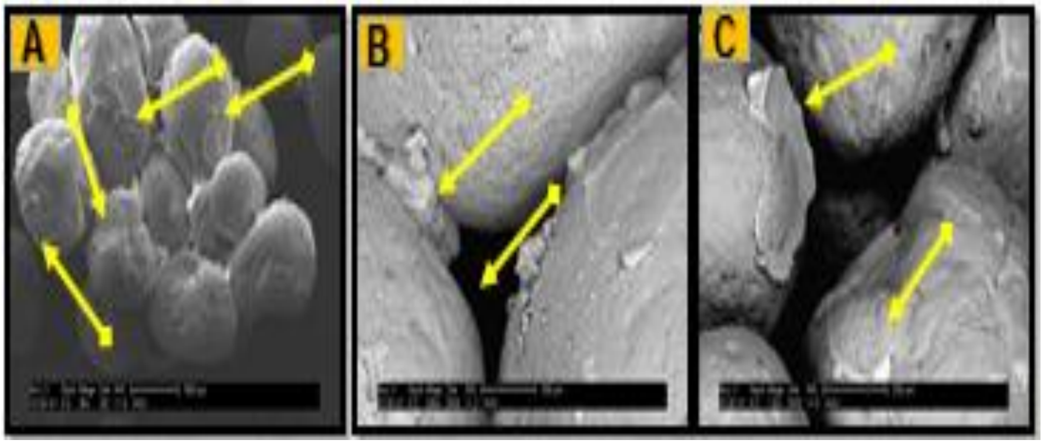
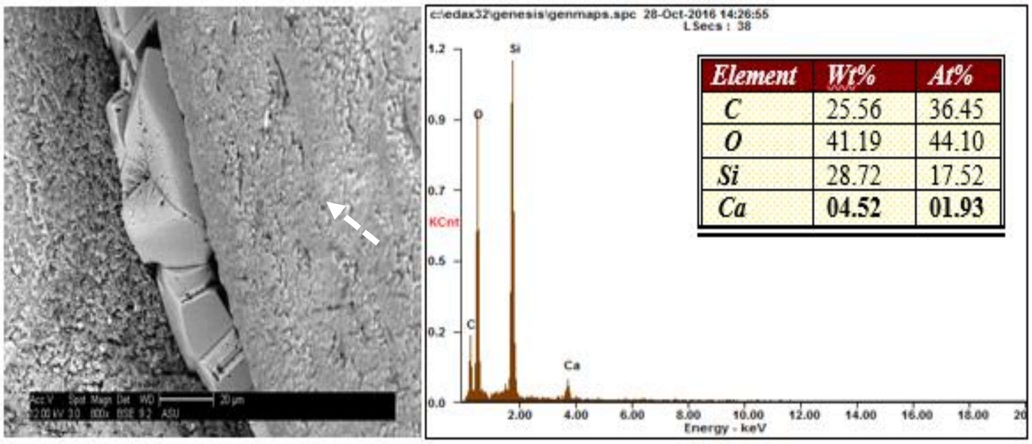


Figure 30. SEM images using EICP Solution 2: (A) $CaCO_3$ at inter-particle contact points, (B) Inter-particle cementation, (C) Broken $CaCO_3$ at inter-particle contact.



Element	Wt%	At%
C	25.56	36.45
O	41.19	44.10
Si	28.72	17.52
Ca	**04.52**	**01.93**

Figure 31. Rhombohedral calcite crystals at inter-particle contact points (left). The results of EDX analysis confirm the presence of the elements constituting calcium carbonate and silica (right).

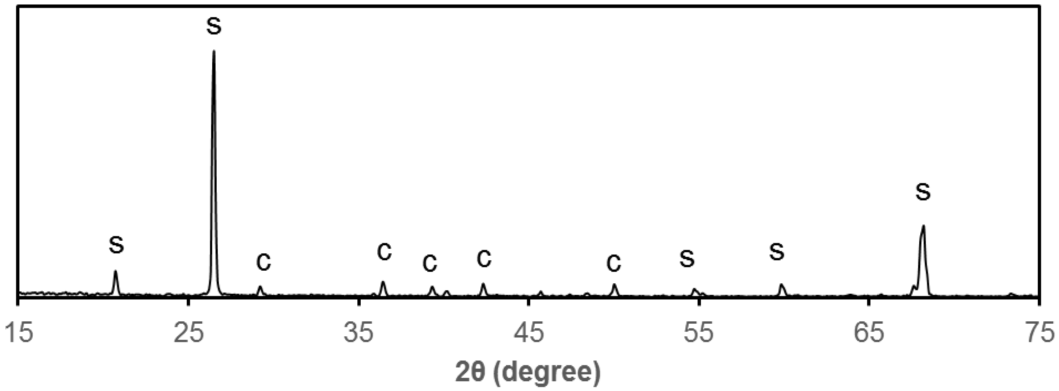


Figure 32. XRD spectrum showing the corresponding peaks of calcite crystal phase and silica sand. (S: silica sand and C: the calcite).

Tests Using EICP Solution 3

Two columns were prepared by adding cabbage juice to the baseline solution (Solution 1) containing 1 M of urea: 0.67 M of $CaCl_2$, and 3 g/l of urease enzyme. Neither visual observation nor strength testing showed a significant change compared to specimens prepared with EICP Solution 1. Acid digestion also yielded similar values to tests on specimens using EICP Solution 1. The UCS tests and carbonate content of the tests using Solution 3 are presented in Table 12.

Table 12. Results of UCS and Carbonate Precipitation Using EICP Solution 3

Column	$CaCO_3$ (%)	Peak Strength (kPa)
5-9	1.21	177
5-10	1.47	119

SEM images were taken of the samples prepared using Solution 3 to provide visual evidence of $CaCO_3$. Figure 33 shows that the calcium carbonate precipitate was spread around the surface of the sand particle, similar to the samples prepared suing Solution 1.

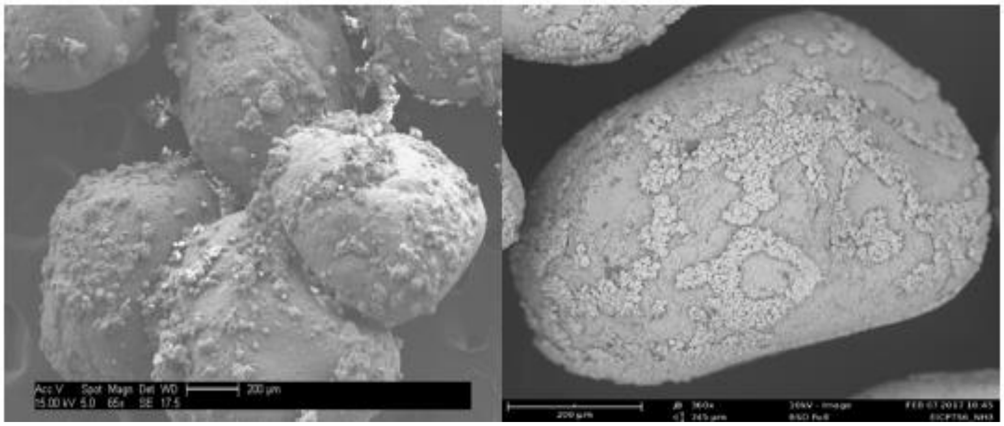


Figure 33. SEM of samples treated using EICP Solution 3

Tests Using EICP Solution 4

A series of specimens were prepared using EICP Solution 4, a solution containing 1 M of urea: 0.67 M of $CaCl_2$, green cabbage juice, 3 g/l of urease enzyme, and 4 g/l of dried non-fat milk. Several different concentrations of cabbage juice were used. This solution is the same as Solution 2 plus the addition of the cabbage juice. The results from the tests using Solution 4 were similar to the results from Solution 2, the solution with powdered dried non-fat milk but no cabbage juice (including the large variability in strength). The UCS tests and carbonate content of the tests using Solution 4 are presented in Table 13. SEM images of the samples prepared using Solution 4, presented in Figure 34, were similar to those prepared suing Solution 2, with concentrated precipitation at the inter-particle contacts.

Table 13. Results of UCS and Carbonate Precipitation Using EICP Solution 4

Column	Peak Strength (kPa)	$CaCO_3$ (%)	Amount of cabbage juice (μL)	Rinsed or Soaked
5-11	705	0.714	50	Rinsed in Column
5-12	899	0.44	87	Soaked for Half hour
5-13	2336	1.16	150	Rinsed in Column
5-14	1079	**	150	Rinsed in Column
5-15	1540	0.65	150	Soaked for Half hour
5-16	1767	0.91	150	Rinsed in Column
5-17	1478	0.89	150	Soaked for Half hour

5-18	1279	0.60	175	Rinsed in Column
5-19	802	0.75	200	Rinsed in Column
5-20	1135	**	225	Rinsed in Column
5-21	1032	1.02	300	Rinsed in Column
5-22	548	0.54	347	Rinsed in Column
5-23	1079	0.94	600	Rinsed in Column
5-24	1091	1.3	1000	Soaked for Half hour
5-25	922	0.91	1500	Soaked for Half hour

**specimen not tested for $CaCO_3$.

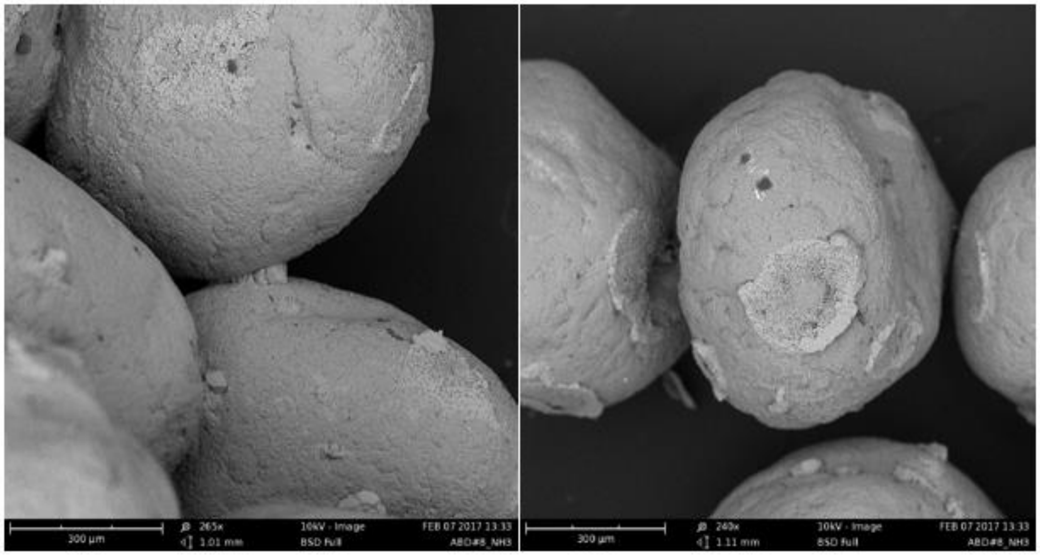


Figure 34. SEM of Sample Treated with Using EICP Solution 4

Tests Using EICP Solution 5

Less $CaCO_3$ than the theoretical maximum (the theoretical maximum is the stoichiometric maximum based on the initial concentrations of $CaCl_2$) was obtained from the treatment solution containing 1 M urea and 0.67 M $CaCl_2$. The Falcon test tube tests reported in Chapter 3 suggest that a treatment solution with a lower concentration of urea and $CaCl_2$ may have a higher precipitation efficiency and still attain significant strength. Therefore, two samples were treated using Solution 5, a solution with a lower concentration of constituents than Solution 2. Solution 5 contained 0.375 M urea, 0.25 M $CaCl_2$, 0.8 g/L enzyme, and 4 g/L of powdered dried non-fat milk (37.5% of the urea and calcium in the other solutions). The results of the tests on these specimens, shown in Table 14, still yield a strength of over 1 MPa at very low $CaCO_3$ content. Furthermore, the amount of $CaCO_3$ precipitated was around the theoretical maximum.

Table 14. Results of UCS and Carbonate Precipitation Using Solution 5

0.375 M Urea, 0.25 M CaCl2, 0.85 g/L of urease, 4 g/L dried non-fat milk			
Column	Peak Strength (kPa)	$CaCO_3$ %	Rinsed or Soaked
5-26	1000	0.57	Soaked overnight
5-27	1396	0.71	Rinsed in Column

SEM images of the specimens prepared using Solution 5, the lower concentration of urea, $CaCl_2$, and urease enzyme, are shown in Figure 35. These images shows the same pattern of concentrated precipitation at inter-particle contacts than seen when the higher concentration of urea, $CaCl_2$, and urease enzyme was used with dried non-fat milk.

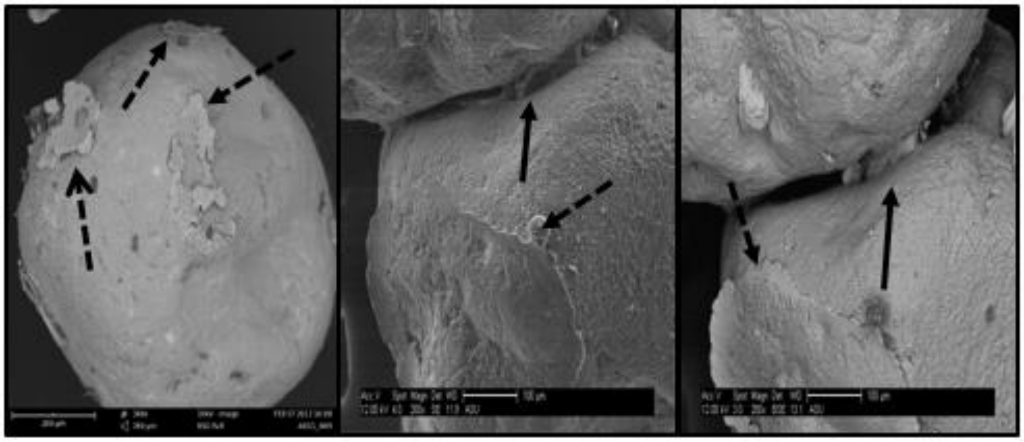


Figure 35. SEM images Using Solution 5, showing the $CaCO_3$ cementation at inter-particle contact points (solid-arrow), and broken $CaCO_3$ at inter-partilce contact (dotted line).

Triaxial Testing

In order to evaluate the shear strength of EICP-treated specimens, a series of treated samples were prepared using Solution 2 for undrained triaxial testing. Samples were prepared by the percolation method using Ottawa 20-30 sand. However, unlike the earlier samples described in this Chapter, these samples were prepared at a relative density of 40% to facilitate comparison to samples prepared using MICP with similar carbonate content. Tests were conducted at three confining pressures: 50 kPa, 100 kPa, and 150 kPa. Three samples were tested at 150 kPa to assess the repeatability of the treatment method. The results of these tests yielded a tensile failure pattern the treated samples tested at 50 kPa. At 100 kPa and 150 kPa, shear failure was observed.

Table 15 summaries all the results from the undrained triaxial tests different confining pressures. There was noticeable variability in pore pressure generation among the three samples at the 150 kPa. However, there was relatively little variability if the value of the major principle effective stress (σ'_1) at failure among the samples at 150 kPa.

Table 15. Summaries the results from undrained triaxial tests

Confining Pressure (kPa)	σ_1 (kPa)	σ_3 (kPa)	σ'_1 (kPa)	σ_3' (kPa)	Excess pore water(kPa)	$CaCO_3$ %
150	2107.32	150	2508.66	551.72	-401.3	0.66
150	2869.93	150	2896.06	184.1	-34	0.72
150	2456.83	152	2775.56	471.2	-319	0.8
100	1574.50	100	1553.11	78.68	-21.38	0.67
50	1342.47	52	1337.94	47.655	4.53	0.77

In evaluating the shear strength, the average results were taken for the three specimens at 150 kPa confining pressure. The left side of Figure 36 shows the average-stress strain curves (Panel A), effective stress path (Panel B), and excess pore pressure (Panel C) for the tests at all the three confining pressures (50 kPa, 100 kPa, and 150 kPa). The right side of Figure 36 shows the behavior of untreated sand at the same relative density and a confining pressure of 100 kPa (note the difference in vertical scales). The interpreted shear strength shown in panel A was established by extrapolating the linear trend of the p'-q plots in panel B back to the origin of the plot. As shown in Figure 36, panel A, when the confining pressure was increased, the effective vertical stress and the interpreted cohesion of the soil increased.

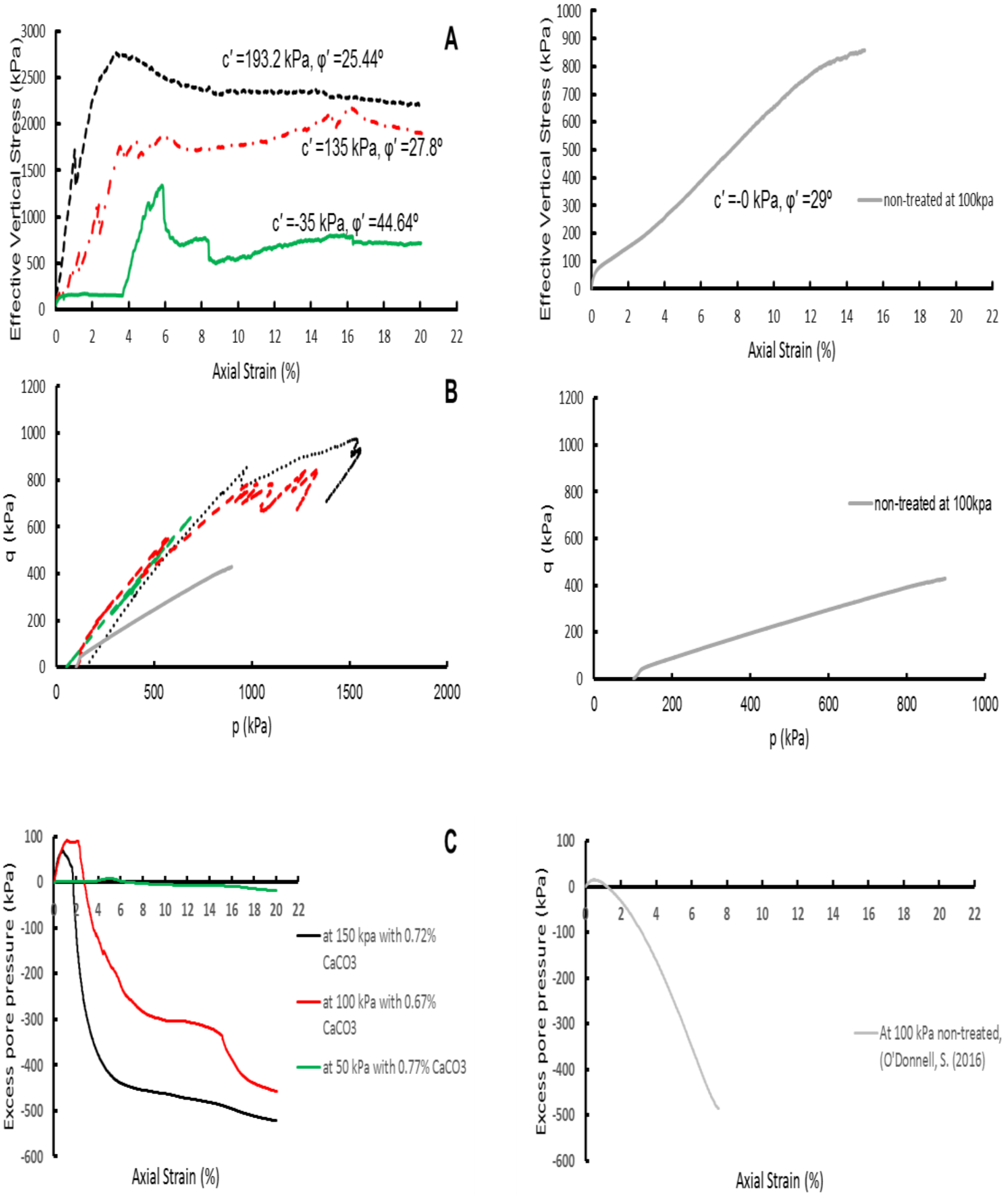


Figure 36. Effective vertical stress (A), q Vs. p (B), and excess pore pressure (C) from undrained testing specimens at an initial relative density of 40% and a different confining stress of 50, 100, and 150 kPa.(Non-treated sample results were taken from O'Donnell, S. (2016).

Mohr's circles drawn for all the three-confining pressure using the effective stress at failure (defined as the peak mobilized shear stress) are shown in Figure 37. Based upon these test, the effective stress cohesion (c') and internal friction angle (φ') were calculated as: c'= 332.96 kPa and φ'= 34.58°, as shown in Figure 37.

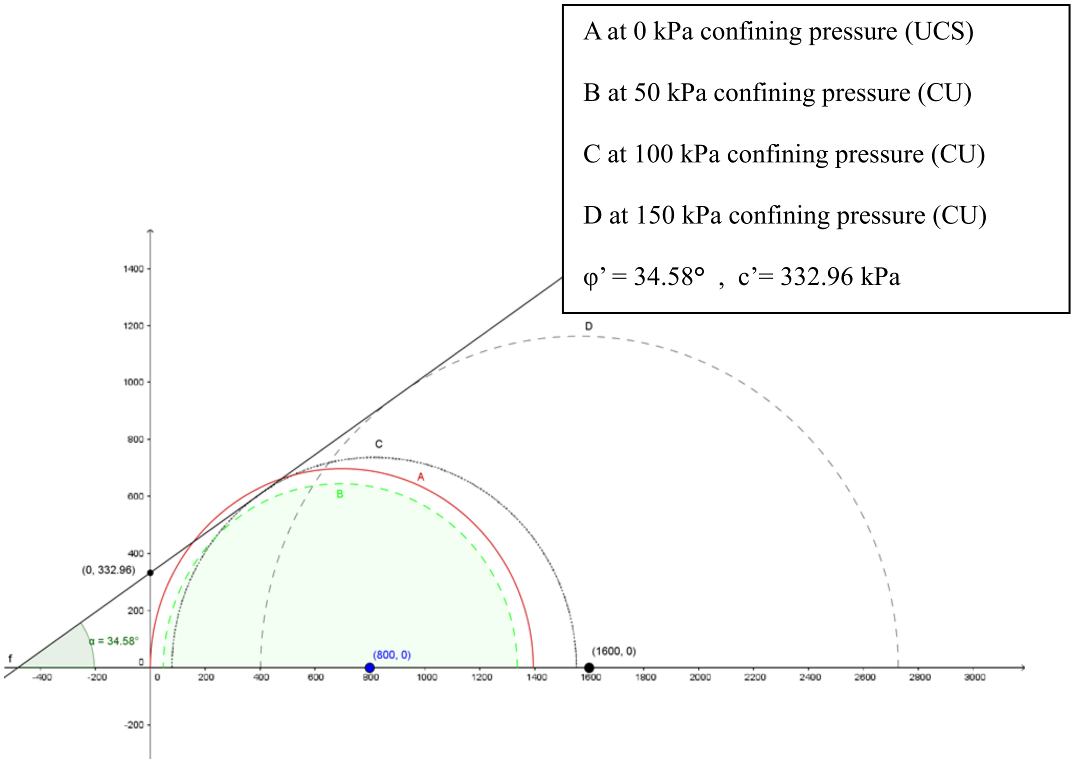


Figure 37. Mohr circles for different confining pressure

$\tau_f = c' + \sigma' \tan \varphi'$ (5)

τ_f= Maximum shear stress the specimen (kPa)

c'= Cohesion (kPa)

σ'= normal effective stress (kPa)

φ'= Internal friction angle (degree°)

INTERPRETATION OF RESULTS

Table 16 summarizes the results obtained using the 5 different EICP solutions described in this chapter in terms of UCS and carbonate content. The values in this table are average values for the specimens prepared using each EICP solution. Figure 38 shows the carbonate content versus UCS for specimens prepared with fresh powdered dried non-fat milk (Solutions 2, 4, and 5) and samples prepared without dried non-fat milk (Solutions 1 and 3). The UCS of the specimens prepared using fresh dried non-fat milk as a stabilizer is significantly greater than specimens of similar carbonate content prepared without fresh powdered dried non-fat milk. The UCS was also significantly greater than that of specimens prepared via EICP without dried non-fat milk. SEM images of the samples treated with Solutions 2, 4, and 5 showed concentrated precipitation at particle contacts rather than distributed carbonate precipitation.

Table 16. Summary of EICP solution tests

Sol. #	Urea (M)	$CaCl_2$ (M)	Dried non-fat milk (g/L)	Urease enzyme (g/L)	Cabbage juice (µL)	# of Tests		Average Strength for UCS tests (kPa)	Average $CaCO_3$ %
						UCS (kPa)	CU (kPa)		
1	1	0.67	0	3	0	2	0	145	1.305
2	1	0.67	4	3	0	6	5	1270	0.9
3	1	0.67	0	3	150	2	0	148	1.34
4	1	0.67	4	3	50 -1000	15	0	1180	0.94
5	0.37	0.25	4	0.8	0	2	0	1200	0.62

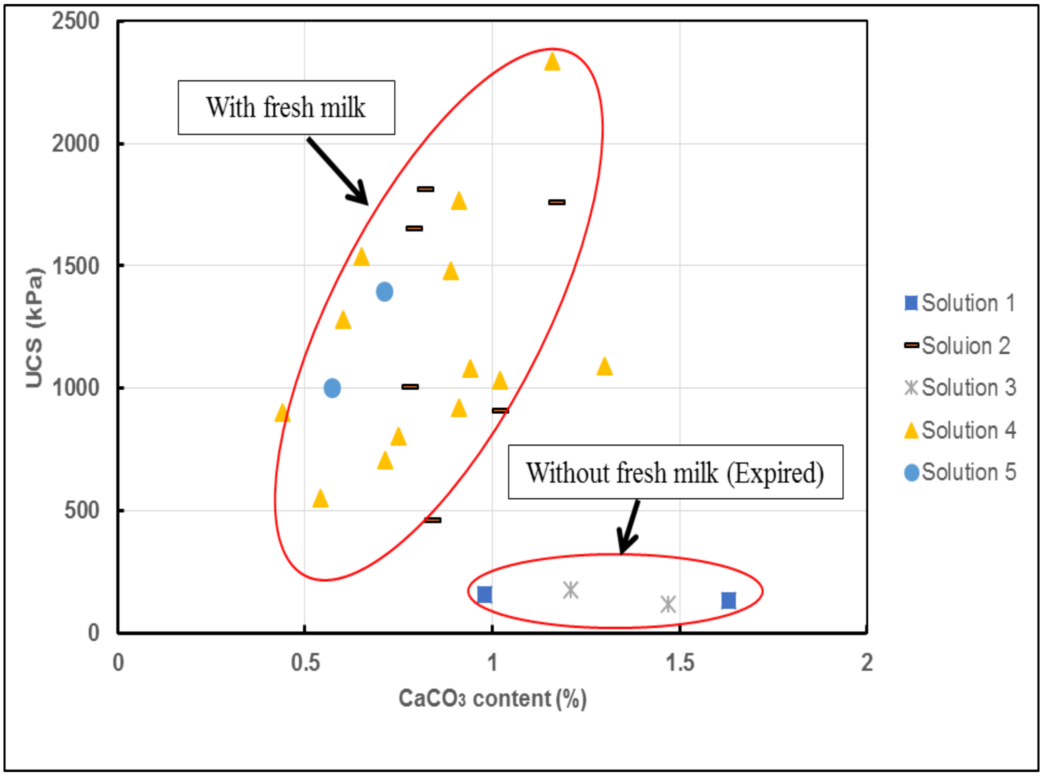


Figure 38. UCS versus $CaCO_3$ content for Solutions 1-5

Figure 39 is a plot of carbonate content versus USC for samples tested in this testing program to those reported in the literature prepared by either EICP or MICP. The UCS reported here for samples prepared using Solutions 2, 4, and 5 is also significantly greater than specimens prepared by either EICP or MICP with the same carbonate content as reported in the literature. The strengths reported here are also unprecedented for a $CaCO_3$ content less than 1% and for samples that were subject to only one cycle treatment. Thus, adding fresh dried non-fat milkto the EICP solution leads not only to a significant improvement in the strength of the soil but also to the morphology of the precipitates. Figure 39 shows carbonate content vs UCS for both EICP as reported by Yasuhara et al. (2012) and as reported herein along with MICP results from Gomez and DeJong (2017) and van Paassen (2010). For the EICP technique, Yasuhara et al. (2012) reported four cycles of treatment were required to achieve a UCS of 1.62 MPa at 5.1% of $CaCO_3$. In this study, one cycle of treatment reached a UCS of up to 2.3 MPa at less than 1% $CaCO_3$. For the MICP technique, Gomez and DeJong (2017) reported that the solution was injected 22 times to reach a UCS of 5.3 MPa with 13.2% $CaCO_3$. Also, the vertical failure plane for the unconfined compression tests on specimens prepared using Solution 2, 4, and 5 shown in Figure 40, indicates a tensile failure pattern.

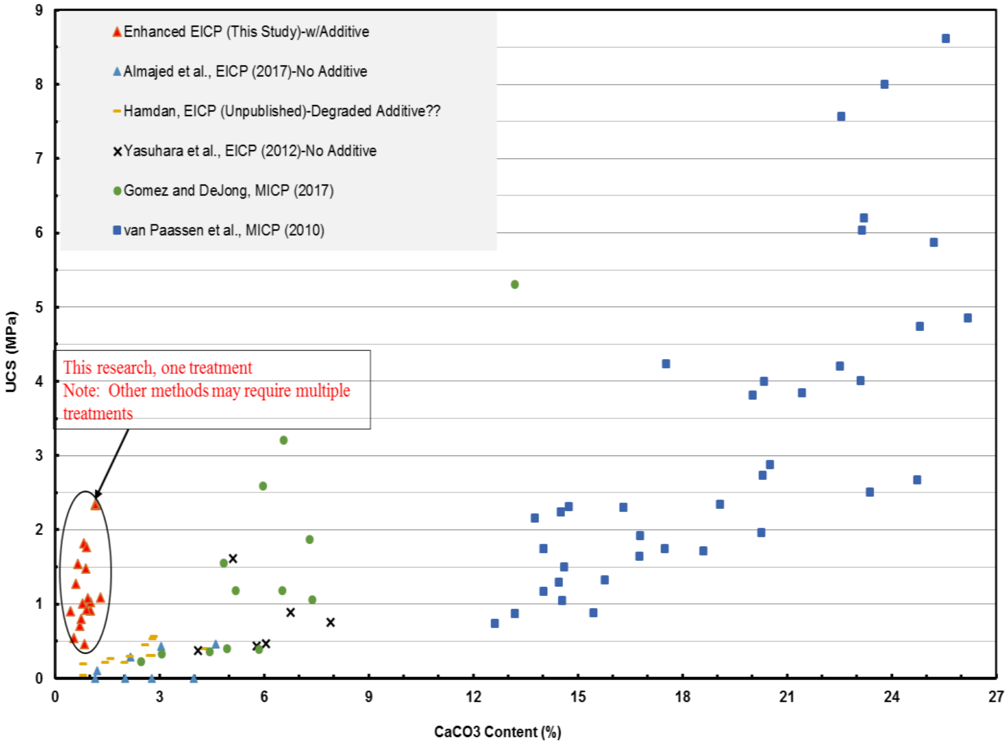


Figure 39. Relationship between UCS Vs CaCO3 for EICP, MICP, and Enhanced

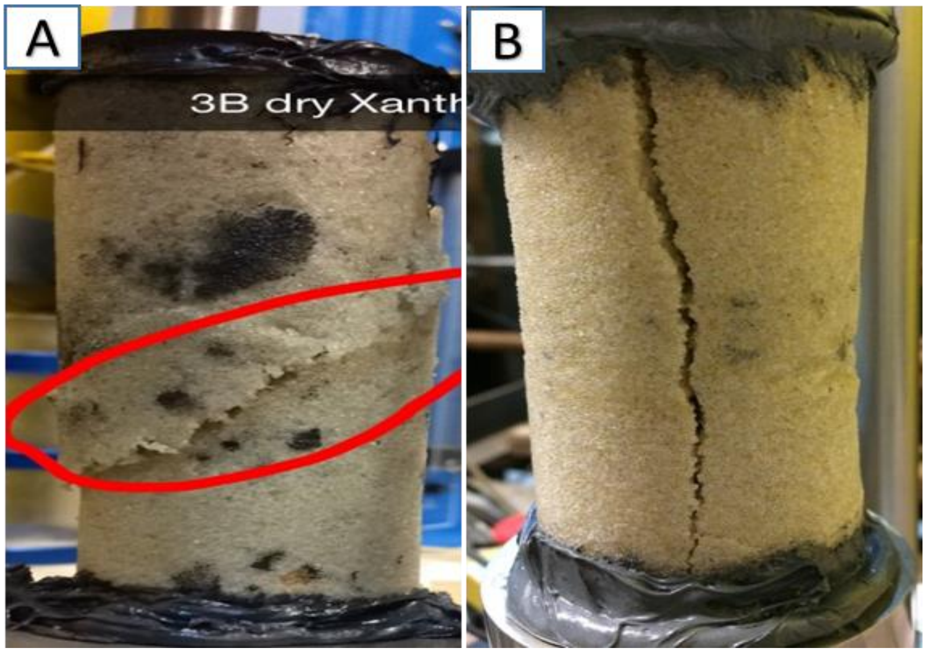


Figure 40. A: Shear failure pattern from unconfined compression when fresh dried non-fat milk not used, B: Vertical failure plane from unconfined compression. As shown, top and bottom surface of the sample were flattened by using plastic steel putty.

The reason for the surprising effects of adding fresh powdered dried non-fat milk to the EICP solution is not clear yet. One hypothesis is that adding fresh dried non-fat milk to the EICP solution may lead to an increase in the viscosity of the EICP solution, since milk has organic agents such as casein and clayton (Zoller, H.F, 1921). Higher viscosity may lead the precipitated calcium carbonate to more readily convert to the calcite, which is the strongest and most stable phase of calcium carbonate. Increasing the viscosity of the EICP solution may also cause calcite crystal formation to focus at the contact points (not on the particle surface), resulting in the significant increase in the strength seen in the UCS tests. Another hypothesis is dried non-fat milk act as nucleation points. These hypotheses explain what has been observed through SEM and EDX analysis. Furthermore, the results from adding green cabbage juice did not have any effect on the strength that was obtained.

These results clarify that fresh dried non-fat milk is the governing element that changes the mechanism of precipitation.

Overall, the key advantage of the enhanced EICP method using powdered dried non-fat milk presented here include is a higher strength with only a single cycle of treatment and much less precipitated carbonate then reported previously. The benefits of this formulation include lower concentrations of substrate and enzyme and production of a lower amount of the undesirable ammonium by product. This makes the process cheaper, more technically feasible, and more environmentally friendly than other EICP formulations and MICP methods. Moreover, the results obtained using the enhanced EICP treatment described herein demonstrates that the quality of precipitation is more important than the quantity of precipitation in strengthening soil via cementation.

CONCLUSION

The results in this chapter represent an important advance for the biogeotechnical improvement technique of induced carbonate precipitation. The addition of dried non-fat milk to the EICP solution resulted in significant benefits with respect to the strength that was achieved and the quantities of substrate and enzyme required to achieve that strength. The results observed were unprecedented for all biogeotechnical carbonate precipitation techniques. The results from the UCS and the undrained triaxial tests for treated samples showed significant improvement in the soil properties with a tiny amount of calcium carbonate (2 MPa UCS with less than 1% $CaCO_3$). Through SEM and EDX, it was demonstrated that adding dried non-fat milk caused the calcite to focus at the contact point. This is believed to be a major contributor to the increased strength. The advantages of the enhanced EICP are that it reduces the number of cycles of treatment and the concentration of substrate and enzyme, resulting in reduced cost. Furthermore, this study proved that the quality of the precipitation is more important than the quantity.

CHAPTER 6

COLUMNAR IMPROVEMENT OF SOIL AND INVESTIGATE DIFFERENT PREPARTION METHODS FOR CREATING COLUMN

INTRODUCTION

This chapter demonstrates at the bench scale the use of Enzyme Induce Carbonate Precipitation (EICP) to create sub-horizontal columns for soil nails and vertical columns of cemented soil similar to vertical columns created using deep soil mixing. The EICP solution was injected through flexible perforated tubes pushed into the soil by a metal pilot tube (which was subsequently removed) to create the sub-horizontal columns. For the vertical columns, the EICP solution was injected through a small diameter vertical tube inserted down the axis of PVC for 101.6 mm x 254 mm (4” x 10”) and acrylic columns for 50.8 mm x 101.6 mm (2” x 4”) prior to placement of the soil around it.

The sub-horizontal column experiment was performed using F-60 Ottawa silica sand. The vertical columns experiments were performed by using both Ottawa 20-30 and F-60 silica sand. The EICP solution for the sub-horizontal consisted of 0.87 M urea, 0.5 M $CaCl_2$-$2H_2O$, 0.85 g/L low-grade urease enzyme (Fisher Scientific, Low Grade Jack Bean Urease), and 4.0 g/L of fresh dried non-fat milk as stabilizer. The vertical column experiments were performed using an EICP solution composed of 1 M urea, 0.67 M $CaCl_2$, 4.0 g/l of old (expired) dried non-fat milk as stabilizer, and 3 g/l of low activity urease enzyme. Two different methods were used to introduce the EICP solution in the vertical columns to investigate the effect of these methods on the strength of the EICP-treated soil.

SUB-HORIZONTAL COLUMNS

Experimental Methods and Procedures

Sub-horizontal columns simulating soil nails were installed in a linear low density polyethylene-lined wooden box 46 cm-long, 22 cm-wide, and 30 cm-tall. One 22 cm x 30 cm side of the box had a removable face with four 1.9 cm-diameter holes drilled through the face at a 15^{o} decline. The drilled holes were approximately evenly spaced on the removable face. The box was filled with approximately 40 kg of F-60 silica sand (mean grain size 0.275 mm, coefficient of uniformity 1.74) and lightly compacted by tamping. Four thin walled metal tubes 38 cm-long x 1.3 cm-inner diameter were inserted through the holes in wooden face of the box at a 15^{o} decline and pushed ≈ 23 cm into the soil. Next, polyvinyl Chloride (PVC) tubes (9.5 mm-ID x 33 cm-long) perforated on the top of the tubes over the last 20 cm of their length with 11 equally-spaced holes (0.4 mm-diameter) and wrapped in 250μm polypropylene mesh were inserted into the four metal tubes. The metal tubes were then slowly pulled out of the box leaving the perforated PVC tubes in the soil. The perforated tubes were connected to flexible tubing and EICP solution was injected into the tubes using a 60 ml syringe. The tubes were injected one at a time with approximately 155 mL of EICP solution and then the mouths of the tubes were covered with aluminum foil for three days to minimize evaporation.

A week after injection of the EICP solution, the wooden face of the box was removed exposing a stable vertical face. The box sat undisturbed for five days at room temperature and then a load was applied to the soil surface to further assess the stability of the exposed face. After load testing, loose soil was excavated to expose the cemented soil along the length of the injection tubes and elsewhere in the box. Samples were collected from near each of the four injection tubes at approximately 0 cm, 8 cm, 15 cm and 23 cm from face of the box. The soil samples were analyzed by acid digestion, X-ray diffraction (XRD), and scanning electron microscopy (SEM).

RESULTS AND DISCUSSION

Figure 41a shows the front of the soil nailing box after removal of the face, with the four injection tubes protruding from the face. Five days after the face was removed a 7.5 kPa normal stress was applied to the top of the stabilized mass via a wooden plate. The mass remained stable with no visual evidence of cracking or instability, as shown in Figure 41a. Upon excavation of the loose soil within the box, it was observed that soil was continuously cemented in a roughly cylindrical manner around each injection tube, there was no continuity between the cemented zones around the tubes, and a continuous vertical cementation structure had formed 23 cm in back of the face (the approximate penetration length of the injection tube) behind which the sand was uncemented, as shown in Figures 41b and 41c. No $CaCO_3$ was detected at the face of the box and the $CaCO_3$ content at 8 cm and 15 cm from the face of the box were found to be 2.0 % and 2.9% by acid digestion. The $CaCO_3$ content of a specimen from the continuous vertical mass ≈ 23 cm from the face was 0.9%. XRD and SEM results, shown in Figure 42, indicated that calcite phase $CaCO_3$ was present in the soil specimens.

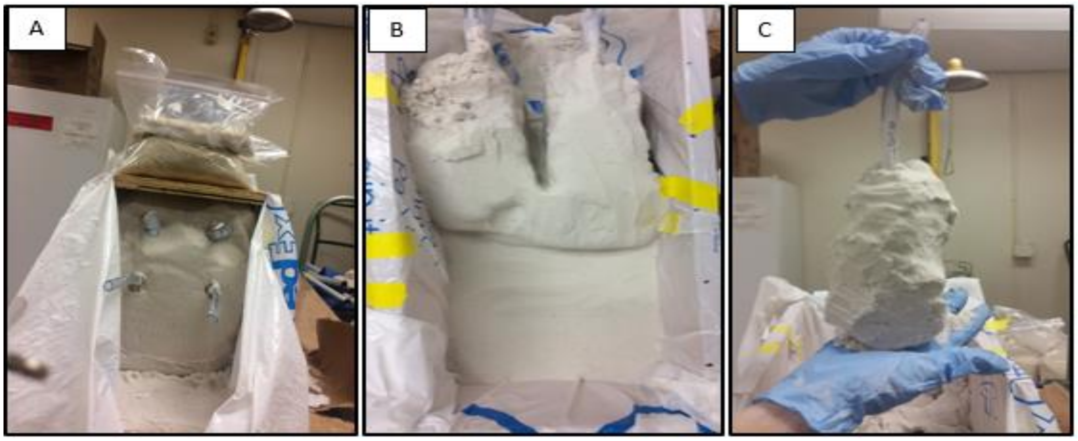


Figure 41. Soil nail experiment: (A) Application of normal load; (B) Top view of cemented tubes and vertical cemented zone near 23 cm; (C) Cylindrically cemented soil mass around (clear) injection tube.

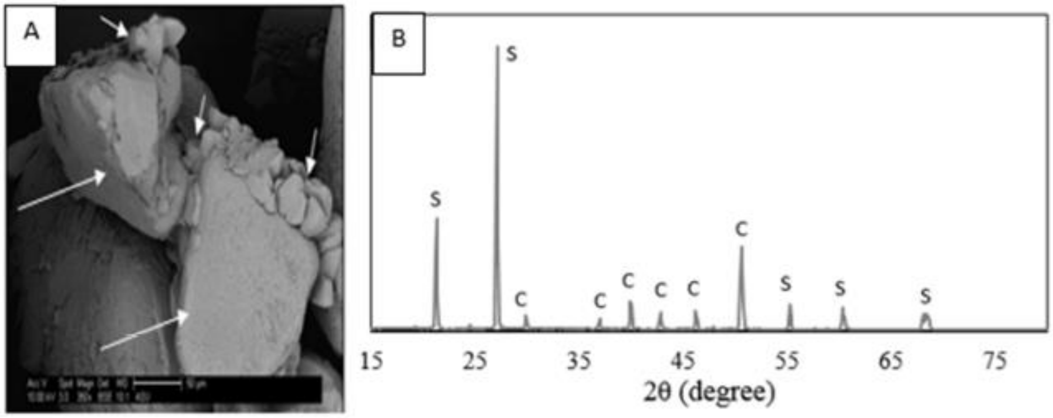


Figure 42: Soil nail experiment results: (A) SEM image showing the calcite phase (small arrow) and silica sand (large arrows) (B) XRD results showing evidence of calcite.

VERTICAL COLUMNS

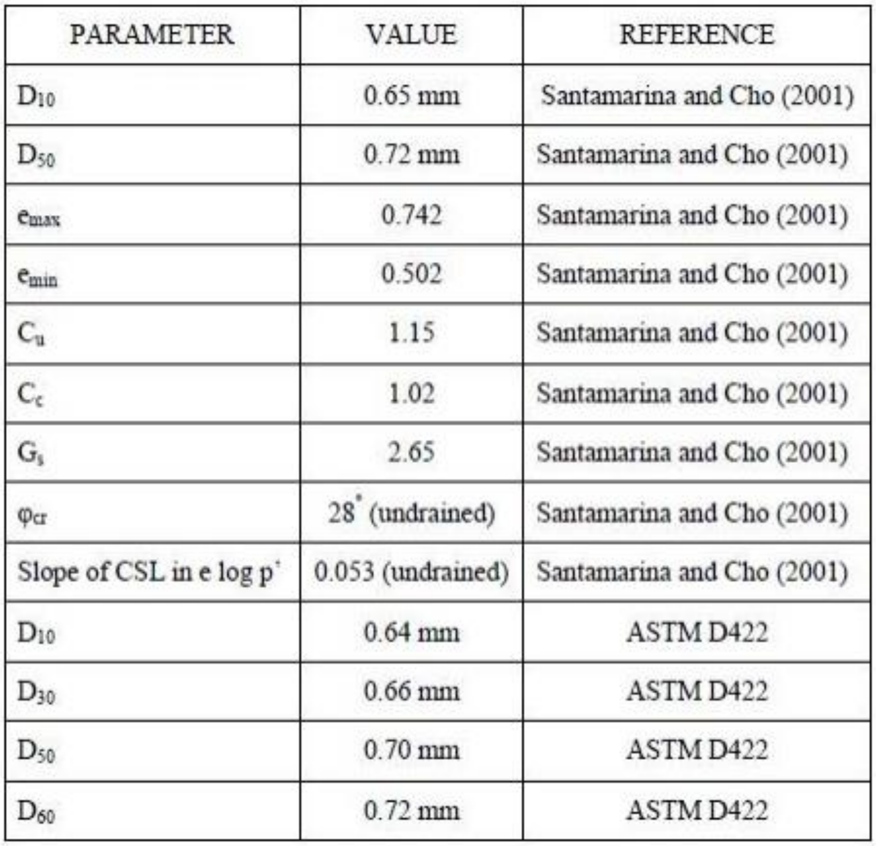
Experimental Methods and Procedures

Two types of soil were used in the vertical column experiments to investigate the effect of soil gradation on the cementation using EICP. Moreover, two preparation methods, percolation and injection, were used to create the vertical columns. Samples were also prepared using mix and compact and the same EICP solution used to create the vertical columns as a control on the properties of the cemented sand in the vertical columns.

Soil A: Ottawa 20-30 Silica Sand

Vertical cylindrical soil columns were created using Ottawa 20-30 silica sand and then stabilized by using the EICP solution. Table 17 presents the physical characteristics of the Ottawa 20-30 sand. An initial batch of three 101.6 mm x 254 mm (4” x 10”) columns was made in PVC cylinders. Afterward, three 50.8 mm x 101.6 mm (2” x 4”) columns were made in acrylic cylinders. Two different preparation methods, percolation and injection, were employed for both sizes of columns to mimic how columns might be created in situ. A third set of samples using columns of both sizes was prepared by the mix and compact method as a control on the EICP solution and for comparison to columns created in earlier work (i.e., in Chapters 3 and 5).

Table 17. Physical characteristics of Ottawa 20-30 (Czupak 2011).



PARAMETER	VALUE	REFERENCE
D_{10}	0.65 mm	Santamarina and Cho (2001)
D_{50}	0.72 mm	Santamarina and Cho (2001)
e_{max}	0.742	Santamarina and Cho (2001)
e_{min}	0.502	Santamarina and Cho (2001)
C_u	1.15	Santamarina and Cho (2001)
C_c	1.02	Santamarina and Cho (2001)
G_s	2.65	Santamarina and Cho (2001)
φ_{cr}	28° (undrained)	Santamarina and Cho (2001)
Slope of CSL in e log p'	0.053 (undrained)	Santamarina and Cho (2001)
D_{10}	0.64 mm	ASTM D422
D_{30}	0.66 mm	ASTM D422
D_{50}	0.70 mm	ASTM D422
D_{60}	0.72 mm	ASTM D422

Each 101.6 mm x 254 mm (4” x 10”) Ottawa 20-30 sand column had a fixed dry mass of soil of approximately 3415 gm, corresponding to a target relative density of 70%. 766 ml of EICP solution (slightly more than one pore volume, such that the solution was approximately 2 mm above the soil in the vertical columns after sample preparation was complete) was used in forming 101.6 mm x 254 mm (4” x 10”) columns. 82 ml of EICP solution was used in forming the 50.8 mm x 101.6 mm (2” x 4”) columns (such that the solution was ≈ 2 mm above the soil in the column). Each of the three 50.8 mm x 101.6 mm (2” x 4”) columns had a fixed dry mass of soil of 350 gm corresponding to the same target relative density, 70%, as the 101.6 mm x 254 mm (4” x 10”) columns. All columns of both sizes were capped at the bottom to contain loose sand and the EICP solution. The

concentration of the EICP solution reagents used in these experiments were those determined to be optimal in Chapter 3 except that old (expired) of dried non-fat milk was used.

Soil B: F-60 Silica Sand

Six samples were created using EICP solution and F-60 silica sand (e_{max}=0.823, e_{min}=0.529, D_{10}=0.15, D_{30}=0.19, D_{50}=0.23, D_{60}=0.26, Cu= 1.73, Cc= 0.926, G_s=2.65, SiO_2>99%). Like the Ottawa 20-30 sand, an initial batch of three 101.6 mm x 254 mm (4" x 10") columns was made in PVC cylinders and three 50.8 mm x 101.6 mm (2" x 4") samples were made in acrylic cylinders. Each of the three 101.6 mm x 254 mm (4" by 10") columns had a fixed dry mass of soil of 3415 gm, corresponding to a target relative density, 90%. A relative density of 90% was chosen for the F-60 silica sand to obtain the same void ratio (0.56) as the Ottawa 20-30 silica sand at relative density of 70%, allowing for the same amount of EICP solution to be used. Two different EICP solution treatment methods, injection and percolation, were employed for both sizes of the columns to mimic how vertical columns might be created in situ. Samples were also prepared for both sizes of columns using the mix and compact method for comparison to earlier work in Chapters 3 and 5.

766 ml of EICP solution (slightly more than one pore volume such that the solution was ≈ 2 mm above the soil in the column) was put into the 101.6 mm x 254 mm (4" x 10") columns. 82 ml of EICP solution (slightly more than one pore volume, such that the solution was ≈ 2 mm above the soil in the column) was put into the 50.8 mm x 101.6 mm (2" x 4") columns. Each of the three 50.8 mm x 101.6 mm (2" x 4") columns had a fixed dry mass of soil of 350 gm at the target relative density, 90%, the same relative density as

the 101.6 mm x 254 mm (4” by 10”) columns. All columns of both sizes were capped at the bottom to contain loose sand and the EICP solution. The concentration of the EICP solution reagents used in these experiments were those determined to be optimal in Chapter 3 except that old (expired) of dried non-fat milk was used.

One 101.6 mm x 254 mm (4” x 10”) column and one 50.8 mm x 101.6 mm (2” x 4”) column of each soil type (Ottawa 20-30 sand and F-60 sand) were prepared by the percolation method. For the 101.6 mm x 254 mm (4” x 10”) columns, dry sand was poured into the columns to a height of approximately 10” (254 mm) using funnel and a height of fall selected to yield the target relative density of 70% for the Ottawa 20-30 column and 90% for the F-60 column. Then, the EICP solution was gradually dripped into the top of the column using a 60-ml syringe to gently disperse the solution into the sand, as opposed to poring it in quickly, which could erode depressions in the soil. 766 ml of EICP solution (slightly more than one pore volume, such that the solution was ≈ 2 mm above the soil) was injected into the Ottawa 20-30 and F-60 sand column in this manner. These processes were repeated with for the 50.8 mm x 101.6 mm (2” x 4”) columns except that 82 ml of EICP solution was put into both the Ottawa 20-30 sand and the F-60 sand.

One 101.6 mm x 254 mm (4” x 10”) column and one 50.8 mm x 101.6 mm (2” x 4”) column for each type of soil (Ottawa 20-30 and F-60 sand) were prepared with the injection method. To inject the EICP solution into the 101.6 mm x 254 mm (4” x 10”) column, a 330 mm (13”)-long Tygon laboratory tube (R-3603 PVC; 3/8” ID) was perforated with two lines of holes 11/64” in diameter, diametrically opposed and spaced at 12.5 mm (0.5”) center-to-center. The tube was wrapped with 250 µm polypropylene mesh (29% open area) to mitigate the potential for clogging of the holes with sand. Silicon glue was used to seal the bottom of the tube. In each column, 175 gm of the silica sand was gently poured into the column to a height approximately 1” (25.4 mm) and then densified gently by tapping the PVC cylinder with a small hammer. The tube was then placed along the central axis of the PVC column with the bottom of the tube on the densified sand. Then, 3240 gm of sand was added to the column to a height of approximately 10” (254 mm). The EICP solution was injected into the open top end of the Tygon tube using a 60 ml syringe. 766 ml of EICP solution was injected into the 101.6 mm x 254 mm (4” x 10”) columns.

To inject the EICP solution into the 50.8 mm x 101.6 mm (2” x 4”) column, a 153 mm (6”) long Tygon laboratory tube (R-3603 PVC; 3/8” ID) was perforated with two lines of holes 11/64” in diameter, diametrically opposed and spaced at 12.5 mm (0.5”) center-to-center. Just as described above for the larger columns, the tube was wrapped with 250 µm polypropylene mesh (29% open area) to mitigate the potential for clogging of the holes with sand. Silicon glue was used to seal the bottom of the tube. In each column, 88 gm of the silica sand was gently poured into the column to a height of approximately 1” (25.4 mm) and then densified gently by tapping the cylinder with a small hammer. The tube was then placed along the central axis of the acrylic column with the bottom of the tube on the densified sand. Then, 262 gm of sand was added to the column to a height of approximately 4” (101.6 mm). The EICP solution was injected into the open top end of the tube using a 60 ml syringe. 82 ml of EICP solution (approximately one pore volume, the solution was above the soil by ≈ 2 mm) was injected into the 50.8 mm x 101.6 mm (2” x 4”) columns of both the Ottawa 20-30 and F-60 sand columns.

One 101.6 mm x 254 mm (4” x 10”) column and one 50.8 mm x 101.6 mm (2” x 4”) column of both types of soil (Ottawa 20-30 and F-60 sand) were prepared with the mix and compact method. For the 101.6 mm x 254 mm (4” x 10”) columns, 766 ml of the EICP solution (slightly more than one pore volume) was poured into a bowl containing the Ottawa 20-30 sand. Then the sand and solution were mixed and placed into the PVC column in three layers. Each layer was compacted very gently with a small hammer to achieve the target relative density of 70% for Ottawa 20-30 sand. The hammer weighed 11.56 N (2.6 lb) and had a diameter of approximately 0.4” (10.2 mm). The three layers of sand and solution filled up the columns to a height of approximately 10” (254 mm) after compaction. The same procedure was used for the F-60 silica sand, except that the target relative density was 90%

For the 50.8 mm x 101.6 mm (2” x 4”) columns, 82 ml of the EICP solution (slightly more than one pore volume) was poured into a bowl containing Ottawa 20-30 sand. Then the mixed sand and solution were placed into the acrylic column in three layers and each layer was compacted very gently with a small hammer to achieve the target relative density of 70% for Ottawa 20-30 sand. The hammer weighed 11.56 N (2.6 lb) and had a diameter of approximately 10.2 mm (0.4”). The three layers of sand and solution filled up the columns to a height of approximately 102mm (4”) after compaction. The same procedure was used for the F-60 sand, except that the target relative density was 90%.

Once the columns were treated with the EICP solution, they were left to rest for three days with aluminum foil as a top cover to minimize solution evaporation. During this period, the samples were monitored for color change and the smell of ammonia (indicators of enzyme activity and cementation occurring). On day four, a 20-gauge needle was poked through the bottom cap, creating a hole big enough for drainage and small enough to stop sand from falling out. Then, 800 ml of DI water was added slowly to the top of each of the

101.6 mm x 254 mm (4” x 10”) columns to flush residual EICP solution and soluble salts from the column. The same procedure was repeated for the 50.8 mm x 101.6 mm (2” x 4”) columns except that only 100 ml of DI water was used. The columns were then left to rest for three more days at room temperature with the 20-gauge needle in place and a container underneath to collect the drained liquid. On day seven, the column was placed in an oven at 40 degrees Celsius for five days. After oven drying, the columns were prepared for the test procedures described below

Unconfined compressive strength (UCS) tests were performed only the 50.8 mm x 101.6 mm (2” x 4”) columns, which were fitted with polypropylene lines to facilitate extraction. UCS tests could not be performed on the 101.6 mm x 254 mm (4” x10”) columns because polypropylene liners were not placed in the acrylic cylinders and the silica sand adhered to the inner surface of the PVC cylinder. This adhesion made the cemented sand extremely difficult to extract from the PVC column. A hammer had to be used to extract the cemented sand columns, breaking the columns into many pieces of varying size, as illustrated in Figure 43, which were unsuitable for mechanical testing.

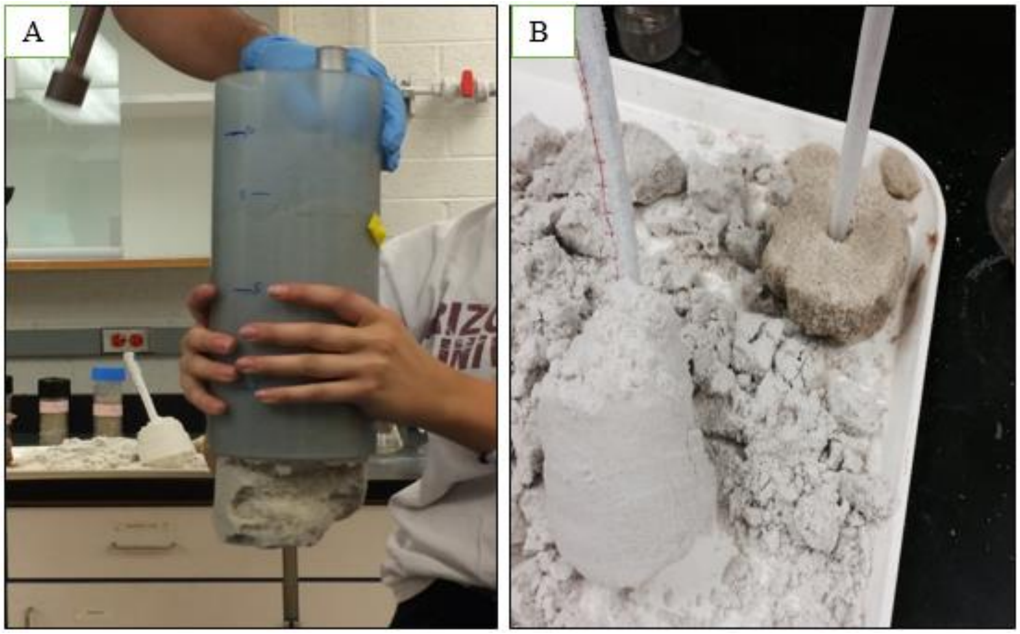


Figure 43: A) Extraction of cemented F-60 silica sand from the 101.6 mm x 254.8 mm (4” x10”) column, B) Pieces of cemented sand after extraction

Selected small cemented fragments from all columns were saved for SEM image analysis (for the small columns, this was done once mechanical testing was completed). The SEM images provided insight into the morphological features of precipitated $CaCO_3$. In order to calculate the percentage of $CaCO_3$, selected intact chunks of cemented sand from all twelve (12) columns involved in these experiments were dried, weighed, washed with 4M hydrochloric acid (HCl), and then dried in the oven at 105 degrees Celsius for 24 hours. The differences in mass before and after washing the samples with HCl indicate the mass of precipitated $CaCO_3$.

Results of EICP Column Experiments

The results and observations from the EICP vertical column experiments are described below based on the preparation method used (i.e., Percolation, Injection, and Mix-and-Compact). As noted above, it was not possible to measure the strength of the

101.6 mm x 254 mm (4” x 10”) columns due to breakage of the cemented columns during extraction. However, the UCS was measured on the 50.8 mm x 101.6 mm (2” x 4”) columns.

Weaker cementation was observed for the F-60 silica sand compared to Ottawa 20-30 sand based upon visual observations from percolation method. The F-60 sample was easily disintegrated into loose sand by hand. The measured UCS for the 50.8 mm x 101.6 mm (2” x 4”) columns supported the visual observation that the F-60 sample had less strength than the Ottawa 20-30 sand samples. Percolation columns of 101.6 mm x 254.8 mm (4” x 10”) were extremely weakly cemented for the first ≈1” from the top of the column. Also, the 50.8 mm x 101.6 mm (2” x 4”) columns easily disintegrated at the top. Additionally, there was not much visual evidence of mineral precipitation (i.e., visual evidence of a white precipitate) in these columns except at the very bottom of the Ottawa 20-30 columns. The weak strength at the top of the columns and the white color at the bottom may be indicative of a non-uniform improvement process, with more cementation at the bottom than at the top of the columns. Acid digestion test results, presented in Table 18, indicated higher carbonate content in the F-60 sand sample than in the Ottawa 20-30 sand sample, despite the lower strength. Table 18 presents the results of tests conducted on samples prepared using the percolation method.

Table 18 Results from columns using the Percolation preparation method

Sand	101.6 mm x 254.8 mm (4” x 10”) Columns			50.8 mm x 101.6 mm (2” x 4”) Columns		
	UCS (kPa)	% $CaCO_3$	Odor (NH_3)	UCS (kPa)	% $CaCO_3$	Odor (NH_3)
Ottawa 20-30	N. A	1.79	Very strong	110	1.21	Very strong
F-60	N. A	2.7	Very strong	60.9	1.39	Very strong

N.A: not available

Visual observations on samples prepared using the injection method indicated that the 101.6 mm x 254.8 mm (4” x 10”) columns had the strongest cementation. Furthermore, based upon the color change, the largest amount of mineral precipitation occurred around the injection tube. The strengths measured on the 50.8 mm x 101.6 mm (2” x 4”) columns indicated a higher strength for the Ottawa 20-30 samples and higher strengths (essentially double) than achieved using the percolation method. However, since the UCS tests were conducted with the injection tube still seated in the sample, the measured strengths may not be the true UCS of the cemented sand. The higher strength achieved using the injection method compared to the percolation method is hypothesized to be either because of: (a) that in the percolation method, the enzyme may become denatured as it percolates down through the specimen (as opposed to the injection, which allows fresh EICP solution to spread throughout the entire specimen, top to bottom), or (b) that the tube inside the injection samples may increase the measured strength. Acid digestion results, reported as shown in Table 19, indicated similar carbonate content than reported for the samples prepared by percolation.

Table 19. Results and observations columns prepared using the Injection method

Sand	101.6 mm x 254.8 mm (4” x 10”) Columns			50.8 mm x 101.6 mm (2” x 4”) Columns		
	UCS (kPa)	% $CaCO_3$	Odor (NH_3)	UCS (kPa)	% $CaCO_3$	Odor (NH_3)
Ottawa 20-30	N. A	1.80	Very strong	332	1.78	Very strong
F-60	N. A	2.31	Very strong	128	0.8	Very strong

N.A: not available

The two 50.8 mm x 101.6 mm (2” x 4”) columns prepared using mix and compact were more uniformly cemented compared to columns prepared using injection or percolation. Once again, it appeared that the F-60 silica columns had weaker cementation than the columns with Ottawa 20-30 sand, as the cemented F-60 sand samples were relatively easily disintegrated into loose sand particles. The measured UCS strength on the small samples supported the observation that the F-60 sand samples were weaker than the Ottawa 20-30 sand samples despite a higher calcium carbonate content. The measured UCS values on the mix and compact samples were close to those of the baseline experiments performed in Chapter 3 using the same EICP solution and preparation method. Table 20 indicates similar carbonate content for the mix and compact samples as that reported for the samples prepared by percolation or injection.

Table 20: Results from columns prepared using mix-and-compact

Sand	101.6 mm x 254.8 mm (4” x 10”) Columns			50.8 mm x 101.6 mm (2” x 4”) Columns		
	UCS (kPa)	% $CaCO_3$	Odor (NH_3)	UCS (kPa)	% $CaCO_3$	Odor (NH_3)
Ottawa 20-30	N. A	1.80	Very strong	52.79	1.91	Very strong
F-60	N. A	2.31	Very strong	42.54	1.56	Very strong

N.A: not available

SEM imaging was used to observe the shape and locations of calcite precipitation occurring on the Ottawa 20-30 and F-60 sand grains. Figure 44 shows images of $CaCO_3$ precipitation for all the three preparations method using Ottawa 20-30 sand grains. The SEM images from the mix-and-compact specimens indicate that most of the calcium carbonate precipitation is on the sand particles’ surfaces, not between or bridging the soil particles. For the percolation specimens, the calcium carbonate precipitation appears to have occurred along a predominantly vertical path that follows the injection tube (see the remnant of the tube pathway in the center of the image) but in an irregular manner and there is also precipitation on the particle surface and at the inter-particle contacts. The images from samples prepared using the injection method show less calcium carbonate precipitation on the surface of the sand compared to another perpetrations method. However, SEM Analysis of F-60 silica sand specimens was performed but the images were not clear because the particle was charged and there was an issue with the machine itself.

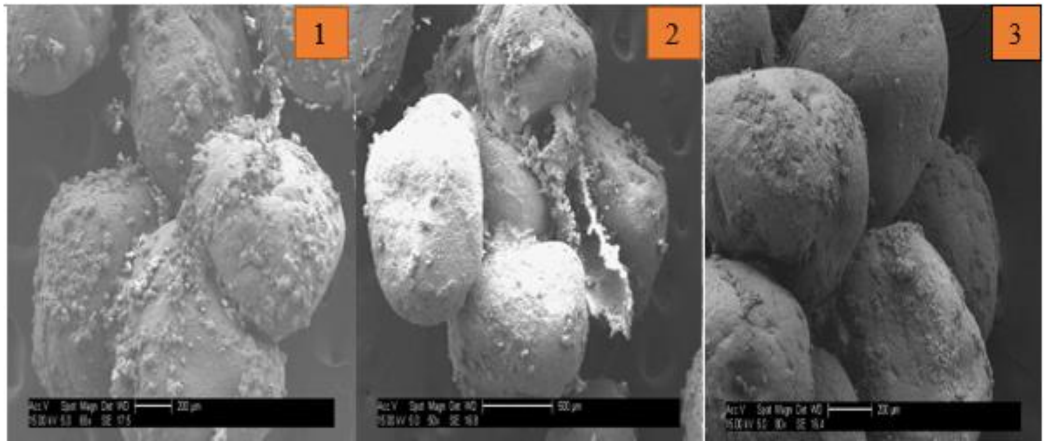


Figure 44: SEM images for samples prepared using the three treatments methods; 1) Mix-and- compact; 2) Percolation; and 3) Injection.

DISCUSSION

Differences in EICP Cementation in Ottawa 20-30 versus F-60 Silica Sand

Testing described in this Chapter indicated that F-60 sand will generally have less strength when treated using EICP compared with Ottawa 20-30 sand treated in the same manner, even when the F-60 sand has a higher relative density and a higher carbonate content than the Ottawa 20-30 sand. The reason for this is unclear, but may be related to grain size, particle angularity, and/or surface texture effects.

Also, while there was a significant variation in strength among samples prepared using the three methods described in this chapter, the $CaCO_3$ obtained using the three different preparation methods were relatively consistent. Therefore, $CaCO_3$ content alone does not seem to be a good indicator of cementation strength.

The method of treatment does appear to impact the strength of the treated samples**.** As shown in Figures 45 and 46, the UCS values from tests on the 50.8 mm x 101.6 mm (2” x 4”) columns, the injection method of preparation yielded the highest strength among the three treatment methods (assuming the tube left in the sample was not a major factor).

Based upon both UCS values and visual observations, the mix-and-compact method produced the lowest strength. The UCS was 52.79 kPa for Ottawa 20-30 sand and 42.54 kPa for F-60 silica sand for samples prepared by mix and compact. The relative similarity in strength between samples of different grain size prepared using the mix and compact method may be because most of the calcium carbonate precipitation in samples prepared in this manner was on the sand particle surfaces and was not at contacts or bridging between soil particles (based upon the SEM imaging). The difference in strength between Ottawa 20-30 and F-60 sand samples prepared using the percolation method was more significant compared to mix-and-compact method in strength values. The strength values differed from 110 kPa for Ottawa 20-30 sand to 60.9 kPa for the F-60 silica sand. The difference in the strength value between the two types of preparation may be due to the grain size effect or it may be due to the surface texture of the particles. The injection method produced the largest value of strength for both the types of sand (Ottawa 20-30, and F-60 silica sand) compared to the other preparation methods. The strength was 332 kPa for the sample of Ottawa 20-30 prepared by injection and 128 kPa for the sample of F-60 silica sand prepared by injection. Two reasons are hypothesized for the greater strength of sample prepared by injection: (1) leaving the injection tube in the sample increased the strength of the sample, and/or (2) the cementation solution was diffused through the entire specimen at about the same time as it was introduced to the soil, minimizing loss of efficiency due to denaturation of the urease enzyme. Note that all of the tests described in this chapter were conducted without the addition of fresh dried non-fat milk as a stabilizer for the enzyme.

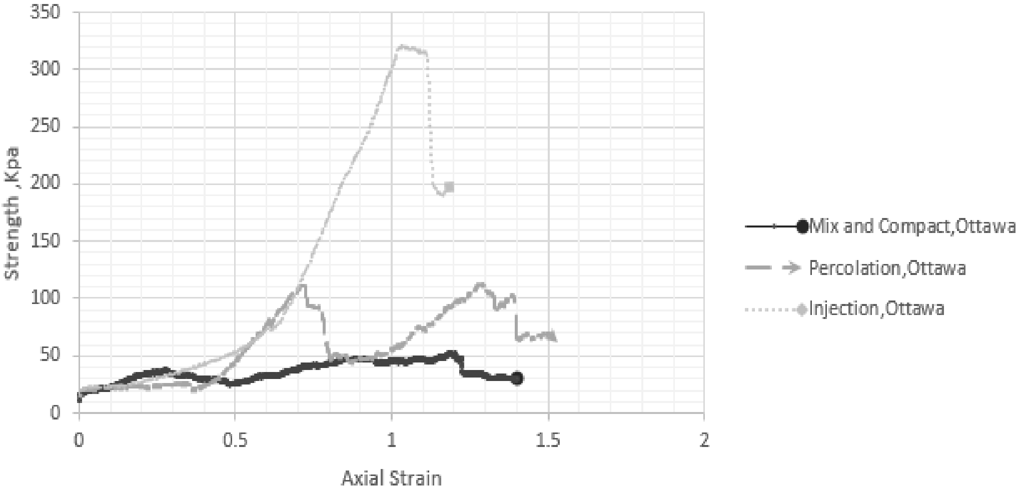


Figure 45. Stress-strain behavior in unconfined compression of Ottawa 20-30 sand for three preparation methods.

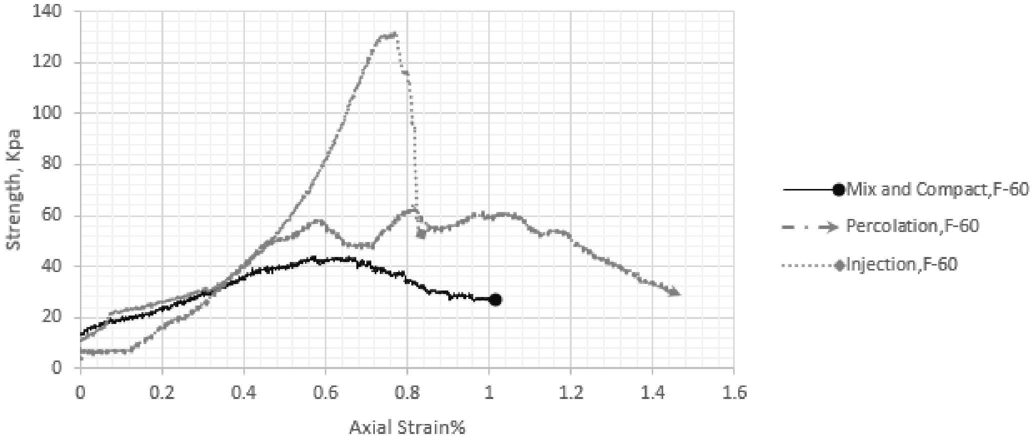


Figure 46. Stress-strain behavior in unconfined compression of F-60 sand for three preparation methods

CONCLUSION

Experiments performed in this chapter describe sub-horizontal and vertical columns prepared by treating soil with EICP solutions. For the sub-horizontal column experiment, inclined columns were installed in a box filled with dry Ottawa F-60 silica sand. The columns were installed by injecting the EICP solution through perforated tubes. After allowing the EICP solution to cure, removing the face of the box, and applying a load to the soil surface, there was no visual evidence of cracking or instability. SEM imaging and XRD analysis were performed to elucidate the pattern of $CaCO_3$ precipitation and the morphology of the carbonate phase. The results indicated that EICP holds promise as a ground improvement technique for creating sub-horizontal columns for soil reinforcement (e.g., soil nailing).

Two types of sand (Ottawa 20-30, and F-60) and two different preparation methods (percolation and injection) were used with two different dimension columns to investigate the effect gradation and the method of preparation on vertical column formation. The results showed that the injection method had the highest UCS compared to the percolation method and that both methods produced higher strength than was achieved using the mix-and-compact method. Analysis of SEM images showed a difference in the pattern of $CaCO_3$ precipitation for the different methods of preparation.

CHAPTER 7

CONCLUSIONS AND RECOMMENDATIONS FOR FUTURE WORK

SUMMARY

This dissertation examines the feasibility of enzyme induced carbonate precipitation (EICP) as a soil improvement technique. The mechanical properties of EICP-treated soil were studied with various admixtures and subject to various treatment conditions. Chapter 2 of this dissertation describes relevant work performed by other researchers using both microbial induced carbonate precipitation (MICP) and EICP.

Chapter 3 presents the results of Falcon tube tests to study the influence of the concentration of constituents on carbonate precipitation. Over 60 tests using different concentrations of urea, calcium chloride, and urease enzyme were conducted. The reusability of effluent from these tests was investigated in order to reduce the cost of EICP treatment of soil. The addition of calcite seeds to the EICP solution was also investigated to see the effect of calcite seeds on the strength of the treated soil and the mineralogy of the precipitates. The optimum EICP recipe deduced from the Falcon tubes was used to treat Ottawa 20-30 sand in in acrylic columns using two different preparation methods: mix-and-compact and percolation. The effect of water rinsing on EICP-treated soil was also examined in this chapter.

Chapter 4 investigated the potential for enhancing the strength of EICP-improved Ottawa 20-30 silica sand by adding supplemental materials. Two different natural materials, xanthan gum and sisal fiber, were evaluated as supplements. Xanthan gum was applied in two ways: mixed as a powder with dry soil and mixed with EICP solution. The fibers were simply mixed with the dry soil. Three different concentrations of urea to calcium chloride were also investigated. The mix-and-compact method was used for all samples. Testing included unconfined compressive strength (UCS) strength tests, scanning electron microscope (SEM) imaging to look at evidence of the phase of calcium carbonate precipitate and the pattern of precipitation, and acid digestion to measure the amount of calcium carbonate precipitate.

In Chapter 5, acrylic columns filled with Ottawa 20-30 sand were prepared using the mix-and-compact method with two organic additives, dried non-fat milk and cabbage juice, in order to enhance the mechanical behavior of the EICP-treated soil. Five different EICP solutions were used in the experiments described in this chapter to evaluate the effect of these additives, including one with a very low concentration of constituents (0.37 M urea, 0.25 M $CaCl_2$, 0.8 g/l of urease enzyme, and 4 g/l of dried non-fat dry milk) to evaluate the potential for minimizing the cost of EICP treatment and its environmental impact. *UCS,* Energy Dispersive X-*ray (*EDX*),* SEM, X-ray Powder Diffraction (XRD), and acid digestions tests were used to characterize the results on the specimens discussed in this chapter.

Tests described in Chapter 6 investigated the potential of using EICP to create sub-horizontal and vertical columns for soil improvement. For the sub-horizontal columns experiment, F-60 silica sand was used. The EICP solution for the sub-horizontal column consisted of 0.87 M urea, 0.5 M $CaCl_2$-$2H_2O$, 0.85 g/L low-grade urease enzyme. For vertical columns, two types of sand (Ottawa 20-30, and F-60) and two different preparation methods (percolation and injection) were used with two different dimension columns to investigate the effect gradation and the method of preparation on vertical EICP column formation. The vertical column experiments were performed using an EICP solution composed of 1 M urea, 0.67 M $CaCl_2$, 4.0 g/l of dried non-fat milk as a stabilizer, and 3 g/l of low activity urease enzyme.

CONCLUSIONS

In Chapter 3, the optimal recipe for EICP based on a high mass of precipitation, high efficiency (with respect to the percent of calcium in the substrate precipitate as carbonate), and a low concentration of enzyme was investigated using Falcon tube tests. This optimal recipe for EICP treatment established on this basis was found to be a solution consisting of 1.00 M urea, 0.67 M $CaCl_2$ and 3.00 g/l urease enzyme. The tests described in this chapter also showed that the effluent from EICP treatment without the use of an organic stabilizer (i.e., dried non-fat milk) cannot be used as a source of enzyme for additional treatment. However, the unconsumed calcium chloride and urea in the effluent from an incomplete treatment process still can be precipitated by adding fresh urease enzyme. The Falcon tube tests suggested that when using the optimal recipe approximately 93% of the calcium in the substrate was converted to calcium carbonate. However, less than 30% of calcium in the substrate was converted to calcium carbonate when the optimal recipe was applied to acrylic columns filled with Ottawa 20-30 silica sand. This reduction in precipitation ratio may be ascribed to insufficient interaction between urease molecules and substrate. In the Falcon tube tests, the solution containing substrate and enzyme was shaken for 72 hours at 200 rpm. However, the acrylic columns filled with sand could not be shaken. Rinsing the treated soil with deionized (DI) water caused a loss of precipitate. The lost precipitate is assumed to be residual salts and organic material and soluble non-calcite phases of calcium carbonate such as vaterite and amorphous. Thus, to properly evaluate the effect of carbonate precipitation on strength, the treated soil should always be thoroughly rinsed to remove residual non-carbonate material.

In Chapter 4, the potential for enhancing the strength of EICP-improved Ottawa 20-30 silica sand by adding supplemental materials was evaluated. Tests were conducted in which xanthan gum was used as a supplemental material. The optimum amount of xanthan gum for enhancing unconfined compressive strength was 1.1% of the dry weight of the soil, i.e., 1.1% (w/w). Mixing the xanthan gum, as powder, into dried soil yielded a higher strength and greater ductility than mixing the xanthan gum into the EICP solution. For both methods of application of xanthan gum, it was very hard to dry rinsed samples, even in an oven at 105 °*C*. Acid digestion of oven-dried samples prepared using both methods yielded a higher percentage of mass loss than the amount (weight) of $CaCO_3$ that could be precipitated based upon the amount of calcium in the EICP solution. The extra mass loss is assumed to represent both residual salts and organic material in the column and as well as xanthan gum that was not removed by rinsing. SEM imaging of rinsed samples showed that xanthan gum still covered the particle surfaces and bridged between particles after rinsing. The SEM images also indicated improvement in the morphology of the precipitated carbonate, a change attributed could be to the change of the kinetic reaction rate and/or delay of the precipitation reaction. Experiments were also performed in acrylic columns that were prepared in which sisal fibers 10 mm and 20 mm in length were added to Ottawa 20-30 sand at fiber contents up to 0.85% (w/w) prior to preparing samples using mix-and-compact method. The longer (20 mm) fibers were not effective. UCS tests showed increases in strength on the order of 380% for the sisal fiber-enhanced samples at up to 0.3% (w/w) fiber content with fibers that were 10 mm length. At fiber contents greater than

0.3% (w/w), there was a sharp decline in strength. The failure strain of the EICP-treated sample with sisal fiber at 0.3% (w/w) was almost three times higher than samples prepared without addition of sisal fiber.

In Chapter 5, enhancement of EICP treatment by adding two organic additives, dried non-fat milk and green cabbage juice, to the EICP solution was evaluated. The results from adding green cabbage juice, reported by others to inhibit ureolysis, did not show any response, i.e., it did not inhibit or slow down the reaction (thereby enhancing the EICP process by facilitating precipitation of calcite phase calcium carbonate) either with or without dried non-fat milk in the solution. On the other hand, adding fresh dried non-fat dry milk to the EICP solution showed a significant improvement for the properties of soil. The results observed after adding fresh dried non-fat dry milk from UCS and undrained triaxial tests for treated samples showed significant improvement in the soil strength with a relatively small amount of calcium carbonate (2 MPa UCS with less than 1% $CaCO_3$). These results were unprecedented for all biogeotechnical carbonate precipitation techniques. Through SEM testing, calcite crystals were found to be focused at the inter-particle contact points. This is believed to be the source of the increased strength. In an attempt to reduce the cost of treatment, urea and $CaCl_2$ concentrations were reduced from relatively high concentrations (1 M urea, 0.67 M $CaCl_2$, and 3 g/l of urease enzyme) to a much lower concentrations (0.37 M urea, 0.25 M $CaCl_2$, and 0.8 g/l of urease enzyme). This corresponded to a reduction in mass of 63% for urea and $CaCl_2$ and 74% for the mass of urease enzyme in the EICP solution. The findings from these tests were: (1) the reduced concentrations resulted in a reduced but still significant strength (above 1 MPa), (2) there was no difference in morphology observed in SEM images of specimens treated using the high concentration solution and specimens treated using the reduced concentration solution, (3) the carbonate content was close to the theoretical maximum for the reduced concentration solution, and (4) the amount of unwanted (harmful) NH_4Cl by-product was reduced using the reduced concentration solution. The results of these tests suggest that the strength of an EICP-treated soil sample is less dependent on how much $CaCO_3$ is precipitated than by the location of precipitation, i.e., whether the precipitation is concentrated at the particle contacts or spread over the surface of particles. In general, using dried non-fat milk was observed to eliminate or establish control over the drawbacks of EICP and enhancing its effectiveness.

In Chapter 6, the results from vertical and sub-horizontal column experiments showed that EICP holds promise as a ground improvement technique for creating stabilized vertical columns and for soil nailing in cohesionless soils. The simulated soil nails were installed by injecting the EICP solution through four equally-spaced sub-horizontal perforated tubes. Moreover, the results from vertical columns showed there was a significant difference in the pattern of precipitation between the methods of column formation. The reason for these differences is not clear. However, the results from these experiments demonstrated that the way a vertical column is made, i.e., via injection or via percolation, is important as it affects the pattern of precipitation and the resulting strength of the column.

RECOMMENDATIONS FOR FURTHER STUDY

The work conveyed in this dissertation presents systematic studies of the factors affecting enzyme induced carbonate precipitation (EICP) as a potential technique for soil improvement. While the work presented herein provides substantial insight into EICP, additional investigations focusing on significant parameters of interest would be helpful in gaining further understanding of the EICP technique, ultimately leading to application of this technology on a field scale. These parameters include the mechanism by which the addition of powdered milk enhances the precipitation process, grain size effects, and the effects of soil mineralogy, soil and pore water chemistry, and soil surface texture. Furthermore, there may be a benefit to slowing down the rate of precipitation by finding an agent that inhibits the EICP reaction in order to get more uniform cementation and a high percentage of calcite precipitate. In addition to understanding these effects on EICP, substantial work will be needed on the best method(s) with which to employ EICP in the field. Therefore, more testing under many different conditions is highly desired to determine the feasibility of EICP soil improvement technology for field applications.

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