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Additive Manufacturing of Soil Using Bio-Cementation

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Additive Manufacturing of Soil using Bio-Cementation

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ABSTRACT

Microbially Induced Calcite Precipitation (MICP) is an emerging soil treatment technique that is proven to increase strength, decrease erosion, reduce liquefaction risk, enhance slope stability, decrease compressibility, decrease swelling potential, and overall create a more competent soil. The benefits and applications of MICP are broad, and this research seeks to broaden them further by developing a single-phase additive manufacturing application with no treatment time delay. This is done by analyzing layering behaviors of five USCS soil classifications (100 % Ottawa sand, sand clay mixtures, and 100% lean clay) which provides insight into process variables such as the solution volume and layer thicknesses for the additive manufactured specimens. Cuboidal specimens were produced using a layering approach where both bacterial and cementation solutions were applied on the surface of every layer using a volume-controlled spray system. The cuboidal specimens were tested in unconfined compression and the results indicated a notable increase in soil strength for clay soils using this treatment method. This application method evades some complexities commonly faced with fine-grained soils. In addition, the potential of utilizing gel spray solutions for higher levels of control when applying solutions in a defined pattern to create mechanically advantageous shapes were considered. The addition of gel to the treatment solutions reduced bleed and allowed for more control. Both potentials show promise but require more examination. All application specifics are highly variant depending on soil type and would need to be calibrated for site-specific projects.

ACKNOWLEDGEMENTS

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1. INTRODUCTION

1.1 Objectives

The objective of this research is to develop a new approach to Microbially Induced Calcite Precipitation (MICP) soil treatment using an additive manufacturing technique. The specific goals include:

a) Examine the possibility of a single spray application method of MICP completed in a layer-by-layer process.
b) Gain an understanding of cementation behavior of different soil classifications and explore soil types that are not typically suitable for injection MICP methods, yet exist in much of the world.
c) Determine strength benefits of this new application method and consider potential applicability.
d) Perform a preliminary assessment of spray gel solutions for a more refined application alternative.

1.2 Overview

After the introductory Section 1, Section 2 provides a literature review summarizing the MICP process, what factors can influence the efficacy of the process, the current methods of application, the effects of MICP on engineering properties of soils, field-scale applications, and cost effectiveness of this type of treatment. Section 3 outlines the methodology for the process including how the bacterial and cementation solutions are developed, the spray system developed for the process, how layer parameters were determined for different soil types, how the unconfined compression test specimens were prepared, the development of gel spray solutions, and the method chosen for stencil usage. Next, Section 4 includes the results and discussion for the cementation penetration tests, unconfined compression tests, and the alternative application options of gel sprays and stencils. Finally, the conclusions of all experimentation, and the viability of this new method of MICP application, is stated in Section 5.

2. LITERATURE REVIEW

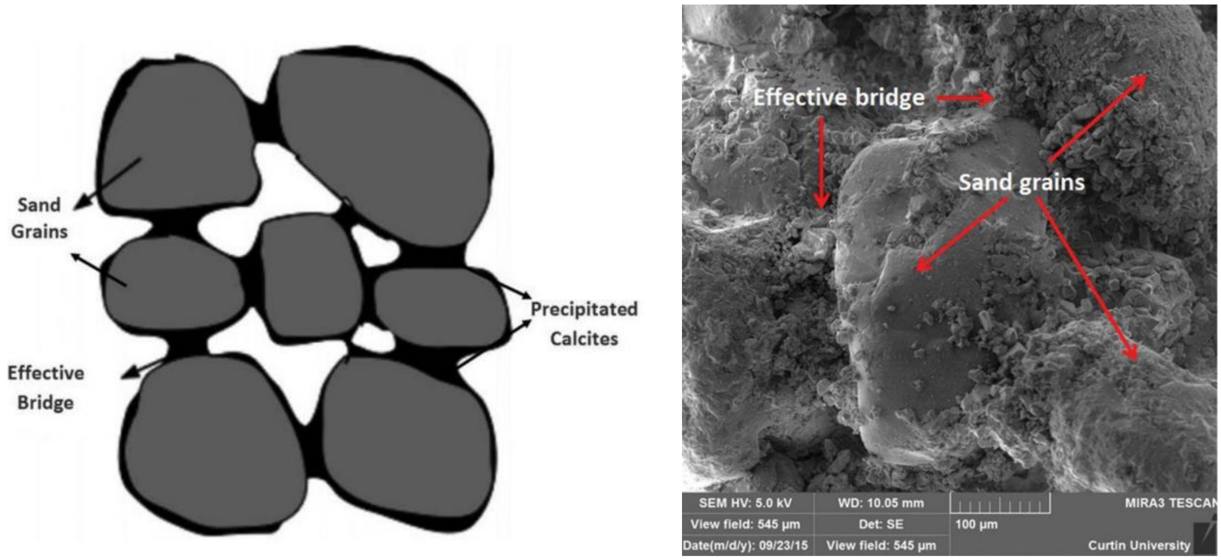
2.1 Microbially Induced Calcite Precipitation (MICP)

Calcium carbonate is a natural byproduct of microbial metabolic activity. The relationship has led to a commonly used system of leveraging microbial activity known as Microbially Induced Calcite Precipitation, or MICP. *Sporosarcina Pasteurii* is generally considered the most reliable and consistent source of microbial activity. Other bacteria options exist, such as *Bacillus Sphaericus*, but result in lower final specimen strengths (Sharma et al., 2021). Several methods exist for activating calcite precipitation, the most common method is urease hydrolysis which behaves according to Equation 1. Once carbonate ions precipitate, the ions react with calcium ions to form calcite (Equation 2).

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

$$Ca^{2+} + CO_3^{2-} \rightarrow CaCO_3 \tag{2}$$

The resulting precipitation provides increased stiffness due to the cementation bridges formed between sand grains. In the typical injection-style application, cementation occurs both around the sand grains and as cementation bridges between grains (Figure 2.1.1). This means that while all precipitation that occurs will affect the soil, not all precipitation will form effective bridges between soil grains and cannot be described thus. Calcite is the most stable form of precipitate, however vaterite crystals are also a viable, less stable precipitate. The stability of the precipitation environment and the type of nutrient used can affect which minerals precipitate (Omoregie et al., 2019).



(a) (b)

Figure 2.1.1 Effective bridge formation: (a) schematic diagram; and (b) Scanning electron microscopy (SEM) (Mujah et al., 2017)

2.1.1 Factors that affect the MICP process

The efficacy of the chemical processes that produce calcite can be affected by several factors, with the primary factor being the molar concentrations of all reagents. The activity level of the bacteria can alter reaction time, which also affects the overall process. Another important factor is the grain size of the soil being treated. The injection form of treatment (i.e., the most common method developed to date) is performed with the most ease on well-graded and coarser sands due to their higher permeability and greater void space which allows for higher activity levels (Arpajirakul et al., 2021; Mortensen et al., 2011; Zhao et al., 2014)**.** Finer sands, silts and clays can be treated but the lower permeability limits the penetration of the solutions and often results in lower levels of uniformity. An increase in specific surface, as occurs with a decrease in particle size, allows for higher levels of bacteria attachment which can lead to higher calcium carbonate activity (Hushmand et al., 2017; Konstantinou et al., 2021; Sun et al., 2019). Having less void space to fill can also aid the bacteria in successfully cementing soil. Figure 2.1.1.1 illustrates how void spaces in finer grains restrict the precipitation create a more overall uniform precipitation. The Scanning Electron Microscope (SEM) images in Figure 2.1.1.2 also demonstrate the effects of a change in specific surface by showing images of coarse, medium and fine sands treated with MICP. The coarse uniformly-graded sand shown in Figure 2.1.1.2.a and Figure 2.1.1.2.d, Ottawa 20-30, has a lower specific surface and fewer contact points than the other two sands shown which noticeably affects the bonding ability of the cementation precipitate. There are, therefore, contrasting benefits both for coarse grained soils and fine-grained soils and results of any given application may be highly variable depending on the specifics of the soil under consideration.

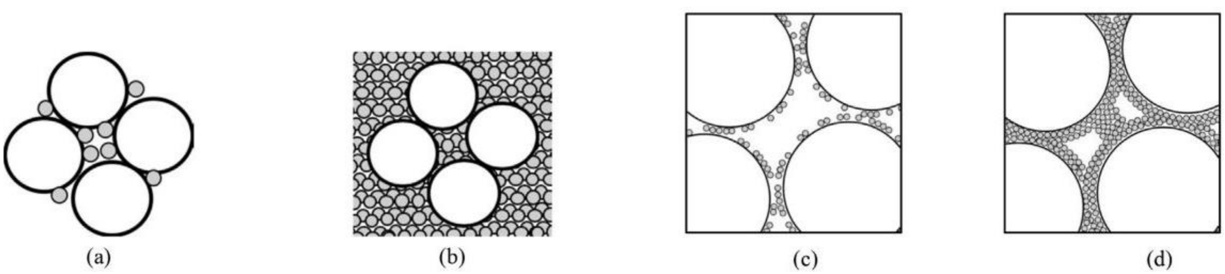


Figure 2.1.1.1 Calcite precipitation in: (a) lightly cemented fine sand; (b) highly cemented fine sand; (c) lightly cemented coarse sand; and (d) highly cemented coarse sand (Konstantinou, Biscontin, et al., 2021).

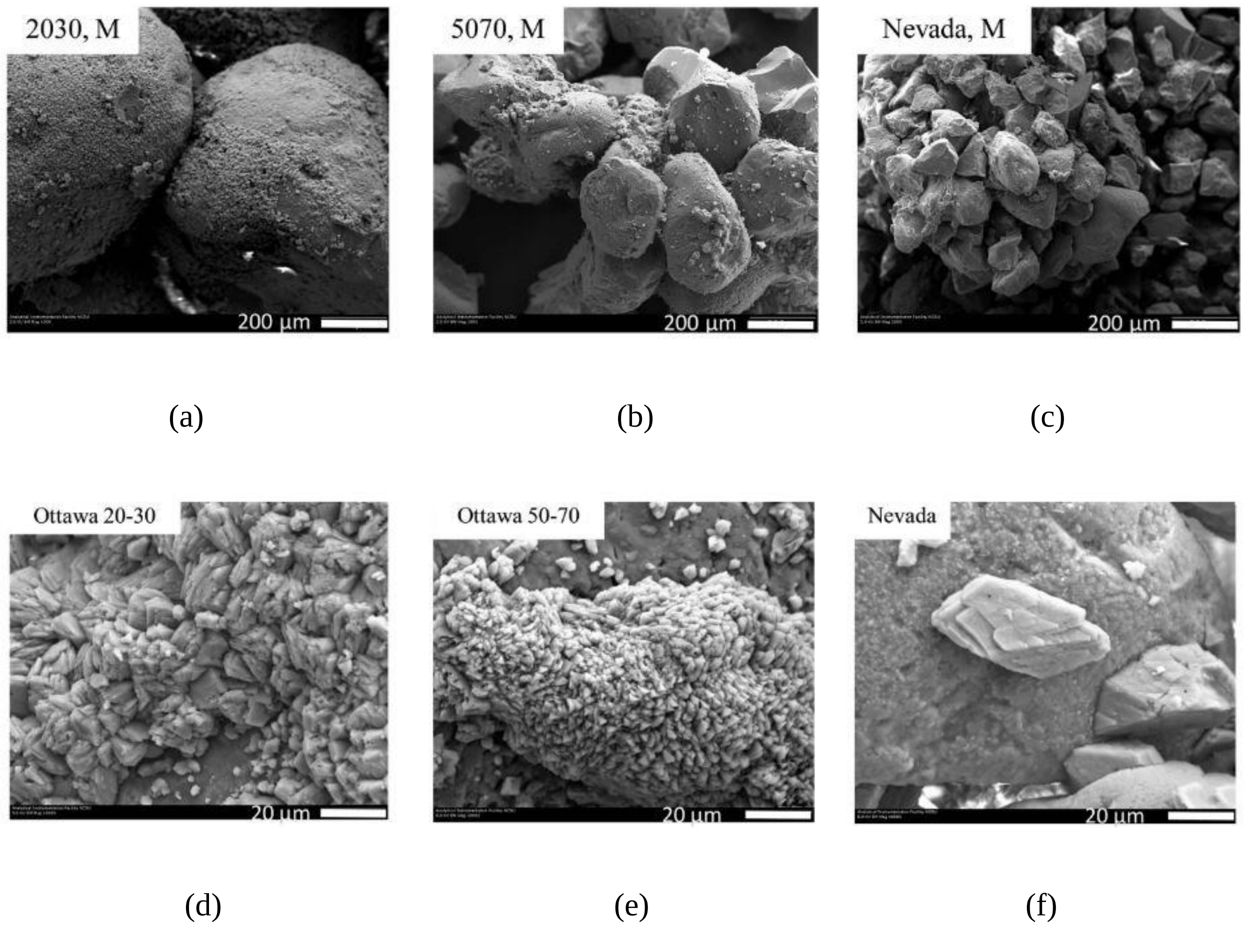


Figure 2.1.1.2 SEM images of: (a) coarse Ottawa 20-30 sand; (b) medium Ottawa 50-70 sand; (c) fine Nevada sand; and (d-f) higher magnifications of the three (Nafisi et al., 2020a).

One benefit of MICP application is that there is no notable variance in performance due to curing environments. Positive results can be achieved in up to 100% salinity, below the groundwater table, and in poor weather conditions (Kumar et al., 2020; Mortensen et al., 2011).

While there are many advantages to using a bio-cementation soil treatment, there are also some difficulties. It is difficult to sustain steady bacteria activity which causes the mass of precipitated calcium carbonate to fluctuate (Zhao et al., 2014). In part due to the fluctuation caused by bacteria activity, the bacteria concentrations of injected solutions also varies within specimens which causes a lack of uniformity (Wang et al., 2017). The issue of uniformity has been somewhat successfully addressed by adjusting the bacterial urease activity (Konstantinou, Wang, et al., 2021) and retarding the bacteria activity with lower temperature to allow for uniform dispersion prior to precipitation (Y. Xiao, Wang, et al., 2021)**.**

2.1.2 Application methods

Bio-cementation has been examined as a soil treatment option considering three methods: injecting, pre-mixing, and surface spraying. Injecting is the most common method used in academic experimentation, pre-mixing is only used for special applications, and surface spraying has been used to prevent erosion of soil surfaces in field-scale applications. These three methods are the foundation for a broad spectrum of specific preparation and application methods performed for different purposes. One facet that all methods have in common is that post a singular placement of bacteria into the soil, multiple treatments of cementation solution are applied.

2.1.2.1 Injection method (or traditional method)

Traditional MICP is performed by injecting the bacteria solution to create a saturated environment, draining the solution, and repeating the process with the urea solution (Fujita et al., 2000; Stocks-Fischer et al., 1999). The urea solution is often injected over repeated cycles to generate more desirable results (e.g., Akimana, 2016; DeJong et al., 2006; Montoya et al., 2021; A. Zamani & Montoya, 2018). Figure 2.1.2.1.1 shows the steps of this method, starting with the introduction of bacteria into the sample and followed by introducing the cementation solution for an extended period of time. Injection is generally controlled by peristaltic pump. The specific time of retention and specific number of cementation solution applications vary across the literature (e.g., Bagriacik, Sani, Uslu, Yigittekin, & Dincer, 2021; Nafisi et al., 2020b; Qian et al., 2021; Whiffin et al., 2007). Once treatment is finished, the sample is generally flushed with deionized water to remove any remaining bacteria and reaction byproducts, namely ammonia. The results can take loose sand and form it into a sandstone like material (Figure 2.1.2.1.2).

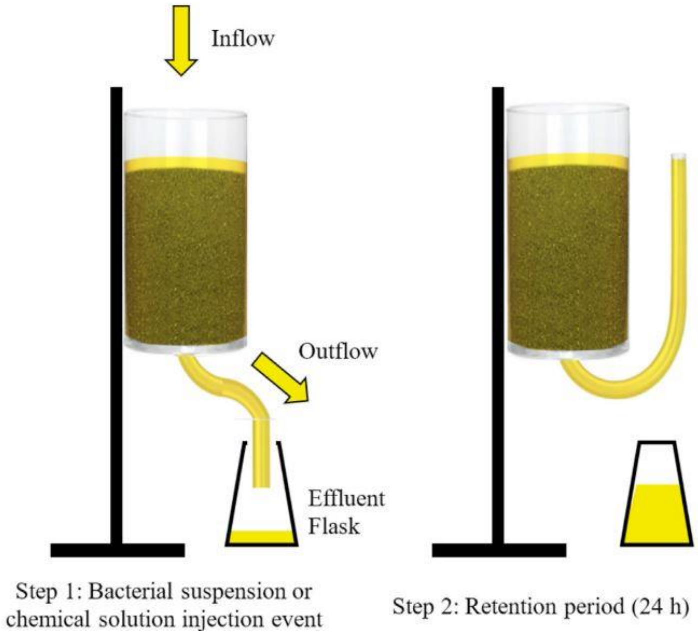


Figure 2.1.2.1.1 A traditional injection method setup (Konstantinou et al., 2021).

(a) (b)

Figure 2.1.2.1.2 MICP transformation: (a) loose sand; and (b) bio-cemented sand (Mujah et al., 2017)

2.1.2.2 Pre-mixing method

Pre-mixing involves directly mixing the bacteria into the soil specimen prior to applying cementation solution (Sun et al., 2019). For field scenarios, this would require removing the soil to be treated, mixing it with the bacteria solution, replacing the soil, and applying the cementation solution. Premixing examples are more limited than other methods, but this method has been applied to soft clay treatment and a type of additive manufacturing (Arpajirakul et al., 2021; Kannan et al., 2020; Nething et al., 2020; Teng et al., 2021; Tiwari et al., 2021). For the soft clay, premixing the bacteria allowed for more uniform spatial distribution which resulted in higher levels of induced calcite (Teng et al., 2021; J. Z. Xiao et al., 2020). Premixing allowed for soft clay to be more effectively treated and be transformed into a useful construction material. While premixing provides more uniform cementation than injecting, it can require natural soil to be disturbed and is not as popular as the injecting method.

2.1.2.2.1 Premixed additive manufacturing

Forays into additive manufacturing using MICP have relied upon selectively premixing a urease active calcium carbonate powder with soil. The 3D printer layers pure sand and selective areas of sand mixed with the urease active calcium carbonate powder created from a bio slurry of calcium carbonate powder (Nething et al., 2020). Once 3D printing was completed with dry pure sand and dry biopowder-sand mix, the entire build volume was treated with cementation solution for 5 minutes every 8 hours for a total of 5 treatments (Figure 2.1.2.2.1.1). Results of this method can be seen in Figure 2.1.2.2.1.2 and Figure 2.1.2.2.1.3.

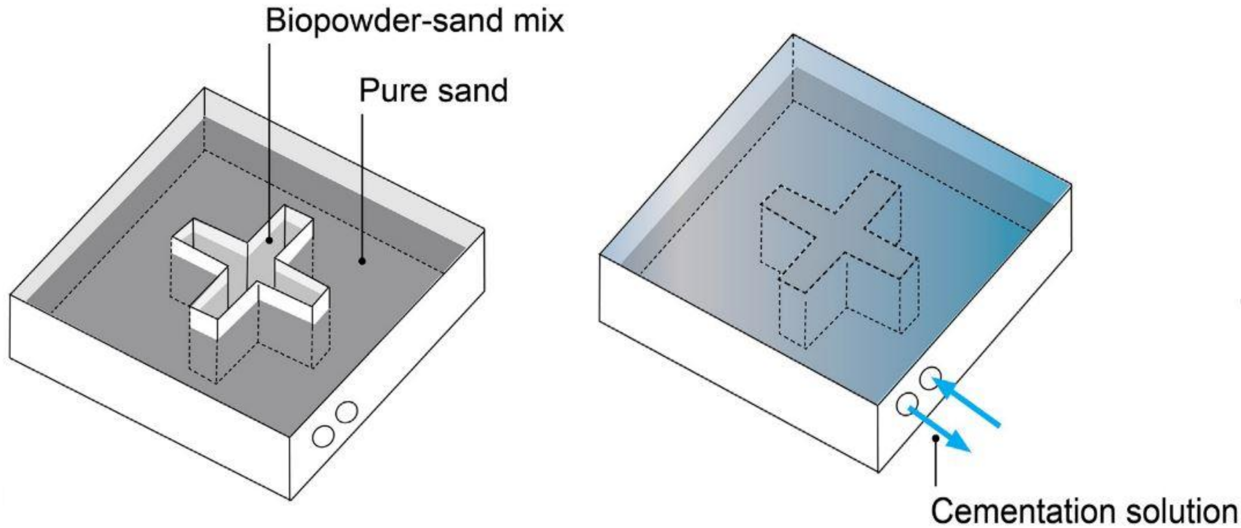


Figure 2.1.2.2.1.1 Additive manufacturing using urease active calcium carbonate powder (Nething et al., 2020).

Figure 2.1.2.2.1.2 Results of selective soil treatment additive manufacturing (Nething et al., 2020).

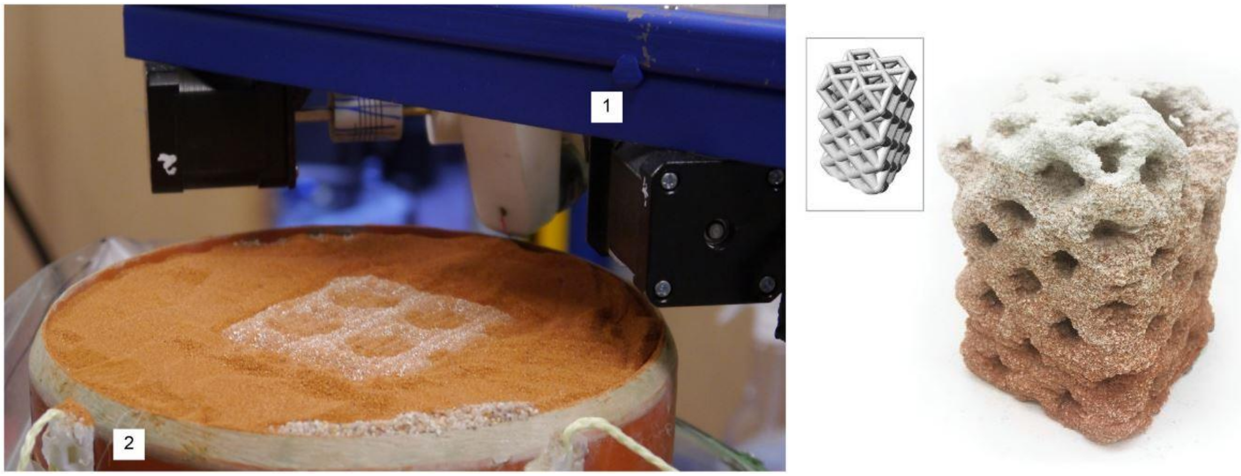


Figure 2.1.2.2.1.3 Additive manufacturing process and results (Nething et al., 2020).

This approach to additive manufacutinr MICP was able to achieve compressive strengths roughly equivalent to weak concrete. The precision of the results was limited by grain size of the soil, and dimension deviations were measured from 2%-43% for the specimen in Figure 2.1.2.2.1.3. Nething et al.'s research demonstrates a successful method of 3D printing using the MICP procedure, but is complicated by the addition of the urease active calcium carbonate powder. The process for creating this powder is likely not field-scale friendly. Requring multiple cementation solution applications over a total of 40 hours also increases the required volume of solution and the time required for treatment application.

2.1.2.3 Surface spraying method

Surface spraying of both solutions has proven to be effective, but without disturbing the soil, the method is limited to the first several meters of the subsurface and can be highly dependent on soil type and grain size. While greater depths may be reached, the level of cementation decreases with depth. It is the bacteria's need for space and oxygen which can limit both the depth of application and the suitable grain size for treatment (van Paassen, 2009). Surface application of MICP treatment has been proven to help prevent erosion in highly susceptible areas such as coastal and desert sand dunes (Do et al., 2020; Katebi et al., 2021; Kumar et al., 2020; Shanahan & Montoya, 2014). Applying MICP to the surface of sand dunes creates a biocrust and effectively increases the angle of repose (Shanahan & Montoya, 2014). When applied in the boreholes of foundation systems, MICP also aids in scour resistance (Do et al., 2020). Surface application methods use multiple applications of cementation solution to achieve the desired calcite content. Applying in-situ surface treatment results in greater precipitation at depths closer to the surface, but can successfully protect the soil's crust (Figure 2.1.2.3.1).

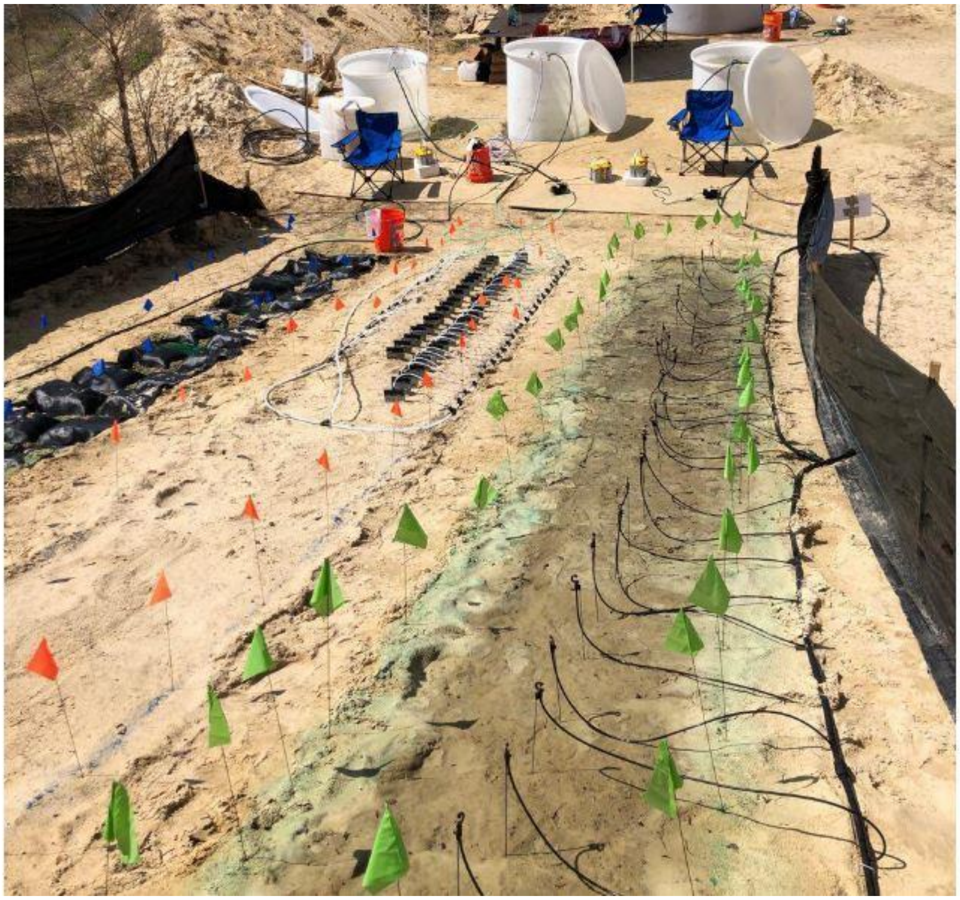


Figure 2.1.2.3.1 MICP treatment on a sandy slope (Kumar et al., 2020).

2.1.3 Effects on engineering properties

Multiple cementation solution injections results in higher percentages of calcite which leads to higher compressive strength (Islam et al., 2020; Muhammed et al., 2021; Mujah et al., 2019; Whiffin et al., 2007; J. Z. Xiao et al., 2020). The increase in calcite causes increase in soil stiffness (Feng & Montoya, 2015; Mortensen & DeJong, 2011). Shear strength of the treated soil also increases from silty-sand to sand (Feng & Montoya, 2015; Ghasemi et al., 2019; Swan, 2011; Wu et al., 2021; A Zamani & Montoya, 2015). The increase in calcite produces decreased pore space and lower permeability (Akimana, 2016; Almajed et al., 2021; Islam et al., 2020; Muhammed et al., 2021; Sun et al., 2019; Atefeh Zamani et al., 2019). The sand bridges formed by the precipitate connect sand grains and increase roughness and therefore increase overall strength (Nafisi et al., 2018). This also effectively increases the cohesion and internal angle of friction which increases slope stability (Almajed et al., 2021; Wang et al., 2017; Wu et al., 2021). The precipitation decreases the void ratio and therefore decreases the compressibility of the soil (Y. Xiao, Zhao, et al., 2021). Further benefits can be gained with the addition of synthetic fibers to the soil specimens (Li et al., 2016).

2.1.3.1 Sand

MICP treatment on sand has a few unique components. Sand that has been treated with bio-cementation is more prone to dilation compared to untreated sand at similar void ratios and applied stress levels (Wu et al., 2021). As levels of cementation increase, dilation potential for treated sand increases. Increases in confining pressure can result in degradation of cemented sand specimens (Nafisi et al., 2021). Degradation of cementation also occurs at higher levels of strain (Wu et al., 2021).

2.1.3.2 Clay

Treating clay specimens with MICP presents many unique challenges. Since the permeability of clay is much lower than coarser grained soils, premixing is often employed to evenly distribute bacteria prior to treatment (Arpajirakul et al., 2021; Teng et al., 2021; Tiwari et al., 2021). While MICP benefits the physical properties of clay soils, it also affects the chemical properties. High-swelling montmorillonite clay has been observed to convert to low-swelling illite clay (Wei et al., 2021). Changes like these result in soil classification changes like fat clay to elastic silt or highly plastic clay to low plastic clay (Islam et al., 2020; Kannan et al., 2020; Tiwari et al., 2021). Due to the chemical nature of clay environments, the surrounding pH affects the MICP activity more than it does with sand. A pH of 9 is ideal as a lower pH, specifically 5, can hinder the process (Keykha et al., 2017; Teng et al., 2021). While applying MICP to clayey soil introduces more complexities, it still results in higher compressive strength, increased shear strength, lower compressibility, and reduced swelling (Cardoso et al., 2018; Islam et al., 2020; Kannan et al., 2020; Tiwari et al., 2021). Surface spraying applications are limited by soil type; the application process proposed in this research will overcome many of the complexities associated with the treatment of clayey soils.

2.1.4 Field-scale applications

Competent soil is in high demand due to continued global construction, and soil treatments like MICP allow for improvement of existing soil without having to import alternative soil (Swan, 2011). Many locations have prohibited synthetic soil treatments, which delivers more value to the environmentally friendly non-synthetic nature of MICP (Swan, 2011). Large scale experimentation has been performed using surface spraying treatment (previously discussed) and injection style treatment. Upscaled surface injection experimentation performed by van Paassen

(2009) successfully demonstrated cementation but presented complexities in creating uniformity (Figure 2.4.1.1).

Figure 2.4.1.1 Large scale soil treatment using injection MICP: a) during treatment; and b) after treatment (van Paassen et al., 2009).

Further development of the process by van Paassen (2011) led to the investigation of borehole stability using MICP. Stability of the soil structure was achieved even with the inclusion of gravel-sized particles (Figure 2.4.1.2). Once it was determined to be an appropriate method, MICP was used for a field scale borehole stability project (van Paassen, 2011).

Figure 2.4.1.2 Borehole stability with gravel: (a) drilling into the prepared soil; and (b) the stable borehole (van Paassen, 2011).

Figure 2.4.1.3 The field where borehole stability was performed (van Paassen, 2011).

In addition to borehole stability, MICP treatment has been investigated as a means of increasing the scour resistance of foundation systems supporting structures on or near waterways (Do et al., 2020). Treatment for increased scour resistance was assessed using 5 cm piles with injection sources inserted at intervals (Figure 2.4.1.4). Tests were performed on 3 ft large scale test boxes and treatment was applied at intervals for almost a month. Surface behavior was noticeably affected, and erosion levels decreased.

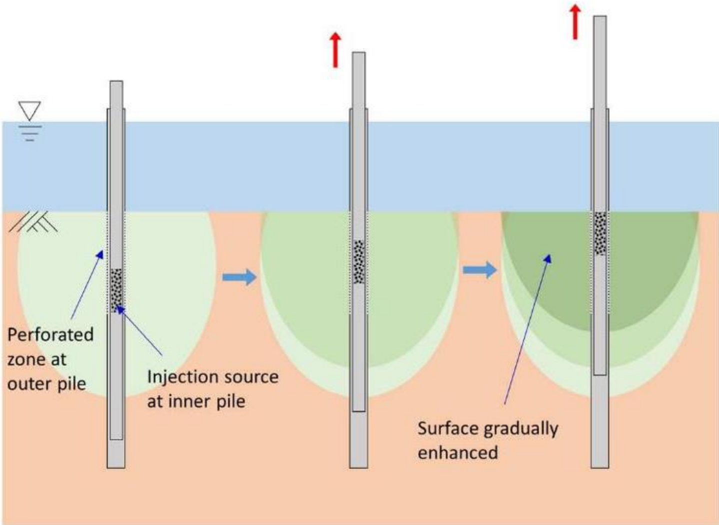


Figure 2.4.1.4 Scour resistance by pile MICP injection treatment (Do et al., 2020).

Many field-applied experimentation has been aimed at decreasing erosion. Erosion control is currently the most predictable use for practical application of MICP. With the further development of MICP procedures and understanding of its benefits, the scope of application will likely continue to grow.

2.1.5 Cost reduction potential

All methods of soil treatment using MICP are economically competitive or similar compared to alternative treatments such as deep soil mixing, jet grouting, or chemical grouting (Wang et al., 2017). While MICP soil treatment has proven to be a comparable economic alternative, further economizing the process dramatically increases the scope of application. Further economic benefits can be achieved by replacing some components of the treatment solutions with effective alternatives. Current forays into cost-effective mass application have focused on alternative sources for bacteria nutrients. The standard food for bacteria growth is industrial grade yeast. The change from laboratory grade chemicals to industrial grade for all chemicals was a crucial change for field-scale applications, but further savings can be accomplished by implementing food grade yeast (Kumar et al., 2020; Omoregie et al., 2019). Omoregie (2019) tested the use of food grade yeast in place of industrial grade yeast. The ideal, most stable calcium carbonate precipitate occurs as calcite, but change in the equation variables can cause calcium carbonate to precipitate as vaterite, a less stable and more soluble condition of calcium carbonate. Omoregie (2019) determined that switching to a food grade yeast alternative caused an increase of around 5% vaterite compared to using industrial grade yeast. Despite the noted increase of vaterite, food grade yeast delivers a dramatic decrease in cost and a viable option for some applications. Many other potential nutrient alternatives have been determined as effective including potato solution, corn pulp, lactose mother liquor, whey, and CSL (Chaparro et al., 2021; Katebi et al., 2021; Wang et al., 2017). Replacing industrial grade urea with pig urine has proven to be another cost-effective replacement for standard MICP components, as it successfully completes the MICP process and puts a waste product to good use as well (Chen et al., 2019).

3. METHODOLOGY

The additive manufacturing method adapted in the following pages is based on a powder bed, or binder jet, style 3D printing application. This method of 3D printing involves a printing bed that is lowered as each layer of dry powder is added. The layers of dry powder are selectively sprayed with a binder solution to create the desired patterns. The process is continued with layering of dry powder and applications of binder solution until the desired part is completed. For this research, powder bed style additive manufacturing will be adapted for bio-cementation of dry soils, where the binder solution consists of the bacterial and cementation solutions applied consecutively.

3.1 Spray solution preparation

Equal amounts of bacterial solution and cementation solution were prepared prior to the additive manufacturing procedures. The entire process followed for this research can be seen in Figure 3.1.1 and will be discussed in the subsequent pages. Cementation solutions were prepared less than 24 hours before application, and bacterial solutions were used directly after optical density was read. Compared to alternative application methods (e.g., injection, premixing, surface spraying), significantly lower volumes of solutions were required, although the concentration of the cementation solution was higher than most.

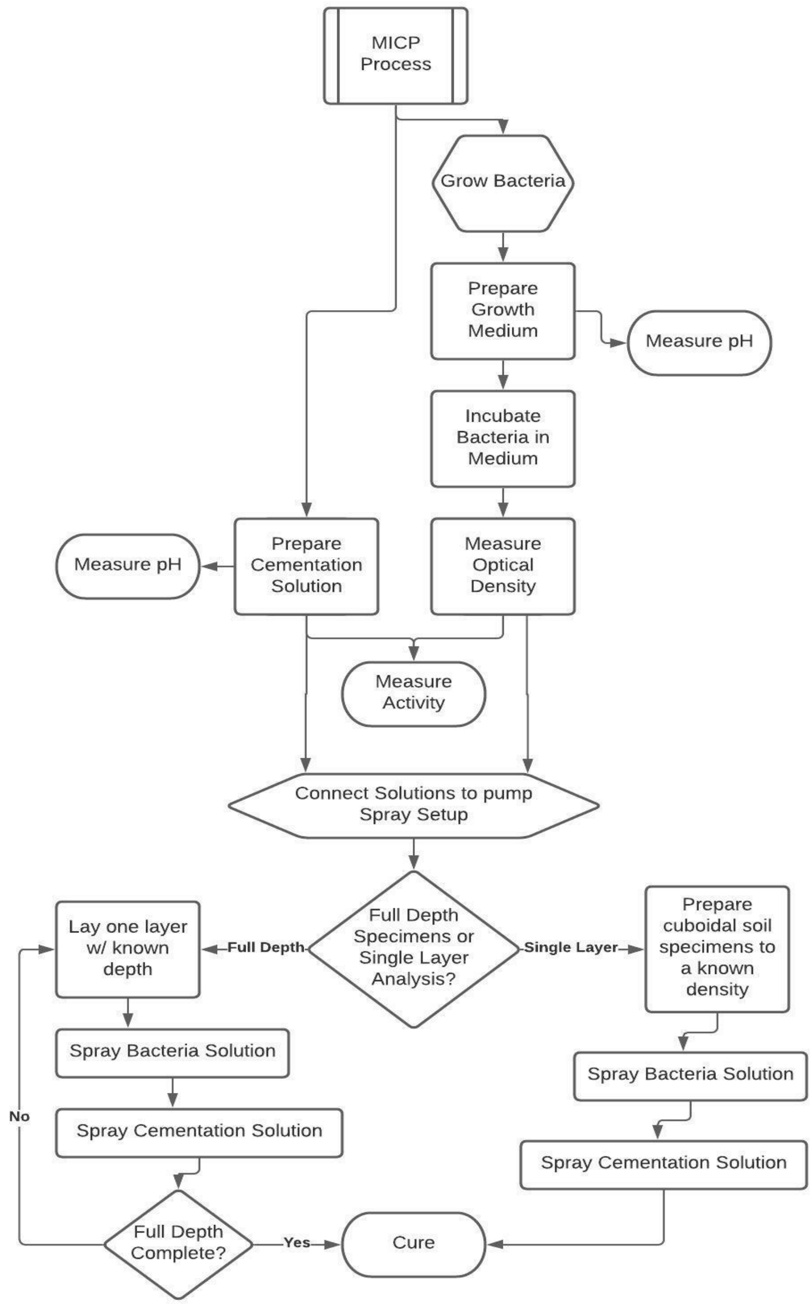


Figure 3.1.1 Flowchart of research process.

3.1.1 Bacterial solution

The behavior of the bacteria, *Sporosarcina Pasteurii*, is well-defined as it relates to urea hydrolysis (DeJong et al., 2006; Mortensen et al., 2011). For this reason, it was chosen as the bacterium for all applications herein. The Sporosarcina Pasteurii bacteria was received in a freeze-dried state as part of the type species from a culture collection (DSM 33 / ATCC 11859). Each step in the bacteria preparation procedure was performed in sterile and aerobic conditions. Bacteria culture was inoculated in growth medium plates prepared with 20 g/L yeast extract, 10 g/L ammonium sulfate, 20 g/L agar, and 0.13 M tris buffer (base). Plates were kept in a warm room at 30°C for 48-72 hours and then stored at 4°C for up to two weeks. Bacteria from one petri dish was removed and incubated in the growth medium (growth medium plate's ingredients minus the agar) at 30°C and 150 rpm for 48-72 hours. 500 grams of medium was prepared in each batch. The growth medium had a pH of 9.1 prior to the addition of bacteria and a pH of 8.0 once the bacteria had grown. Optical density (OD) was measured using a scanning spectrophotometer with a final $OD_{600\ nm}$ of 0.6-1.85. Higher optical densities were found at wavelength values of less than 600, however 600 was used as a standard value which allows for ease of comparison with previous literature.

3.1.2. Cementation solution

The urea-calcium cementation solution was prepared at a 1.5:1.0 ratio of urea and calcium, respectively. A high concentration of solution was prepared due to the single treatment application method of additive manufacturing MICP. The ideal concentration of calcium chloride has been found to be 1.0 M (Lai et al., 2021), which is used here. The pH of the urea cementation solution was measured to be 7.1. Equivalent volumes of cementation solution and bacterial solution were prepared for each batch.

3.1.3 Urease activity measurement

The level of urea hydrolysis was calculated using the electrical conductivity method (Whiffin et al., 2007). For this method, a 1:9 volume combination of bacteria solution (OD_{600} = 1.0) and urea solution (1.11 M concentration), respectively, were combined and activity was measured over a 5 minute interval using an electrical conductivity probe. Hydrolyzed urea can be calculated using equation 3 (Bagriacik, Sani, Uslu, Yigittekin, Chu, et al., 2021). The pH of the combined solutions was measured to be 7.7.

$$\text{Urea Hydrolyzed (mM)} = \text{Conductivity (mS)} * 11.11 \quad (3)$$

3.2 Spray setup

Initial trials to cement sand using a spray MICP technique were performed using three different renditions of spray apparatuses. The first set of spray bottles used were all-purpose plastic spray bottles with an adjustable spray nozzle (Figure 3.2.1.a). While this preliminary version confirmed the potential for an additive manufacturing application of MICP the generic bottles allowed for too much variability in spray volume and direction. In an effort to maintain an enhanced level of control on solution output, a new set of hand-held pressure pump sprayers that allowed for continuous spraying were obtained (Figure 3.2.1.b). These new sprayers allowed for more consistent spraying than the previous set, however the pressure decreased during spraying which varied the applied solution volume slightly. An attempted solution was a set of spray gel bottles for the gel MICP solutions. These bottles were chosen for the specific application but posed similar issues to the first set of spray bottles.

(a) (b)

Figure 3.2.1 Initial spray bottles: (a) all-purpose plastic spray bottle; and (b) hand-held pressure pump sprayer.

The final spray application setup for both liquid and gel was developed with a Raspberry Pi controlling two small peristaltic pumps. Separate pumps and lines were used for the two solutions so that precipitation would not occur prior to application and the lines would remain unclogged. The pumps were connected to drip irrigation micro sprinklers (foggers) with flex tubing and a 360° spray pattern that includes a mechanism to break liquid into micro-sized drops. The nozzles also are adjustable so that the volume and spray diameter can be controlled. The dispensed solutions were controlled by time of spray; a relationship was developed between time of spray and mL of solution dispelled. A rate of 1.3 mL/s was dispensed for each cementation procedure used in this research. The final spray set up can be seen in Figure 3.2.2.

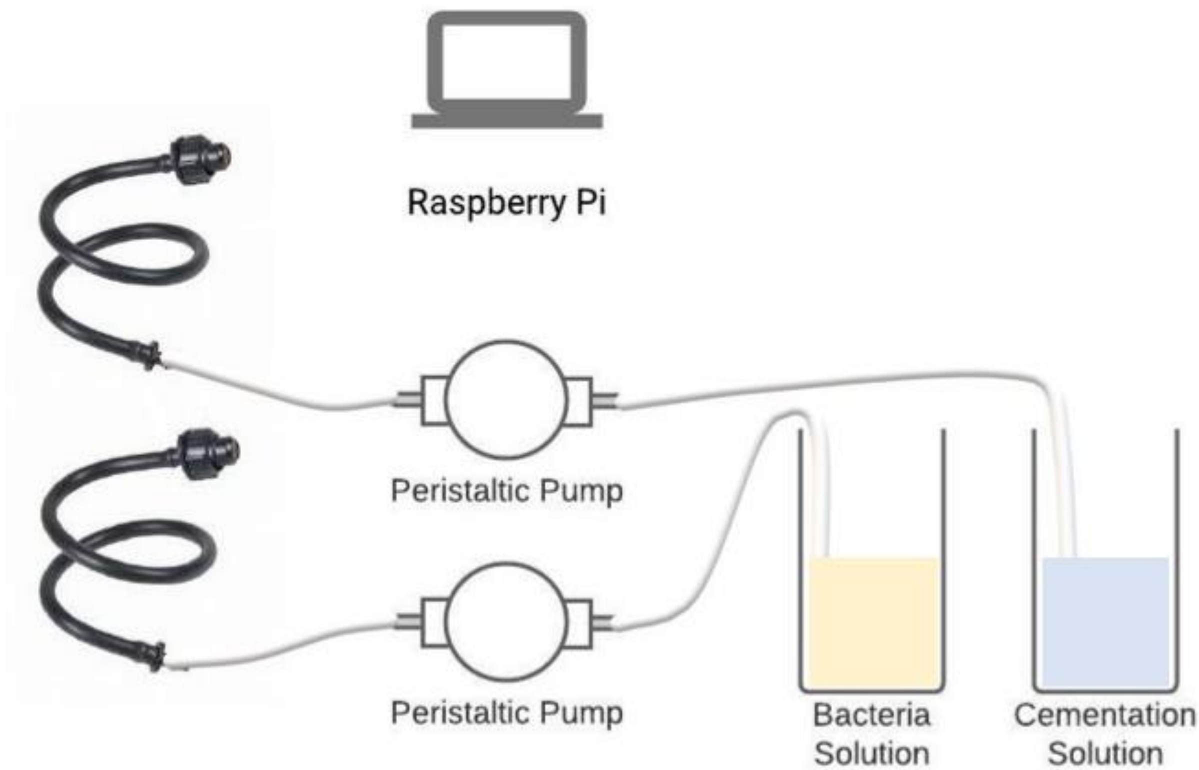


Figure 3.2.2 Final spray setup.

During the development of the final spray setup, many specimens were cemented and the results analyzed. The analyses of the intermediary results provided essential insight into the project that ultimately led to testing that was performed using the final spray setup. Table 3.2.1 includes all specimens cemented throughout the process, both preliminary and final. With the initial multi-use spray bottle setup, both small and large molded cylinders were cemented with Ottawa 20-30 sand. The large cylindrical molds proved an issue as the soil cemented to the walls of the mold and proved difficult to extract. After initial testing, large cylinders were created with a stencil in the middle of a larger square mold to remove any complexities with cementation to the mold itself. Once the cuboidal molds were acquired, they were used for both surface cementation and cubic specimens to allow for cohesive results.

Table 3.2.1 Complete research matrix.

Specimen type:	Spray Bottles with Liquid Solutions	Pressure Pump Sprayers with Liquid Solutions	Final Spray System with Liquid Solutions	Final Spray System with Gel Solutions
Small cylinders from molds	45	10		
Large cylinders from molds	5			
Surface cementation in tins	30	20		
Surface cementation, square		12	40	
Surface cementation with stencil			12	48
Large cylinders from stencil layering		2	3	
Cuboidal		10	15	
Surface cementation with plant stalk stencil			1	

3.3 Layer penetration parametric study

3.3.1 Soil mixtures

In order to perform MICP with additive manufacturing where the two solutions required for the process are sprayed consecutively for each layer, it is crucial to understand how far the given volume of solution permeates through each soil layer. The depth of solution penetration varies depending on the soil properties such as hydraulic conductivity, void space, and soil type. Two standardized soil types were chosen to represent a range of soil types: Ottawa 20-30 sand

and Red Art clay. Ottawa 20-30 sand is a poorly graded sand with a specific gravity of 2.65 and a D_{50} of 0.71 mm (Polito et al., 2013). The gradation for Ottawa 20-30 Sand is shown in Figure 3.3.1.1. Ottawa 20-30 is considered a coarse sand and is comprised of spherical sand grains. The combination of sphericity and size of the sand particles results in a soil that is more difficult to bio-cement and requires higher levels of precipitation. As a stand-alone soil, finer sands, well-grade sands, or more angular sands would allow for more efficient cementation. Red Art clay, a commercially available pottery clay, is lean clay with a liquid limit of 38, plasticity index of 19, and specific gravity of 2.77. A standard clay and a clean sand were chosen to represent two distinctly different soil classifications to prove that cementation using the method described herein is possible with both classifications and for the range of soils in-between. Incremental combinations of these two foundational soils were also considered using percent by volume (Table 3.3.1.1). All soil mixes were classified according to the USCS soil classification method (American Society for Testing and Materials International, 2000). A void ratio of 0.70 was chosen to for soil mixes, and the soil was considered in a dry state. Phase diagrams shown in the appendix provide the results of the individual mix analyses. Initially incremental combinations of soil were created at every 10% of volume; however, the lack of disparity between the results did not justify the use of twelve soil mixtures and therefore the soil mixes were reduced to every 25% of volume resulting in five different soil mixes. Future work could focus on more specifically desired soils, or soils more likely to be encountered in the field.

Table 3.3.1.1 USCS Classifications of soil combinations.

% OTTOWA SAND	% RED ART CLAY	CLASSIFICATION
100	0	SP – poorly graded sand
75	25	SC – clayey sand
50	50	CL – sandy lean clay
25	75	CL – lean clay with sand
0	100	CL – lean clay

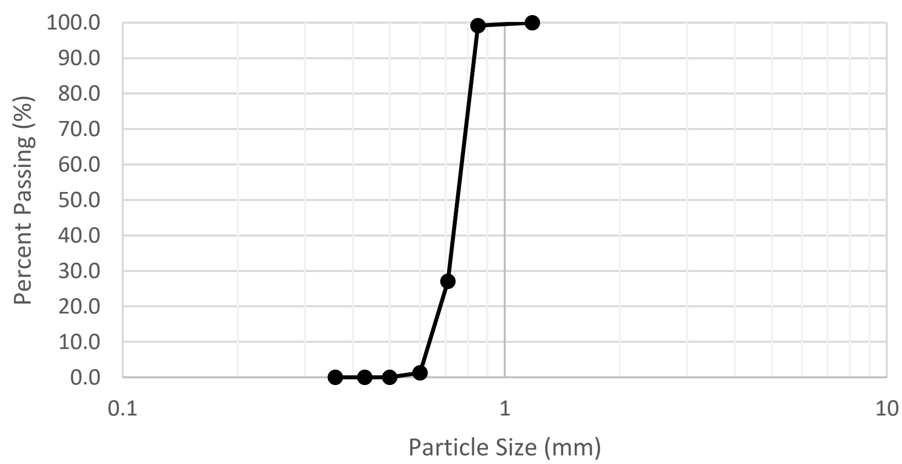


Figure 3.3.1.1 Soil gradation for Ottawa 20-30 sand.

3.3.2 Single layer application

Specimens were prepared in two-inch cubic silicon molds with all soil mixtures compacted to equal void ratios of 0.70. Single applications of 2.6 mL of bacterial solution and 2.6 mL of cementation solution were applied sequentially to the entire surface area of each prepared specimen. Specimens were then left to cure for at least 24 hours to allow precipitation to occur and cemented locations to air dry. This process was performed with a constant volume of both solutions so that the relationship between the volume of solution and the depth of cementation penetration for each respective soil type could be established.

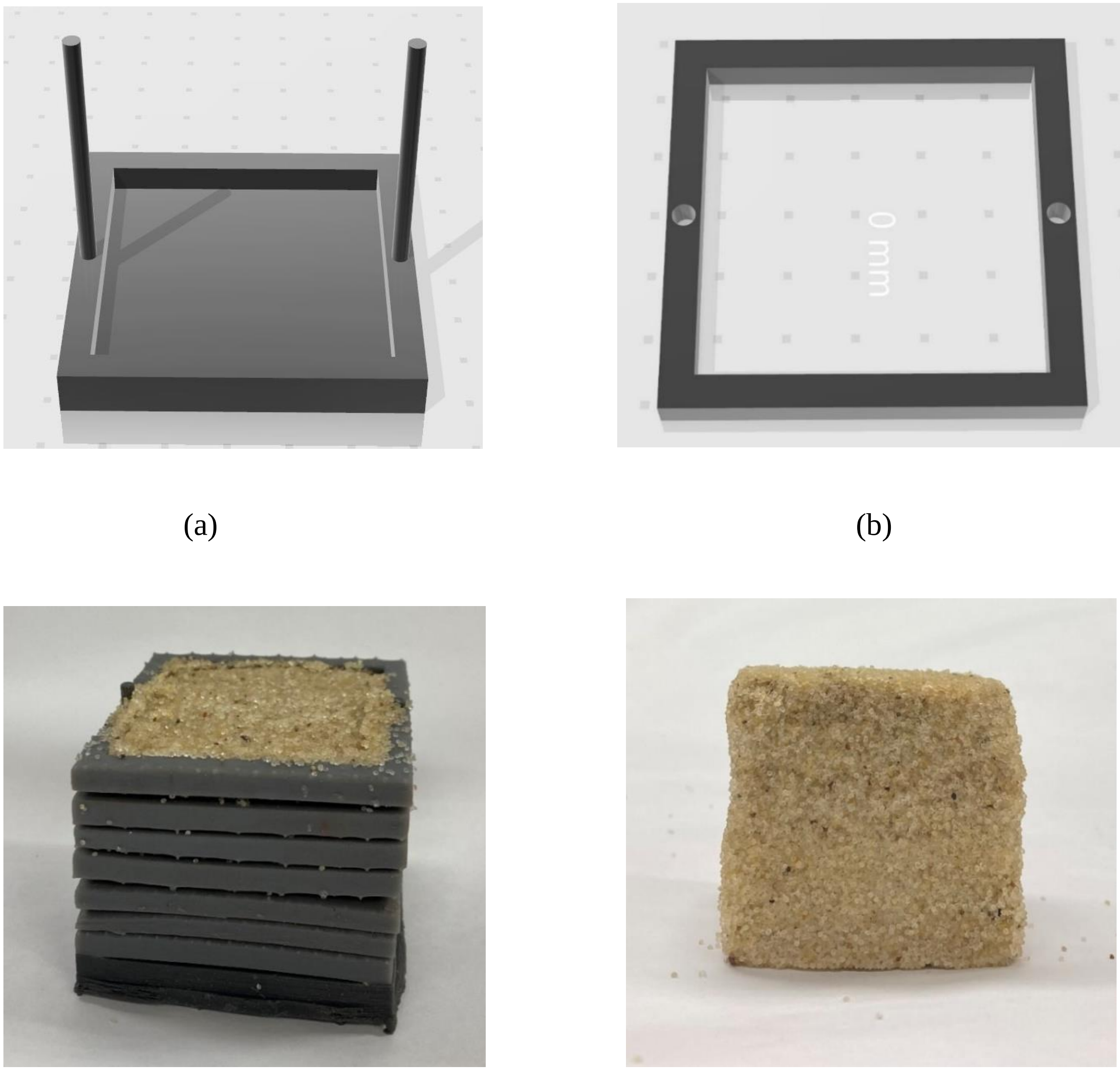
3.4 Cubic strength specimens

Once an understanding of cementation penetration depth was achieved for the soil mixtures, layered specimens could be created. By limiting the height of each layer to less than the penetration depth determined in the single layer application trials, the potential of layer delamination should be reduced. Unconfined compression strength (UCS) test specimens were prepared using a 50% Ottawa sand and 50% Red Art clay mix, a sandy lean clay (CL). One 100% Ottawa sand specimen was produced as a baseline comparison, although it was determined that the clean Ottawa sand was more difficult to cement than the mixture soils. The sandy lean clay specimens were prepared in a 2-inch cubic silicon mold by gently compacting each individual layer prior to applying bacterial solution and cementation solution. Specimens were prepared with various optical densities of bacteria and various solution volumes; a five second pause was always allotted between applying the bacteria solution and the cementation solution. Once specimens were complete, they were placed in a 62°C oven until dry. Notable urease activity occurs in a temperature range of about 10 °C to 70 °C, after which activity significantly declines (van Paassen et al., 2009). Therefore, urease activity was still able to occur as specimens dried in the oven. A baseline strength for this preperation method was determined by preparing specimens with the sand/clay soil mixture with the additive manufacturing provess replacing both solutions with water. The wet, compacted specimens were then dried in the oven prior to testing. Both untreated and MICP treated specimens can be seen in Figure 3.4.1. Unconfined compression tests were performed on a hydraulic frame at a rate of 1% strain/minute.

(a) (b)

Figure 3.4.1 Oven-dried specimens: (a) MICP treated; and (b) untreated

From the penetration experiements, approximate depths of cementation were determined for the different soil mixes. Pure Ottawa sand cemented in more shallow layers than when mixed with any amount of clay; the limited cementation depth required a method of controlling layer depth during specimen construction so as to avoid potential layer delamination. For this purpose a 3D printed mold was designed with stackable, equal height layers of 5 mm (Figure 3.4.2).



(a) (b)

(c) (d)

Figure 3.4.2 3D printed stackable mold for MICP treated sand specimen: (a) mold base; (b) mold layer; (c) stached mold with specimen; and (d) sand sample.

3.5 Gel spray solutions for clean sand samples

Soils with higher hydraulic conductivity values exhibit less uniform distribution of cementation through the additive manufacturing process. In other words, the solutions dispersed throughout the soil in a less controlled manner. In order to address this issue, it was determined that increasing the viscosity of both spray solutions and creating spray gels could reduce the dispersion of the solutions and allow them to concentrate at the grain contacts, ultimately providing a more efficient cementation and a way to spray a defined pattern. The spray gel solutions were prepared using the same concentrations as the original solutions, but with the addition of 2g/L agar. The addition of agar increased the density of the solution to 1.31 g/cm^3. Agar was sterilized and dissolved by bringing it to a boiling point with deionized water. The agar solution was allowed to cool before combining it with the other ingredients. For the cementation solution, the total concentrations of urea and calcium chloride were adjusted such that when combined with the agar, they reached the proper concentrations. For the bacteria solution, optical density was read after the addition of agar with the base solution (i.e., growth medium with agar). The initial optical density of the bacteria solution was 1.83; after the addition of the agar solution, an optical density of 1.60 was measured. The effects of adding agar to the solutions was analyzed by single layer applications with a circular stencil such that both spread and penetration could be measured (Figure 3.5.1).

Figure 3.5.1 Circular layer of 100% Ottawa sand created using spray gels with the pump spray system.

3.6 Stencil specimens

One benefit of additive manufacturing construction is the ability to create shapes and structures that provide benefits such as increased stiffness, load transfer ability, or mechanical behavior. Small-scale assessment of the possibility for dual-spray MICP to perform well when applied to specific surface areas was evaluated by circular stencils and by a 3D printed stencil representing an idealized plant stalk cell structure. A circular stencil was applied to every layer of clay/sand soil within a preparation box to create a column (Figure 3.6.1). Plant stalk stencil applications were performed on surface layers only (Figure 3.6.2).

Figure 3.6.1 A layer in the column construction using a stencil.

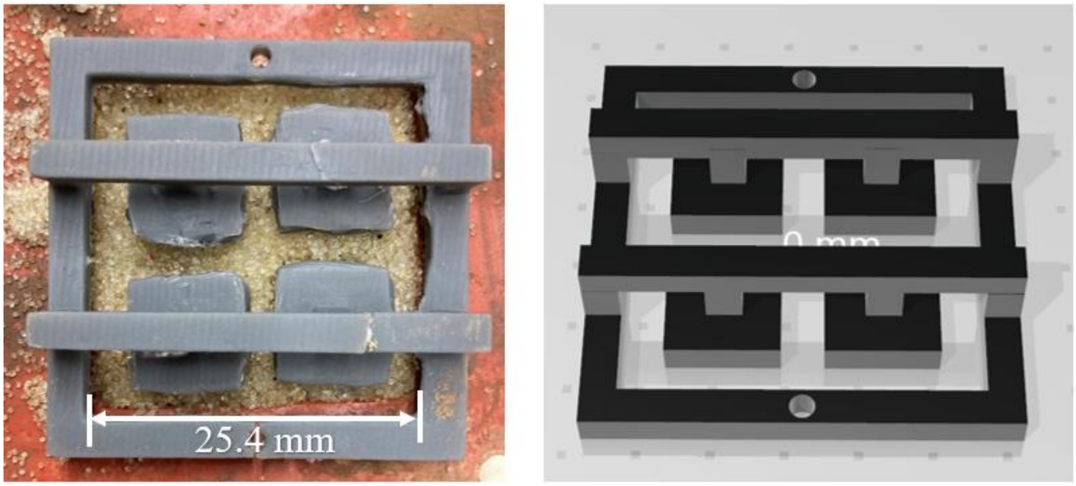


Figure 3.6.2 3D printed plant stalk stencil: (a) surface layer application; and (b) design.

For the circular stencil tests, three different scenarios were considred: (1) one spray of each solution per layer placed in the oven to cure, (2) two sprays of each solution per layer placed in the oven to cure, and (3) two sprays of each solution cured at room temperature. This was performed to assess the effect that changing the viscocity of the solutions might have on the cemenetation in terms of uniformity, depth, and spread. Preliminary experimentation indicated potential issues with the solutions mixing in-situ, which is why tests were run with two sets of sprays and with oven-curing.

4. RESULTS AND DISCUSSION

4.1 Layer penetration calibration

The cementation behavior of MICP is known to be affected by the type of soil being treated. This variance is largely due to grain size affecting the specific surface of the soil. Other factors that influence cementation include: the angularity of the grains, the volume of the void space, and the mineralogy of the soil. In the style of application examined herein, the hydraulic conductivity of the soil also impacted the results, as the solutions were applied to the surface without any significant treatment pressure. Table 4.1.1 provides average data from the set of three tests performed. Both weight and depth of cementation increase when clay is mixed with sand, but begin to decrease with the further addition of clay.

Table 4.1.1 Average penetration for different soil mixtures at equal treatment volume.

% OTTOWA SAND	% RED ART CLAY	CLASSIFICATION	WEIGHT OF CEMENTATION (g)	DEPTH OF CEMENTATION (mm)
100	0	SP – poorly graded sand	46.0	7.0
75	25	SC – clayey sand	95.8	16.9
50	50	CL – sandy lean clay	73.7	12.3
25	75	CL – lean clay with sand	45.4	8.7
0	100	CL – lean clay	31.2	7.1

All of the soil types successfully cemented to varying degrees. As shown in Figure 4.1.1, the top layers of each soil combination were placed on their side such that a section view could be observed. The 100% Ottawa sand specimen provided less specific surface than all other specimens and therefore the bacteria had fewer connection points allowing for cementation. This is expected to be the reason for the lower depths of cementation for the pure sand. The 75% Ottawa sand 25% clay blend gave the largest values for both depth and weight of cementation. The decline in depth and weight of cementation with the addition of more clay is most likely due to the decrease in hydraulic conductivity and the soil's ability to hold moisture. For optimal performance, the 75% Ottawa sand appears to balance the effects of specific surface and hydraulic conductivity. A 50-50 soil blend also performs comparatively well. Despite some soils appearing to have superior cementation, all soils were able to be cemented successfully without additional pressure and providing only one treatment application.

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

(d) **(e)**

Figure 4.1.1 MICP penetration for: (a) 100% Ottawa sand; (b) 75% Ottawa sand 25% clay; (c) 50% Ottawa sand, 50% clay; (d) 25% Ottawa sand, 75% clay; and (e) 100% clay.

While soil type has substantial affect on cementaiton results, the optical density of the bacteria solution is also a significant variable. The higher the optical density, the more active the cementation. Since the additive manufacturing method considered herein consists of only one treatment with the urea cementation solution, higher optical densities of the bacteria provide necessary benefits. Figure 4.1.2 exemplifies the increase in cementation volume by weight as optical density increases. While 0.58 and 1.0 are not likely ideal for field applications, the data demonstrates the benefit of increased optical density. Figure 4.1.2 also shows the trend of increased cementeation with the addition of clay. Corresponding trends can be observed for the relationships between optical density and depth of cementation with respect to soil type (Figure 4.1.3).

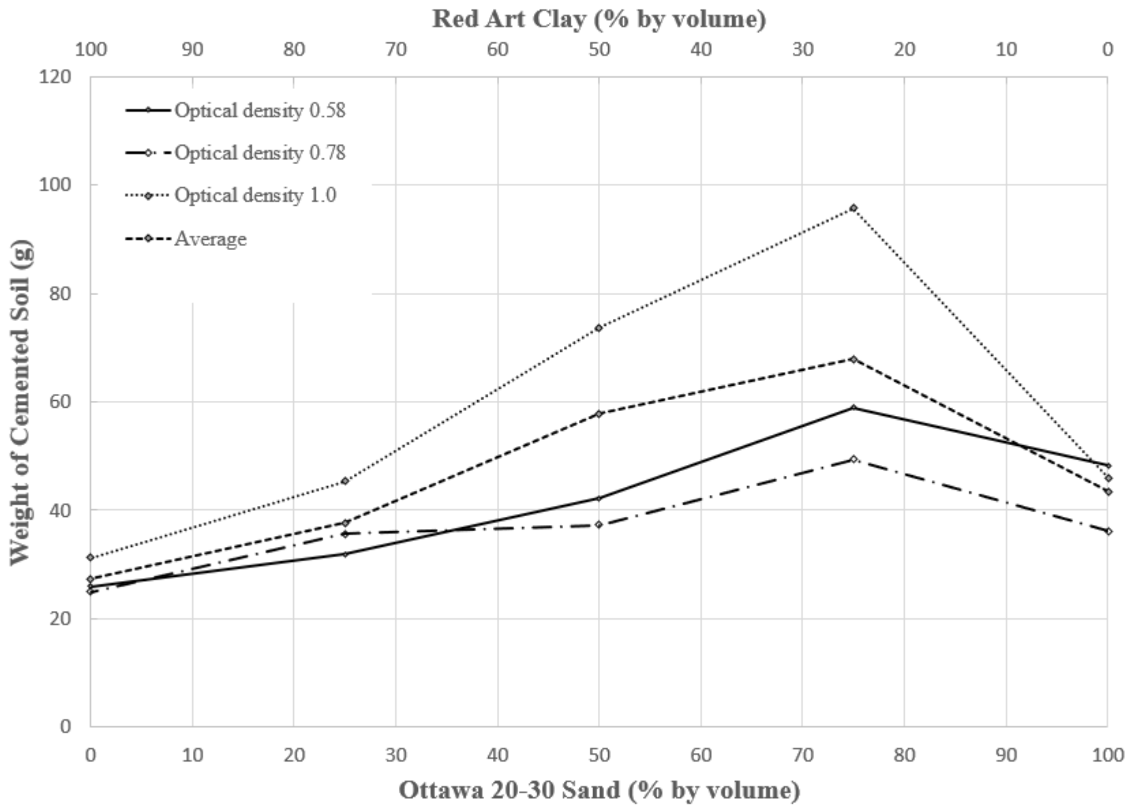


Figure 4.1.2 Weight of cemented soil volume for different soil combinations with constant spray solution volumes of 2.6 mL/2.6 mL.

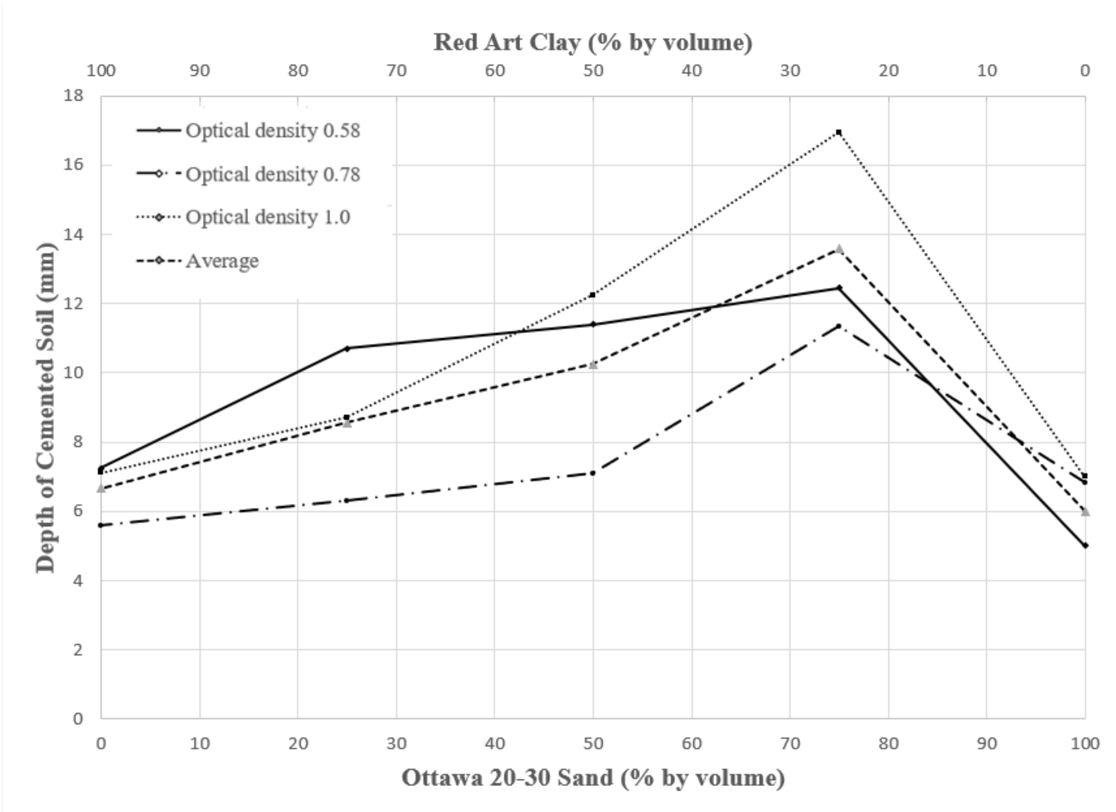
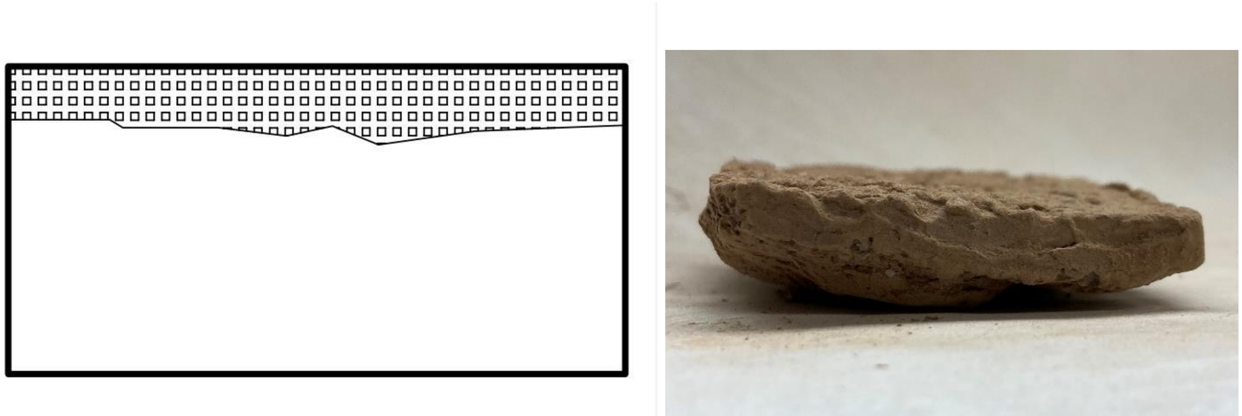


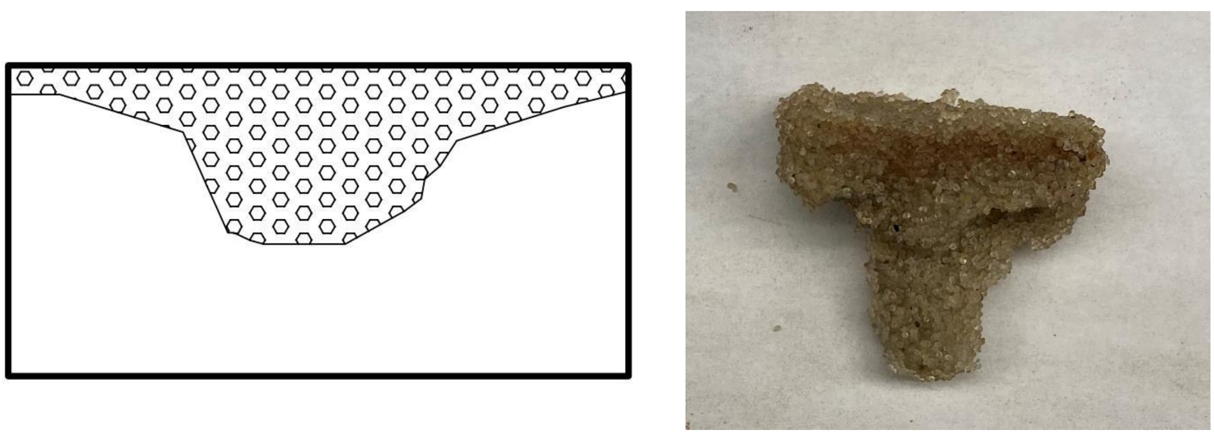
Figure 4.1.3 Depth of cemented soil for different soil combinations with constant spray solution volumes of 2.6 mL/2.6 mL.

The hydraulic conductivity of soil affects the final infiltration of solution leading to cementation. Additionally, the hydraulic conductivity had observable effect on cementation uniformity. When the solutions were applied to the surface of the samples with clay, permeation was not immediate. The increased time of infiltration allowed the solutions to cover the surface area more evenly resulting in relatively homogenous depth across the surface (Figure 4.1.4). Even application of solution across the surface area was more challenging for the 100% sand samples due to the almost immediate permeation of the liquids into the soil samples. This difficulty often resulted in conical cementation of the sand layers (Figure 4.1.5).



(a) (b)

Figure 4.1.4 Penetration behavior of CL - lean clay: (a) schematic; and (b) physical soil sample.



(a) (b)

Figure 4.1.5 Penetration behavior of SP - gap graded sand: (a) schematic; and (b) physical soil sample.

Layer penetration can be highly variable. However, with some soil specific calibration, an understanding of an acceptable layer depth range can be achieved. Due to the complexity of this method of application, it is recommended that site-specfic testing be performed prior to any field application.

4.2 Cubic strength specimens

All traditional methods of MICP application require several treatments with cementation solution. Increase in cementation solution treatments or exposure time leads to increase in calcite precipitation and thus strength of the treated soil. Relying on one application of both bacteria and cementation solution limits the resulting strength. One treatment application will not be able to provide the same strength as numerous treatments, but it does increase the strength compared to untreated soil (Islam et al., 2020; Muhammed et al., 2021; Nafisi et al., 2019). Table 4.2.1 provides the results of unconfined compression strength tests performed at a rate of 1%/min (ASTM, 2013). Variability in the peak stress is observable, even among specimens made with the same optical density and solution volumes, but a general trend of higher strength with higher density can be observed (Figure 4.2.1). This may be due to the slight variances in density and layer thicknesses. Table 4.2.2 provides the UCS results of the soil cubes prepared in the same method as the MICP treated cubes but with water in place of the MICP solutions. The untreated cubes unfortunately have a larger prestress value than the cemented specimens, however initial poundage that the two specimens experienced was only 13 pounds and 29 pounds.

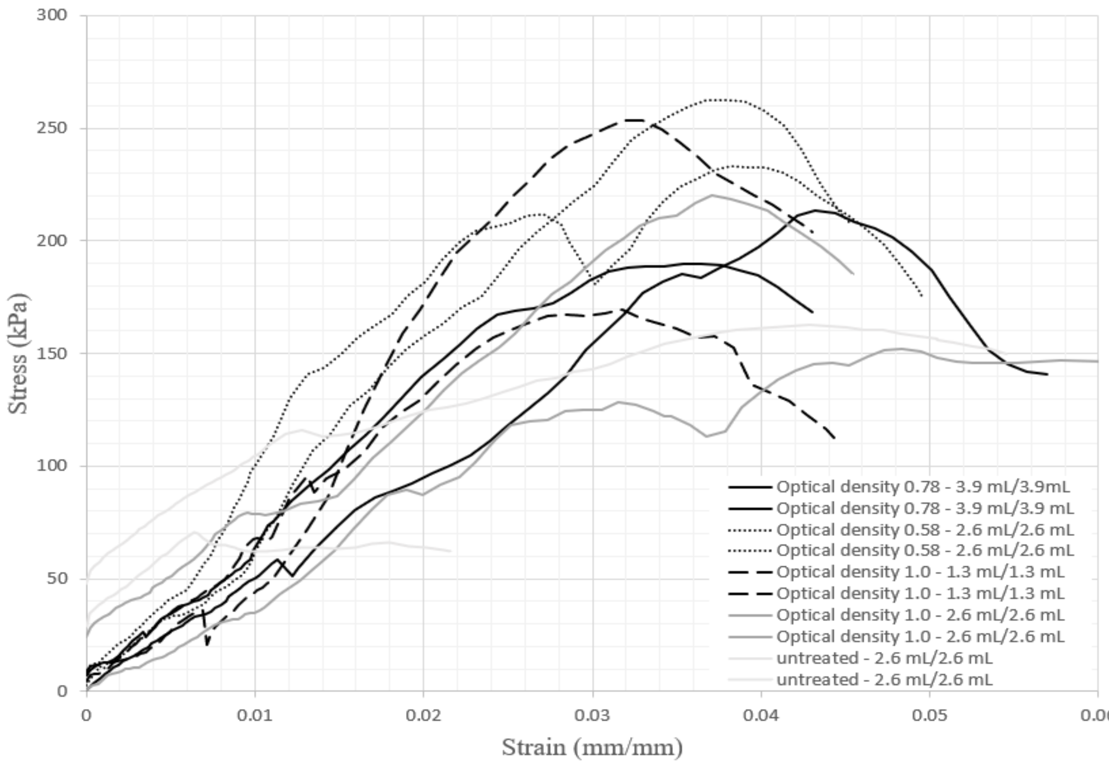


Figure 4.2.1 Stress-strain curve from UCS tests of 50-50 specimens.

Table 4.2.1 Sample properties and UCS results for treated sand-clay specimens.

Optical density	Spray volume (mL)	Soln. volume/surface area (L/m^2)	Density (g/cm^3)	Peak stress (kPa)	Strain at peak stress (%)
0.58	**2.6**	**1.05**	**1.588**	**233.3**	**3.8**
0.58	**2.6**	**1.05**	**1.594**	**262.6**	**3.8**
0.78	**3.9**	**1.63**	**1.615**	**213.2**	**4.3**
0.78	**3.9**	**1.67**	**1.604**	**190.0**	**3.6**
1.00	**1.3**	**0.506**	**1.608**	**253.4**	**3.3**
1.00	**1.3**	**0.504**	**1.566**	**169.6**	**3.2**
1.00	**2.6**	**1.07**	**1.605**	**152.2**	**4.8**
1.00	**2.6**	**1.08**	**1.583**	**220.1**	**3.7**

Table 4.2.2 Sample properties and UCS results for untreated specimens.

Optical Density	Spray volume (mL)	Density (g/cm^3)	Peak Stress (kPa)	Strain at peak stress (%)
n/a	**2.6**	**1.627**	**162.9**	**4.3**
n/a	**2.6**	**1.566**	**70.3**	**0.61**

Considering the average peak stress of the untreated cubes compared to the MICP treated cubes, an increased strength up to 2.25 times was observed for the MICP treated cubes, although only a slight improvement occurred for several specimens. Because of the current unpredictability in the results, it is recommended that additional testing be conducted to further understand the influence of these parameters on the resulting cementation and compressive strength. . In some cases, additional applications of the cementation solution could be a feasible method of increasing the calcite content and thus the strength of the treated soil.

The failure mechanisms were quite similar for every specimen. What appears to be tension cracking can be observed (Figure 4.2.1). These cracks could potentially be due to tension failure as a result of a significantly weaker tension strength in the specimens. However, it seems likely that the failure mechanisms are a result of uneven loading surfaces. Future testing could attempt to create level surfaces for strength testing. The untreated cubes also presented similar failure mechanisms (Figure 4.2.3). Once the specimens failed and broke apart, the layers of construction were discernible within. Figure 4.2.2 exposes the interior of a cemented cube and labels the thicknesses of the layers within. There is some variation in the layer thicknesses, but all thicknesses are substantially within the appropriate range determined during the layer penetration calibration process. Calcium carbonate precipitate can be observed at the layer interfaces which is likely due to seconds that the solutions remained on top of the soil prior to fully permeating the surface. While layer delamination was not a common issue, it was observed within one specimen and is a potential issue that should be considered in any field applications of this method.

Figure 4.2.2 Failure behavior of cemented soil cubes.

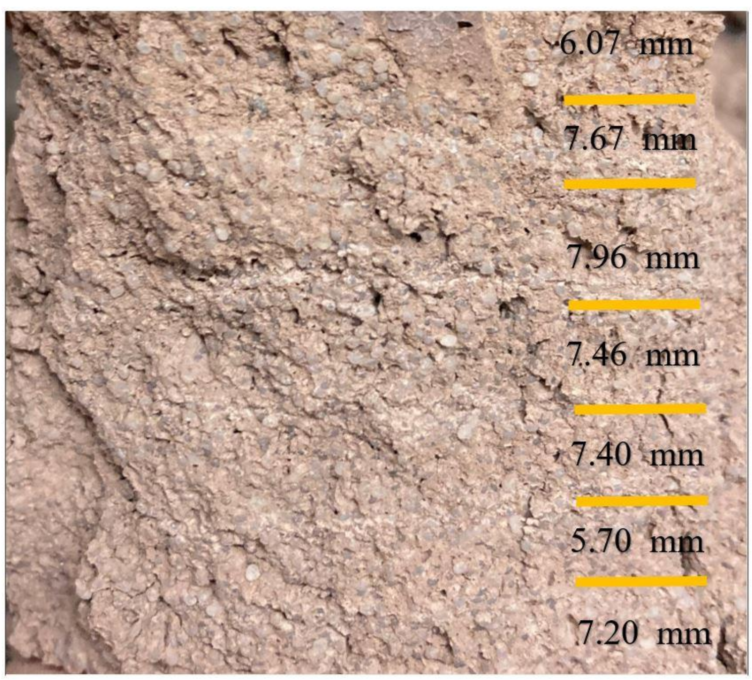


Figure 4.2.3 Observable layers in a cemented specimen.

Figure 4.2.4 Failure behavior of an uncemented cube.

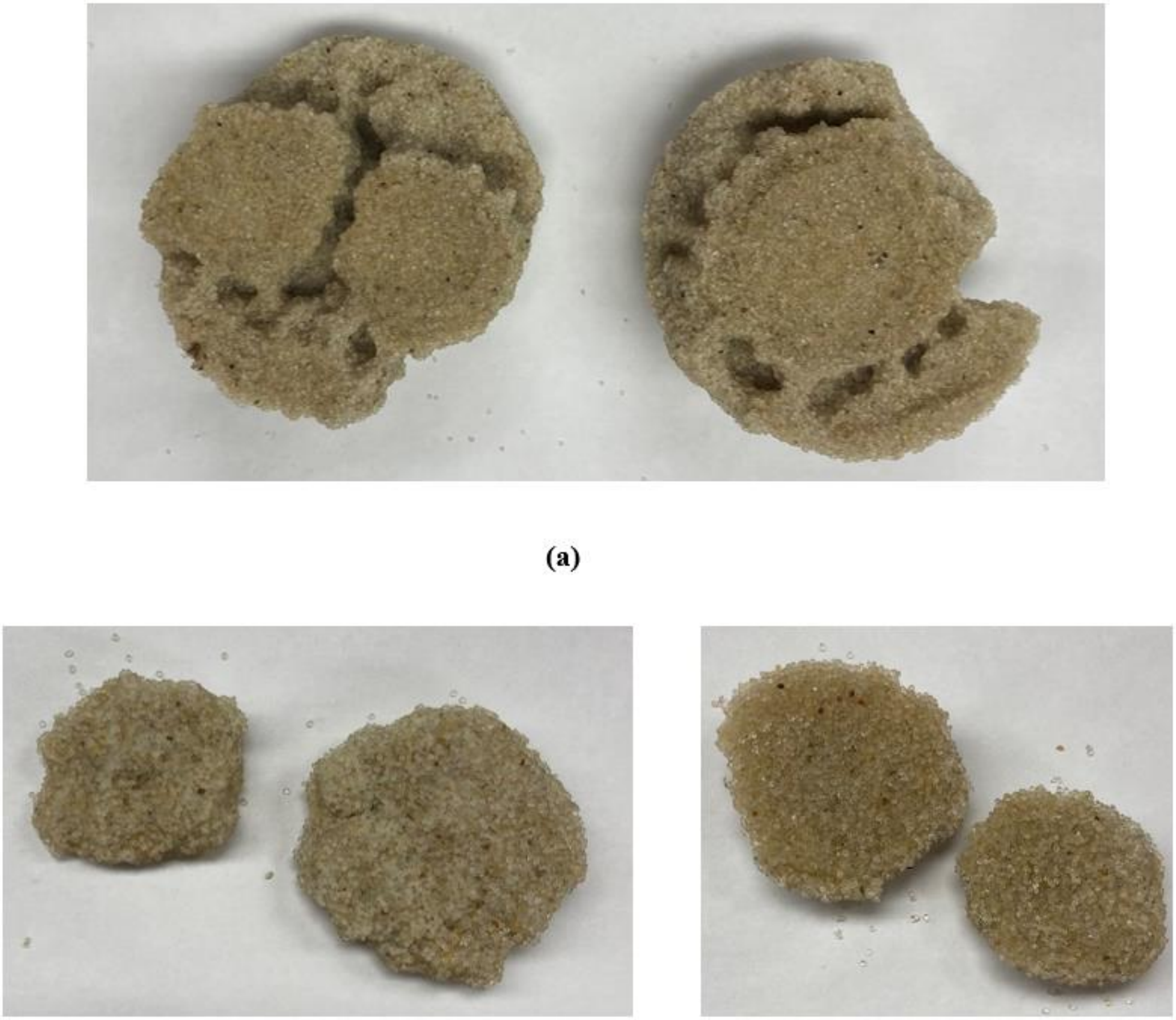
Due to the cohesion of clay specimens, an unconfined test of soil that has not been bio-cemented is possible. Comparisons between treated and untreated UCS strengths for 50% clay specimens were made, but no similar comparison could be performed for 100% sand specimens. Although no viable direct comparison, the sand cube prepared with 5 mm layers was tested in compression and reached a peak stress of 496 kPa. The failure mechanism for the pure sand sample was similar to the 50-50 samples and resulted in release of individual grains from the cemented body.

Figure 4.2.5 Failure behavior of a 100% Ottawa sand specimen.

The results of all the unconfined compression testing suggest notable increase in soil strength after layered MICP application. This MICP application method is a single-phase method that does not require several treatments of cementation solution or long retention periods of either solution. Other MICP methods increase the strength by repeated cementation solution treatment, up to around 100 treatments. Repeated treatment methods can result in strengths similar to weak concrete or sandstone for sand specimens. For the method discussed herein, notable strength increases occurred in a sandy lean clay with only one application of the cementation solution.

4.3 Gel spray solutions for sand samples

In an effort to establish a higher level of control for soils with higher permeability, spray gel solutions were developed for both the bacteria and cementation solutions. Initial single applications applied and cured at room temperature resulted in weakly cemented soil. The addition of the agar seemed to cause issues in allowing the solutions to combine and react appropriately. Furthermore, once the specimens had cured, the agar remained throughout the cemented area and seemed to behave as somewhat of a lubricant. In an effort to address these issues, two additional methods of application were considered. First was applying each solution twice to each layer. Applying the solutions twice was performed and the specimens were cured at room temperature (Figure 4.3.1.c) or cured in a 62°C oven (Figure 4.3.1.a). Initial results show the spray gel in the oven-cured circle-stencil specimens spread and significantly affected the surrounding soil. The room temperature cured specimens cemented similarly to the initial efforts although slightly improved in apparent strength. A single application of each solution was also applied, and oven cured and resulted in results similar to the double-application cured at room temperature, but with somewhat higher depths of penetration (Figure 4.3.1.b). These results appear to convey that if gel solutions are to be oven cured there is an appropriate volume limit for solution application to have control over the results. Note that required oven curing is less desirable for field applications because it complicates the method; however, it was explored here to examine the effects on the spray gel cementation levels.



(a)

(b) (c)

Figure 4.3.1 Gel spray results for: (a) double sprayed and oven cured for (2) 25.4 mm diameter stencils and (1) 31.75 mm diameter stencil; (b) Single sprayed and oven cured for (2) 25.4 mm diameter stencils; and (c) double sprayed and room temperature cured for (2) 25.4 mm diameter stencils.

While the spray gel solutions show success, there are still unpredictable issues involved with the process. In Figure 4.3.2.a the initial results of an oven cured spray gel specimen can be observed. However, Figure 4.3.2.b shows the same sample one week later. The sample that was once clearly cemented seemingly dissolved over time. This may be a side effect of adding agar to the solutions. Potentially the addition of agar, or some other variable chosen in this process, led to the development of vaterite in place of calcite, which is a much more unstable precipitate. While there is potential for success in creating gel spray solutions for specific MICP applications, there are still considerable efforts required to create a beneficial and reliable process. Another application option would be to try simultaneously spraying the solutions for each layer instead of the traditional sequential style.

(a) (b)

Figure 4.3.2 Effects of time on 25.4 mm diameter cemented sand specimens: (a) 2 days after initial cementation; and (b) 1 week after initial cementation.

4.6 Stencil specimens

Stencil specimens were investigated as an accessible technique for creating specific, beneficial shapes using additive manufacturing MICP. The column created by layering with a circular stencil is shown in Figure 4.6.1. While the column cemented successfully, the results are somewhat disfigured and do not demonstrate pristine clarity of form. Diameters measured along the height of the column were found to be up to 1.5 times the diameter of the circular stencil. Spread values were ranging from 4.7 mm 7.1 mm to (18% to 28% of the intended dimensions) for each exterior surface. For the overall diameter, the sum of the spread therefore ranged from 9.4 mm to 14.2 mm. Such seemingly high spread values are ostensibly problematic, but this variability was based on a sample with an intended diameter of 25.4 cm. If upscaled to field scale, the likelihood of 7 mm spread on intended dimensions is likely much less significant. The variability and the actual spread is, of course, highly dependent on soil type.

Figure 4.6.1 Cemented column, 50% sand 50% clay.

The plant stalk inspired stencil was applied to the surface layer of a 100% Ottawa sand specimen with liquid solutions (Figure 4.6.2). The depth of cementation increased compared to the penetration experiments due to the decrease in treated surface area. Variability between the dimensions of the stencil and the dimensions of the cemented layer ranged from 18%-60%. This variability is considering the intended dimensions of 3.75mm-5.55mm. While not displaying particular clarity, the results of the stencil layer application show promise for upscaled applications.

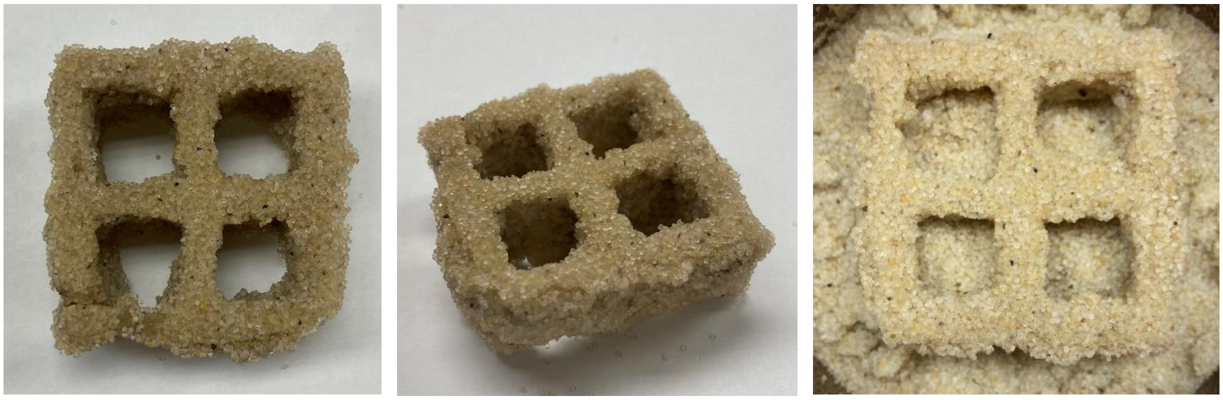


Figure 4.6.2 Cemented plantstalk layer, 100% Ottawa sand.

5. CONCLUSIONS

The feasibility of layer-by-layer single spray application additive manufacturing MICP soil treatment was evaluated. The behavior of different soil classifications were assessed by comparing the cemented zones formed through surface layer parametric studies. Cubic UCS specimens comprised of 50% Ottawa sand and 50% red art clay were prepared with variant optical densities and volumes of applied solutions. The results of this study indicate the viability of a new approach to MICP soil treatment using additive manufacturing. Additive manufacturing allows for leveraging shapes that provide enhanced engineering properties with less material. Using this method, most soil types could be enhanced to any excavated depth. The depth of cementation per layer will increase with an increase in hydraulic conductivity, but is also begins to decrease with the lower specific surface area of coarser grained soils. For field scale applications, depth of penetration could also be increased with pressure applied to the spray applications. Particular parameters of field applications would need to be analyzed for each individual soil application to determine the site-specific parameters (i.e., soil type affects both the depth and efficacy of additive manufacturing MICP treatment). Gel spray treatment solutions may be applicable in situations where higher control is desired. The gel in the solution does not completely inhibit precipitation, but likely debilitates precipitation to some degree. Initial mixing of the gel solutions may be necessary prior to application to ensure calcite precipitation forms effectively. Soil enhancement with the method described herein provides lower levels of strength increased compared to more traditional methods, but the range of soil types that can be treated and the efficiency of the treatment application make it a promising technique.

Future work on this project will include both broadening the work presented herein and expanding it further. For the unconfined compression specimens, a method for creating level surfaces in the cemented specimens should be developed. UCS testing should also be performed on cylindrical cemented specimens and full-depth gel solution specimens. Further understanding of the specimens created with this new method of additive manufacturing MICP can be obtained by acquiring true calcite content and performing an X-ray CT scan. For the gel solutions, full depth specimens should be cemented and a relationship between the increased viscosity of solution and higher control of application developed, as well as UCS comparison for gel solution versus non-gel solution cemented sands. For the stencil specimens, full-depth plant stalk specimens should be created and tested in unconfined compression, and other stencil shapes should be created for comparison. Finally, this new process should be applied to a larger scale experimentation, namely 1-ft square.

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7. APPENDIX

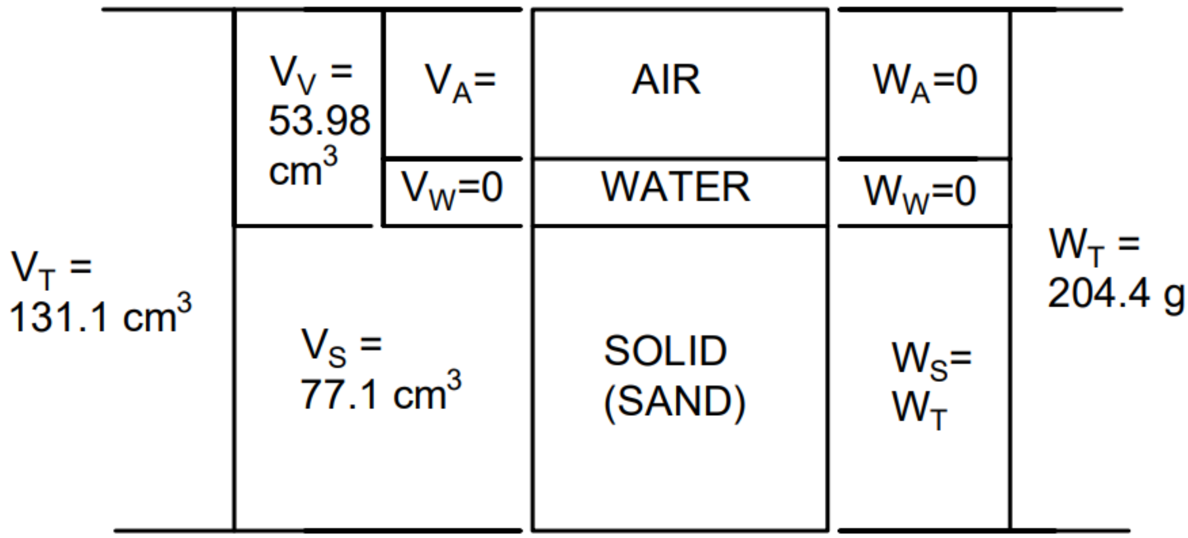

Figure 7.1 Soil phase diagram for 100% 20-30 Ottawa sand.

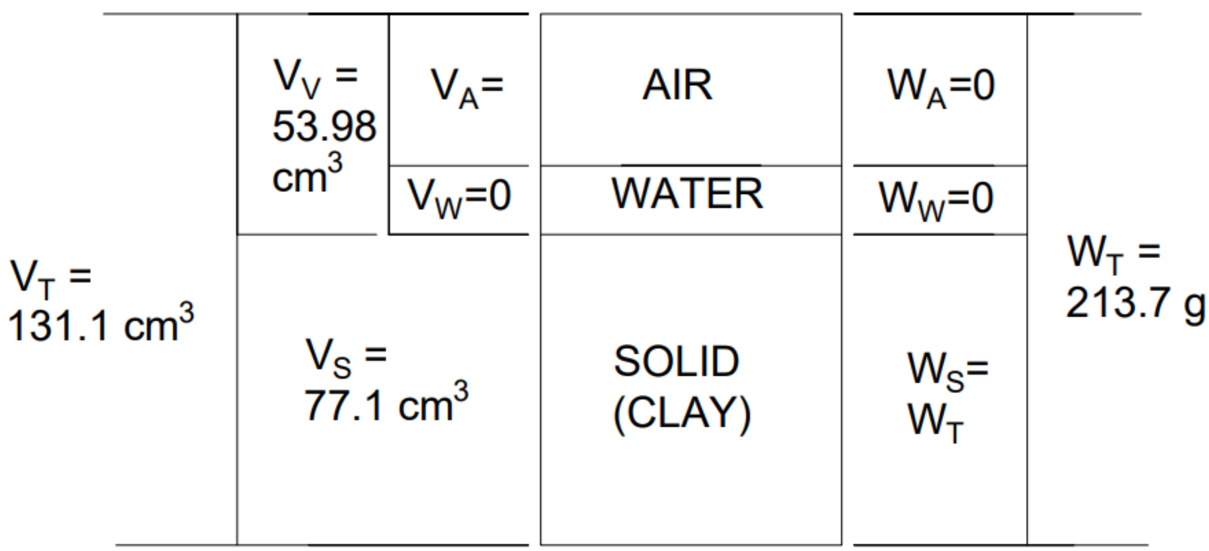

Figure 7.2 Soil phase diagram for 100% Red Art clay.

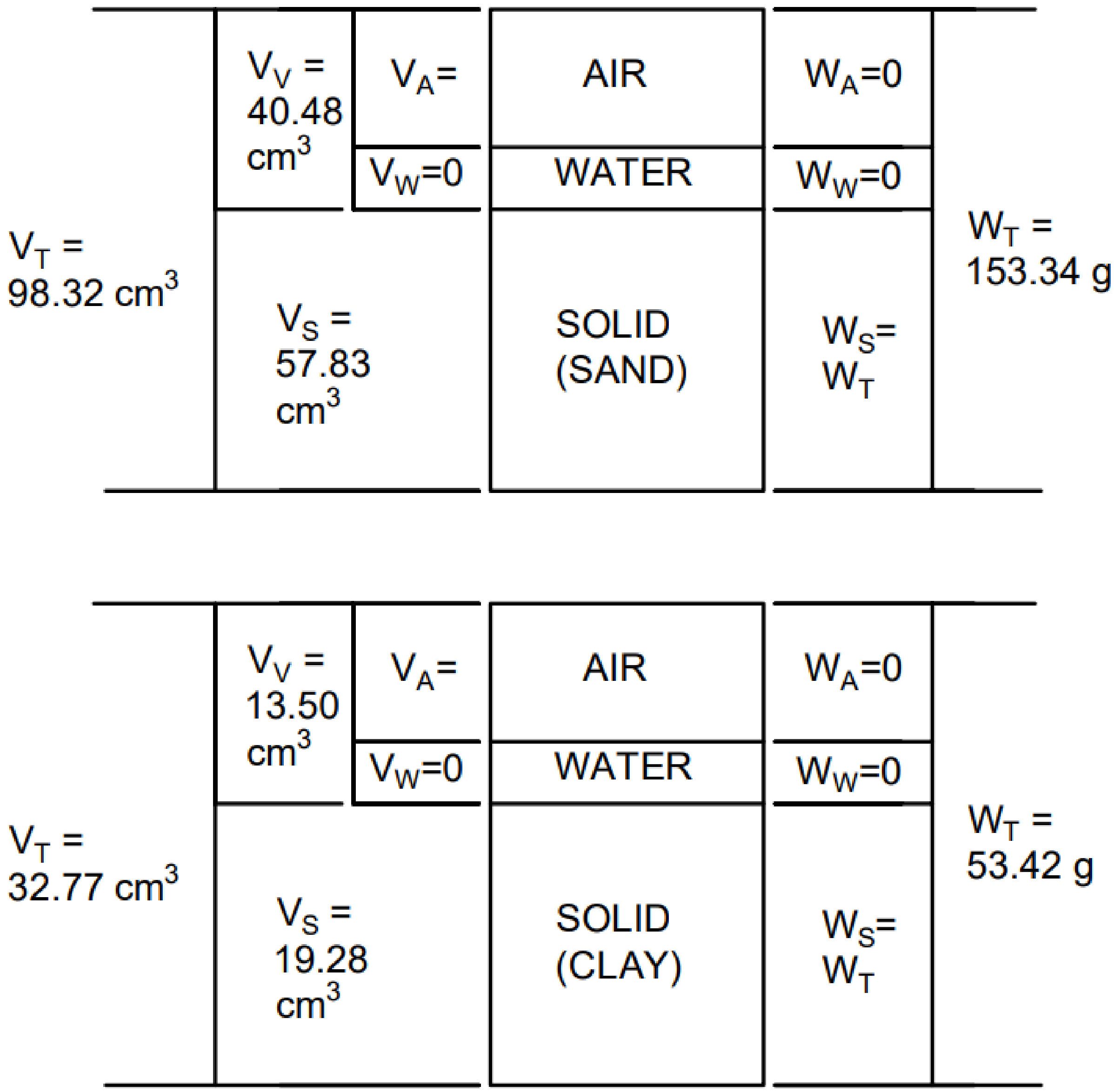

Figure 7.3 Soil phase diagrams for 75% Ottawa sand and 25% Red Art clay by volume.

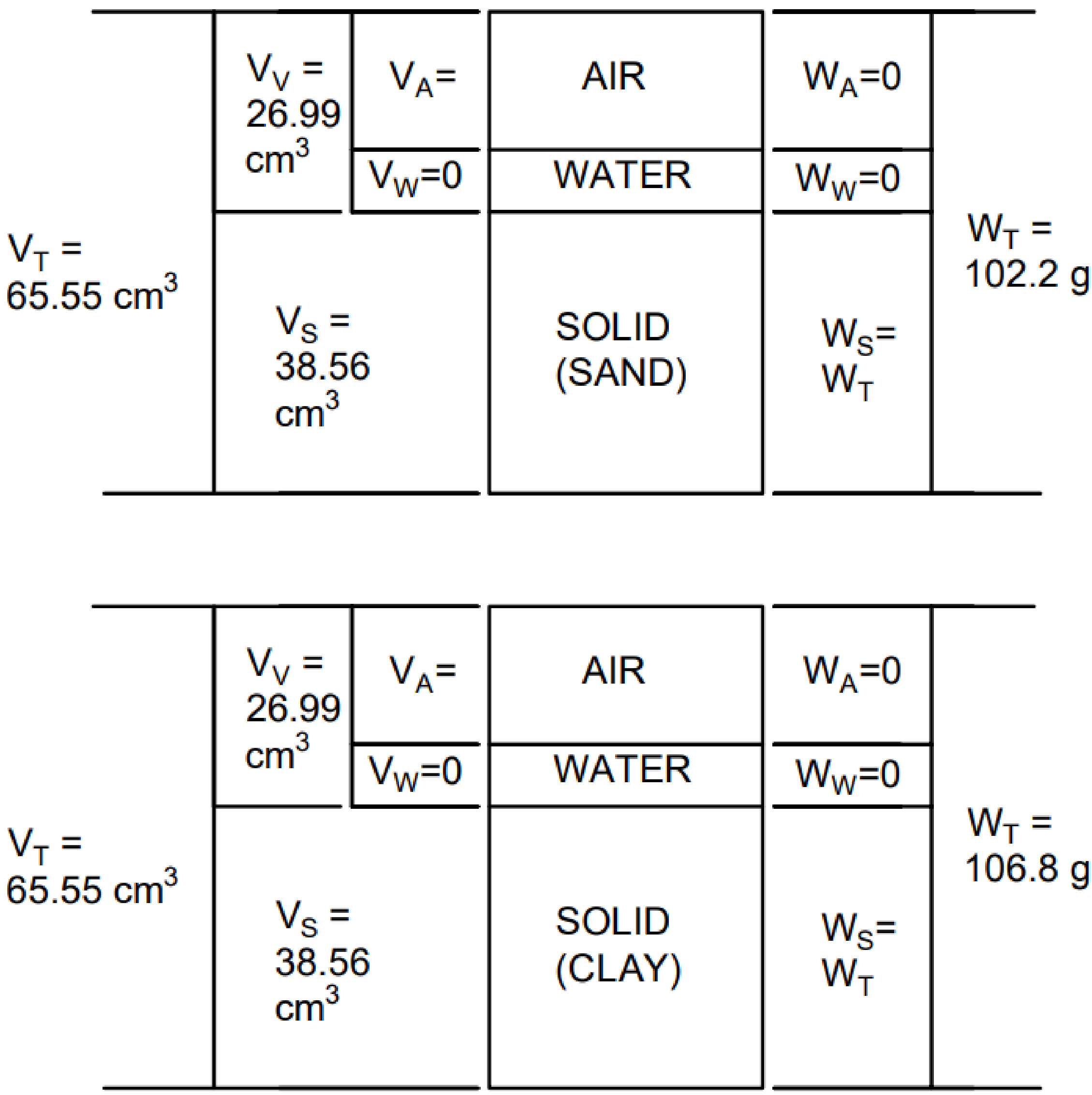

Figure 7.4 Soil phase diagrams for 50% Ottawa sand and 50% Red Art clay by volume.

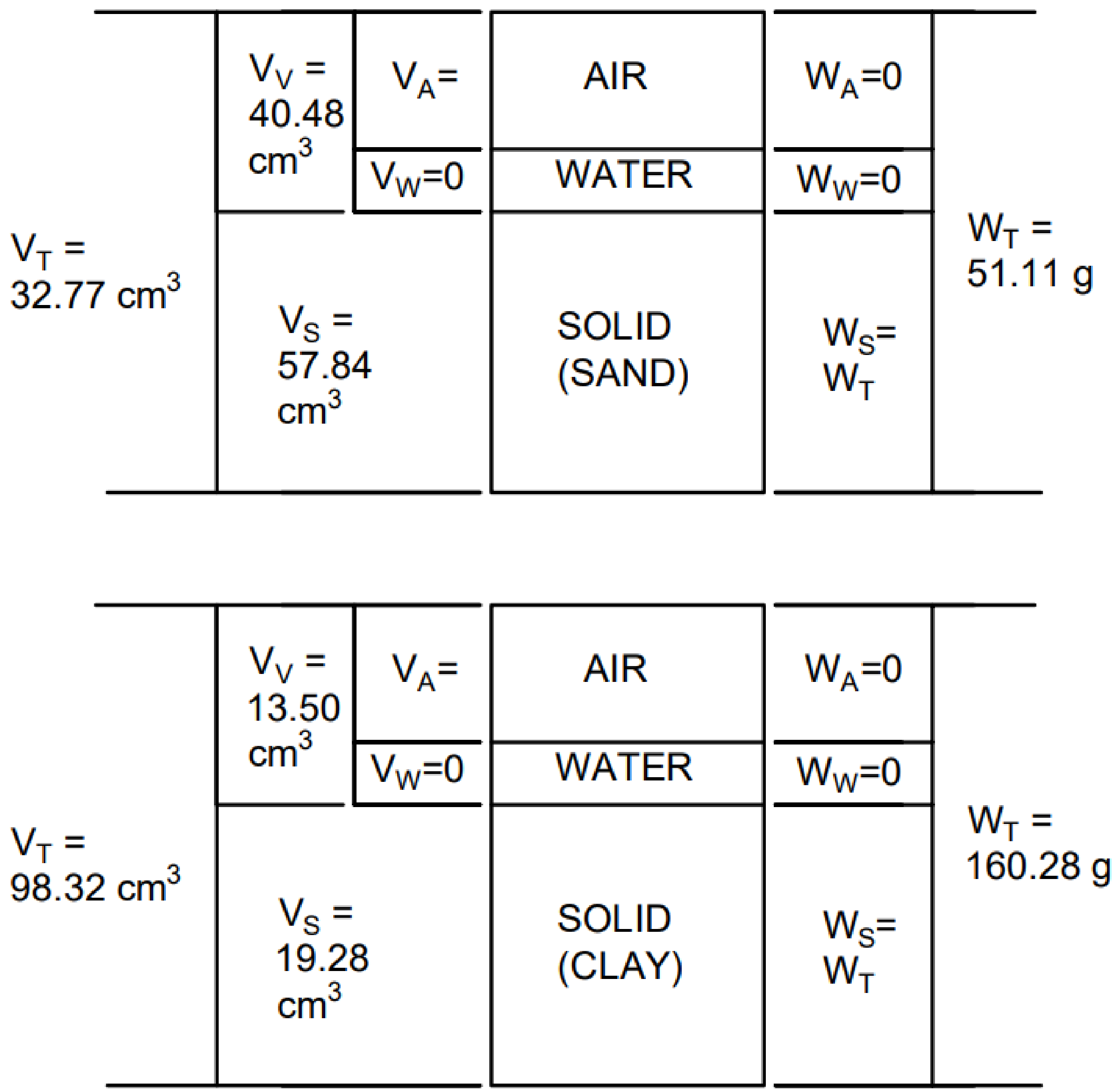

Figure 7.5 Soil phase diagrams for 25% Ottawa sand and 75% Red Art clay by volume.