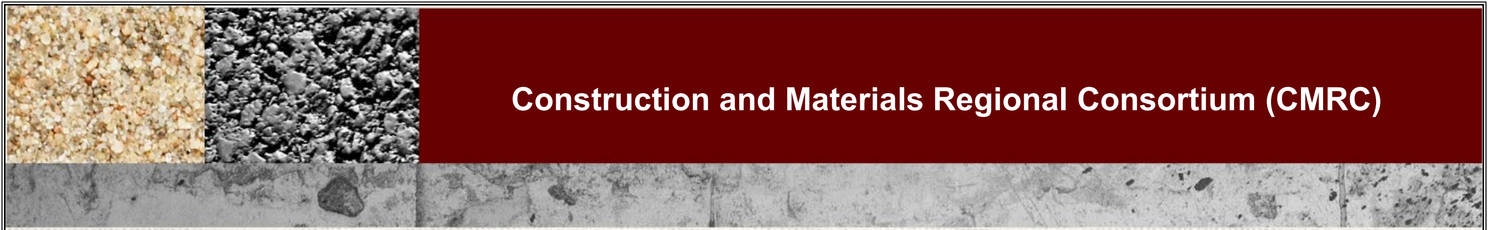

Construction and Materials Regional Consortium (CMRC)

CMRC

Review of Bio-Stabilized Construction Materials

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16. Abstract

This report summarizes over 250 references to identify the current state of practice when using biomaterials as a sustainable construction material. This report primarily focuses on the use of microbially induced calcite precipitation (MICP) to stabilize soils. This report is generally broken down into four evaluations of MICP: 1) an overview of the chemical mechanisms, 2) evaluation of laboratory scale characterization efforts, 3) evaluation of field scale characterization efforts, and 4) applications of MICP technology. This report concludes with a Strengths, Weaknesses, Opportunities, and Threats (SWOT) analysis of MICP technology.

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EXECUTIVE SUMMARY

Soil stabilization techniques have been heavily studied for decades by the Engineer Research and Development Center (ERDC) where non-traditional stabilizing materials have been an area of interest. Bio-stabilization methods have gained popularity in the last 10 to 15 years due to its potential for sustainability and could be of interest to ERDC. The primary objective of this report is to survey available literature to understand the current state of bio-stabilized materials for soil stabilization. In total, 265 references were used to comprehensively evaluate the mechanisms of microbially induced calcite precipitation (MICP) in soils as well as its potential applications.

To date, hundreds of laboratory scale experiments have been conducted to understand the influence of control variables (e.g., soil type, pH, temperatures, oxygen availability, etc.) on calcium carbonate precipitation during the MICP process. Physical properties of laboratory scale MICP stabilized soils are also frequently studied. Literature was evaluated to provide holistic analysis of physical properties and quantify the range of expected properties in MICP stabilized soils. In addition to laboratory scale efforts, some research has been conducted to evaluate MICP stabilization techniques in larger, field-scale applications.

MICP stabilization has been successfully implemented as a bio-remediation technique for immobilizing heavy metals. Additional applications include erosion control and dust abatement as surface applications of MICP can create a hardened top layer of soil. These techniques have been evaluated both in controlled, laboratory environments as well as in field trials.

This holistic evaluation of literature shows that there is still work needed to fully understand MICP stabilization processes, especially in field scale applications where engineering properties are difficult to characterize. Based on this literature review, there are potential applications for MICP technologies including stabilizing base material for traffic (e.g., low volume roads in rural area, beach front arterials). However, there is a need for both design standards as well as quantitative methods to evaluate in-situ strength of MICP treated soils in field applications.

CHAPTER 1 - INTRODUCTION

1.1 Background

The Engineer Research and Development Center (ERDC) has been evaluating potential soil stabilization techniques since the late 1940's (Oldham et al., 1977). Several studies have focused on stabilizing silty and clayey sands as well as coarse aggregate to increase load bearing capabilities for use in low volume roads (Santoni et al., 2002; Tingle and Santoni, 2003; Santoni et al., 2005; Barbieri et al., 2022). Soil stabilization efforts have also been an integral piece of the Joint Rapid Airfield Construction (JRAC) Program which has utilized cement-polymer stabilized soil surfaces as well as polymer emulsion surfaces for the rapid airfield construction projects capable of supporting aircrafts as large as C-17 transport aircraft (Anderton et al., 2008; Anderton et al., 2021). Additional efforts have also evaluated non-traditional stabilizers to quantify stabilization mechanisms and optimum application scenarios considering soil type, strength improvement, volume stability, and waterproofing (Tingle et al., 2004a; Tingle et al., 2007). This research into non-traditional soil stabilization has continued, but efforts have begun to shift to investigating the potential of biological based stabilizing materials.

In recent years, multidisciplinary efforts have led to the development of bio-stabilizing materials that can be used in place of traditional portland cements (Choi et al., 2020; Bhutange and Latkar, 2020; Mujah et al., 2017; Naveed et al., 2020; Ng et al., 2012). The demand for bio-stabilizing materials has increased in response to the large amounts of carbon dioxide (CO_2) and nitrogen oxides (NO_x) emissions that occur during cement production (Worrell et al., 2001). Several bio-based soil improvement methods have been evaluated that utilize different mechanisms to produce meaningful amounts of stabilization. Some of these bio-based methods do not lead to meaningful mechanical property improvement (e.g., unconfined compressive strength or shear strength) but instead lead to favorable permeability or workability characteristics.

- **Enzyme induced calcite precipitation (EICP):** This bio-based method uses the same chemical reactions as MICP to produce calcite; however, instead of using bacteria, enzymes from bacterial solutions or plants are used (Almajed et al., 2018; Muhammed et al., 2021; Park et al., 2014; Putra et al., 2020).

- **Microbial biopolymer accumulation (MBA):** This bio-based method stimulates microbes that over time produce biopolymers which can be used to stabilize the surrounding materials. This method has been successfully implemented by Kim et al. (2019) for fermentation based bacteria.

- **Biofilm formation (BF):** This bio-based method is a collective of one or more types of microorganisms that are capable of growing in natural environments and protecting these microorganisms from external attacks (Roeselers et al., 2008; Ta et al., 2014; DeJong et al., 2014b).

- **Biogas generation (BG):** This bio-based method utilizes insoluble gas bubbles of nitrogen or carbon dioxide produced by microbes to improve liquefaction resistance of soils (He et al., 2015; Rebata-Landa and Santamarina, 2012).

- **Biopolymer treatment (BPT):** This bio-based method involves utilizing biopolymers as a cementing material in soils. These biopolymers can be produced by bacteria (e.g., xanthan gum, gellan gum, and beta-glucan), algae, plants, shellfish (e.g., chitosan), and dairy agar produces (e.g., casein) (Sharma et al., 2018). Biopolymers are typically dried and added to soil during mixing similar to portland cement (Chang et al., 2020; Choi et al., 2020).

- **Microbially induced calcite precipitation (MICP):** This bio-based method decomposes urea into ammonium and carbonate ions. The ammonium leads to an increase in pH while the carbonate ions combine with calcium to form calcium carbonate (i.e., calcite) which stabilizes the surrounding material. The remainder of this report provides an extensive literature review on the uses of MICP for construction applications.

Bio-stabilizing methods have gained popularity in the last 10 to 15 years due to its reduced carbon footprint compared to traditional stabilization techniques and potential for sustainability. A histogram of literature referenced in this report on MICP stabilization efforts was created to show the consistent increase in publications focusing on characterizing and implementing MICP technology (Figure 1).

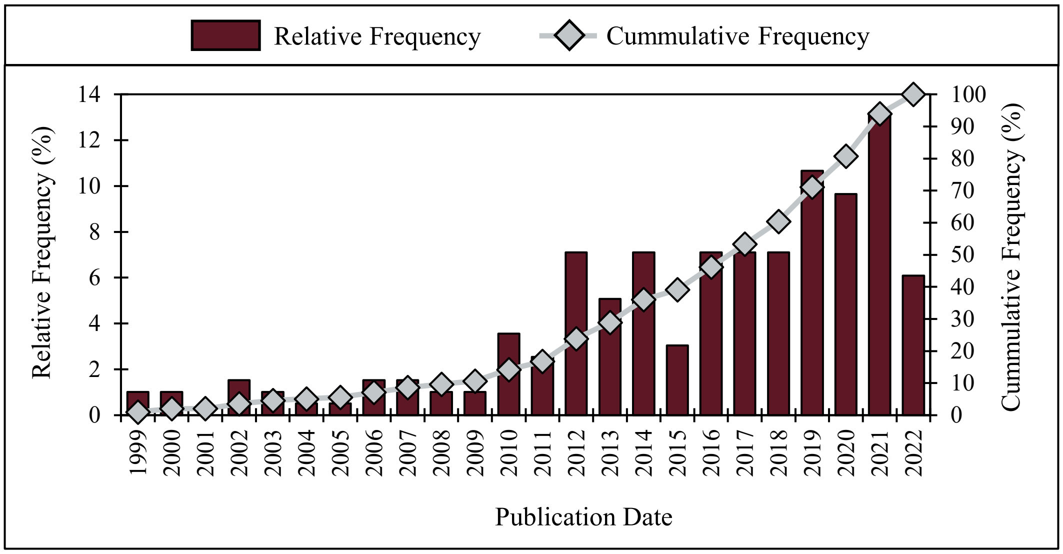


Figure 1. Frequency of publications focusing on implementing MICP technology

1.2 Objectives and Scope

This report details efforts by the Engineer Research and Development Center (ERDC) and Mississippi State University (MSU) to evaluate and to the extent applicable implement both traditional and bio-stabilization techniques to stabilize poorly graded sand. The primary objective of this report is to survey available literature to understand the current state of bio-stabilized materials for soil stabilization. This literature review is intended to be used as a basis of knowledge for bio-stabilized material research efforts by the ERDC, the Mississippi State University (MSU) Center for Advanced Vehicular Systems (CAVS), and the Richard A. Rula School of Civil and Environmental Engineering (CEE).

1.3 Notation

1.3.1 Symbols and Acronyms

BF	biofilm formation
BG	biogas generation
BPT	biopolymer treatment
c	cohesion
CAVS	Center for Advanced Vehicular Systems
CBR	California bearing ratio
CEE	Civil and Environmental Engineering
COV	coefficient of variation
DCP	dynamic cone penetrometer
DOE	Department of Energy
DSS	direct shear strength
E	elastic modulus
E:UCS	ratio of elastic modulus to unconfined compressive strength
EICP	enzyme induced calcite precipitation
ERDC	Engineer Research Development Center
H	hardness
IDT	indirect tensile strength
IDT:USC	ratio of indirect tensile strength to unconfined compressive strength
JRAC	Joint Rapid Airfield Construction
k	thermal conductivity
k(S)	thermal conductivity as a function of degree of saturation
k_{dry}	thermal conductivity of dry soil
k_r	normalized thermal conductivity
k_{sat}	thermal conductivity of saturated soil
k_{soil}	thermal conductivity of soil
k_{water}	thermal conductivity of water
K_{IC}	fracture toughness
L:D	length to diameter ratio
MBA	microbial biopolymer accumulation
MEPDG	mechanistic-empirical pavement design guide
MICP	microbially induced calcite precipitation

MSU	Mississippi State University
N	number of cementation solution cycles
OD_{600}	bacteria concentration
P	permeability
P_{norm}	normalized permeability
R_c	ratio of cementation solution to sample volume
RMSE	root mean square error
S	degree of saturation
UCS	unconfined compressive strength
USACE	United States Army Corps of Engineers
V_s	shear wave velocity
w/v	weight in grams divided by volume in milliliters
XRD	x-ray diffraction
γ_{dry}	dry density
φ	friction angle
η	material parameter for thermal conductivity calculation
κ	material parameter for thermal conductivity calculation
χ	material parameter for thermal conductivity calculation
τ	shear stress

1.3.2 Chemistry Notation

Ca^{2+}	calcium ion
$CaCl_2$	calcium chloride
$CaCO_3$	calcium carbonate or calcite
Cd	cadmium
CH_2O	formaldehyde
CO_2	carbon dioxide
CO_3^{2-}	carbonate
Cu	copper
H^+	hydrogen
Hg	mercury
HS^-	bisulfide
H_2S	hydrogen sulfide
NH_3	ammonia
NH_4^+	ammonium
NO	nitric oxide
NO_x	nitrogen oxides
O_2	oxygen
OH^-	hydroxide ion
Pb	lead
Zn	zinc

CHAPTER 2 – OVERVIEW OF MICROBIALLY INDUCED CALCITE PRECIPITATION (MICP)

This section introduces and explains the reactions that occur during microbially induced calcite precipitation (MICP). A detailed explanation of the varying chemical mechanisms in which MICP occurs as well as different microorganisms capable of calcite precipitation is provided.

2.1 Chemical Mechanisms

As the microorganisms begin to undergo metabolic activity, they begin to produce carbonate ions which are precipitated as calcium carbonate ($CaCO_3$) when exposed to calcium ions (Ca^{2+}). MICP is a natural process, and the microorganisms has little to no control over the precipitation (Bazylinski et al, 2007; Frankel and Bazylinski, 2003). Hammes and Verstraete (2002) note that there are four key influencing factors in the precipitation of calcium carbonate: 1) Ca^{2+} concentration, 2) concentration of dissolved inorganic carbon, 3) medium pH of the environment, and 4) availability of nucleation sites.

There are many chemical mechanisms through which calcium carbonate precipitation can occur, and these mechanisms can generally be categorized as either heterotrophic or autotrophic. Heterotrophic mechanisms utilize microorganisms that cannot produce their own food and get nutrients from other sources. Examples of heterotrophic mechanisms include ammonification, denitrification, sulfate reduction, and urea hydrolysis. Autotrophic mechanisms utilize microorganisms that can produce their own complex organic compounds using carbon. Examples of autotrophic mechanisms are methane oxidation and photosynthesis. Table 1 summarizes MICP mechanisms that have been seen in published literature. Although these processes are generally considered to be more environmentally friendly than traditional cement stabilization of soils (Wong, 2015), there are undesirable by-products produced during calcite precipitation in each of these mechanisms.

Ammonification of amino acids commonly takes place in soils rich with organic material, within a pH of 5 to 8, and have access to oxygen (Dawid, 2000). During the process, amino acids metabolism produces carbonate (CO_3^{2-}) and ammonia (NH_3). The hydrolysis of NH_3 then leads to ammonium (NH_4^+) and hydroxide (OH^-) ions leading to supersaturation and ideal conditions for calcite precipitation. Harmful by-products of ammonification include ammonia (a toxic gas) as well as ammonium which can form toxic salts; however, nitrogen is well bound in the soil. Ammonification commonly occurs with aerobiosis bacteria such as *Myxococcus xanthus* and *Alcanivorax borkumensis*, which are naturally abundant and utilize amino acids as their sole energy source (Gerth et al., 2003; Jian et al., 2021).

Denitrification generally happens when organic matter is available to produce alkalinity, CO_2, and nitrogen gas (Martin et al., 2013). This process initiates with the consumption of H^+ which increases the surrounding pH and allows for the production of CO_2 which in turn is used to precipitate calcite. Denitrification produces the least harmful final by-products of the chemical mechanisms defined in Table 1 (i.e., nitrogen gas and small amounts of carbon dioxide); however, its intermediate products (i.e., nitric oxide and nitrogen dioxide) are greenhouses gases and can cause acid rain. There are several types of bacteria that are capable of reducing nitrate including *Achromobacter*, *Alcaligenes*, *Bacillus*, *Denitro bacillus*, *Micrococcus*, *Pseudomonas*, *Spirillum*, and *Thiobacillus* (Karatas, 2008).

Table 1. MICP mechanisms reported in published literature

Mechanism	**Primary Chemical Reaction**	**Reaction By-Products [1]**	**References**
Ammonification (Heterotrophic)	*Amino Acids* + O_2 + Ca^{2+} → NH_4^+ + H^+ + $CaCO_3$ ↓	Ammonia (NH_3) Ammonium (NH_4^+) Hydrogen (H^+)	Castro-Alonso et al. (2019) González-Muñoz et al. (2010) Zhu and Dittrich (2016)
Denitrification (Heterotrophic)	$(CH_3COOH)_2Ca$ + NO_3^{-} → $0.8N_2$ + $3CO_2$ + $3H_2O$ + OH^{-} + $CaCO_3$ ↓	Carbon Dioxide (CO_2) Nitric Oxide (NO) Nitrogen Dioxide (NO_2) Nitrogen (N_2)	Bu et al. (2022) Jain et al. (2021) Pham et al. (2018) Hamdan et al. (2017)
Methane oxidation (Autotrophic)	CH_4 + SO_4^{2-} + Ca^{2+} → H_2S + H_2O + $CaCO_3$ ↓	Hydrogen Sulfide (H_2S)	Castro-Alonso et al. (2019) Mondal and Ghosh (2019) Zhu and Dittrich (2016)
Photosynthesis (Autotrophic)	$2HCO_3^{-}$ + Ca^{2+} → CH_2O + O_2 + $CaCO_3$ ↓	Formaldehyde (CH_2O) Oxygen (O_2)	Achal et al. (2015) Castro-Alonso et al. (2019) Dhami et al. (2014)
Sulfate reduction (Heterotrophic)	SO_4^{2-} + $2[CH_2O]$ + OH^{-} + Ca^{2+} → CO_2 + $2H_2O$ + HS^{-} + $CaCO_3$ ↓	Bisulfide (HS^{-}) Carbon Dioxide (CO_2)	Castro-Alonso et al. (2019) Seifan and Berenjian (2019) Warthmann et al. (2000)
Urea hydrolysis (Heterotrophic)	$CO(NH_2)_2$ + $2H_2O$ + Ca^{2+} + *Cell* → $2NH_4^+$ + *Cell*-$CaCO_3$ ↓	Ammonia (NH_3) Ammonium (NH_4^+)	Mondal and Ghosh (2019) Naveed et al. (2020) Seifan and Berenjian (2019)

[1] Reaction by-product can be either occur during an intermediate stage or as a final by-product

Anaerobic methane oxidation largely occurs in bodies of water where methane is converted to methanol in the presence of oxygen (Seifan et al., 2016). Methane oxidation produces hydrogen sulfide as a by-product which is a toxic gas that can be damaging to the ecosystem. Aerobic methane oxidation is another form chemical mechanism; however, it largely leads to the dissolution of carbonates due to increasing acidity (Reeburgh, 2007). Naturally occurring aerobic methanotrophs have been discovered and reported in literature, but no anaerobic methanotrophs had been discovered as of a few years ago (Hinrichs et al., 1999).

Photosynthesis produces calcium carbonate precipitation by exchanging bicarbonate (HCO_3^-) and hydroxide (OH^-) ions which in turn raises the pH around the microorganism. The HCO_3^- is then broken into CO_2 and OH- which will form carbonate minerals (Castanier et al., 1999; Miller and Colman, 1980). Formaldehyde is one by-product of this MICP mechanism that can be hazardous to the health of the environment. Cyanobacteria and microalgae are the main microorganisms capable of using photosynthesis to precipitate calcite in an aquatic environment. It is estimated that approximately 70% of carbonate rocks on Earth were formed by MICP of cyanobacteria (Altermann et al., 2006), and have been found in freshwater, salt water, and terrestrial areas (Krumbein and Giele, 1979; Wright, 1989; Goh et al., 2010; Rodriguez-Martinez et al., 2012). Additionally, cyanobacteria has been shown to absorb CO_2 from the atmosphere without the need for externally supplied urea or carbon sources (Chuo et al., 2020).

Sulfate reduction occurs in an anaerobic environment with ample amounts of organic matter. This MICP method is a combination of three mechanisms: 1) dissolution, 2) diffusion, and 3) calcium carbonate precipitation. As microorganisms remove sulfates from the environment, the surrounding pH is raised. Calcium ions are released through the dissolution of gypsum and then react with carbon dioxide which, when exposed to the elevated pH levels, can lead to the formation of calcite (Baumgartner et al., 2006; Perito and Mastromei, 2011). Hydrogen sulfide is released as a by-product of the calcium carbonate precipitation. Since the anaerobic condition needed to maintain sulfate reduction is quite difficult to achieve in field applications, sulfate reduction is not practical for engineering applications (Jain et al., 2021).

Of the Table 1 MICP mechanisms, urea hydrolysis is reported to be the most commonly implemented due to is efficiency to precipitate more calcium carbonate in a short period of time than other precipitation methods (Al-Thawadi, 2011; Dhami et al, 2013; Whiffin, 2004). Microorganisms hydrolyze urea to yield ammonia and carbonic acid. The ammonia then undergoes hydrolysis which produces hydroxide ions and increases the pH of the environment. Additionally, the carbonic acid is dissociated, and bicarbonates are produced which are then free to react with available calcium to precipitate calcite (Bachmeier et al, 2002; De Muynck et al., 2013; Knoll, 2003). Urea hydrolysis is meaningfully influenced by temperature, pH (optimal of 7 to 9), concentration of available urea, and incubation period (Hasan, 2000). Bacteria from the genus *Bacillus* are the most common microorganisms used for urea hydrolysis (Martin et al., 2012; Wong, 2015). Specifically, *Sporosarcina pasteurii* (formerly denoted as *Bacillus pasteurii* in earlier taxonomies) has been shown to optimize ureolytic activity and yield a higher calcite precipitation rate (DeJong et al., 2006; Whiffin et al., 2007). Several different microorganisms have also been successfully used for MICP in construction and engineering applications (Lian et al., 2006; Mitchell et al., 2019). Although the use of urea hydrolysis is a key piece in the implementation of bio-stabilized construction materials, the by-products of ammonia and ammonium can be harmful to the environment.

2.2 Bacteria Types

Several types of microorganisms have been identified as being capable of undergoing MICP – some of which were mentioned in the previous section. Several literature review papers have presented lists of commonly used bacteria for different MICP mechanisms to stabilize soils (Bu et al., 2022; Choi et al., 2020; Seifan and Berenjian, 2019). Similarly, all literature complied in this report was used to create a comprehensive list of bacteria used to induce calcite precipitation through urea hydrolysis (Figure 2). Bacteria used to induce calcite precipitation through other MICP mechanisms are discussed in the previous section in addition to the literature reviews referenced in this section.

Almost two thirds of the references evaluated used *Sporosarcina pasteurii* (also known as *Bacillus pasteurii* in earlier taxonomies) to induce calcite precipitation through urea hydrolysis. As noted in the previous section, this bacterium is commonly used due to its high calcite precipitation rate. Two other bacteria, *Bacillus sphaericus* and *Bacillus megaterium*, were also used consistently in literature. There were several bacteria strains that were used in only one or two reported sources but are reported herein for consistency.

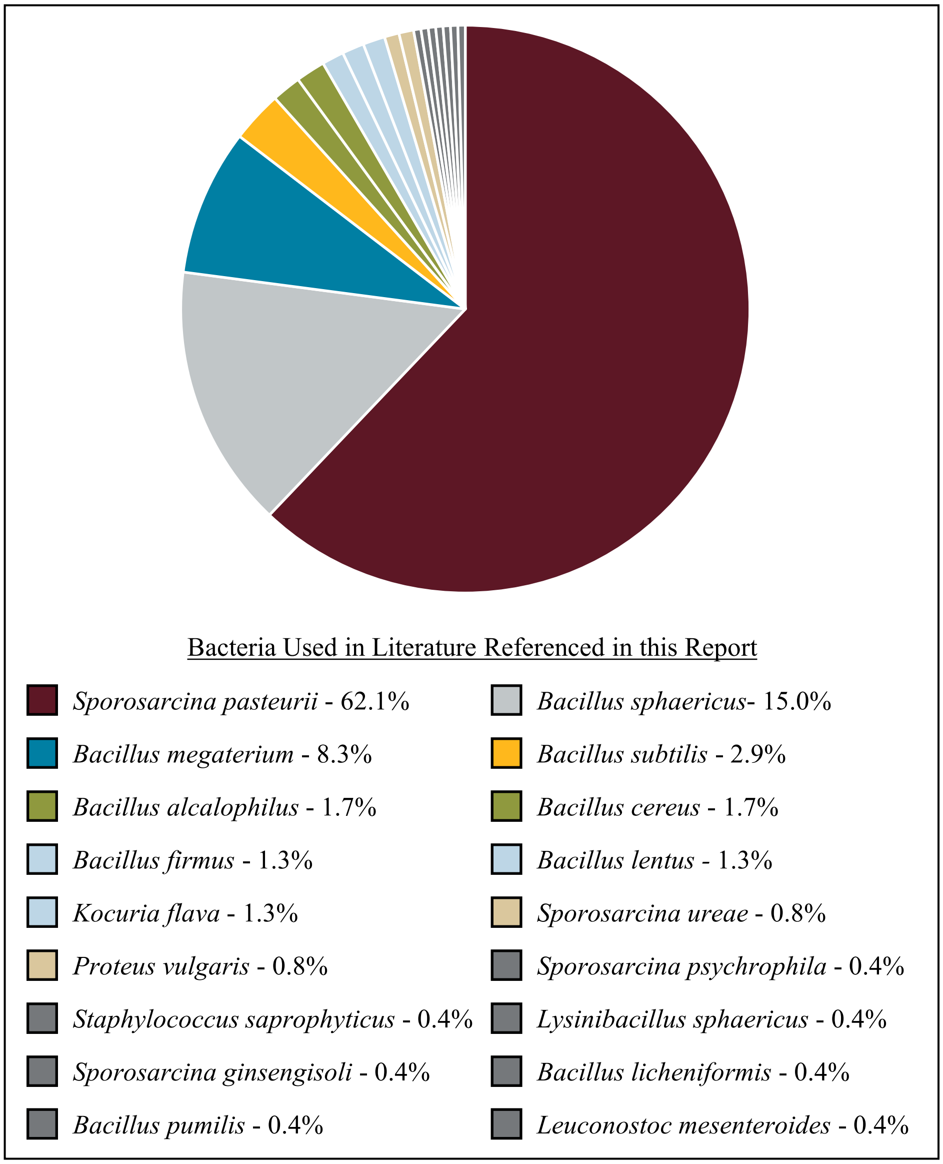


Figure 2. Overview of bacteria used in literature studies

CHAPTER 3 – LABORATORY SCALE EFFORTS TO CHARACTERIZE MICP STABILIZED SOILS

MICP has been the focus of many laboratory-based studies. This chapter details studies from five perspectives: 1) influence of different variables on the development of MICP, 2) effect of MICP stabilization on physical properties of stabilized soils, 3) quantification of failure mechanisms of MICP stabilized soils, 4) implementation of MICP in large scale laboratory tests, and 5) comparisons of MICP to traditional stabilizing materials (e.g., lime, cement, gypsum, etc.).

3.1 Influence of Control Variables on MICP Development

3.1.1 Soil Type and Condition

Soil gradation and morphology has been shown to be a key factor in the development of MICP where particle size changes, the number of particle contacts, amount of precipitated calcite, and the calcite needed to effectively bind particles together changes MICP development (Nafisi et al., 2020a). There has been some debate among what soil gradation is best for promoting calcite precipitation as grain size has been shown to largely influence calcite precipitation (Inagaki et al., 2011; Ismail et al., 2013). Table 2 summarizes optimal gradation findings reported in literature.

Table 2. Optimal soil gradation and morphology for calcite precipitation from literature

Optimal Gradation/Morphology	Soil Classification [1]	Findings	References
Coarse-grained, round soil	AASHTO: N/A USCS: SP	Allowed cementation solution to flow freely through the soil for uniform calcite precipitation.	Arpajirakul et al. (2021) Nafisi et al. (2020a)
Poorly-graded soil	AASHTO: A-3 USCS: SP	The higher permeability allowed for cementation solution to permeate throughout specimens	Zhao et al. (2014a)
Fine, rounded soil	AASHTO: N/A USCS: SP	Retained more bacteria than rounded and angular coarse sand for calcite precipitation.	Nafisi et al. (2018)
Manufactured, rounded soil	AASHTO: N/A USCS: SP	Calcite precipitates formed a homogenous shell which uniformly coated rounded particles.	Song et al. (2019); (2022)

[1] In some cases, there was not enough information to determine the AASHTO Soil Classification

Rebata-Landa (2007) reported limitations for both fine and coarse soils for MICP stabilization – in fine soils, MICP is hindered by permeability while in coarse soils thin layers of calcite precipitates cannot bind soil particles together. This finding was quantified by

Mortensen et al. (2011) where extreme gradations (i.e., very coarse or very fine) had difficulties precipitating calcite. Although fine materials can hinder MICP, Xiao et al. (2021b) reported that using a soil with some fines can increase interparticle bonds formed during MICP.

Even though many sources report a meaningful relationship between particle gradation and calcite precipitation, Cheng et al. (2017) found that at calcite precipitation levels less than 5%, the effects of sand gradations were negligible. However, at higher calcite precipitation levels, the influence of sand gradation was noticeable.

Another factor that has been indicated as potentially having influence over MICP is the mineralogy of the soil particles (Zhao et al., 2014a). Mortensen et al. (2011) evaluated four sands with mineralogy of silica, calcite, feldspar, and iron oxide. Results showed that MICP can successfully occur in a range of soil minerals. It is noted that different available minerals could lead to more favorable conditions for calcite precipitation as the mineralogy of the soil particles can influence the thermodynamics of the precipitation/dissolution reactions in the system. For example, soils that are naturally high in calcium carbonate provide ideal nucleation sites for calcite precipitation.

The relationship between soil saturation and calcite precipitation has also been studied (Cheng et al., 2013). The general trend found was that as soil saturation increased, the amount of precipitated calcite meaningfully increased.

3.1.2 Bacteria Type and Concentration

Several types of bacteria have been used to precipitate calcite (see Section 2.2); however, some studies have systematically studied the effects of different microorganism on MICP development. Sharma et al. (2021) evaluated *Sporosarcina pasteurii*, *Bacillus sphaericus*, and *Bacillus subtilis* as potential bacteria for MICP. The study found that as long as the cementation solution concentration is accurate, *Bacillus sphaericus* and *Bacillus subtilis* are capable of performing just as effectively as *Sporosarcina pasteurii.* Sun et al. (2019) directly compared *Sporosarcina pasteurii* and *Bacillus megaterium* and determined that the two bacteria produced comparable precipitation rates at high temperatures (i.e., 30°C); however, at low temperatures (i.e., 15°C) *Sporosarcina pasteurii* exhibited a much higher precipitation rate than *Bacillus megaterium.* Additional efforts have isolated naturally occurring bacteria strains and evaluated their ability to precipitate calcite when exposed to urea (Burbank et al., 2012; Omoregie et al., 2017).

Bacteria concentration has also shown to be an influential parameter on calcite development. Cheng et al. (2017) evaluated the effects of varying bacteria amounts on calcite precipitation when exposed to a constant quantity of cementation solution. Higher amounts of bacteria produced small calcite crystals ranging in size from 2 to 5 µm as seen in SEM images. However, calcite crystals formed when exposed to low amounts of bacteria were seen in SEM images to have agglomerated and formed large clusters sized at approximately 20 to 50 µm. Al-Thawadi and Cord-Ruwisch (2012) found that the average size of calcite precipitates increased with increased bacteria concentrations which could be useful for the production of crystals of a designed size. Additionally, Zhao et al. (2014a) found that as bacteria concentration increased, the percent of calcite found in soil samples increased from approximately 5% calcite at a concentration of 0.3 to approximately 14% calcite at a concertation of 1.5. Okwadha and Li (2010) found that increasing bacteria concentration from 10^6 to 10^8 cells per mL of solution, increased the amount of precipitated calcite (p-value <

0.01). Bacteria retention in porous media is a coupled process that is heavily dependent on solution chemistry, pore structure, and system hydrodynamics (Torkzaban et al., 2008).

3.1.3 Cementation Solution Type, Concentration, and Input Rates

Composition of the cementation solution controls the interaction between the bacteria and urease as well as the type and morphology of calcite crystals precipitated (Tang et al., 2020). Mortensen et al. (2011) evaluated three different cementation solutions that had varying ratios of chemicals. It was determined that by increasing the amount of ammonium chloride (i.e., a salt) in the cementation solution can slow the rate of calcite precipitation along the flow path and allow for a more homogenous calcite precipitation distribution. The delaying effect of ammonium chloride was also validated through other published references (Nemati et al., 2005; Rivadeneyra et al., 2000). Gorospe et al. (2013) included seven different calcium salts into a urease extract to determine their influence over the overall urease activity. Overall, most salts noticeably decreased the urease activity when compared to a control solution; however, calcium silicate performed significantly better than all other salts evaluated. Additional research by De Muynck et al. (2010) and Zhang et al. (2014) found that different types of calcium salts affected the morphology of calcite crystals.

The concentration of cementation solution has also been shown to have a meaningful influence on the efficiency of calcite crystal precipitation in addition to the calcite crystal structure and spatial distribution (Tang et al., 2020). Nemati et al. (2005), Okwadha and Li (2010), Soon et al. (2013), Sun et al. (2019), and Zhao et al. (2014a) found that increasing the concentration of cementation solution yielded more precipitated calcite. Additionally, Al-Thawadi and Cord-Ruiwisch (2012) noted that as cementation concentration increased, the size of precipitated calcite crystals also increased. However, at higher concentrations, calcium ions are not being fully utilized as the MICP process has been optimized and the additional Ca ions are not needed (Whiffin, 2004). Omoregie et al. (2017) reported an optimal urea concentration between 6% (w/v) and 8% (w/v) to facilitate calcite precipitation where urea concentrations greater and lower than this range produced less precipitates. Although a higher concentration of cementation solution typically led to a higher amount of precipitated calcite, the spatial distribution of these calcite crystals has been shown to be non-uniform (Al Qabany and Soga, 2013). This finding was validated using SEM images from Al Qabany et al. (2012) which showed that lower concentrations of cementation solution applied over a longer duration resulted in more homogenous calcite precipitation.

Naeem et al. (2022) found that lower cementation solution concentrations yielded higher strength gain as the number of treatment cycles increased. This was attributed to increased calcite crystals distribution at lower but more frequent cementation solution application that allows the crystals to bridge soil particles.

The input rate of cementation solution has been shown to influence precipitation of calcite. Al Qabany et al. (2012) reported an optimal effective input rate of 0.042 mol/L/h which yielded a chemical efficiency of >90%. However, when doubling the input rate to 0.084 mol/L/h the average efficiency decreased to an average of 50%.

3.1.4 Soil Treatment Processes

A key factor in MICP development is the application of the microorganisms and cementation solution into the soil. If the bacteria and cementation solution are not introduced to the soil in an efficient manner, then it could lead to non-uniform precipitation of calcite within the stabilized soil (Mujah et al., 2017). There are three commonly used methods to treat soils: 1) injection, 2) surface percolation, and 3) premixing.

3.1.4.1 Injection

In the injection method, bacteria are flushed into a specimen for some duration of time (i.e., a retention period) to allow for the bacteria to attach to the soil grains. Once bacteria have attached to soil grains in the specimen, the cementation solution is injected into the specimen to begin the process of calcite precipitation. Gebru et al. (2021) notes that there are three ways of injecting bacteria and cementation solutions into soil samples:

- **Single Phase Injection:** Both the bacteria solution and the cementation solution are injected simultaneously into the soil. Cheng et al. (2019) notes it is difficult to predict the performance of the calcite precipitation with this injection method. Additionally, single phase injection has been shown to cause clogging at the injection site (Stocks-Fischer et al., 1999; Mahawish et al., 2018).

- **Two Phases:** First the bacteria solution is injected into a soil specimen for some duration of time followed by a second injection of cementation solution. This method has been shown to minimize flocculation and pore clogging (Cheng et al., 2019; Akiyama and Kawasaki, 2012). This method prevents pore clogging at the injection site and yields a more uniform distribution of calcite over a greater area of the soil sample (Mathur and Patel, 2018; Harkes et al., 2010; Tobler et al., 2011).

- **Three Phases:** Bacteria is first injected into the soil specimen followed by an injection of calcium chloride ($CaCl_2$) which acts as a bonding agent between the bacteria and soil. Finally, cementation solution is added to the soil to induce calcite precipitation (Arab, 2019). van Paassen (2009) used this injection method on a five meter long specimen and the sand column showed meaningful improvement in mechanical properties due to successful calcite precipitation. This technique was also successfully used in DeJong et al. (2006).

A study by Martinez et al. (2011) evaluated two injection techniques to find which one was most conducive to uniform calcite precipitation in 0.5 meter columns. Stopped flow injection was utilized where nutrients were intermittently injected at a high flow rate and then allowed to rest. Continuous flow injection occurred at a much slower rate for the entire duration of testing. Both columns received the same amount of total cementation solution over the testing duration. Results showed that stopped flow injection produced a more uniform distribution of calcite precipitation in comparison to continuous injections.

3.1.4.2 Surface Percolation

Surface percolation is a soil treatment method where bacteria and cementation solution are spread over the exposed soil surface and allowed permeate through the soil. This technique has been used in laboratory experiments and is being evaluated for use in field-scale applications (Cheng and Cord-Ruwisch, 2014). Cheng and Cord-Ruwisch (2012) determined that surface percolation was capable of stabilizing a one meter column by alternating applications of bacterial and cementation solution. Percolation was also shown to be a viable low-cost application method for different strains of bacteria capable of producing calcite precipitation (Omoregie et al., 2017; Omoregie et al., 2019). This application method is also being studied for use to stabilize soil with a goal of minimizing erosion (Chek et al., 2021; Kou et al., 2021).

3.1.4.3 Premixing

Premixing appears to be the least common soil treatment process for MICP applications as it alters the local soil which can lead to changes in properties. Premixing typically involves mixing bacteria with the soil to form one homogenous mixture prior to applying the cementation solution. Arpajirakul et al. (2021) found that premixing bacteria and soil prior to compaction of laboratory specimens followed by the addition of the cementation solution yielded evenly distributed MICP precipitation throughout laboratory specimens. Yasuhara et al. (2012) notes that premixing of bacteria and sand resulted in successful stabilization and meaningfully improved compressive strength compared to non-stabilized sand. However, it was determined that injection of the bacteria would lead to better stabilization results.

Zhao et al. (2014a) premixed 330 grams of sand with 85 mL of urease bacteria to form specimens that were molded into full contact, flexible molds. These specimens were then submerged in a cementation solution and allowed to cure. SEM images revealed that precipitated calcite crystals were larger than the typical size reported in literature. This was attributed to the premixing of the sample. The development of the full contact, flexible molds produced using geotextiles was also shown to produce uniform calcite precipitation in laboratory scale specimens (Zhao et al., 2014b). Although these geotextiles are successful in inducing calcite precipitation in laboratory scale studies, the implementation of these geotextiles in a large-scale application is unlikely to be successful in a large-scale effort. Ultimately, this method is feasible for laboratory experiments but is unlikely to be implemented in large-scale applications.

3.1.5 pH

Soil pH values have a direct influence on the development of calcite precipitation rates. Soon et al. (2014) and Martinez et al. (2013) showed that the pH of the environment will increase over the duration of MICP treatment as urea hydrolysis increases the pH of the environment due to the production of ammonium ions which increases the rate of urea hydrolysis. This will continue until an optimum pH is reached. Generally speaking, a pH in the range of 7 to 8 has been shown to produce the highest percentages of calcite (Stocks-Fischer et al., 1999; Ng et al., 2012; Omoregie et al., 2017). For example, Keykha et al. (2017) reported that an increase in pH from 5 to 9 increased the percentage of precipitated calcite from 8 to

20%. Kim et al. (2018) found that a pH of 7 yielded the highest of precipitated calcite based on a range of bacteria type, curing durations, and curing temperatures.

Environmental pH values not only influence the efficiency of urea hydrolysis, but also can alter the metabolism of bacteria which can alter the ability of the bacteria to decompose urea (Tang et al., 2020). Whiffin et al. (2007) determined that a pH of 7-8 produced the highest urea decomposition rates. Additionally, pH has been shown to alter the quantity of NH_3, NH_4^+, CO_3^{2-}, and HCO_3^- in pore solution of the soil (Stocks-Fischer et al., 1999), change the crystallization rate and size (Cheng et al., 2014a; Rodriquez-Navarro et al., 2003), and alter the morphology of precipitated calcite (Cheng et al., 2007).

3.1.6 Temperature

Changes in temperature can directly affect the activity of urease producing bacteria. For example, Peng and Liu (2019) found that higher temperatures yielded higher urease activity within the first 24 hours of testing; however, this precipitation rate also decreased noticeably faster at higher temperatures. As temperatures lowered, there was a lower overall peak in urease activity, but the rate did stay noticeably higher for longer. An optimum temperature seen in literature ranged from 20 to 40°C with most sources reporting 30°C (Huang et al., 2009; Keykha et al., 2007; Kim et al., 2018; Ng et al., 2018; Omoregie et al, 2017; Sun et al., 2019). Sun et al. (2019) reported a precipitation rate of 25% for both *Sporosarcina pasteurii* and *Bacillus megaterium* when tested at 30°C.

Changes in temperature can also affect the morphology and size of precipitated calcite. Both Cheng et al. (2017) and Wang et al. (2005) studied calcite morphology and size at 4-5°C (e.g., cold regions), 25°C (e.g., tropical regions), and 50°C (arid regions); findings are summarized in Table 3. Kralj et al. (1994) reported that changing temperature affected the crystallization rate of calcium carbonate but not crystal type; however, Somani et al. (2006) found that temperature had no influence on crystal type.

Table 3. Influence of temperature on calcite size and morphology

Testing Temperature	Size (Chang et al., 2017)	Morphology (Wang et al., 2005)
4-5°C	2-5 μm	Amorphous, poorly aggregated
25°C	20-50 μm	Spherical, uniformly distributed, well crystalized
50°C	2-5 μm	Spherical, square, & spindle shaped crystals w/ poor stability

3.1.7 Oxygen Availability

Oxygen availability has been shown to have a large influence over the development of MICP. Mortensen et al. (2011) found that urease activity was not influenced by anoxic (i.e., limited oxygen) conditions. This finding suggested that MICP could occur below the groundwater table where there is limited or even no oxygen availability. However, a study by Martin et al. (2012) reported that *Sporosarcina pasteurii* bacteria was unable to grow in anaerobic (i.e., no oxygen) conditions and that de novo synthesis of urease is unlike to occur in anoxic conditions. Li et al. (2018) evaluated the development of MICP in submerged specimens in an open, air restricted, and aerated testing set ups. Aerated conditions yielded the most $CaCO_3$ (4 to 9%) followed by open conditions (3 to 5%) and then air restricted conditions (1 to 2%). Additionally, Jain and Arnepalli (2019) found that ureolytic activity and precipitation of bacteria was negligible in anerobic conditions compared to aerobic conditions. These studies trend with the findings seen in Martin et al. (2012) highlighting the influence of oxygen availability on MICP development.

3.2 Physical Properties of MICP Stabilized Materials

3.2.1 Density

Dry density (γ_{dry}) of bio-stabilized sands increases due to the precipitation of calcite crystals; however, the increase in γ_{dry} varies based on number of treatments, bacteria concentration, and cementation solution. Dry density values of MICP stabilized soils have been reported as ranging from 1.11 to 2.40 g/cm^3 with the majority those densities ranging from 1.70 to 1.90 g/cm^3 (Fang et al., 2020; Ma et al., 2022; Sharaky et al., 2018). Venuleo et al. (2016) reported an average increase of 0.13 g/cm^3 for MICP treated specimens (1.81 g/cm^3 compared to 1.68 g/cm^3). Xiao et al. (2019) reported a similar increase in γ_{dry} of 0.11 g/cm^3 for specimens with high treatment cycles; however, for specimens with lower treatment cycles the average increase in γ_{dry} of 0.06 g/cm^3.

Some authors reported the initial density of laboratory scale specimens prior to MICP treatment as a percentage of the maximum dry density (γ_d) calculated from AASHTO T99 or equivalent test methods. As seen in Figure 3, a wide range of target percent of γ_d were targeted in literature referenced in this review. As soils were treated with bacteria and cementation solution, the percent of γ_d for each specimen increased as calcite precipitated. Soon et al. (2013) did report that a 90% of γ_d produced the most precipitated calcite. As a reference, the Unified Facilities Guide Specifications (UFGS) Section 32 11 33.13 states that traditional stabilized soils (i.e., soil-cement) must have a field density of 98% of maximum dry density (USACE, 2020).

Table 4 presents three studies (Wang et al., 2018; 2019; 2020) where density was calculated after each cycle of cementation solution to quantify the effects of calcite precipitation on density. Using the data in Table 4, a relationship was developed between dry density and the number of cementation solution cycles applied to stabilized sand specimens (Equation 1). Generally speaking, for each cycle of cementation solution added to a stabilized sand specimen, dry density will increase by approximately 0.03 g/cm^3.

$$\gamma_{\mathrm{dry}} = 0.032(\mathrm{N}) + 1.56\ {}^{\mathrm{g}}/_{\mathrm{cm}^3} \tag{1}$$

Where:

γ_{dry} *dry density*
N *number of cementation solution cycles*

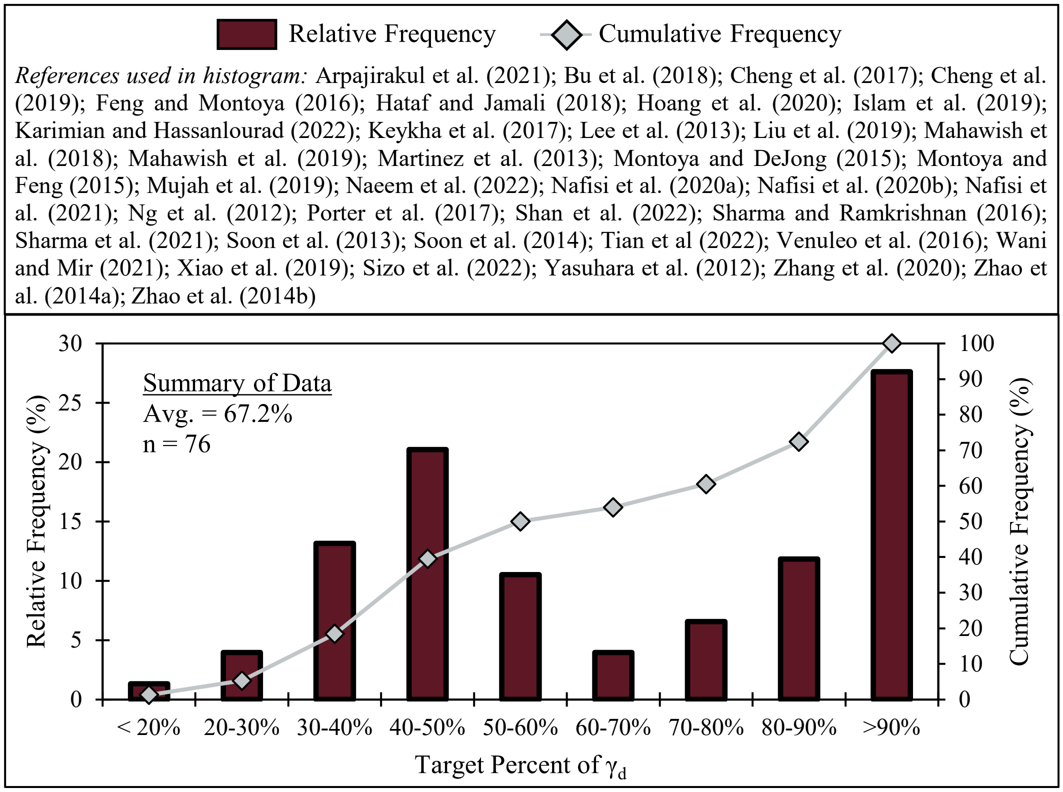
An additional relationship shown in Equation 2 was developed by Liu et al. (2019) yielded a linear relationship between dry density and the ratio of cementation solution to sample volume ratio (R_c). For each increase of 1 R_c, dry density increased by approximately 1.1 g/cm^3.

$$\gamma_{\text{dry}} = 0.096(R_c) + 1.08\ {}^{g}/_{cm^3} \tag{2}$$

Where:

γ_{dry} *dry density*

R_c *ratio of cementation solution to sample volume*



References used in histogram: Arpajirakul et al. (2021); Bu et al. (2018); Cheng et al. (2017); Cheng et al. (2019); Feng and Montoya (2016); Hataf and Jamali (2018); Hoang et al. (2020); Islam et al. (2019); Karimian and Hassanlourad (2022); Keykha et al. (2017); Lee et al. (2013); Liu et al. (2019); Mahawish et al. (2018); Mahawish et al. (2019); Martinez et al. (2013); Montoya and DeJong (2015); Montoya and Feng (2015); Mujah et al. (2019); Naeem et al. (2022); Nafisi et al. (2020a); Nafisi et al. (2020b); Nafisi et al. (2021); Ng et al. (2012); Porter et al. (2017); Shan et al. (2022); Sharma and Ramkrishnan (2016); Sharma et al. (2021); Soon et al. (2013); Soon et al. (2014); Tian et al (2022); Venuleo et al. (2016); Wani and Mir (2021); Xiao et al. (2019); Sizo et al. (2022); Yasuhara et al. (2012); Zhang et al. (2020); Zhao et al. (2014a); Zhao et al. (2014b)

Figure 3. Summary of target percent of maximum dry density in laboratory specimens prior to MICP treatments

Table 4. Influence of cementation solution cycles γ_{dry} (g/cm^3) on of MICP stabilized sand

Reference	**γ_{dry} Control**	**γ_{dry} after 1 Cycle**	**γ_{dry} after 2 Cycles**	**γ_{dry} after 3 Cycles**	**γ_{dry} after 4 Cycles**
Wang et al. (2018)	1.58	1.59	1.61	1.65	1.73
Wang et al. (2019)	1.50	1.58	1.61	1.62	1.65
Wang et al. (2020)	1.59	1.64	1.66	1.70	---

3.2.2 California Bearing Ratio

California bearing ratio (CBR) is a metric commonly used to assess bearing capacity of roadway materials. Some studies have quantified CBR values for MICP stabilized soils where a range of bacteria, cementation solutions, curing conditions, and testing ages were used (Mohapatra et al., In Press; Wani and Mir, 2021; Wani et al., 2021). It should be noted that

CBR is often used on well-graded material systems and MICP stabilized sand systems do not qualify as such. Conducting CBR tests on MICP stabilized soil should be approached with caution.

Reported CBR values from each source were normalized to the CBR value of untreated soil so that comparisons across literature could be easily drawn and plotted as a function of penetration depth (Figure 4). Generally speaking, MICP stabilization increased CBR values of soils at all penetration depths. The effects of MICP stabilization were very noticeable in the first 2 mm and then leveled off to a steady level of improvement. A power function was optimized to data reported in literature by minimizing the root mean square error (RMSE) between predicted values and reported values. Additionally, an upper boundary line is shown to quantify the best-case scenario of MICP stabilization on CBR values.

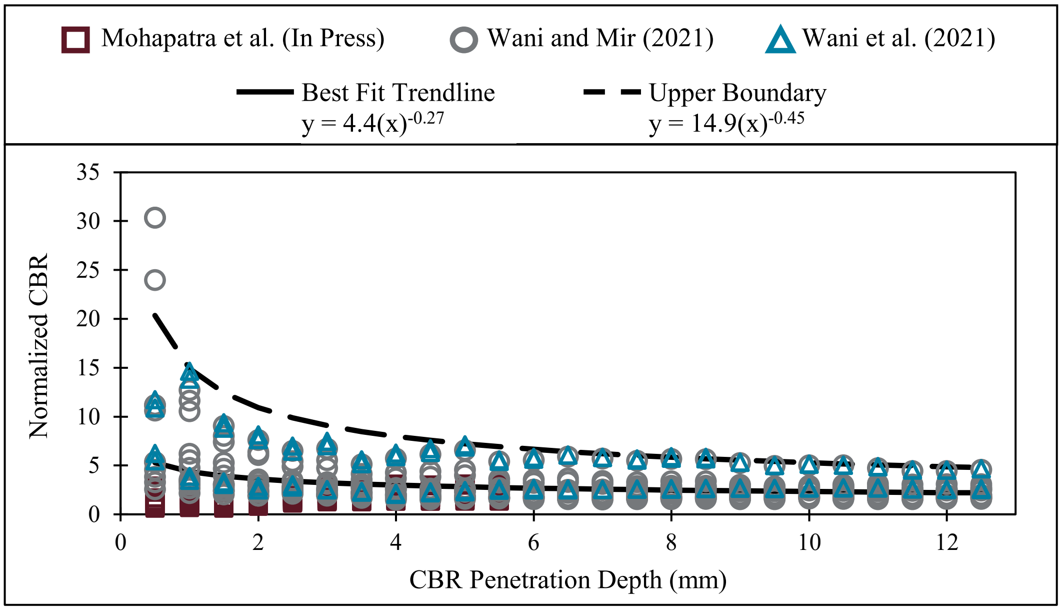


Figure 4. Normalized CBR values as a function of penetration depth

Xiao et al. (2022) tested 1 meter by 0.4 meter by 0.32 meter specimen intended to represent a roadway base. CBR results of these specimens revealed that soil strength increased to a certain depth then began to decrease due to clogging in the upper layers of the specimen (Figure 5). This depth varied depending on material and cementation solution. CBR values of high treatment specimens reported CBR values ranging from 60 to 80% which could have a high enough bearing capacity to serve as a road base.

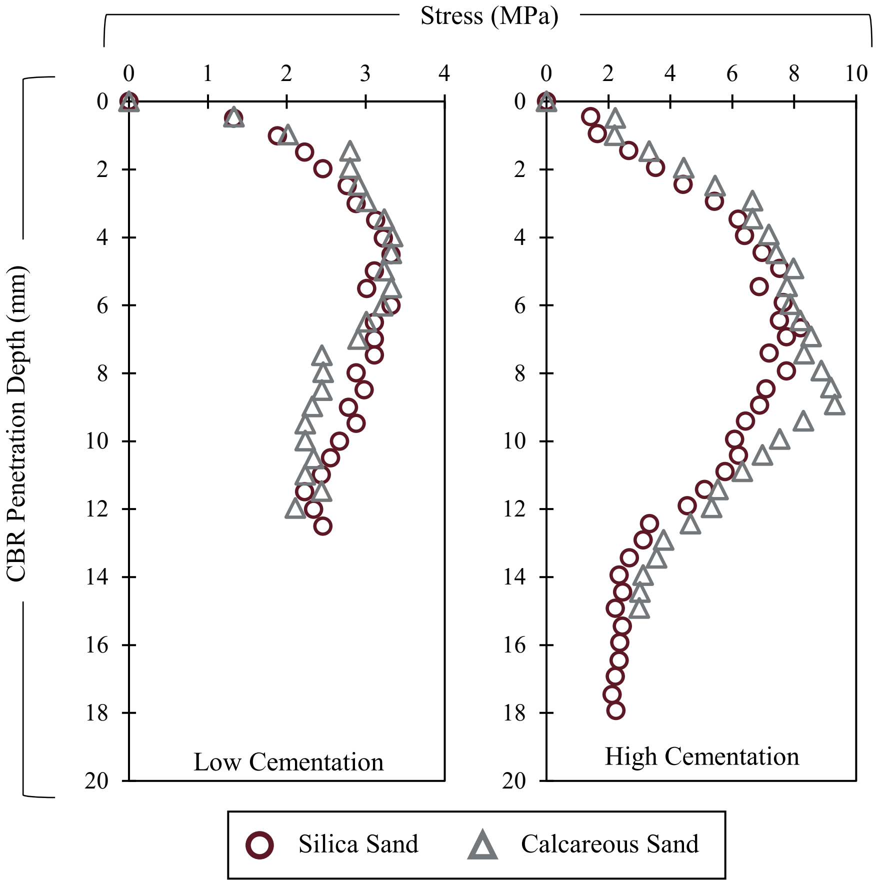


Figure 5. Comparison of CBR values as a function of depth for silica and calcareous sands at low and high cementation application (adapted from Xiao et al., 2022)

3.2.3 Unconfined Compressive Strength

Arpajirakul et al. (2021) reported that treating sand with MICP can improve unconfined compressive strength (UCS) values by as much as 320 kPa. UCS as a function of calcite ($CaCO_3$) precipitation has been reported in several references (e.g., Yu et al., 2019). Over 500 data points were collected from 21 references evaluated herein and are summarized in Figure 6. Data was collected using several cementation solutions/concentrations, bacterium types, and testing ages; however, this provides an opportunity to holistically evaluate UCS trends of laboratory produced MICP soils. The average UCS of all MICP stabilized sands summarized from literature was determined to be 1,120 kPa with a standard deviation of 1,507 kPa. Additionally, it was determined that, on average, a 1% increase in $CaCO_3$ yielded a UCS increase of 190 kPa.

Trendlines were utilized in Figure 6 to evaluate the ratio of UCS:$CaCO_3$. Additionally, the percentage of data within each envelope of UCS:CaCO3 ratio is provided as a bracketed number. 73% of the Figure 6 data had a UCS:$CaCO_3$ ratio less than 200:1. When isolating data with UCS less than 3,000 kPa and calcite percentages less than 15%, the majority of the data can be seen in a higher resolution.

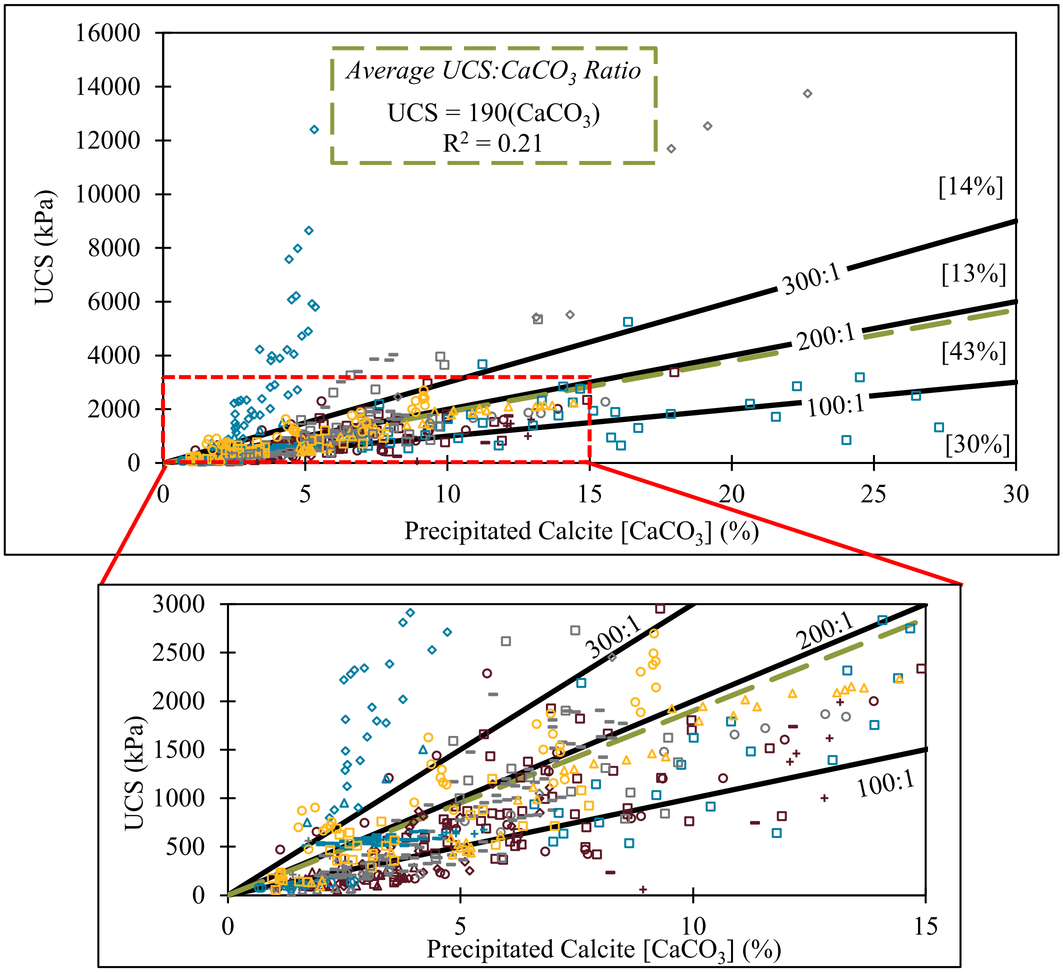


Figure 6. Unconfined compressive strength (UCS) as a function of precipitated calcite ($CaCO_3$) from laboratory specimens reported in literature

The size of UCS specimens can meaningfully influence the reported UCS value. The majority of the UCS specimens reported in Figure 6 utilized specimens with a diameter of 50 mm and a height of 100 mm; however, there were other sizes utilized but in most cases the length to diameter ratio (L:D) was 2:1 as shown in Table 5. Analysis of variance (ANOVA) tests were conducted at a 0.05 significance level to determine if any size or UCS:$CaCO_3$ ratio was significantly significant. On average, 50 mm by 100 mm and 25 mm by 50 mm were significantly stronger than most other specimen sizes used in literature (p-value < 0.01). To remove the influence of precipitated calcite, the UCS:$CaCO_3$ ratio was evaluated. On average, 50 mm by 100 mm specimens had the statistically highest UCS:$CaCO_3$ ratio (p-value < 0.01).

The influence of bacteria type on UCS was evaluated in Figure 7 and it was seen that *Sporosarcina pasteurii* produced the highest average ratio of UCS to precipitated calcite (197:1) followed by *Bacillus subtilis* (173:1), *Bacillus sphaericus* (164:1), and *Bacillus megaterium* (99:1). The relationships for *Bacillus megaterium* and *Bacillus subtilis* were developed with modestly sized data sets and are not robust like the relationships for *Sporosarcina pasteurii* and *Bacillus sphaericus*.

Table 5. Effect of specimen size on unconfined compressive strength (UCS)

Specimen Size	L:D Ratio [A]	Average UCS (kPa)	Average UCS:$CaCO_3$	References
25 mm by 50 mm	2:1	1,712	128	Song et al. (2014)
38 mm by 76 mm	2:1	553	191	Wani and Mir (2021)
39 mm by 80 mm	2.1:1	1,249	99	Cui et al. (2017)
45 mm by 90 mm	2:1	762	143	Cheng et al. (2017); Mujah et al. (2019)
50 mm by 100 mm	2:1	1,753	313	Cheng et al. (2014b); Choi et al. (2016); Gomez and DeJong (2017); Hoang et al. (2020); Lee et al. (2013); Mahawish et al. (2018); Naeem et al. (2022); Soon et al. (2014); van Paassen (2009); Zhao et al. (2014a)
55 mm by 110 mm	2:1	876	163	Cheng et al. (2013)
100 mm by 250 mm	2.5:1	916	135	Al Qabany and Soga (2013)
30 mm by 30 mm by 60 mm	2:1 [B]	489	156	Xiao et al. (2022)
No Specimen Size Information	N/A	1,197	256	Stabnikov et al. (2013); Wani (2021); Yang et al. (2020)

[A] Length to diameter ratio

[B] Specimen was a prism and the ratio of length to width and length to thickness is 2:1.

UCS has also been evaluated as a function of dry density as well as ratio of cementation solution to sample volume ratio (R_c). Fang et al. (2020) reported that, on average, as dry density increased by 1 g/cm^3, UCS increased by 6700 kPa. Liu et al. (2019) reported an exponential relationship between UCS and R_c.

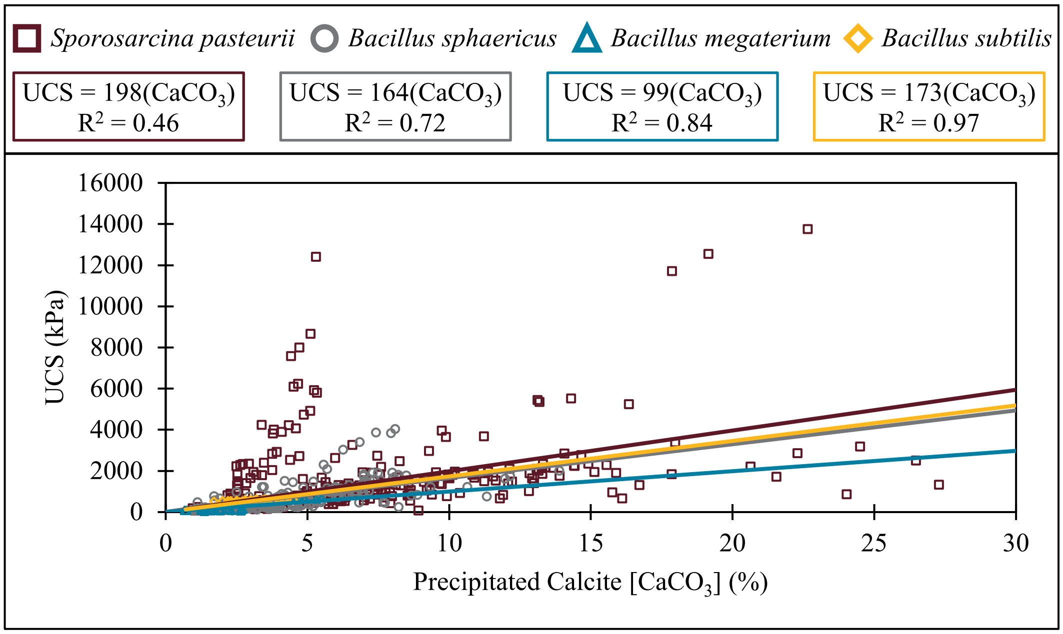


Figure 7. Unconfined compressive strength versus precipitated calcite for different strands of bacteria

3.2.4 Triaxial Strength Parameters

Triaxial compression tests measure soil shear strength parameters including cohesion (c) which is the force that holds together molecules within soil and friction angle (φ) which describes the friction shear resistance of soils together with mean effective stress. Triaxial tests at multiple confining pressures are used to create a Mohr-Coulomb failure criterion diagram where c and φ can be calculated (Figure 8). Failure modes experienced by soils in triaxial compression tests changed from ductile (bulging failure mode) to brittle (shear bands) due to increases in precipitated calcite (Do et al., 2019).

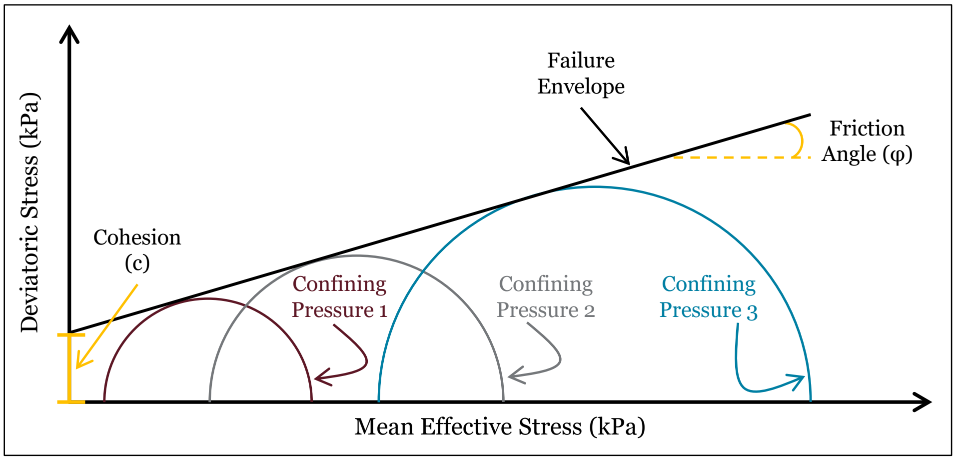


Figure 8. Example Mohr-Coulomb failure diagram generated using data from triaxial test to determine cohesion (c) and friction angle (φ) of soil

The effects of precipitated calcite percentage on cohesion and friction angle in MICP treated sands are described in several references and were combined to create Figure 9. Cohesion as a function of precipitated calcite formed clear trends where, as calcite increased, cohesion also increased (Liu et al., 2019). As precipitated calcite increases due to MICP treatment, precipitated crystals fill available pore space, increasing the cohesion of soil particles. A relatively strong quadratic trend was seen in the data from literature. When data was compared to another quadratic trend reported in Yu et al. (2021), the trend did not reasonably predict cohesion. Friction angle was not largely affected by precipitated calcite percentage. When looking at individual references, friction angle does appear to slightly increase as precipitated calcite increases; however, there is no strong global trend.

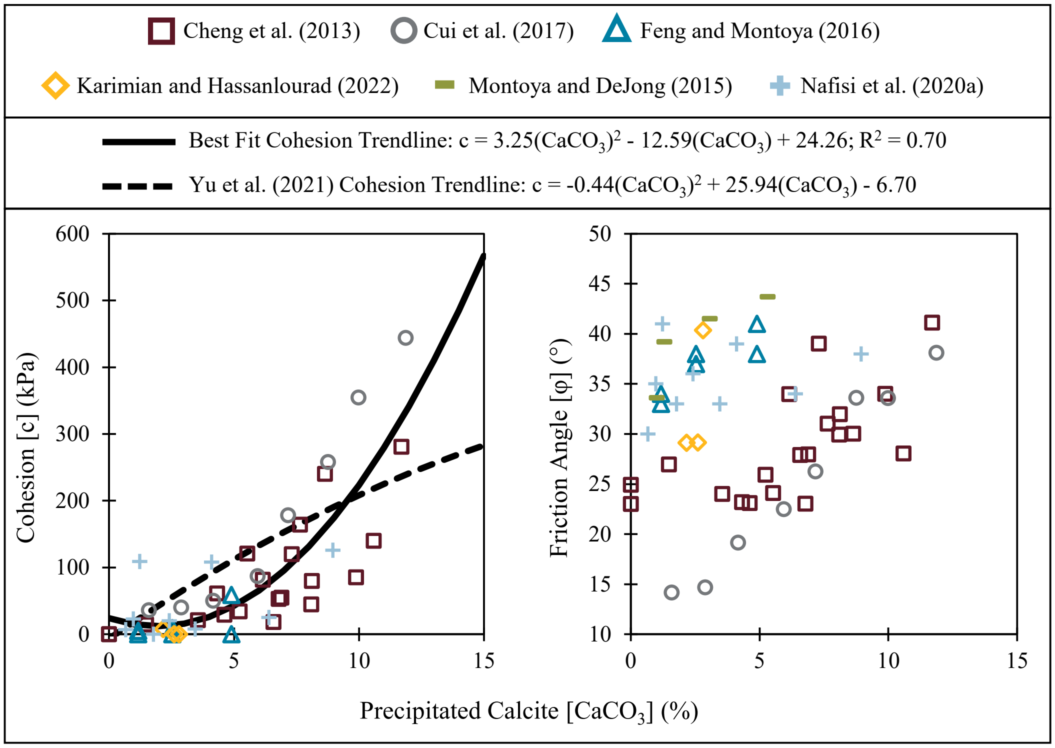


Figure 9. Cohesion (c) and friction angle (φ) as a function of precipitated calcite ($CaCO_3$) from laboratory specimens reported in literature

One study did evaluate the effects of including a polypropylene multifilament fiber to sand prior to MICP treatments on triaxial properties (Li et al., 2016). Generally speaking, a fiber percentage of 0.2 to 0.3% yielded optimum shear strength, friction angle, and cohesion. The inclusion of fibers increased friction between fibers and soil particles making it difficult for soil particles surrounding fibers to move – thus increasing shear strength, cohesion, and friction angle.

Additionally, dynamic triaxial tests were conducted to evaluate the number of cycles to liquefaction for MIPC treated soils. Karimian and Hassanlourad (2022) reported that MICP treated soils reported 81 cycles to induce liquefaction compared to 15 cycles for untreated soils. In other words, MICP treated soils were able to withstand approximately 5 times as many cycles to liquefaction as untreated soils. Xiao et al. (2019) evaluated the dynamic triaxial properties of soils at different levels of MICP treatment and found that increasing biocementation produced had a similar effect on cycles of liquefaction as increasing relative density.

3.2.5 Direct Shear Strength

Direct shear strength (DSS), also known as a box shear test strength, is used to determine shear strength of a soil. This test can be used as an alternative way to determine shear strength parameters (i.e., cohesion and friction angle); however, most researchers use triaxial tests to determine these parameters. Figure 10 summarizes available literature to show holistic trends between shear stress (τ) and precipitated calcite ($CaCO_3$). Although there were no holistic trends seen with the available data, in individual cases, as calcite percentage increased, shear strength also increased. Canakci et al. (2015) noted that MICP treated specimens behaved similarly to dense granular soils compared to untreated specimens which were loose sands. Pakbaz et al. (2018) found that as the duration of MICP treatments increased shear strength of the soil increased by 44 to 86%.

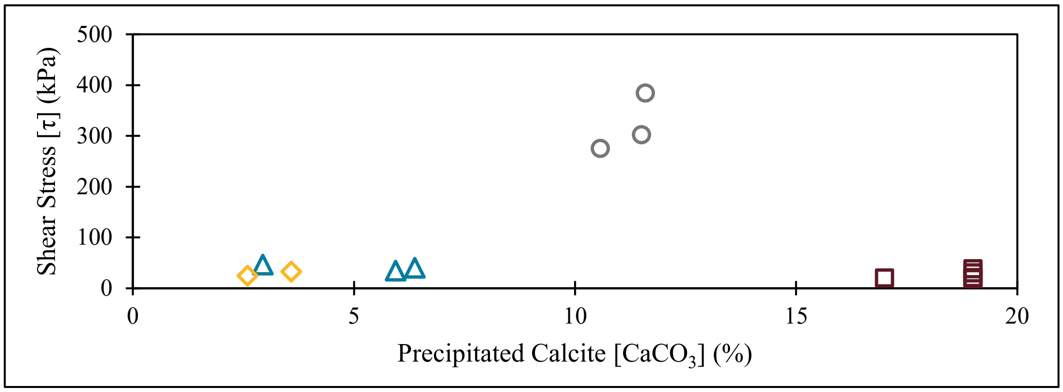


Figure 10. Shear strength (τ) as a function of precipitated calcite ($CaCO_3$) from laboratory specimens reported in literature

Riveros and Sadrekarimi et al. (2020) performed cyclic direct shear tests on MICP treated soils and found that shear modulus increased from 46 to 123 MPa. MICP treatment also improved sand’s resistance to cyclic liquefaction indicated that failure changed from liquefaction failure for untreated sand to cyclic mobility in MICP treated sands.

3.2.6 Indirect Tensile Strength

Indirect tensile strength (also called splitting tensile strength) can be used to estimate tensile strength of materials where it is difficult to test in direct tension. Liu et al. (2019) reported an exponential relationship between indirect tensile strength and the number of treatment applications. When plotting available data from literature to holistically evaluate the trend between indirect tensile strength (IDT) and precipitated calcite ($CaCO_3$) was seen to be polynomial (Figure 11). When regression through the origin (RTO) was used with a linear trendline it was found that for a 1% increase in precipitated calcite, IDT, on average, increased by 42.8 kPa (R^2 of 0.80).

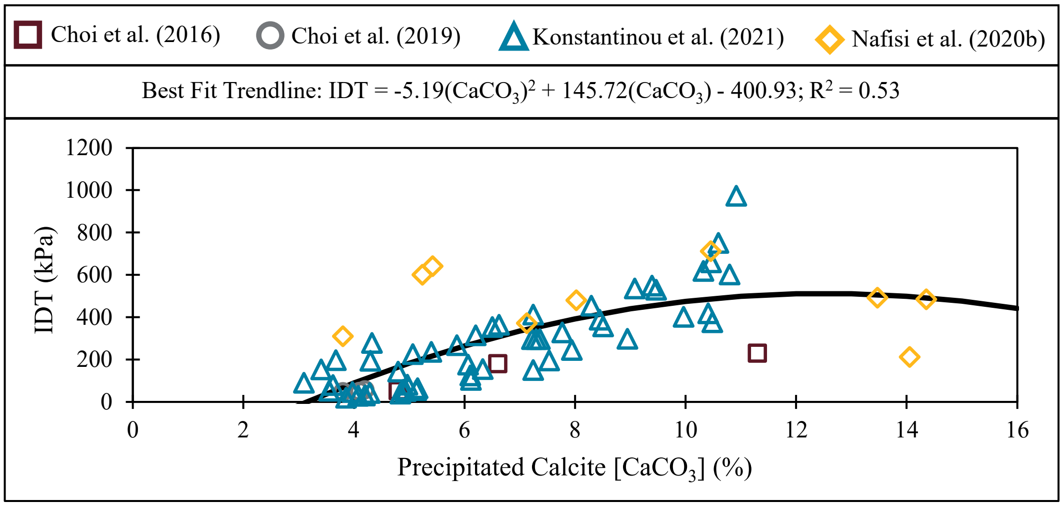


Figure 11. Indirect tensile strength (IDT) versus precipitated calcite (CaCO$_3$) reported in laboratory specimens from literature

Another relationship of interest is the ratio of indirect tensile strength to unconfined compressive strength (IDT:UCS). Using linear regression through the origin, it was found that as UCS increases by 1 kPa, IDT increases by 0.17 kPa (Figure 12). This ratio is in the range of IDT:UCS ratios reported for traditionally stabilized soils IDT:UCS ratios for soil-cement over a range of ages are typically 0.15:1 (Carey et al., 2022) while, for full depth reclamation, IDT:UCS ratios have been reported to be as high as 0.22:1 (Carey et al., 2023).

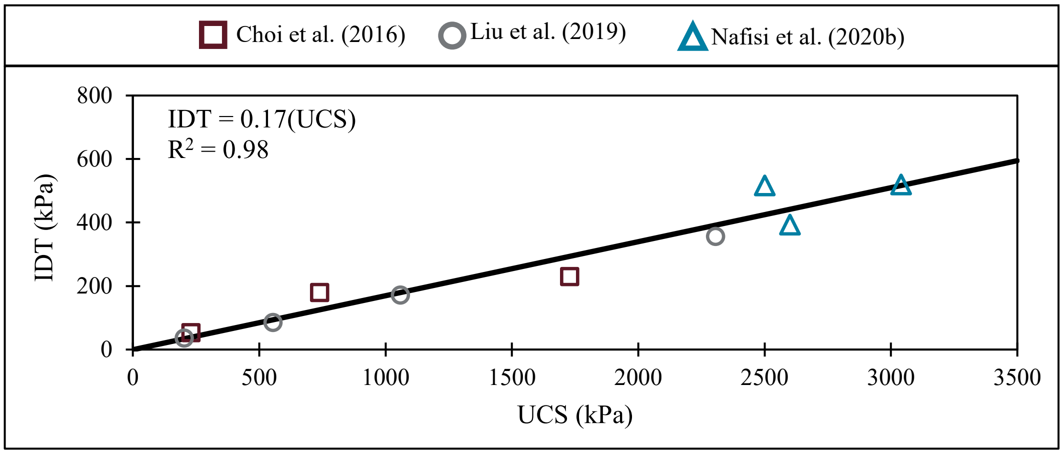


Figure 12. Relationship between indirect tensile strength and unconfined compressive strength ratio (IDT:UCS ratio)

The inclusion of fibers in MICP treated soils has also been shown to improve IDT strengths. Using data reported in Choi et al. (2016) and (2019), linear regression through the origin showed an average increase in IDT of 31 kPa for each 0.1% increase in fiber content. Fang et al. (2020) evaluated different fiber lengths and found an optimal fiber length of 9 to 12 mm as shorter fibers the number of fibers per unit volume weakened the ability to absorb tensile stress while long fibers were easily twisted together leading to decreased tensile strength.

3.2.7 Elastic Modulus

Elastic modulus (E) measurement of non-permanent deformation and can be calculated as the slope of the initial linear portion of a stress-strain curve. Arpajirakul et al. (2021) evaluated E of different MICP treated soils and found that reported elastic modulus varied by as much as 470 MPa depending on the soil type. However, for all soils, MICP treatment increased elastic modulus when compared to non-treated soils. When plotting data from literature, a clear polynomial trend was found between precipitated calcite and elastic modulus (Figure 13). When regression through the origin was utilized, it was found that, on average, a 1% increase in precipitated calcite increased elastic modulus by 12 MPa.

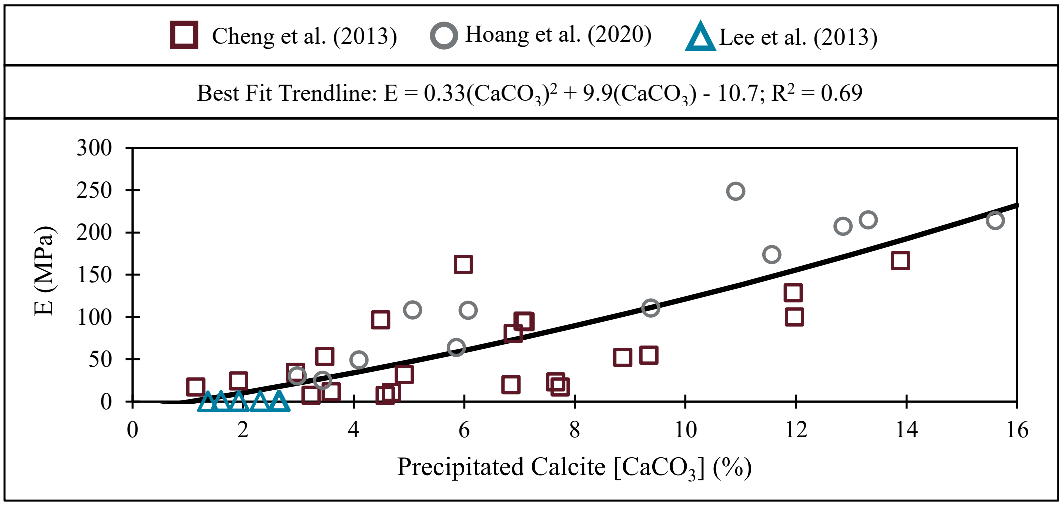


Figure 13. Elastic modulus (E) versus precipitated calcite ($CaCO_3$) reported in laboratory specimens from literature

One relationship of interest from a design standpoint is the ratio of elastic modulus to unconfined compressive strength (E:UCS). This ratio is a design parameter used in the mechanistic-empirical pavement design guide (MEPDG) for using chemically stabilized base materials when building pavement base layers. As seen in Figure 14, for MICP treated soils from literature had an E:UCS ratio of 85:1 (i.e., an increase of 1 unit of UCS resulted in an average increase in E of 85 units). This number is meaningfully lower than E:UCS ratios of traditionally stabilized soils; however, this low E:UCS ratio for MICP stabilized soils have been due to the soil gradation. For example, the MEPDG specifies an E:UCS ratio of 1200:1 for cement stabilized base material. The lowest E:UCS ratio recorded by MSU personnel was 500:1 on 4.5 year old cores from a full depth reclamation project with very high fines content (10% on average but ranging to 30%) from US Highway 49 (Howard and Cox, 2016). Additional information regarding E:UCS ratios of traditionally stabilized base materials can be found in Carey et al. (2023) and Carey et al. (2022).

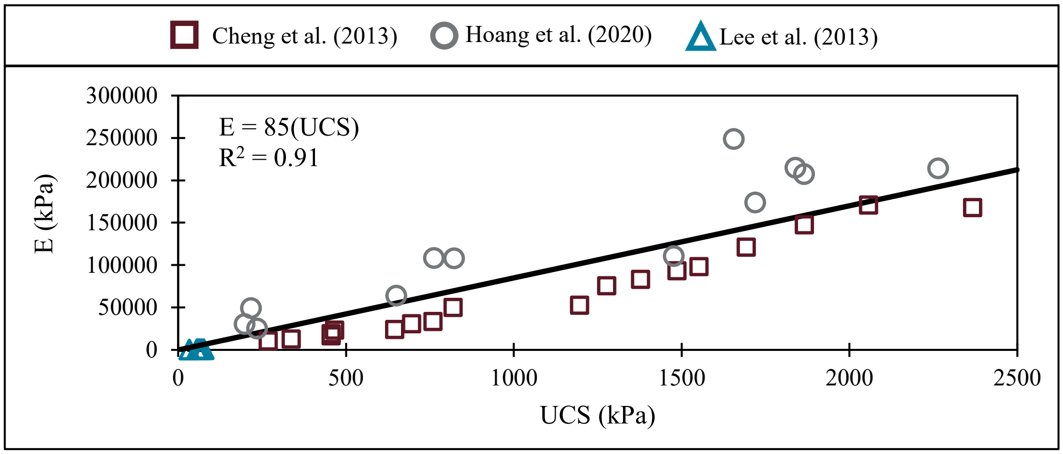


Figure 14. Relationship between elastic modulus and unconfined compressive strength ratio (E:UCS ratio)

Li et al. (2016) studied the influence of fiber content on elastic modulus in MICP treated soils. The inclusion of fibers increased elastic modulus to approximately 450,000 kPa at a fiber content of 0.20%. Increases in fiber content above 0.20% led to decreases in recorded elastic modulus.

3.2.8 Stress-Strain Relationships

Stress-strain relationships of MICP treated soils have been evaluated from many perspectives including influence of MICP treatment, fibers, confining pressures, treatment durations, flow pressures, and sand gradations. Table 6 summarizes the influence of these parameters on stress-strain relationships reported in literature.

Table 6. Influence of testing parameters on stress-strain relationships

Parameter	General Trend	References
MICP Treatment	MICP increased the total amount of axial stress that specimens could withstand.	Arpajirakul et al. (2021) Feng and Montoya (2016) Montoya and DeJong (2015)
Cementation Media Concentration	Additional MIPC treatments & cementation media concentration increased stress material could withstand but did not appear to influence strain.	Hoang et al. (2020) Lee et al. (2013) Shan et al. (2022)
Fiber Content	Increased fiber content increased overall stress and stain of MICP stabilized specimens. Optimal fiber content varied from study to study.	Choi et al. (2016) Choi et al. (2019) Fang et al. (2020)
Flow Pressure	A flow pressure of 0.2 bar produced an optimal stress value and increases in flow pressure decreased the overall stress.	Lee et al. (2013)
Soil Type	Soils with higher percentages of fines had lower stress values. Overall, soil characteristics meaningfully impacted stress-strain profiles.	Arpajirakul et al. (2021) Karimian & Hassanlourad (2022) Xiao et al. (2021b)

3.2.9 Shear Wave Velocity

Shear wave velocity (V_s) is a non-destructive measurement widely used to estimate the in-situ elastic properties of soils (Yu et al., 2021). In MICP treated soils, Vs is mostly influenced by particle-particle stiffness that depends on cementation level as well as soil density, confining pressure, and degree of saturation. Generally speaking, lightly cemented soils have a shear wave velocity of 400 m/s, moderately cemented soils have a V_s of 700 m/s and heavily cemented soils have a V_s of 1200 m/s (Do et al., 2019).

Shear wave velocity of MICP stabilized soils is different than a pulse velocity test used commonly with concrete (i.e., ASTM C597, 2016). Shear wave velocity of soils is measured using piezo-ceramic bender elements as described in ASTM D8295 (2019). A shear wave is generated at one boundary of a soil specimens and is received at the opposite boundary. Shear wave travel time is measured over a known distance (i.e., distance between the two bender elements) and then shear velocity is calculated. All references in this review where shear wave velocity measurements are reported used bender elements as described by ASTM D8295.

The influence of precipitated calcite on shear wave velocity as reported in literature was evaluated in Figure 15. On average, a 1% increase in $CaCO_3$ resulted in an increase in shear wave velocity of 147 m/s. When defining a general upper and lower bound it was found that the majority of shear wave velocity data fell within 50($CaCO_3$) and 800($CaCO_3$).

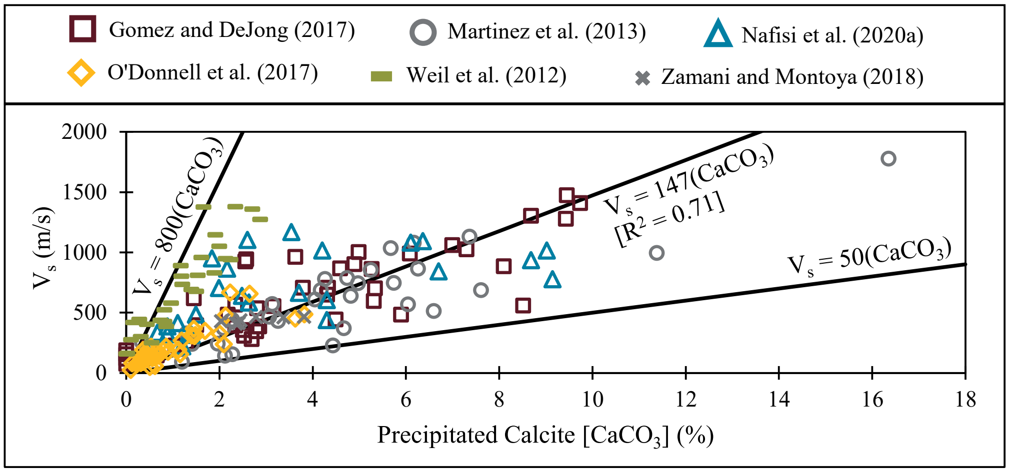


Figure 15. Shear wave velocity (V_s) versus precipitated calcite ($CaCO_3$) reported in laboratory specimens from literature

Shear wave velocity changes over time at early ages while calcite precipitation is ongoing. Changes in shear wave velocity at early ages can be an indication for the amount of precipitation as MICP stabilization has been shown to increase V_s (Karimian and Hassanlourad, 2022; Nafisi et al., 2021). Figure 16 shows shear wave velocity versus time (t) reported in literature for MICP stabilized soils. On average, shear wave velocity increased by 7.9 m/s for each hour of time that passed from the introduction of cementation solution; however, V_s was seen to increase as much as 45 m/s per hour and as little as 2.5 m/s per hour depending on the testing parameters. In most tests, as V_s began to plateau, another round of cementation solution was added to soils to continue to increase V_s and the total amount of precipitated calcite.

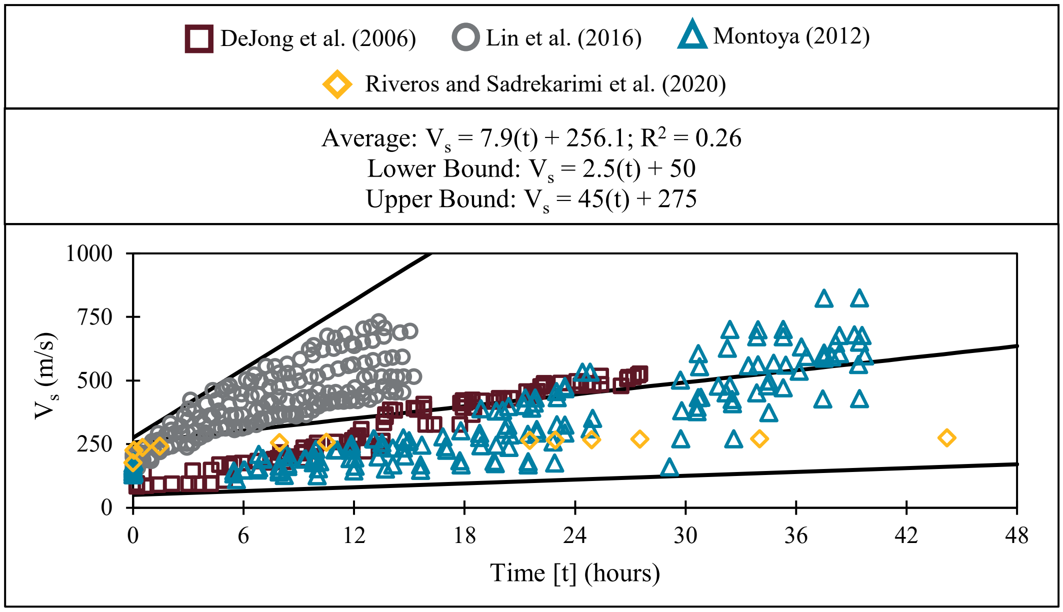


Figure 16. Shear wave velocity (V_s) as a function of time (t) as reported in literature

3.2.10 Nanoindentation

Nanoindentation testing allows for fundamental mechanical properties of materials on the nanoscale. Typically, nanoindentation is used to estimate elastic modulus (E), hardness (H), and fracture toughness (K_{IC}) of materials. In terms of elastic modulus, Dhami et al. (2016) reported values between 35 and 65 GPa, Huang et al. (2021) reported values between 5 and 55 GPa, and Lee et al. (2016) reported an average value of approximately 60 GPa. This is a wide range of reported modulus values which highlights the inherent variability of finding nano-scale properties on a material such as sand and calcite. Hardness values reported in Huang et al. (2021) ranged from 0 to 4 GPa while in Dhami et al. (2016) they ranged from 2 to 4 GPa. Lee et al. (2016) reported an average hardness of 5.2 GPa which was noticeably higher than other sources. Fracture toughness values were also variable as Huang et al. (2021) reported values ranging from 0.1 to 1.3 MPa * $m^{1/2}$.

Additionally, Huang et al. (2021) developed equations using least-square regression analysis to define fracture toughness (K_{IC}) as a function of elastic modulus (E) and hardness (H). Both relationships have strong correlations with R^2 values greater than 0.90. Equations 3 and 4 show these relationships.

$$K_{IC} = 0.3(E) + 0.2 \qquad \textbf{(3)}$$

$$K_{IC} = 0.05(H) - 0.25 \qquad \textbf{(4)}$$

3.2.11 Permeability

Permeability (P) is a geotechnical parameter that quantifies how easily fluids flow through soils (Yu et al., 2021). Chu et al. (2013) showed that permeability values decreased by a factor of 10^3 due to MICP stabilization. Additionally, several studies have quantified the influence of precipitated calcite on permeability of soils (Al Qabany and Soga, 2013; Cheng et al., 2013; Cheng et al., 2014b; Cheng et al., 2017; Hoang et al., 2020; Shan et al., 2022; Song et al., 2019; Soon et al., 2014; Zamani and Montoya, 2017). Figure 17 utilizes data from these studies to holistically evaluate the relationship between calcite content and permeability for several different soils, bacteria, and cementation solutions. Normalized permeability (P_{norm}) was plotted on the y-axis where the permeability after each treatment of cementation solution was normalized to its initial permeability prior to treatment (i.e., permeability of the untreated soil). Untreated permeability for soils in Figure 17 were typically 10^{-4} to 10^{-5} m/s however, untreated permeability ranged from $1.0 * 10^{-3}$ to $5.4 * 10^{-8}$ m/s.

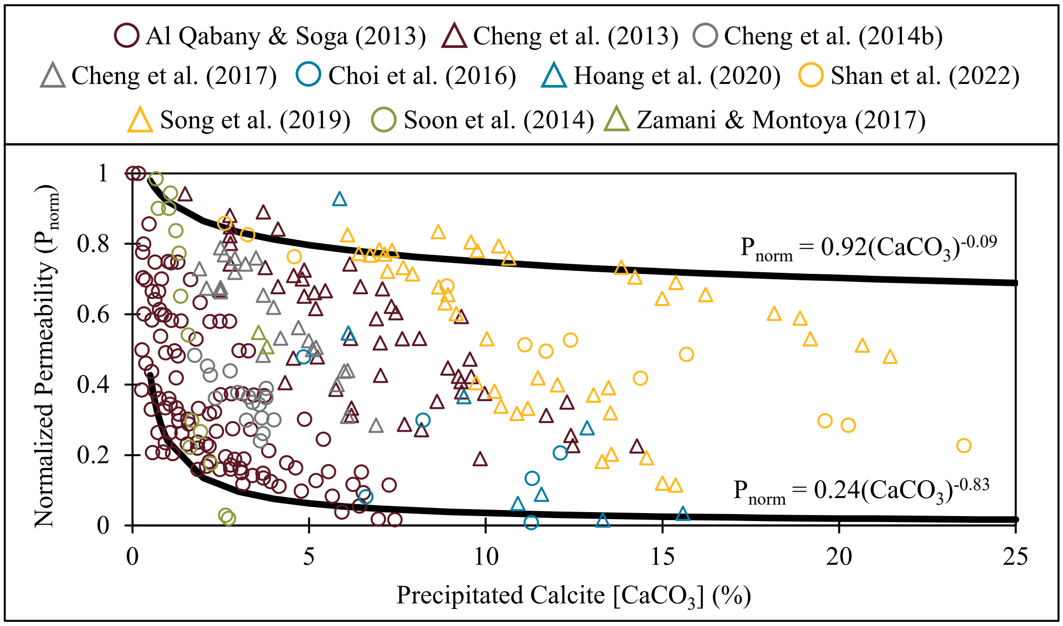


Figure 17. Normalized permeability (P_{norm}) as a function of precipitated calcite ($CaCO_3$) available in literature

Although there was a fair amount of scatter in Figure 17 when holistically viewing all data reported in literature, the general trend within each data source is that as calcite content increases, permeability decreases. This scatter can partly be attributed to the differences in soil, cementation solution and concentration, bacteria type and concentration, etc. Boundary trendlines were added to the figure to show the extreme ratios of normalized permeability to percent of precipitated calcite. Power functions have been used in literature to quantify the influence the effects of precipitated calcite on soil permeability (Gao et al., 2019; Mahawish et al., 2018). Approximately 86% of data fell within the boundaries in Figure 17 with 8% falling above the upper bound and 6% falling below the lower bound.

3.2.12 Thermal Conductivity

Thermal conductivity (k) is defined as the rate at which heat passes through a material and is expressed in units of watts per meter times kelvin (W/m*K). Several studies have evaluated the effects of MICP on thermal conductivity of sand. Sand that has been stabilized using MICP methods having higher k values than non-MICP stabilized sand, and as number of cementation solution treatments increase thermal conductivity increases (Cheng et al., 2021; Martinez et al., 2019; Venuleo et al., 2016; Wang et al., 2019; Wang et al., 2020; Xiao et al., 2021a). This can be attributed to the calcite crystals serving as "thermal bridges" and offering larger surface area for heat exchange (Figures 18 and 19). In dry sands, calcite filled the pore space typically occupied by air which has a low thermal conductivity (k = 0.025 W/m*K) compared to calcite (k ≈ 5.0 W/m*K) meaning calcite is capable of transferring heat roughly 200 times more efficiently than air.

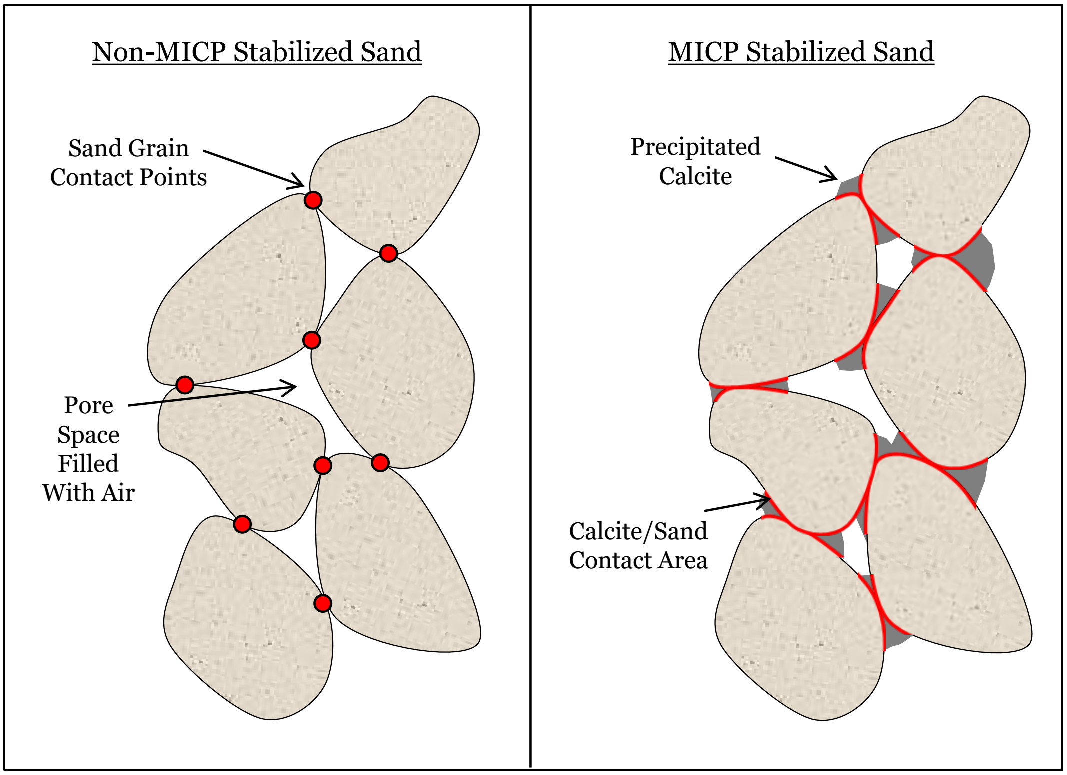


Figure 18. Visual of contact points in non-MICP stabilized sand compared to calcite "thermal bridges" of MICP stabilized sand

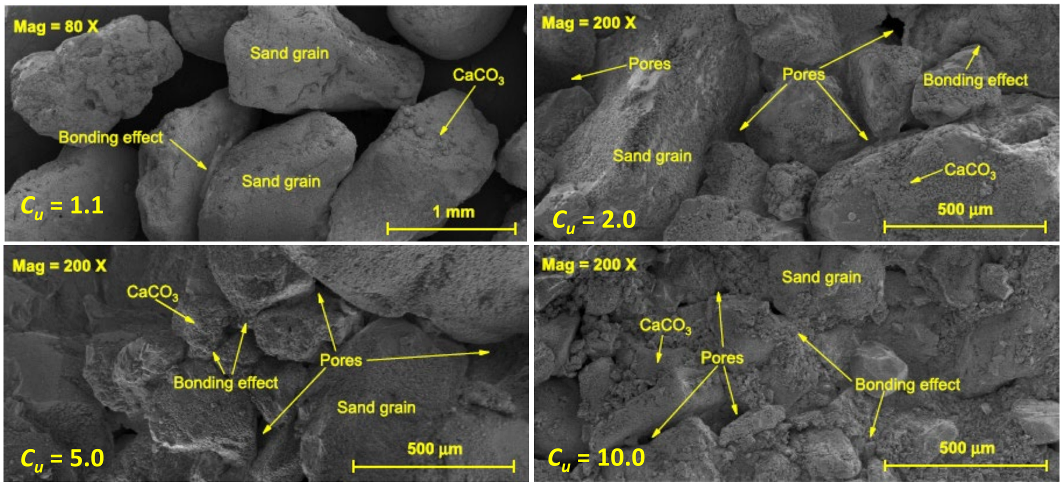


Figure19. SEM images from Xiao et al. (2021b) showing MICP precipitation for sands of varying gradations

When sand becomes saturated, the thermal conductivity of soil continues to increase as water is filling pore spaces (k = 0.59 W/m*K) rather than air (k = 0.025 W/m*K). Although thermal conductivity is higher at higher soil saturations, the difference between MICP stabilized and non-stabilized soil specimens was less (Venuleo et al., 2016; Martinez et al., 2019; Wang et al., 2019; Xiao et al., 2021a). In other words, at higher saturation values, the water into pore space is more influential on thermal conductivity than at lower soil saturation values where calcite is the main avenue of heat transfer as there is still air in some pore spaces. Venuleo et al. (2016) noted that for unsaturated soils thermal conductivity of MICP stabilized sand were 250% greater than non-stabilized soils; however, for saturated soils MICP stabilized soils were only 25% higher. Martinez et al. (2019) reported similar percentages with MICP stabilized sand being 328% higher in unsaturated soils and 13% higher in saturated soils.

A relationship was proposed by Côté and Konrad (2005) to predict thermal conductivity of different soils as a function of soil saturation (Equation 5).

$$\mathrm{k(S)} = \left(\mathrm{k}_{\mathrm{water}}^{\mathrm{n}}\mathrm{k}_{\mathrm{soil}}^{\mathrm{n}} - \chi 10^{-\eta \mathrm{n}}\right)\left[\frac{\kappa \mathrm{S}}{1 + (\kappa - 1)\mathrm{S}}\right] + \chi 10^{-\eta \mathrm{n}} \qquad \textbf{(5)}$$

Where:

k(S)	*thermal conductivity as a function of degree of saturation*
k_{water}	*thermal conductivity of water*
k_{soil}	*thermal conductivity of the soil*
χ	*material dependent parameter*
η	*material dependent parameter*
n	*porosity*
κ	*material dependent parameter*
S	*degree of saturation*

The following constant values were used in Equation 5: k_{water} was 0.59 w/m*K; k_{soil} was 4.26 W/m*K; χ was 0.75 W/m*K; η was 1.20; n was 0.35; and κ was 3.55.

This equation is referenced frequently in literature as a well-accepted relationship; however, this equation was not developed using MICP stabilized soils. This relationship was compared to data sets found in literature (i.e., Martinez et al., 2019; Venuleo et al., 2016; Wang et al., 2020) where sand at various levels of saturation were stabilized with MICP. As seen in Figure 20, Equation 5 predicted the lower bound of literature data. When Equation 5 was offset by 1.1, the equation loosely defined the upper boundary of reported k values. When comparing the predicted k values from Equation 5 to reported values in literature, predicted k values were on average 30% lower than reported k values (Figure 21). The relationship did reasonably predict data from Wang et al. (2020); however, it noticeably underpredicted thermal conductivity of data from Martinez et al. (2019) and Venuleo et al. (2016). More refinement is needed to optimize material dependent constants (e.g., χ, η, and κ) for MICP stabilized sands prior to use.

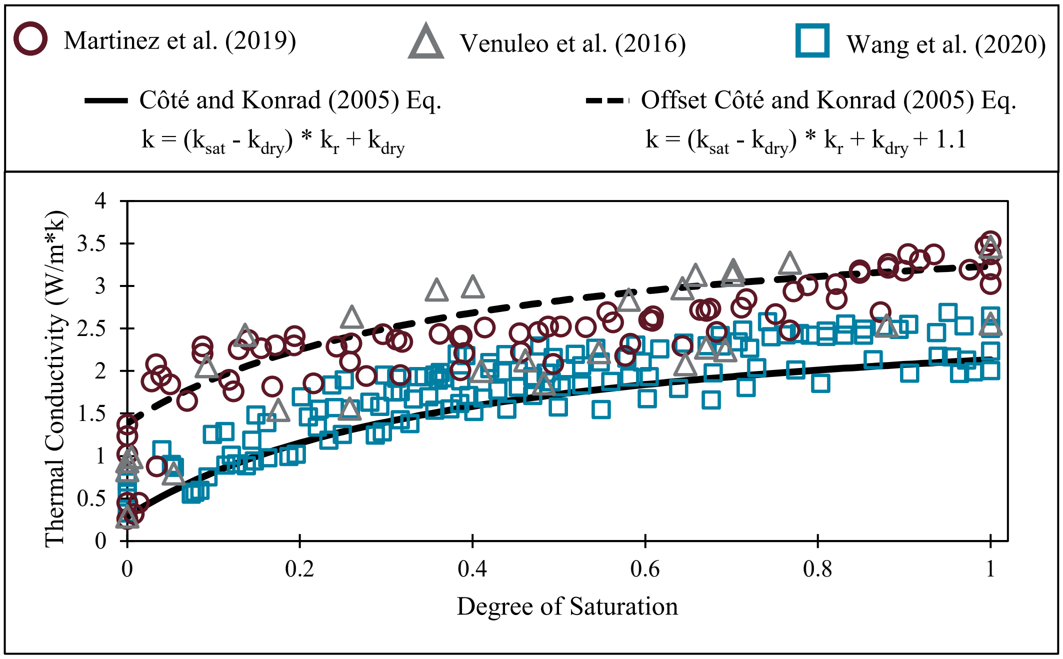


Figure 20 Thermal conductivity versus degree of saturation trends from literature

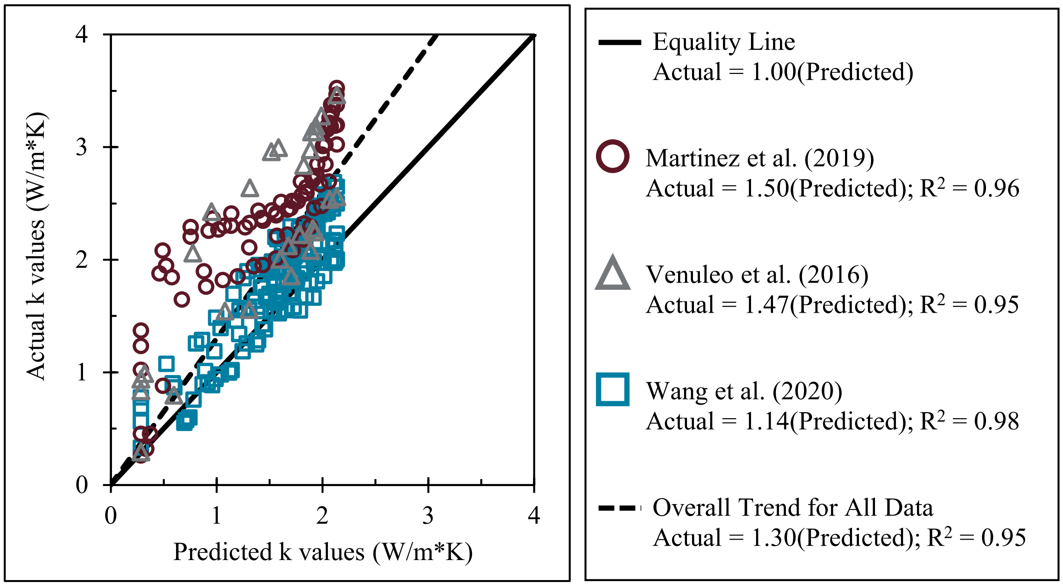


Figure 21. Comparison of Equation 3 predicted thermal conductivity to reported thermal conductivity of MICP stabilized soils data in literature

3.3 Uniformity of MICP Precipitation

Unlike traditional soil stabilization techniques where the cementitious material is commonly dry mixed with soil and assumed to be uniform, MICP cementation is typically induced in a laboratory using an injection method and uniformity must be evaluated (Feng and Montoya, 2016). Several references show relationships between precipitated calcite and depth from the injection point of stabilized specimens. The data from these plots was mined and percentage of precipitated calcite at each location in a specimen was normalized to the average value of precipitated calcite of the specimen (Figure 22). Normalized calcite percentages ranged between -1 and 1 where a negative value indicated that precipitated calcite at a location was less than the average precipitated calcite and a positive value indicated that precipitated calcite at a location was more than the average precipitated calcite.

Uniformity trends shown in Figure 22 generally fell into four categories: 1) high scatter, 2) low scatter, 3) increased calcite with depth, 4) decreased calcite with depth. Do et al. (2019) showed high amounts of scatter with no general trends while Karimian and Hassanlourad (2022) showed little scatter with no clear trends. Nafisi et al. (2018) and (2020a) reported calcite content generally increasing as the distance from the injection point increased. This trend is counterintuitive as sand is a naturally filtering material. However, Feng and Montoya (2016) and Song et al. (2019) reported that as distance from the injection site increased, the percentage of precipitated calcite decreased.

Li et al. (2016) evaluated MICP stabilized specimens who were fully submerged in cementation solution and reported differences in precipitated calcite percentages of 1.3% between the exterior and interior of the sand specimens. The top and bottom 18 mm of specimens had precipitated calcite contents of 8.2%. The middle of the specimen was in contact with the cementation solution reported a precipitate calcite percentage of 7.6% while the interior of the middle of the specimen had 6.9% precipitated calcite.

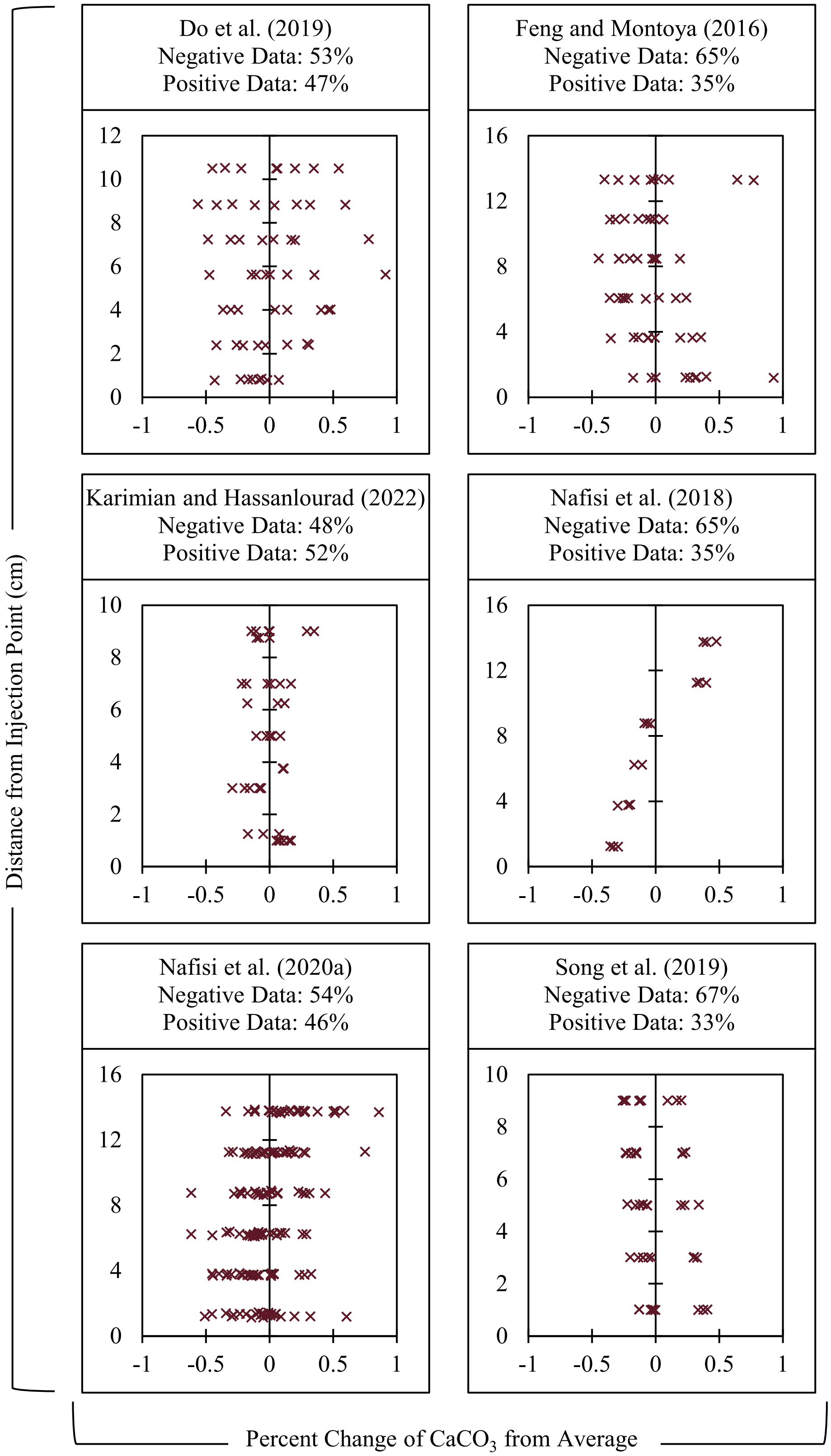


Figure 22. Evaluation of uniformity of precipitated calcite from literature

3.4 Failure Mechanisms for MICP Stabilized Materials

Calcite precipitates are deposited between soil particle voids and adjacent calcite precipitates form agglomerates due to volume expansion which cements soil particles together. Additionally, calcite crystals adhere to soil particle surfaces which increases surface roughness and creates an interlocking effect between soil particles (Tian et al., 2022a). DeJong et al. (2010) initially proposed that failure of MICP stabilized soils occur as fractures within the calcite or between the precipitated calcite and soil particle (Figure 23). However, scanning electron microscope and X-ray computed tomography images of fracture plans in MICP stabilized specimens showed that a layer of calcite remained on both surfaces of sand particles where a particle contact once was and small particles of calcite were noticeable especially in shear and UCS loading modes. In other words, it was not a clean break within the calcite bond or between the precipitated calcite and sand particles. These observations suggest that the calcite aggregate form a rather heterogeneous structure that is weak relative to the sand particles.

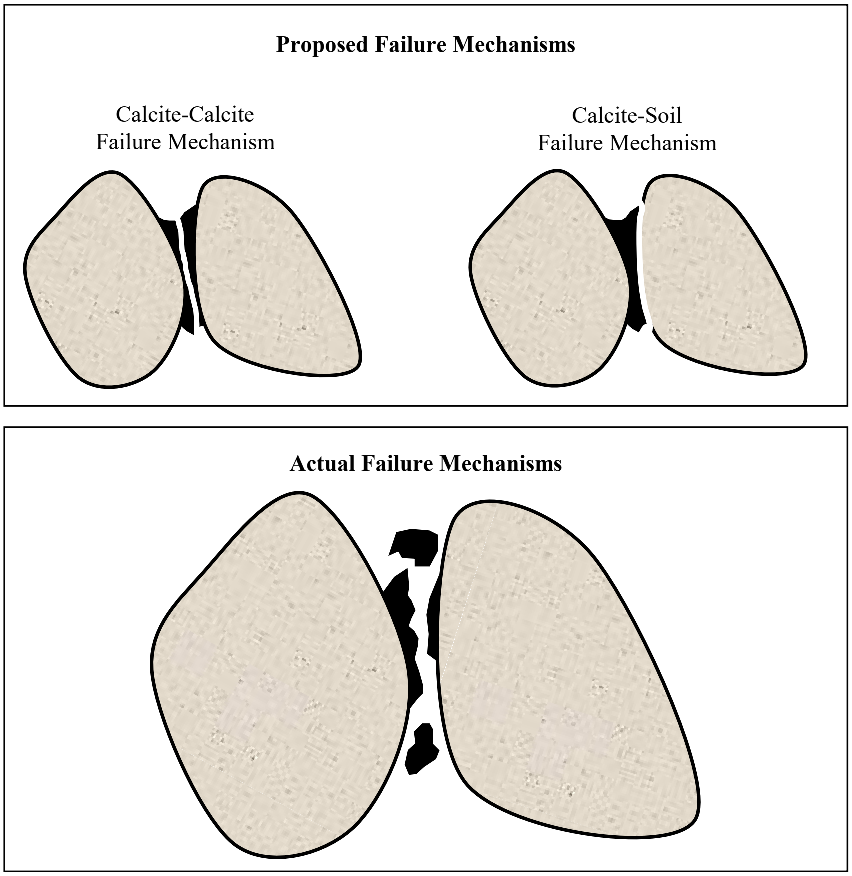


Figure 23. Proposed and actual failure mechanisms reported in DeJong et al. (2010)

Strength of a calcite bond was directly measured by Montoya and Feng (2015) by measuring the tensile and torsional strength of two pea gravel particles bonded by MICP. Two different sized pea gravel particles were used in this experiment (5 mm and 10 mm); however, there was not a meaningful difference in reported strength due to particle size. Tensile strength was reported as approximately 3.5 N and torsional strength was 0.35 N*m. The failed surface between the particles was evaluated after loading and illustrated that failure occurred within the calcite phase of the bond and not the calcite-silica boundary. This is expected as the bond between precipitated calcite and soil particles is a mechanical bond.

On a macroscale, failure mechanisms have been reported to vary depending on the level of cementation. Nafisi et al. (2020a) reported that untreated and lightly cemented specimens typically had a bulging type of failure while moderately and heavily cemented specimens produced a shear band during failure. Thickness of the shear band depended on the type of sand used as it was influenced by particle size. Feng and Montoya (2016) reported gradual strain-softening behavior in MICP stabilized specimens. Additionally, a numerical analysis of MICP stabilized specimens showed that under different stress paths, bond breakage rate was different, and the location of the broken bonds varied (Feng et al., 2014).

3.5 Large-Scale MICP Laboratory Tests

Most MICP laboratory scale efforts focus on using small scale specimens; however, some studies have evaluated larger, laboratory scale specimens in an attempt to understand the effects of upscaling MICP treatment. Literature reports strength and precipitated calcite data for 1 meter (Cheng and Cord-Ruwisch, 2012), 2 meter (Cheng and Cord-Ruwisch, 2014), and 5 meter sand columns (Whiffen et al., 2007). The 1 and 2 meter sand columns were stabilized using surface percolation while the 5 meter column was stabilized using a series of injections that lasted approximately 216 hours (9 days).

When evaluating the distribution of strength throughout each sand column it was clear that in some cases strength was consistent, but in some columns the variation in strength was significant (Figure 24). The average strength in each sand column and coefficient of variation (COV) was calculated to quantify the range of properties within each column. For example, the 1 meter column and 2 meter column stabilized with 22 treatments had low COV values indicating low strength variability throughout each column. Cheng and Cord-Ruwisch (2014) capture the potential for clogging in sand columns where fine sands are used (e.g., 10 treatments in the 2 meter column) as the permeability is low meaning cementation solution cannot fully permeate through the entirety of the sand column.

When looking at the distribution of precipitated calcite in each sand column, it was clear that for columns that utilized fine sand (i.e., all specimens except for Cheng and Cord-Ruwisch (2014) with 22 treatments) the calcite distribution was noticeably variable (Figure 25). For a coarse sand, the COV in precipitated calcite was 12%; however, for all other sand columns where fine sands were used, calcite COVs varied from 39% to 76%. This again indicates that the fineness of the sand has a meaningful impact on the distribution of precipitated calcite as well as in-place properties.

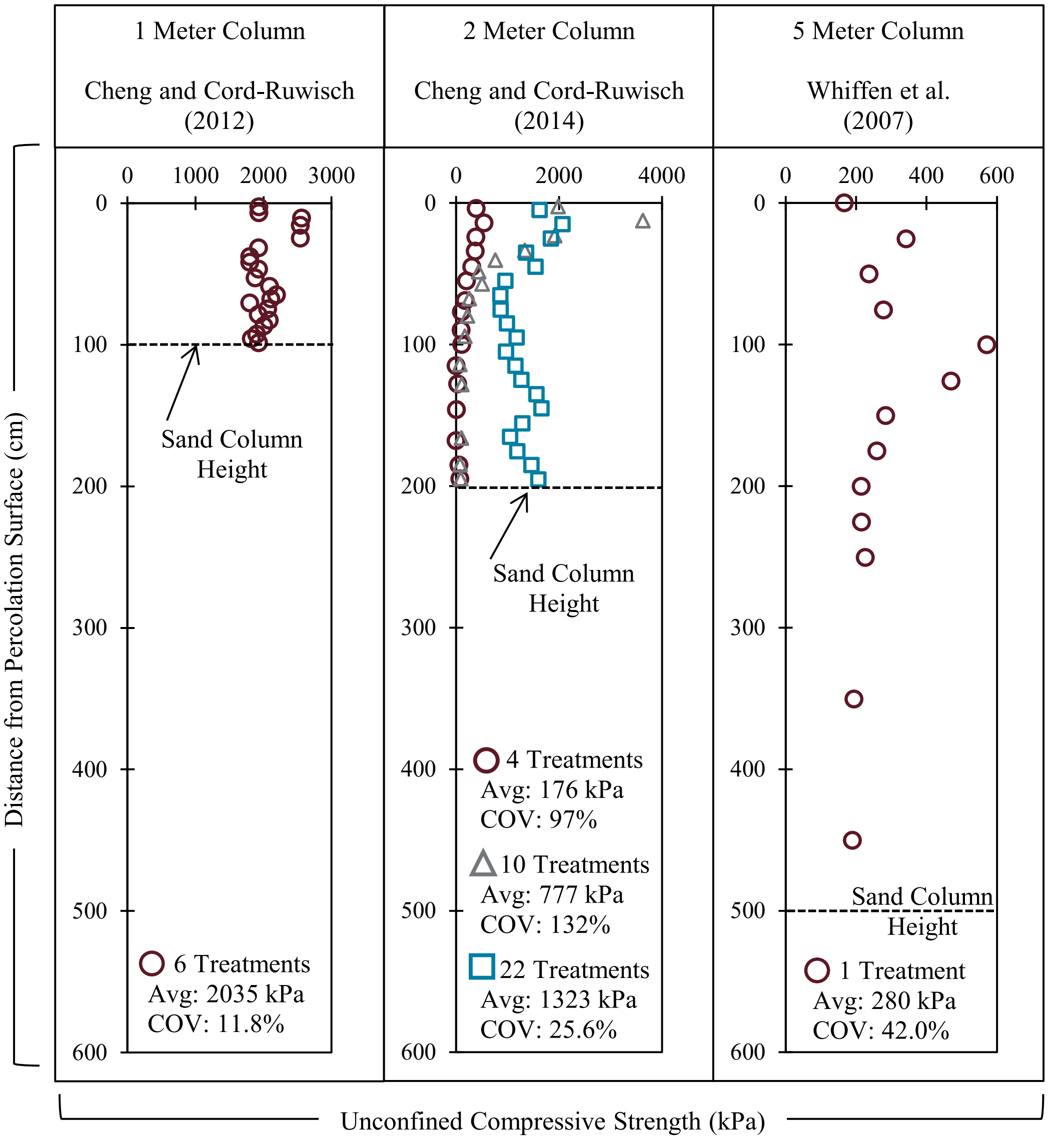


Figure 24. Variations in compressive strength over the length of large-scale laboratory specimens stabilized with MICP

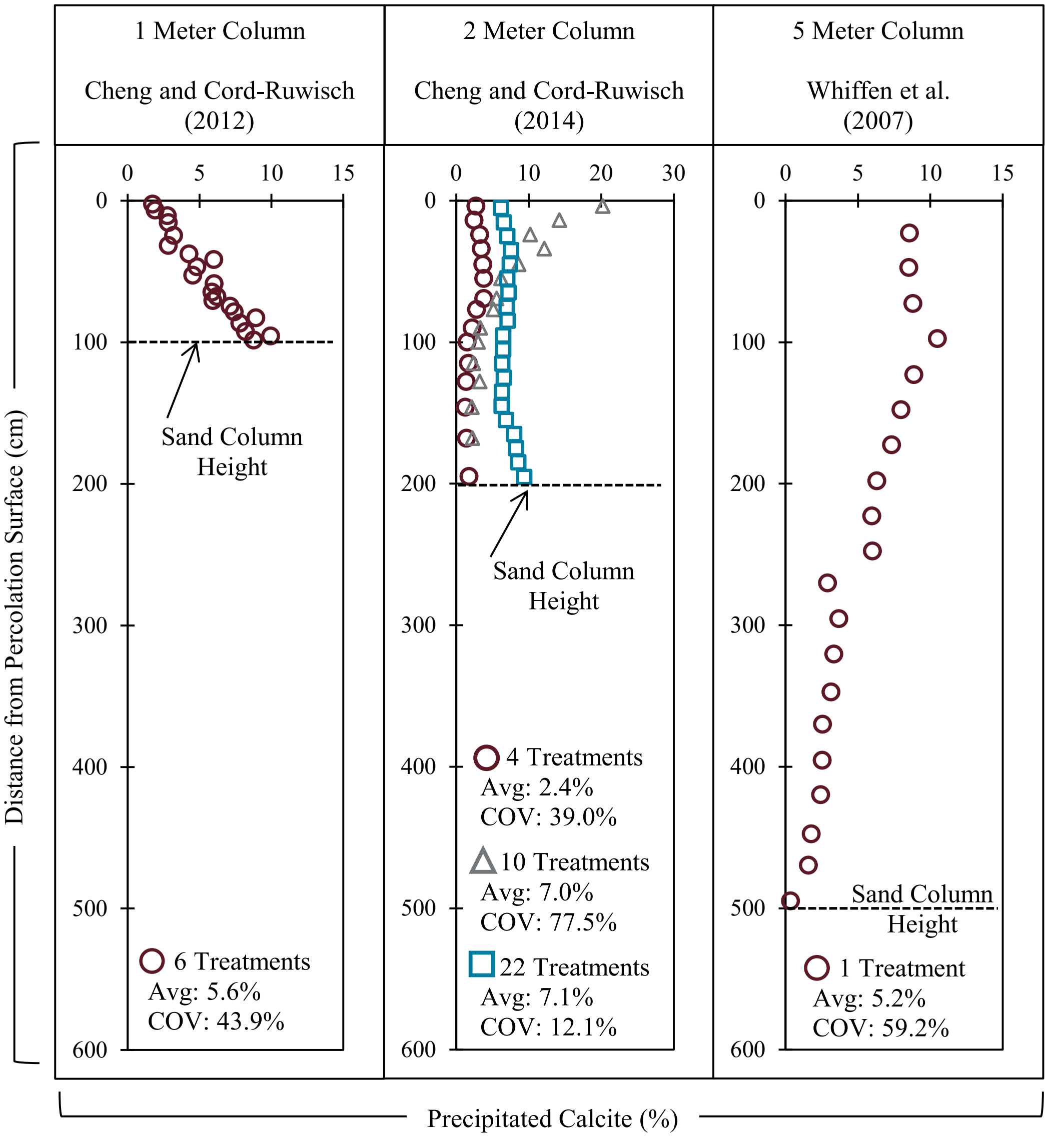


Figure 25. Variations in precipitated calcite over the length of large-scale laboratory specimens stabilized with MICP

3.6 Comparison of MICP to Traditional Stabilizing Materials

Some studies have compared bio-stabilized sands to more traditional stabilizing materials such as portland cement, hydrated lime, metakaolin, and rice husk ash. Reported comparisons are summarized in Table 7. Strength ratios reported in Table 7 were then plotted in Figure 26 to provide a general overview of MICP's performance compared to traditional stabilizing materials. On average, MICP strengths were 64% stronger than corresponding strengths of traditionally stabilized materials. When evaluating specific traditional stabilizing materials, the ratio of MICP to traditional strength for cement specimens was 0.68:1, hydrated lime was 0.07:1, rice husk ash was 0.97:1, and metakaolin was 0.58:1.

Table 7. Summary of MICP versus traditional stabilizing material strength in literature

Reference	Traditional Stabilizer	Mechanical Property	Strength Ratio of MICP to Traditional Stabilizer
Bu et al. (2018)	10% Cement	Flexural Beam	8.6
	10% Cement	UCS Cube	0.7
	10% Cement	UCS 2:1 Cylinder	1.4
	10% Hydrated Lime	Flexural Beam	18.8
	10% Hydrated Lime	UCS Cube	1.6
	10% Hydrated Lime	UCS 2:1 Cylinder	32.4
Mujah et al. (2019)	2% Cement	UCS 2:1 Cylinder	2.1
	4% Cement	UCS 2:1 Cylinder	2.3
	6% Cement	UCS 2:1 Cylinder	1.2
	8% Cement	UCS 2:1 Cylinder	1.7
	10% Cement	UCS 2:1 Cylinder	1.4
Oyediran and Ayeni (2020)	0% Cement	UCS 2:1 Cylinder	0.9
	5% Cement	UCS 2:1 Cylinder	0.9
	10% Cement	UCS 2:1 Cylinder	0.8
	15% Cement	UCS 2:1 Cylinder	1.0
	0% Rice Husk Ash	UCS 2:1 Cylinder	1.0
	5% Rice Husk Ash	UCS 2:1 Cylinder	1.1
	10% Rice Husk Ash	UCS 2:1 Cylinder	0.2
	15% Rice Husk Ash	UCS 2:1 Cylinder	1.7
Porter et al. (2017)	5% Cement	UCS 2:1 Cylinder	8.6
	5% Metakaolin	UCS 2:1 Cylinder	0.7

Notes: Flexural beam tests followed ASTM D6272; UCS Cube tests followed ASTM C67; UCS 2:1 Cylinder tests followed ASTM D2166. All traditional stabilizer and MICP comparisons are at the same percent stabilizer (e.g., 10% cement versus 10% MICP)

Although traditional stabilizing materials can produce optimal strengths in some cases compared to MICP stabilized soils, there are still benefits to using MICP stabilization. Cheng et al. (2013) notes that the permeability of MICP is much higher than traditional portland cement which can lead to reduction in installation and construction costs of drainage systems.

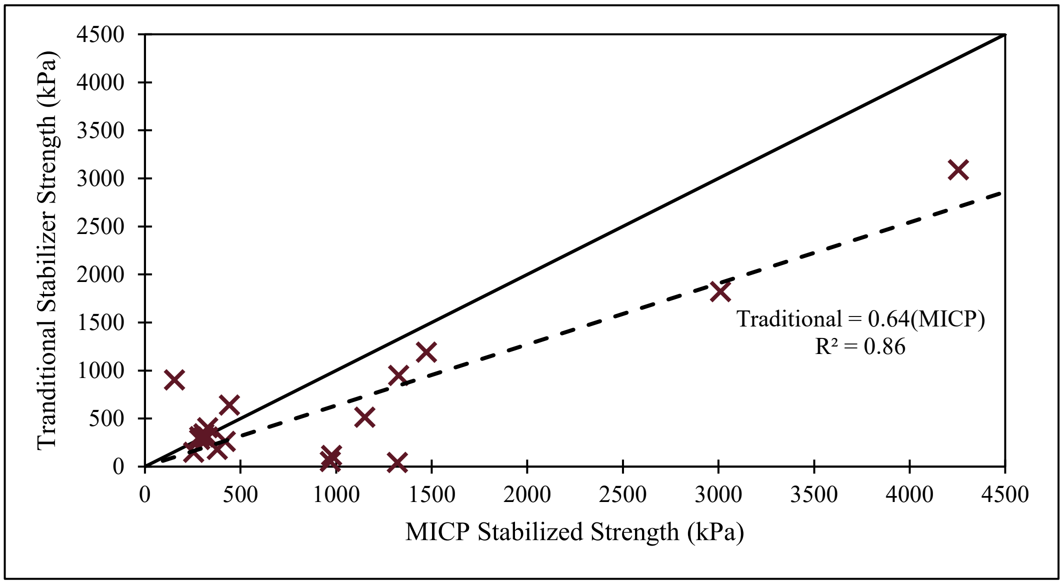


Figure 26. Comparison of strength for traditional stabilized materials and MICP stabilized material

3.7 Comparison of MICP Stabilized Sands to Naturally Stabilized Carbonate Soils

In addition to MICP stabilization as described in this report, particle bonding due to calcite precipitation has also been shown to occur naturally in carbonate soils. These soils are commonly found in sea floors and are high in carbonates due to the skeletal remains of marine organisms which leads to a high variability in cementation (Airey, 1993). The properties of these calcite stabilized soils were compared to typical MICP properties in Table 8. Generally speaking, carbonate soils had noticeably higher UCS values, friction angles, IDT values, and E values. Permeability and density values were similar to reported MICP stabilized values.

Table 8. Comparison of naturally stabilized carbonate soils (i.e., calcarenite) to MICP stabilized sands from literature

Reference	Density (g/cm^3)	UCS (kPa)	Friction Angle (°)	IDT (kPa)	E (MPa)	Permeability (cm/s)
Airey (1993)	1.52	700 – 2,000^A	44.8	276	780	---
Chang-qi et al. (2016)	1.64	7,762	---	1,157	7,067	$5.08 * 10^{-3}$
Cuccovillo (1995)	1.72	---	36	---	---	---
Sangrey (1972)	---	---	31	---	---	---
Zimbardo (2016)	1.67	11,377	---	---	---	---
MICP Literature	1.60	1,118	31.4	285	67.9	$1*10^{-3}$ to $10*10^{-3}$ ^{B}

Notes: All reported values are averages from reported values in each reference; --- indicates no data

^{A} Only a range of values was provided in literature

^{B} Values varies with $CaCO_3$ content

CHAPTER 4 – FIELD SCALE EFFORTS TO STABILIZE SOIL USING MICP

Although the majority of studies of MICP stabilized soil focuses on laboratory experiments, there are a small number of studies that have deployed MICP stabilization techniques in field applications. Additionally, several researchers have developed numerical models to quantify MICP reactions and stabilization of large-scale placements.

4.1 Field Studies

Most field scale efforts of MICP are rooted in laboratory experiments where fundamental mechanisms were first understood. For example, van Paassen (2011) details gradual size increases in MICP stabilized soils to quantify the potential of a MICP grout to stabilize soils. Over a five year period, experiments went from 10 cm laboratory scale experiments to a 100 m^3 large-scale experiment. Based on these experiments, MICP was successfully used to stabilize a coarse gravel that was ultimately bored for underground pipes on a project in the Netherlands.

When evaluating the homogeneity of calcite precipitation in large scale efforts, there was noticeable variation as shown in Table 9. Variations in precipitated calcite ranged from 0 to 25% in the largest volume of sand evaluated in Table 9 (van Paassen et al., 2010). The variability in precipitated calcite was seen to be a function of injection location and flow velocity among other variables.

Table 9. Variability of calcite precipitation in large scale MICP applications

Reference	Application Size	Range of $CaCO_3$	Commentary
DeJong et al. (2014)	0.04 m^3	650 mol/m^3 [50 to 700 mol/m^3]	$CaCO_3$ precipitation was highest at the injection well and decreased as distance from the well increased.
Gomez et al. (2017)	1.13 m^3	5% [0.5 to 5% $CaCO_3$]	$CaCO_3$ was highest along the injection path between Well 1 and Well 2 with low $CaCO_3$ around Well 3.
van Paassen et al. (2009)	1 m^3	17% [0 to 17% $CaCO_3$]	$CaCO_3$ was lowest near injection point and along principal aces. Highest $CaCO_3$ was in lower half.
van Paassen et al. (2010)	100 m^3	24% [0 to 24% $CaCO_3$]	$CaCO_3$ was lower around injection points due to high flow velocities that hampered bacteria attachment.

In addition to precipitated calcite percentages, van Paassen et al. (2009) also reported a range of UCS from 0 to 9 MPa for the 1 m^3 placement. UCS values were also reported at select locations within the 100 m^3 placement (van Paassen et al., 2010) ranging from 0.7 to 12.4 MPa which corresponded to locations with 12.6 and 24.8% precipitated calcite, respectively. Filet et al. (2012) also attempted to stabilize a 100 m^3 volume of soil using MICP stabilization. Cores from this placement tested for UCS showed a range of 0 to 0.9 MPa with the majority of core strengths between 0.05 and 0.5 MPa. This wide range of UCS values highlights the variability in strength due to MICP application processes on a large scale.

Another aspect that has been evaluated on a field scale is the effects of bio-stimulation versus bio-augmentation. Bio-augmentation utilizes the addition of bacteria and other biological materials to soil for stabilization. Bio-stimulation utilizes the native ureolytic microorganisms in a soil for stabilization. Bio-stimulation has the potential to for significant reductions in environmental and ecological impacts (Gomez et al., 2014). In a field-scale comparison of these two stabilization techniques, Gomez et al. (2017) found that these two techniques produced similar properties of interest (e.g., cone tip penetrometer resistance, calcite content, shear wave velocity). Graddy et al. (2018) compared the ureolytic bacteria of these field scale experiments and found that the native bacteria in the bio-stimulation soil had a very high diversity with 20 different strains belonging to 5 species identified. However, Graddy et al. (2021) notes that deciding which technique to use is more complex than simply evaluating the material, environment, and time-cost.

4.2 Numerical Modeling Efforts

Several studies have attempted to numerically model MICP reactions using laboratory scale experiments as a basis for quantifying MICP performance on a large scale. These models typically couple several mechanisms (e.g., flow, transport, chemical reactions) in an attempt to numerically define complex relationships. Table 10 summarizes literature that presents numerical models to quantify MICP development. From a USACE perspective, many more field scale experiments are needed to fully understand how MICP performs in large-scale applications. Although these models are validated using experimental data, there are still several outstanding questions and concerns about potentially using MICP on a large-scale, let alone relying on a numerical model to accurately predict the complex relationships that are present during MICP reactions.

Table 10. Summary of MICP numerical models

Reference	Model Characteristics	Verification Against Field Data
Cunningham et al. (2019)	Couples flow, transport, and relevant biogeochemical reactions	Model agreed well with corresponding field observations of a subsurface stabilization effort
DeJong et al. (2014a)	Well-to-well up-scaled treatment model	Model was capable of capturing complex treatment scenario
Fauriel and Laloui (2012)	Bio-chemo-hydro-mechanical	Model was able to reproduce all mechanisms of interest and their couplings
Landa-Marban et al. (2021)	Single-phase field-scale that includes transport, biofilm activity, and calcite production	Model was able to show that MICP can potentially be used to stop CO_2 leakage from subsurface
Martinez (2012)	One dimensional bio-geochemical reactive transport	Model was able to reasonably predict calcite content of stabilized sand columns
Nassar et al. (2018)	Transient flow and reactive transport	Model reasonably predicted precipitated calcite levels within large-scale experiment

CHAPTER 5 – APPLICATIONS OF MICP STABILIZED SOIL

This chapter summarizes how MICP stabilization techniques have been successfully implemented for soils in applications such as bioremediation (Section 5.1) as well as erosion control and dust abatement (Section 5.2).

5.1 Bioremediation of Heavy Metals

Heavy metals are typically defined as any metallic chemical element that has a relatively high density and is toxic at low concentrations. Examples of heavy metals include lead (Pb), cadmium (Cd), copper (Cu), mercury (Hg), and zinc (Zn). These metals can enter the environment as pollution from mining and metal refining, sewage and wastewater, or energy production (Kumari et al., 2016; Mani and Kumar, 2014).

Researchers have begun to evaluate the potential of biological processes such as MICP to stabilize heavy metals in soils. In a traditional MICP process, calcium ions are added to a solution and precipitate calcium carbonate; however, in MICP bioremediation, calcium carbonates incorporate heavy metals into their surfaces via substitution of suitable divalent cations in the carbonate lattice. This alters the chemical form of the carbonates and alters the heavy metals from soluble to insoluble forms which reduces potential toxicity (Rajasekar et al., 2017). One major advantage of MICP compared to other traditional methods to stabilize heavy metals is its resistance to redox-sensitive solution which allows heavy metal carbonates to remain non-toxic and insoluble. Additionally, heavy metals are less mobile at high pH levels meaning that even if they did reenter solution, they would not be transported until the pH of the solution decreased which would require a large percentage of the carbonates to dissolve (Rajasekar et al., 2021).

One method to evaluate the effectiveness of MICP to immobilize heavy metals in soil is to determine the metal concentrations within different soil fractions (e.g., soluble exchange, carbonate bound, Fe-Mn oxides bound, organic bound, and residual). In several references, soil fractions were reported for control samples and MICP samples. This data was synthesized into Table 11 where the difference between MICP stabilized and control samples were reported. On average, soluble exchange fraction of heavy metal decreased by 26% while the carbonate bound, Fe-Mn oxides bound, and organic bound fractions increased by 19%, 3%, and 2%, respectively. This indicates that a large percentage of heavy metals were successfully immobilized due to the MICP treatment.

An alternative method to evaluate the effectiveness of MICP treatment to immobilize heavy metals is x-ray diffraction (XRD). XRD tests are used to assess the crystallinity and structure of different materials. During XRD tests, an x-ray is used to generated in a cathode ray tube which exposes samples to electrons which produces a characteristic x-ray spectrum. As the sample and detector are rotated, the intensity of the reflected x-rays is recorded and reported as "counts per second". This intensity vs degrees plot can then be interpreted to determine key minerals within the sample. Several references report meaningfully increased amounts of carbonate bound heavy metals (Achal et al., 2012; Kang and So, 2016; Kang et al., 2014; Kang et al., 2016; Mwandira et al., 2017).

Overall, MICP treatment has been shown to immobilize heavy metals found in soils. Its success has been documented in multiple references where different test methods led to the same conclusion. More research efforts are needed to evaluate its effectiveness in full scale efforts as all studies referenced herein occurred at the laboratory scale.

Table 11. Changes in heavy metal distribution coefficients due to MICP bioremediation of soils

Reference	Heavy Metal	Soluble Exchange	Carbonate Bound	Fe-Mn Oxides Bound	Organic Bound	Residual
Achal et al. (2012)	Pb	- 50.2%	+ 20.5%	+ 0.2%	+ 2.1%	+ 27.3%
Chen et al. (2021)	Pb	- 0.8%	+ 0.7%	+4.8%	- 0.6%	- 4.2%
Ghorbanzadeh et al. (2020)	Cd	- 62.5%	+ 56.3%	+ 5.3%	+ 0.5%	+ 0.3%
Liu et al. (2021a)	Pb	- 20.1%	+ 13.7%	+ 2.6%	+ 2.1%	+ 1.9%
Liu et al. (2021a)	Zn	- 17.3%	+ 12.9%	+ 1.6%	+ 1.1%	+ 2.3%
Liu et al. (2021a)	Cd	- 19.5%	+ 6.9%	+ 3.6%	+ 6.0%	+ 3.3%
Yang et al. (2016)	Cu	- 21.2%	+ 25.7%	+ 1.0%	+ 1.2%	- 6.3%
Yang et al. (2016)	Pb	- 18.8%	+16.2%	+ 2.9%	+ 1.0%	- 1.5%
Yang et al. (2016)	Cd	- 21.0%	+ 20.2%	+ 2.1%	+1.2%	- 2.5%
Average	All	- 25.7%	+ 19.2%	+ 2.7%	+1.6%	+ 2.3%

Notes: Cd = cadmium; Cu = copper; Pb = lead; Zn = zinc; change in distribution coefficient defined as coefficient of bio-stabilized sample minus distribution coefficient of control sample.

5.2 Erosion Control and Dust Abatement

Erosion control and dust abatement have emerged as promising potential applications for MICP treatment. Literature generally evaluates erosion from one of three perspectives: 1) resistance to water on a laboratory scale (e.g., rainfall or scouring), 2) resistance to wind on a laboratory scale, and 3) field scale trails to evaluate overall erosion resistance.

5.2.1 Erosion Potential Due to Water

Artificial rainfall is used in laboratories to evaluate the susceptibility of soils to erosion. MICP treatment was applied to several types of soils and subjected to artificial rainfall that ranged in intensity from 13.5 mm/h (light rain) to 300 mm/h (heavy rainstorm) depending on the study. Data was mined from these papers to evaluate the rate of soil erosion as a function of precipitated calcite (Figure 27). Generally, as precipitated calcite increased, erosion rate decreased logarithmically. Due to the spread in data, a global trend defining erosion rate as a function of precipitated calcite could not be identified. Liu et al. (2021b) noted that as MICP treatments increased, a uniform crust began to form on the outermost layer of soil that was resistant to erosion from artificial rainfall. In a study by Chek et al. (2021), crust depth was shown to increase by approximately 7 mm with each 1% increase in precipitated calcite when a surface percolation method was used.

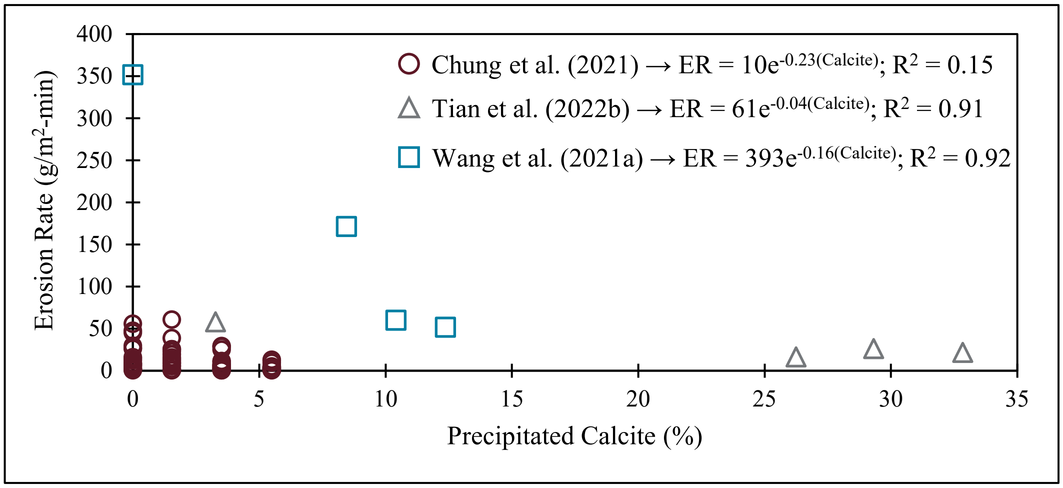


Figure 27. Erosion rate as a function of precipitated calcite from literature

Flume experiments have been used to simulate coastal erosion due to waves as well as scouring around bridge piles. The inclusion of MICP treatment was seen to drastically improve performance of sand in multiple studies (e.g., Kou et al., 2021; Tsai et al., 2022). Application method has been reported as a potential influence on the performance of MICP treated sands. Kou et al. (2021) used a surface treatment on a sand slope and found a modest increase in crust depth at the toe of the slope compared to the crest. Additionally, there was a clear increase in precipitated calcite at the toe of the slope compared to the crest. This can potentially be attributed to gravity and the sloped surface resulting in more bacteria and reactant solution at the toe of the slope. When comparing surface application to an injection method, surface applications produced a more uniform erosion compared to injections which left irregular erosion patterns due to the uneven stabilization of sand (Tsai et al., 2022).

MICP treated sands have also been reported to reduce scouring in laboratory scale experiments with flumes (Bao et al., 2017). Li et al. (2022) reported a reduction in maximum scour depth of 84% after 2 MICP treatments and no noticeable scouring after 4 MICP treatments. Similarly, Do et al. (2021) noted that MICP treatment increased erosion resistance by 2.5 orders of magnitude and decreased erosion rate by 5 orders of magnitude when compared to non-treated sand. It was also noted that during laboratory tests, MICP treated surfaces became brittle and eroded in agglomerated pieces rather than the smooth particle transport of untreated sands.

5.2.2 Erosion Potential Due to Wind

The influence of wind on sand erosion has typically been studied in laboratories using wind tunnels. When comparing typical wind erosion rates of MICP treated soils and untreated soils, it was found that on average, untreated sands erode almost 150 times faster than MICP treated sands (Figure 28). Wang et al. (2021b) reported that MICP treatment drastically improved wind erosion by forming a crust that resulted in small erosion holes rather than large erosion pits seen in untreated sand. There are factors that affect the overall wind erosion rate such as particle size, bacteria and reactant concentrations, and wind speed. For example, Chae et al. (2021) reported that as particle size decreases, the amount of calcite needed to mitigate wind erosion is less due to the interparticle cohesive forces.

Naeimi and Chu (2017) evaluated several potential dust suppressants such as MICP treatments, calcium lignosulfonate, calcium chloride ($CaCl_2$), and water. MICP treatments yielded the least erosion with an average mass loss of approximately 1.5% compared to water (approximately 12.5% mass loss), $CaCl_2$ (approximately 7% mass loss), calcium lignosulfonate (5% mass loss), and untreated soil (27% mass loss). When the top layer of sand was evaluated for each suppressant, MICP treated sands had the largest agglomerated pieces of sand followed by calcium lignosulfonate, $CaCl_2$, and water.

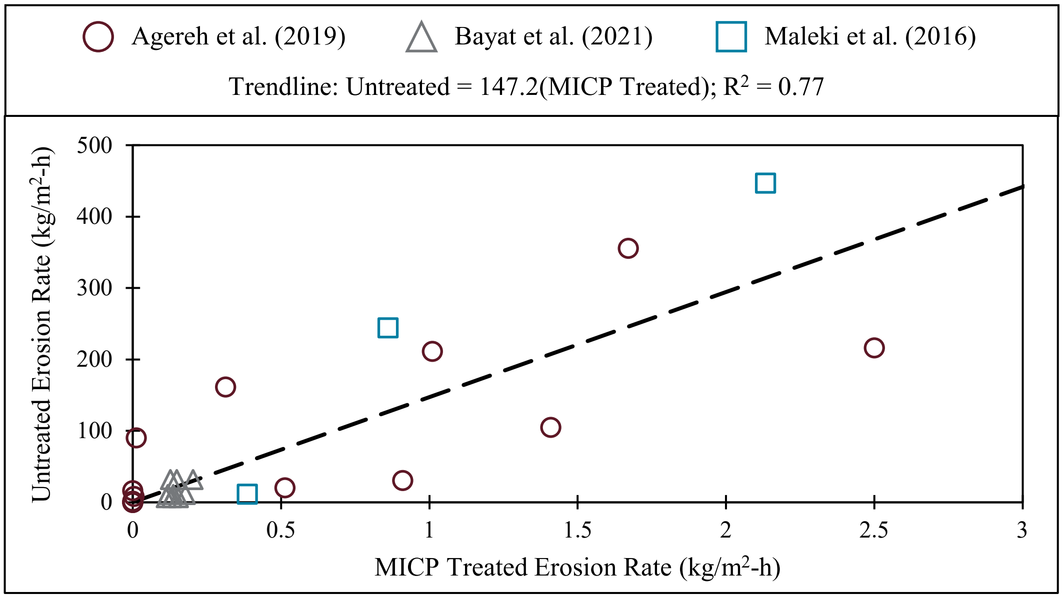


Figure 28. Comparison of wind erosion rates for untreated and MICP treated soils reported in literature

Field scale dust abatement strategies have been a heavily researched by ERDC for several decades in applications ranging from helipads and airfields to lines of communication and base camps (White Jr. and Decell, 1969). A dust control handbook was published by the USCAE-ERDC to provide guidance for effective stabilization techniques (Rushing and Tingle, 2006). However, most of this research evaluated the use of chemicals such as polymer emulsions, chloride salts, synthetic fluids, and asphalt emulsions for dust mitigation (Rushing et al., 2005; Rushing et al., 2006a; Rushing et al., 2006b; Tingle et al., 2004b).

The Department of Energy (DOE) recently published a dust control guide for construction activities that occur in the Hanford Central Plateau area in southern Washington state (Yonkofski et al., 2019). This area has a large number of heavy metal contaminants as it was a former plutonium refinement facility for nuclear weapons during World War II and the

Cold War. It is critical to keep heavy metals out of groundwater sources in the adjacent area. Unlike published ERDC dust control guidance, the DOE report does mention the use of MICP was a promising method for dust abatement.

5.2.3 Field Scale Evaluation of Erosion Potential

Field scale studies have been conducted to evaluate overall erosion characteristics of MICP stabilized soils in field applications. Each field scale trial is summarized below to present the key findings of interest relative to this report.

Ghasemi and Montoya (2022) evaluated three application methods when stabilizing a coastal sandy slope: surface spraying, prefabricated vertical drains, and shallow trenches. Surface spraying yielded a wide treatment zone that could be optimal in applications such as sand dune protection, dust and erosion control, and scour mitigation. Prefabricated vertical drains were capable of treating sand further below the surface and could be optimal in deep soil applications such as slope stability. Shallow trenches improved both surface and depth stabilization in a more localized area and could be ideal for stabilizing ditches or canals. Lower-grade chemicals and water from a nearby pond were used in this field study during application, and improvements in soil performance were still reported. Performance was monitored over a year long period and during this timeframe MICP treated soils were exposed to freeze-thaw cycles, heavy rain, and a hurricane. Even with the exposure to extreme environmental conditions, MICP treated soil showed no significant degradation.

Hodges and Lingwall (2020) present a case study of three field scale projects chosen to fully understand the capabilities of MICP stabilization. Relevant details about each test site are summarized in Table 12. One key finding was that MICP treatment worked under many conditions; however, there were observed limitations. For example, MICP treatment needs time to react and form a crust prior to extreme environmental exposure (e.g., heavy rainfall). Another limitation was seen at test site 3 where there were high concentrations of deicing chemicals in the soil killed many microbes before they were able to react and form calcite.

Table 12. Summary of test sites in Hodges and Lingwall (2020)

Test Site	Site Description	Soil Type	Justification for Site Selection
1	Slope of an excavation site at a landfill	Weathered shale with sand, cobbles, boulders, and 15-30% fines	Chosen as an extreme case to evaluate MICP in "worst case" environment and soil
2	Burn scar on a gentle slope in state park	50% sand, 40% gravel, 10% fines, and thin layer of ash on top	Burned soil has high levels of calcium in soil available for microbial use
3	Embankment on side of major interstate	Loose sandy top soil over a lean clay embankment fill	Large sheet flow during summer rain and exposure to deicing chemicals in winter

Gomez et al. (2015) applied MICP treatment via surficial application to evaluate its effectiveness for dust control. The best performing test plot received lowest concentrations of urea and calcium chloride and reported strengths up to 28 cm deep (only 2 cm short of the target depth). This is an encouraging finding as lower quantities of chemicals may be needed than anticipated to adequately stabilize soil with MICP treatments. Overall, MICP crust thickness varied by as much as 2 cm depending on the concentrations of reactants used. There were also no significant signs of deterioration after 44 days of exposure. It is noted that there are currently qualitative tests to evaluate soil improvement from MICP treatment; however, the development of a quantitative test would be beneficial going forward.

CHAPTER 6 – STRENGTHS, WEAKNESSES, OPPORTUNITIES, AND THREATS (SWOT) ANALYSIS

This section aims to evaluate the strengths, weaknesses, opportunities, and threats of MICP treatment of soils. These observations are based on findings from literature thoroughly reviewed in this report.

6.1 Strengths

A strength of MICP treatment of soils is that it is a naturally occurring reaction that is generally considered environmentally friendly. MICP treatment has also been shown to successfully immobilize heavy metals during precipitation making them insoluble as detailed in Chapter 5.1. MICP treatment is far more environmentally friendly compared to traditional remediation treatments that are still used today such as ion-exchange, adsorption, and membrane filtration (Fu and Wang, 2011).

Another strength is the ability of MICP treatment to form a crust of stabilized soil that can mitigate the effects of environmental erosion. This technology has been successfully implemented in multiple field projects where erosion resistance was meaningfully improved (e.g., Gomez et al., 2015; Ghasemi and Montoya, 2022).

6.2 Weaknesses

One key weakness of MICP treated soils is the lack of a clear quantitative method to evaluate in-situ strength. Unconfined compressive strength (UCS) measurements are difficult to obtain from field trials as MICP treated sand is difficult to successfully core. As a result, the use of dynamic cone penetrometer (DCP) testing (ASTM D6951) is commonly used as a tool to approximate soil strength as well as pocket penetrometer tests (ASTM D6169) which can yield a crude approximation of in situ strength. Additionally, the variations in the amount of precipitated calcite seen in field scale efforts can be a source of concern as this directly influences the variability in mechanical properties.

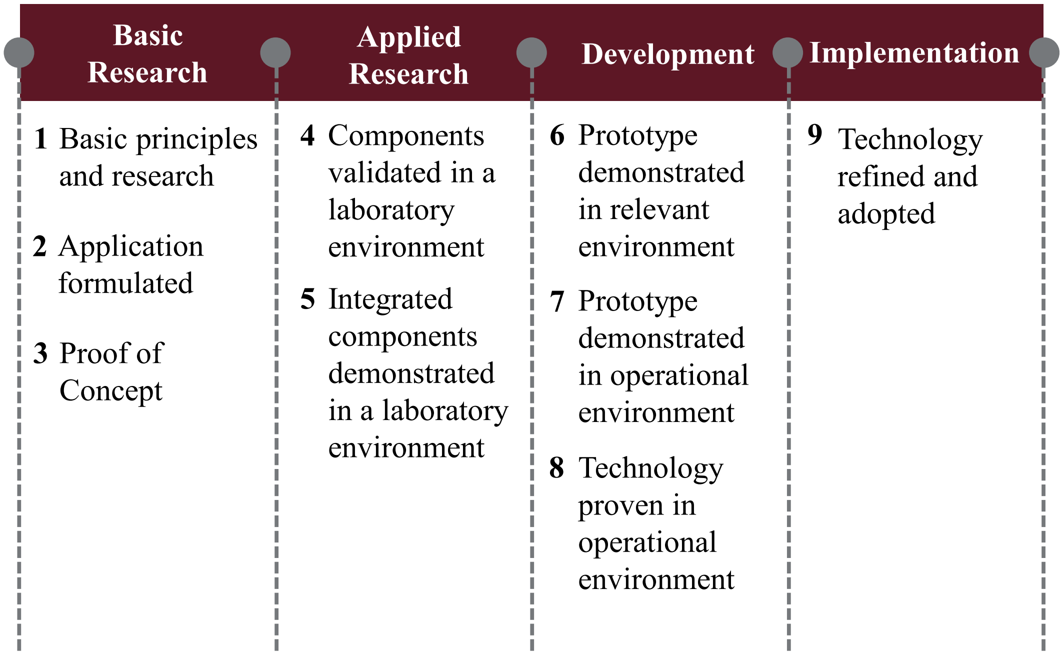
Some have suggested the use of modeling efforts to quantify in situ strengths of MICP treated soils. One method is to use a representative volume element (RVE) with computational modeling that allow for a micromechanical analysis of materials. Representative volume elements (RVE) have previously been used to evaluate heterogenous materials such as concrete (Shahzamanian et al., 2014) and fish scales (Nelms et al., 2017). However, the central criticism of using RVEs to understand properties of MICP treated soils is that microscale properties typically differ from macroscale properties. In other words, it is unknown how much material would need to be used to get an accurate understanding of the representative engineering behaviors.

6.3 Opportunities

There are several potential applications for MICP treated sands based on the findings reported herein. One potential application is using MICP treated sands to stabilize base material for traffic (e.g., low volume roads in rural areas, military facility roadways used by civilians, beach front arterials). More research is needed to define an upper bound of vehicle weight, tire pressure, repeated passes, and so forth for a MICP stabilized roadway. Another application could be to stabilize a helipad in an appropriate climate condition. MICP treated material has been shown to have significantly improved erosion rates which could be an optimal for applications with helipads.

Additionally, there is a need for design standards and testing methods for MICP stabilized materials. Currently there are no design standards for the use of MICP treated materials. A Department of Energy dust mitigation manual does mention MICP treatment as a potential dust abatement technique but does not provide any guidance for optimal implementation methods (Yonkofski et al., 2019). Testing protocols such as DCP are commonly used to approximate strength of MICP treated soils; however, there is not set testing protocol as defined by the USACE. Detailed instrumentation, similar to what has been implemented previously on airfield pavements, may also be beneficial to understanding strength development and performance over time (Doyle et al., 2021).

When evaluating MICP stabilization with the technology readiness level (TRL) assessment (Towery et al., 2017) it can be seen there are opportunities for further development of this technology for large scale implementation (Figure 29). Based on the current state of literature, it appears that research into MICP stabilization for field scale efforts is at roughly a TRL3. There are several literature sources that have detailed MICP stabilization on field scale projects. Although these studies have shown that MICP stabilization is feasible when scaled up, there are still several questions regarding logistics of large scale MICP stabilization efforts.



Basic Research	Applied Research	Development	Implementation
1 Basic principles and research **2** Application formulated **3** Proof of Concept	**4** Components validated in a laboratory environment **5** Integrated components demonstrated in a laboratory environment	**6** Prototype demonstrated in relevant environment **7** Prototype demonstrated in operational environment **8** Technology proven in operational environment	**9** Technology refined and adopted

Figure 29. Summary of technology readiness level (TRL) assessment

6.4 Threats

There are concerns over the biological safety of using bacteria in large quantities to stabilize soil. Naveed et al. (2020) notes that certain strains of bacteria used for MICP stabilization have been shown to cause gastric ulcers, urinary tract infections, and lung infections to humans. Some alternatives that have been evaluated include using dead but urease active cells in place of live bacteria as well as the selection of alkaliphilic, psychrophilic, and halotolerant microorganisms. A full environmental impact study is needed to fully assess the safety implications of using bacteria to stabilize large areas of soil.

An additional threat is the price of materials for MICP stabilization. As of January 2023, the current price of urea is \$450 per metric ton whereas the price of portland cement is approximately \$130 per metric ton. Additionally, MICP stabilization typically performs best with multiple applications of bacteria and nutrients driving the total costs upward.

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