

## **Microbially induced calcite precipitation in fine-grained soils through mechanical mixing**

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### **ABSTRACT**

Microbially induced calcite precipitation (MICP), recognized as an eco-friendly method, has gained considerable attention in recent years. However, a primary obstacle to its widespread use is its application in fine-grained soils such as clays due to their very small pore sizes and low permeability. The small pore size and low permeability prevent the efficient delivery of bacteria, along with their required nutrients and also calcium sources, for calcium carbonate in the soil. This study aims to address these challenges by investigating a mechanical mixing method where the fine-grained soil is blended with bacteria and nutrients/calcium sources. The proposed method can be used in the Deep Soil Mixing method to reduce the use of cement grout. In this study, clayey samples were prepared, and solutions containing bacteria suspension and substrate solution were incorporated into the soil using the proposed mechanical mixing method. The precipitated carbonate content was measured using the Calcium Carbonate Content Chamber (ASTM D4373). Furthermore, Scanning Electron Microscopy (SEM) tests were conducted to examine the precipitated carbonates. Finally, soil strength changes were evaluated through Unconfined Compressive Strength (UCS) Tests. A comprehensive discussion of the results, the observed challenges during the lab experiments, and their implications for possible future field applications are presented.

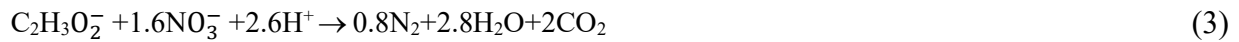
### **INTRODUCTION**

In recent decades, improving soil behavior using bio-geotechnical methods has gained significant attention due to the economic and environmental advantages of these approaches. Microbial Induced Calcite Precipitation (MICP) is one of the most popular methods in this area. The fundamental principle of MICP involves the presence of bacteria that are capable of producing compounds that react with other agents to form calcium carbonate. Two well-known pathways leading to MICP are ureolysis and denitrification. In the ureolysis pathway, bacteria such as *Sporosarcina pasteurii* hydrolyze urea, producing ammonia and carbamic acid, which further decomposes into additional ammonia and carbonic acid. The ammonia hydrolyzes to yield ammonium and hydroxide ions, raising the system's pH. This alkaline condition favors the conversion of carbonic acid into bicarbonate and carbonate ions. When calcium and carbonate ions reach sufficient concentrations, they precipitate as solid calcium carbonate. These reactions are

summarized as follows (Eq. 1 and 2) (Khaleghi et al., 2018; Lin et al., 2021; Prajapati et al., 2023; Whiffin et al., 2007):



In the denitrification pathway, denitrifying bacteria utilize nitrate for respiration. These bacteria employ an organic carbon source, such as acetate, as an electron donor. The carbon source provides the necessary electrons, which are transferred to nitrate, reducing it stepwise to nitrogen gas through a series of reactions. During this process, carbonate ions are generated as byproducts. These carbonate ions subsequently react with calcium ions, often supplied from a source such as calcium acetate, leading to the precipitation of calcium carbonate due to the following the reactions: (3, 4, and 5) (Lin et al., 2021; van Paassen et al., 2010).



The produced calcium carbonate in each of these methods can fill the soil pores, enhancing soil stability and strength. Many studies have been conducted on different types of soil, resulting in increased soil strength and stability (Almajed et al., 2021; DeJong et al., 2022; Thomas O'Donnell & Kavazanjian, 2015; Wang et al., 2017; Zhang et al., 2023; Kavazanjian et al., 2016). However, one of the most challenging issues associated with this (MICP) method is the slow rate of percolation, especially in fine-grained soils, and the uniformity of calcium carbonate distribution within the treated soil matrix as well as clogging due to cementation near the injection port which inhibit proper infiltration of the treatment solutions into the lower depth (Liu et al., 2020; Mujah et al., 2017). Not only does the MICP method face challenges due to the low permeability of fine-grained soils, but other soil enhancement techniques, such as cement mixing, also struggle with this limitation. However, the Deep Mixing Method (DMM) addresses these challenges effectively (Arasan et al., 2017). DMM not only facilitates the mixing of fine particles such as clay and silt with the desired stabilizing agents but also ensures a uniform distribution of the mixed materials throughout the soil matrix.

The effectiveness of DMM can vary depending on soil composition and treatment method. For example, study by Pakbaz & Farzi (2015) showed that in saturated bentonite-sand mixtures, wet cement treatment yielded higher strength than dry cement treatment, while the opposite was true for lime treatment (Pakbaz & Farzi, 2015). For soft soils, DMM has been extensively used to improve bearing capacity and reduce settlement. In Shanghai's soft silty clay, DMM with a higher water-to-cement ratio of 1:6 reduced the UCS from 1.54 MPa to 1.38 MPa (Chen et al., 2013). Arulrajah et al. (2018) used Deep Soil Mixing (DSM) to stabilize soft marine clay using both traditional binders (cement, lime, and cement-lime combinations) and geopolymer binders (fly ash and slag) in different ratios. Geopolymer binders achieved the highest UCS values, with 20% binder content reaching 5.1 MPa after 28 days, compared to 3.1 MPa for cement alone. Lime and cement-lime mixtures had much lower UCS values (0.3 MPa and 1.2 MPa, respectively), making them less effective for stabilization (Arulrajah et al., 2018). As a result, although the DMM method can be highly effective for soil improvement, it is essential to consider the soil type and treatment method to achieve desirable results.

Another challenge with the MICP method is its need for specific strains of microorganisms and their requirement for restricted environmental conditions, such as maintaining a sterile ambient environment. Maintaining these conditions is relatively straightforward in laboratory settings but can be problematic during site implementation. To overcome this challenge, some studies have utilized sources of wastewater treatment, such as activated sludge, which is rich in a diverse type of microorganisms (Pham et al., 2018; Yang et al., 2020).

In this study, the MICP (ureolysis and denitrification) was implemented using activated sludge as a bacterial resource, and the DMM was used to blend solutions to stabilize two type of clayey soils. The results of this application are thoroughly analyzed and discussed, highlighting the effectiveness and implications of combining these advanced techniques.

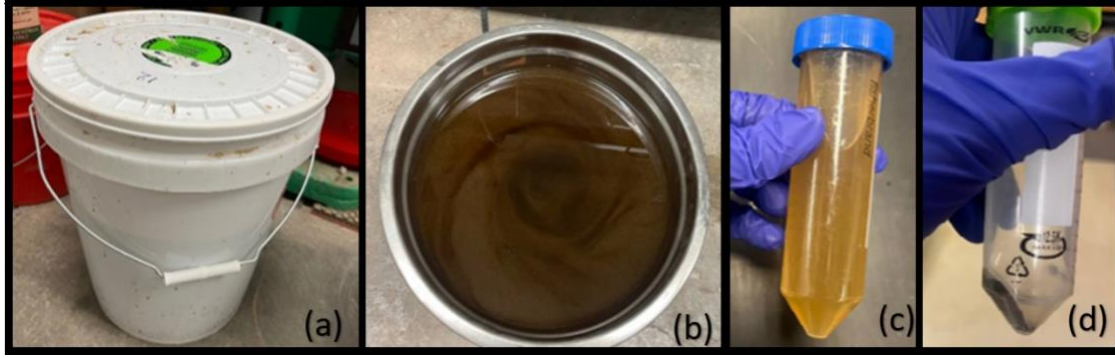
## MATERIAL AND METHODS

**Bacteria cultivation.** In this study, non-sterile bacteria solutions, specifically Activated Sludge (AS), were sourced from local water waste treatment near Houghton, Michigan, USA. Two different pathways were applied to the AS solution to cultivate the desired bacterial species to obtain their expected behavior.

To cultivate denitrifying bacteria, the optimal conditions and methods described in the study by Pham et al. (2018) were employed. In step 1, bacteria inoculation, substrates, and nutrients were mixed with the activated sludge according to Table 1 and poured into a container, which was then allowed to settle for 6 days at  $25 \pm 1$  °C (Figure 1a). After this period, the solids and deposited materials were discarded, and the transparent suspension was used as the bacterial resource. In step 2, soil treatment solution, a solution consist of substrates and nutrients was prepared according to Table 1 and mixed with the bacteria solution in an equivalent volume.

To cultivate bacteria with ureolysis capability, the method outlined by Yang et al. (2020) was utilized. In step 1, bacteria inoculation, and activated sludge were mixed with nutrients according to Table 1, and the pH was adjusted to 10 using NaOH. The entire suspension was then stirred with a magnetic stirrer for 36 hours at  $25 \pm 1$  °C (Figure 1b). After this period, the suspension was centrifuged at 5000 rpm for 5 minutes. Figure 1c shows the solution before centrifuge and Figure 1d shows the bacteria pellet after centrifuge. The supernatant was discarded, and the bacterial pellet was thoroughly mixed with a 0.9% saline solution. In step 2, soil treatment solution, this bacterial suspension was then combined with substrates according to Table 1 in an equivalent volume.

It is worth mentioning that, in field applications, preparing all the necessary requirements simultaneously can be challenging and should be carefully considered. It is vital to ensure that the bacteria have adequate nutrients to maintain their viability.



**Figure 1. a) Denitrification bacteria inoculation, b) Ureolysis bacteria inoculation, c) Bacteria solution before centrifuge, d) Bacteria pellet after centrifuge**

**Table 1. Substrates and nutrients concentrations**

Composition	Pathway	Concentration	Step
Ammonium Sulfate	Denitrification	0.00039 g/L	1&2
Magnesium Sulfate	Denitrification	0.00029 g/L	1&2
Monopotassium Phosphate	Denitrification	0.00082 g/L	1&2
Dipotassium Phosphate	Denitrification	0.00244 g/L	1&2
Trace Element Solution (SL12B)	Denitrification	1ml/L	1&2
Calcium Nitrate	Denitrification	8.205 g/L	1&2
Calcium Acetate	Denitrification	9.49 g/L	1
Calcium Acetate	Denitrification	12.65 g/L	2
Ammonium Chloride	Ureolysis	5 g/L	1
Yeast Extract	Ureolysis	20 g/L	1
Nickel Chloride	Ureolysis	0.01 g/L	1
Calcium Chloride	Ureolysis	111 g/L	1
Urea	Ureolysis	10 g/L	1
Urea	Ureolysis	60 g/L	2

**Laboratory scale Deep Mixing experiments.** In this study, two different fine-grained soils were utilized. The first was Varved Clay (VC) soil, obtained from a natural landslide site located in Ontonagon, Michigan, USA. The soil chunks were air-dried, crushed, and sieved through a No. 200 sieve. The second clay soil used in this study was a mixture of 70% Bentonite and 30% Kaolinite (BK). The Atterberg limits associated with these soils were LL: 31% and PI:11% for VC soil and LL: 77% and PI: 27% for BK soil. Plastic molds were prepared using binding covers, designed with a diameter of 1.27 cm and a height of 15.24 cm. The samples tested were prepared with a height of 2.6 cm. It is important to note that finding the optimal proportion of soil to treatment solution can be quite challenging and often requires trial and error. It is strongly suggested that, before any field application or extensive laboratory experiments, trial samples be prepared to determine the best proportion, which depends on the soil type and its properties. The optimal moisture content (OMC) for the DMM, based on the liquid limit (LL) of the soil, depends on the specific soil properties and binder used. Various studies have explored OMC values in the range of 0.75LL to 1.25LL. Wassie et al. (2023) investigated metakaolin-based

geopolymer stabilization within this range and identified 0.75LL as providing the best performance. Arulrajah et al. (2018) tested moisture contents of 0.75LL, 1.0LL, and 1.25LL to replicate field conditions. These findings, supported by studies such as study by Bhavita Chowdary et al. (2020) suggest that while the optimal OMC may vary based on the soil and binder, the range of 0.75LL to 1.25LL serves as a reliable guideline for DMM applications (Arulrajah et al., 2018; Bhavita Chowdary et al., 2020; Wassie et al., 2023). It is crucial to conduct laboratory tests to determine the ideal moisture content for each study, as factors such as binder type, soil properties, and desired strength characteristics can influence the optimal moisture content for DMM treatment. In this study, different volumes of water were mixed with a specified amount of soil to achieve a homogeneous soil-water mixture. The results showed that a moisture content of 37.5% for VC and 63.5% for BK produced a practical mixture that can be mixed with a mixer. After pouring the soil into the molds, a bacterial solution was mixed using the Deep Mixing Method. In addition, distilled water was mixed with the soil using the same mixing method to prepare untreated samples. The solutions were divided into three portions. The first portion was poured on top of the soil, and a mixer was used to blend the soil with the solution by moving up and down while rotating throughout the soil. Afterward, the second and third portions were added to the mold. Since the initial portion had made the soil matrix viscous, the subsequent portions could be easily mixed with the solution using the same technique. Figure 2-Left shows DMM in the prepared mold and Figure 2-Right shows the soil samples in the molds. After 8 days of treatment, samples were placed in an oven at a controlled temperature of 50°C for 48 hours to ensure uniform drying and minimize any variations in moisture content that could potentially affect the results.

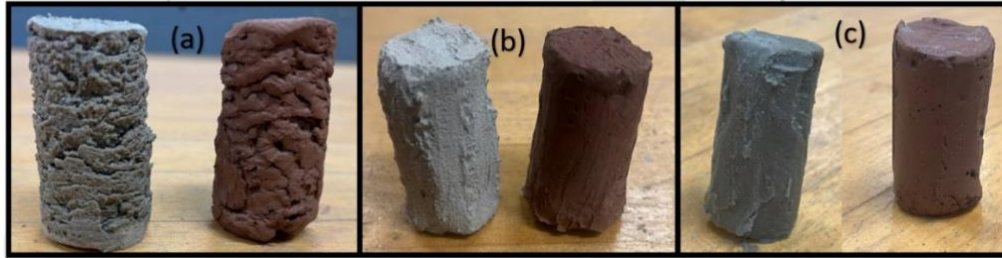


**Figure 2. Left: DMM in the prepared mold, Right: Prepared soil samples**

**Strength and characterization tests.** In addition to the initial identification test described in the previous section, Unconfined Compression Strength (UCS) (in accordance with ASTM D2166), Calcium Carbonate Content Chamber (in accordance with ASTM D4373), and Scanning Electron Microscopy (SEM) tests were conducted on samples.

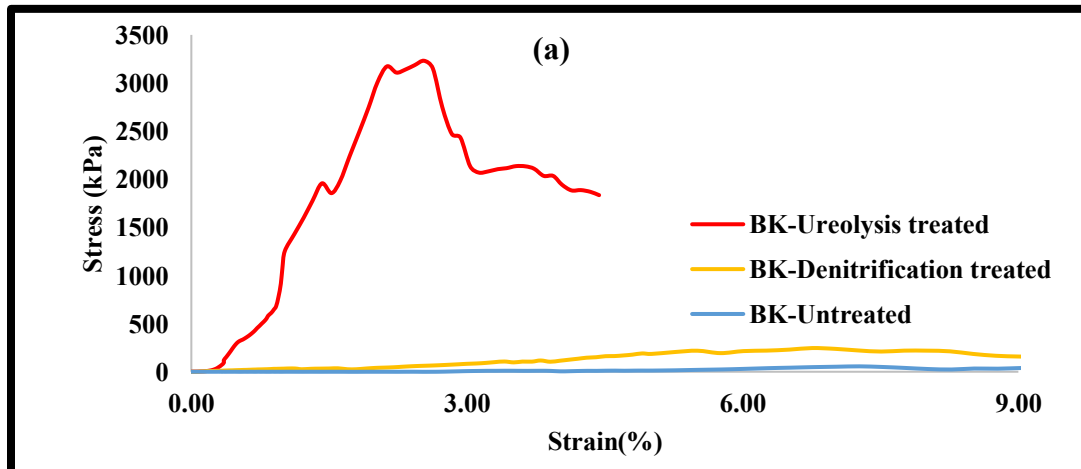
## RESULTS AND DISCUSSION

Figure 3 displays untreated, denitrification-treated, and ureolysis treated samples. In each subfigure, the gray sample (on the left) corresponds to bentonite-kaolinite soil, whereas the brown samples (on the right) denote varved clays.

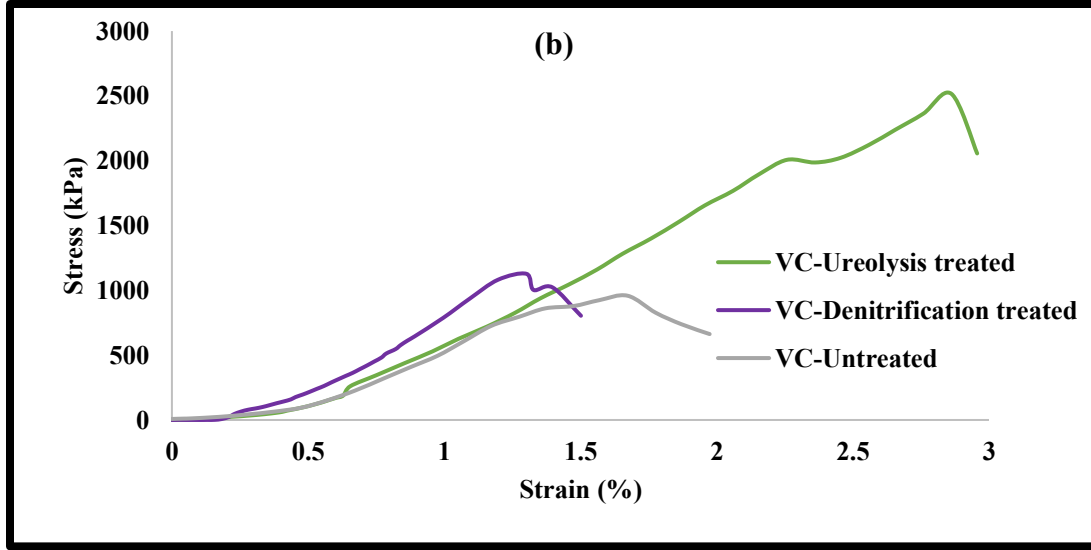


**Figure 3. a) Denitrification treated samples, b) ureolysis treated samples, c) Untreated samples**

Clay materials are challenging for MICP due to low permeability, leading most studies to focus on clay-sand mixtures. An example of clay-MICP treated is the study by Arpajirakul et al. (2021) that explored MICP for three fine-grained soils (Kaolin clay, Laterite, Bangkok clay) using *Sporosarcina pasteurii* as ureolysis bacteria. To enhance cementation solution diffusion, they employed an air compressor and pressure regulator. UCS improvements of 189%, 278%, and 106% were achieved, demonstrating MICP's potential for strengthening fine-grained soils (Arpajirakul et al., 2021). The stress-strain plots of the UCS tests on treated and untreated samples of this study are presented in Figures 4a and 4b. It should be noted that all UCS tests were conducted in triplicates and only the results of one of them are shown in the figure as an example. The MICP treatment resulted in a notable increase in soil strength. The strength gained in ureolysis MICP-treated samples was significantly higher than those in denitrification MICP-treated samples. The bentonite-kaolinite soil strength was increased by 339% and 5669% for denitrification-MICP and ureolysis-MICP treatment, respectively. The strength increase after treatment for varved clay samples was 41.5% and 349% for denitrification-MICP and ureolysis-MICP, respectively.

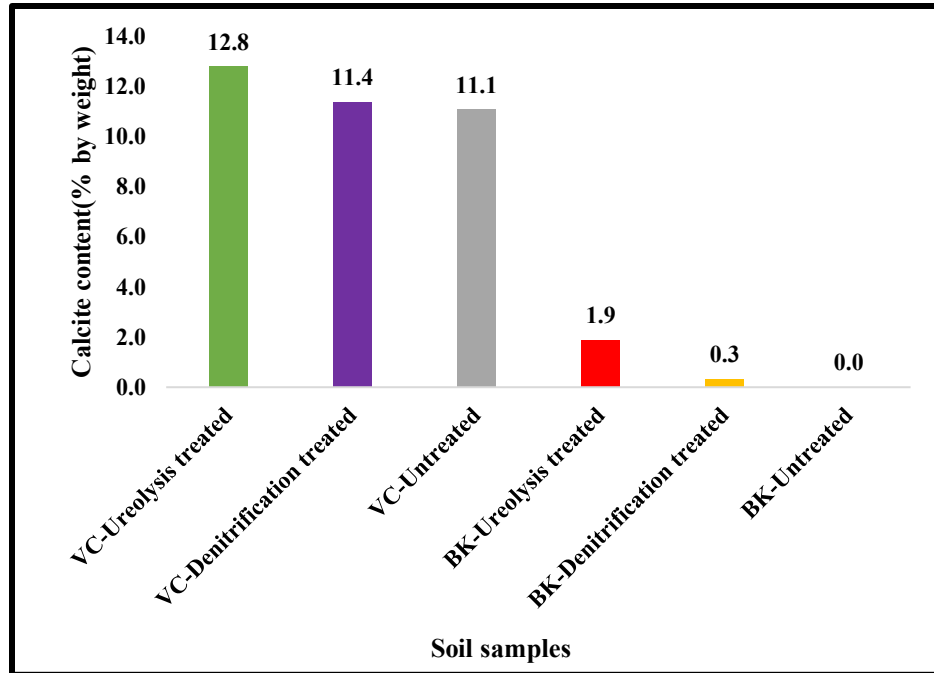






**Figure 4. Load-displacement plots; a) Bentonite-Kaolinite (BK) samples, b) Varved Clay (VC) samples**

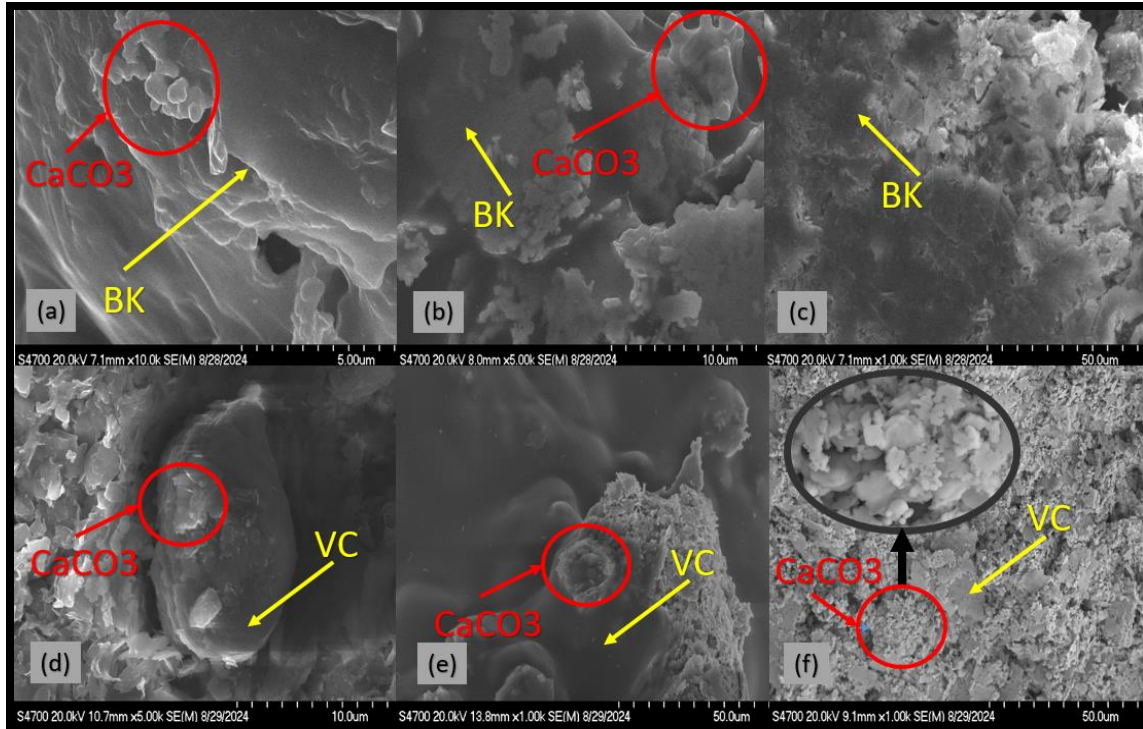
This substantial difference in increased strength may be attributed to the lower amount of precipitated calcium carbonate after the denitrification treatment compared to the higher calcium carbonate content precipitated after the ureolysis-treated treatment (Gao et al., 2022). Figure 5 shows the calcium carbonate content of treated and untreated samples. As can be seen, there is natural calcium carbonate in the untreated varved clay soil but the Bentonite-Kaolinite mixture does not have any calcium carbonate before treatment. The results show that about 0.3% calcium carbonate was precipitated in both soils after denitrification-MICP treatment while 1.7 to 1.9 % calcium carbonate was precipitated after ureolysis-MICP treatment. It is also worth mentioning that the amount of increase observed in the strength of varved clay is less than that of the bentonite-kaolinite samples. This is potentially partly due to the pre-existing natural calcium carbonate in varved clays used in this study (Fu et al., 2023).



**Figure 5. calcium carbonate content measurement in treated and untreated samples (VC: varved clay, BK: bentonite-kaolinite)**

Figure 6 shows the result of the SEM test on untreated and MICP-treated (both denitrification and ureolysis pathways) samples. Subfigures 6a, 6b, and 6c show the ureolysis-MICP treated, denitrification-MICP treated, and untreated bentonite-kaolinite samples, respectively. The precipitate calcium carbonate and the soil particles (clay sheets) are specified in the figure. Subfigures 6d and 6e, show the varved clay treated via MICP-denitrification and MICP-ureolysis pathways, respectively. Subfigure 6f shows the untreated varved clay soil confirming the presence of calcium carbonate within the soil matrix before treatment (this preexisting calcium carbonate was observed during the calcite content tests).





**Figure 6. SEM of: a) BK-Denitrification treated, b) BK-Ureolysis treated, c) BK-Untreated, d) VC-Denitrification treated, e) VC-Ureolysis treated, f) VC-Untreated (BK: bentonite-kaolinite mixture, VC: varved clay)**

## CHALLENGES AND ENVIRONMENTAL CONSIDERATION

In the soil improvement via biological treatments, selecting and introducing bacteria capable of performing desired soil reactions involves key challenges. It is essential to choose strains or microbial communities that are non-pathogenic and environmentally safe. One practical approach can be utilizing indigenous bacteria already present in the soil, as they are more likely to be ecologically compatible and less disruptive to the native ecosystem.

When working with microbial communities and scaling up treatments for field applications, additional treatment and modifications to land application might be needed to prevent the risk of pathogen transfer and spread in the environment. Furthermore, the harmful byproducts can cause environmental issues that need to be addressed. For instance, the ureolysis pathway that produces ammonia, which can pose environmental risks. Future research should focus on minimizing harmful byproducts production and ensuring the sustainability and environmental safety of bacterial soil improvement methods.

## CONCLUSION

In this study, laboratory experiments were conducted to investigate the possibility of using MICP treatment via two different pathways for deep mixing of fine-grained soils. Two soil types, a natural varved clay with preexisting calcium carbonate and a mixture of bentonite-kaolinite prepared in the laboratory, were used for these experiments. Aerobic denitrification and nonsterile

ureolysis pathways were used in the MICP treatments. The results indicate that MICP treatments effectively enhanced soil strength in both samples, with the ureolysis pathway being more efficient than denitrification. Higher strength gains were observed in samples treated via the ureolysis pathway which is attributed to higher amounts of precipitated calcium carbonate in this pathway. The strength gain was lower in the natural varved clays compared to the bentonite-kaolinite mixtures. This lower strength can be at least partially attributed to the preexisting calcium carbonate in the natural varved clays used in this study, which may influence calcium carbonate precipitation. SEM analysis confirmed the presence of calcium carbonate in both denitrification and ureolysis treatment pathways. Finally, this study confirms that the two less-known pathways/practices, i.e., aerobic denitrification and nonsterile inoculation of ureolytic bacteria, have the potential to be used in ground improvement methods including deep mixing of fine-grained soils and should be investigated in more detail in future.

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