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ORIGINAL ARTICLE

Investigating MICP sand stabilization with Bacillus thuringiensis: nutrient concentration and availability

Sumonthip Kongtunjanphuk[a], Piti Sukontasukkul[b*], Satharat Pianfuengfoo[b], Hexin Zhang[c], Worathep Sae-Long[d], Pithaya Jamsawang[e]

[a]Department of Biotechnology, Faculty of Applied Science, King Mongkut's University of Technology North Bangkok, Bangkok 10800, Thailand
[b]Construction and Building Material Research Center, Department of Civil Engineering, Faculty of Engineering, King Mongkut's University of Technology North Bangkok, Bangkok 10800, Thailand
[c]School of Computing, Engineering and the Built Environment, Edinburgh Napier University, Edinburgh EH11 4BN, Scotland, United Kingdom
[d]Department of Civil Engineering, Faculty of Engineering, Phayao University, Phayao 56000, Thailand
[e]Soil Engineering Research Center, Department of Civil Engineering, Faculty of Engineering, King Mongkut's University of Technology North Bangkok, Bangkok 10800, Thailand
[*]Corresponding Author: Piti Sukontasukkul. Email: piti.s@eng.kmutn.ac.th

Abstract: This study investigates Bacillus thuringiensis TISTR 126 in Microbially Induced Calcium Carbonate Precipitation (MICP) for sand stabilization in neutral pH environments. It addresses a gap in research on non-alkali-tolerant bacteria. Most MICP studies focus on ureolytic bacteria, which thrive in alkaline conditions. B. thuringiensis is cost-effective, widely available, non-pathogenic, and suitable for neutral pH, making it a promising alternative. The study investigates the effects of nutrient availability and calcium chloride ($CaCl_2$) on calcium carbonate formation and the mechanical properties of sand, including water permeability, unconfined compressive strength (UCS), and internal friction angle. Results show that optimizing the cementation solution enhances sand properties, leading to a 12.1% increase in density, a ten-fold reduction in water permeability, and a UCS of approximately 339.6 kPa. The highest cementation ratio produced an internal friction angle of 48°, indicating a dense structure. This research addresses the critical gap in nutrient optimization for MICP processes. It introduces B. thuringiensis as a viable, sustainable, and non-pathogenic alternative to traditional ureolytic bacteria for sand stabilization, broadening the scope of MICP applications.

Keywords: Microbially induced calcium carbonate precipitation, sand stabilization, cementation solution, calcium carbonate preparation

1 Introduction

Sand stabilization refers to the process of improving the engineering properties and stability of sand, particularly in construction and geotechnical applications. Sand, being a loose and granular material, can pose challenges due to its low shear strength, poor load-bearing capacity, and susceptibility to erosion or shifting. Sand stabilization techniques aim to enhance the cohesion, shear strength, and overall stability of

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sand to ensure it can support structures, prevent erosion, or provide a stable foundation. Sand stabilization techniques are commonly used in applications such as road and pavement construction, building foundations, coastal erosion control, and land reclamation projects.

Various methods can be employed for sand stabilization, including mechanical methods such as compaction, vibro-compaction, or soil reinforcement techniques like geotextiles, geogrids, or soil nails. Chemical methods involve the use of additives or binders, such as cement, lime, or chemical stabilizers, to improve the bonding and cohesion within the sand particle [1-2].

Cement is widely recognized as one of the most commonly used construction materials worldwide, finding applications in various construction types, including soil and sand stabilization [3-5]. However, the production of cement is associated with significant environmental concerns. According to the IEA's 2020 report, cement production accounts for approximately 8% of global greenhouse gas emissions from fuel combustion [6-7], contributing to the annual increase in global warming.

Therefore, several research studies have been conducted to mitigate the impact on global warming by reducing the usage of cement in construction. For instance, investigations have explored the use of mineral admixtures to replace a portion of cement in concrete and the development of geopolymers as complete substitutes for cement. Similarly, in the domain of soil or sand stabilization, microbial-induced calcite sedimentation has been employed to enhance soil or sand stabilization, shear strength, and stiffness [8-10]. The MICP process relies on the metabolic activities of bacteria that produce enzymes to catalyze the urea hydrolysis reaction, resulting in the production of carbonate, as depicted in Eq. 1. Subsequently, a reaction occurs between the carbonate and calcium ions on the surface of bacterial cells (derived from a calcium source), leading to the formation of calcium carbonate sedimentation, as illustrated in Eq. 2 [14]. Importantly, this process does not involve the use of cement as a component.

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

$$Cell + Ca^{2+} + CO_3^{2-} \rightarrow Cell + CaCO_3 \quad (2)$$

One of the advantages of Microbially Induced Calcium Carbonate Precipitation (MICP) for sand stabilization is that it is a biological and environmentally friendly process, as it utilizes naturally occurring bacteria and avoids synthetic additives or chemical binders. The technique can be applied in situ, reducing the need for excavation and sand replacement. Additionally, MICP-treated materials benefit from self-healing properties, as the bacteria thrive in moist environments, enabling cracks to repair themselves and improving durability. The method forms resilient calcium carbonate bonds capable of withstanding harsh conditions, such as chemical exposure and extreme temperatures. However, its effectiveness depends on factors like the calcium source, bacterial type, nutrient concentration, sand composition, and environmental conditions. Limitations include lower initial mechanical properties, longer processing times, and the need for precise control of bacterial stability and curing environments.

The calcium source used in MICP plays a crucial role in the effectiveness of sand stabilization. Calcium ions are essential for the formation of calcium carbonate, which acts as the binding agent for the sand particles. The choice of calcium source can impact the rate and extent of calcium carbonate precipitation, as well as the strength and durability of the stabilized sand. Common calcium sources include calcium chloride ($CaCl_2$) and calcium nitrate ($Ca(NO_3)_2$), among others. Previous studies indicate that calcium chloride is considered one of the most suitable calcium sources due to its solubility, high concentration of calcium ions, high compatibility, rapid reaction, and cost-effectiveness. Research by Achal [15] and Gorospe et al. [16] confirmed the stability of calcium chloride as a calcium source for the MICP process.

In terms of bacteria types used in MICP for sand stabilization, ureolytic bacteria are commonly employed. These bacteria possess the enzyme urease, which catalyzes the hydrolysis of urea, leading to the release of carbonate ions and ammonium. The carbonate ions then react with calcium ions to form calcium carbonate, which precipitates and binds the sand particles together. Some examples of ureolytic bacteria frequently used in MICP for sand stabilization include: Sporosarcina pasteurii (formerly Bacillus pasteurii) [17], Bacillus sphaericus [18], and Bacillus pseudofirmus [19].

As for the concentration and availability of nutrients, both play a crucial role in microbial-induced calcite precipitation (MICP) for sand stabilization. Nutrients, such as nitrogen and carbon sources, are essential for the growth and metabolic activities of the bacteria involved in MICP. Nitrogen, typically provided in the form of urea, serves as a crucial nutrient for bacteria. Carbon sources, such as organic compounds or simple sugars, provide the bacteria with the necessary carbon for their metabolic activities. The concentration and availability of these nutrients are important factors to consider in MICP sand stabilization. Insufficient nutrient supply can limit bacterial growth and activity, resulting in inadequate calcium carbonate precipitation [20].

Conversely, an excessive nutrient supply may lead to overgrowth of bacteria [21], affecting the overall performance and efficiency of MICP. Previous research studies dedicated to optimizing nutrient concentrations and availability in microbial-induced calcite precipitation (MICP) for sand stabilization included the following. In a study conducted by Lee et al. [22], Sporosarcina pasteurii KCTC 3558 bacteria were utilized to induce sand bio-clogging and stabilization. The sand was shaped using a layer-by-layer technique, with each layer being 10 mm in height. The fixation solution (calcium source) and bacterial solution were added and mixed with each layer until all six layers, totaling 60 mm, were completed. The nutrient supply of 10 ml was applied twice a day to the top and bottom of the sample, achieved by rotating the sample upside down, for a period of 20 days until it could no longer permeate the coagulated surface.

Choi et al. [23] investigated the use of Sporosarcina pasteurii ATCC 11859 bacteria in a medium comprising yeast extract, ammonium sulfate, and tris buffer to achieve bio-clogging of sand. For bacteria and nutrient supply, they continuously applied both bacteria and nutrients (urea and calcium chloride) to the sand sample in 2 cycles daily, with each cycle lasting 12 hours. Essentially, the bacteria and nutrients were circulated within the sand sample continuously for 10 days.

Li et al. [24] explored the use of Sporosarcina pasteurii ATCC 11859 bacteria for sand bio-clogging and stabilization. The cementation solution included urea, hydrated calcium chloride, ammonium chloride, sodium bicarbonate, and Nutrient Broth. To avoid shortage of nutrient supply, the sand samples were placed in a batch reactor filled with the cementation solution and incubated for 7, 14, and 21 days, corresponding to single, double, and triple treatments, respectively.

While previous studies have extensively investigated the use of various ureolytic bacteria, such as Sporosarcina pasteurii, Bacillus sphaericus, and Bacillus pseudofirmus, in Microbially Induced Calcite Precipitation (MICP) for sand stabilization, there remains a significant research gap regarding the exploration of alternative, non-alkali tolerant bacteria for this application. The commonly studied bacteria often thrive in highly alkaline conditions, which may limit their effectiveness in non-coastal, neutral pH environments typically found in many construction and geotechnical settings. Bacillus thuringiensis, known for its cost-effectiveness, availability, and non-pathogenic nature, presents a promising alternative due to its ability to thrive in neutral pH conditions. However, its potential in MICP sand stabilization remains largely unexplored.

Moreover, while the influence of nutrient supply and calcium sources has been explored in MICP studies, the specific relationship between nutrient concentration, availability, and the resulting mechanical properties of sand has not been sufficiently investigated. Previous research often lacks a clear definition of nutrient supply volumes and patterns, which could be crucial for optimizing MICP efficiency. This study addresses these gaps by investigating both the feasibility of Bacillus thuringiensis and the impact of nutrient availability, aiming to provide novel insights into improving MICP techniques for sustainable sand stabilization.

The primary objective of this study is to investigate the potential of Bacillus thuringiensis as a bacterial strain for MICP in sand stabilization, with a focus on its suitability for neutral pH environments typically found in non-coastal sand. This research also aims to explore the impact of nutrient concentration and availability on the effectiveness of MICP in improving the mechanical properties of sand, such as shear strength, unconfined compressive strength, and water permeability.

2 Materials & Method

2.1 Bacteria strain cultural & calcium carbonate sediment

The bacterial strain used in this research is *B.* thuringiensis TISTR 126 (Bt), a pure non-pathogenic strain obtained from the Thailand Institute of Scientific and Technological Research (TISTR). The selection of Bt is based on findings from our previous study [25], summarized as follows: Bt was compared with four other bacterial strains in terms of urease activity and $CaCO_3$ precipitation. Bt exhibited high urease activity (1722 U/ml), second only to *P.* mirabilis TISTR 100 (2189 U/ml), and produced a $CaCO_3$ yield of 3.080 mg/ml at an optimum $CaCl_2$ concentration of 250 mM. These results highlight Bt's superior performance as a non-pathogenic bacterium suitable for this application. The detailed comparison is summarized in **Table 1.** Additionally, the sedimented $CaCO_3$ from Bt appeared as flaky, light-brown crystals, distinguishing it from the white crystals reported in other studies [22].

Table 1. Urease activity of each bacteria strain [25]

Bacteria	Urease activity (U/ml)
Bacillus thuringiensis TISTR 126	1722
Bacillus megaterium TISTR 067	456
*Bacillus sp. TISTR 658	522
*Proteus mirabilis TISTR 100	2189
*Staphylococcus aureus TISTR 118	722

* Pathogenic bacteria

The bacteria were cultured using a two-step method, starting with a liquid stock solution with an initial cell concentration of 1% in selective media composed of Nutrient Broth (13 g/l) and Urea (20 g/l). The culture was incubated for 32 hr. in a multi-stack refrigerated shaking incubator with a shaking rate of 250 rpm at 37 ℃.

The study of calcium carbonate sedimentation was conducted by adding calcium chloride solution to the bacterial-suspended solution with concentrations ranging from 0.1 to 0.3 M, followed by incubation at 37 ℃ for 7 days. Subsequently, the solution was filtered using a Büchner funnel equipped with Whatman No.4 filter paper (pore size: 15 µm). After filtration, the residues were dried in a hot-air oven at 60 ℃ for 24 hr., and the weight of the obtained sediments was measured. Using this method, the researcher selected the calcium chloride concentration that resulted in the highest yield of calcium carbonate sediments by the B. thuringiensis TISTR 126 strain for further subsequent.

2.2 Materials

The cohesion-less sand, with a particle size ranging from 425 to 600 µm, a bulk specific gravity (dry condition) of 2.46 and an absorption of 0.78%. When conducting this sand to the maximum dry density test according to ASTM D698 [26] (standard proctor), it was found that the sand in this research study exhibited a maximum dry density of 1.60 g/cm3 at an optimum moisture content of 17.5%.

The cementation solution used in the study comprised Nutrient Broth at a concentration of 13 g/l (without bacterial suspension), urea at 20 g/l, and calcium chloride solution at 36.76 g/l (equivalent to 0.25 M). The solution was thoroughly stirred using a magnetic stirrer and then sterilized by autoclaving at 121 ℃ with a steam pressure of 15 psi for 15 minutes. To prevent early sedimentation from their reactions, nutrient broth containing urea and calcium chloride were mixed separately. The cementation solution was prepared under sterile conditions to ensure the integrity and reliability of the experimental results.

2.3 Sand sample preparation

Before preparing the sand samples, the optimum moisture content, which resulted in the maximum dry density, was determined using the Standard Proctor Test. The results of the Proctor test revealed an optimum moisture content of 17.5%, yielding a maximum dry density of 1.6 g/cm^3.

The process of preparing the sand samples began with the mixture of sand and bacterial-suspended solution. The volumes of the bacterial-suspended solution represented the total liquid content of the mixture and were maintained at 17.5% of the sand weight, in accordance with the optimum moisture obtained from the earlier Proctor test. Once the sand was thoroughly mixed with the bacterial-suspended solution, it was compacted into cylindrical molds with dimensions of diameter 50 mm x 100 mm for unconfined compression tests, and into prism molds with dimensions of 60 mm x 60 mm for direct shear tests. The final step involved the addition of the calcium chloride solution to the sand specimens. The entire process, carried out at a controlled temperature of 25 ℃ [27], is illustrated in **Fig. 1.**

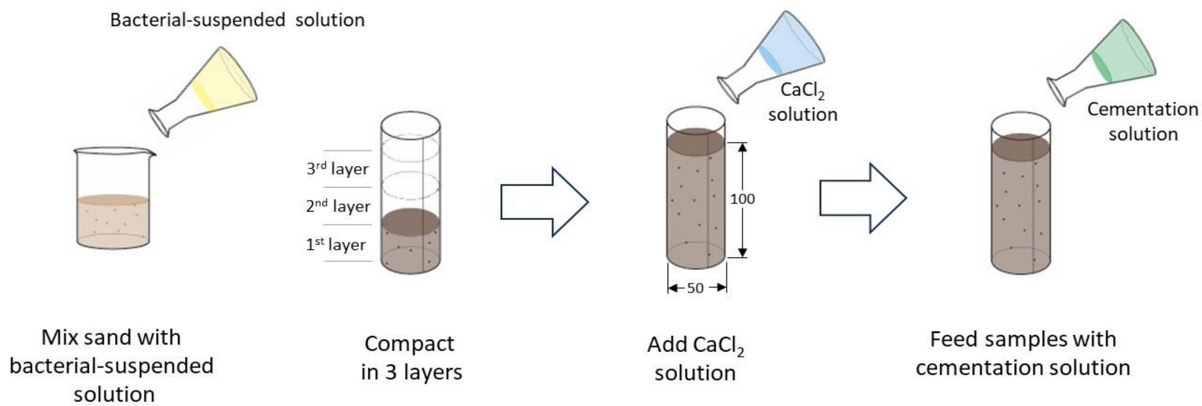


Fig. 1. Sand sample preparation.

Two investigations were conducted to examine the effects of repetitive cementation supply and levels as follows:

1) Investigation on repetitive cementation supply: Two feeding types were administered:
   - Single-feed type: The cementation solution, with a volume equivalent to 17.5% of the sand weight, was supplied to the samples once, with no further supply for 27 days.
   - Multiple-feed type: The cementation solution, with a volume similar to the single-feed type, was added daily to the samples at the same time for a duration of 28 days.

The results between these two feeding types were compared to assess the impact of repetitive nutrient supply on the properties of the sand samples.

2) Investigation on cementation levels:

The test was conducted on samples subjected to the multiple-feed type feeding regimen. The volume of cementation solution for daily dosage varied at 8.75, 13.13, 17.5, and 21.88% of the sand weight. These values corresponded to 50, 75, 100, and 125% of the optimum moisture content, respectively (**Table 2**).

Table 2. Sand sample mix proportions.

Designation	Sand (%)	Bacterial Solution (%)	$CaCl_2$ Volume (%)	Daily Treatment Solution (%)	Remarks
Control	100	0	0	0	No bacteria
N17.5/1				0.00	One-time feed
N8.75/28				8.75	Daily feed 28 days
N13.12/28	100	17.5	17.5	13.13	Daily feed 28 days
N17.50/28				17.50	Daily feed 28 days
N21.88/28				21.88	Daily feed 28 days

2.4 Test method

2.4.1 Direct shear

The Direct Shear test was conducted in accordance with the ASTM D3080 standard [28]. The procedure involved preparing test specimens in prism molds and placing them between two horizontal rigid plates of a direct shear apparatus. A vertical (normal) load was applied through the movable lower plate to ensure proper contact and eliminate any gaps between the specimen and the plates (the upper plate remained stationary). Subsequently, a horizontal shear force was gradually applied by moving the lower plate horizontally at a constant rate of 1 mm/min until failure occurred or the displacement reached 10%. Once either of these conditions was met, the test procedure was completed. The shear strength of the specimen (τ) was then calculated by dividing the peak shear force at failure (F) by the cross-sectional area of the specimen (A) (Eq. 3).

$$\tau = F / A \tag{3}$$

Using the shear strength, the angle of internal friction (φ) can be calculated by dividing the shear force (F) with the applied normal load (N) (Eq. 4).

$$\tan(\varphi) = F / N \tag{4}$$

2.4.2 Water permeability

The water permeability test was conducted in accordance with ASTM D2434 standard [29]. Sand samples were placed in a permeameter mold and secured to the test apparatus, which includes a constant head reservoir, a sample cell, and a water collection tank. The samples were saturated with water until a constant water level was maintained at the top. Subsequently, the permeability of the soil was measured by allowing water to flow through the saturated sample under a constant head condition. Flow rate and corresponding hydraulic gradients or heads were recorded at multiple points, ensuring the flow rate remained relatively constant throughout the test. The coefficient of permeability (k) was calculated using Eq. 5.

$$k = \frac{\Delta V \times L}{A \times \Delta h \times \Delta t} \tag{5}$$

Where: k is hydraulic conductivity (m/s), ΔV is Quantity of the permeated water (m^3), L is specimen height (m), A is sand sample cross-sectional area (m^2), Δh is sand samples' head loss (m), and Δt is experimental time frame (s).

2.4.3 Unconfined compressive strength

The unconfined compression test was conducted following the ASTM D2166 standard [30]. The test began by demolding the test sample, and since it was in a moist condition, it had to be handled with care to avoid excessive disturbance. The sample was then carefully placed in the unconfined compression test apparatus. Before compression, ensure that the sample is centered and aligned with the loading platens. Apply a vertical axial load to the soil sample at a constant rate (e.g., 0.5 to 2.0 mm/min). Record the axial load and deformation (strain) during the test. The test was performed until the sample reached failure, or when the displacement reached 10% of its initial height. The compressive strength is calculated by dividing the peak axial load by the cross-sectional area of the sand sample.

2.4.4 Microstructure

The microstructural analysis of the specimens was performed using a Scanning Electron Microscope (FEI QUANTA 450) equipped with a backscattered electron detector, operating at a magnification of 1000x. Additionally, the elemental composition of the samples was examined using the Energy-dispersive X-ray spectroscopy (EDS) technique. This analysis was conducted on the samples' control and the top layer of sand treated with MICP.

2.4.5 Chemical composition

The chemical composition was determined using X-ray Diffraction (RIGAKU Smart Lab) with a scanning 2θ range of 20-60° at a scanning 2θ rate of 1°/min. This test investigated the structure of calcium carbonate formed within the sand samples. Additionally, tests were performed to study the continuity of the mechanism that induces the formation of calcium carbonate from microbial activity. These tests were conducted on both control samples and samples taken from the top, middle, and bottom layers of the N17.50/28 sample.

3 Results & Discussion

3.1 Bacterial $CaCO_3$ sediment vs $CaCl_2$ concentration level

The study was conducted as a preliminary test to determine the optimum concentration of the Calcium Chloride solution that would yield the highest amount of calcium carbonate sedimentation. The calcium chloride solution was prepared under sterile conditions at various concentrations: 14.70 g/l (0.1 M), 22.05 g/l (0.15 M), 29.40 g/l (0.2 M), 36.76 g/l (0.25 M), and 44.11 g/l (0.3 M). The solution was also stirred well using a magnetic stirrer and then subjected to autoclaving at 121 ℃ with a steam pressure of 15 psi for 15 minutes.

Fig. 2 illustrates the correlation between the quantity of calcium carbonate sediments produced by *B.* thuringiensis TISTR 126 at different concentrations of calcium chloride. At a calcium chloride concentration of 0.10 M, the average sediment formed is 2.405 mg/ml. As the concentration increases to 0.15 M, the sedimentation increases to an average of 2.665 mg/ml. Continuing the trend, a calcium chloride concentration of 0.20 M results in an average sediment of 2.82 mg/ml. The highest concentration of 0.25 M yields the greatest sediment formation, with an average of 3.08 mg/ml. Finally, at a concentration of 0.30 M, the average sediment is slightly reduced to 2.715 mg/ml.

This trend suggests that there is a positive correlation between the concentration of calcium chloride and the amount of calcium carbonate sediment produced by *B.* thuringiensis TISTR 126. The increase in calcium chloride concentration seems to enhance the microbial-induced calcite precipitation process, resulting in greater calcium carbonate sediment formation. However, with further increase of calcium chloride concentration higher than 0.25 M, a decline in the calcium carbonate sediment yield was observed. This decrease can be attributed to the inhibitory impact of elevated calcium chloride concentrations on urease activity [31].

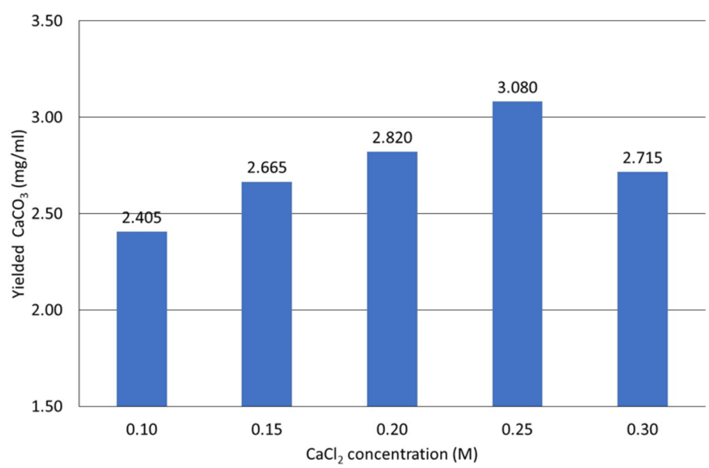


Fig. 2. Calcium carbonate sediments by *B. thuringiensis* TISTR 126.

3.2 Properties of sand mixed with bacteria

3.2.1 Weight increment percentage

This section discusses the effect of cementation supply on the weight increment percentage of sand mixed with bacteria.

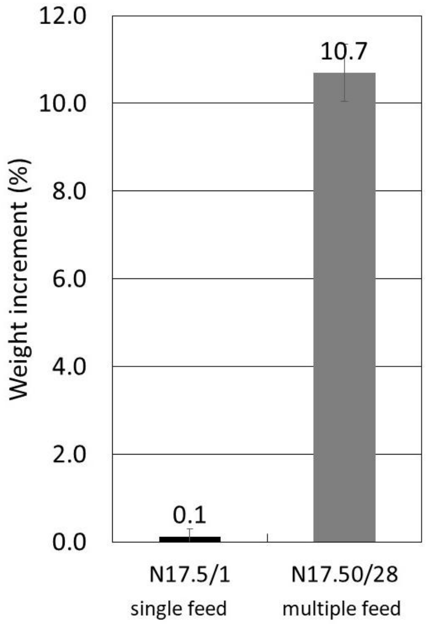
The physical appearance of the sand samples, as shown in **Fig. 3,** demonstrates the impact of the Microbially Induced Calcium Carbonate Precipitation (MICP) process under different feeding conditions. While the untreated control sample exhibits a uniform loose structure, MICP-treated specimens show notable changes on the outer surface. For the sample treated with the optimal feeding condition (N17.5/28), the surface appears denser and more consolidated compared to other specimens. This denser outer layer is attributed to the sufficient and balanced calcium carbonate precipitation induced by MICP over 28 days.

For the sample treated with excessive cementation solution (N21.88/28), the surface appears slightly porous. This porosity likely results from overproduction of calcium carbonate, which can lead to crystal overgrowth and reduced compaction efficiency. Such overgrowth may also hinder proper bonding between sand particles, contributing to the observed porous appearance.

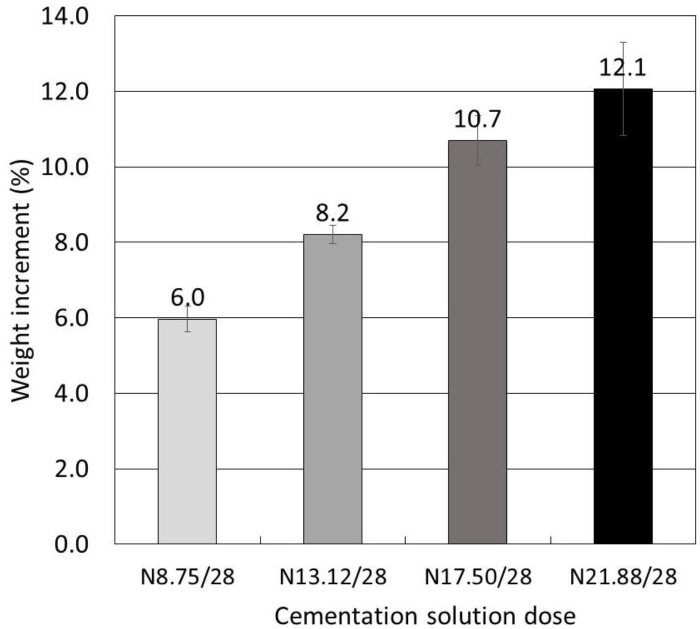
The bottom portions of the MICP-treated specimens display a darker color compared to the rest of the sample. This discoloration could be attributed to limited bacterial activity at the bottom, potentially caused by insufficient oxygen levels or an uneven distribution of nutrients and bacteria. Such conditions may have hindered calcium carbonate precipitation, leaving behind organic residues or unutilized nutrients, which contribute to the darker appearance. Additionally, the uneven distribution of the cementation solution—supplied from the top surface—might have resulted in reduced penetration and less effective precipitation at the lower regions of the specimen.

Fig. 3. Structure of each sand samples.

Regarding repetitive cementation supply, a comparison is made between samples subjected to single-feed and multiple-feed regimens over 28 days. The single-feed samples received a cementation solution equal to 17.5% of the sand weight once. In contrast, the multiple-feed samples were supplied with the same amount of cementation solution daily for 28 days, as described in Section 2.3. The results are depicted in **Fig. 4a.** Based on the test results, the weight of the sand sample subjected to single-feed hardly changed from its original weight. This lack of change is perhaps attributed to the limited urease activity resulting from insufficient nutrient supply. Conversely, with nutrients being supplied daily to the sand for 28 days, the weight of the sample increased by approximately 10.7%. This indicates a significant rise in urease activity with continuous daily nutrient supply, resulting in a substantial deposition of calcium carbonate sediment and subsequently a weight increase of about 10.7%.



(a) Different feed frequencies



(b) Different cementation dosages

Fig. 4. Weight increment percentage of sand samples.

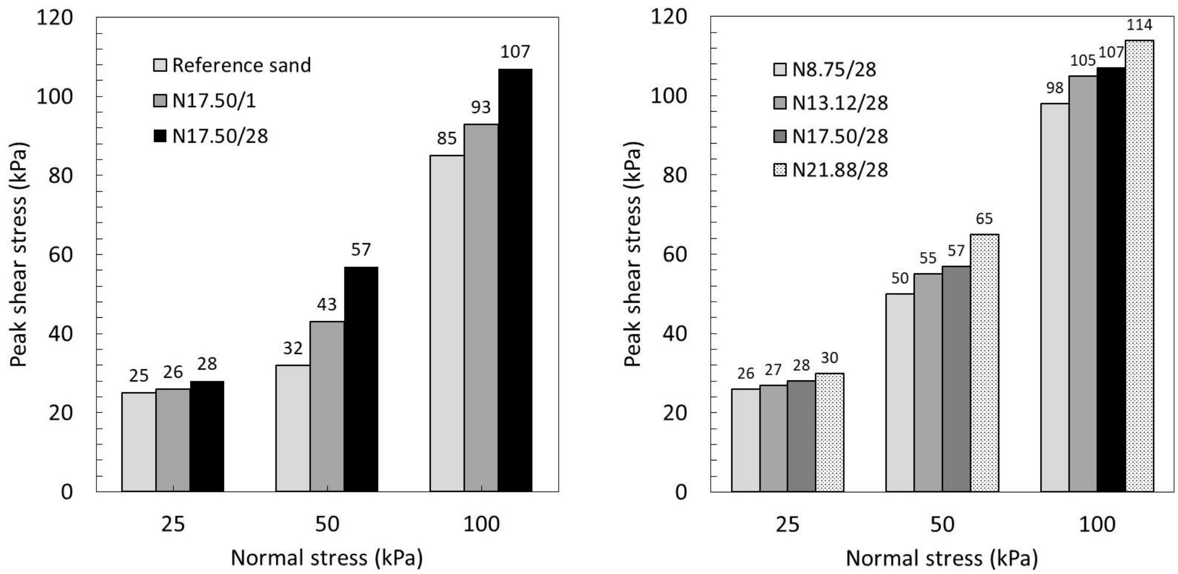
In the examination of the impact of different cementation volumes, four distinct volumes of cementation solution (representing 50, 75, 100, and 125% of the optimum content) were daily supplied to the sand sample over a period of 28 days. The results, as depicted in **Fig. 4b,** indicate that as the cementation solution volume increased from 50% to 125%, there was a corresponding increase in weight, ranging from 6 to 12.1%. This indicates that as the volume of cementation solution increases, it provides more nutrients, urea, calcium, and carbonate ions for the MICP process and supports higher bacterial activity. All these factors combined contribute to an increase in the formation of calcium carbonate within the sand matrix, resulting in a corresponding increase in weight.

3.3 Shear strength & Angle of internal friction

The shear strength results for each sand type are depicted in **Fig. 5.** The findings reveal a trend: an increase in the number of feeding times led to an increase in shear strength across all ranges of applied normal forces. For instance, when subjected to a normal force of 100 kN, the shear strength is as follow: 28 kN for the untreated sand sample, 57 kN for the sand sample with a single bacterial treatment and a single feed of cementation solution, and 107 kN for the sand sample treated with bacteria and subjected to multiple feeds of cementation solution over a 28-day period (**Fig. 5a**). This pattern points out that providing bacteria with a daily supply of cementation solution resulted in a considerable enhancement of the sand's shear strength.

Fig. 5b illustrates the influence of varying cementation solution volumes on the shear strength of the sand samples. Like the influence on density, it is evident that as the quantity of cementation solution in the multiple feeding pattern increases, the shear strength of the sand also increases. This phenomenon is attributed to the rise in urease activity with increasing cementation volume, resulting in a greater deposition of calcium carbonate. This leads to the better compaction of the sand, resulting in higher density and thus, an increase in shear strength.

This finding aligns with the study by Karimian et al. [32], which reported that shear strength improves with higher calcium carbonate content in samples. Furthermore, the shear strength of sand treated with the multiple-feed method increased as the daily cementation solution volume rose, leading to higher calcium carbonate content in the samples. This observation supports prior research indicating that increased calcium carbonate content enhances the sand's shear strength.



(a) Different feed frequencies (b) Different cementation dosage

Fig. 5. Shear strength of sand samples.

In terms of sand internal friction, it refers to the internal resistance or frictional force that sand particles exert on each other when subjected to an applied force or load or the resistance to sliding or shear deformation within a mass of granular material, such as sand. This angle quantifies how easily the sand particles can move past each other when subjected to external forces, such as the weight of a structure or the forces generated by an earthquake or other external loads. The angle of internal friction is a useful parameter for classifying the packing characteristics of sand and granular materials, as illustrated in **Table 3** [33].

Table 3. Classification of soil based on friction angle [33]

Description	Soil Friction Angle (°)
Very loose sand	<30
Loose sand	30-35
Compacted sand	35-40
Dense sand	40-45
Very dense sand	>45

Results on the friction angle are depicted in **Fig. 6.** The data obtained demonstrates that the application of bacterial treatment and cementation processes significantly improved the internal friction angle of the sand. The reference sand, which received no bacterial treatment, exhibited the lowest internal friction angle at 39°, classifying it as 'Compacted Sand.' In contrast, the sand samples subjected to single and multiple-feed MICP treatments with same volume of cementation solution, such as N17.5/1 and N17.5/28, displayed an increase in internal friction angles to 42° and 46°, categorizing them as 'Dense Sand' and ‘Very Dense Sand’, respectively (**Fig. 6a**).

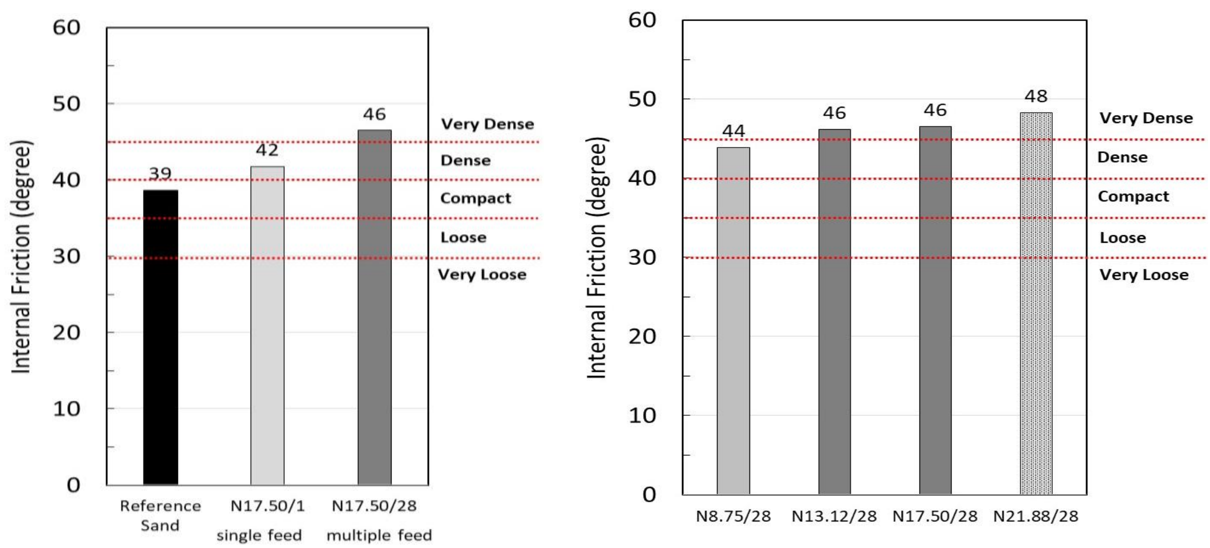
Regarding the multiple-feed treatments (N8.75/28, N13.12/28, N17.5/28, N21.88/28), the results revealed that higher volumes of cementation solutions generally led to increase in the internal friction angle, ranging from 44 to 48. These angles can be categorized as 'Dense Sand' and 'Very Dense Sand' (**Fig. 6b**).

Changes in the internal friction angle were attributed to the increased calcium carbonate content in the sand, which correlated with the volume of daily cementation solution. Untreated sand was categorized as compacted sand, while sand treated with the single-feed MICP process transitioned to dense sand, and sand treated with the multiple-feed process became very dense sand. Additionally, higher volumes of daily cementation solution in the multiple-feed process contributed to a more pronounced increase in the internal friction angle. This finding aligns with previous research suggesting that increasing calcium carbonate content enhances both the density and internal friction angle of sand. For instance, the study by Karimian et al. [32] utilized the bacterium Sporosarcina pasteurii PTCC 1645 in the MICP process to improve the bonding of loose sand. The process involved injecting bacterial and cementation solutions from the bottom for 5 days, followed by surface percolation for another 5 days. After curing at room temperature for 14 days, the internal friction angle of the sand increased from 34.5 degrees (loose sand) to 40.4 degrees (dense sand), representing an approximate 17% improvement.

In comparison, the multiple-feed technique (N17.50/28) in this study increased the internal friction angle of sand from the compacted reference sand (39 degrees) to 46 degrees, representing an approximately 18% improvement, which is consistent with the results of Karimian et al. [32]. The multiple-feed technique was more effective than the single-feed technique (N17.50/1), which raised the angle to 42 degrees (from reference sand), reflecting a 10% increase. These results clearly demonstrate that the calcium carbonate content generated through the multiple-feed process significantly enhances the stabilization and strength of the sand.

This study found that the MICP process effectively enhances shear strength by forming calcium carbonate bonds between sand particles, resulting in increased density. Untreated sand exhibited a friction angle of 39°, classifying it as compacted sand. After MICP treatment, the friction angle increased to 44-48°, categorizing the sand as dense to very dense. In comparison with the conventional soil-cement method, the findings from Boutouba et al. [34] indicated similar friction angles (40-48°) with a cement content of 2-10%, depending on the mix proportions.

In conclusion, it can be inferred that with the appropriate supply of nutrients (both in terms of volume and frequency), the sand can indeed undergo a transformation, evolving from a compacted state to becoming very dense through the proposed Microbially Induced Calcium Carbonate Precipitation (MICP) process.



(a) Different feed frequencies (b) Different cementation dosage

Fig. 6. Internal Friction angle of sand samples.

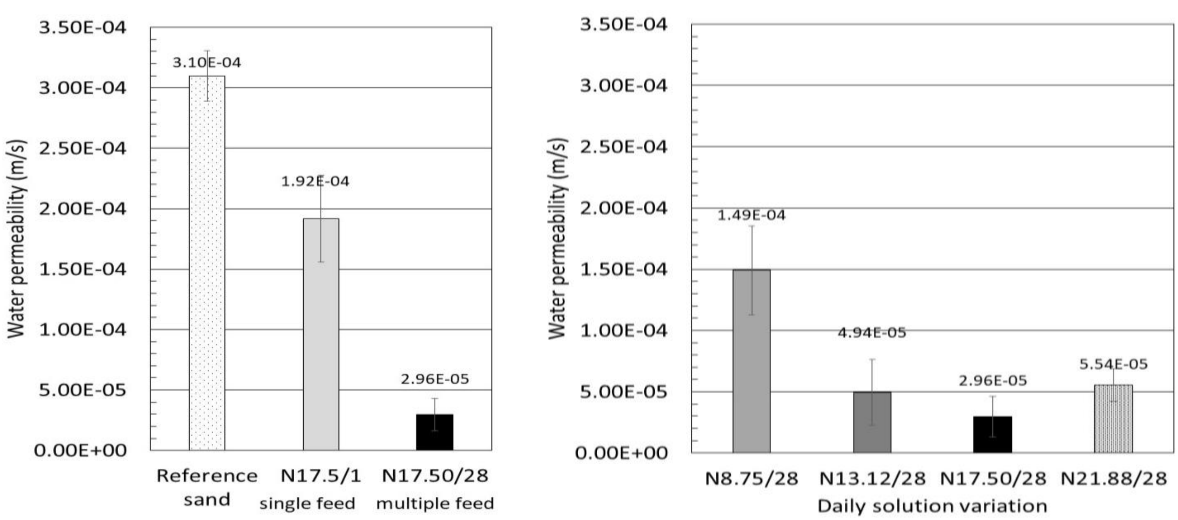
3.4 Water permeability

Fig. 7 illustrates the correlation between the supply of cementation solution and the water permeability coefficient (k) of sand samples. In relation to supply frequency (**Fig. 7a**), an increase in the number of feedings from 1 to 28 days results in a reduction of the water permeability coefficient (k) in sand samples by approximately 85%. This reduction is attributed to the calcium deposits generated through the MICP process, which effectively fill the voids between sand particles, leading to increased sand density and consequently, a decrease in permeability.

In terms of supply volume, it was observed that an increase in the daily proportion of cementation solution led to a reduction in water permeability (k). Among the multiple-feed samples, the one with a 100% volume ratio (N17.50/28) displayed the lowest water permeability coefficient, measuring 2.96×10^{-5} m/s. This indicates the highest resistance to water flow compared to the control sand, which had a water permeability coefficient of 3.10×10^{-4} m/s, roughly ten times higher.

This reduction in water permeability can be attributed to the effective filling of voids between sand particles with calcium carbonate sediments. However, it is noted that in the case of sample with 125% volume ratio (N21.88/28), there was a trend towards a higher water permeability coefficient, measuring 5.54×10^{-5} m/s. This addition could be attributed to overgrowth or premature crystallization, leading to the crystals' lower stability (as observed in microscope image). Consequently, the low-stability crystal structures were more easily dissolvable in water, generating voids in the sand structure [35].

According to the result from this study, it was found that the increase in calcium carbonate content led to higher water tightness of sand, both in the single-feed (N17.50/1) and multiple-feed (N8.75/28, N13.12/28 and N17.50/28), with the calcium carbonate content in the sand samples ranging from 0.1 to 10.7%. These findings align with research suggesting that increasing calcium carbonate content enhances the sand's water tightness. According to Li et al. [36] experiments, which investigated the effect of cementation solution quantities on the efficiency of the MICP process in sand using Bacillus pasteurii ATCC 11859 bacteria. Additionally, the study by Yuan et al. [37], which conducted similar experiments but substituted bacterial solution with pure urease enzyme solution (Enzyme-Induced Calcium Carbonate Precipitation), also found that an increase in cementation solution quantities resulted in higher water tightness of the sand, corresponding to the increase in calcium carbonate content in the sand samples.



(a) Different feed frequencies (b) Different cementation dosage

Fig. 7. Water permeability of sand samples.

Compared to traditional methods such as soil-cement, which uses 3–7% cement to improve sand stability, the water permeability of sand can be significantly reduced. Typically, cemented sand exhibits water permeability values ranging from 10^{-7} to 10^{-8} m/s. However, for certain types of sand with uniform particle sizes, permeability may be higher, ranging from 10^{-5} to 10^{-6} m/s, reflecting the variability in results obtained from the soil-cement treatment [3], [38].

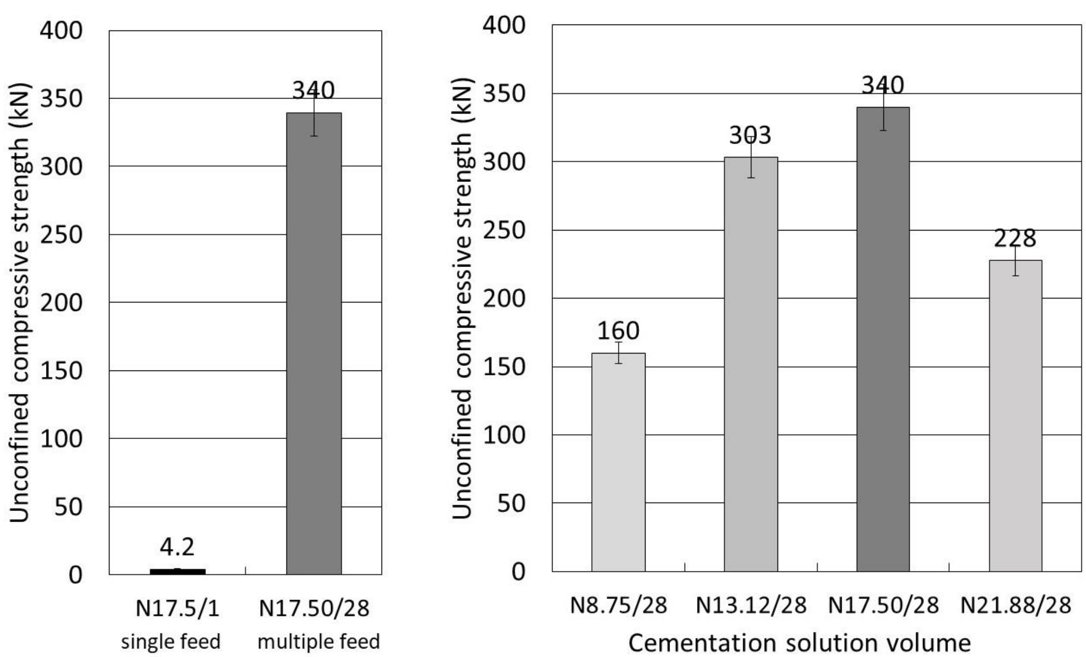
In this study, uniform particle sizes sand treated with the multiple-feed process, such as N17.50/28, exhibited a water permeability of 2.96×10^{-5} m/s, which falls within the range of cemented sand with uniform particle sizes. Achieving this level of water permeability without using cement demonstrates the potential of the MICP process in reducing sand's water permeability.

However, differences between the water permeability of MICP-treated sand and cemented sand may arise from variations in the pore-filling mechanisms. Cemented sand generally exhibits a denser structure, resulting in more continuous bonding within the sand matrix. In contrast, MICP-treated sand relies on calcium carbonate's localized and non-uniform precipitation. This precipitation accumulates more on the surfaces than within the matrix. Therefore, regarding water permeability, the MICP process may not yet fully match to the performance of cemented sand.

3.5 Unconfined compressive strength

Fig. 8 illustrates the results on the unconfined compressive strength (UCS) of sand samples with different feed frequencies and cementation volumes. In terms of feed frequency, the results shown in **Fig. 8a** reveal a substantial increase in compressive strength when comparing single-feed and multiple-feed samples, with values ranging from 4.2 kPa to 340 kPa. This rise in compressive strength corresponds with the findings related to water permeability. The samples subjected to multiple-feed patterns exhibited significantly lower water permeability, indicating a denser microstructure, and consequently, higher compressive strength. The increased frequency of feedings enhances urease activity and allows for a greater deposition of calcium, contributing to the observed improvements.

In terms of supply volume, the incremental increase in cementation volume, starting from 8.75% to 17.50%, exhibited a gradual increase in compressive strength from 160 to 340 kPa. However, with a further increase in the cementation volume to 21.88%, the compressive strength decreased to approximately 228 kPa.



(a) Different feed frequencies (b) Different cementation dosage

Fig. 8. Unconfined compressive strength of sand samples.

This trend aligns with the findings related to permeability, where permeability decreases as the cementation volume increases up to around 17.50%, after which it begins to increase. The increase in permeability was attributed to potential overproduction of calcium carbonate sediment, creating voids, and leading to the development of porosity and debonding between sand particles [39]. Confirming evidence for this conclusion can be observed in the forthcoming microstructural analysis, as discussed in the next section.

Based on the study of the effect of the MICP process on the unconfined compressive strength of sand, it was found that both the type of sand and dry density influenced the compressive strength in the MICP process [40]. Choi et al. [19] used Sporosarcina pasteurii ATCC 11859 bacteria to precipitate calcium carbonate in Ottawa sand, with a controlled dry density of 1.7 g/cm^3. The samples were treated using a recirculated MICP process (each feeding solution flows through the sample from top to bottom via a peristaltic pump) for a period of 14 days (converted to surface percolation time). The results showed that the bacteria could precipitate approximately 5.5-8.5% of calcium carbonate and achieved an average compressive strength of 1000 kPa. In contrast, this study's maximum compressive strength is 340 kPa, with a calcium carbonate content of 10.7% (N17.50/28), which may differ due to dry density and the type of sand used.

Conversely, the use of natural desert sand in the MICP process results in a significant reduction in compressive strength. This can be attributed to the unique physical characteristics of desert sand, such as its well-rounded particle shape and narrow particle size distribution, which reduce the interparticle friction and bonding efficiency. For instance, in the study by Li et al. [33], desert sand with a calcium carbonate content of 12-20% achieved compressive strength values of only 200-300 kPa. These results highlight the inherent limitations of desert sand in achieving high compressive strength compared to sands with more angular particles and broader size distributions.

In terms of the feeding cycle, increasing the number of feeding cycles resulted in a higher compressive strength of the sand. This finding aligns with the research suggesting increased feeding cycle increases calcium carbonate content. Li et al. [24] found that increasing the feeding cycle from single to double to triple feeding cycles, with each cycle lasting 7 days, led to an increase in calcium carbonate precipitation and higher compressive strength. The maximum compressive strength was observed at the triple feeding cycle, approximately 21 days, with a compressive strength of 1600 kPa (artificial sand). This experiment confirmed that increasing the number of bacteria or nutrients enhances compressive strength, and the number of feeding cycles also significantly contributes to increasing the compressive strength of the sand [23], [24], [35-37].

Compared with the conventional soil-cement treatment, the 28-day UCS achieved in this study was 340 kPa, with a calcium content of 10.7%. Traditional soil-cement stabilization typically achieves UCS values of 2,000-4,000 kPa with 8-10% cement [41]. While MICP-treated sand demonstrates lower strength, it offers significant sustainability benefits by avoiding cement use. This reduces greenhouse gas emissions and aligns with global efforts to minimize the environmental impact of construction.

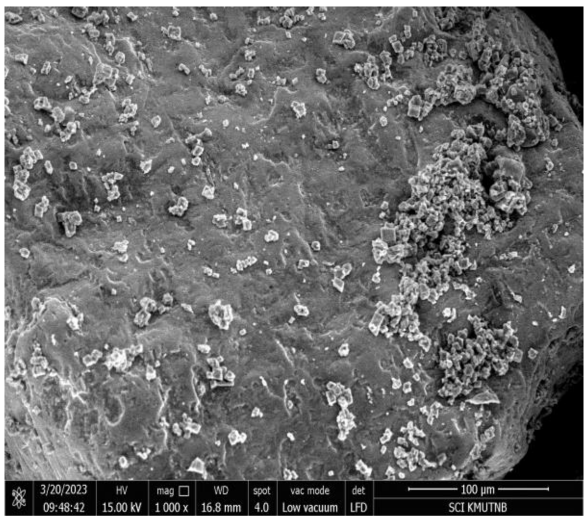
The tradeoff between strength and sustainability is evident. Although the UCS of MICP-treated sand is lower than that of cement-stabilized sand, the absence of cement reduces environmental impacts. The MICP process relies on naturally occurring bacteria and renewable materials, making it a more sustainable alternative for certain applications. Further studies are needed to enhance the scalability and uniformity of MICP treatment, particularly in addressing challenges like uneven calcium carbonate precipitation at sample bottoms.

3.6 Microstructure analysis

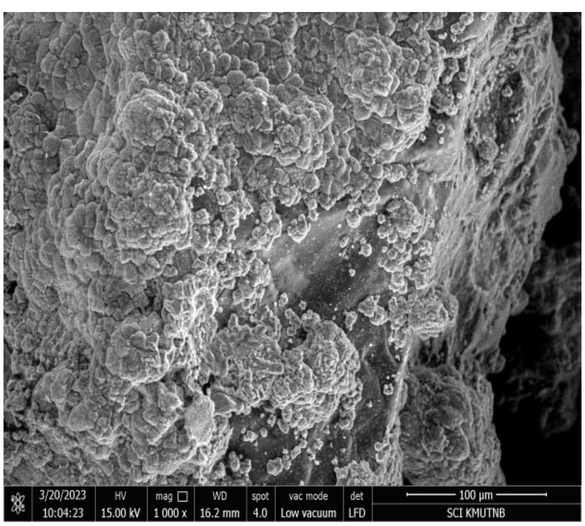
The SEM images and EDX analysis results are presented in **Fig. 9.** Both investigations yielded results that can be categorized into two distinct groups. Group 1 comprises single-feed samples (N17.5/1) (**Fig. 9a**), while Group 2 consists of samples subjected to multiple feeds for 28 days with varying cementation supply volumes, namely N8.75/28, N13.12/28, N17.50/28, and N21.88/28 (**Fig. 9b-e**).

It is evident that Group 1 exhibited lower calcium carbonate sedimentation compared to Group 2, as indicated by the sparser distribution of calcium carbonate particles on the surface of sand particles (**Fig. 9a**). This discrepancy can be attributed to the limited calcium carbonate sedimentation due to the single feeding and the absence of further cementation solution supply. EDX analysis further confirmed that the surface of the N17.5/1 sample contained a lower calcium phase content than that with daily feed (N17.5/28) (**Fig. 9a**). Additionally, the more exposure of sand particles in Group 1 resulted in a higher silica phase presence compared to the other samples.

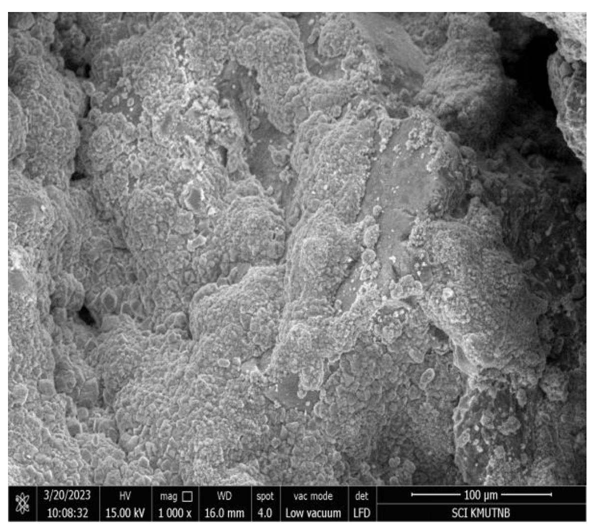
In Group 2, as the number of feedings and cementation volume increased, the sedimentation of calcium carbonate also increased gradually to the extent that particles collided, eventually covering most of the sand particle surfaces (**Fig. 9b-d**). EDX analysis further supports this observation, revealing an increase in the calcium phase and a decrease in the silica phase of the sand particles. Based on this study's results, increasing the feeding cycle affects calcium carbonate coverage. This finding aligns with Fouladi et al. [39], who identified optimal feeding conditions for the MICP process. Their SEM images illustrated calcium carbonate precipitation during the MICP process. The surface coverage of calcium carbonate on sand particles becomes more evident with additional feeding cycles. However, excessive feeding solution volumes or accelerated precipitation reactions lead to overgrowth crystallization, resulting in unstable crystal structures. This negatively impacts the mechanical and physical properties of MICP-treated sand.



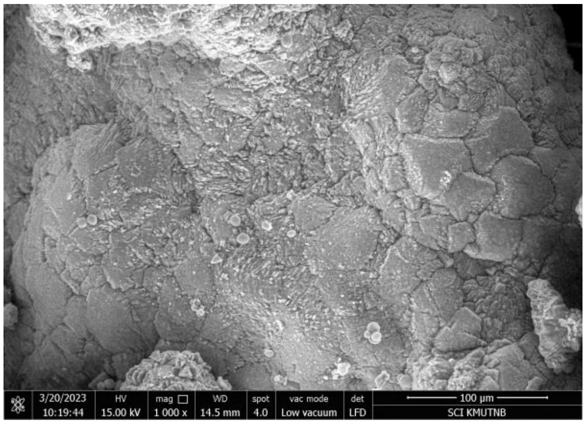
（a）N17.50/1



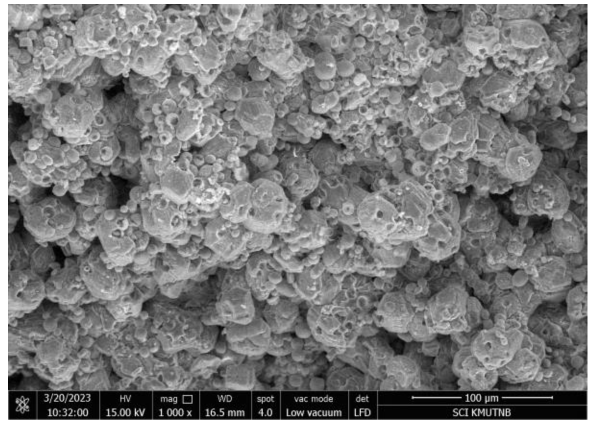
(b) N8.75/28



(c) N13.12/28



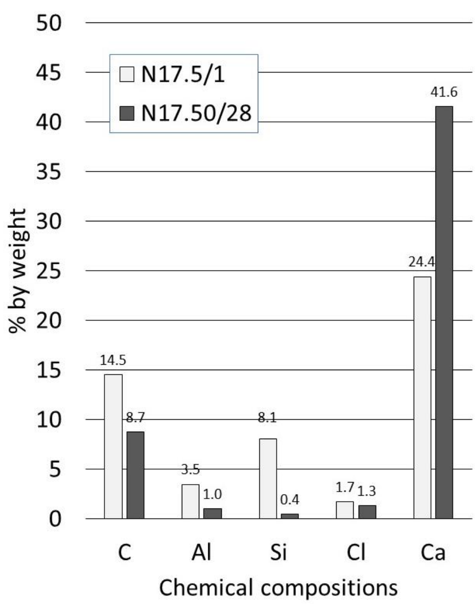
(d) N17.50/28



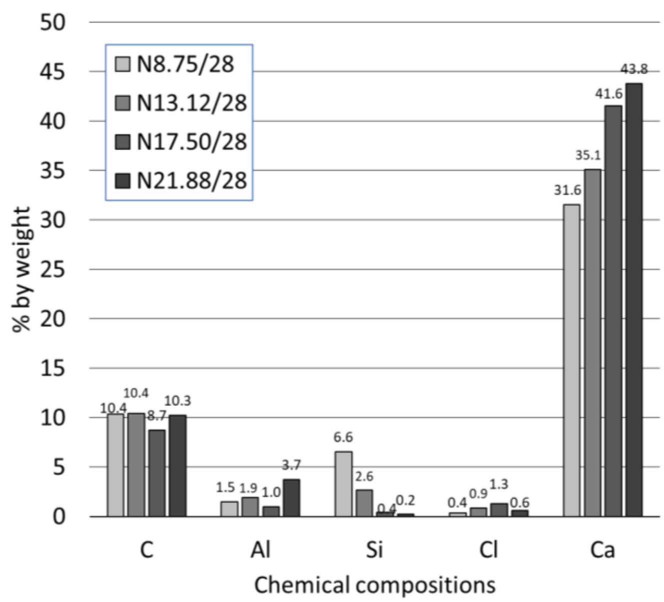
(e) N21.88/28

Fig. 9. SEM images of all MICP-treated sand samples.

However, it's worth noting that in the case of N21.88/28, which received the highest daily supply of cementation volume (approximately 125% of the optimum volume), the calcium carbonate appeared to be overly produced, and the large number of excess calcium carbonate particles were observed on the sand surface. This leads to the formation of voids and high porous (less compacted) sand samples (**Fig. 9e**). This finding is consistent with the observed decrease in compressive strength and the increase in permeability that occurs when the cementation volume exceeds the optimum content of 17.5%. The EDX analysis further supports this finding, revealing the highest calcium phase and the lowest silica phase for N21.88/28 (**Fig. 10b**).



(a) Different feed frequencies



(b) Different cementation dosage

Fig. 10. EDX analysis of sand samples.

4 Conclusions

The study investigated the impact of nutrient supply, calcium chloride (cementation solution), and B. thuringiensis TISTR 126 on Microbially Induced Calcium Carbonate Precipitation (MICP) in sand stabilization. The choice of B. thuringiensis TISTR 126 as the bacterial strain offers advantages including availability, non-pathogenic nature to humans, and cost-effectiveness. The investigation included the effects of both the quantity and frequency of the cementation solution on sand properties, including water permeability, unconfined compressive strength, internal friction angle, microstructural characteristics, and calcium carbonate content. Results showed that increased cementation solution supply, in terms of both quantity and frequency, led to improvements in sand properties.

In the case of internal friction and shear stress, the highest cementation ratio (N21.88/28) with a multiple feeding pattern exhibited the most substantial internal friction angle of 48°, indicating a very dense sand structure. A proper supply of nutrients and cementation solution can transform sand from compacted to very dense through MICP.

Regarding permeability and unconfined compressive strength, increasing the number of feeding times and cementation volume up to the optimum level (17.5%) led to improvements in both properties. However, excessive cementation volumes, as observed in N21.88/28, resulted in overgrowth of calcium carbonate, internal cracks, reduced compressive strength, and increased permeability.

Both SEM and EDX analyses supported these findings by demonstrating an increase in $CaCO_3$ formations and variations in calcium and silica phases with different cementation volumes.

Limitations and Future Studies: This study is limited to laboratory-scale experiments, which may not fully capture the complexities of real-world applications. Field-scale studies are essential for advancing the practical implementation of the MICP process. These studies must account for additional factors, such as varying soil characteristics, climatic conditions, curing environments, and interactions with local microorganisms. Addressing these challenges will be critical to ensuring the effective and scalable use of MICP in diverse, real-world settings.

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Credit authorship contribution statement

Sumonthip Kongtunjanphuk : Supervision, Conceptualizing, Writing – Original Draft. **Satharat Pianfuengfoo**: Investigation, Formal analysis, Writing – original draft, Review and Editing. **Hexin Zhang:** Validate, Review and Editing. **Worathep Sae-Long**: Validate. **Pithaya Jamsawang:** Validate. **Piti Sukontasukkul**: Conceptualization, Funding acquisition, Supervision, Writing – original draft, Review and Editing.

Conflicts of Interest

The authors declare that they have no conflicts of interest to report regarding the present study.

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