

# High-level of bio-cementation by microbially induced carbonate precipitation technique with nano calcite seeds

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## KEYWORDS

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Bacteria  
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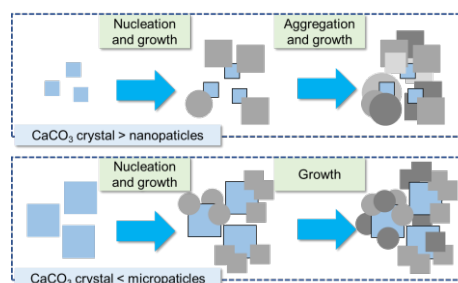
## ABSTRACT

The bio-cementation process through bacterial mineralization has emerged as an eco-friendly solution for soft or loose sandy soils, particularly in areas with ground slopes and high rainfall. This approach is aligned with sustainable development goals, promoting environmentally responsible and long-lasting solutions. In this context, ureolytic bacteria capable of decomposing urea for calcium carbonate precipitation are the primary agents for bio-cementation. This study explores the effects of nano calcite as nucleation sites on the MICP capacity to enhance the bio-cementation process. The microstructure of the precipitation was analyzed alongside its capacity for MICP. In addition, a simulated rainfall model was set up to evaluate the erosion resistance of sand samples. The findings of this study can guide the development of eco-friendly solutions for soil stabilization in environmentally vulnerable areas.

## 1. Introduction

Microbially Induced Carbonate Precipitation (MICP) is a promising eco-friendly solution for soil stabilization, especially in areas with high rainfall and unstable ground slopes. In recent years, researchers have explored using ureolytic bacteria to induce calcium carbonate precipitation to strengthen sandy soils. However, to enhance the effectiveness of this process, the addition of nano calcite as nucleation sites has been proposed [1–7]. Several MICP-based projects have been explored for civil engineering applications in developing and developed countries. For instance, spore-forming *Bacillus subtilis* was examined to form calcite for concrete repair and soil-sand treatment based on its capacity for calcite precipitation in lab-scale experiments [8–10]. Since the previous results significantly improved soil's physical and geotechnical properties, MICP using bacteria shows promising ability for large-scale applications. As a preliminary study, *Bacillus subtilis* [11, 9, 12] was used as a treatment technique for sand and soil-sand materials in the LAB scale. Soil-isolated *Bacillus* species were tested for sand stiffening ability using a syringe set-up with daily nutrient addition. This treatment method for soil and sandy soil shows benefits with simple technique and preparation. However, there is still a lack of knowledge on bio-mineralization in different environments. On the other hand, increasing calcite precipitation capacity was the focus. MICP involves using bacteria to facilitate the precipitation of calcium carbonate ( $\text{CaCO}_3$ ) in soils or sand, forming a bio-cement. One of the challenges in MICP is accelerating the nucleation and crystal growth of

$\text{CaCO}_3$  [13–16]. This challenge can be addressed by adding nano calcite seeds to the solution. Nano calcite seeds act as nucleation sites for  $\text{CaCO}_3$  crystals, providing a surface on which the crystals can grow. The presence of these seeds results in a more efficient and faster precipitation process. In addition to accelerating nucleation, nano calcite seeds can facilitate crystal growth by providing a framework for the  $\text{CaCO}_3$  crystals to grow around. Adding nano calcite seeds to MICP allows greater control over the resulting bio-cement. By manipulating the seeds' size and quantity, the resulting bio-cement's properties can be tailored to meet specific engineering requirements. This study utilized *Sporosarcina ureilytica* on sand samples to gauge the degree of bio-cementation via water prevention testing. Nano calcite was employed to expedite and enhance biom mineralization. Laboratory-scale erosion and wash-out experiments were carried out to visually evaluate the effectiveness of bio-cementation.



**Figure 1.** The process of accelerating nucleation and facilitating crystal growth in MICP using nano calcite seeds.

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## 2. Materials and Experiments

For this study, the biomineralization agent was *Sporosarcina ureilytica*, a gram-positive, aerobic, non-pathogenic bacterial strain that breaks down urea. The bacteria were sourced from corals in Kien Giang province, Vietnam, and used in their spore form, which can withstand high alkalinity and urea concentrations up to 3M, with a urea hydrolysis rate of 0.76 Mm/min. The density of the bacteria was 108-109 colony-forming units per milliliter (CFU/mL). Urea was the nutrient source, and calcium chloride was used as the calcium source for mineralization. Saigon Nanomat Co., Ltd in Vietnam supplied the nano calcite, which had a purity of 98% and an average particle size of about 52 nm. The bacterial solution with and without nano calcite was analyzed to prepare bio-cementitious sand samples. Microstructural analyses were conducted post-precipitation. Sand with a density of 1400 kg/m<sup>3</sup> was segregated into three series based on particle size. It was mixed with bacterial solution and nano calcite (3 wt% concentration) before being placed in a mold. Light compaction is then applied to give initial bonding during the forming process. The sand samples were then cured in an airtight container with a constant humidity of about 80%. After a specified period, the sand samples were removed from the mold for mechanical testing. Three groups of samples were divided: the bacterial treatment group without nano calcite (ordinary MICP), the bacterial treatment group with nano calcite added (modified MICP), and the control group without bacteria (reference).

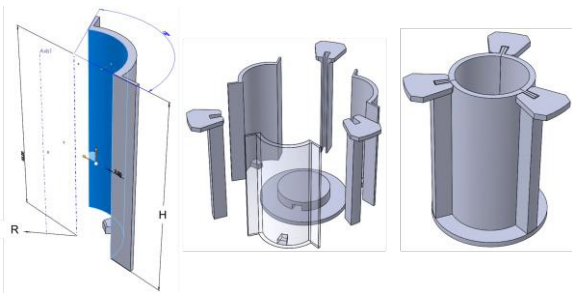


Figure 2. Specific-design mold for shaping preparation of sand samples.

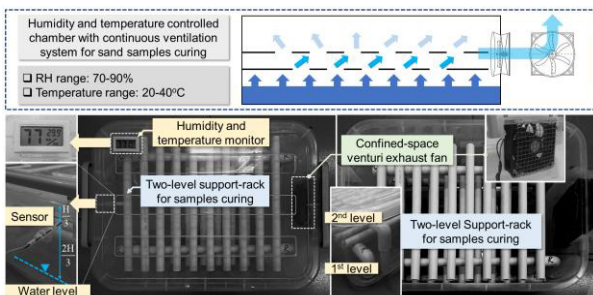


Figure 3. Humidity and temperature controlled cabinet for sand samples curing.

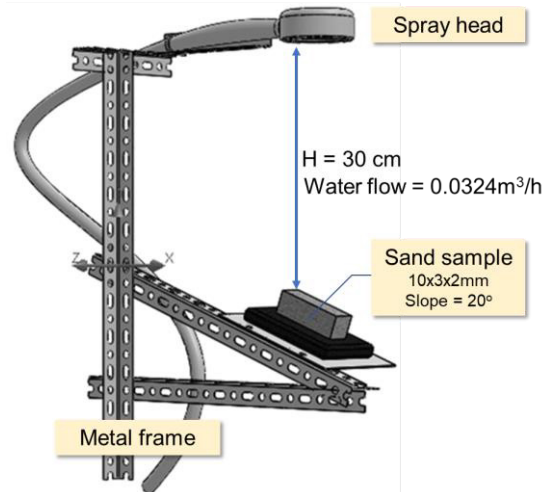


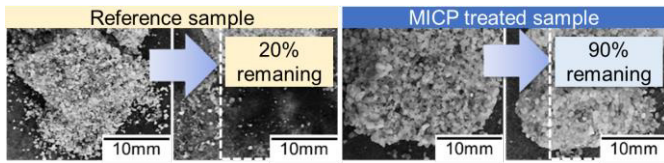
Figure 4. Testing system to simulate rain-fall water erosion and resistance of MICP-treated ability.

In the context of conducting MICP testing, using molds specifically designed (Figure 3) to shape sand samples is of utmost importance. In order to prevent the MICP solution from escaping during the testing process, these molds should be leak-proof. Furthermore, it is essential to ensure that the molds are easy to assemble and disassemble to facilitate the removal of the sand sample once it has been set. The use of molds that meet these requirements above is crucial in ensuring the accuracy and reliability of MICP testing. The shape and size of the original and post-molded samples were compared. It is imperative to note that proper ventilation is critical for the success of the MICP process. However, if appropriate precautions are not taken, pests such as insects can invade the chamber, causing potential damage to the sample. Nevertheless, researchers in this study have addressed these concerns by developing an innovative chamber system and utilizing a low organic nutrient composition, effectively resolving the pest infiltration issue (Figure 3). To assess the ability of bio-cement sand grains, a series of tests are conducted, including those designed to evaluate resistance to water column pressure and runoff erosion via a simulated rainwater system (Figure 4).

## 3. Results and discussions

### 3.1. Strength restoration

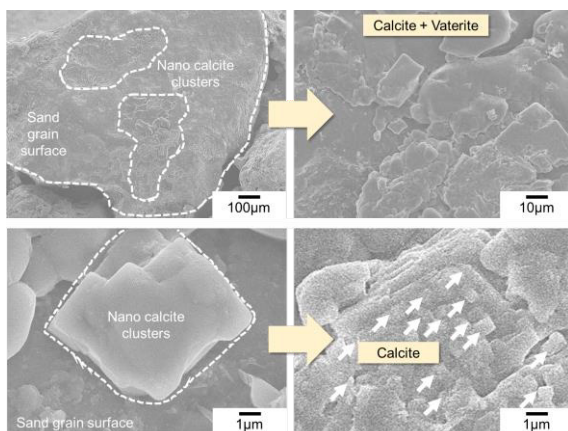
After conducting the water (Figure 4), a small amount of reference sand sample was observed to remain (20%). The sample consisted mainly of refined grains and was slightly damp. Although a few larger particles were present, the sample had a relatively uniform appearance. In contrast, sand samples treated with MICP maintained their volume (90%), shape, and compatibility, highlighting the effectiveness of this method in preventing erosion and maintaining the sample's integrity.



**Figure 5.** Comparison of sand samples pre- and post-rainfall water erosion test.

### 3.2. Microstructure analysis

The presence of nano calcite seeds can significantly influence the nucleation and growth process of  $\text{CaCO}_3$  crystals (Figure 6). Nanocalcites provide a surface for the  $\text{CaCO}_3$  ions to adsorb onto, promoting the formation of crystalline structures [17, 3]. The size and distribution of the nano calcite seeds can also affect the resulting crystal size and morphology. During the nucleation process,  $\text{CaCO}_3$  ions come together on the surface of the nano calcite seeds, forming small clusters of ions. As more ions join the clusters, they become denser, eventually forming a crystalline nucleus. The presence of nano calcite seeds can increase the nucleation rate, resulting in a more significant number of nuclei formed and a shorter nucleation time. Once the nucleation process is complete, the growth of  $\text{CaCO}_3$  crystals begins. The nano calcite seeds act as a template for the growth of the crystals, with the  $\text{CaCO}_3$  ions depositing onto the seed surface and extending outwards to form the crystal structure. The size and morphology of the nano calcite seeds can affect the orientation and arrangement of the  $\text{CaCO}_3$  crystals that grow on them, resulting in crystals with different shapes and sizes.



**Figure 6.** Nucleation and growth process of  $\text{CaCO}_3$  crystals with nanocalcites seeds presence.

## 4. Conclusion

The laboratory-scale experimental results have shown promising outcomes of the MICP-treated sand against washout and erosion. Utilizing a solution of urea-degrading bacteria in soil, combined with

nano calcite, has proven to be an effective technique in improving the properties of bio-cement sand. Moreover, the results of the tests conducted against water washout and wind erosion have been favorable, highlighting the effectiveness of nano calcite in accelerating the MICP process. Overall, the addition of nano calcite seeds to the MICP process offers a promising approach to accelerating nucleation and facilitating crystal growth, ultimately leading to the formation of stronger and more durable bio-cements. However, further experiments with larger-scale samples and under more realistic conditions are necessary to obtain more reliable data for field trials and practical applications.

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