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The influence of soil type on the effectiveness of biocementation

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ABSTRACT: Biocementtion, specifically Microbially Induced Calcite Precipitation (MICP), is a complex, environmentally friendly method for improving soil properties. While the process is influenced by many factors, this study focuses on the critical role of soil grain size in treatment efficacy. This study aims to investigate the influence of soil grain size on the efficacy of MICP by applying an immersing treatment with *Sporosarcina pasteurii* to two soils: a fine sand and a medium sand. The resulting mechanical strength was quantified by Unconfined Compressive Strength (UCS) tests and correlated with microstructural observations from Scanning Electron Microscopy (SEM). The results demonstrate that the treatment was significantly more effective in medium sand, which achieved a mean UCS of 275 kPa, approximately 2.3 times higher than the 119 kPa achieved by the fine sand. SEM analysis revealed this difference was due to the formation of robust, inter-particle calcite bridges in the medium sand, whereas the fine sand developed a less effective, surficial microcrystalline crust.

KEYWORDS: MICP, SEM images, biocemented sand, UCS.

1 INTRODUCTION

Traditional methods of soil stabilization, such as cement grouting, chemical injection, and deep mixing, can be effective but come with significant drawbacks. These include high energy consumption, a substantial carbon footprint, and the risk of groundwater contamination. In recent years, particular importance is given to environmentally friendly and sustainable solutions. Nature-inspired technologies have emerged as promising alternatives. Microbially Induced Calcite Precipitation (MICP) is a promising technique for soil modification, utilizing a biological and environmentally friendly process. Through the MICP process, the biocementation of soil and thus soil stabilization is achieved (Whiffin et al. 2007; Al Qabany and Soga 2013). The biocementation of soil is the process in which living microorganisms, under appropriate conditions, induce calcium carbonate precipitation. Precipitated calcite creates bonds between soil particles and fills the pores (DeJong et al. 2010), thereby improving its mechanical properties. The method has been studied as a soil improvement since the 2000s (Dejong et al. 2006; Whiffin et al. 2007) and remains an active area of research. The method connects knowledge from chemistry, microbiology and geotechnics, which makes it complex and brings a lot of challenges.

Microogranisms which might be used as an initiators of the calcium carbonate ($CaCO_3$) precipitation are ureolytic bacteria. One of the most frequently used for soil improvement is bacteria strain *Sporosarcina pasteurii* which naturally exists in soils (Whiffin et al. 2007), has high urease activity (Wu et al. 2021), and tolerate extreme conditions (Anbu et al. 2016). *Sporosarcina pasteurii* hydrolyse urea producing ammonia and carbon dioxide (Ferris et al. 2004) – Equation (1). The bacterial cell surface serves as a nucleation site with a negative charge, where positively charged calcium ions bind. Elevated pH and presence of calcium ions leads to calcium carbonate precipitation, what Equation (2) presents.

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

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

Calcium carbonate crystals form on the surface of bacteria, surrounding them.

In most of the cases, soil is subjected to MICP method by introduction bacterial solution, and cementation/mineralization solution with Ca^{2+} ions - to provide nutrients to the bacteria and allowed calcium carbonate precipitation.

There are many factors influencing precipitation of $CaCO_3$ in soils: soil type, injection method, bacterial concentration and cementation solution concentration. Most of the research are focused on biocementation of sandy soils, which is due to larger pores than in fine-grained soils (Dejong et al. 2006; Whiffin et al. 2007; Al Qabany and Soga 2013).

This study aims to investigate the influence of soil gradation on the effectiveness of MICP by comparing the treatment outcomes in two distinct sandy soils: a fine sand and a medium sand. Using a submerging treatment protocol, the macroscopic improvement was quantified by Unconfined Compressive Strength (UCS) tests. To provide a microstructural explanation for the mechanical results, the morphology of the precipitated calcium carbonate was analyzed in detail using Scanning Electron Microscopy (SEM).

2 MATERIALS AND METHODS

2.1 *Materials*

2.1.1 *Soil*

Two sandy soils, classified as medium sand (mSa) and fine sand (fSa) according to EN ISO 14688-1, were selected for this investigation. The grain size distribution for each soil, determined by sieve analysis, is presented in Figure 1. Key physical and index properties, including the unit weight of soil solids (γ_s), median particle diameter (D_{50}), coefficient of uniformity (C_U), and coefficient of curvature (C_C), are summarized in Table 1. Based on the calculated C_U values, both soils are classified as uniformly graded according to EN ISO 14688-2.

Table 1. Samples parameters.

Sample	γ_s (kN/m^3)	D_{50} (mm)	C_U (–)	C_C (–)
Fine sand (fSa)	26.0	0.18	2.44	0.73
Medium sand (mSa)	26.0	0.35	2.28	1.06

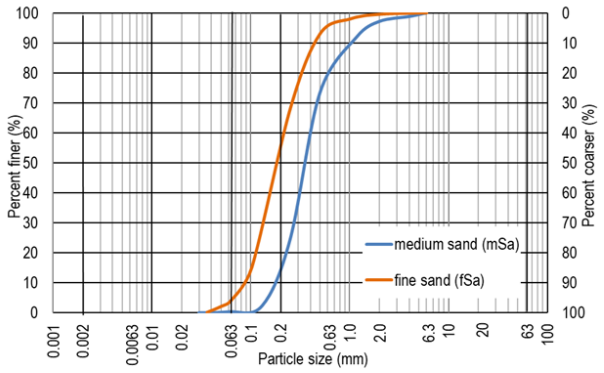

Figure 1. Grain size distribution curves of tested sands.

2.1.2 *Microbial treatment solutions*

The ureolytic bacterium *Sporosarcina pasteurii* (DSM 33) was selected for this study due to its well-documented high urease activity.

The bacterial culture was initiated by rehydrating freeze-dried *S. pasteurii* and plating it onto solid nutrient agar supplemented with urea. Following a 48-hour incubation period at 25°C, visible colonies were harvested and transferred to a flask containing 50 mL of a sterile liquid growth medium. This flask was then incubated in a rotational shaker at 30°C and 200 rpm for 48 hours to produce a concentrated stock culture. The bacterial concentration was quantified by measuring the optical density at a wavelength of 600 nm (OD_{600}) using a UV-Vis spectrophotometer, which yielded a value of 1.695, indicating a high cell density suitable for treatment.

To prepare the final treatment solutions, the stock culture was centrifuged at 2000 rpm and 4°C for 10 minutes. The supernatant was discarded, and the resulting bacterial pellet was resuspended in a fresh solution to create the bacterial solution. A second solution, the cementation solution, was prepared with the same composition but without the bacterial pellet. Both solutions contained nutrient broth, urea ($NH_2(CO)NH_2$), ammonium chloride (NH_4Cl), sodium bicarbonate ($NaHCO_3$), and a calcium chloride ($CaCl_2$) stock solution, following the formulation adapted from DeJong et al. (2006). The final molarity of the cementation solution was 1.0 M.

A preliminary test was conducted to assess the viability of using a refrigerated bacterial pellet for subsequent experiments. A pellet, stored at 4°C for several days, was resuspended in fresh growth medium and incubated under standard conditions (30°C, 200 rpm). The OD_{600} was monitored over time. After 24 hours, the OD_{600} reached 0.865. However, despite visual cloudiness suggesting cell multiplication, the OD_{600} measurement after 48 hours decreased to 0.445. This decline indicated a significant loss of bacterial viability. Consequently, to ensure consistent and high metabolic activity for all experiments reported in this study, each batch of bacterial solution was prepared fresh from newly activated colonies grown on Petri dishes, and refrigerated pellets were not used for the main treatment procedures.

2.2 *Methods*

2.2.1 *Sample preparation*

Cylindrical samples with a diameter of 38 mm and a height of 76 mm were prepared for Unconfined Compressive Strength (UCS) testing. This geometry yields a height-to-diameter ratio of 2.0, which complies with the range of 1.8 to 2.5 indicated by the PN-EN ISO 17892-7:2018-05 standard.

The sample preparation procedure was adapted from Zhao et al. (2014). First, the sand was oven-dried at 105°C for 24 hours without prior sterilization. The dried sand was then carefully placed into a geotextile mold using the air pluviation technique with a funnel to ensure consistent initial density.

Following the MICP treatment and curing period, the biocemented specimens were subjected to UCS testing. The tests were performed under strain-controlled conditions at a constant axial strain rate of 1.5% per minute, as specified in PN-EN ISO 17892-7:2018-05.

2.2.2 *Sample treatment*

The successful and homogeneous application of MICP treatment to soil specimens is a challenge (Wasil et al., 2023). While various techniques exist, including injection and surface spraying, the immersing method was selected for this study due to its suitability for ensuring thorough contact between the treatment solutions and the soil samples.

For the treatment procedure, an adapted submerging method from Zhao et al. (2014) was used. The process began with placing the soil samples on a perforated surface in a container and submerging them in a *Sporosarcina pasteurii* solution to allow for bacterial attachment. The bacterial solution was then drained and substituted with a cementation solution for a one-week immersion. During this time, an air pump provided aeration and fluid circulation in place of a mechanical stirrer. Once the treatment was complete, the samples were removed and air-dried at 21°C for seven days. The final step before UCS testing involved removing the geotextile molds and oven-drying the samples at 105°C.

3 RESULTS

3.1 *UCS test*

The mechanical strength of the MICP treated samples was assesed via Unconfined Compression Strength (UCS) tests. The results from three replicate samples for each tested soil type are presented in Table 2. A graphical comparison of the mean UCS values is presented in Figure 2.

Table 2. UCS test results for biocemented sand samples.

Sample	Fine sand fSa UCS (kPa)	Medium sand mSa UCS (kPa)
1	126	285
2	120	276
3	110	263
Mean (average)	118.7	274.7
Standard Deviation	8.1	11.1

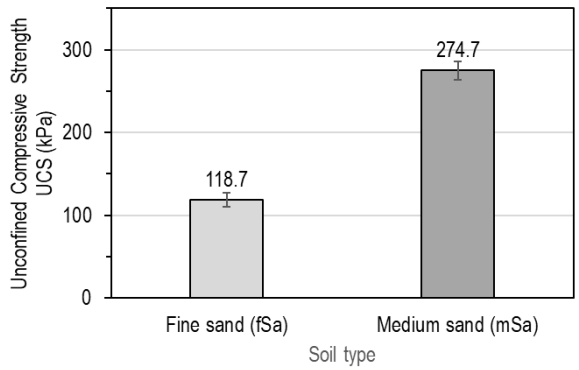

Figure 2. Grain size distribution curves of tested sands.

A notable difference in the degree of improvement was observed between the two soil types. The samples of medium sand (mSa) exhibited a mean unconfined compressive strength

(UCS) of 274.7 kPa, whereas the fine sand (fSa) samples produced a mean UCS of 118.7 kPa. The error bars shown in Figure 2 represent the standard deviation of three replicate samples. These standard deviations were small for both tests, with ±11.1 kPa for medium sand and ±8.1 kPa for fine sand. These results demonstrate that the Microbially Induced Calcite Precipitation (MICP) treatment was approximately 2.3 times more effective at increasing compressive strength in mSa compared to fSa under the same laboratory conditions.

3.2 *SEM analyses*

The microstructure of the treated soils and control precipitates was analyzed using Scanning Electron Microscopy (SEM). To characterize the morphology of the calcium carbonate formed in the absence of a soil matrix, a control experiment was conducted, following a procedure from previous work (Wasil et al., 2023). In this experiment, the bacterial and cementation solutions were combined in a flask, and the resulting pure precipitate was collected for analysis. Figure 3 shows a representative SEM micrograph of this precipitate at 5000x magnification. A higher magnification micrograph (10 000x) of the top-right area indicated in Figure 3 is presented in Figure 4, providing a more detailed view of the individual crystal morphology.

Analysis of the pure precipitate reveals a complex mixture of crystal morphologies and biological features. The dominant morphology, seen clearly in Figure 3 and Figure 4, consists of spherical aggregates characteristic of vaterite. Smaller, well-defined rhombohedral crystals, identified as the more stable calcite, are also visible on the right side.

The sample provides direct evidence of the bacteria's role in this precipitation. Numerous elongated, rod-shaped imprints, consistent in size (1-5 µm) and shape with *S. pasteurii* (Ghosh et al., 2019), are present on the surfaces of the vaterite spheres. In some locations, intact or partially embedded bacterial cells appear to be entombed within the mineral structure. This confirms that the bacterial cells served as primary nucleation sites for mineral growth (Marvasi et al. 2020). The mixture of crystal types, including both stable calcite and metastable vaterite, is significant because the type of polymorph directly affects the mechanical strength of the biocemented soil (Anbu et al., 2016).

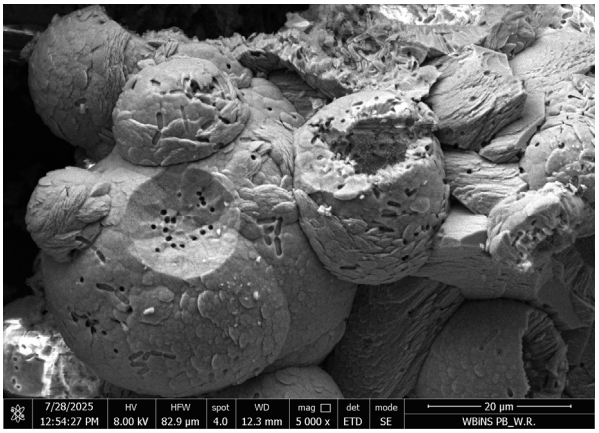

Figure 3. Pure calcium carbonate precipitated in the flask (5000x).

Figure 5 and Figure 6 present the microstructure of the untreated fine sand (fSa) and medium sand (mSa), respectively. The SEM analysis confirms that the grains in both soils are free from any surficial precipitates or coatings. The grains of fine sand are predominantly angular to sub-angular. The grains of medium sand are predominantly sub-angular to sub-rounded. In both samples, the grains remain separate, with no crystalline material filling the pore spaces or coat the grains, and there is a complete absence of inter-particle cementation or bridging.

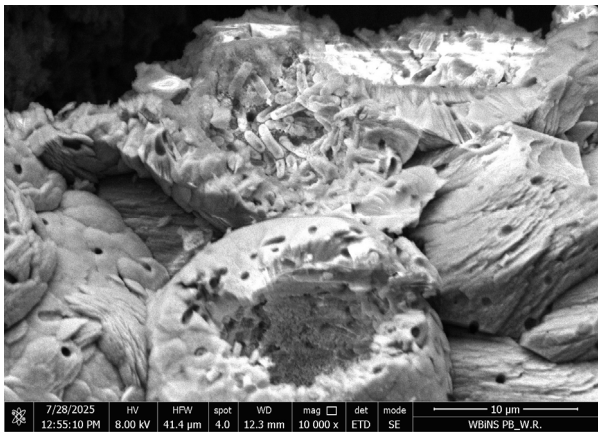

Figure 4. Pure calcium carbonate precipitated in the flask (10000x).

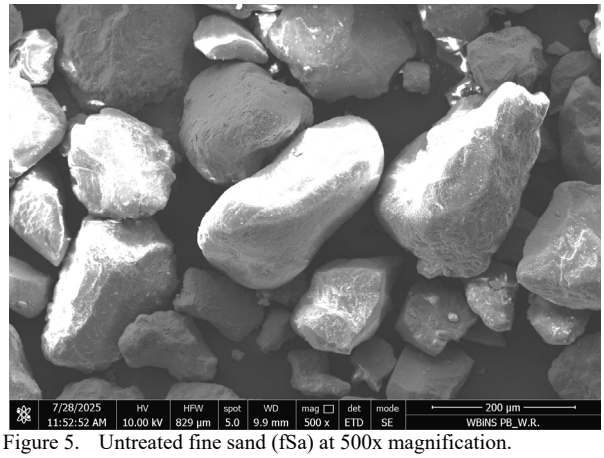

Figure 5. Untreated fine sand (fSa) at 500x magnification.

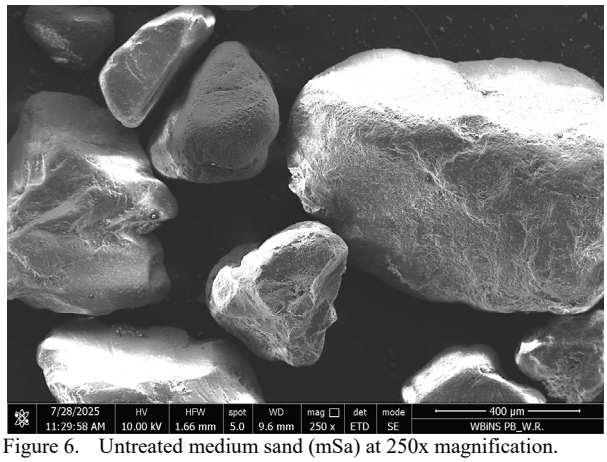

Figure 6. Untreated medium sand (mSa) at 250x magnification.

Figure 7 and Figure 8 illustrate the successful application of the MICP method to the medium sand. This process has produced a substantial amount of rhombohedral calcite crystals (ranging from 5-10 µm in size) that coats the sand grains. The sand grain surface is covered with small, well-defined blocky crystals with flat faces and sharp angles. Most importantly, it forms strong crystalline bridges between the grains, transforming the loose sand into cemented material.

The microstructure of the fine sand following MICP treatment, presented in Figure 9, differs from that of the medium sand. The precipitated calcium carbonate is characterized by significantly smaller crystals, typically ranging from 1-3 µm. Some of the microcrystals show hints of a rhombohedral shape, although many are less defined.

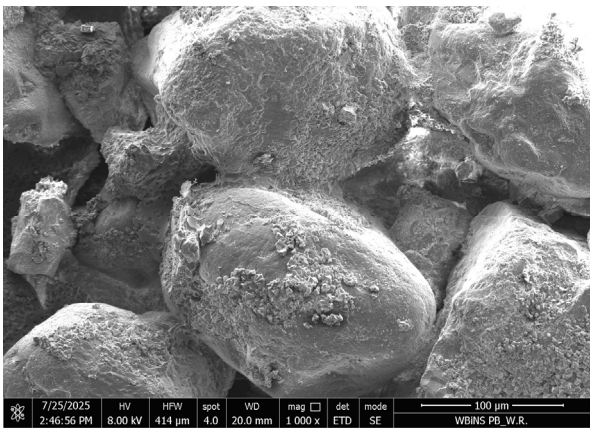

Figure 7. SEM micrograph of mSa after MICP treatment (1000x).

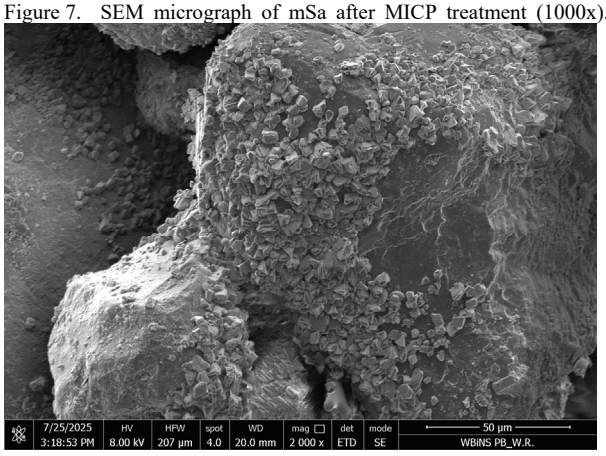

Figure 8. Medium sand (mSa) grain covered with calcite crystals with visible bond between grains (2000x).

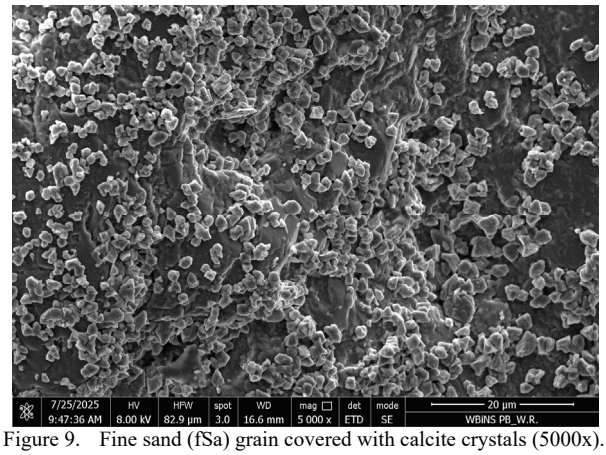

Figure 9. Fine sand (fSa) grain covered with calcite crystals (5000x).

4 CONCLUSIONS

This study investigated the influence of soil gradation on the efficacy of Microbially Induced Calcite Precipitation (MICP) using an immersing treatment method. Through a combined analysis of mechanical strength tests and microstructural observations, several key conclusions can be drawn.

The initial particle size of the soil dictates the macroscopic strength development of the biocemented material. Under identical treatment conditions, the medium sand (mSa) resulted in a mean UCS of 275 kPa, which was approximately 2.3 times higher than the 119 kPa in the case of the fine sand (fSa). Coarser, well-graded sands are significantly more amenable to strength enhancement via the immersion method.

SEM analysis explained the difference in mechanical strength between the two soils. In medium sand, the lower surface area and larger pores allowed for the growth of large, well-defined calcite crystals that formed effective inter-particle bridges. This resulted in a much stronger cemented soil matrix compared to the fine sand.

Despite using a high 1.0 M cementation media concentration, the final UCS values were moderate. This suggests the immersing treatment method was a limiting factor, likely causing rapid precipitation on the sample exterior and leading to surface clogging (pore-plugging). This highlights that high reagent concentrations may require a method like injection to overcome such transport limitations.

Ultimately, these results confirm that MICP is a complex process where the treatment protocol must be carefully matched to the soil type to be effective.

5 ACKNOWLEDGEMENTS

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