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Enhancing sandy soils of varying densities via microbially induced calcite precipitation

Kağan Eryürük[1]✉, Yavuz Yenginar[1], İlyas Özkan[1] and Hatice Türk Dağı[2]

[1] Necmettin Erbakan University, Faculty of Engineering, Department of Civil Engineering, Demeç St., 44/1, 42005, Konya, Türkiye

[2] Selçuk University, Faculty of Medicine, Department of Microbiology, Celal Bayar St., 313, Zip Code, Konya, Türkiye

Corresponding author:
Kağan Eryürük

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Faculty of Civil Engineering and Architecture Osijek
Josip Juraj Strossmayer University of Osijek
Vladimira Preloga 3
31000 Osijek
CROATIA

Abstract:
Interest in microbially induced calcite precipitation (MICP) has grown due to the demand for sustainable and energy-efficient soil improvement methods. This study explored the potential of using Sporosarcina pasteurii to enhance the engineering properties of sandy soils with varying grain sizes and relative densities. Calcium carbonate precipitation induced by bacterial activity was assessed under different bacterial concentrations (10^7, 10^8, and 10^9 cells/mL) and temperatures (16 °C, 30 °C, and 45 °C). The improvements were evaluated using unconfined compressive strength (UCS) tests and microstructural analyses using SEM, EDS, and XRD techniques. The results indicated that MICP significantly increased soil strength, with the highest UCS values observed for medium and coarse sands under optimal conditions. Fine sand exhibited limited improvement owing to lower permeability, which hindered bacterial distribution. SEM and XRD analyses confirmed the presence of calcium carbonate polymorphs, such as calcite and vaterite, enhancing intergranular bonding. The optimal conditions for bacterial activity and calcium carbonate precipitation were a concentration of 10^9 cells/mL and temperatures of 30 °C for fine sand and 45 °C for coarse sand. This research underscores the potential of MICP as a sustainable soil stabilisation technique while highlighting challenges in bacterial distribution and bonding across different sand types.

Keywords:
microbially induced calcite precipitation; sand; soil improvement; sporosarcina pasteurii; strength

1 Introduction

Conventional mechanical techniques (such as compaction, vibro-compaction, and drainage) and chemical and synthetic additions (such as cement, lime, fly ash, industrial wastes, organic compounds, and geosynthetics) can be used to strengthen the soil [1-5]. Notwithstanding their demonstrated efficacy, these conventional techniques have been increasingly criticised for their high costs and energy usage, as well as their detrimental environmental impacts, including the emission of carbon dioxide and release of hazardous materials [6-9]. Therefore, in addition to successfully fortifying the soil, innovative methods that are both economically and environmentally feasible and energy-efficient must be developed. Using microorganisms guided by microbiology and biochemistry is one such method [10]. The technique of creating a biochemical reaction network in the soil, which employs bacteria and chemical reactants, is known as microbially induced calcite precipitation (MICP)-based soil strengthening; the $CaCO_3$ produced as a result of this process enhances the soil matrix [11]. The MICP development phases are as follows [11]:

$$NH_2CONH_2 + H_2O \xrightarrow{urease} 2NH_3 + CO_2 \tag{1}$$

$$2NH_3 + 2H_2O \rightarrow 2NH_4{}^{+} + 2OH^{-} \tag{2}$$

$$CO_2 + OH^{-} \rightarrow HCO_3{}^{-} \tag{3}$$

$$CaCl_2 \rightarrow Ca^{2+} + 2Cl^{-} \tag{4}$$

$$Ca^{2+} + HCO_3{}^{-} + OH^{-} \rightarrow CaCO_3 + H_2O \tag{5}$$

The overall reaction is as follows:

$$NH_2CONH_2 + CaCl_2 + 2H_2O \rightarrow 2NH_4{}^{+} + 2Cl^{-} + CaCO_3 \tag{6}$$

Bacteria and reactants are usually added to the soil in water-based solutions. Two processes occur when bacteria are introduced into the soil: bacterial adsorption onto soil particles and bacterial movement (via advection and diffusion) within the soil pores. Some of the factors that influence these two processes are geometry of the soil pores [12-14]; bacterial cell shape, surface charge, and hydrophobicity [15-17]; soil particle surface roughness and mineralogy; and temperature, chemistry, and flow regime of the fluid in the voids [12-14; 18-20]. Studies have been conducted using MICP to fix fractures [21], plug fractures [22], harden cementitious materials [23], reduce hydraulic conductivity [11; 24-26], seal ponds [27], improve undrained shearing behaviour [28], explore techniques for restoring ceramic cultural relics [29], and improve the durability of concrete [30].

Studies on the compaction behaviour of soils with MICP revealed that the compressibility decreased as the amount of CaCO3 increased [31; 32]. Lee et al. [33] reported that the total slump during the one-dimensional compaction of soil samples with MICP was reduced by up to 23 % compared to samples without MICP. This study aimed to enhance the engineering properties of sandy soil using a microbiological approach. Specifically, this study explored the potential of microbially induced calcite precipitation (MICP), facilitated by Sporosarcina pasteurii, to improve the mechanical strength and stability of sandy soils with varying grain sizes, relative densities, and bacterial concentrations. The goal is to develop an environmentally friendly alternative to conventional soil-stabilisation techniques.

2 Methodology

2.1 Materials and methods

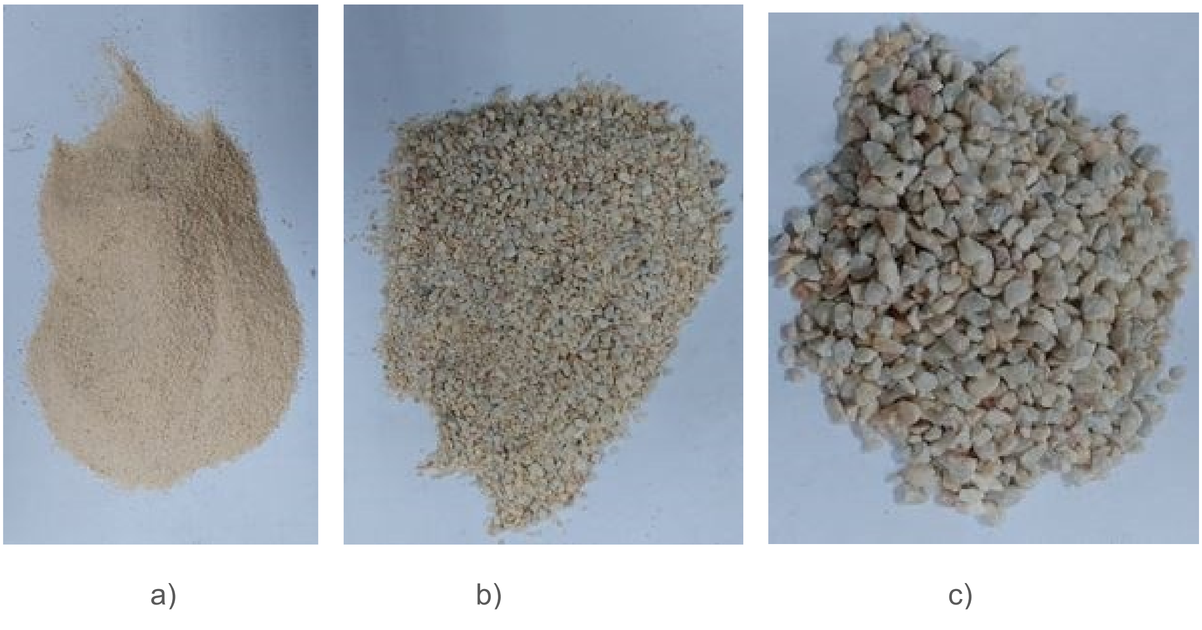
In the bacterial soil stabilisation method, a range of quantities of the bacteria Sporosarcina pasteurii (ATCC 11859) of varying grain sizes and relative densities (the index property that expresses the compactness of a granular soil relative to its loosest and densest possible states) were added to sandy soil samples. The effect of calcium carbonate precipitation, a byproduct of bacterial activity, on the geotechnical properties of the soil was examined. The parameters under investigation included the grain size of the sand (0,0-0,6 mm, 0,6-2,0 mm, 2,00-4,76 mm), relative density (0,3; 0,6; and 0,9), and bacterial concentration (10^7, 10^8, and 10^9 cells/mL). Additionally, samples were prepared and cured at 16 °C, 30 °C, and 45 °C to evaluate the effects of temperature.

This study comprised four fundamental stages, which are briefly summarized as follows:

- The initial stage of the experiment involved the preparation of sand samples at specific relative densities for unconfined compressive strength (UCS) tests. At this stage, the sand samples were arranged at different densities to make them suitable for this test.
- The second stage involved the multiplication and preparation of bacteria at specific concentrations. This process was conducted at the Microbiology Laboratory of Selçuk University, Faculty of Medicine. The cultivation of bacteria under favourable conditions enabled the preparation of bacterial solutions at different concentrations required for subsequent stages.
- The third stage of the experiment was to perform UCS tests. The purpose of these tests was to determine the mechanical strength properties of the stabilised sand samples.
- The final stage of the study consisted of analyses to support the results obtained from the uniaxial tests and explain the bacterial treatment process in more detail. The purpose of these analyses was to study the effects of the bacterial treatment process and microstructural changes in the sand grains.

2.2 Sandy soil

The sands were sourced from a single origin and classified into three categories based on their particle sizes. Sandy soils have grain diameters of 0,075-4,750 mm according to the Unified Soil Classification System. To investigate the grain size distribution of soil on microbial stabilisation, sandy soil was sieved using varying sieves and separated into fine sand, medium-coarse sand, and coarse sand with grain diameters of 0,075-0,600 mm, 0,6-2,0mm, and 2,00-4,76mm, respectively (Figure 1a, 1b, and 1c). These samples underwent a thorough sieving and classification process in accordance with standard protocols.



a) b) c)

Figure 1. Grain sizes: a) fine sand (0,075-0,600 mm); b) medium-coarse sand (0,6-2,0 mm); c) coarse sand (2,00-4,76 mm)

The specific gravity of the samples was determined according to the guidelines set out in ASTM D854 [34]. Following the classification process, the maximum and minimum void ratios were calculated according to ASTM D4253 (for maximum density) [35] and ASTM D4254 (for minimum density) [36], respectively. These void ratio values were then used to calculate the relative densities of sands required for bacterial treatment, ensuring precise preparation for subsequent experimental applications. The physical properties of the sand samples are listed in Table 1.

Table 1. Properties of unstabilised sand specimens

Type of sand	Specific gravity	e_{min}	e_{max}	Relative density (%)	Density (g/cm^3)
fine	2,69	0,499	0,989	30	1,460
				60	1,587
				90	1,738
medium-coarse	2,69	0,551	0,998	30	1,443
				60	1,555
				90	1,681
coarse	2,69	0,585	1,025	30	1,421
				60	1,528
				90	1,651

In this study, 81 sand samples were prepared with three soil grain sizes (fine, medium-coarse, and coarse) and three relative density levels for each sand type (30 %, 60 %, and 90 %). Additionally, three bacterial concentrations (10^7, 10^8, and 10^9 cells/mL) and three temperature settings (16 °C, 30 °C, and 45 °C) were utilised.

2.3 Preparation of inoculum and cultivation

Sporosarcina pasteurii (ATCC 11859) was used to precipitate the calcium carbonate in the experiments. Tris-YE medium containing 20 g/L yeast extract, 10 g/L ammonium sulphate, and 130 mM Tris buffer (pH 9,0) was used to cultivate the cultures. To prepare a solid medium for stock cultures, 2 % agar was added to the liquid medium. Before use, all medium ingredients were combined and individually sterilised for 15 min at 121 °C [25]. Resting cells of Sporosarcina pasteurii were obtained by inoculating the strain in Tris-YE medium and shaking it at 120 rpm overnight at 30 °C. Following 10-min centrifugation at 10.000 g, the cells were once again resuspended in 1 L of distilled water to reach a concentration of 109 cells/mL [25] (Figure 2a). After cell suspension, which was initially prepared at a concentration of 109 cells/mL, cells were diluted to achieve concentrations of 108 cells/mL and 107 cells/mL (Figure 2b and 2c).

a) b) c)

Figure 2. Bacteria concentrations: a) 109 cells/mL; b) 108 cells/ mL; c) 107 cells/mL

2.4 Experimental study

Sand grains are categorised as cohesionless soils with no self-bonding properties. Because of these characteristics, uniaxial compression tests cannot be performed directly on sand samples.

The binding of sand grains is facilitated by the precipitation of calcium carbonate ($CaCO_3$) as a consequence of a pozzolanic reaction. At the initial stage of the experiment, a bacterial suspension was added to the sand samples according to their void volume, after which the bacteria were allowed to adhere to the sand surface for 2 h. Thereafter, a precipitation solution was added daily for one week. The purpose of this process was to increase the bacterial count to promote $CaCO_3$ formation and strengthen the bonds between the grains. Finally, uniaxial compression tests were performed in accordance with the ASTM D2166 [37] standard to determine the strength properties of the sand samples after mechanical bonding was achieved via calcium carbonate precipitation.

2.5 Analyses

All analyses were performed in the laboratories of Necmettin Erbakan University Science and Technology Research and Application Centre.

Scanning electron microscopy (SEM) was used to examine the surface morphology of the sand samples. This analysis was conducted to observe the microstructural changes in the surface of the bacteria-treated sands. The process involved analysing the mechanism of bacterial attachment to sand grains. Additionally, this analysis provided information about some compounds that form bonds between the grains, such as calcium carbonate ($CaCO_3$).

Energy Dispersive X-ray Spectroscopy (EDS) was employed to analyse the elemental composition of sand samples stabilised using bacteria. This analysis revealed chemical changes in mineral formation. This analysis was performed in conjunction with SEM.

XRD (X-ray Diffraction) was used to analyse the crystal structures of the treated samples. The presence of new minerals, such as $CaCO_3$ crystals formed as a result of bacterial activity in the sand samples, was determined by this analysis.

3 Results and discussion

This study evaluated UCS tests conducted in an experimental study. Additionally, data obtained from a range of analytical techniques (SEM, EDS, and XRD) were examined in detail. This section explores the effect of changes in the material microstructure on the physical and chemical properties.

3.1 Observations on stabilized soils

A total of 81 specimens were prepared for UCS tests. However, UCS tests could not be performed on most of these specimens. Nevertheless, the analysis was based on successfully tested specimens across the full range of conditions, ensuring reliable conclusions. The primary reasons for this are outlined as follows:

- Bacteria were not homogeneously distributed in the sand samples. The lack of a homogeneous distribution of bacteria in the sand samples can be summarised by two factors. First, bacteria accumulated in the upper layers of the fine sand samples because of their low permeability. Thus, they could not move towards the lower layers (Figure 3a). Second, the bacteria were unable to adhere to the upper layers because of the higher permeability of the medium and coarse sand samples. Therefore, they accumulated in the middle and lower regions of the sample (Figure 3b, 3c, and 3d), and the volumetric integrity of the stabilised samples could not be obtained in such cases. These factors indicate that bacterial distribution varies depending on the sample characteristics.
- In the medium-coarse and coarse sand samples, the bacterial densities of 107 and 108 cell/mL levels were insufficient. Therefore, UCS experiments could not be conducted

on these sets. The low bacterial concentration was insufficient to induce the required level of microbial activity. Consequently, the bacterial population was inadequate to improve the sand properties, thereby hindering the attainment of reliable and meaningful results.

- Local burns were observed on the surfaces of some specimens (fine sand) during the drying process. Consequently, the nutrients that were not consumed by the bacteria were combusted during the drying process, resulting in localised burns (Figure 3e). These were surface effects and did not significantly affect UCS results, as only intact samples were tested.

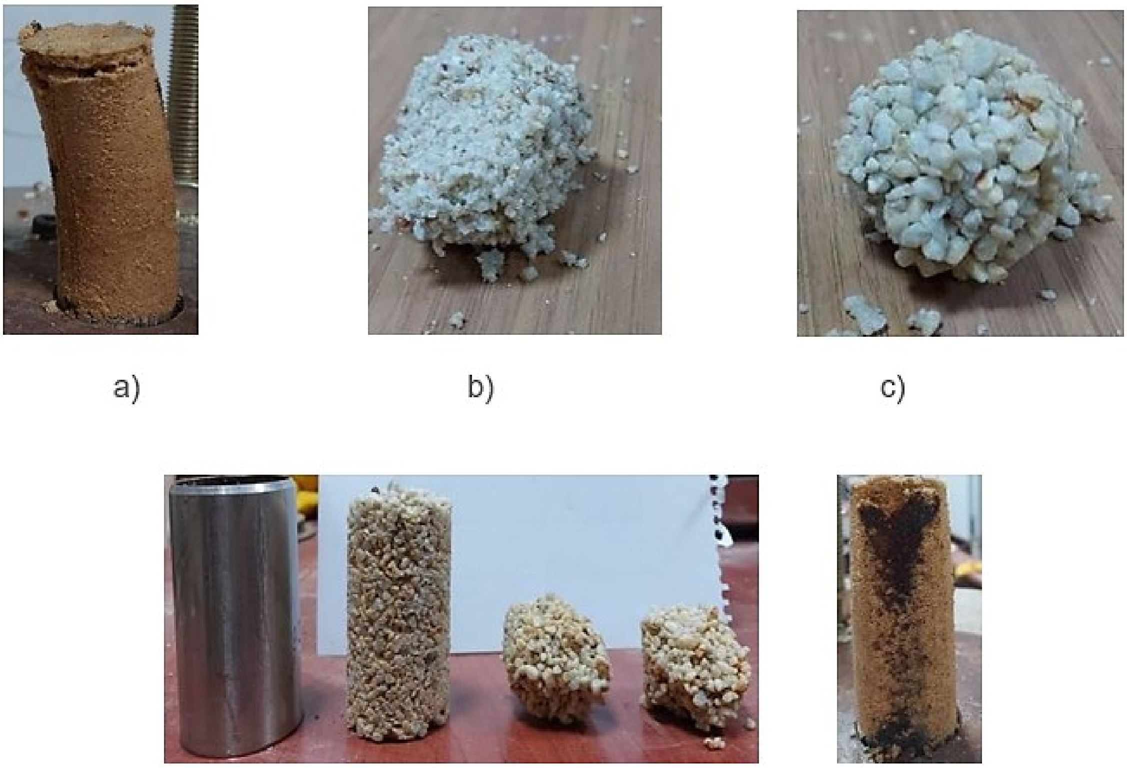

a) b) c)
d) e)

Figure 3. Figure 3. Sand specimens treated with bacteria: a) fine sand; b) medium-coarse sand; c) coarse sand; d) coarse sand with varying densities; e) local burns in fine sand

3.2 Unconfined Compressive Strength (UCS) test

Strengthening of sand specimens was achieved via bacterial remediation. Intergranular bonding was achieved through calcium carbonate precipitation. The effectiveness of this treatment was evaluated by UCS tests.

A series of unconfined compression tests were conducted on a selection of specimens that had been cured using bacteria, as mentioned in the preceding section. The stress–strain curves of the fine sand specimens are shown in Figure 4.

The stress–strain behaviour of fine sand varied significantly depending on temperature, bacterial concentration, and relative density. These results indicate the existence of an optimal temperature (30 °C) for the bacterial treatment of sand specimens. At this optimal temperature, specimens with higher bacterial concentrations (10^9 cells/mL) demonstrated greater strength than those with lower concentrations. At the optimum temperature of 30 °C, the strength of the prepared specimen (with 90 % relative density and 109 cells/mL) was approximately 30 % of the strength of the most effective specimen in this set. These findings indicate that an inverse correlation exists between bacterial concentration and relative density.

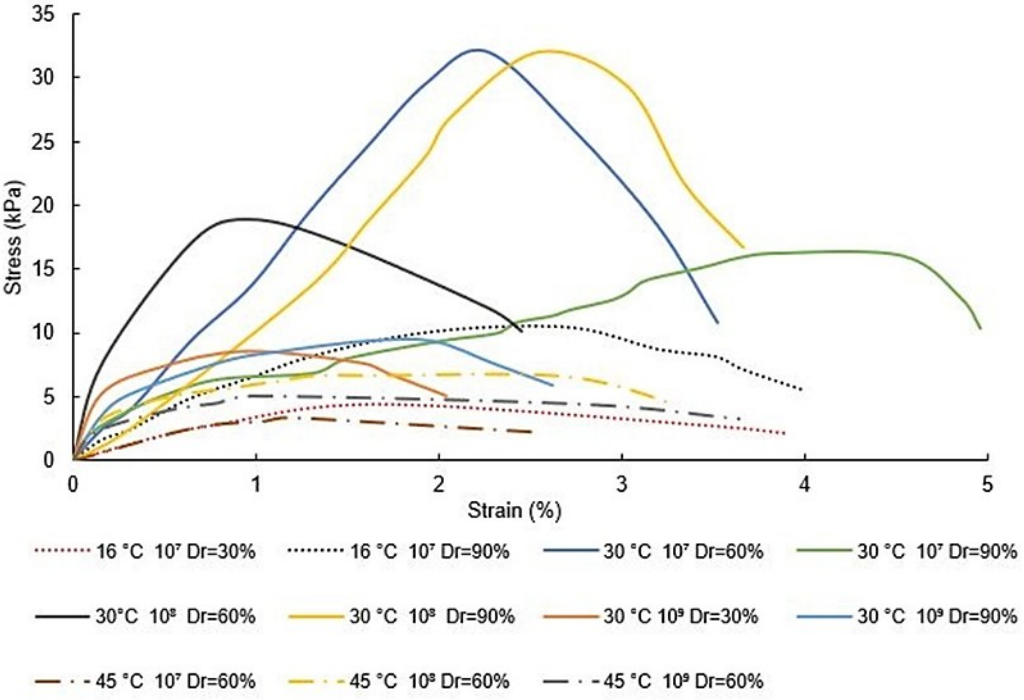


Figure 4. Stress–stain curves of bacterially stabilized fine sand specimens

In the fine sand samples, the highest values of UCS were obtained for samples with bacterial densities of 10^7 and 10^8 cells/mL. However, the measured compressive strength was approximately 33 kPa. Thus, this value was not sufficient for soil stabilisation.

The UCS test was performed on medium-coarse sand specimens with a bacterial density of 10^9 cells/mL. As the specimens did not show sufficient improvement, testing them with other bacterial densities was not possible. The stress–strain curves obtained from the test results are presented in Figure 5.

The highest measured strength (approximately 1200 kPa) was obtained in the medium-coarse sand specimens at a temperature of 16 °C and relative density (Dr) of 90 %. These conditions were determined to provide the optimum environment for medium-coarse sand. At a temperature of 45 °C, the recorded strength value was approximately 800 kPa. By contrast, the strengths of the specimens at Dr = 30 % and 60 % remained consistently low, irrespective of the applied temperature.

Medium-coarse sand samples achieved their highest strengths at 16 °C. This is attributed to favourable $CaCO_3$ crystal growth at lower temperatures, whereas coarse sands required higher bacterial activity at 45 °C owing to larger void spaces. Although temperatures above 30 °C are more suitable for bacterial survival and growth, lower temperatures (less than 30 °C) are more appropriate for the formation of calcium carbonate. At 16 °C, the 109 cells/mL bacteria concentration provided sufficient healing by filling the voids in the sand sample with calcium carbonate. However, at 45 °C, the rapid bacterial growth resulted in the replacement of calcium carbonate with bacteria in some of the voids, which led to the formation of weaker zones.

For a coarse sand specimen at 45 °C and a relative density of 90 %, a maximum strength of approximately 1200 kPa was achieved (Figure 6). Because high temperature increases the growth and metabolic activity of the bacteria, temperature conditions in microbial experiments must be monitored. Thus, this scenario resulted in more effective bonding between the sand grains. However, bacterial activity decreased with a decrease in both temperature and relative density of the coarse sand specimens.

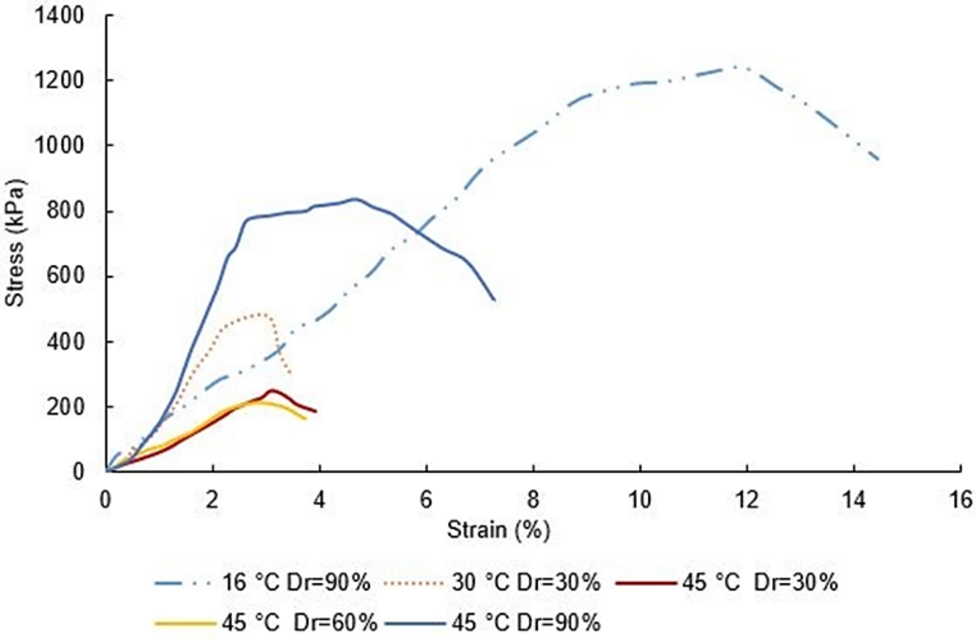


Figure 5. Stress–stain curves of bacterially stabilized medium-coarse sand specimens

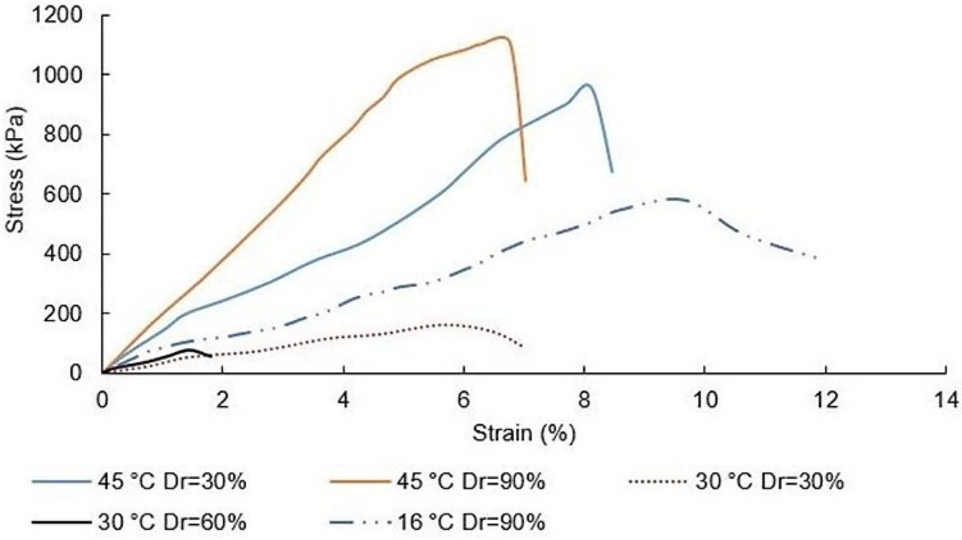


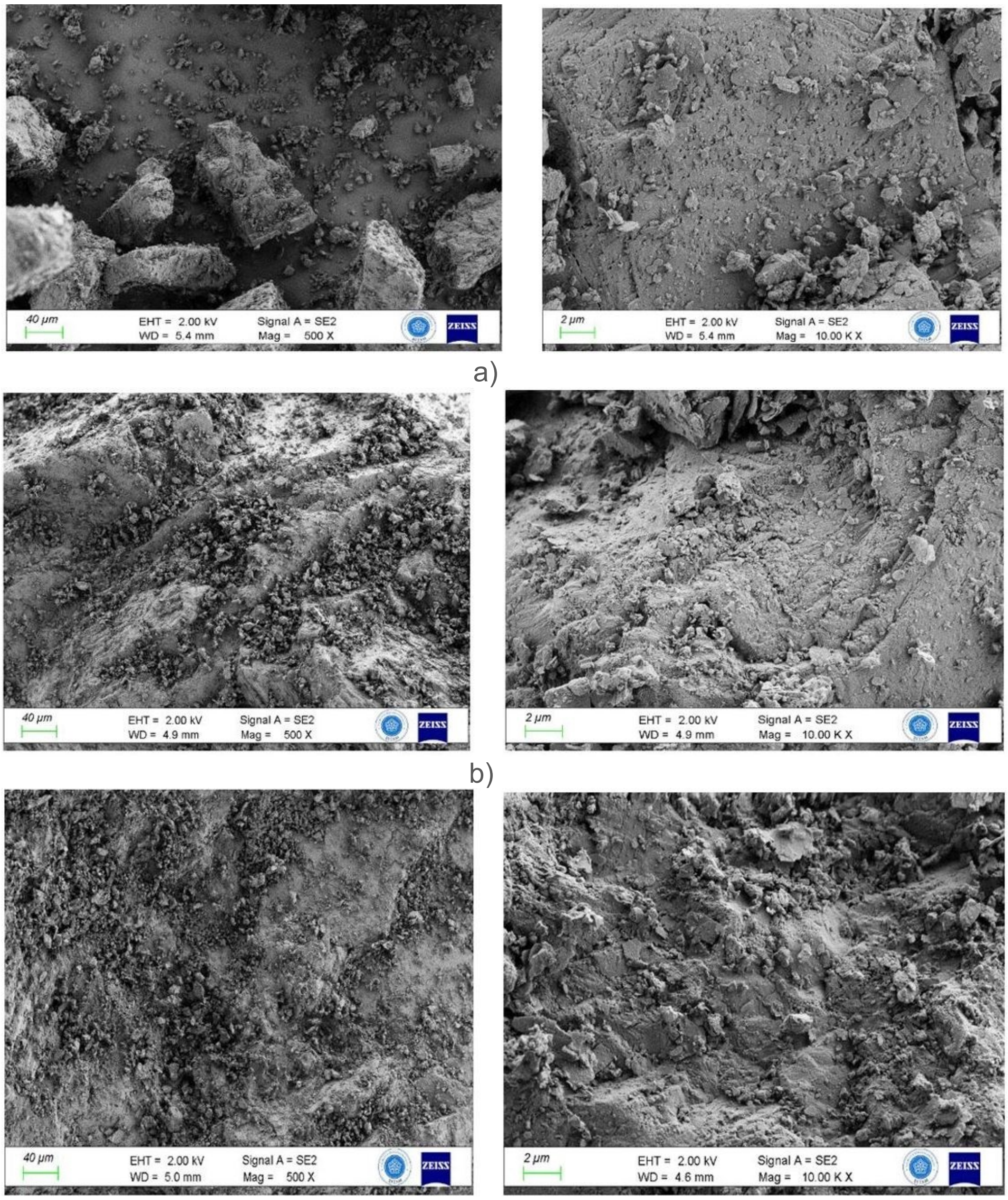
Figure 6. Stress–stain curves of bacterially stabilized coarse sand specimens

In the coarse sand samples, the void sizes were larger than those of the medium and fine sand samples. Tests conducted at low temperatures showed that the number of bacteria (10^9 cells/mL) was insufficient to fill these large voids; thus, more bacteria were required for effective filling. Additionally, the results showed that a temperature of 45 °C provides a more suitable environment for bacterial growth and survival than other temperatures.

3.3 SEM Analysis

Bacterial remediation involves microbially induced calcium carbonate precipitation. Because calcium carbonate precipitates as a result of the metabolic activity of bacteria, it can be used in various applications. This process can produce various crystal forms of calcium carbonate, such as calcite and vaterite [38].

SEM analysis was performed at both 500x and 10,000x magnification. SEM images obtained from this analysis are shown in Figure 7 a-c.



a)

b)

c)

Figure 7. SEM images of unstabilised sand: a) fine sand; b) medium-coarse sand; c) coarse sand at 500x and 10.000x magnification

Fine-grained particles or powders were observed on the surfaces of the raw sand samples in the SEM images. This situation arose from the sieving of sand samples based on their sizes, without washing. However, the absence of wet sieving analysis may have facilitated bacterial adhesion to the surfaces of the sand particles.

SEM images of the specimens treated with bacterial remediation are presented in Figure 8 a-c.

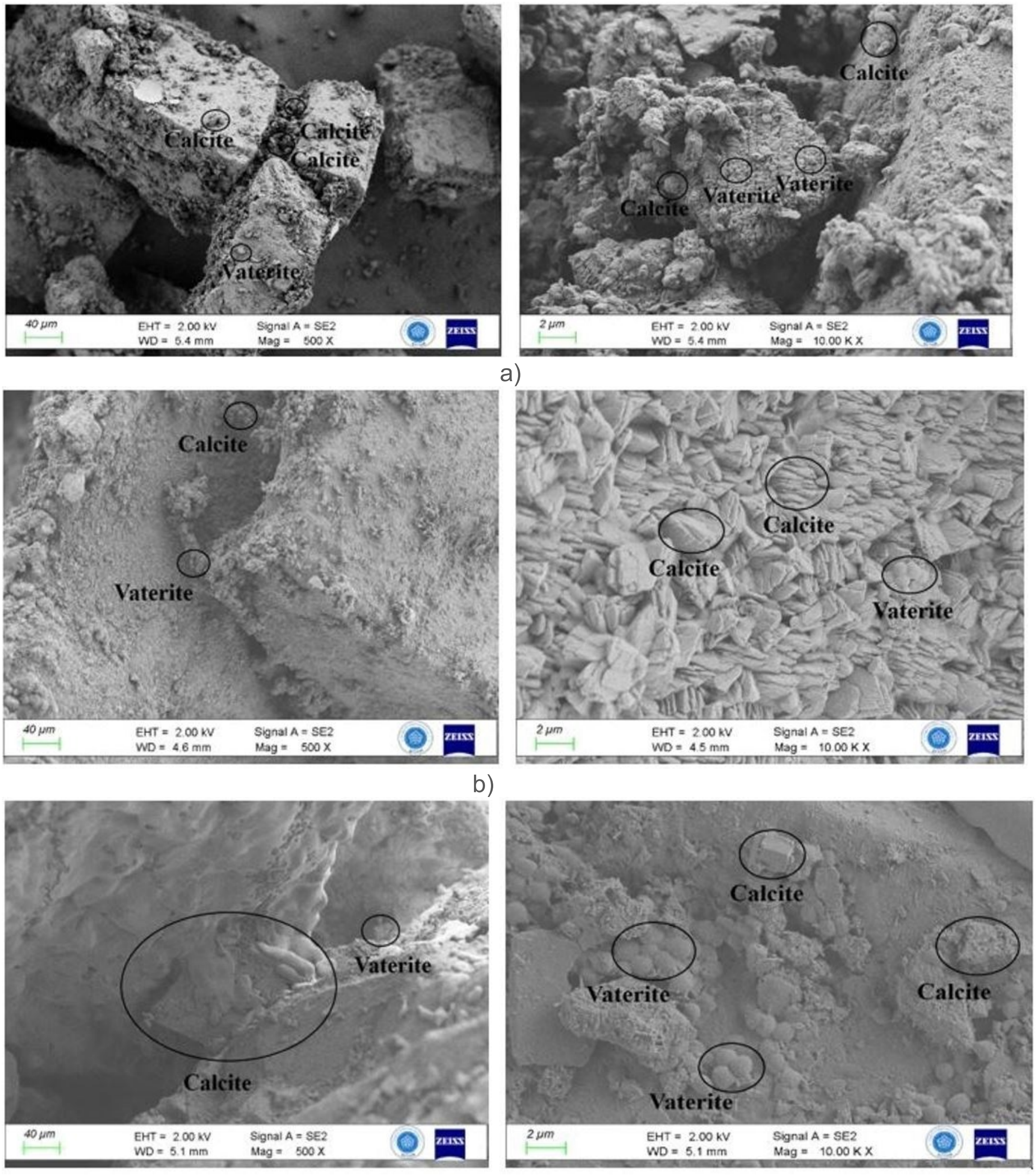


Figure 8. Sand specimens stabilized with bacterial remediation: a) fine sand; b) medium-coarse sand; c) coarse sand at 500x and 10.000x magnification

This analysis revealed the formation of calcium carbonate polymorphs, such as calcite and vaterite, as a result of bacterial-induced mineralisation. Moreover, as the most effective remediation occurred in the medium-coarse and coarse sands, formations such as calcite and vaterite were more clearly visible in these specimens.

The environment necessary for bacterial growth must provide high temperatures and a large surface area. Although the temperature conditions were met under laboratory conditions, the bacteria could not find sufficient space for proliferation in both fine and coarse sands. The optimal surface area for bacterial growth was found in the coarse sand specimens. Bacteria were observed more clearly in the 500x magnified SEM images of the coarse sand samples.

3.4 XRD Analysis

XRD analysis was conducted to investigate the mineralogical composition of the samples. This method provides detailed information on the crystalline phases present in the material.
XRD analysis could not be performed on the coarse sand because of its large particle size. However, this analysis was successfully conducted on fine and medium-coarse sands. Although XRD was not possible for the coarse sands, SEM and EDS confirmed $CaCO_3$ precipitation, reinforcing the mineralogical conclusions. XRD patterns are shown in Figure 9a and 9b.

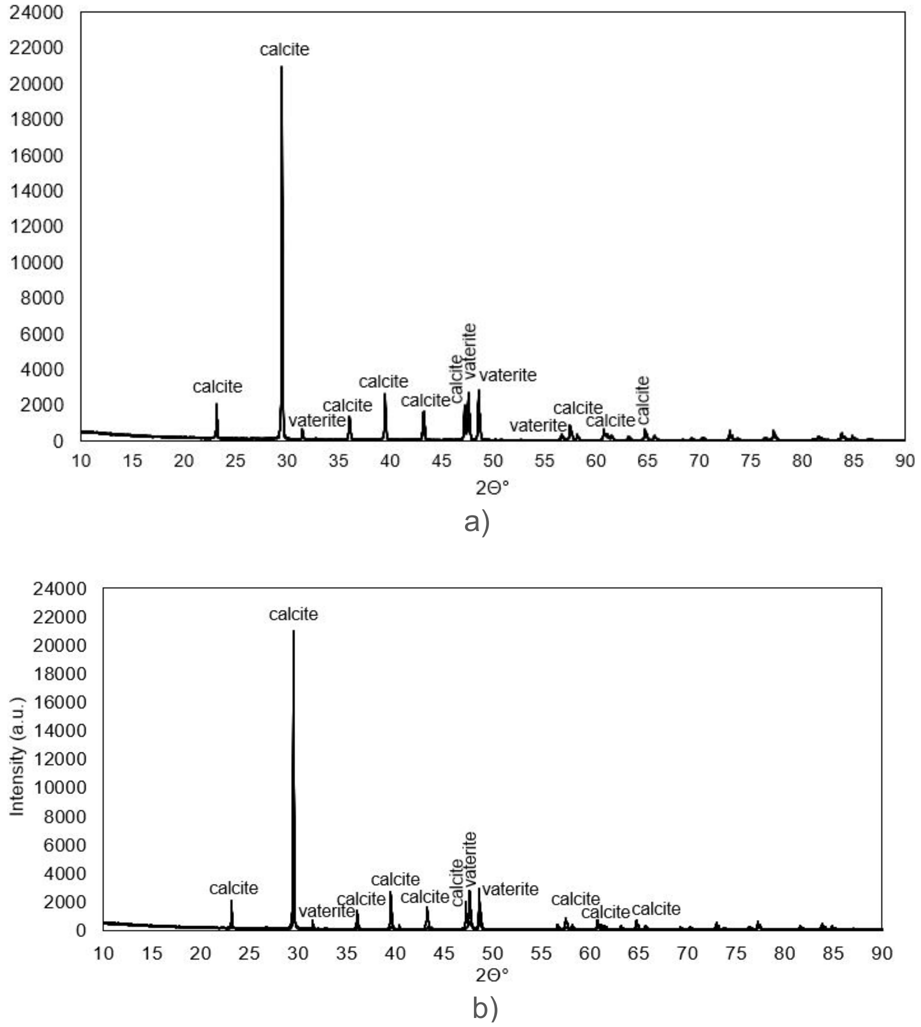


Figure 9. XRD diffraction patterns of specimens with bacterial remediation: a) fine sand; b) medium-coarse sand

The calcite phase of calcium carbonate was visible with diffraction peaks at 2Θ = 29,4°; 36,0°; 39,4°; 43,1°; 47,5°; and 48,5°; 57,5°; 60,0°; 65,0°; and the vaterite phase of calcium carbonate was observed at 2Θ =24,9°; 27,1°; 32,8°; 48,3°; 50,1°; and 55,6° in all specimens' XRD patterns [39-41].

4 Conclusions

This study explored the use of MICP to enhance the engineering properties of sandy soils, offering an environmentally friendly alternative to conventional soil stabilisation methods.

- The experimental investigation revealed that bacterial remediation effectively induces calcium carbonate precipitation, which strengthens the soil matrix via intergranular bonding. UCS tests demonstrated that the effectiveness of this method depends on factors such as bacterial concentration, sand grain size, relative density, and temperature.
- Optimal results were observed in medium-coarse sand samples treated at a bacterial density of 109 cells/mL and temperature of 16 °C, achieving a maximum strength of approximately 1200 kPa. In the coarse sand specimens, the highest strength was obtained at 45 °C and 90 % relative density, confirming the significant influence of temperature on bacterial activity and calcium carbonate formation. Conversely, fine sand samples exhibited challenges owing to insufficient bacterial distribution and lower void volumes, which limit their strength improvement.
- Future work could employ pressure-assisted injection, pre-flushing with nutrient-rich solutions, or combining MICP with biopolymers to overcome the bacterial distribution limitations in fine sands. For example, fine sands reached approximately 33 kPa UCS, whereas medium and coarse sands achieved approximately 1200 kPa under optimal conditions, indicating a disparity in performance.
- Microstructural analyses, including SEM, EDS, and XRD, provided detailed insights into the mineralisation process and formation of calcium carbonate polymorphs, such as calcite and vaterite. SEM images confirmed effective bacterial adhesion and mineralisation, especially in medium-coarse and coarse sand specimens, whereas XRD analysis verified the presence of calcite and vaterite phases, highlighting successful bacterially induced mineralisation. Calcite contributes primarily to long-term strength, whereas vaterite provides initial bonding before transformation into calcite, ensuring both short- and long-term stabilisation.

This study underscores the potential of MICP as a sustainable soil stabilisation technique. By optimising the bacterial concentration, temperature, and soil properties, MICP can be tailored to achieve substantial improvements in soil strength, thereby presenting a viable solution for environmentally conscious geotechnical applications. Further research should focus on scaling this approach up for field applications and exploring its long-term durability under various environmental conditions. At the field scale, the heterogeneity, injection methods, and environmental variability must be addressed. Future work will include pilot-scale trials to assess the practical feasibility.

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