

APPLICATION OF MICROBIALLY INDUCED CALCITE PRECIPITATION TO IMPROVE POORLY GRADED SANDY SOIL

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Abstract

The challenges associated with improving or strengthening poor soil consistently necessitated investigation to establish a novel, environmentally acceptable, and sustainable soil improvement method. The MICP (microbially induced carbonate precipitation) approach is one method in which microorganism metabolic pathways are used to create calcite precipitation inside the soil matrix, improving soil engineering qualities. This study aims to examine the efficiency of the MICP approach in improving the California Bearing Ratio value and reduction in permeability of poorly graded sand. Aerobic bacteria, namely *Bacillus Subtilis*, was used in this investigation for ureolysis and calcite precipitation. Three distinct concentrations of urea-calcium chloride solution (50, 300, and 600 mmol/L) treatment conditions were explored in the experimental program, and the California Bearing Ratio (CBR) value and permeability of the soil was determined for the targeted soil. This investigation indicated that the MICP has caused a significant quantity of calcite precipitation, resulting in a substantial improvement in the strength and a reduced permeability of the poorly graded sandy soil. Scanning electron microscopy (SEM) examination testing of the produced CaCO_3 crystals indicated that the CaCO_3 predominantly created connections from point to point between the particles of sand throughout the process of precipitation.

Keywords: Bio-cementation; California Bearing Ratio (CBR); Permeability; Poorly-Graded Sand; *Bacillus Subtilis*

Introduction

Soil is a living ecosystem that is characterized by biogeochemical activities such as mineral precipitation, gas production, biofilm development, and biopolymer creation. Microbially induced calcite precipitation (MICP) is a bio-geochemical process that causes calcium carbonate (or calcite) precipitation in the soil matrix as a result of

microbial metabolic activity. It has recently received a lot of attention from geotechnical engineering researchers all around the world. The MICP occurs naturally and is produced by non-pathogenic organisms found in the soil environment (Whiffin *et al.*, 2007; Cheng *et al.*, 2014; Mujah *et al.*, 2017; Ersan *et al.*, 2018).

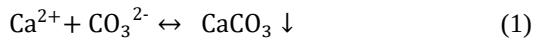
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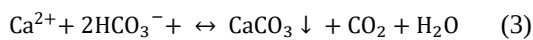
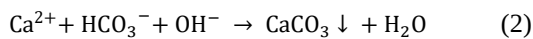
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The hydrolysis of urea is a relatively advanced process to causes calcite precipitation, and it is the most often described in the literature due to its simplicity (Okwadha and Li, 2010; Mujah *et al.*, 2017). The formation of calcium carbonate is a result of bacterial metabolic processes that lead to an increase in the pH of the surrounding environment. The increase in pH in the local environment can be attained by the enzymatic hydrolysis of urea, which leads to the generation of ammonia (Mujah *et al.*, 2017). This process is commonly referred to as urease activity. *Bacillus pasteurii* utilizes urea as a source of energy and generates ammonia, resulting in an increase in pH in the nearby surroundings. This rise in pH leads to the precipitation of calcium ions (Ca^{2+}) and carbonate ions (CO_3^{2-}) as calcium carbonate (CaCO_3). The increase in pH within the immediate environment frequently leads to the microorganisms themselves acting as nucleation sites for the process of crystallization (Dejon *et al.*, 2010; Okwadha and Li, 2010; Maleki *et al.*, 2016). The activity of the urea enzyme is maximized within a certain pH range, often ranging from 7 to 8.0, which is considered the ideal pH for enzyme activity. According to the literature, the optimal pH for the activity of the majority of microbial ureases is about 7 (Sidik *et al.*, 2014; Mujah *et al.*, 2017).

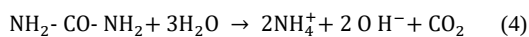
In calcite precipitation, the overall equilibrium reaction is (Whiffin *et al.*, 2007; Cheng *et al.*, 2014; Mujah *et al.*, 2017):



Microbiologically induced calcite precipitation occurs according to the reactions:

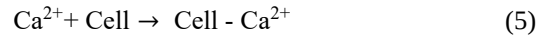


Equation (2) occurs due to changes in pH caused by the metabolic activity of bacteria. The alkaline pH environment is established by the process of urea breakdown, as indicated by the following chemical reaction (Dejon *et al.*, 2010).



In addition to the calcite precipitation process described above, calcium ions settle onto the surfaces of microorganisms with a negative cell surface charge. The equations governing the precipitation of calcite at the cell surface, which acts as the nucleation site, may be expressed as follows

(Dejon *et al.*, 2010; Dessy *et al.*, 2011; Maleki *et al.*, 2016):



The durability of soil is the resistance when exposed to declining conditions during its service life. The arrangement of particles and the interconnection of the precipitated calcite within the soil matrix play a role in determining the soil's mechanical properties and ability to withstand stresses (Andalib *et al.*, 2014). The long-term viability of the microbial community responsible for calcite precipitation is crucial for maintaining the durability of MICP-treated soils. The susceptibility of calcite to undergo disintegration in acidic environments is a prominent challenge in terms of the long-term durability of soil treated with calcite. According to Cheng *et al.*, (2014), the high bridging points and high soil permeability in MICP-treated soils are the reasons for their strong durability against freeze-thaw cycles. The current preference for the MICP procedure over alternative approaches is due in part to the dependability of the MICP treatment. This is attributed to the MICP treatment's ability to mechanical and biological adjustments, which enables it to provide the best treatment possible for the type and state of the local soil. Additionally, the adoption of MICP treatment in soil improvement is flexible since the use of bacteria to regulate the treatment process is simple, which promotes the potential utilization of this method in the renovation of construction sites (Andalib *et al.*, 2014). The *Bacillus* strains can produce CaCO_3 as a bio-product material as part of the MICP process to fill the pores and increase the durability of the soil (Whiffin *et al.*, 2007; Maleki *et al.*, 2016; Mujah *et al.*, 2017). The pH is another important component that greatly affects MICP, with an alkaline surrounding known to promote the process. Studies on the impact of chemicals on the geotechnical characteristics of fine-grained soils have been studied recently as a result of the rising rate of soil contamination (Kim *et al.*, 2018). Many studies have focused on the effects of acid rain on the mechanical properties of different soils (Bakhshipour *et al.*, 2016). It is necessary to choose a microbe that can produce CO_2 in tandem with an increase in the pH of the surrounding soil to an alkaline level that causes calcium carbonate to precipitate for microbially induced calcite precipitation (MICP) to be successful (Nemati *et al.*, 2005; Whiffin *et al.*, 2007; Dessy *et al.*, 2011; Maleki *et al.*, 2016; Mujah *et al.*, 2017). Because

they offer two sources of CO₂, respiration by the cell and urea decomposition, aerobic bacteria that can utilize urea as a source of energy are particularly excellent choices. Such an aerobic bacteria is *Bacillus subtilis*, a common bacterium that naturally exists in the subsoil.

The subgrade layer is the bottommost layer of pavement and is a compacted layer of soil with the requisite cross slope and gradients (Schaefer *et al.*, 2008). This compacted layer of earth, known as the subgrade, is an important aspect of road construction and must fulfil certain specifications to receive pavement layers. Sometimes, this layer may not be strong enough to support the weight passed through the layers of pavements. In this instance, subgrade reinforcement is required, which is accomplished by compaction, and the addition of admixtures. When soil lacks appropriate qualities such as dry density and California Bearing Ratio (CBR) value, it must be treated such that it meets subgrade criteria. Reduced CBR values result in reduced road load-bearing capability. As a result, the soil may be treated and improve the CBR value. The fundamental issue with most highway developments is a poor subgrade. There are many strategies for increasing the CBR value of subgrade for different types of soil (Schaefer *et al.*, 2008; Soni and Varshney, 2021).

Current studies on the MICP have concentrated on diverse aspects, including its underlying mechanics, practical applications, and improving for various engineering purposes. Extensive research has been conducted to investigate the process of MICP to improve the mechanical characteristics of soil, particularly by enhancing the Unconfined Compressive Strength (UCS) and bearing capacity (Tang *et al.*, 2020). This characteristic makes it suitable for application in ground enhancement and foundation reinforcement applications. During the process of MICP, the formation of calcite precipitates can effectively reduce the mobility of heavy metals in soils, improve the binding of scattered sands, and facilitate the repair of fractures in concrete (Chang *et al.*, 2024). Wilcox *et al.*, (2024) employed the MICP mechanism as a bioremediation method for mining waste. Zhu *et al.*, (2024) identified a relationship between the scale size and the initial dispersivity. In addition, it has enhanced the theoretical framework for multi-process reactions in MICP. As the scale size increases exponentially from 10⁻² m to 10⁵ m, the longitudinal dispersivity also increases proportionally from 10⁻³ m to 10⁴ m. Also, the maximum amount of carbonate increases in direct proportion to the initial porosity, whereas the average amount of carbonate shows a significant increase with the rise in bacterial injection rate.

Moreover, the use of fibers in concrete for civil engineering applications has received considerable attention in recent years. This is because it has the potential to improve the mechanical properties of concrete, specifically its tensile strength, durability, and resistance to cracking (Li *et al.*, 2016; Srinivasan *et al.*, 2024; Kumar *et al.*, 2024; Parthasaarathi *et al.*, 2024). In a study conducted by Siddique *et al.*, (2016), the characteristics of concrete made from bacterial rice husk ash (RHA) were examined. The researchers discovered that incorporating bacteria into the mixture resulted in higher compressive strength, decreased water absorption, porosity, and permeability, and improved the long-lasting nature of the concrete.

Previous studies have demonstrated that incorporating bacteria into soil has enhanced its mechanical properties. However, the number of research conducted in India using the MICP technique is limited. The soil of Jote, Arunachal Pradesh, is predominantly characterized by poor grading. This study aimed to examine the influence of MICP on the mechanical characteristics of a poorly graded sandy soil in Jote Village, located in the Papum Pare district of Arunachal Pradesh.

Materials and methods

Study area

Arunachal Pradesh is recognized as an area with high precipitation levels within the northeastern part of India, with an average annual rainfall ranging from 3000 to 5000 mm (Anshu *et al.*, 2022). It has been observed that the Papum Pare District exhibits a favourable precipitation pattern, characterized by an average annual rainfall of 3200 mm. Jote is a growing village in Arunachal Pradesh which is about 58 km from the capital city, Itanagar. It has a tropical climate characterized by substantial precipitation during the Southwest monsoon season (June-September) and the receding Northeast monsoon season (December-January) (Anshu *et al.*, 2022). Roads constructed in such regions are susceptible to water damage because of the concentrated rainy duration and relatively high rainfall volume. Taking into account the local climate and environmental conditions, geotechnical reinforcement and engineering applications are required to enhance the strength of the soil.

Field soil samples obtained from Jote were used in this study. The particle size distribution curve of the untreated soil is shown in Figure 1. The D₁₀, D₃₀, and D₆₀ are 0.10, 0.23, and 1.02 mm, respectively. The coefficient of uniformity (Cu) is 0.51 and the coefficient of gradation (Cc) is 10.2. The soil was found to be non-plastic and the

optimum moisture content was found to be 13.25%. It was classified as poorly graded sand based on the Unified soil classification system.

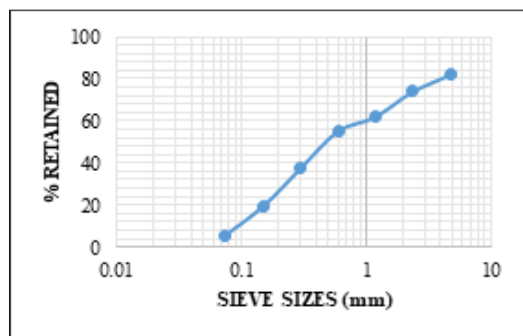


Figure 1. Grain size distribution curve of the soil used

Bacterial culture and cementation solution

Since forming carbonate by hydrolysis of urea in the presence of the urease enzyme was the chosen reaction, the microorganism must be able to discharge the urease enzyme. A bacteria species called *Bacillus subtilis* (MTCC NO. 441), procured from Microbial Type Culture Collection and Gene Bank (MTCC) by CSIR-Institute of Microbial Technology, Chandigarh, was used in this study. The growth media for *Bacillus subtilis* is tabulated in Table 1. The bacteria culture was cultivated aerobically at 30°C as shown in Figure 2. The optical density at 600 nm (OD_{600}) of the bacterial culture was between 2.0-2.5 which was determined using a spectrophotometer. Urea-calcium chloride of 1 M was used as the cementation solution to facilitate the $CaCO_3$ production as depicted in Figure 3.

Table 1. Growth media of *Bacillus subtilis*

Growth media name	Nutrient
GR1	Beef Extract 1.0g
GR1	Yeast Extract 2.0g
GR3	Peptone 5.0g
GR4	NaCl 5.0g
GR5	Distilled water 1.0L



Figure 2. Cultured bacteria broth solution of *Bacillus subtilis*



Figure 3. Cementation solution (Urea-Calcium chloride)

Preparation of slurry for MICP

The slurry was formed by mixing a specific amount of bacterial culture with a mixture of urea and calcium chloride cementation solution in equal moles. The aim was to achieve target concentrations of 50, 300, and 600 mmol/L. The mixture was then stirred at a speed of 600 rpm for approximately 24 hours in a shaker incubator. The $CaCO_3$ crystals were generated as a result of urea hydrolysis by the ureolytic bacteria, *Bacillus subtilis*, due to the presence of urea and calcium ions as depicted in Figure 4.



Figure 4. Precipitated slurry

California Bearing Ratio (CBR) test

By removing air from the soil, soil densification increases its strength properties and decreases permeability and compressibility. For a given amount of energy, the amount of moisture in the soil influences its density. In the present study, standard compaction tests were conducted to determine the Optimum Moisture Content (OMC) and Maximum Dry Density (MDD) of the treated soil with different targeted concentrations of urea and CaCl_2 . With the OMCs obtained from different concentrations, the California Bearing Ratio (CBR) test samples were prepared to determine the strength and load-bearing capacity of the soil.

Permeability test

Permeability is the measure of a material's ability to allow flow to pass through its pores. Permeability is an essential property of soil in projects where the movement of water through soil is a concern, such as seepage through or under a dam and drainage from subgrades or backfills. The falling-head method, as specified by ASTM D 2435, was employed in this study to investigate the impact of MICP on the permeability of poorly graded sandy soil.

Scanning Electron Microscopy (SEM)

The bio-cemented particles of soil are held together more firmly by the process of particle-to-particle contact. Researchers may directly and closely examine the CaCO_3 connections formed at the inter-particle soil grains using Scanning Electron Microscopy (SEM), which gives insight into the process by which bio-cemented soils improve (Maleki *et al.*, 2016). This work focuses on the examination of the microstructural characteristics of the soil matrix by the utilization of scanning electron microscopy (SEM).

Results and Discussion

Effect of concentration of cementation solution on the optimum moisture content of the soil

A standard proctor test has been carried out to determine the OMC of the treated and untreated soil samples. The OMC of the untreated soil was found to be 13.25% with a maximum dry density (MDD) of 1.75 g/cc. For the treated soil samples, the slurry was added at different percentages by weight of the water content. At 20% addition of slurry into the soil sample with 50 mmol/L of urea-calcium concentration, no change in the OMC is depicted in Table 2. Further, the percentage of slurry was increased to 50% and 70% and OMC was determined. It was observed that with the increase in the percentage of slurry addition to the soil samples with 50 mmol/L, there was no impact on the OMC of the soil. At lower concentrations, the efficiency of bacterial activity in producing the necessary carbonate ions may be reduced, hence limiting the formation of calcite. In addition, decreased calcite formation may not sufficiently fill the voids between larger soil particles, requiring more water to lubricate the remaining pores and produce optimum compaction.

For 300 and 600 mmol/L urea-calcium chloride concentration, the same procedures were repeated to determine the OMC by adding different percentages of slurry to the soil. It was observed that there was no such improvement in the OMC with 50 and 300 mmol/L concentration of urea- CaCl_2 as tabulated in Table 2. However, as the concentration increased to 600 mmol/L, a significant decrease in the OMC was observed. According to the results, a minimum OMC of 12% was achieved in the presence of 70% bio-slurry at 600 mmol/L concentration of urea- CaCl_2 . Figure 5. shows the relationship between the OMC and urea-calcium chloride concentrations. As the slurry with concentrations of cementation solution at 50 and 300 mmol/L did not result in any increase in the optimum moisture content (OMC) of the soil, the OMC achieved with a concentration of 600 mmol/L at 70% slurry was chosen for further investigation.

An optimization study was subsequently performed using various concentrations of urea- CaCl_2 . The slurry was prepared by combining bacteria with a cementation solution containing urea- CaCl_2 . After the complete utilization of urea and calcium chloride, the mixture was left undisturbed for 6 hours to allow for settling. The settled crystals were then subjected to centrifugation

to separate the solid content, and the weight of the resulting crystals was measured. It was observed that at higher concentrations (800 mmol/L and 1000 mmol/L), the production of calcite decreased. This decrease was attributed to the formation of a thick layer of crystals around bacterial cells, which hindered the diffusion of urea to the bacteria.

Table 2. The OMC and MDD were obtained from 50, 300, and 600 mmol/L of urea- CaCl_2 concentration at different percentages of slurry addition

Urea- CaCl_2 Concentration (mmol/L)	Addition of Slurry (%)	OMC %	MDD (g/cc)
50	20	13.45	1.75
	50	13.0	1.88
	70	13.0	1.87
300	20	13.0	1.88
	50	13.0	1.88
	70	13.0	1.88
600	20	12.85	1.89
	50	12.6	1.89
	70	12.25	1.90

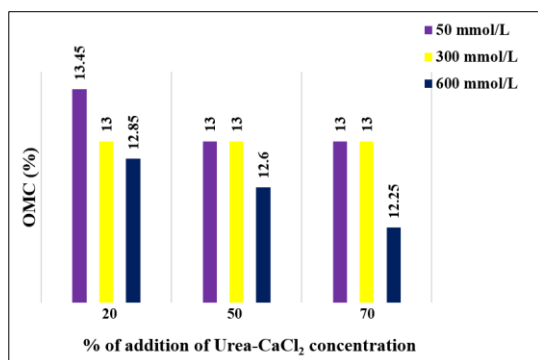


Figure 5. Variation of optimum moisture content (OMC) with the addition of different concentrations of urea-calcium chloride concentration at varying percentages

Effect of MICP on California Bearing Ratio value of soil subgrade

Before the treatment, the CBR test for the untreated soil was conducted. With the OMC obtained at 600 mmol/L of urea-calcium concentration at 70% addition of slurry by weight of water content, the California Bearing Ratio (CBR) test sample was prepared to determine the strength of the subgrade soil. The test was conducted for both conditions (soaked and unsoaked) and values were obtained. Figure 6 shows the variation of California

bearing ratio (CBR) value with OMC obtained at 600 mmol/L of urea-calcium concentration at 70% addition of slurry for treated and untreated soil.

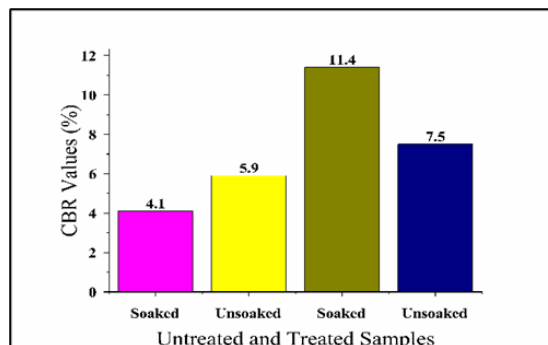


Figure 6. Variation of California bearing ratio (CBR) value with OMC obtained at 600 mmol/L of urea-calcium concentration at 70% addition of slurry for treated and untreated soil samples

According to the findings of the CBR test as shown in Figure 6., a remarkable improvement in the CBR value was obtained. The soaked sample gave a promising result as compared to the unsoaked one. The results of the CBR test indicate a clear enhancement of the engineering performance of poorly graded subgrade soil by improving its strength from 4.1% to 11.4% and 5.9% to 7.5% in soaked and unsoaked conditions respectively. However, it was observed that the soaked samples showed more strength when compared to unsoaked samples. The strength improvement was attributed to the fact that the bacterial slurry consisting of urea- CaCl_2 reacted with water to form calcite precipitation. This shows that the subgrade will be sustained even in the monsoon season of the year.

Effect of MICP on the permeability of soil subgrade

The Optimum Moisture Content (OMC) determined at a urea-calcium concentration of 600 mmol/L and a slurry addition of 70%, was used to determine the permeability of the poorly-graded sandy soil. A falling head permeability test was conducted to examine the drainage properties of the soil. The samples were prepared by adding 70% of slurry by weight of water content and kept undisturbed for 1, 5, 10, and 15 days. After the completion of respective days, the water was allowed to flow for 24 hours and the head differences were noted.

Table 3. Permeability test values for treated and untreated soil

Condition	OMC	Permeability, k (cm/s)			
Untreated soil	13.25	6.52×10^{-3}			
Treated soil	12.0	Reaction period (days)	1	5	10
			5.67×10^{-3}	4.72×10^{-3}	4×10^{-3}
					3.9×10^{-3}

From the test results as shown in Table 3, it was seen that the permeability of the soil has reduced as the reaction period increases. The permeability of natural soil was 6.52×10^{-3} cm/s, which was reduced to 5.67×10^{-3} cm/s, 4.72×10^{-3} cm/s, 4×10^{-3} and 3.9×10^{-3} after a reaction period of 1, 5, 10 and 15 days respectively. With time, the calcite formation forms an effective bridging between the soil grains, known as clogging. The interaction among bacteria, urea, and calcium chloride in the soil leads to microbial processes that contribute to clogging. The bacteria, *Bacillus subtilis*, utilized urea as an energy and nutrition source, increasing the formation of biofilms that restrict the pore openings in the soil. Due to this clogging, the movement of water through the soil was slowed down, leading to the reduction of permeability of the soil. However, the rate of reduction of permeability has reduced after a reaction period of 15 days. This can be concluded by the fact that as the bio-cementation progresses, the soil becomes more solidified, resulting in a reduction in pore space that bacteria can inhabit and where minerals can form.

The presence of an excessive amount of minerals in the soil might impede the transport of nutrients and metabolic waste, resulting in a decrease in bacterial activity and the rate at which bio-cementation occurs. To address this issue, it is essential to optimize the concentration of the cementation solution, which consists of urea and CaCl_2 . Higher concentrations can increase soil strength but also lead to excessive calcite precipitation. This precipitation can hinder the movement of nutrients and metabolic waste by forming a thick crystal layer over bacterial cells. Hence, it is crucial to achieve an optimal balance by regulating the concentration of the cementation solution to ensure sufficient formation of calcite without causing excessive hardening of the soil matrix. This provides sustained bacterial activity and a consistent rate of biological cementation, hence maintaining the desirable properties of the soil.

Effect of pH of soil subgrade

The pH of rainwater at the site varied from 6.9 to 7.5 with an average of 7.2 which reflects to be in the alkaline range. The pH of the natural soil varied

from 5.5 to 7.2 with an average of 6.3. The pH elevation seen can be attributed to the production of hydroxide ions (OH^-) resulting from the process of ureolysis, leading to an alkaline shift in the soil's pH. Before the implementation of the treatment, the soil's pH level was measured to be 6.9, which then increased to 8.1 after the treatment. The bacteria generated an alkaline microenvironment by the consumption of H^+ in the presence of soluble calcium ions which increases the pH of the soil. This alkaline environment facilitated to formation of more calcite precipitation in the soil matrix.

Morphology of biologically cemented samples

The evaluation of the uniformity of CaCO_3 crystal precipitation was conducted using scanning electron microscopy (SEM). The morphology of the soil sample treated with 600 mmol/L of urea-calcium concentration at 70% addition of slurry was consolidated and inspected using a scanning electron microscope (SEM) is illustrated in Figure 7. In this image, it can be observed that distinct calcium carbonate (CaCO_3) crystals have formed and developed between the sand grains. Upon closer examination at a higher magnification, a multitude of crystal forms, sizes, and distributions were identified.

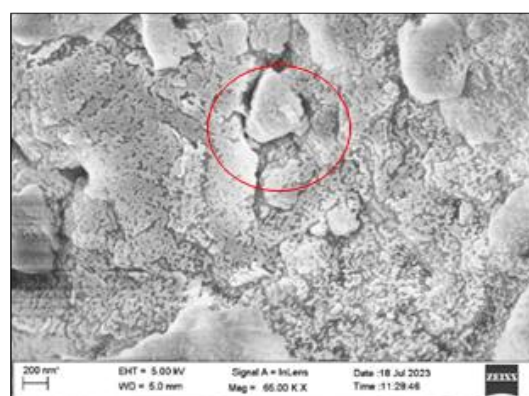


Figure 7. SEM images of soil treated with 70% addition of slurry of 600 mmol/L of urea-calcium concentration

Conclusion

In this research, based on microbial-induced carbonate precipitation (MICP), a solution for poorly graded sandy subgrade soil was investigated. Since Arunachal Pradesh receives heavy yearly rainfall, it is more prone to soil erosion. The results indicated that the application of MICP in subgrade soil can be an effective alternative for the poorly graded subgrade which is susceptible to soil erosion, especially at monsoon. The CBR value obtained from the soaked soil sample was higher compared to the unsoaked soil sample. This indicated that the *Bacillus subtilis* along with urea-calcium carbonate solution will sustain even in monsoon season. The pH of natural soil was found to be 6.9 before the treatment, which reflects the moderately acidic range. However, the pH increased to 8.1 after the treatment. During the urea hydrolysis process, *Bacillus subtilis* utilizes urea as a source of energy and generates ammonia, resulting in an increase in pH in the nearby surroundings. The increase in the pH makes the surroundings alkaline and leads to the formation of calcite precipitation. This relationship between MICP treated soil and rainfall occurrence suggested that the formation of the CaCO_3 by the MICP process could reduce the erosion amount remarkably by forming a bridging between the soil grains. The application of MICP enhanced the CBR of the subgrade soil, resulting in an increase in the soil strength. The CBR test shows a comprising improvement in CBR value with treated soil samples as compared to the untreated soil. Further, the permeability of the treated soil also showed a notable reduction as compared to the untreated soil. Thus, it implies that MICP-reinforced soil will perform better than natural soil and increase load carrying capacity of soil. The process of calcite precipitation resulted in the filling of soil pores, leading to the formation of a firm soil matrix, as indicated by the SEM micrograph. More research should be focused on optimizing the MICP process at both the micro and macro levels before its direct application in the field. Specific challenges need to be investigated including the flow and transport of medium through the heterogeneous media, durability of treatment, permanence of the mixing technique, and mapping of the subsurface stratigraphy at the particle level.

Conflict of interest The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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