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Improving Geotechnical Properties Of Sands Using Microbially Induced Calcite Precipitation (Micp): Influencing Factors And Laboratory Evaluation

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Abstract
Microbially Induced Calcite Precipitation (MICP) is a sustainable method for soil strengthening, which offers energy efficiency, and protection of the ecological environment. It successfully enhances sand strength and stiffness, erosion resistance, and permeability, without the limitations and pollution associated with cement and chemical materials. In this paper Laboratory tests were conducted to evaluate the impact of ureolysis-catalyzed calcium precipitation by "Sporosarcina Pasteurii" on the geotechnical properties of sand. The study investigates the treatment process and identifies key factors that influence the outcomes. The key influence factors like Degree of Saturation, Optical Density of Bacterial Solution, Molarity of Cementation Solution, Particle Size, Distance from Point of Inoculation, Curing Period and pH is maintained 7.5-8.5 were assessed. The mass of Calcite precipitation was measured through the Gravimetric Acid Washing method. Additionally, Scanning Electron Microscope (SEM), Energy Dispersive X-ray Analysis (EDX) and X-Ray Diffraction (XRD) analyses were carried out to examine the bonding between the sand particles.
Keywords: *Microbially Induced Calcite Precipitation (MICP), Soil Reinforcement, Sustainable Geotechnical Properties, Environmental Friendliness.*

1.0 INTRODUCTION

With Rapid growth in population and urbanization, there is a rising demand for urban infrastructure construction, demanding the improvement of unfavorable soils. However, the adverse effects of using conventional methods like cement, lime, and fly ash for soil treatment, poses threat to environment, such as increased greenhouse gas emissions, vegetation loss, and pH variations in soil and water, have prompted researchers to explore environmentally friendly alternatives to conventional admixtures [Joga, J. R et al. 2020; Blanck, G et al. 2014]. Therefore, the discovery of energy-efficient and environmentally friendly soil improvement technologies, like Microbially Induced Calcite Precipitation (MICP), is crucial.

Currently, the most effective way to use MICP is to use bacteria or enzymes to break down urea, which then reacts with a soluble calcium source to form insoluble calcium carbonate crystals. These crystals bind individual sand grains together, making the soil stronger and stiffer. The basic mechanism of the MICP process can be described through the urea hydrolysis pathway, which can be represented by the following equations (1-2) [Qabany, A. A., & Soga, K. 2014].

$CO\,(NH_2)_2 + 2H_2O \rightarrow 2NH_4^{+} + CO_3^{2-}\ pH\uparrow$

$Ca + CO_3^{2-} \rightarrow CaCO_3\ pH\downarrow$

Several researchers have conducted studies over the past few years to explore the potential of MICP in geotechnical applications, specifically focusing on its ability to enhance strength and reduce permeability. These studies have highlighted the adaptability of MICP, as it can be customized for different applications through various injection methods [Ivanov, V et al. 2008; Whiffin, V. S et al. 2007]. The MICP process is considered 100% efficient when all the urea available is hydrolyzed by the bacteria, leading to the precipitation of carbonate and calcium as calcium carbonate at the intended volume. On the other hand,

chemical efficiency is defined by the main injected chemicals (calcium and urea) precipitating as calcium carbonate. However, it's important to note that achieving 100% chemical efficiency does not guarantee 100% overall process efficiency unless the precipitation occurs in the desired location [Al Qabany, A et al. 2012].
Sporosarcina and Bacillus are commonly found strains of urease-producing bacteria that exhibit superior adaptability to the environment, increased specific surface area, and reduced cell aggregation compared to other bacterial species [Jiang, N. J et al. 2019]. Apart from their ability to hydrolyze urea, they can utilize urea as both an energy and nitrogen source through respiration. This process generates CO_2 and converts it into $CO3^{2-}$ in alkaline conditions, facilitating the metabolic production and deposition of calcium carbonate. Notably, Bacillus and Sporosarcina exhibit rapid and high-yield calcium carbonate production. Consequently, they are primarily employed for initiating MICP to enhance the stability and quality of weak geomaterials [Qian, C. X et al.2015; De Muynck, W et al. 2010]. Previous researchers have conducted many tests on MICP by considering various factors such as type of bacteria, concentration of chemicals, bacterial solution, pH of soil, curing period which influence the quantity and degree of improvement. Many of researchers mainly concentrated on fine sand and not mentioned anything regarding coarse sand. However, not much information is available on effect of each factor as well as interaction effect of factors on Calcite precipitation. In this investigation, a series of test were conducted by considering the factors especially degree of saturation, type of sand and the point of inoculation in addition to bacterial solution, cementation solution, Optical density and bacteria. The amount of calcite precipitated in coarse sand is also studied in this investigation.

2.0 MATERIALS AND METHODS

2.1 Sand

The sand used in this study was collected from the banks of the Swarna-Mukhi River near Chandragiri, Tirupati, Andhra Pradesh, India as shown in Figure 1. The field-obtained sand was then divided into two fractions, coarse sand and fine sand, using a dry sieve analysis. The coarse sand fraction was obtained by passing the sand through a 4.75mm sieve and retaining the particles that were larger than 2mm. The fine sand fraction was obtained by passing the sand through a 425-micron sieve and retaining the particles that were smaller than 75 microns. The particle size distribution curves for the fine and coarse sand fractions are shown in Figure 2. According to the International System for Classification of Soils (IS: 1498-1970, reaffirmed 2007), both sands belong to the category of "Uniformly Graded Sand."

Figure 1: Soil Location

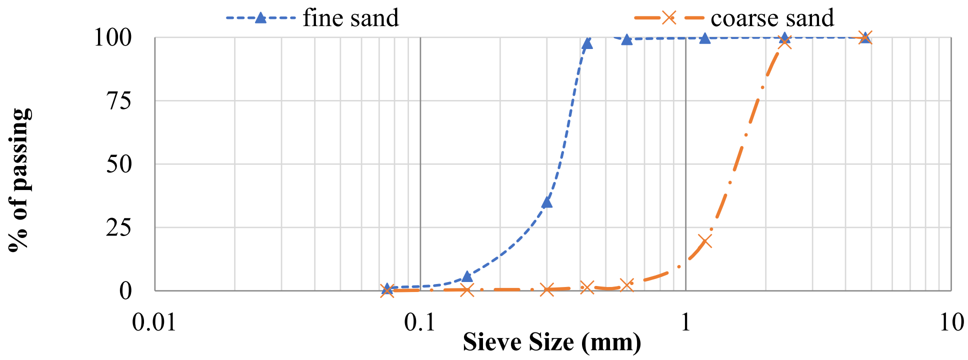

Figure 2: Grain Size Distribution Curves

2.2 Bacteria

Sporosarcina Pasteurii is a commonly found soil-dwelling microorganism that is non-toxic to humans and does not pose any health risks. It exhibits the highest urease activity among alkalophilic soil bacteria, making it widely distributed. While it has been isolated from human feces, it is considered more of a contaminant of the intestine rather than a native inhabitant. The specific strain of bacteria, MTCC-1761, can be obtained from the Gene Bank at CSIR-Institute of Microbial Technology in Chandigarh. To further investigate the obtained culture, it was cultivated in a nutrient broth under optimal conditions. By utilizing a UV-visible spectrophotometer, the concentration of bacterial cells was determined to be highest at 600nm. The composition of the culture medium ingredients is presented in Table 1.

Table 1: Composition of the Culture Medium

S. No	Composition	
	Component	Concentration (g/l)
1	Nutrient Broth	13
2	Urea	7.5
3	Calcium Chloride	13.88

2.3 Sample Preparation

All the identical cylindrical containers are loosely (Coarse and Fine Sands) placed in a cylindrical plastic container such that dry unit weight of the sample is 14.3 kN/m^3 which corresponds to a very loose state. The bacterial concentrations are inoculated into samples based on the Degree of Saturation. The solution was kept for about 4-6 hours to allow uniform bacterial attachment of the particles. After completion of 6 hours, the Cementation Solution was inoculated at an interval of 12 hours for a period of 1 week. After completion of a week, the samples are tested for the amount of Calcium Carbonate precipitation. Mass of calcite measurement using acid washing technique.

2.4 Mass of Calcite Measurements:

The amount of calcite presents in the MICP treated soil was measured by a simple technique called gravimetric analysis [Soon, N. W et al. 2014]. A 20g dry soil sample was taken and reacted with hydrochloric acid, which caused the calcite in the soil to release carbon dioxide, leading to effervescence. The remaining residue was collected, dried again, and its weight loss was used to determine the percentage of calcite in the soil on a dry-weight basis (percentage of the 20g dry soil).

3.0 RESULTS AND DISCUSSIONS

3.1 Processes Responsible for Calcite Precipitation

The tests were conducted to investigate the factors that influence Calcite Precipitation through biochemical processes. The factors examined included the Molarity of the Cementation Solution, the Optical Density of the Bacterial Solution, the Degree of Saturation of the soil, the type of Soil, and the location of the sample in relation to the point of inoculation. After 7 days, soil samples are extracted from a cylindrical plastic container. Approximately 15g of soil from the top and bottom portions are used to determine the amount of calcite that has precipitated. The results of the first series of tests, as shown in

Table 2, include 18 tests with only the addition of Bacterial Solution (referred to as case-1), 18 tests with only the addition of Cementation Solution (referred to as case-2), and the remaining tests with both Bacterial Solution and Cementation Solution (referred to as case-3).
The purpose of case 1 and case 2 tests is to investigate whether the bacterial process alone or the Cementation Solution alone can produce calcite. Plate 2 displays photographs of the soil samples after 7 days of curing corresponding to case 1, case 2, and case 3 tests.
After completion of 7 days, the samples are taken out of a cylindrical plastic container, and about 15g of soil from top and bottom are used for determination of the amount of calcite precipitated. The amount of Calcite Precipitated for all the tests conducted in the first series of tests is summarized in Table 2. In this series 18 tests are conducted by adding Bacterial Solution only without Cementation Solution (case-1 in Table 2), 18 tests are conducted by adding Cementation Solution only without Bacterial Solution (case-2 in Table 2), and the remaining tests are conducted by adding both Bacterial Solution and Cementation Solution (case-3 in Table 2). Case 1 and case 2 tests are aimed to identify if bacterial process alone or Cementation Solution alone can produce calcite or not respectively. Figure 3 shows the photographs of the soil sample after 7 days of curing corresponding to case 1, case 2, and case 3 tests.

Table: 2: Percentage of Calcite Precipitated for the 1st Series of Tests

S. No	**Optical Density of Bacterial Solution (OD)**	**Molarity of Cementation Solution (M)**	**Degree of Saturation of soil (S_r %)**	**Soil**	**$CaCO_3$ (%)**	
					Top	**Bottom**
Case 1- Bacterial Solution without Cementation Solution						
1	0.561	0	10	Coarse Sand	0	0
2	0.523	0	10	Coarse Sand	0	0
3	0.438	0	10	Coarse Sand	0	0
4	0.561	0	25	Coarse Sand	0	0
5	0.523	0	25	Coarse Sand	0	0
6	0.561	0	10	Fine Sand	0	0
7	0.523	0	10	Fine Sand	0	0
8	0.438	0	50	Fine Sand	0	0
9	0.561	0	25	Fine Sand	0	0
10	0.523	0	25	Fine Sand	0	0
Case 2- Cementation Solution without Bacterial Solution						
1	0	0.5	10	Coarse Sand	0	0
2	0	0.75	10	Coarse Sand	0	0
3	0	0.5	25	Coarse Sand	0	0
4	0	0.75	25	Coarse Sand	0	0
5	0	0.5	50	Fine Sand	0	0
6	0	1.0	10	Fine Sand	0	0
7	0	0.5	25	Fine Sand	0	0
8	0	0.75	25	Fine Sand	0	0
Case 3- Both Cementation Solution and Bacterial Solution						
1	0.438	0.75	10	Fine Sand	3.0	2.3
2	0.561	0.75	25	Fine Sand	4.3	4.0
3	0.438	0.75	50	Fine Sand	4.7	3.0
4	0.523	0.5	10	Coarse Sand	0.33	0.8
5	0.523	0.5	25	Coarse Sand	0.67	1.0

S. No	Optical Density of Bacterial Solution (OD)	Molarity of Cementation Solution (M)	Degree of Saturation of soil (S_r %)	Soil	$CaCO_3$ (%)	
					Top	Bottom
6	0.438	0.5	50	Coarse Sand	0.87	1.0

The results of the experiment indicate that the biological process alone, represented by the Bacterial Solution, does not lead to any binding of soil particles in either Coarse Sand or Fine Sand, suggesting that it cannot produce calcite as shown in Figure 3(b). Additionally, the use of a chemical solution, represented by the Cementation Solution, in Case-2 does not result in any Calcite Precipitation in 8 tests, indicating that it alone cannot produce calcite as shown in Figure 3(b). However, when the biochemical processes from the Bacterial culture are combined with the Cementation Solution in Case-3, Calcite Precipitation is observed in 6 tests as shown in Figure 3(c), suggesting that the combination of biochemical and chemical processes is necessary for calcite production.

a b c

Figure 3: (a) Biological process only (Case 1, Test No. 8) (b) Chemical process only (Case 2, Test No.4) (c) Bio-Chemical process (Case 3, Test No. 2)

From the experimental results it has been observed that Fine Sand shows higher calcium precipitation at its top side and lower precipitation at its bottom side, while the opposite trend was observed in Coarse Sand. This difference can be attributed to the difference in microbial transport rates between the two types of sand.

In Fine Sand, where the void size is smaller, microbial transport is slower, leading to higher bacterial activity at the top and consequently more calcite precipitation in that region. On the contrary, in Coarse Sand, with its larger void size, microbial transport is faster, resulting in higher bacterial activity at the bottom and more calcite precipitation there.

The formation of calcite precipitation is influenced by several factors, including Optical Density, Molarity, Degree of Saturation, Particle size, and the point of inoculation. Although some calcite is precipitated in Coarse Sand, there is no binding of particles observed, suggesting that the process of calcite precipitation may be distinct from the binding mechanism in this type of sand.

3.2 SEM Analysis:

The test and the formation of calcite crystals after microbial treatment were of particular interest. SEM analysis was carried out on the selected soil samples. SEM analysis shows the microstructure of calcium carbonate crystals between the soil particles. Figure 4(a) depicts the structure of a sand grain of an untreated sample, it is clear that the surface is free from crystallization, Figure 4(b) depicts the sand particle surface precise an interparticle bond between calcite crystals and sand grains, Figure 4(c), depicts the formation of larger size calcite crystal. Figure 4(d) shows the sand particle surface which can see environs for bacterial growth and microbe beds in 20μm magnification.

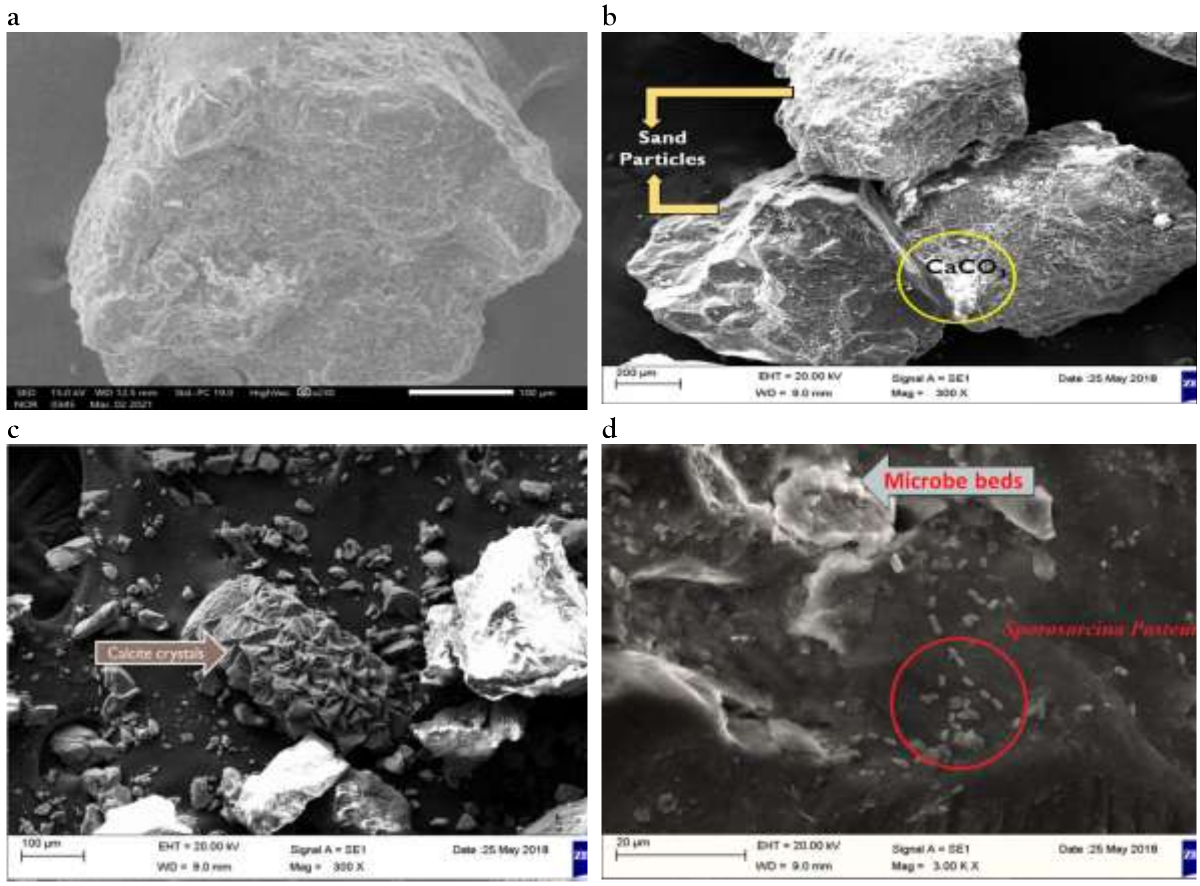

Figure 4: SEM analysis

3.3 EDAX Analysis:

Figure 5 shows the Energy-dispersive X-ray analysis (EDAX) was employed to analyze the chemical composition of the samples. From the EDAX results clear that carbon 16.01%, oxygen 45.39%, Aluminium 7.71%, and Silica 19.17% are the main minerals in the sand. Mineral composition calculated in table 3. The treated soil present in calcium and mineral composition calculated in table 4.

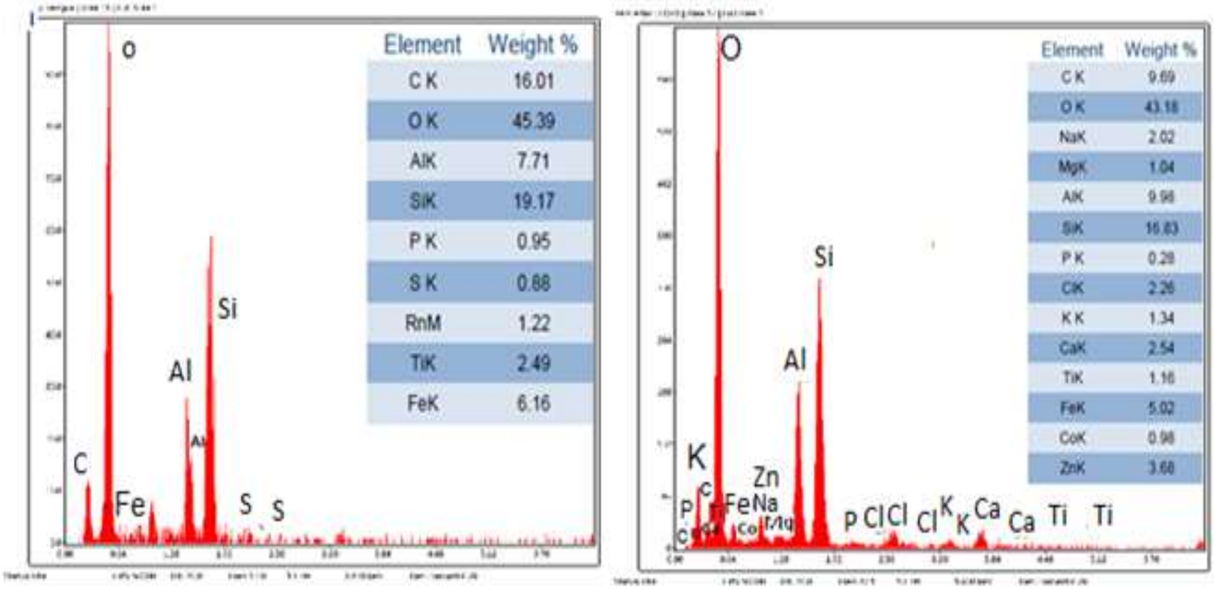

Figure 5: Energy-dispersive X-ray analysis (EDAX) for Untreated and Treated sand

Table 3: Chemical Composition of untreated sand

Oxide	%Weight of Oxide
SiO_2	41.01
Al_2O_6	21.43
CO_2	20.34
Fe_2O_3	8.80

Table 4: Chemical Composition of sand treated with s-pastueri and 0.75M cementation solution (7 Days)

Oxide	%Weight of Oxide
SiO_2	36.00
Al_2O_3	18.85
CO_2	12.31
Fe_2O_3	7.17
CaO	4.3
Na_2O	2.72
MgO	1.72
ZnO	4.58
Cl_2O	4.3

3.4 XRD Analysis:

The XRD analysis was conducted on 0.75M concentration cementation media treated samples with S-Pasteria bacteria. Calcite cyrtals were present in 7 days treated sample in XRD data. Calcite was not present in the untreated sand as shown in Figure 6 after MICP treatment clearly seen Calcium in the XRD analysis as shown in Figure 8.

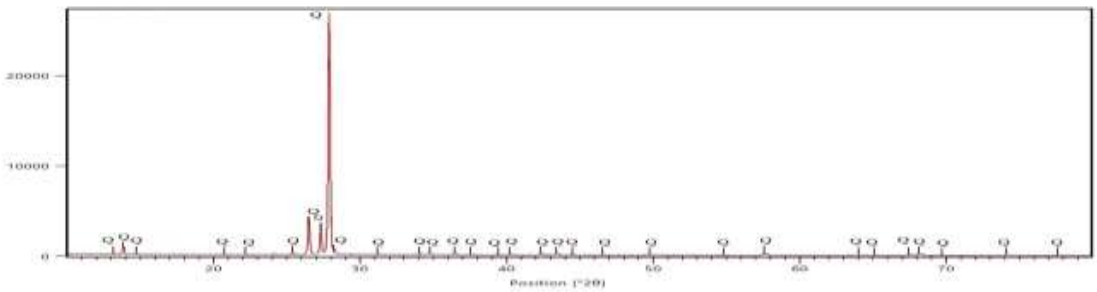

Figure 7: Untreated sand

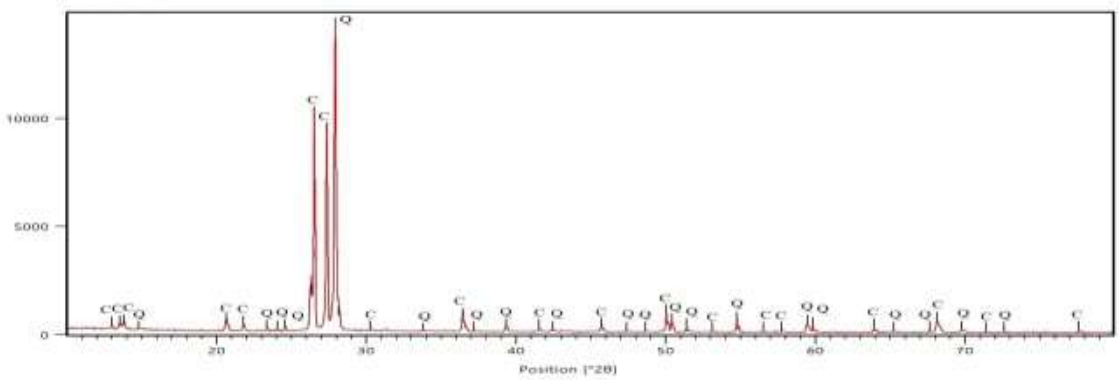

Figure 8: MICP-treated sand

4.0 CONCLUSIONS

- Calcite Precipitation is observed in both Fine Sand and Coarse Sand treated with both Cementation Solution and Sporosarcina Pasteurii Bacterial Solution.
- No Calcite Precipitation is observed when these soils are treated with either Cementation Solution or Bacterial Solution alone.
- This implies that biochemical processes alone can produce calcite, while neither chemical process alone nor biological processes alone can produce calcite.
- Cementation of soil particles is observed only in Fine Sand and not in Coarse Sand.
- Calcite Precipitation is higher at the point of inoculation of bacterial and Cementation Solution and lower at the bottom for Fine Sand, and vice versa for Coarse Sand.
- The order of bio-cement produced ranges from 0.33% to 4.7%, depending upon factors such as the point of inoculation of Bacterial Solution and Cementation Solution, Optical Density of Bacterial Solution, Degree of Saturation of soil, Molarity of Cementation Solution, and Curing Period.
- SEM analysis shows clear visible bonding between the soil particles.

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Declarations

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