

Estimation of Shear Strength Properties of Bio-treated Sand

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Abstract. Majority of the available competent soils have exhausted with the growing trend in population and urbanization which necessitates to improve the properties of existing weak soils. Many of the conventional ground improvement techniques found to be environmentally unsound (For e.g. Chemical grouts). Thus the need for techniques which can modify those unsuitable soils through more natural and sustainable means are gaining momentum. The current research article presents the investigations carried out by treating the locally available sand (poorly graded) around the surroundings of NIT Warangal using Microbial Induced Calcite Precipitation (MICP) through urea hydrolysis. An attempt is made to estimate the shear strength properties of the sand strengthened with calcite formed through the MICP process using Direct Shear Test (DST). The volume change characteristics of both treated and untreated sand at different relative densities of 35% and 85% were studied and presented. The volume change behaviour of the loose specimen showed dilative behaviour upon MICP treatment which reveals the densification through calcite precipitation. The XRD and SEM analyses were used to study the microstructure of the treated sand specimens. Cohesion intercept is developed significantly in the treated specimens with a slight reduction in the angle of shearing resistance. The XRD spectrum revealed the formation of calcite in different amorphous forms and the SEM images depicts the specific patterns of the calcite precipitation.

Keywords: MICP, Calcite, Microstructure, XRD, SEM.

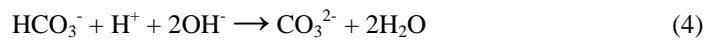
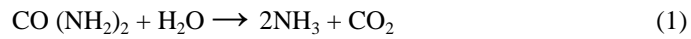
1 Introduction

Majority of civil engineering constructions require essentially, the adequate ground conditions so as to provide sufficient bearing capacity and limited settlements. The overpopulation all around the world urges the engineers for construction of earth structures such as embankments which uses the locally available soils with some initial treatment if required, to make it suitable for that particular construction [8]. The

usage of the conventional ground improvement techniques have been limited in many circumstances since these techniques possess high variations in availability, constructability, suitability and sometimes even found to be environmentally toxic, though they are proven to be quite effective [17]. Hence there is a requirement to search for sustainable and environmental friendly techniques to achieve the ground improvement. In recent years, the techniques that utilizes the biological means have gained prominence since all the additives used in such techniques are environmental friendly [4-6, 16, 18, 21]. The byproducts produced during the metabolic activities of bacteria are used to treat the soil in this technique.

Among various techniques that utilize the biological activities, Microbial Induced Calcite Precipitation (MICP) has grabbed the attention of researchers. MICP treatment could be achieved through many processes such as Urea Hydrolysis, Iron Reduction and Sulfate Reduction, etc. [25]. Among these processes, Urea Hydrolysis is found to be most effective because of its high calcite precipitation efficiency and simplicity in application [7]. Urea hydrolysis which is actually a slow process, can be catalyzed in the presence of urease enzyme. MICP process through urea hydrolysis uses indigenous microorganisms to secrete urease enzyme to catalyze the urea hydrolysis process. An alkalophilic bacteria, *Sporosarcina Pasteurii* (formerly known as *Bacillus Pasteurii*) is found to be highly urease positive [9]. Most researchers tried to evaluate the potential of MICP through urea hydrolysis by supplying urea and calcium chloride solutions along with the ureolytic bacteria [26-27]. The calcite precipitated through this process forms bridges between the soil particles this densifying the soil with a limited reduction in the permeability [1, 4, 16, 18].

The MICP process is controlled by various environmental factors like the injection conditions [11], aqueous conditions, particle sizes, gradation [19], degree of saturation [2], bacterial cell count [20] and flow pressure [23]. The curing time was most contributing factor in the treatment process followed by bacterial count, nutrient concentration and the flow rate of solutions [24]. The process of MICP through urea hydrolysis could be explained through a series of reactions (that occur in the presence of urease enzyme) shown below in equations 1 to 5 [25].



In the present study, an attempt is made to apply this technique to the sand specimens to modify its properties. Direct Shear Tests (DST) were carried out on the treated specimens to understand the improvement in the shear strength properties.

2 Materials

2.1 Sand

Bacterial activity requires a favorable pore size of about 50 – 400 μm [22] and it should be able to allow for the bacterial movement of size 0.5 – 3 μm [18] which is available in soils with grain size matching silt and above. Hence the study is carried out in sand to evaluate the applicability of this method. The sand used in the current study was collected from a local site near NIT Warangal, Telangana, India.

2.2 Bacterial Culture

In the present study, an indigenous soil bacterium, *Sporosarcina Pasteurii* (formerly known as *Bacillus Pasteurii*) cells (freeze dried cells), were procured from Microbial Type Culture Collection (MTCC), India. This microorganism has biosafety level 1 rating given by United States Centre for Disease Control and Prevention (CDCP). This microorganism is gram-positive, i.e., its properties will not vary with temperature and pressure. As per the MTCC, the optimum conditions for bacterial growth are 37°C temperature, aerobic environment and proper nutrient medium (Nutrient Broth). The cells are revived from the freeze-dried pellet and stored for future use. The microscopic view of the bacterial cells at 400x magnification is shown in figure 1.

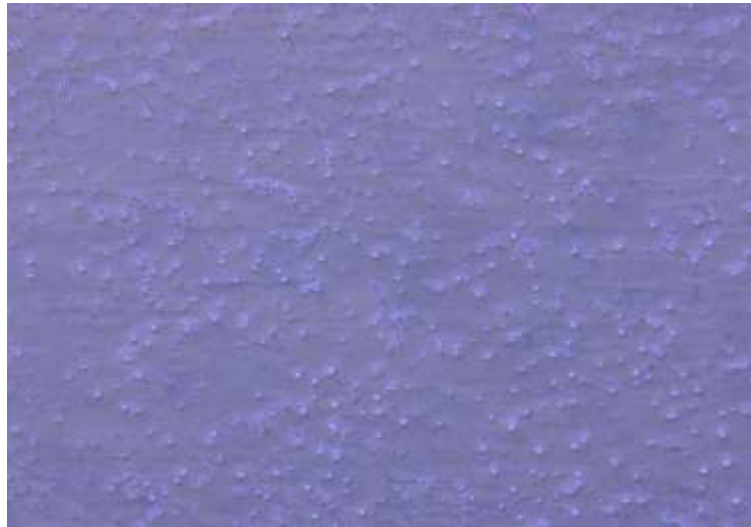


Fig. 1. Microscopic View of *Sporosarcina Pasteurii*

3 Methodology

3.1 Preliminary Studies on Sand

Experiments were carried out on the collected sand to identify the basic index properties as per IS: 2720. The Specific Gravity, Grain Size Analysis and Relative Density tests were carried out according to IS: 2720 [12-13, 15]. In the present study, the specimens are prepared at loose and dense states, i.e., at 35% and 85% relative densities respectively. The shear strength parameters of the specimens prepared were found from Direct Shear Tests (DST) carried according to IS: 2720 [14].

3.2 MICP Treatment

3.2.1 Preparation of Bacterial Cell Solution. A starter culture was prepared from the stored culture to bring the culture to active state. The main culture medium (NB Media) was prepared and autoclaved. It is then inoculated with starter culture at a dilution of 1:100 and incubated at 37°C for 30 hours by the end of which the cells would have reached its maximum population. The cells are centrifuged at 7000rpm and 4°C and washed twice with Sodium phosphate buffer (pH = 7) to remove any metabolic waste generated during the bacterial growth. The bacterial cell solution is prepared by dissolving 10g of NB, 20g of Urea, 10g of Ammonium Chloride and 2.12g of Sodium bicarbonate in small quantity of double distilled water and then made up to one litre. The pH of solution is brought down to 8.5 using 4M Hydrochloric (HCl) acid [1] and is autoclaved. The centrifuged cells are transferred into the autoclaved bacterial cell solution and stored at 4°C for further use.

3.2.2 Sample Preparation and Injection. Sample extractor with dimensions 6cm x 6cm x 2.5cm was used for preparing the DST specimens. It was placed on a scouring pad (Scotch Brite) and then on a cardboard piece of same size wrapped with cello tape. The openings were closed with bentonite and sealed with wax to avoid evaporation of bentonite paste. The sand specimen is now prepared by pouring the known quantity of sand by changing the height of fall so as to achieve densities of loose and dense states (35% and 85% respectively).

For successful MICP treatment, proper injection strategy is essential. In the present study, multi-stage injection suggested by Inagaki et al., [11] was followed. This process involves the injection of bacterial cell solution first and followed by a series of injections of cementation solutions (Urea and CaCl₂). After the specimen preparation, the bacterial cell solution was injected into the sand specimen at a rate of 5-10ml/min immediately followed by a high saline solution (0.1M CaCl₂) to fix the bacterial cells to the surface of sand grains [10]. It was allowed to stand for 24 hours and then drained off under gravity. Cementation solution concentration, to provide high calcite precipitation, was chosen as equimolar solution of 0.5M Urea and CaCl₂ [11] and NB media was also added to supply nutrients. The cementation solution was injected at a

frequency of 12 hours for two consecutive days. Before every injection the retained solution was drained off. The injection of solutions into the specimens prepared was shown in figure 2. Finally the specimens were allowed for a curing period of 21 days at an ambient room temperature of 25-35°C.

3.3 Strength Testing

The samples were extracted from the moulds and direct shear testing is carried out on treated sand as per IS: 2720 (Part 13) [14]. Three different normal stresses of 50, 100 and 150 kPa were used and corresponding failure shear stresses were found so as to plot the Mohr's failure envelope and determine the shear strength parameters of treated specimens. The volume change behaviour of specimens were calculated at 50kPa normal stress. The specimen after the shearing was shown in figure 3. The calcite precipitation can be observed clearly at the failure plane in figure 3.



Fig. 2. Injection of Solutions into the Specimens



Fig. 3. MICP Treated Specimen after Shearing

3.4 Morphological Studies

The morphology needs to be studied to confirm the precipitated mineral was calcite and for understanding the precipitation pattern of the calcite on the sand surface. Powder X-Ray Diffraction (XRD) tests were performed on the treated and untreated specimens and the minerals matching the peaks were identified using X'Pert Highscore software. The Scanning Electron Microscope (SEM) analysis was carried out on the treated specimens to analyze the microstructure of the treated specimens.

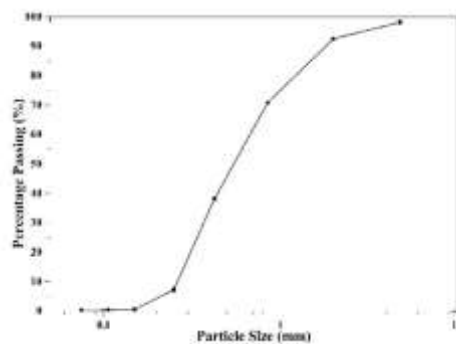
4 Results and Discussions

4.1 Preliminary Studies on Sand

The basic properties of the sand were presented in table 1 and the grain size distribution curve was shown in figure 4. Based on the grain size distribution and grain size analysis, the sand used in the study was found to be poorly graded sand (SP).

Table 1. Geotechnical Properties of Sand

Sl. No.	SOIL PROPERTY	VALUE
1.	Specific Gravity, G	2.65
2.	% Gravel	1.81
	% Sand	97.95
	% Fines	0.24
	D ₁₀	0.34 mm
	D ₃₀	0.52 mm
	D ₆₀	0.97 mm
3.	C _u	0.85
	C _c	0.82
4.	IS Soil Classification	SP
5.	Relative Density Test:	
	$\gamma_{d, \min}$	14.06 kN/m ³
	$\gamma_{d, \max}$	19.51 kN/m ³

**Fig. 4.** Grain Size Distribution Curve

4.2 Strength Testing

The Mohr's failure envelopes of the treated and untreated specimens were presented in figure 5 and the shear strength parameters were presented in table 2. It can be observed that the cohesion intercept was developed in the MICP treated specimens which could be credited to the precipitation of calcite. The angle of shearing resistance values of the treated specimens were found to be a little less than those of the untreated specimens. Similar findings were observed with the studies of Chou et al., [3]. The volume change behaviour with shear strain was studied at both loose and dense states, before and after MICP treatment and was shown in figure 6.

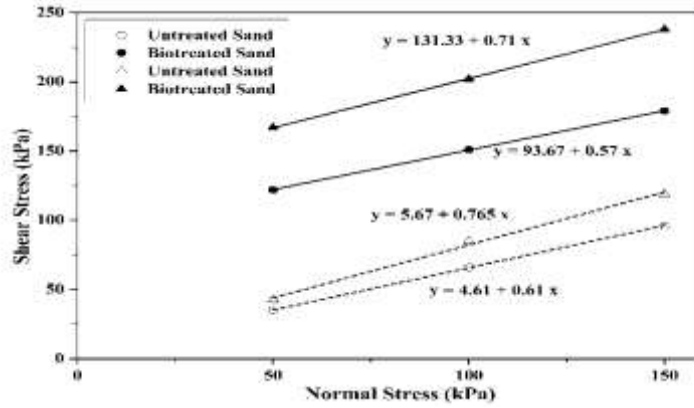


Fig. 5. Mohr's Failure Envelopes of Treated and Untreated Specimens

Table 2. Shear Strength Parameters of Treated and Untreated Specimens

Relative Density (%)	Untreated Sand		Bio-treated Sand	
	Cohesion Intercept, c(kPa)	Angle of Shearing Resistance, ϕ	Cohesion Intercept, c(kPa)	Angle of Shearing Resistance, ϕ
35	4.67	31.4°	93.67	29.7°
85	5.67	37.4°	131.33	35.4°

The volume change behaviour of the loose specimen was observed to be dilative upon shearing with the MICP treatment. This proves the densification of the loose specimen and the specimen with initial high density showed much more dilative behaviour.

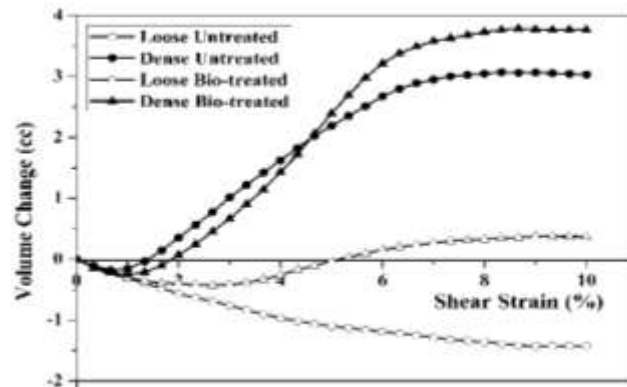


Fig. 6. Volume Change Behavior of Treated and Untreated Sand

4.3 XRD Spectrum Analysis

The XRD spectra of both Untreated and Treated specimens were plotted in figure 7. Figure 7 confirms the presence of calcite which is identified to exist in various amorphous forms with crystalline structures changing as rhombohedral, hexagonal and orthorhombic systems.

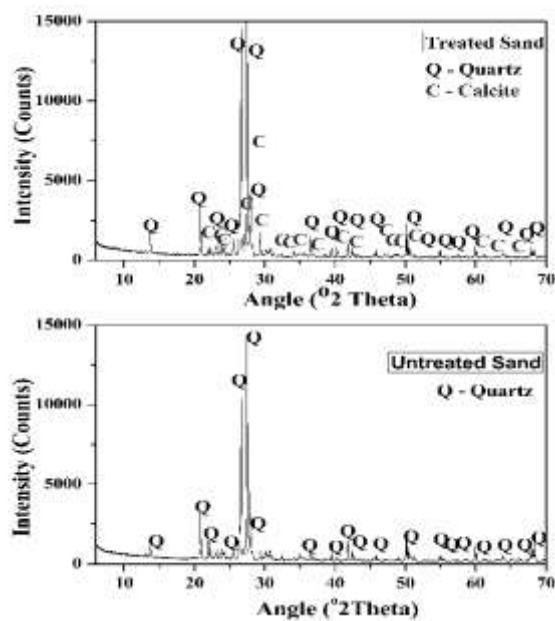


Fig. 7. XRD Spectrum of Untreated and Treated Sand

4.4 SEM Analysis

The SEM images of the treated specimens at various magnifications were shown in figure 8 which clearly depicts the precipitation of calcite at the particle joints. At some locations, multiple layers of calcite precipitation was also observed.

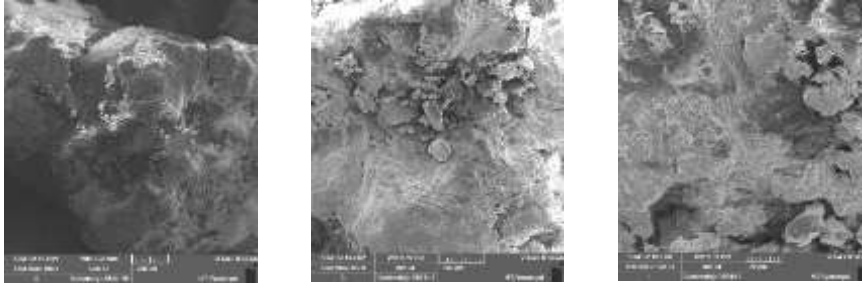


Fig. 8. SEM images of Treated Sand

5 Conclusions

Based on the observations of the tests performed, it is concluded that the MICP produced significant improvement in the poorly graded sand. The cohesion intercept produced by the precipitation of calcite that binds the solids was found to be significant. The angle of shearing resistance values were found to be almost close to the initial values with a minor reduction that is matching with the studies of Chou et al., [3]. The microstructure of the specimens studied through the XRD and SEM analyses confirmed that the increased strength was the contribution of calcite precipitation at preferred locations.

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