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Influence of Single-Dose Biocement Treatment on the Hydraulic Conductivity of the Riverbank Sand

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Abstract **-** Biocementation through Microbially Induced Calcite Precipitation (MICP) is proven for amending the geotechnical properties of soil without disturbing the innate soil structure due to the water like consistency of cementation solutions. This study emphasizes the effect of single dosing of bio-cementation on the hydraulic conductivity of the Brahmaputra sand by one of the prominent soil bacterium *Bacillus megaterium* (NCIM 5472). The bio-calcification potential of *Bacillus megaterium* (BM) is first evaluated by its ability to produce urease and calcium carbonate. This is followed by the application of the biocementing culture into sand columns via injecting the cementation solution. The changes in the microstructure and mineralogy of the treated specimen were also analyzed. The saturated hydraulic conductivity is evaluated as a measure of change in pore space of treated sand at two different relative densities of soil. The major findings of the present study are that the change in the pore space via precipitation is more evident in low-density soil and at higher concentrations of the cementation solutions.

Keywords**:** *Bacillus megaterium*, Microbial Induced Calcite Precipitation (MICP), Urea hydrolysis, riverbank soil stabilization.

1. Introduction

The Brahmaputra riverbank region in Assam (India) is known for the geotechnical challenges in terms of seismic hazards and high rainfall. The huge hydromorphic changes and high rainfall intensity make this region prone to soil liquefaction and riverbank erosion [1]. The existing techniques to mitigate these geohazards includes cement grouting, lining, and chemical methods, which have limitation at the front of ecological sustainability and cost-effectiveness [2]. Therefore rather than focusing on the hard structures on the banks, it is necessary to have an alternative stabilization method that can be ecologically sustainable for the riparian zone [3]. An emerging field known as bio-mediated soil improvement has been claimed by many researchers to have the potential for sustainable and eco-friendly hydraulic erosion control and liquefaction mitigation [4]–[6]. The primary theory with bio-mediated soil improvement is that the soil media hosts several biological activities in the soil pore spaces, which may alter the soil pore network by means of bio-mineralization, biofilm formation, and biogas generation [7]–[10]. Therefore, engineering these biological processes may provide potential solutions for geotechnical challenges. One of the prevalently explored bio-geo-engineering processes is Microbial Induced Calcite Precipitation (MICP), which utilizes bacterial urease to precipitate calcite in the soil pores and alters the shear strength and hydraulic conductivity of the soil. Thus, this process has been proposed for tackling various geotechnical challenges such as weak ground properties, liquefaction, and soil erosion [2]. MICP can be deciphered as catalysis of urea hydrolysis reaction employing ureolytic bacteria *Sporosarcina pasteurii*. The urea hydrolysis reaction is shown in Eq. (1).

$$\mathbf{CO(NH_2)_2 + H_2O \xrightarrow{\textit{Ureolytic microbe/Urease}} H_2CO_3 + 2NH_3} \tag{1}$$

The produced ammonia further yields to ammonium hydroxide ions in the aqueous solution, which results in the increase of the alkalinity of the solution, as shown in Eq. (2).

$$NH_3 + H_2O \rightarrow NH_4^+ + OH^- \quad (2)$$

The produced bicarbonate further disassociates further to form carbonate ions in the solution, as described in Eq. (3). The precipitation of $CaCO_3$ crystals occurs in the presence of Calcium ions (Dissolved Calcium salt such as $CaCl_2$) in the alkaline environment, as shown in Eq. (4).

$$H_2CO_3 \rightarrow 2H^+ + 2\ CO_3^{2-} \quad (3)$$

$$Ca^{2+} + CO_3^{2-} \rightarrow CaCO_3 \downarrow \quad (4)$$

The formation of calcite crystal is observed in three steps which can be described as the development of supersaturated conditions, initiation of calcite crystal formation at critical saturation and crystal growth in stable conditions [11]. Saturated hydraulic conductivity can be evaluated as dependent on the pore size and network [12]. That makes saturated hydraulic conductivity one of the key geotechnical parameters to investigate in the case of MICP treatment, as the precipitation alters the pore network. Whiffin et al. (2007) have reported that the saturated hydraulic conductivity of the soil decreased to 9×10^{-6} m/s upon treatment from 2×10^{-5} m/s of the untreated soil [13]. The saturated hydraulic conductivity was reported to reduce 20% when the precipitated $CaCO_3$ content was 2% of the sample weight [14]. It has been reported that the application of MICP treatment leads to clogging of the soil pores with calcite crystals precipitates. That eventually causes a considerable reduction in the pore volume leading to the decrease of the saturated hydraulic conductivity of the soil. It is to be noted that most of the studies on bio-mediated soil improvement have considered *Sporosarcina pasteurii* for biocementation due to its high urease activity [15]. However, there are few studies that have also recommended other microbes such as *Bacillus subtilis*, *Bacillus megaterium, Proteus vulgaris* and *Sporosarcina ureae* [15]–[18]. Most of these microbes are found considerably in the soil environment [19].

One of the abovementioned microbe, *Bacillus megaterium* (BM), can survive well in toxic conditions, including a wide range of temperature from 3°C to 45° C and on a variety of carbon sources [20]. Its large size provides prevention against being flushed out, and the influence of oxygen on the urease activity of this strain has been found to be marginal [21]. The capability of this culture to grow in a wide range of environmental conditions make it an ideal candidate for riverbank soil stabilization. Therefore, BM can be a viable alternative to *Sporosarcina pasteurii* for biocementation applications.

The objective of this study is to evaluate the potential of BM for bio-calcification and to investigate the influence of initial soil density on the treatment level by evaluating the saturated hydraulic conductivity. The bacteria BM was characterized for its ureolytic and calcite precipitation ability of this culture before investigating the effect of bio-cementation on microstructure and saturated hydraulic conductivity of Brahmaputra Riverbank sand at different densities. This preliminary study will provide useful insights on the influence of the initial density of the soil on the BM mediated MICP treatment for future investigations on liquefaction mitigation and erosion control in the Brahmaputra basin.

2. Materials

2.1 Soil

Brahmaputra riverbank deposited sand was collected from the river basin nearby the Indian Institute of Technology (IIT) Guwahati, Assam, India. The grain size distribution, specific gravity, relative density, organic content, and pH of the soil were evaluated by following Standard test methods described by the American Society for Testing and Materials (ASTM).

2.2 Bacterial and cementation solutions

In this study, the ureolytic bacteria *Bacillus megaterium* (BM) with accession number NCIM 5472 was procured from from the National Collection of Industrial Microorganisms (NCIM), India. Bacterial culture was grown in Nutrient Broth (NB) media in conical flasks, as shown in figure 2(a) at pH 8 and 37°C temperature in a shaking incubator at 200 rpm. Single dosing of the bio-cementation solution is proposed for the bio-calcification process in this study to keep minimal chemical and microbial interferences to the river ecology. Cementation solution was prepared by mixing 60 g urea and 147 g $CaCl_2.2H_2O$ along with 1 g of NB in 1 litre of ultrapure water.

2.3 Preparation of soil specimen

First, the sterilized sand required for the desired density was mixed with one pore volume of bacterial solution. The sand was sterilized to highlight the influence due to the applied microbe BM only. The sand columns with two different relative densities (R.D.) of 35% and 85% were prepared by the wet sedimentation method in an acrylic cylindrical tube of 40 mm diameter and 100 mm length. Porous stones of 10 mm thickness and Whatman grade 42 filter paper was used on the top and bottom of the column to prevent the possible migration of soil particle and bacteria. After 24 hours of bacterial application, the cementation solution (one-pore volume) was injected through a burette. The sample was washed multiple times with de-ionized water prior to and after the treatment to replace air voids with water prior to the measurement of hydraulic conductivity.

3. Methodology

3.1 Biocementation Characterization of bacteria and precipitates

The growth was evaluated by measuring its optical density at 600nm ($O.D._{600}$) in the spectrophotometer over a time duration of 48 hours. The biocementation potential of BM was performed by evaluating the qualitative & quantitative urease activity and calcite precipitation potential. The qualitative urease activity of the bacteria is tested on Urea Agar Base (UAB). The quantitative urease activity was measured by the phenol-hypochlorite method [19]. One unit of urease is defined as the amount of enzyme hydrolyzing one micromole urea/min/ml. Specific urease activity is defined as urease activity at unit optical density [19]. The calcite precipitation potential was evaluated with a gravimetric method [19].

3.2 Measurement of saturated hydraulic conductivity

The saturated hydraulic conductivity of Brahmaputra sand before and after MICP treatment was measured with the help of falling head permeability test according to ASTM standard procedure [22] at loose (R.D. 35%) and dense (R.D. 80%) states. After one week of the reaction time for precipitation, the saturated hydraulic conductivity of the soil was measured. After the hydraulic conductivity test, the MICP treated soil was extruded. All experimental trials were evaluated in triplicates, and the results were also analyzed by analysis of variance (ANOVA).

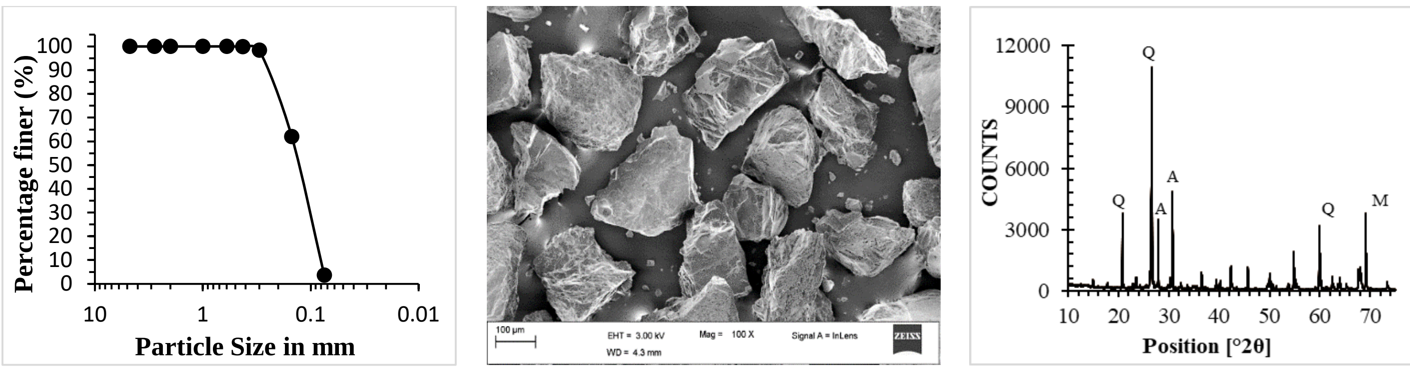
3.3 Microstructural and Mineralogical analysis

The microstructural and mineralogical analysis of the bacteria, precipitates and treated soil were carried out using Field Emission Scanning Electron Microscope (FESEM) and EDX (Energy-dispersive X-ray spectroscopy). The FESEM and EDX analysis was performed on sigma 300, ZEISS (Carl Zeiss Pvt. Ltd.). A small quantity of the specimens (precipitates and soils) was sprayed on carbon tape mounted aluminium stubs. The microbial samples (BM) were prepared by the drop-casting method [23]. The specimens were sputtered with a 10-nm thick platinum coating to provide conductivity through the sample. The imaging was conducted, varying the electron beam intensity up to 20 kV. X-ray powder diffraction (XRD) was also performed on Rigaku, Model No: TTRAX III (5 kW) to determine the mineralogical properties of the untreated and treated sand. The input for step size was given as 0.03°, and the range of 2θ was selected from 10 to 75°. The minerals present were analyzed using Match software to determine the mineralogical content of the soil.

4. Results and Discussion

4.1 Characterization of Brahmaputra riverbank sand

The grain size distribution curve of the Brahmaputra riverbank sand is presented in Fig. 1 (a). The FESEM image of the oven-dried sand grains illustrated as Fig. 1 (b) revealed that Brahmaputra sand has angular particles with rough surfaces. The particle size varied from 150 microns to 350 microns in dimension. X-ray diffraction revealed quartz (Q) dominance in the mineralogy of the Brahmaputra riverbank sand. Notable peaks of Albite (A) and Mica (M) were also observed in the XRD plot.



(a) (b) (c)

Fig. 1 (a). Grain Size Distribution Curve, (b). SEM micrograph, and (c). XRD plot of the Brahmaputra riverbank sand.

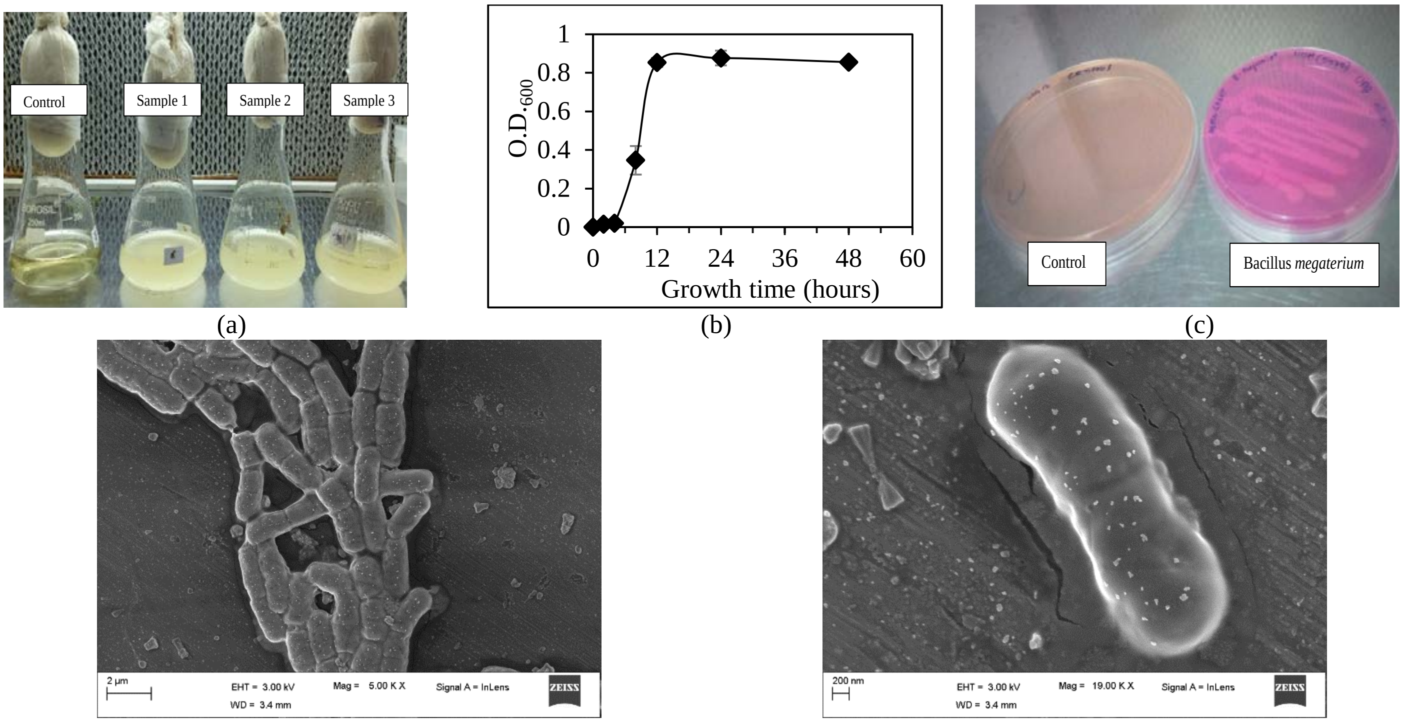
The engineering properties of Brahmaputra Riverbank sand are given in Table 1. It is classified as poorly graded fine sand (SP) as per the USCS classification. The Brahmaputra riverbank sand can be characterized as a neutral pH soil with negligible organic content.

Table 1: grain size characteristics of Brahmaputra riverbank sand

Characteristics	Values
D_{60} (mm)	0.15
D_{30} (mm)	0.1
D_{10} (mm)	0.08
Coefficient of uniformity, C_u	1.875
Coefficient of curvature, C_c	0.833
Specific Gravity, G_s	2.7
pH	7.2
Minimum dry unit weight (kN/m^3)	13.09
Maximum void ratio at minimum dry unit weight	1.02
Maximum porosity (%)	50.5
Maximum dry unit weight (kN/m^3)	15.6
Minimum void ratio at maximum dry unit weight	0.69
Minimum porosity (%)	41.1
Organic content (%)	0.3

4.2 Calcification potential of bacteria and characterization of the precipitates

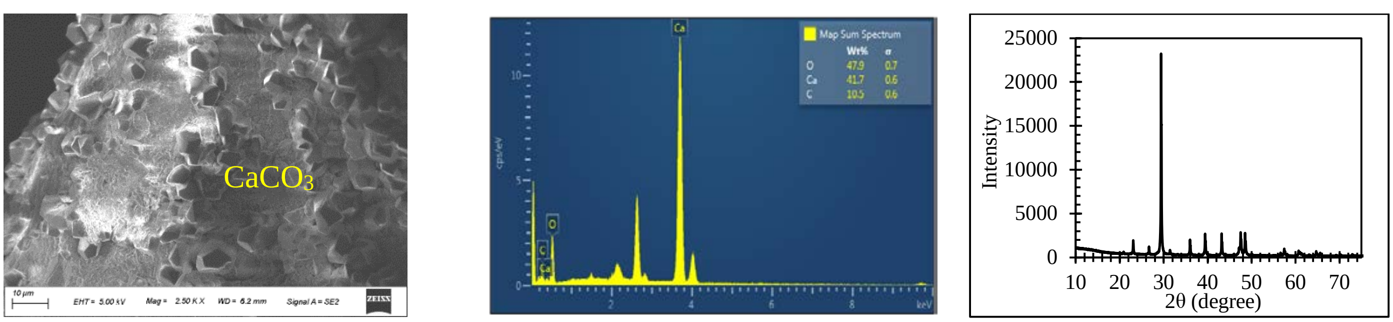
The growth characteristics of BM are shown in Figs. 2 (a, b). A change in colour due to the alkalinity induced is noted within 24 hours to 36 hours on Urea agar base plates and shown in Fig. 2 (c). The micro-graphical analysis of BM cells is shown in Fig. 2 (d-e). The cells were recorded to be rod-shaped. The length of bacterial cells was in the range of 2 microns (μm) to 5 microns (μm). The mean specific urease activity of the bacterial culture was evaluated to be 300±50 U/ml for unit optical density. It is to be noted that Dhami et al. 2013 reported the urease activity of *Bacillus megaterium* (isolated from the soil) to be around 300 U/ml for 24 hours of inoculation. The maximum urease activity of BM was reported as 690 U/ml at 96 hours of the growth period [19]. The precipitated calcite was oven-dried and weighed to evaluate the calcite precipitation potential of the culture. The average calcite precipitation potential of BM was observed as 450±25 mg per 100 ml of cementation solution.



(d) (e)

Fig. 2 Observations on the bacteria, (a). Visual growth, (b). Growth characteristics, (c). Qualitative urease activity, (d). Colonies, and (e). Single-cell (2-3μm length) of *Bacillus megaterium* observed under FESEM.

The morphology of the recovered precipitates was then analyzed by FESEM as reported in Fig. 3 (a). The rhombohedral precipitates were lumped together. To confirm the mineralogy of precipitates, EDX and XRD analyses were also performed on the samples, and the results are shown in Fig. 3 (b) and 3 (c). With EDX analysis, it was confirmed that the precipitated crystals consisted of calcium, carbon, and oxygen. Later, with XRD analysis, the precipitated crystals were confirmed to be calcite.

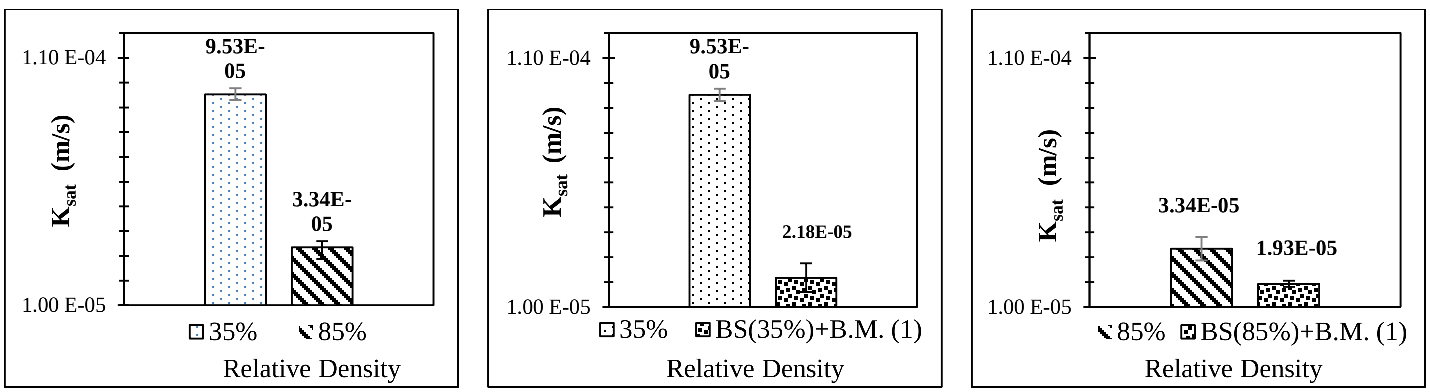


(a) (b) (c)

Fig. 3 Crystal precipitation potential of *Bacillus megaterium*, (a). Photographic image of precipitates, (b). FESEM image of precipitates, (c). EDX analysis of precipitates, and (d). XRD pattern of the precipitates.

4.3 Saturated hydraulic conductivity of amended Brahmaputra riverbank soil

The average saturated hydraulic conductivity values of untreated sand columns at loose (35%) and very dense state (85%) by falling head permeability test is shown in Fig. 4 (a). After treatment of the soil with the Bacterial solution (at O.D.600 =1) and the equimolar cementation solution, the saturated hydraulic conductivity of the sand at a very loose and dense state is evaluated after seven days and represented in Fig. 4 (b) and 4 (c). The saturated hydraulic conductivity of the loose soil sample (relative density=35%) is 9.53×10^{-5} m/s, which got reduced by 87% to 2.18×10^{-5} m/s upon the MICP treatment. At the same time, the saturated hydraulic conductivity of the dense soil samples (relative density=85%) was 3.3×10^{-5} m/s which got reduced by 40% to 1.98×10^{-5} m/s upon treatment. These values suggest that the magnitude of reduction in the hydraulic conductivity value of the bio-treated soil is more in the loose soil when compared with the dense soil. As the saturated hydraulic conductivity is a function of the pore space and particle size of the soil, it is obvious that the extent of treatment is more in loose sand due to ease of bacterial and cementation solution transfer in the porous media. In dense soil, the available pore space is relatively smaller than the dense sand, as discussed in Table 1, and hence the bacterial transport for the cementation becomes difficult, leading to non-uniform cementation and marginal influence on the saturated hydraulic conductivity. It is to be noted that though in the previous studies[14], the influence of MICP treatment has been reported with cementation solution concentration, calcite content and distance from the injection, while, in this experimental study, the influence of relative density of the soil on the saturated hydraulic conductivity of the treated sample has been reported.

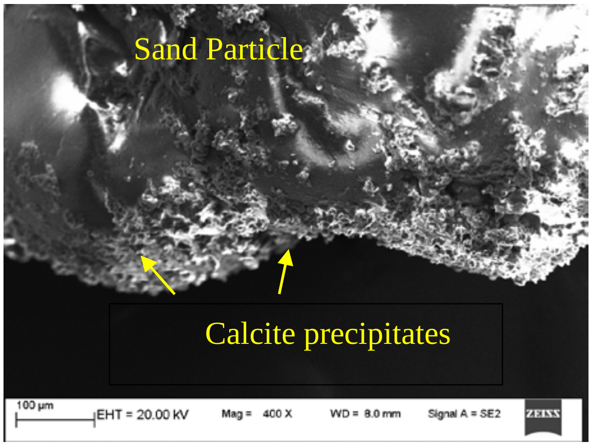


(a) (b) (c)

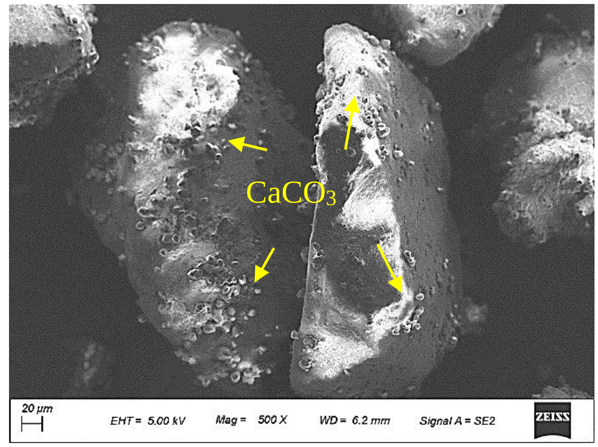
Fig. 4 Comparison of average saturated Hydraulic conductivity of the untreated and treated sand at (a). Different densities, (b). Loose state (untreated and treated), and (c). Dense state (untreated and treated).

The extruded sand column for loose sand failed during the compression at negligible load due to low calcite content. The microstructure of the cemented loose sand was examined by FESEM analysis, as shown in Fig. 5 (a-b). The FESEM on the treated sand revealed precipitation of calcite crystals of approximate size 10 µm over the sand particles. The FESEM indicates the absence of soil bridging at low cementation among the sand particles, as illustrated in Figure 5 (b), which is the reason for insignificant compressive strength.

It is to be noted that the single dosed cement treatment produced 3.3% $CaCO_3$ content on loose sand and 3.7% $CaCO_3$ content in the dense sand, filling 1.85% of the total voids in both cases. The saturated hydraulic conductivity of the loose sand upon MICP treatment is reduced to equivalent to the hydraulic conductivity of the dense sand, which indicates densification can be carried out on the loose sand by a single dose of bio-cementation solution. The densification of loose riverbanks may provide resistance against erosion and liquefaction. However, further investigation on the improvement in strength with multiple treatment strategies is necessary.



(a)



(b)

Fig. 5 (a). The precipitated calcite over sand surface, and (b). sand grains without bridging.

5. Conclusion

In this study, the potential of the bacteria *Bacillus megaterium* as a mediator for urease-based bio-mediated soil improvement techniques was evaluated. This study is a preliminary investigation on the influence of single-dose biocementation on the saturated hydraulic conductivity of the loose Brahmaputra riverbank sand. The major conclusion from this study may be encapsulated as follows-

- The Brahmaputra riverbank sand is characterized as quartz dominant poorly graded fine sand with insignificant organic content and neutral pH. The saturated hydraulic conductivity of the sand at different density states has been evaluated to be in the degree of 10^{-5} m/sec, which decreased around one-third with densification.
- *Bacillus megaterium* (NCIM 5472) is observed to have considerable potential as a bio-mediator for soil improvement with a specific urease activity of 300 U/ml. This culture has considerable potential for average calcite precipitation potential 450 mg/100 ml of equimolar cementation solution for bio cementation purposes.
- The saturated hydraulic conductivity of *Bacillus megaterium* mediated bio-treatment of loose Brahmaputra riverbank sand decreases equivalent to dense sand; however, a comparatively smaller change was observed in the permeability of the dense sand upon the same treatment.

Further exploration with multiple treatment cycles is required to study the shear strength characteristics and the factors influencing the process. However, this study will provide valuable insights for the characterization of *Bacillus megaterium* as an alternative biocement potent microbe to *Sporosarcina pasteurii* for its utilization in the ground improvement techniques.

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