Investigation of the Micro-Structure of Brahmaputra Sand treated with Bacillus *megaterium* Mediated Single Dosed Bio-Cementation

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Abstract. The bio-mediated soil improvement has promising capabilities to provide sustainable aid to the geotechnical challenges. The geotechnical behavior of the soil can be modified utilizing bio-mediated processes. Most of the studies on the Bio-mediated soil improvement focuses on a particular bacteria Sporosarcina *pasteurii.* This study utilizes urease positive bacteria Bacillus *megaterium* (NCIM 5472) as an alternative to Sporosarcina *pasteurii* and Brahmaputra riverbank sand for the investigation of bio-cementation in the soil. In this study, the primary characterization of Bacillus *megaterium* has been reported. The qualitative urease activity of the bacteria has been assessed with Urea agar base (Christensen), and quantitative analysis of urease activity assay has been evaluated by the phenol-hypochlorite method. Quantitative calcite precipitation has been evaluated at equimolar cementation solution. After the characterization, single dosing of bacterial broth solution mixed along with cementation solution (one pore volume) is introduced to Brahmaputra riverbank sand, and the microstructure of the sand has been investigated with the help of FESEM (Field Emission Scanning Electron Microscope) images. The influence of bio-cementation was observed significantly on the microstructure of Brahmaputra riverbank sand in the form of bridging of the calcite precipitated. This study is a preliminary study to investigate the applicability and potential of the bacteria Bacillus *megaterium* for bio-mediated soil improvement. The study concludes that the bacteria Bacillus *megaterium* is moderately urease active, and it has the potential for bio-cementation.

Keywords: Bio-mediated soil improvement, Urea hydrolysis, Bio-calcification, Bacillus megaterium, Microbial Induced Calcite Precipitation (MICP).

1 Introduction

There are various biological activities occurring in the soil media, which may alter the soil pore network [1] and therefore, utilization of the biological processes in the soil media holds promising aid for the geotechnical engineering applications such as

in ground improvement, liquefaction mitigation, erosion control and hydraulic barrier materials [2, 3].

The most prevalently used bio-mediated soil improvement is catalysis of urea hydrolysis process by utilizing ureolytic bacteria such as Sporosarcina *pasteurii*.[3–6]. The urea hydrolysis reaction can be explained as follows-

$$CO(NH_2)_2 + H_2O \xrightarrow{\text{bio-mediator}} H_2CO_3 + 2NH_3 \quad (1)$$

The ammonia further reacts in water and yields in ammonium and hydroxide ions leading to the rise of the pH of the solution, as shown in equation 2.

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

The bicarbonate breaks further into carbonate ion, as shown in equation 3 and in the presence of Calcium ion, this leads to precipitation of the Calcium Carbonate crystals, as shown in equation 4.

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

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

The rise in the alkalinity is caused by the yield of ammonium and hydroxide ions as shown in equation 2, and the accelerated carbonation in the presence of urease enzyme leads to an accelerated rate of the calcite precipitation [7].

After MICP treatment the soil pore gets filled with calcite crystals, which leads to a decrease in pore volume, eventually results in a decrease of saturated hydraulic conductivity of soil and an increase in shear strength properties. The bridging among the particles due to bio-cementation also influence the engineering properties of MICP treated soil, apart from precipitated calcite crystals itself. The order of change in hydraulic conductivity and shear strength of the soil depends on various other parameters like the uniformity and morphology of precipitated $CaCO_3$ crystals [8].

This study is an investigation of Bacillus *megaterium* (NCIM 5472 equivalent collection number ATCC 14581) mediated MICP and its influence on the microstructure of the Brahmaputra river bank sand. The MICP related primary characterization of the bacteria Bacillus *megaterium* is accomplished, including quantification of its urease activity and calcite precipitation capacity.

This study accentuates on the bacteria Bacillus megaterium as it can grow well in a range of temperature of 3°C to 45°C , in toxic situations and can survive on a variety of carbon sources [9] and the influence of oxygen on the urease activity of Bacillus *megaterium* is insignificant [10]. As most of the MICP studies emphasize on conventionally popular ureolytic bacteria Sporosarcina *pasteurii*, there are very few studies available on Bacillus *megaterium* mediated MICP [11, 12].

2 Materials and Methodology

Brahmaputra river bank deposited sand was collected from the river basin nearby Indian Institute of Technology Guwahati, Assam, India. The soil is poorly graded fine sand as per USCS classification. The fine content was observed to be less than 4%.

The coefficient of uniformity, coefficient of curvature, D_{60},D_{10},and D_{30} are shown in table 1.

Table 1. Grain Size Characteristics of Brahmaputra river bank sand

Characteristics	Values (Unit)
C_u	1.875
C_c	0.833
D_{60}	0.15 mm
D_{30}	0.1 mm
D_{10}	0.08 mm

The ureolytic bacteria Bacillus ***megaterium*** (NCIM 5472 equivalent collection number ATCC 14581) is grown in a Nutrient Broth (NB) media in conical flasks at pH 8 and 30°C temperature in a shaking incubator at 200 rpm. The growth characteristics of Bacillus *megaterium* was evaluated by measuring its optical density at 600 nm in the spectrophotometer over a time duration of 48 hours. Urease activity of the bacteria is one of the indicators of the potency for bio-cementation. The qualitative urease activity of the sample was evaluated using Urea Agar Base, Christenson (UAB), and the quantitative urease activity of the strain was evaluated by Phenol-Hypochlorite method [13–15]. The qualitative urease test the bacteria was evaluated by the capacity of the bacteria to turn yellowish UAB plates to pink. The Calcite producing capacity of the bacteria was also evaluated in the flask tests after mixing the culture broth with the equimolar cementation solutions.

Then one pore volume of the bacterial broth (optical density 1) and equimolar cementation solution (Urea and $CaCl_2$) was mixed in equal proportions with the Brahmaputra river bank sand in a cylindrical column of diameter 40 mm and length 80 mm. The samples were extruded for the evaluation of their geotechnical and microstructural properties.

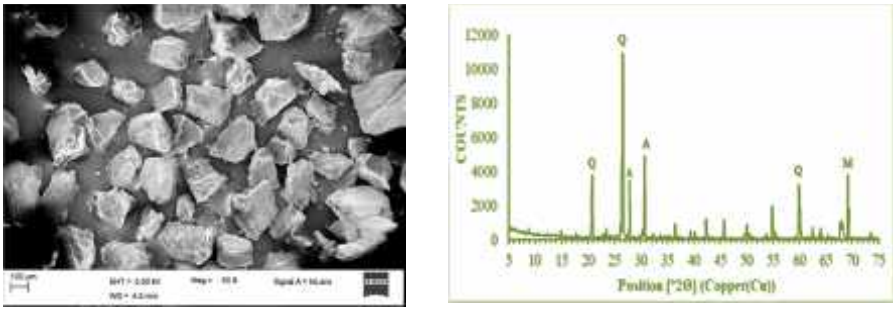
3 Results and Discussion

The geotechnical properties of Brahmaputra River bank sand are given in table 2 as follows-

Table 2. Geotechnical properties of Brahmaputra Sand

Geotechnical Properties	Values (Unit)
Specific Gravity	2.7
pH	7.2
Minimum density	13.1 ($kN/m^{3)}$
Maximum density	15.4 (kN/m^3)

The FESEM and XRD of the Brahmaputra Sand (BS) revealed that the BS is smooth surfaced, angular, and Quartz dominant, as shown in Figure 1.



(a) (b)

Fig. 1. (a). FESEM (Field Emission Scanning Electron Microscope) and (b). XRD (X-ray Diffraction) graph of Brahmaputra Sand (Q denotes Quartz)

The qualitative urease activity of the bacteria Bacillus *megaterium* was observed using Urea Agar Base (UAB), Christensen (HiMedia lab.). The change in color from yellowish to pink is due to the alkalinity caused by urea hydrolysis and is noted within 24 hours, as shown in Figure 2.

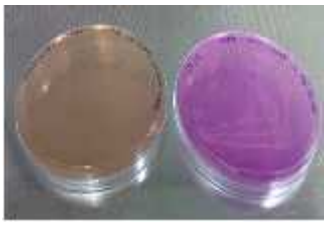


Fig. 2. Qualitative Urease test for Bacillus *megaterium* on Urea Agar Base

The Urea Agar base took 24 to 36 hours for turning the plate's color from yellow to pink. Indicating that the bacteria is a moderate urease producing bacteria.

The quantitative urease activity of the bacteria was 247 U/min. One unit of bacterial urease is termed as the quantity of enzyme required to hydrolyze 1 micromole urea/min/ml [15]. The Calcite producing capacity of bacteria in the flask at temperature 30° Celsius and pH 8.0 was observed to be 504 mg/100 ml of cementation solution. The XRD of precipitates revealed it to be calcite.

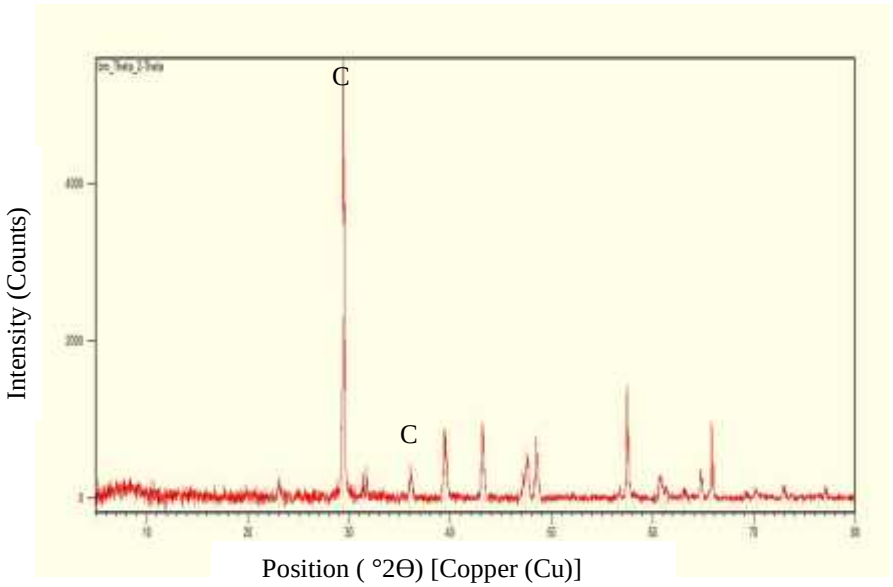
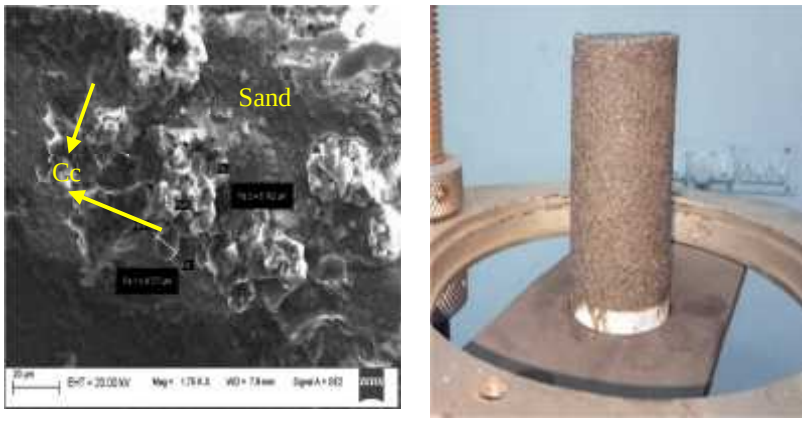


Fig. 3. The XRD (X-ray Diffraction) graph of Brahmaputra Sand of precipitates in flask test (C denotes Calcite)



(a) (b)

Fig. 4. FESEM (Field Emission Scanning Electron Microscope) of the lumps collected from treated soil column (b) Treated soil column

The soil columns were kept for 1 week for bio-cementation. The sample was oven-dried and then extruded before testing it for geotechnical and microstructural characterization. The extruded soil (sand) sample was able to stand due to the bonding induced by the calcite produced. However, the sample didn't show sufficient strength for Unconfined compressive strength, which may be due to two reasons, 1. Less calcite content due to a single number of dosing and 2. Heterogeneous calcite precipita-

tion. The lumps of broken soil sample were taken for microstructural analysis (FESEM and XRD). FESEM revealed that there are precipitates in between the soil pores.

4 Conclusion

In this study, the geotechnical properties of the Brahmaputra river bank sand and the potential of the bacteria Bacillus *megaterium* as a mediator for urease based bio-mediated soil improvement techniques were evaluated. The major conclusion from this study may be encapsulated as following-

- Bacillus *megaterium* (NCIM 5472) is observed to have significant potential as a bio-mediator for soil improvement with a moderate urease activity of 247 U/ml. Bacillus *megaterium* also yields a significant amount of calcite precipitation potential (509 mg/100 ml of equimolar cementation solution) for bio cementation purposes.
- The FESEM and XRD of treated sand reveal the presence of bonding in forms of precipitated calcite, which indicates that the Bacillus *megaterium* can be used effectively for Bio-mediated soil improvement.

The bio-mediated ground improvement certainly holds possibilities as eco-friendly ground improvement technique; however, to utilize the process for engineering applications, in-depth exploration, and the limitations such as ammonia generation must be addressed. This study is a primary study on the potential of Bacillus *megaterium* mediated soil improvement and its potential for engineering the hydraulic conductivity behavior of the soil. Further exploration is required to study the shear strength characteristics and the factors influencing the process. However, this study will provide significant insights for the characterization of Bacillus *megaterium* as an alternative and potent urease based bio-mediator for its utilization in the ground improvement techniques.

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