Kochi Chapter

Indian Geotechnical Conference
IGC 2022
15th – 17th December, 2022, Kochi

Influence of Microbial Treatment on the Stiffness and Strength Enhancement of Standard Ennore Sand

[1] Nilanjana Banik [000-0001-6535-2443] and Rajib Sarkar [2][0000-0002-7900-3890]

[1]Research Scholar, Dept. of Civil Engineering IIT(ISM) Dhanbad, Jharkhand-826004, India
[2]Associate Professor, Dept. of Civil Engineering IIT(ISM) Dhanbad, Jharkhand -826004, India
rajib@iitism.ac.in

Abstract. Engineering properties of natural sand can be improved by bio-cementation process. In the present study, microbial induced carbonate precipitation (MICP) technique with bacillus sp. bacteria was adopted for bio-cementation of standard Ennore sand. The morphological and chemical composition of the bio-cemented sand are investigated through scanning electron microscope (SEM) and X-ray diffraction (XRD) tests respectively. Stress-strain behaviour of bio-cemented sand with different pore volumes of cementation solution has been investigated through unconfined compression strength (UCS) testing. Bender element testing has also been carried out to investigate the improvement in low-strain stiffness indicated by shear wave velocity of bio-cemented sand. Results indicate good enhancement in the stiffness and strength characteristics of bio-cemented sand.

Keywords: Ennore sand; MICP treatment; UCS test; Bender element test

1 Introduction

In recent years microbially induced calcite precipitation (MICP) technique has emerged as one of the environmentally friendly techniques in the area of geotechnical engineering. This technique is adopted to improve the bonding between the soil particles of weaker soils. As a result, the mechanical properties of the soils get improved. Several investigations have been conducted to study the engineering properties of bio-treated geomaterials [1,2]. Stabilization of loose sand has generally been done through ureolysis in presence of calcium chloride to induce calcite precipitation. Calcite precipitation held the loose sand particles together and as a result strong bonding occurs between the sand particles. There are two mechanism of calcite precipitation. First is the attachment of bacterial cells to the nucleation site for the precipitation of $CaCO_3$ and second is the hydrolysis of urea which increases the pH by producing carbonate ions around the cells [3,4]. In this study, microstructural characteristics and chemical composition of bio-treated sand has been investigated through SEM and XRD analysis. Strength properties has been studied through UCS tests and bender element testing has been carried out for assessing the shear modulus. For microbial calcite precipitation, urease producing bacteria s. pasteurii was used. Cementation solution was varied by pore volumes (1 PV, 0.5 PV) considering the treatment period and frequency of treatment cycles. Comparison between the strength properties of different composition of sand samples has been analyzed. Application of bio-cementation methods such as MICP technique includes various engineering applications like improvement in bearing capacity, oil recovery enhancement, restoration of old buildings, reduction of hydraulic conductivity of fractured rocks, reduction of permeability, erosion control, enhancement of liquefaction resistance of poorly graded sands.

2 Material of the Study

Ennore standard sand collected from Chennai, India is used in this study and the sand conforms to Grading Zone-III sand following Indian standard [5]. The sand is classified as poorly graded sand as per relevant Indian standard [6] implying that the sand may be liquefiable under saturated condition. Various geotechnical and chemical properties of the sand are listed below in Table 1. The particle size distribution curve is presented in Fig. 1.

Table 1. Properties of Ennore sand

Geotechnical Properties	
Coefficient of uniformity, C_u:	1.711
Coefficient of curvature, C_c :	0.837
Diameter corresponding to 10% finer D_{10} (mm):	0.445
Specific gravity, G_s:	2.65
Maximum void ratio, e_{max}:	0.74
Minimum void ratio, $e_{min:}$	0.56
Chemical Properties	
Silicon dioxide (SiO_2)	99.30%
Aluminium oxide (Al_2O_3)	-
Ferric oxide (Fe_2O_3)	0.10%
Calcium Oxide (CaO)	-
Loss on extraction with hot HCl	0.11%
Loss on ignition	-

3 Microbial Culture

Bacillus sp. also known as s. pasteurii (NCIM 2477) is collected from NCIM, Pune (National Collection of Industrial Microorganisms). The bacterial strain is stored at -20ºC. Bacterial culture is prepared in nutrient broath solution under laminar air-flow condition. The bacterial strain is placed in orbital shaking incubator for 24 hours at a rotating speed of 120 rpm at 30ºC. The optical density is measured using spectrophotometer at 580 nm. OD is measured to be approximately 1.0.

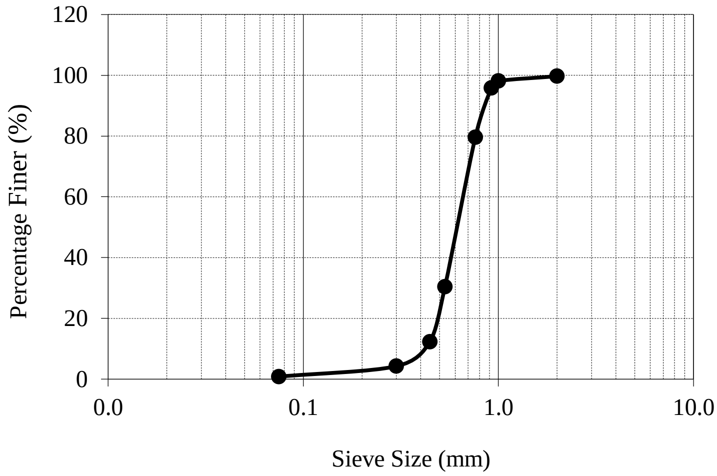

Fig. 1. Particle size distribution curve of standard Ennore sand

4 Methodology

4.1 Preparation of Bio-cemented Sand Samples

Ennore standard sand was oven dried at 105ºC for 24 hours before the test. Standard sand samples were prepared at a relative density of 40%. Plastic spray bottles of size 50 mm diameter and 100 mm height is taken to prepare the samples maintaining length to diameter ratio of 2.0. Bottle caps were used as a nozzle to control the drainage of the arrangement. Sand samples were poured in the moulds using funnel to achieve relative density of 40% through pluviation technique. 325 grams of sand samples were poured in 5 layers. Bacterial solution was sprayed in the sand sample uniformly. 1 pore volume (PV), 0.5 PV of bacterial solution was sprayed in the sand samples at an interval of 12 hours and 24 hours respectively. Bacterial solution was held for 15 hours since most of the bacterial growth was during this phase. After 15 hours of attachment period without draining the bacterial solution cementation solution was sprayed in the sand samples. Cementation solution was sprayed without calcium chloride di-hydrate and kept for 24 hours, so that urea hydrolysis takes place. This period is commonly known as simulation period [7]. After this simulation period, the cementation solution was drained and fresh cementation solution with calcium chloride di-hydrate at an interval of 12 hours and 24 hours respectively was provided to observe calcite precipitation for 7 days. The composition of the cementation solution is presented in Tables 2 and 3. and it may be mentioned that nutrient broath was not autoclaved. Table 4 presents the samples prepared for testing with different treatment period and treatment intervals.

Table 2. Composition of the cementation solution (1:1) used in the study [8,9]

Material	**Mass (gm/ml)**
Urea	30.03
$Cacl_2$. $2H_2O$	73.5
NH_4Cl	10.0
$NaHCO_3$	2.12
Nutrient broth	3.0

Table 3. Composition of the cementation solution (2:1) used in the study

Material	**Mass(gm/ml)**
Urea	30.03
$Cacl_2$. $2H_2O$	36.75
NH_4Cl	10.0
$NaHCO_3$	2.12
Nutrient broth	3.0

Table 4. Sample prepared for evaluation of UCS test

Sample Designation (Treatment Period in days)	Cementation Concentration	Pore Volumes (PV)	Treatment Interval (in hours)
B_1 (7 days)	1:1	1.0	12
B_2 (7 days)	1:1	0.5	24
B_3 (7 days)	1:1	0.5	12
B_4 (7 days)	1:1	1.0	24
B_5 (7 days)	2:1	1.0	12
B_6 (7 days)	2:1	0.5	24
B_7 (7 days)	2:1	0.5	12
B_8 (7 days)	2:1	1.0	24

4.2 Details of UCS Test

After microbial treatment, unconfined compressive strength tests were done for treated samples. Before the test the samples were oven-dried at 105ºC for 24 hours. Unconfined compressive strength is measured as the peak stress or the stress that yields 20% of axial strain whichever is lower. UCS tests were carried out at a strain rate of 1.25 mm/min in accordance of ASTM standard [10] test method.

4.3 Details of Bender Element Test

In this study, bender element testing has been carried out on MICP treated sand for evaluation of the low-strain shear wave velocity values. A bender element test setup consists of transmitter and receiver piezoelectric bender elements for transmitting and receiving signals. The setup is attached with a function generator for generation of input waves with variables such as frequency, amplitude and waveform. The input and output waves are monitored through the oscilloscope. In this study, the excitation voltage 20 V (peak to peak i.e. 20Vpp) with frequency of 1 kHz is used as input wave through the transmitting bender element. For the bender element testing, only the samples with cementation concentration of 1:1 (samples B_1 to B_4) were considered.

5 Results and Discussions

5.1 Microstructure Analysis

Microscopy analysis has been done to investigate about the location and structure of $CaCO_3$ precipitation and to determine the bonding between the grain hosts and bonding agents. Scanning electron microscopy is done to analyze the microstructure of the treated and un-treated samples. Scanning electron microscope (SEM) is a type of microscope that produces images of a sample by scanning the surface with a focussed beam of electrons [11]. Irregular, discrete and bigger size of particles were observed in untreated sand whereas the size of the particles was smaller and agglomeration of particles has been observed in treated samples. Small crystals of calcite precipitation have been observed in 7 days treatment analysis and the voids were significantly lower than the untreated silica sand. Figures 2a and 2b depict the SEM images of treated and un-treated samples. On the other hand, chemical composition of the treated and un-treated samples has been investigated through XRD analysis. From the XRD analyses, it was evaluated that the un-treated sand consists of only silica and the treated samples showed calcium composition along with silica showing precipitation calcium carbonate after MICP treatment. Figure 2c presents the XRD analyses results of treated sand with 1:1 cementation solution.

5.2 Strength of Bio-Cemented Sand

Figure 3 shows the failure patterns of the treated sand sample obtained from the UCS testing. It is observed that the major failure patterns of the bio-cemented samples are either by shearing or bulging.

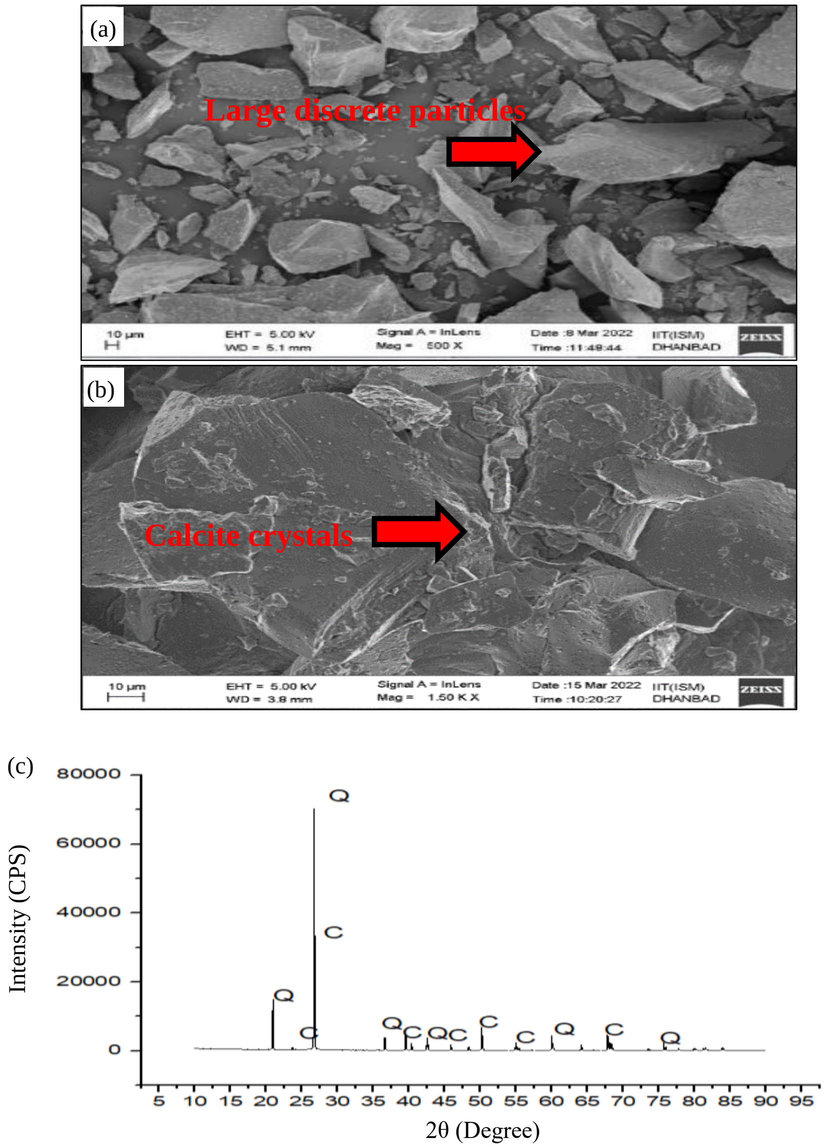

Fig. 2. (a) SEM image of un-treated sand, (b) SEM image of treated sand with 1:1 cementation solution, (c) XRD analyses results for treated sand with 1:1 cementation solution

(a) (b)

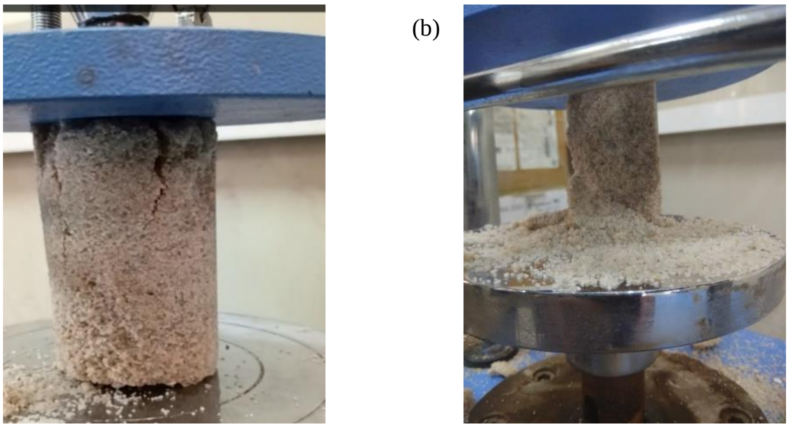

Fig. 3. Failure patterns of treated sand: (a) Shear failure, (b) Bulging failure

Treated samples for 7 days period with varying cementation solution of 1:1 and 2:1 concentration were tested to determine the unconfined compressive strength. Figures 4 and 5 compare the stress-strain curves for soil specimens treated with s. pasteurii and cementation reagents. Effect of concentration of calcium carbonate and urea in the cementation solution has been observed. The samples treated with 1:1 cementation solution showed greater strength and cementation was also high whereas the samples treated with 2:1 cementation solution showed lesser strength.

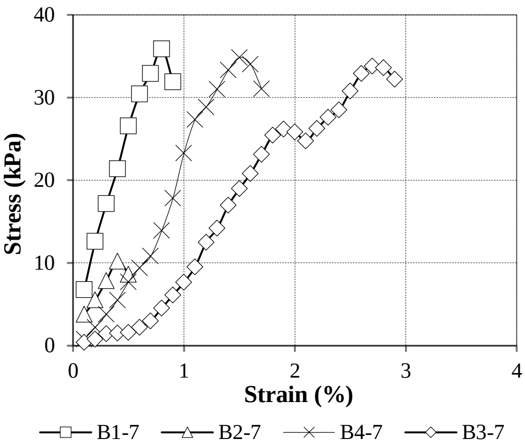

Fig. 4. Stress strain behaviour for 1:1 treatment solution

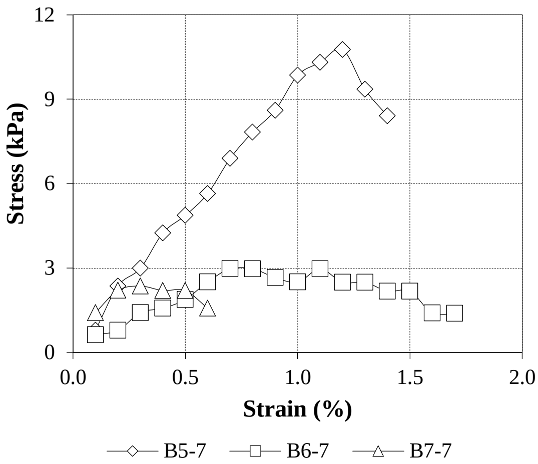

Fig. 5. Stress strain behavior for 2:1 cementation solution

This higher strength is because of the proportion of calcium chloride di-hydrate in the cementation solution of 1:1 concentration (refer to Fig. 4). It is implied from the tests results that uniform and good cementation concentration of calcium source in the cementation solution plays an important role. Gandhi *et al.* (1995) [12] discussed the differences in the crystal precipitation patterns and it has been attributed between crystal growth and crystal nucleation. In their study, it was reported that nucleation of new crystals compete with the process of crystal growth if nucleation of new crystals prevails over the growth of those already existed. If the urease production is low, this may be due to precipitation of crystals over the initial existing crystals and this leads to formation of larger crystal sizes rather than small crystals [13]. Shear strength of soil may not be directly proportional to amount of calcite content. Improved strength and stiffness depend on the percentage of gaps filled by the crystals between the sand grains [14]. In this line, samples treated with 2:1 cementation solution showed lesser strength after treatment for 7 days. It may be mentioned here that some samples could not be tested because of extremely low cementation even after 7 days. It may also be noted here that though higher concentration of urea lead to higher urease activity but in few past studies it was observed that the slower hydrolysis of urea can form more effective bridges between the sand particles as compared to faster hydrolysis of urea [15,16].

In the present study, it is observed that 7 days treated samples within the interval frequency of 12 hours for 1:1 cementation concentration showed higher strength and stiffness as compared to 2:1 cementation concentration. In this study, 1:1 treated samples showed almost similar strengths for samples B_1 to B_4 while B_1 yielded at a strain of 0.9% and B_3 yielded at a strain of 2.9% and B_4 yielded at a strain rate of 1.7%. However, sample B_2 showed very less strength. This may be due to the effect of pore volume in the treatment hours. For the sample B2 0.5PV pore volume was used (refer to Table 4). It means that 50% of total pore volume implying that although the sample showed calcite precipitation but the pores were not filled to form strong bonding between the soil particles indicating lesser strength. On the other hand, Sample B_3

showed higher strength despite of 0.5 PV of cementation solution. This is because sample B_3 has been treated with cementation solution for twice a day within the treatment period of 12 hours. It implies along with the pore volume; frequency of treatment also plays an important role in determining the strength characteristics of the samples. Figure 6 compares the dry density and the % of calcium carbonate of the MICP treated sand samples.

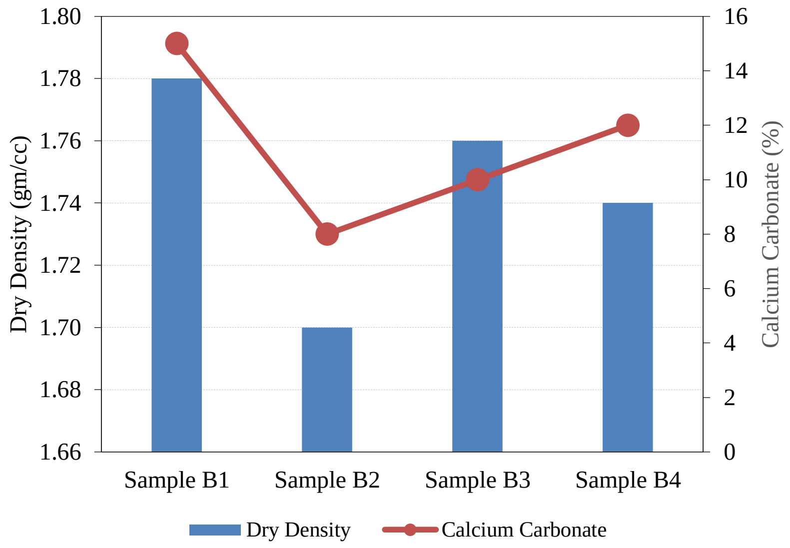

Fig. 6. Dry density and percentage of calcite precipitation for 1:1 cementation solution

5.3 Low-Strain Shear Wave Velocity of Bio-Cemented Sand

In this section, the shear wave velocity of the bio-cemented sand for different treatment periods are reported through bender element tests. The shear wave velocity of bio-cemented sand is determined for wide range of confining pressure varying from 0.5 to 3.0 kg/cm^2. Figure 7 presents the shear wave velocity of bio-cemented sand for 7 days of treatment for different levels of confining pressure.

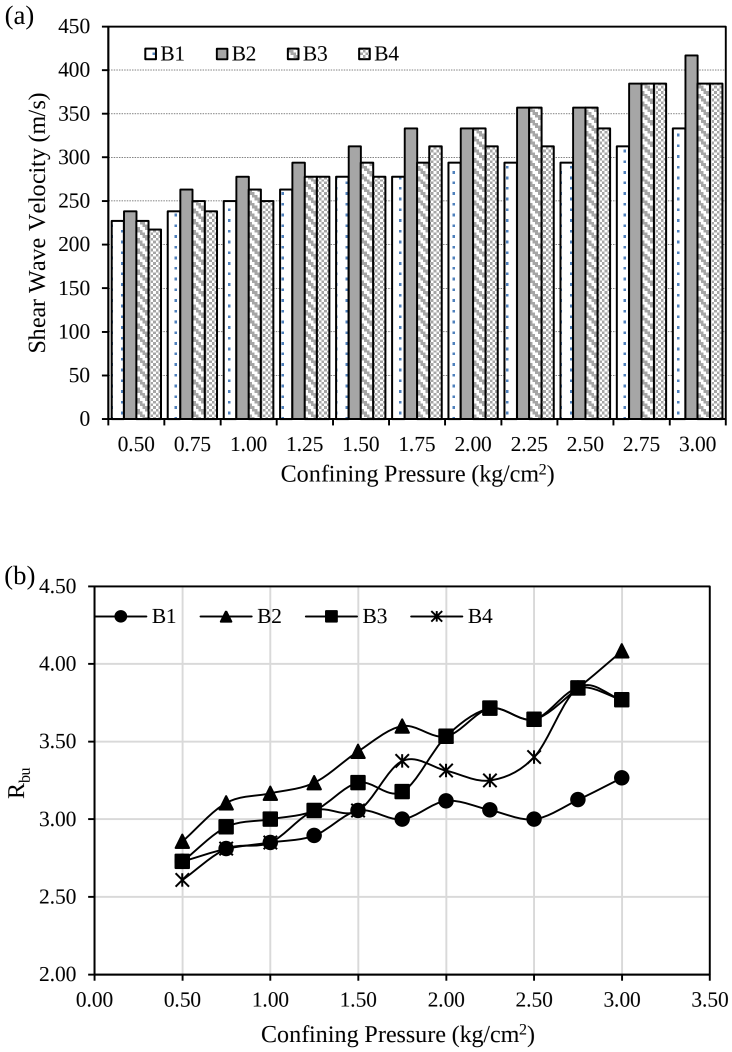

Fig. 10. (a) Variation of shear wave velocity of bio-cemented sand (b) Ratio of shear wave velocity of bio-cemented sand with untreated sand (R_{bu}) for different confining pressure considering 7 days of treatment

It is observed that the shear wave velocity increases due to bio-cementation of the sand. For 7 days of treatment, bender element testing shows that the shear wave velocity of bio-cemented sand varies from 200 m/s to about 400 m/s for different confining pressure. It may be noted that the maximum shear wave velocity of the untreated standard sand for 3.0 kg/cm^2 was assessed to be 102 m/s. This indicates the significance of bio-cementation in improving the low-strain stiffness values of the sand. For quantification of the improvement ratio of the shear wave velocity of the bio-cemented sand to that of untreated sand (R_{bu}) was determined and presented in Fig. 10b. This indicates that the minimum improvement in case of bio-cemented sand is more than 2.0 and maximum improvement for 3.0 kg/cm^2 confining pressure is about 4.0.

6 Summary and Conclusions

The present study on strength and stiffness enhancement of bio-cemented sand may be summarized as following.

a) The strength of the bio-cemented sand increases with the frequency of treatment.
b) Concentration of urea in the cementation solution is significant in determining the strength characteristics of bio-cemented sand.
c) Shear wave velocity evaluated through bender element testing indicates that the bio-cemented sand transforms in to dense soil or stiff soil condition as per NEHRP (2003) guidelines. The improvement of 2.0 to 4.0 times was observed for shear wave velocity values of bio-cemented sand in comparison to the un-treated sand considering different confining pressure.

References

1. Harkes, M.P., Van Paassen, L.A., Booster, J.L., Whiffin, V.S., and Van Loosdrecht, M.C.M.: Fixation and distribution of bacterial activity in sand to induce carbonate precipitation for ground reinforcement. Ecol. Eng. 36(2), 112-117 (2010).
2. Van Paassen, L.A.: Biogrout, ground improvement by microbial induced carbonate precipitation. Doctoral dissertation, Delft University of Technology, TU Delft, South Holland. (2009)
3. Dejong, J.T., Fritzges, M.B., and Nüsslein, K.: Microbially induced cementation to control sand response to undrained shear. J. Geotech. Geoenviron. Eng. 132 (11), 1381-1392 (2006).
4. Stocks-Fischer, S., Galinat, J.K., and Bang, S.S.: Microbiological precipitation of CaCO3. Soil Biol. Biochem. 31(11), 1563-1571(1999).
5. IS 383: Indian Standard Specification for Coarse and Fine Aggregates from Natural sources for Concrete. Bur. Indian Stand. New Delhi 1-19 (1970).
6. IS 2720 -Part 4: Indian Standard: Methods of test for soils, Part 4: Grain size analysis. Bur. Indian Stand. New Delhi 1-39 (1985).
7. Cheng, L., Shahin, M.A., and Mujah, D.: Influence of Key Environmental Conditions on Microbially Induced Cementation for Soil Stabilization. J. Geotech. Geoenv. Engg. 143(1) (2016).
8. Whiffin, V.S., Van Paassen, L.A., and Harkes, M.P.: Microbial Carbonate Precipitation as a Soil Improvement Technique. J. Geomicrobiology (24), 417-423(2007).
9. Sharma, M., and Satyam, N.: Strength and durability of bio-cemented sands: Wetting-drying cycles, ageing effects and liquefaction resistance J. Geoderma (402),115359 (2021).
10. ASTM D 2166: Standard test method for unconfined compressive strength of cohesive soils, American Society for Testing and Materials, Philadelphia (2006).
11. Kalantary, F., Govanjik, D.A., and Gonbad, M.S.S.: Stimulation of Native Microorganisms for Improving Loose Salty Sand. J. Geomicrobiology. ISSN: 0149-0451 (2019).
12. Gandhi, K.S., Kumar, R., and Doraiswami, R.: Some basic aspects of reaction engineering of precipitation processes. Ind. Eng. Chem. Res. 34(10), 3223–3230 (1995).
13. Otterstedt, J.E., and Brandreth, D.A.: Small particles technology, Springer, Berlin (2013).

14. Martinez, B.C., and DeJong, J.T.: Bio-mediated soil improvement: Load transfer mechanisms at the micro- and macro-scales. Advances in Ground Improvement. 242–251(2009).
15. Cheng, L., Shahin, M.A., and Mujah, D.: Influence of Key Environmental Conditions on Microbially Induced Cementation for Soil Stabilization. J. Geotech. Geoenv. Engg. 143(1) (2016).
16. Van Paassen, L.A., Ghose, R., van der Linden, T.J.M., van der Star, W.R.L., and van Loosdrecht, M. C. M.: Quantifying biomediated ground improvement by ureolysis: Large-scale biogrout experiment J. Geotech. Geoenviron. Eng.1721-1728. (2010b).