



## Effect of Bio-Cementation with Rice Husk Ash on Permeability of Silty Sand

Martina Gumsar Sorum <sup>1\*</sup>, Ajanta Kalita <sup>1</sup>

<sup>1</sup> Department of Civil Engineering, North Eastern Regional Institute of Science and Technology (NERIST), Nirjuli, India.

Received 14 June 2023; Revised 30 September 2023; Accepted 09 October 2023; Published 01 November 2023

### Abstract

The scarcity of competent soils in the desired locations has forced geotechnical engineers to look for soil stabilization that is sustainable and environment-friendly. In this regard, bio-cementation technology has received a lot of interest in this area because of its benefits over traditional soil stabilization techniques. The present study aims to examine the influence of the bio-cementation technique with and without Rice Husk Ash (RHA) on the permeability property of silty sand. Biocemented soil samples were prepared with various combinations of the bacterial solution (0.5, 1.0, and 1.5 optical density (OD)) and cementation solution (0.5, 1.0, and 1.5 molarity) at 0, 3, 7, 14, and 28 curing days. The RHA, an agricultural waste with good pozzolanic qualities, was added to the control soil and the biocemented soil samples at 5, 10, and 15% by weight. A falling head permeability test was employed in this study. The test results showed that the permeability of the soil decreased when the bio-cementation technique, with or without RHA, was applied. The permeability of the soil decreased with increasing BS and CS concentrations in all curing days. A greater decrease in the permeability value was seen when the RHA additive was added to the bio-cemented soil. The results of the micro-analysis tests were also in support of this reduction. Overall, the addition of RHA up to 10% with 1.0 OD BS and 1.0M CS at a 14-day curing period was noted to optimally reduce the permeability property of the soil.

**Keywords:** Bio-Cementation; Permeability; Rice Husk Ash; Silty Sand; Bacterial Solution.

### 1. Introduction

Due to population growth and the rapid expansion of infrastructure, the availability of competent soils (typically characterized by high load-bearing capacity and low compressibility) in the desired location becomes limited. The scarcity of such soils in the desired locations has forced civil engineers to look for economical soil improvement techniques. Conventional soil stabilization techniques such as chemical grouting, stone columns, the blending of natural and manmade materials, and other soil improvement techniques are being used all over the world [1–3]. Nevertheless, the majority of these methods rely on artificial or mechanical components, which require a lot of energy to produce or install. Therefore, looking for innovative, cost-effective, environment-friendly, and overall, more sustainable soil improvement techniques become necessary. The most recent and environmentally friendly soil improvement technique now considered in geotechnical engineering is the bio-cementation process [4].

Bio-cementation is a recent technique for improving soil properties that uses bacteria to create calcium carbonate ( $\text{CaCO}_3$ ) crystals between soil particles [5–7]. The fundamental idea behind the bio-cementation process is that the microbial enzyme urease breaks down urea into ammonia and carbon dioxide. The ammonia released into the environment raises the pH, resulting in an accumulation of insoluble  $\text{CaCO}_3$ . The process through which  $\text{CaCO}_3$  is precipitated by urea hydrolysis involves two steps: (i) Urea hydrolysis and (ii)  $\text{CaCO}_3$  precipitation [8].

\* Corresponding author: [tinagsorum@gmail.com](mailto:tinagsorum@gmail.com)



<http://dx.doi.org/10.28991/CEJ-2023-09-11-016>



© 2023 by the authors. Licensee C.E.J, Tehran, Iran. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC-BY) license (<http://creativecommons.org/licenses/by/4.0/>).

Urease from microorganisms hydrolyzes urea to create ammonium and carbonate ions:



When a calcium source is present, the generated carbonate ions mix with the calcium ions to create calcium carbonate precipitates.



Several previously published investigations have shown that the bio-cementation process is viable for a larger variety of soils, including sandy soils, clayey, silty sand, organic soil, rocks, and concrete and mortar [8–11].

Ivanov et al. (2012) [12] developed two new microbial-based grouting materials: calcium-based biogROUTS and iron-based biogROUTS. The authors found that these materials reduced permeability and increased the UCS values of the biocemented sandy soil samples. Zhao et al. (2014) [13] examined the influence of urease and bacteria concentration, curing conditions, and type of sand on the properties of biocemented-sandy soil. They found that the effectiveness of the bacteria concentration for the enhancement of the MICP process was greater than that of the urease concentration. The curing periods on the properties of biocemented soil had a small effect. Similarly, Chen et al. (2023) [14] examined the effects of relative density, CS concentration, and frequency of consolidation on the mechanical properties of biocemented-sandy soil. The study findings showed that consolidation frequency was the most influential factor on the properties of the soil, with a 5–15 times as effective range. Khaleghi & Rowshanzamir (2019) [15] studied single and mixed cultures by using urease-positive bacteria and non-ureolytic bacteria. The authors observed that the permeability value of the mixed medium was reduced by more than 34% compared to the single culture. In another study, Li et al. (2023) [16] observed that only 35.19% of the initial UCS strength of the biocemented calcareous sand sample remained after 30 days of field marine environment erosion. Jain & Das (2023) [17] found that the amount of biomineral precipitation influenced the permeability of the biocemented sandy soil. The permeability value was reduced sharply initially when the biomineral percentage varied from 0 to 2. There was minimal variation in permeability reduction beyond 10% biomineral dosage. Tabrizi et al. (2023) [18] investigated the strain accumulation pattern in the sandy soil under cyclic traffic loads. They found that accumulated plastic strains were reduced by 95% under the same cyclic loading conditions when the amount of precipitated calcite was increased from 0.38% to 0.83%.

Some studies have investigated the efficiency of adding fiber to the bio-cemented sandy soil [19, 20]. Li et al. (2016) [21] evaluated the influence of randomly dispersed discontinuous fiber on the mechanical properties of the biocemented soil. The study outcomes revealed that the addition of 0.2–0.3% fiber increased the shear strength, ductility, and failure strain of the biocemented soil. In another study, Xiao et al. (2019) [20] observed that the tensile strength and UCS values of basalt fiber-biocemented sandy soil increased with increasing calcite content.

Sharma & Ramkrishnan (2016) [22] evaluated the influence of micro-organisms, CS, and curing times on the properties of the clay. The study findings demonstrated that the UCS value of intermediate and highly compressible clayey soils noticeably improved (1.5 to 2.9 times) with the addition and duration of MICP treatment. Morales et al. (2019) [23] found in their study that the content of  $\text{CaCO}_3$  in the soil was enhanced because of the addition of bacteria to clay phyllites. The increment in plasticity values and specific surface area was also noticed. This finding is similar to the findings of Bindu et al. (2017) [24]. They observed a decrease in the liquid limit value for the biocemented clayey soil.

Sidik et al. (2014) [25] investigated the effect of the bio-cementation process on the permeability property of organic soil. They confirmed that the permeability reduction could be effectively achieved through the application of bio-cementation. Canakci et al. (2015) [26] concluded that the bio-cementation technique was feasible for organic soil. The authors found that the bio-cementation technique decreased the compressibility properties and hence enhanced the shear strength of the soil.

In the bio-cementation process, some researchers experimented with calcium from alternative sources than the calcium chloride ( $\text{CaCl}_2$ ) that is commercially available. Cheng & Shahin (2017) [27] studied the viability of employing seawater as the source of calcium rather than  $\text{CaCl}_2$ . The study findings revealed that the repeated treatment of seawater could considerably enhance sand column strength. Similarly, Choi et al. (2016) [28] and Dayakar et al. (2019) [29] evaluated the UCS and permeability of the sandy soil mixed with eggshell. Calcium from eggshells was shown to be just as effective as calcium from  $\text{CaCl}_2$ .

Some researchers have looked into the impacts of RHA on neat soil and biocemented soil. Rathan Raj et al. (2016) [30] mixed the soil in various proportions (5%, 10%, 20%, 30%, 40%, 50% and 80%) and observed improvement in the soil properties. Adhikary & Jana (2016) [31] stabilized the soil with 5%, 10%, 15%, and 20% of RHA by weight. They observed a considerable enhancement in CBR and UCS with the addition of RHA. Roy (2014) [32] stabilized the soil with different dosages of RHA and a small amount of cement. The findings showed UCS and CBR improved

considerably and optimum value was observed at 10% RHA and 6% cement. Oyediran & Ayeni (2020) [33] examined the influences of RHA, cement, and MICP on the geotechnical properties of soils. The study findings revealed that the addition of 15% of cement and bio-cementation, and 10% of RHA increased the soil properties in optimum level. The workability improved, UCS value increased, and the permeability value get reduced.

Based on the above literature, it was found that many studies have been carried out using bio-cementation techniques on different types of soil, but limited research was done on silty sand soil. Although many research has been conducted on the effect of bio-cementation techniques on various geotechnical engineering (such as consistency limits, permeability, UCS, and shear strength), the effect of bacterial solution (BS), cementation solution (CS), and curing period on bio-cementation process was not completely investigated. Also, the effect of RHA on biocemented soil samples with these parameters was not explored properly. This motivated the authors to investigate the effect of the various concentrations of BS and CS along with RHA on the permeability property of the silty sand soil.

The rest of this manuscript is outlined as follows. Different materials used and methods adopted in the study are presented in section 2. The results and discussion of different laboratory tests are described in section 3. The last section of the manuscript presents the main conclusions drawn from the study. Future recommendation is also presented in this section.

## 2. Materials and Methods

### 2.1. Soil

The soil used in the current study was gathered from Lekhi village along National Highway 415, Arunachal Pradesh, India (Figure 1). It was gathered by open excavation. After drying in an oven for 24 hours, the soil was sieved using an IS sieve size of 425  $\mu\text{m}$ . The category of the soil used in the current study is poorly graded silty sand as per IS classification system (as shown in Figure 2). The basic properties of the soil are illustrated in Table 1.



Figure 1. Image of the soil sample

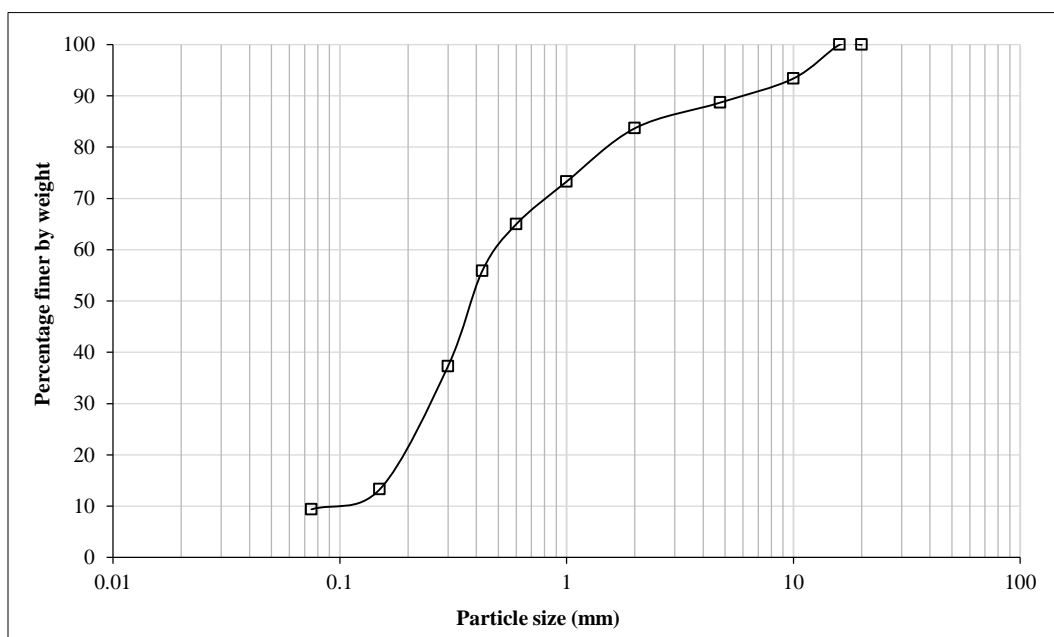


Figure 2. Particle size distribution curve for silty sand

**Table 1. Basic properties of the soil**

Sl. No.	Description	Values
<i>Sieve Analysis:</i>		
1.	Gravel (>4.75 mm)	11.3 %
	Coarse sand (2-4.75 mm)	5%
	Medium sand (0.425-2 mm)	27.8%
	Fine sand (0.075-0.425 mm)	46.5%
	Silt + Clay (<0.75 mm)	9.4%
	CU	5.04
	CC	1.78
2.	Specific Gravity	2.67
3.	Plastic Limit	Non-plastic
4.	Liquid Limit	22.9
5.	MDD	18.4 kN/m <sup>3</sup>
6.	OMC	14.61%
7.	Coeff. of permeability	4.16×10 <sup>-4</sup> cm/s
8.	Classification	SP-SM

## 2.2. Bacteria

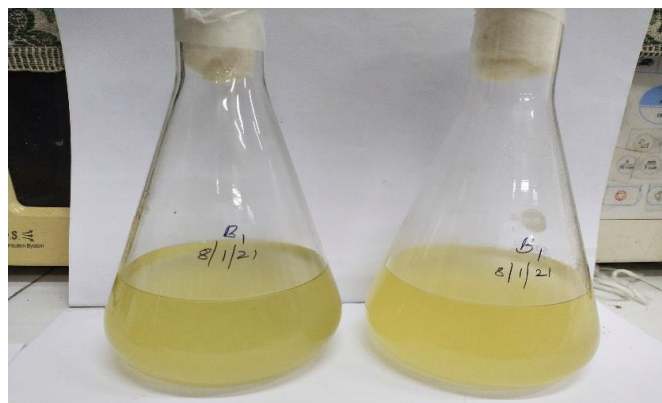
Bacillus Sp (MTCC No. 4445) bacteria was used in the present study and was obtained in powder form in a glass ampule from the Microbial Type Culture Collection and Gene Bank in Chandigarh, India. All chemicals used in the study were ordered from Krishna Enterprise, Guwahati, India.

## 2.3. Culture Media

Nutrient broth was used to cultivate the bacteria. The composition of nutrient broth is presented in Table 2. The growth medium, i.e., the nutrient broth solution, was prepared by maintaining the pH at  $7.0 \pm 0.2$ . It was sterilized for 15 minutes at 15 psi in an autoclave. After sterilization, it was again given ultraviolet rays for 15 minutes to prevent contamination with bacterial growth. Then only the purchased powder-formed bacteria were injected into the growth medium under the laminar flow cabinet. The bacteria and growth medium were incubated at 30°C in an incubator. The optical densities (0.5, 1.0, and 1.5) of the cultivated bacterial solution were determined using a spectrophotometer (at  $\lambda = 600\text{nm}$ ) on different days (Figure 3). The bacterial solution of these 3 different ODs was stored at 4°C until used.

**Table 2. Composition of nutrient broth**

Sl.no.	Composition	Quantity
1.	Peptone	5 g
2.	NaCl	3 g
3.	Yeast extract	1 g
4.	Beef extract	3 g
5.	Distilled water	1000 ml

**Figure 3. Image of bacterial solution**

## 2.4. Cementation Solution

Distilled water was mixed with urea having molar mass of 60 g/mol and calcium chloride with 111 g/mol to create cementation solutions (CS) with different concentrations of molarity (0.5M, 1.0M, and 1.5M). The quantity of urea/CaCl<sub>2</sub> is calculated as per the below formula.

$$\text{Quantity of urea/CaCl}_2 = \text{Strength(w)} = \text{MM} \cdot \text{V} / 1000(\text{g}) \quad (3)$$

where, M is molar mass of urea or CaCl<sub>2</sub>, M' is molar concentration of cementation solution, and V is volume of distilled water.

## 2.5. Rice Husk Ash

Rice Husk Ash (RHA) is an agricultural waste with high silica content that is produced by burning rice husks. [30, 31]. Pozzolana is a siliceous or siliceous and aluminous component that, when combined with calcium hydroxide and water at room temperature, produces cementitious materials. It is produced in enormous quantities and its disposal could be harmful to the surrounding and human health. Many researchers have been attempting to develop uses for it to lessen the environmental risk related to its disposal. By using this as an additive in place of cement, lime, etc., in soil stabilization, both the construction cost as well as the environmental risk will be decreased. Although the burning of this material will also produce CO<sub>2</sub> the use of this material can reduce CO<sub>2</sub> emission compared to cement production. India produces around 20 million tonnes of paddy each year. This yields roughly 4.4 million tonnes of RHA and 24 million tonnes of rice husk annually.

Locally available rice husk was used in the current study (Figure 4). It was burnt in the open air to get RHA. It was sieved via a 425 µm IS sieve. The specific gravity of RHA was 2.03. The chemical composition of the RHA is presented in Table 3. The RHA concentrations employed in the present study were 5, 10, and 15% by weight.



Figure 4. Image of RHA

Table 3. Chemical compositions of the RHA

Compositions	Weight %
O SiO <sub>2</sub>	55.39
Mg MgO	0.42
AL Al <sub>2</sub> O <sub>3</sub>	4.77
Si SiO <sub>2</sub>	31.38
K MAD- 10 Feldspar	1.5
Ca Wollastonite	0.48
TiTi	0.37
Fe Fe	4.54
Cu Cu	0.68
Zn Zn	0.47

## 2.6. Preparation of Soil Sample

Bacterial solution (BS) was used to replace half of the water needed to prepare all of the samples, and cementation solution (CS) was used to replace the remaining half of the water needed. At OMC and MDD, the samples were prepared. First, the required amount of BS was added and evenly mixed with hand-wearing gloves. The required amount of CS was then added and thoroughly mixed. The mixing method was adopted in the preparation of the treated soil sample because the homogeneous distribution of CaCO<sub>3</sub> can easily be achieved by the mixing method. Figure 5 displays the flowchart of the methodology used in the present study.

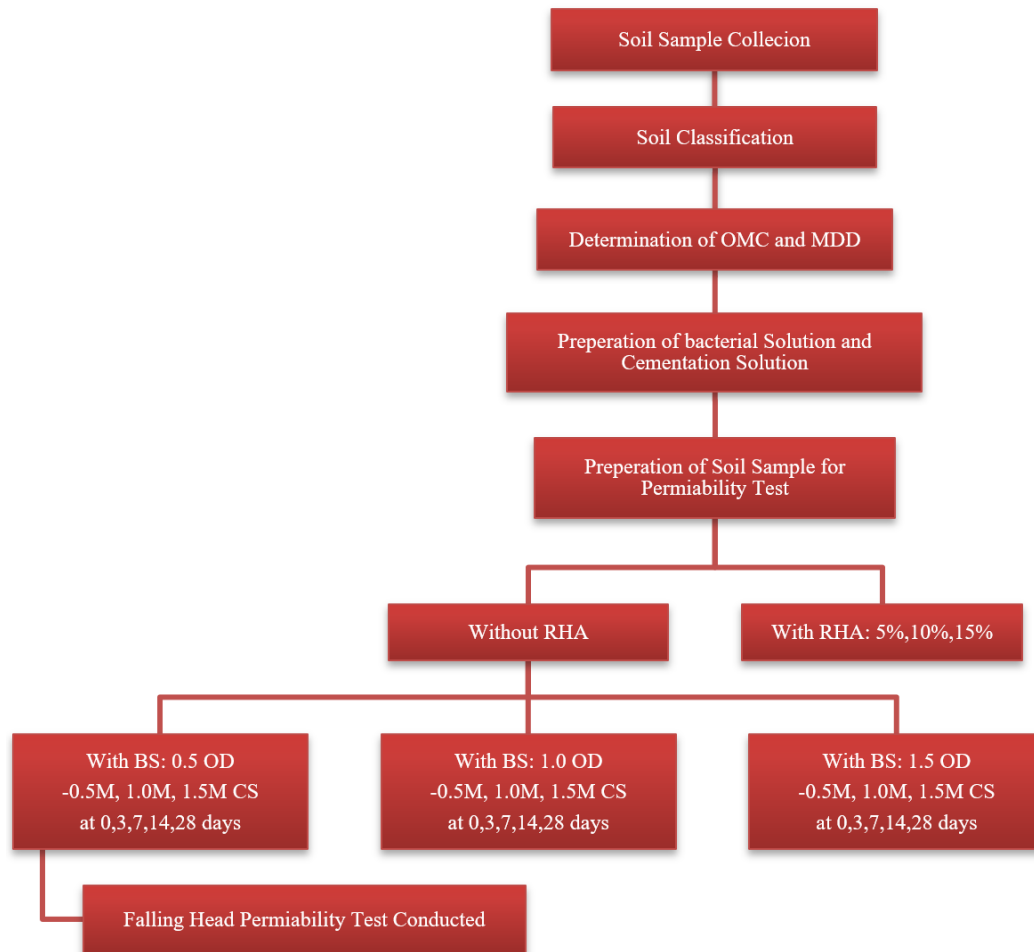


Figure 5. Flowchart of the methodology used in the present study

### 3. Results and Discussion

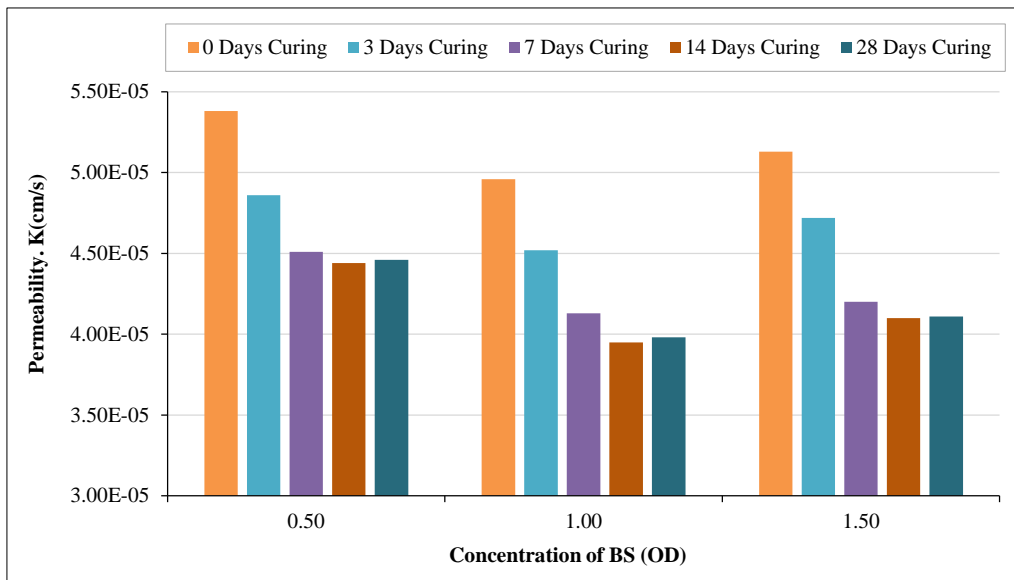
Falling head permeability test was employed for the evaluation of the permeability property of control, biocemented, and biocemented + RHA soil samples. Figure 6 displays the progress of the falling head permeability test. The permeability value for control soil was found to be  $2.49 \times 10^{-4}$  cm/s.



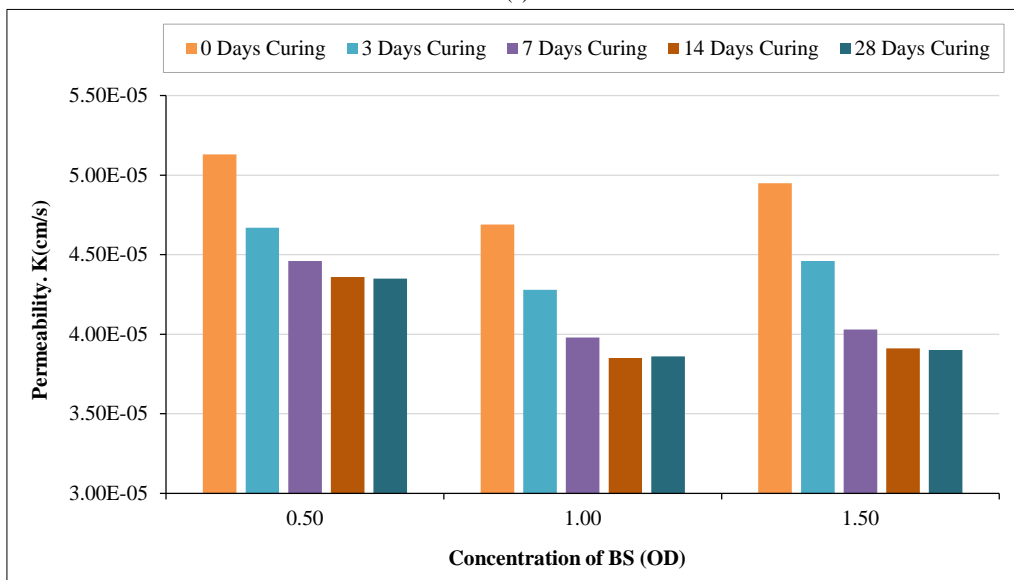
Figure 6. Falling head permeability test in progress

#### 3.1. Effect of Concentration of Bacterial Solution (BS)

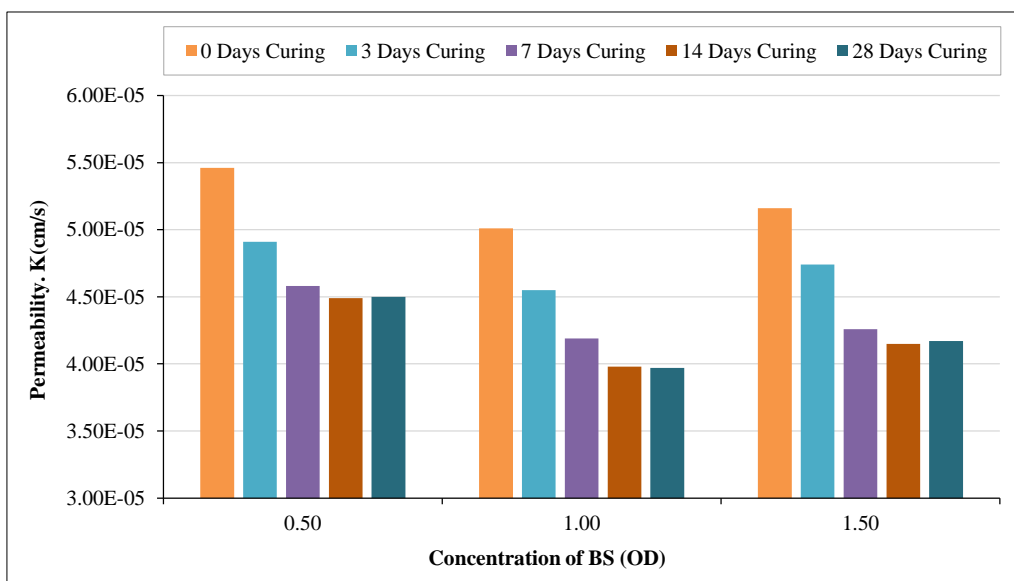
Figures 7-a to 7-c illustrates how the concentrations of BS influence the permeability of the biocemented sample. It was observed from Figure 7-a that at the 0.5M CS concentration, the permeability value of biocemented soil samples decreased initially and then increased with increasing the BS concentration. The same pattern was observed for 1.0M and 1.5M CS concentrations.



(a)



(b)



(c)

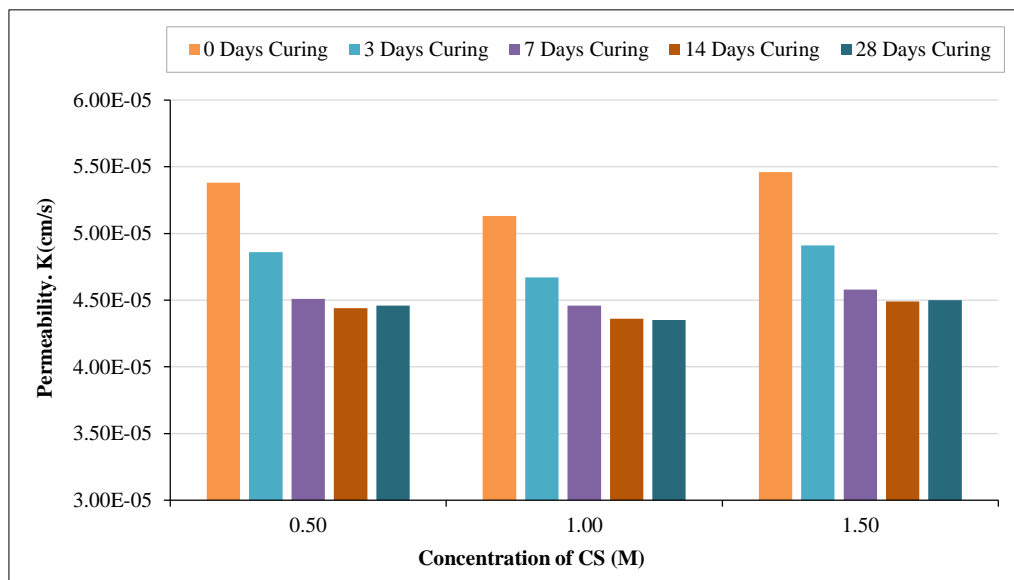
Figure 7. Permeability values of biocemented soil with different concentration of BS and curing periods – with (a) 0.5M CS (b) 1.0M CS (c) 1.5M CS

The maximum reduction of permeability value of biocemented soil samples was observed in 14-day curing period for all BS concentrations irrespective of the CS concentrations. This may be the result of microbial activity producing pore-filling materials. The negatively charged surface of the bacteria caused  $\text{CaCO}_3$  to precipitate when calcium ions in the form of  $\text{CaCl}_2$  were supplied [34], and it provided nucleation sites for the adhesion of  $\text{Ca}^{2+}$ . The  $\text{CaCO}_3$  precipitation blocked the soil pores which reduced the permeability of the soil and limited water flow [35]. The maximum percentage reduction in the permeability value was found in 1.0 OD concentration (20.36%) at 0.5M CS concentration. This was followed by 1.5OD (20.07%) and 0.5OD BS concentrations (17.47%). Similarly, for the CS concentration 1.0M, the maximum reductions were 17.97%, 21.92%, and 19.38% for 0.5OD, 1.0OD, and 1.5OD BS concentrations, respectively. The 17.76%, 20.56%, and 19.57% reduction in permeability value for 0.5OD, 1.0OD, and 1.5OD BS concentration, respectively, were observed in 1.5M CS concentration.

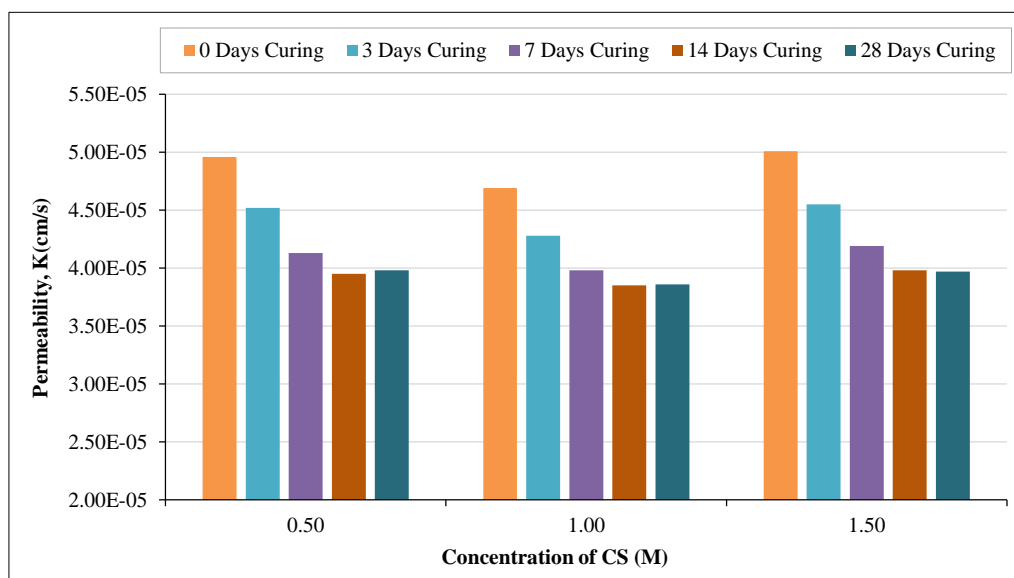
In comparison among BS concentrations, the maximum permeability reduction was associated with the 1.0OD BS concentration (i.e., 20.36%, 21.92%, and 20.56% for 0.5OD, 1.0OD, and 1.5OD, respectively). In addition, the maximum reduction in permeability value of the soil sample prepared at 1.0OD and 1.0 M with 14 days curing time was found to be 84.98% with respect to the control sample.

### 3.2. Effect of Concentration of Cementation Solution (CS)

The variation in the coefficient of permeability values of the bio-cemented samples at different CS concentrations, 0.5, 1.0, and 1.5M, is illustrated in Figures 8-a to 8-c. The results show the permeability values decreased for all the CS concentration regardless of BS and curing periods from the control sample. It could be due to the fact that the precipitation of calcite reduced the permeability values. As precipitation increased, the soil particles filled up and a bio-clogging effect connected the particles, decreasing the permeability.



(a)



(b)

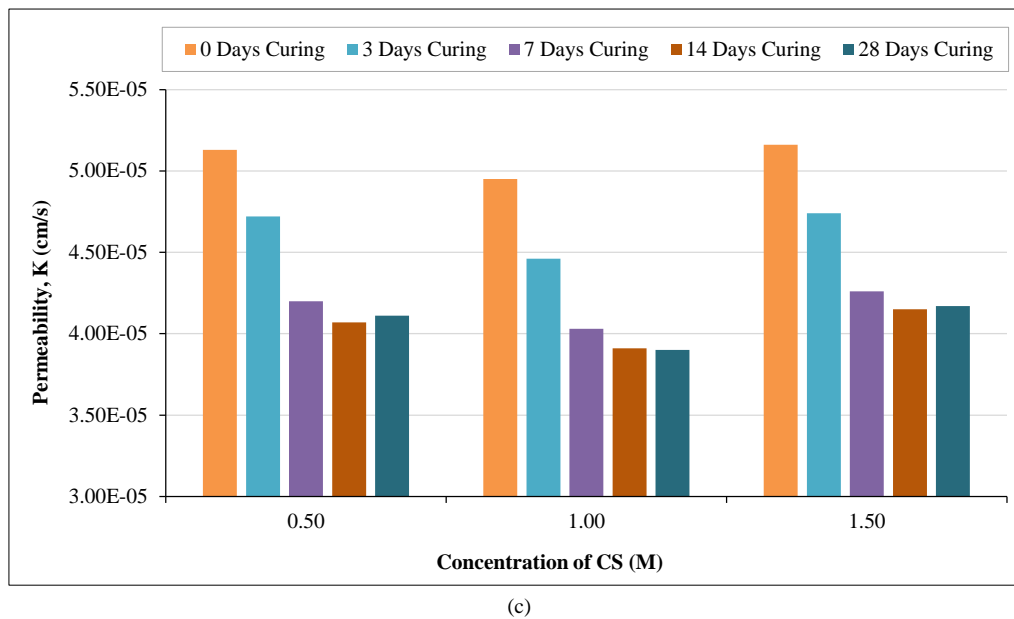


Figure 8. Permeability values of biocemented soil with different concentrations of CS and curing periods – with (a) 0.5 OD BS (b) 1.0 OD BS (c) 1.5 OD BS

It was observed from Figure 8(a) that at the 0.5OD BS concentration, the permeability value of biocemented soil samples decreased initially and then increased with increasing the CS concentration. The same pattern was observed for 1.0OD and 1.5OD BS concentrations. The maximum reduction of permeability value of biocemented soil samples was observed in 14 days curing period for all CS concentrations irrespective of the BS concentrations.

The maximum percentage reduction in the permeability value was found in 1.0M concentration (17.97%) at 0.5OD BS concentration. This was followed by 1.5M (17.76%) and 0.5M CS concentrations (17.47%). Similarly, for the BS concentration 1.0OD, the maximum reductions were 20.36%, 21.92%, and 20.56% for 0.5, 1.0, and 1.5M CS concentrations, respectively. The maximum percentage reduction in the permeability value for 1.5OD BS concentration was observed in 0.5M CS concentration, i.e., 20.08%. This was followed by 19.57% for 1.5M and 19.38% for 1.0M CS concentration. This finding is similar to the results of the studies conducted by Wani and Mir (2021) [7], Dejong et al. (2010) [36], and Qabany and Soga (2013) [37]. When the MICP treatment was performed with a low cementation solution concentration, precipitation happened at pore throats where there was an excessive number of bacteria and nutrients [36]. Qabany and Soga (2013) [37] observed that  $\text{CaCO}_3$  precipitation was more evenly distributed throughout the majority of the samples as a whole and there was no drastic blockage.

In comparison among CS concentrations, the maximum permeability reduction was associated with the 1.0M CS concentration (i.e., 17.97% and 21.92% for 0.5OD and 1.0OD, respectively), and with the 0.5M CS concentration (i.e., 20.08% for 1.5OD).

### 3.3. Effect of Curing Period

Figures 8-a, and 8-c show a sudden decline in the permeability values at the initial stage of treatment (7 days curing) till 14 days curing with respect to BS as well as CS concentration. The reason might be the blocking of pore spaces due to the development of calcite precipitation [35, 37]. Also, at the initial stage, bacteria might be active enough to increase the rate of calcite precipitation while cementation reagents were present and also due to the availability of pore spaces. Similar results were observed in the studies of Dejong et al. (2010) [36] and Al Qabany & Soga (2013) [37]. However, a slight increase after 14 days of curing was observed. The reason might be due to the dormant state of bacteria as there is no nutrition left for consumption after 14 days [7]. Also, there might be limited spaces for calcite precipitation after 14 days. Therefore, the coefficient of permeability values started increasing after 14 days up to 28 days.

### 3.4. Effect of RHA Addition

It was observed from Figure 9-a that at the 0.5M CS concentration, the permeability value of biocemented soil samples decreased initially and then increased with increasing the percentage of RHA additive. The same pattern was observed for 1.0M and 1.5M CS concentrations in all the bacterial solution concentrations (Figures 9-b to 9-c). The maximum permeability reduction was observed when 10% RHA was added to the biocemented sample (12.14% for 0.5M, 13.76% for 1.0M, and 12.29% for 1.5M, respectively) in 0.5OD. Similarly, for the BS concentration 1.0OD, the maximum reductions were 29.37%, 30.39%, and 28.10% for 0.5M, 1.0M, and 1.5M CS concentrations, respectively. The 20.88%, 22.51%, and 21.20% reduction in permeability value for 0.5M, 1.0M, and 1.5M CS concentration, respectively, were observed in 1.5OD. Similar findings were observed in the study of Oyediran & Ayeni (2020) [33]. The decrease in the permeability of the soil (with and without biotreated) mixed with RHA might be due to the fact that the RHA is a pozzolanic additive with a high concentration of amorphous silica that reacts with water and formed

cementitious compounds in soil particles. These cementitious substances bonded the soil particles together, decreasing the permeability of the soil [33].



Figure 9. Permeability values of biocemented soil@ 14 days curing with different percentage of RHA– with (a) 0.50 OD BS (b) 1.0 OD BS (c) 1.5 OD BS

In comparison among CS concentrations, the maximum permeability reduction was associated with the 1.0M CS concentration (i.e., 13.76%, 30.39%, and 22.51%) for 0.5OD, 1.0OD, and 1.5OD, respectively when 10% RHA was added. The maximum reduction in permeability value of the soil sample prepared at 1.0OD and 1.0 M with 10% RHA for a 14-day curing time was found to be 87.3% with respect to the control sample.

The permeability values of the soil samples stabilized with the RHA additive alone decreased initially and started increasing after 14 days curing in all RHA dosages (Figure 10). The maximum permeability reduction of the soil was observed in a curing period of 14 days when stabilized with 5%, 10%, and 15% RHA (as observed in Figure 10). This might be because there was a setting period that allowed urea and bacteria to spread across the entire soil before precipitation occurred. The increase in the permeability of soil or biotreated soil when mixed with 15% RHA might be because there was an excess of RHA in the sample that was not mobilized during the reaction, and as a result, the soil-RHA mixture's binding was weaker since they occupied spaces in the sample [31, 38].

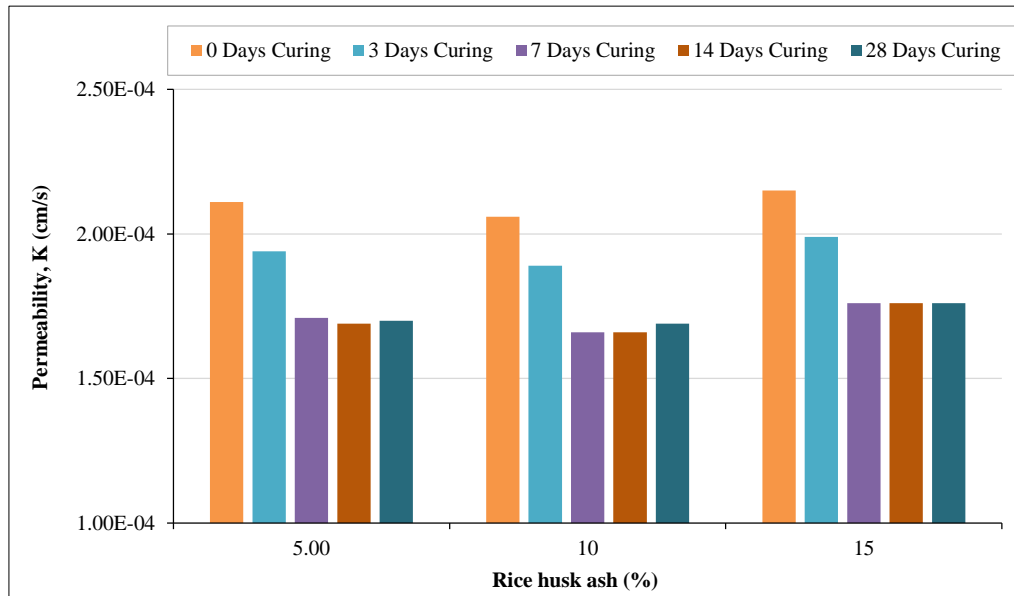


Figure 10. Permeability value of soil treated with only RHA at different curing periods

### 3.5. Microstructural Analysis

Scanning Electron Microscope (SEM) and Energy Dispersive X-ray microanalysis (EDAX) tests were conducted on the untreated and biocemented soil samples. The results of the SEM test are presented in Figures 11-a, and 11-b. It is noted that the formation of calcite is not observed in the untreated soil samples (Figure 11-a). Figure 11-b shows the SEM image of optimum MICP-treated soil. It is observed, from Figure 11-b, that the calcite was formed on the surface of the soil particles and at the contact points between them. This filled the pore spaces between the soil particles and reduced the coefficient of permeability of the soil samples. The results of the EDAX test for untreated and MICP-treated soil samples are presented in Figures 12-a, and 12-b. A higher energy peak of Ca in the treated sample showed the precipitation of calcite crystals in the samples

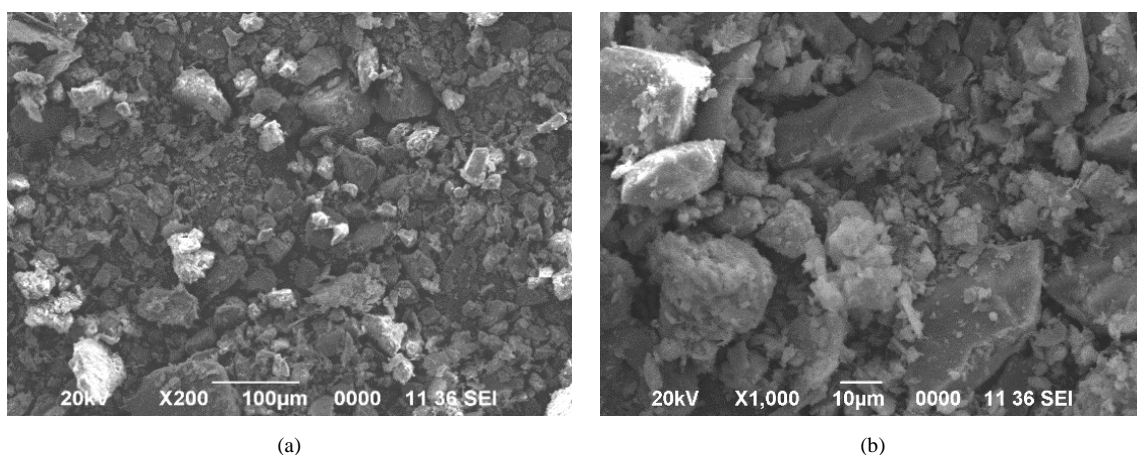


Figure 11. SEM image of (a) untreated soil and (b) MICP treated soil samples

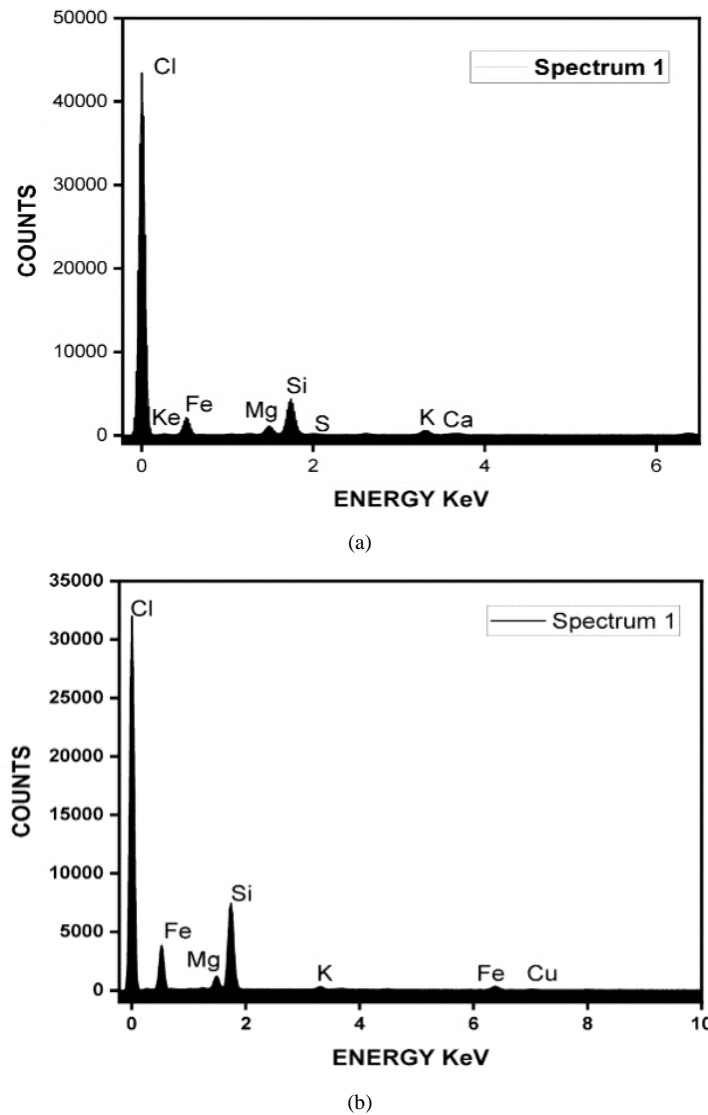


Figure 12. EDAX graph for (a) untreated soil and (b) MICP-treated soil samples

#### 4. Conclusions

The present study has demonstrated the application of bio-cementation techniques using the RHA additive on locally available soil to improve its permeability property. Some conclusions drawn from the present study are listed below:

- The coefficient of permeability value gets reduced due to the introduction of the bio-cementation technique, with or without the RHA additive. The addition of RHA up to 10% with 1.0 OD BS and 1.0M CS at a 14-day curing period was noted to optimally reduce the permeability property of the soil.
- The permeability of the soil decreased with the increase in BS and CS concentrations in all curing days. The maximum permeability reduction was 84.98% when the soil sample was treated with 1.0OD BS and 1.0M CS at 14 days of curing.
- Further, more decrease in the permeability value was seen when the RHA additive was added to the bio-cemented soil. The maximum permeability reduction at 1.0OD and 1.0M with 10% RHA after a 14-day curing time was found to be 87.3%.
- The addition of RHA alone to the untreated soil sample also reduced the permeability value. The maximum permeability reduction of the soil was observed in a curing period of 14 days when stabilized with 5%, 10%, and 15% RHA. The results of the SEM and EDAX tests are also in support of this reduction.

Overall, it can be concluded that the bio-cementation technique can be used as an effective and alternate soil improvement technique. The improvement of other geotechnical properties, like UCS and CBR, of the soil using this technique and RHA addition can be explored in future studies.

## 5. Declarations

### 5.1. Author Contributions

Conceptualization, M.G.S.; methodology, M.G.S.; writing—original draft preparation, M.G.S.; writing—review and editing, A.K. All authors have read and agreed to the published version of the manuscript.

### 5.2. Data Availability Statement

The presented datasets in the manuscript are available from the corresponding author on reasonable request.

### 5.3. Funding

The authors received no financial support for the research, authorship, and/or publication of this article.

### 5.4. Conflicts of Interest

The authors declare no conflict of interest.

## 6. References

- [1] Collins, R. W. (2011). Stabilization of marginal soils using geofibers and nontraditional additives. Master Thesis, University of Alaska Fairbanks, Fairbanks, United States.
- [2] Phummiphan, I., Horpibulsuk, S., Phoo-ngernkham, T., Arulrajah, A., & Shen, S.-L. (2017). Marginal Lateritic Soil Stabilized with Calcium Carbide Residue and Fly Ash Geopolymers as a Sustainable Pavement Base Material. *Journal of Materials in Civil Engineering*, 29(2), 04016195. doi:10.1061/(asce)mt.1943-5533.0001708.
- [3] Jan, O. Q., & Mir, B. A. (2018). Strength Behaviour of Cement Stabilised Dredged Soil. *International Journal of Geosynthetics and Ground Engineering*, 4(2), 1-14. doi:10.1007/s40891-018-0133-y.
- [4] Wani, K. M. N. S., & Mir, B. A. (2021). Influence of microbial geo-technology in the stabilization of dredged soils. *International Journal of Geotechnical Engineering*, 15(2), 235–244. doi:10.1080/19386362.2019.1643099.
- [5] Wani, K. M. N. S., & Mir, B. A. (2022). Application of Bio-Engineering for Marginal Soil Improvement: An Eco-Friendly Ground Improvement Technique. *Indian Geotechnical Journal*, 52(5), 1097-1115. doi:10.1007/s40098-022-00639-7.
- [6] Smith, A., Pritchard, M., Edmondson, A., & Bashir, S. (2017). The Reduction of the Permeability of a Lateritic Soil through the Application of Microbially Induced Calcite Precipitation. *Natural Resources*, 8(5), 337–352. doi:10.4236/nr.2017.85021.
- [7] Wani, K. M. N. S., & Mir, B. A. (2021). An Experimental Study on the Bio-cementation and Bio-clogging Effect of Bacteria in Improving Weak Dredged Soils. *Geotechnical and Geological Engineering*, 39(1), 317–334. doi:10.1007/s10706-020-01494-0.
- [8] Cardoso, R., Pires, I., Duarte, S. O. D., & Monteiro, G. A. (2018). Effects of clay's chemical interactions on biocementation. *Applied Clay Science*, 156, 96–103. doi:10.1016/j.clay.2018.01.035.
- [9] Dhami, N. K., Reddy, M. S., & Mukherjee, A. (2016). Significant indicators for biomineralisation in sand of varying grain sizes. *Construction and Building Materials*, 104, 198–207. doi:10.1016/j.conbuildmat.2015.12.023.
- [10] Arbabzadeh, E., & Cardoso, R. (2019). Efficiency of biocementation as rock joints sealing technique evaluated through permeability changes. *World Congress on Civil, Structural, and Environmental Engineering (ICGRE'19)*, Rome, Italy. doi:10.11159/icgre19.143.
- [11] Al-Salloum, Y., Abbas, H., Sheikh, Q. I., Hadi, S., Alsayed, S., & Almusallam, T. (2017). Effect of some biotic factors on microbially-induced calcite precipitation in cement mortar. *Saudi Journal of Biological Sciences*, 24(2), 286–294. doi:10.1016/j.sjbs.2016.01.016.
- [12] Ivanov, V., Chu, J., & Stabnikov, V. (2014). Iron-and calcium-based biogrouts for porous soils. *Proceedings of Institution of Civil Engineers: Construction Materials*, 167(1), 36–41. doi:10.1680/coma.12.00002.
- [13] Zhao, Q., Li, L., Li, C., Li, M., Amini, F., & Zhang, H. (2014). Factors Affecting Improvement of Engineering Properties of MICP-Treated Soil Catalyzed by Bacteria and Urease. *Journal of Materials in Civil Engineering*, 26(12), 04014094. doi:10.1061/(asce)mt.1943-5533.0001013.
- [14] Chen, Y., Han, Y., Zhang, X., Sarajpoor, S., Zhang, S., & Yao, X. (2023). Experimental study on permeability and strength characteristics of MICP-treated calcareous sand. *Biogeotechnics*, 1(3), 100034. doi:10.1016/j.bgtech.2023.100034.
- [15] Khaleghi, M., & Rowshanzamir, M. A. (2019). Biologic improvement of a sandy soil using single and mixed cultures: A comparison study. *Soil and Tillage Research*, 186, 112–119. doi:10.1016/j.still.2018.10.010.
- [16] Li, Y., Li, Y., Guo, Z., & Xu, Q. (2023). Durability of MICP-reinforced calcareous sand in marine environments: Laboratory and field experimental study. *Biogeotechnics*, 1(2), 100018. doi:10.1016/j.bgtech.2023.100018.

- [17] Jain, S., & Das, S. K. (2023). Influence of size and concentration of carbonate biomineral on biocementation and bioclogging for mitigating soil degradation. *Biogeotechnics*, 1(2), 100021. doi:10.1016/j.bgtech.2023.100021.
- [18] Tabrizi, E. M., Tohidvand, H. R., Hajialilue-Bonab, M., Mousavi, E., & Ghassemi, S. (2023). An investigation on the strain accumulation of the lightly EICP-cemented sands under cyclic traffic loads. *Journal of Road Engineering*, 3(2), 203–217. doi:10.1016/j.jreng.2023.03.002.
- [19] Choi, S. G., Wang, K., & Chu, J. (2016). Properties of biocemented, fiber reinforced sand. *Construction and Building Materials*, 120, 623–629. doi:10.1016/j.conbuildmat.2016.05.124.
- [20] Xiao, Y., He, X., Evans, T. M., Stuedlein, A. W., & Liu, H. (2019). Unconfined Compressive and Splitting Tensile Strength of Basalt Fiber-Reinforced Biocemented Sand. *Journal of Geotechnical and Geoenvironmental Engineering*, 145(9). doi:10.1061/(asce)gt.1943-5606.0002108.
- [21] Li, M., Li, L., Ogonnaya, U., Wen, K., Tian, A., & Amini, F. (2016). Influence of Fiber Addition on Mechanical Properties of MICP-Treated Sand. *Journal of Materials in Civil Engineering*, 28(4). doi:10.1061/(asce)mt.1943-5533.0001442.
- [22] Sharma, A., & Ramkrishnan, R. (2016). Study on effect of Microbial Induced Calcite Precipitates on strength of fine grained soils. *Perspectives in Science*, 8, 198–202. doi:10.1016/j.pisc.2016.03.017.
- [23] Morales, L., Garzón, E., Romero, E., & Sánchez-Soto, P. J. (2019). Microbiological induced carbonate ( $\text{CaCO}_3$ ) precipitation using clay phyllites to replace chemical stabilizers (Cement or Lime). *Applied Clay Science*, 174, 15–28. doi:10.1016/j.clay.2019.03.018.
- [24] Bindu, J., Kannan, K., & Sajana, S. Biocementation in Marine Clays: Effect on Grain Size Distribution. *Indian Geotechnical Conference 2017*, 14-16 December, 2017, IIT Guwahati, India.
- [25] Sidik, W. S., Canakci, H., Kilic, I. H., & Celik, F. (2014). Applicability of biocementation for organic soil and its effect on permeability. *Geomechanics and Engineering*, 7(6), 649–663. doi:10.12989/gae.2014.7.6.649.
- [26] Canakci, H., Sidik, W., & Halil Kilic, I. (2015). Effect of bacterial calcium carbonate precipitation on compressibility and shear strength of organic soil. *Soils and Foundations*, 55(5), 1211–1221. doi:10.1016/j.sandf.2015.09.020.
- [27] Cheng, L., & Shahin, M. (2017). Bacteria induced cementation for soil stabilization. *Proceedings of the 19<sup>th</sup> International Conference on Soil Mechanics and Geotechnical Engineering*, 17-22 September, 2017, Seoul, Korea..
- [28] Choi, S.-G., Wu, S., & Chu, J. (2016). Biocementation for Sand Using an Eggshell as Calcium Source. *Journal of Geotechnical and Geoenvironmental Engineering*, 142(10), 06016010. doi:10.1061/(asce)gt.1943-5606.0001534.
- [29] Dayakar, P., Raman, K. V., Arunya, A., & Venkatakrishnaiah, R. (2019). Study on strength properties of sand by biocementation with eggshell. *International Journal of Civil Engineering and Technology*, 10(1), 2770–2785.
- [30] Rathan Raj, R., Banupriya, S., & Dharani, R. (2016). Stabilization of soil using rice husk ash. *International Journal of Computing Engineering Research*, 6(2), 43-50.
- [31] Adhikary, S., & Jana, K. (2016). Potentials of rice husk ash as a soil stabilizer. *International Journal of Latest Research in Engineering and Technology*, 2(2), 40-42.
- [32] Roy, A. (2014). Soil stabilization using rice husk ash and cement. *International Journal of Civil Engineering Research*, 5(1), 49-54.
- [33] Oyediran, I. A., & Ayeni, O. O. (2020). Comparative effect of microbial induced calcite precipitate, cement and rice husk ash on the geotechnical properties of soils. *SN Applied Sciences*, 2(7), 1157. doi:10.1007/s42452-020-2956-0.
- [34] Nemati, M., Greene, E. A., & Voordouw, G. (2005). Permeability profile modification using bacterially formed calcium carbonate: Comparison with enzymic option. *Process Biochemistry*, 40(2), 925–933. doi:10.1016/j.procbio.2004.02.019.
- [35] Whiffin, V. S., van Paassen, L. A., & Harkes, M. P. (2007). Microbial carbonate precipitation as a soil improvement technique. *Geomicrobiology Journal*, 24(5), 417–423. doi:10.1080/01490450701436505.
- [36] DeJong, J. T., Mortensen, B. M., Martinez, B. C., & Nelson, D. C. (2010). Bio-mediated soil improvement. *Ecological Engineering*, 36(2), 197–210. doi:10.1016/j.ecoleng.2008.12.029.
- [37] Qabany, A. A., & Soga, K. (2013). Session 2: Bio-chemo-mechanical aspects in geomechanics. *Geotechnique*, 63(4), 331-339. doi:10.1680/geot.SIP13.P.022.
- [38] Yadu, L., Tripathi, R. K. & Singh, D. (2011). Comparison of fly ash and rice husk ash stabilized black cotton soil. *International Journal of Earth Sciences and Engineering*, 4(6), 42–45.