

Development of Single-Phase Microbial Cementation Method and to Investigate its Efficacy on Bearing Capacity, UCS, and Permeability of Sandy Soils

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Highlights:

- Contribution to the development of environment-friendly material to reduce the consumption of cement in civil engineering work.
- Use of bio agents in combination with locally available sand/soil.
- Investigation of the increase in ultimate bearing capacity (qu) and the decrease in settlement, and the effect of the size of the bearing plate for sand reinforced with a bacterial culture and cementation (BCC) solution.

Abstract. Microbially induced calcite precipitation (MICP) is a method based on collaborative knowledge of microbiology, chemistry and geotechnical engineering. The objective of this study was to investigate the increase of the bearing capacity and the unconfined compressive strength (UCS) as well as the reduction of the permeability of sandy soil using MICP. Experiments were carried out using Bacillus Pasteurii, on three different types of sand. The admixture of bacterial culture and cementation (BCC) solution all-in-one with sand by singlephase injection was applied to induce cementation. Three samples of the selected sand were treated with varied concentrations of BCC solution, ranging from 0.05 to 0.2 L/kg, with a curing period of 3, 7 and 14 days. The test results indicated an enhancement of 55% in UCS for sand treated with a BCC content of 0.05 to 0.2 L/Kg and a reduction of 40% in permeability for untreated sand with an effective diameter of 0.5 mm treated with 0.2 L/kg of BCC solution after 14 days of curing. The results of a plate load test (PLT) on MICP treated sand showed an increase in the ultimate bearing capacity (qu) by about 2.95 to 5.8 times and a 1.7 to 3.31-fold reduction in settlement corresponding to the same load applied on untreated footing. Further investigation of the size and shape of the bearing plate on bearing capacity and settlement was carried out through a plate load test. The higher and more favorable results shown by a rectangular plate compared to a circular plate indicate that the first is preferable.

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1 Introduction

The community of civil engineers has to deal with field problems associated with dewatering of back-fills, water-bearing strata, water loss from canals, undesirable leakage of liquids, gases, oil recovery wells, excessive seepage through dams and reservoirs constructed on highly permeable soil, contamination of groundwater coming through waste landfills, etc. Useful land for future constructions or renovations at current sites is scarce. All these facts have put forth the need for improvement of the engineering properties of weak soil. Conventional methods to improve the mechanical properties of weak soil are replacement of existing weak soil, grouting, consolidation, the use of chemical admixtures, and the use of geo fibers and polymers. The addition of chemicals and admixtures leads to the creation of unsafe and harmful environments [1]. Nowadays, increasing awareness and consciousness of the environmental aspect of building and construction necessitate the search for ecofriendly and sustainable alternatives for soil improvement.

MICP is a technique that has emerged from interdisciplinary research at the confluence of geochemistry, microbiology and geotechnical engineering. One such emerging technology is bio-cementation, or bio-clogging, to improve the mechanical characteristics of weak soil [2-6]. The laboratory and field application of MICP in various fields includes repair of cracks in concrete [6], liquefaction [2,7], enhancement of mechanical properties of cement mortar [8], the effect of microbial cementation methods and treatment time to improve porosity and mechanical properties of sand [9], oil recovery [10], permeability [11], upscaling and field application of MICP [12-13], and the use of bio-grout for ground improvement [14]. These studies demonstrated the technical feasibility, validity and advancement of the MICP concept.

1.1 MICP Process and Reactions Involved

Through biologically driven urea hydrolysis, MICP enhances soil conditions for calcium carbonate precipitation in particle-to-particle contact by producing calcium carbonate (CaCO₃). The overall equilibrium of the bio-chemical reaction involved in calcium carbonate (CaCO₃) precipitation by bacteria is formulated in Eqs. (1) to (8) [1,15]. This bio-chemical reaction involves the production of CO₂ and NH₄ through microbes. In urea hydrolysis (urea decomposition), aerobic, ureolytic nonpathogenic bacteria *Sporosarcina Pasteurii* (formerly known as *Bacillus Pasteurii*), hydrolyze the urea [2,14,16]. Urease hydrolyzes the urea

substrate, generating ammonia and carbamic acid (NH_2COOH) (Eq. (1)). Spontaneous hydrolysis of NH₂COOH leads to the generation of additional mole of ammonia and carbonic acid (Eq. (2)) [17]. The carbonic acid gives rise to bicarbonate (HCO⁻³) and protons (H⁺) (Eq. (3)). The ammonia generated in Eqs. (1) and (2) reacts with water to give two moles of ammonium ions (NH_4^+) and two moles of hydroxide ions (OH⁻) (Eq. (4)) [17]. The increase of alkalinity of the micro-environment due to a raise in pH is observed on account of the production of hydroxide ions [17]. The available calcium ions (Ca^{+2}) in the solution get adsorbed into the cell wall of the microbes (Eq. 5) and in the presence of CO3⁻², calcite precipitation takes place on the cell wall (a layer located outside the cell membrane) (Eq. (8)) [16]. Also, calcite precipitation due to stimulation of native microbes available at the subsurface takes place [18,19]. All cations (+)and anions (-) produced in the reaction (Eqs. (3)-(4)) are combined to produce carbonate anions (CO_3^{-2}) , ammonium ions (NH_4^+) and water, as can be seen from Eq. (6). The calcium carbonate anions in the reaction come into contact with calcium cations available in the proximal environment or on the cell walls of the microbes to precipitate calcium carbonate in the form of crystals, as can be seen in Eq. (7). Upon completion of the reaction, NH_4^+ evolves as a byproduct, as can be seen in Eq. (8).

$$CO(NH_2)_2 + H_2O \xrightarrow{OFERSE} NH_2COOH + NH_3$$
 (1)

Umagaga

$$NH_2COOH + H_2O \rightarrow NH_3 + H_2CO_3$$
(2)

$$H_2CO_3 \leftrightarrow HCO_3^- + H^+$$
 (3)

$$2NH_3 + 2H_2O \leftrightarrow 2NH_4^+ + 2OH^-$$
(4)

$$Ca^{+2} + microbial cell \rightarrow cell-Ca^{+2}$$
 (5)

$$HCO_{3}^{-} + H^{+} + 2NH_{4}^{+} + 2OH^{-} \leftrightarrow CO_{3}^{2-} + 2NH_{4}^{+} + 2H_{2}O$$
 (6)

$$cell - Ca^{+2} + CO_3^{2-} \rightarrow cell - CaCO_3 \downarrow$$
 (7)

The net reaction, or the sum of Eqs. (1) to (7), is

$$CO(NH^2)^2 + H_2O + Ca^{+2} + \text{microbial cell} \rightarrow$$

microbial cell-CaCO₃ + 2NH₄⁺ (8)

In summary, the MICP mechanism through calcite precipitation by urea hydrolysis in the presence of Ca^{+2} ions consist of: (a) hydrolysis of urea (Eqs. (1)-(3)); (b) an increase in pH and alkalinity of the micro-environment (Eq. (4)); (c) adsorption of Ca^{+2} ions into the surface of the cell (Eq. (5)); and (d) nucleation and crystal growth of precipitated CaCO₃ (Eqs. (6)-(8)). A schematic diagram representing the entire urea hydrolysis process based on the previous literature is shown in Figure 1.



Schematic Representation of MICP-Ureolysis.

Figure 1 Schematic representation of the MICP process.

The calcium carbonate formed sticks/binds the soil particles together and reduces space, voids in the soil mass, which ultimately enhances the strength and reduces the permeability of the soil. The evidence of microbial remnants indicates that the bacterial cell wall acts as nucleation site for calcite precipitation [16].

The rest of this paper is organized as follows. Subsection 1.2 presents the literature review that was conducted in this study. The methodology of the research work is described in Section 2. Section 3 presents the results and elaborates the discussion on the validation and interpretation of the obtained results, followed by the conclusion in Section 4. The major contributions of this research work are:

- 1. Investigation of the suitability of a bio-agent to commonly found sand in the region of Maharashtra, India as an alternative for cement.
- 2. Integrated approach of applying a bacterial cementing solution for the enhancement of the engineering properties of the samples used.
- 3. Elaborative investigation on improving the bearing capacity based on loadsettlement curves using the tangent method.

1.2 Literature Review

Several treatment strategies are available for the improvement of the permeability of soil using MICP. The results of previous research work on MICP are tabulated in Table 1. Ref. [20] found that permeability of porous media can be reduced effectively by the use of enzymatic formation of CaCO₃ in situ. The use of

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multiple injections with increased urease concentration and reactants enhances the formation of CaCO₃ precipitation and leads to a significant decrease in permeability of porous media of up to 98% [20]. This method can be used as a solution for field problems in sand consolidation areas in oil reservoirs, to enhance oil recovery, and to prevent contamination of ground water systems.

Another study claimed that plugging of porous media is possible due to the formation of calcium carbonate either by using bacteria or enzymes, or by using bio-mass as a plugging agent [21]. The permeability ratio obtained by bacteria, enzymes and bio mass as plugging agent was 65%, 62% and 52%, respectively. Formation of a 1-mm thick, strong and watertight bio-cemented coating on the surface of sand reduced the permeability from 10⁻⁴ to 1.6×10^{-7} m/s.

The formation of such an impermeable bio-cemented crust on sandy soil is a beneficial field solution for slope stabilization, sealing of channels, aquacultural ponds and reservoirs [4]. Reduction in permeability of up to 55% is claimed by [3] based on an experiment on a 5-m Itterbeck sand column treated with *Sporosarcina pasteurii* and reagents, representing pragmatic field conditions. Stabilization of dredged soils was achieved by [22] with the help of varied OD of Bacillus Subtilis along with a 0.25 M and 1 M cementation solution of urea and calcium chloride. In this experiment, an unconfined compression test at a controlled strain rate of 1.2 mm/min was conducted on a cylindrical specimen of 38 mm diameter and 76 mm length, prepared from 132 gram of oven-dried dredged soil, uniformly mixed with bacteria and 21 ml of distilled water (0.15 L/kg) and curing for 7 and 28 days at an average temperature of 25-28 °C. The outcome of the research was an increase in UCS by 2.5 times compared to untreated sand.

Martinez, *et al.* [23] studied the load transfer mechanism at the micro and the macro scale for bio-mediated calcite precipitation of sands [23]. The performance of MICP treated Ottawa sand was evaluated through a 1g scale model test on a shallow foundation of size (2B x 1.5B x B). The sand bed was prepared by using dry pluviation [24]) at relative density 35%. The sand was first treated with *Sporosarcina pasteurii* and nutrients, followed by discrete injection of nutrients and calcium for a duration of 40 hours. The test result and the load-settlement curves revealed a five-fold reduction in foundation settlement under the same load compared to the untreated specimen.

Improvement of the bearing capacity of sand in the range of 1.17 to 3.9 was achieved by reinforcing shredded waste tires in [25]. Plate load tests on a strip footing with width 75, 100, and 120 mm rested on unreinforced fine sand and reinforced with geo-grid was conducted to evaluate improvement of the bearing capacity and settlement characteristics in [26]. This study concluded that the gain

in bearing capacity ratio (BCR) relative to that of the unreinforced case was found to be in range of 1.5 to 1.7. In an experimental study on the behavior of a strip footing on sandy soil bounded by walls of different depths and located at different distances from the footing showed that the presence of the wall remarkably affected the bearing capacity due to the increase in soil confinement, leading to an improvement of the bearing capacity in the range of 37% to 59% and a reduction in the vertical settlement ranging from 5 to 160% [27].

D.f	Madarial	Destants	Teste ettere en ettered	Permeability	UCS
Kei.	Material	Bacteria	Injection method	Reduction(%)	(KPA)
20	porous media	Urease	2 injections	98	NR
	Toyoura		4 for permeability,		
5	sand	Urease	8 for UCS	70	1600
			6 sequential		
4	sand	Bacillus pasteurii	treatments	99	NR
	Itterbeck	Sporosarcina			
3	sand	pasteurii	2-phase injection	55	570
					2.5
					times
					that of
			mixing bacteria in		the un-
	dredged		soil followed by 48 hr		treated
22	soil	Bacillus Subtilis	cementation solution	NR	soil
30	sand	Bacıllus pasteurii	single-phase injection	NR	2500
					1.3
					times
					that of
					the
					OPC
	silica				sampl
31	sand	Bacillus pasteurii Sporosarcina	two-phase injection	NR	e
32	sand	pasteurii	4 alternate injections	40	14000
	dune	singal culture of	multiple alternate		
33	sands	Bacillus pasteurii	injections at 12 hr	78	1392
		B. pasteurii+B.			
		subtilis		85	1690
	NR	Not reported			
	Note	Bearing capacity/stre	ngth was not reported		

Table 1Review on MICP research work.

Lin, *et al.* [28] studied the response of MICP bio-grout in large-scale application to improve soil-pile interaction and the pile's ability to withstand axial compression [28]. The successfulness of the experiment was revealed by measurement of S-wave velocity and CaCO₃ content. The experiment included testing of pervious concrete piles of 76 mm diameter and 1.07 m length, one MICP treated and the other one untreated, for load versus settlement.

The load-displacement curve showed that the increase of initial slope (stiffness) and ultimate load (5117 N to 12648 N) was 2.5 times compared to the untreated pile. Ref. [29] studied ground improvement by using pervious concrete piles, which offer more strength and stiffness as compared to granular columns. The load carrying capacity response was observed based on pile type, i.e. pervious concrete pile versus granular column as well as based on installation method, i.e. installed pervious concrete pile versus precast pervious concrete pile. The vertical load tests results indicated a 4.4 ratio of the ultimate load of the pervious concrete pile (9.8 kN) to the ultimate load of the granular column (2.2kN). Further, a load settlement test was conducted on pervious concrete piles to understand the effect of installation method using a precast pile and an installed pile. This test showed that the ultimate load of the installed pile was 2.6 times greater than that of the precast pile.

1.3 Objective of Research

It is necessary to study the improvement of locally available soil properties by applying MICP in order to make any civil engineering project economically viable. The geological nature of the soil and mineral composition change from one location to another and therefore it is necessary to check the feasibility of using different types of soil for MICP. The selection of bacteria was decided based on the availability of cultures from the National Collection of Industrial Microorganism (NCIM), Pune, Maharashtra (India). Most researches conducted on MICP, as mentioned in Table 1, were aimed at finding UCS and permeability reduction by using various injection methods.

The multiple-phase injection methods are somewhat tedious and complex to operate. The present study investigated the efficacy of injecting an admixture of bacterial culture and cementation solution (BCC) using the single-phase injection method. As per the knowledge of the authors, limited research has been carried out on improving the bearing capacity of soil using MICP. The objective of this study was application of a BCC solution in one go on three different selected sand samples with varying curing period and its effectiveness in enhancing UCS, permeability and bearing capacity. The secondary objective was to investigate different sizes and shapes of the bearing plate in a plate load test to determine the difference in bearing capacity.

2 Materials and Methods

This section describes the methodology followed to achieve the objectives of the present research work. The methodology involved collecting three types of material samples, cultivation of bacterial cultures, preparation of the BCC solution, application of the BCC solution to the material samples. The mixture

was kept for curing for three scenarios, viz. 3 days, 7 days and 14 days. The process was repeated for three sets of sand samples for each scenario.

UCS and permeability tests were conducted on the three sand samples with D10 of size 0.1 mm, 0.5 mm and 1 mm. Based on the UCS and permeability results, D10 of size 0.5 mm was selected to determine the bearing capacity using a plate load test. The details of the procedure are explained in the subsections below.

2.1 Sample Collection

Sand samples were collected from Bhima River, Pune, Maharashtra (India), at latitude (18.30337) and longitude (74.762706). Table 2 shows the physical properties uniformity coefficient (Cu) and curvature coefficient (Cc) of the sand before injection.

Sand Sample	D ₁₀ (mm)	Cu	Cc	Soil classification	Specific Gravity	Permeability (mm/sec)
1	0.1	7	3.33	SW	2.69	0.52
2	0.5	12	1.08	Well-graded	2.7	1.60
				SW		
3	1.0	4.5	2	SP	2.74	4.4

 Table 2
 Physical properties of sand samples before injection (pristine).

2.2 Bacterial Culture and Cultivation

Bacterial culture of Bacillus Pasteurii NCIM 2477 was used due to its high urease activity. It is extensively preferred to produce a high amount of precipitate within a short period of time [34]. The mixture of bacterial culture, urea, and CaCl2 all-in-one (BCC) was applied using single-phase injection to achieve cementation of soil.

In this work the single-phase injection method was used to avoid complex multiple-phase injection methods. Due care was taken to distribute the BCC solution inside the soil matrix and to avoid clogging through bio-floc formation [30]. Bacillus Pasteurii was cultivated using nutrient agar media with the protocol and instructions mentioned on the container of the culture medium.

2.3 Preparation of the Cementation Solution

Ureolytic driven calcite precipitation was achieved by using urea-calcium cementation medium. In this process, urea and calcium chloride are used as reagent. From 60.06 g/mole molecular weight of urea (NH₂COH₂) and 111 g/mole of unhydrous calcium chloride (CaCl₂), a cementation solution with a concentration of 0.25 M was made by dissolving 15.1 g of urea (solid) and 27.75 g of unhydrous CaCl₂ (solid) into 1 liter of water. Both solutions were added

together in equal proportion and the cultivated bacteria were released into the solution. The varied quantity of BCC solution (0.05 to 0.2 L/kg of sand) was in line with [34-35]. Mixing 7 ml of bacterial solution in 200 g of sand and addition at 7-ml increments up to 4 times; (0.035 L/kg to 0.14 L/kg) was adopted [34]. Similarly, mixing 900 g with 250 ml of bacteria solution (0.277 L/kg) and 140 g sand with 45 ml of bacteria solution (0.32 L/kg) for the MICP treatment was adopted from [35].

Most researches adopted the injection method, which is similar to the grouting of artificial material for soil improvement. The method of injection could be either parallel or staggered, by surface percolation or by spraying on soil [36]. One-phase injection of bacteria, urea and CaCl₂ all-in-one was used by [30]. The prepared sand samples had small depth, therefore the all-in-one BCC solution was applied by directly spreading over the sand specimen. Uniformity of the spreading solution was ensured by visual observation.

2.4 Experimental Work

Homogeneous dry samples were prepared in three equal layers using the air pluviation method [24] to achieve the desired relative density. Figure 2 shows a photograph of the prepared samples. All samples were then placed in the laboratory for a desired curing period in controlled room temperature (27 °C) for the bacterial action to take place.

Table 3 shows the test specimens with the varying BCC content and curing periods adopted for the tests. After the desired curing period, the samples were removed from the polythene bag and tested for permeability and UCS as per IS 2720 (1986), Part 17 and IS 2720 (1991), Part 10, respectively. A UCS test as per IS standards was conducted on cylindrical specimens of 38 mm diameter and 76 mm in height, which had been cured for 3, 7 and 14 days. It is observed that the sample with 3-day curing period did not gain sufficient strength to stand alone.



Figure 2 Samples in polythene bags.

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Figure 3 Experimental setup for the PLT test.

Table 3Combination	n of test specimens.
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Sr. No.	D10 (mm)	BC solution (L/kg)	Curing period (days)
Sample1	0.1	0.05, 0.10, 0.15, 0.20	3, 7, 14
Sample 2	0.5	0.05, 0.10, 0.15, 0.20	3, 7, 14
Sample 3	1.0	0.05, 0.10, 0.15, 0.20	3, 7, 14

A plate load test (PLT) on the sand treated with and without BCC was conducted to investigate the strength enhancement after treatment. The effect of shape and size of the bearing plate was also investigated by using different shapes and sizes of bearing plate.

The simulated model test pit shown in Figure 3 consisting of a rectangular tank (600 x 600 mm) made from 25-mm thick mild steel plate to accommodate a footing with centrally placed plates was fabricated. The PLT was conducted on the footing using 25-mm thick circular plates of diameter 50 mm, 100 mm, 120 mm and square plates with 50 mm, 100 mm, 120 mm sides. Sand specimens of effective diameter 0.5 mm, with and without 0.2 L/kg BCC solution cured for 14 days with maximum UCS were used. The specimens for the PLT were prepared from dry pluviation to maintain the desired relative density [24]. The BCC solution was uniformly spread over the top surface of the specimens and allowed the desired curing period. A vertical point load was applied at the center of the plate, measured with a proving ring.

Two dial gauges with 25-mm travel capable of measuring with up to 0.01-mm accuracy, were used to measure settlement against applied load. After removal of the applied seating load, the dial gauge readings were noted and further incremental loading equal to one tenth of the estimated failure load was applied. The average settlement was noted at intervals of 0, 5, 10, 20, 40, 60 minutes, until abrupt settlement or failure of the footing occurred or until the rate of settlement was reduced to less than 0.02 mm/min.

The test ended when large displacement occurred without increase of load. The failure evidence of the untreated specimen was recorded by visual inspection, when the sand showed minimum heaving of the surface peripheral of the footing, along with its propagation in the vertical and the horizontal plane; indicative failure by bulging/abrupt settlement and corresponding peak pressure was also noted. The failure of the treated footing was confirmed after development of surface cracks and raising of the surface. The load-settlement curves for the pristine and the treated specimen were plotted to calculate the ultimate bearing capacity (q_u) and the corresponding settlement (δ_u) using the tangent method.

The bearing capacity ratio (BCR), defined as the ratio between the ultimate bearing capacity of the treated sand and the ultimate bearing capacity of the untreated (pristine) sand as well as the settlement reduction factor (SRF), defined as the ratio between the ultimate settlement of the treated sand and the ultimate settlement of the untreated sand, was calculated. To ensure uniformity of precipitation, a gravimetric analysis test was performed on the samples collected from different footing locations. The amount of calcium carbonate precipitation was determined by EDTA method [3]. Dry samples were collected from different locations and weighed as M1. The sample consisted of calcium carbonate precipitate and calcium chloride crystals. To this sample 0.2 M HCl was added. Calcium carbonate dissolves in HCl but calcium chloride does not react with HCl. Dry weight M2 was noted after dissolving and washing. The content of precipitated CaCO₃ was determined using Eq. (9).

%CaCO₃ content =
$$\frac{(M_1 - M_2)}{M_1} \times 100$$
 (9)

3 Results and Discussion

3.1 Effect on Permeability

Table 4 shows the results of the permeability test. Figure 4 (a, b, c) shows the response in terms of reduction in permeability versus the concentration of BCC solution, at 3, 7, and 14 days, for an effective diameter of 0.1 mm, 0.5 mm and 1 mm of sand particles respectively. It was observed that as the BCC content was increased, the coefficient of permeability was reduced with increasing curing period. The decrease in permeability occurs due to the filling of pore spaces in the sand by deposition of calcite crystals surrounding the particles [37]. The decrease in permeability for an effective diameter of 0.1 mm, 0.5 mm and 1.0 mm was 17.31%, 40.62% and 6.81%, respectively. Coarse grained sand has larger pores and spaces between its particles due to which bacteria can pass more easily from one pore to another. This results in a uniform distribution of calcite carbonate in the soil [38]. Precipitation of calcite in MICP treated sand influences

the permeability, UCS and stiffness depending on particle size [37]. Limited entry of BCC solution into the pore spaces of fine sand (0.1 mm) may result in less CaCO₃ precipitation and thus insufficient bridging of gaps. Maximum reduction in permeability was observed for sand of effective diameter 0.5 mm, which is in line with [38]. The smaller reduction in permeability for an effective diameter of 1 mm was also expected. This is attributed to distribution of calcite patterns within pore spaces and the limited effect on the formation of bio film on coarse particles. Also, a marginal difference in percentage reduction of the permeability was observed for 7 and 14 days of curing as compared to 3 days. It was observed that out of the total reduction in permeability, 68% to 94% reduction was attained at the end of the 7th day. The variation in permeability reduction is attributed to the effective size of the sand and the BCC content. Further, a slight improvement was observed in the reduction of permeability from day 7 to day 14. For example, as shown in Table 4, for BCC content 0.2 and D10 effective size 0.5 mm, the permeability reduction on day 7 was 35.63% and 40.62% on day 14 compared to the initial permeability of 1.60 mm/sec; the initial permeability is shown in Table 2. Thus, it can be seen that approximately 87% of the permeability reduction was achieved at the end of day 7 and the rest at the end of day 14.

The results obtained for an effective diameter of 0.5 mm through this experiment are in line with the results obtained in [39], where Azotobacter Chroococcum aerobic bacteria were used along with residual soil in situ effectively acting as subsurface bio-barrier for the permeability reduction by 40%, 42% and 32% after nutrient solution was penetrated for 10, 15, and 20 days respectively. Hence, the authors are of the opinion that an effective diameter of 0.5 mm with 0.2 L/kg of BCC solution is more advisable to achieve maximum permeability reduction as compared to the other effective diameter sizes used in this test.

BCC			% Redu	ction in Per	rmeability	for effecti	ve diamete	er	
Solution	0.1 mm				0.5 mm		1.0 mm		
L/kg	3	7 Days	14	3 Days	7 Days	14	3 Days	7 Days	14
	Days		Days		-	Days		-	Days
0.05	1.93	9.61	11.54	3.13	18.75	25	1.14	3.41	5
0.1	4.81	11.54	13.47	12.5	23.75	26.87	1.82	4.55	5.69
	10.5								
0.15	8	14.43	15.39	15.63	30.63	34.37	2.73	5.23	6.36
0.2	12.5	16.35	17.31	18.75	35.63	40.62	3.41	6.37	6.82

Table 4Summary of % reduction in permeability.



Figure 4 Permeability results for effective diameter a) 0.1 mm, b) 0.5 mm, and c) 1 mm.

3.2 Effect on Unconfined Compression

Table 5 shows a summary of the UCS values obtained through the test and Figure 5 shows the influence of BCC content on UCS for different effective diameters of sand. After 14 days of curing, for effective diameter 0.1 mm, 0.5 mm and 1 mm, the maximum UCS values were 157.6 kN/m², 180.8 kN/m² and 136.8 kN/m² at 0.2 L/kg of BCC solution, while the minimum UCS values were 101.3 kN/m², 114.3 kN/m² and 89.3 kN/m² at 0.05 L/kg of BCC solution. The authors are of the opinion that specimens with effective diameter 0.5 mm had the highest UCS values due to dense and compact formation of calcium carbonate crystals in compatible voids as compared to the other effective diameter sizes. Comparison of UCS values at 0.05 L/kg and 0.2 L/kg after 14 days shows a 1.54 to 1.65 times increase in UCS.

Comparison of UCS values at 0.05 L/kg and 0.2 L/kg after 14 days shows an average 1.55 times increase in UCS. These results are in line with the results obtained in [22]. The specimens with an effective diameter of 0.5mm had the highest UCS values due to dense and compact formation of calcium carbonate crystals in compatible voids as compared to the other effective diameter sizes. Based on the permeability and UCS results obtained, the combination of effective diameter size 0.5 mm with 0.2 L/kg of bacterial solution after 14 days was further considered in the plate load tests.

	UCS (kN/m ²) for effective diameter							
	0.1	mm	0.5	mm	1 mm			
BC solution L/kg	7 days 14 days		7 days	14 days	7 days	14 days		
0.05	80.43	101.3	88.6	114.3	72.8	89.3		
0.1	95.8	119.6	101.3	133.3	83.3	102.7		
0.15	102.2	140.2	108.3	152.2	93.7	123.1		
0.2	118.5	157.6	134.4	180.8	98.3	136.8		
% increase	47.33	55.57	51.70	58.18	35.02	53.2		

Table 5 UCS test results.

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Figure 5 UCS vs BCC solution.

3.3 Effect on Bearing Capacity

The combination of effective diameter 0.5 mm with 0.2 L/ kg BCC solution and 14 days of curing was further considered in the plate load tests, since the maximum decrease in permeability and increase in UCS was 40.62% and 58.18% repectively. Figure 6 shows the load settlement curves for the MICP treated sand specimen using a square bearing plate. Initially, these curves are almost linear, followed by nonlinear behavior until failure. The slope of the load settlement curve shows a larger increase in stiffness of the treated specimen than in the untreated specimen. This increase in stiffness can be attributed to the effect of calcium carbonate precipitation, which binds the soil particles together and reduces space, voids in the sand mass, creating a stiffer sand matrix. The nonlinear behavior of the load-settlement curve may be attributed to the combined effect of two causes. One, the larger the plate size, the larger the deformation of the soil underneath the plate, resulting in larger settlement. The other and opposite cause is the development of deeper and larger stress bulbs on account of the increase in plate size. Confinement of the sand within these bulbs reduces the settlement. The ultimate bearing capacity calculated based on the intersection of the tangent and the corresponding ultimate settlement is shown in Table 6. BCR is the indicative measure of improvement in bearing capacity. Table 6 lists the calculated values of BCR and SRF from the load settlement curves of the treated and the untreated sand. The average BCR value for the circular and the square bearing plate was 4.64 and 4.05, respectively, indicating an increase in q_{μ} for the treated sand as compared to the untreated sand. The improvement in q_u of the treated footing was due to increased stiffness and shearing resistance. Also, the average settlement reduction percentage observed for the circular and the square bearing plate used was 53.76 and 34.9, respectively, indicating an increase in stiffness.



Load - Settlement curve for 120 mm x 120 mm Footing

Figure 6 Load-settlement curves for untreated and treated footing.

Table 6 Summary of BCR and SRF.

Size and shape	Area (sq.mm)	Ultimate bearing capacity and settlement				BCR =	SRF =	Settlement	
of footing (mm)		Untreated		Treated		an/a'n	δ΄μ/δμ	reduction	
or 1000mg ()		$\mathbf{q}_{\mathbf{u}}$	δ_{u}	q ´u	δ'u(mm)	4		(%)	
		(kPa)	(mm)	(kPa)	u(iiiii)				
50 diameter	1963.5	19	13.5	93	7.1	4.89	0.526	53 76	
100 diameter	7853.98	42	9.2	133	5.4	3.16	0.587	55.70	
120 diameter	11309.73	29	7.2	170	3.6	5.86	0.5	average	
50 x 50	2500	25	17.4	111.5	7.3	4.46	0.42	24.0	
100 x 100	10000	49	16.2	166	5.3	3.38	0.327	54.9	
120 x 120	14400	41	14.6	177	4.4	4.31	0.3	average	

3.4 Effect of Size and Shape on Ultimate Bearing Capacity (Qu) & Settlement

From Table 6 it can be seen that the ultimate bearing capacity (q_u) increased with an increase in plate size. This increase in q_u was higher for the rectangular plate in comparison with the circular plate, as can be seen from Figure 7. Further, for the same load intensity, the settlement decreased with an increase in plate size. The higher the plate size, the lower the settlement. Also, for the same settlement, the load increased with an increase in plate size. An experimental investigation was carried out to study the effect of shape (circular, square and rectangle) of the footing on its bearing pressure, a plate load test was carried out on dry river sand with two different relative densities (60% and 80%) to obtain the relation between load and settlement. It was found that the bearing pressure varied as the L/B ratio increased. The results revealed that the shape of the footing had a significant effect on the bearing pressure and settlement characteristics. From the above discussion, the results obtained were in line with [40]. It was validated that the square plate performed better in comparison to the circular plate in terms of enhancement in bearing capacity, BCR and SRF. Hence, the authors suggest that a square plate is preferable over a circular plate in PLT.



Figure 7 Value of q_u versus size of footing.

4 Conclusions

This study investigated two different parameters, i.e. bacterial content and curing period, for the enhancement of UCS, bearing capacity and reduction of permeability of sand using MICP treatment. Based on the experimental investigations carried out in the laboratory, the following conclusions were drawn.

The sand reinforced with bacterial content showed variation in UCS and permeability with respect to D10 effective size, curing period, and concentration of BCC solution. The permeability of the sand was reduced with the addition of microorganisms to the sand. This may be due to filling of the pores and voids by calcite and the covering of particles during the cementation process. The intrusion of microorganisms results in increasing the UCS with an increase of its content. These microorganisms may form a rigid crust on the surface of the sand and bind the sand particles. This results in the formation of a compact mass of sand with increased strength of the sand.

For a D10 effective diameter of 0.1 mm, 0.5 mm and 1 mm, with 0.2L/kg BCC and 14 days of curing, the permeability reduction was 17.31%, 40.62% and 6.82%, respectively, as compared to pristine sand. Also, the increase in UCS was 55.57%, 58.18% and 53.2% respectively as compared to UCS and 7 days of

curing with 0.05L/kg of BCC. The effective size of the sand affects the UCS and permeability of microorganism reinforced sand. Among 0.1 mm, 0.5 mm and 1 mm effective diameter, 0.5 mm was found to be the most effective in terms of reduction of permeability and increase of strength.

The ultimate bearing capacity of a footing of MICP treated sand using a circular plate was found to increase by 2.95 to 5.8 times and for a square plate 3.71 to 4.22 times compared to a footing of untreated sand. The reduction in settlement for a circular plate was found to be 1.7 to 2 times and 2.38 to 3.31 times for a square plate on treated sand as compared to untreated sand. The ultimate bearing capacity (q_u) increased with an increase in plate size. For the same load intensity, the settlement decreased with an increase in plate size. The preferred shape of the bearing plate is a square over a circular plate based on the PLT.

Declarations

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