Engineering properties of bacterially induced calcite formations

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This article presents the engineering properties of bacterially induced calcite formations, which are often referred to as microbially induced calcite precipitation (MICP) via ureolysis on granular formations consisting of loose and collapsible river sand. Two sets of experiments consisting of five sand columns each were treated using urease-producing bacteria, urea and calcium chloride solutions. The reaction produced biomineralized calcium carbonate crystals (referred to as calcite) that bind and stiffen the sand grains. The reaction was checked by measuring the pH level. The pH values of the effluent solution taken from the initial stage to day 14 of the treatment ranged from 7 to 8. When the pH reading was in alkaline range (<7), there was significant calcite formation. The strength gained in the treated specimens was estimated from the unconfined compression tests and obtained in the range 1.1–2.18 MPa based on 4.0–8.0% calcite content at different reaction times. Calcite formation within the biocemented sand was ascertained from scanning electron microscopic images. The grain-size distribution of the untreated and treated formations was compared. It was observed that the increase in grain size of treated formation was a function of MICP. The collapse potential of the formation reduced as a result of bacterially induced calcite precipitation. The strength of the bacterially induced calcite formations was comparable to soft rocks.

Keywords: Bacteria, biocementation, calcite precipitation, soil treatment.

CALCITE is a metabolic by-product of the biogeochemical reaction that results when bacteria release urease enzyme which in turn reacts with cementation reagents resulting in the formation of precipitation which is referred to as microbially induced calcite precipitation (MICP). Urease is an enzyme that catalyses the hydrolysis of urea. Compared to the traditional ground improvement techniques, biocementation through the MICP process is an innovative, environmental-friendly and cost-effective technique for improving the engineering properties of soil.

During the MICP process, bacteria release urease enzymes which can hydrolyse urea to ammonium and carbonate ions in the presence of water. The carbonate ion reacts with the calcium ions from $CaCl_2$ to produce insoluble calcite grains ($CaCO_3$) that bind the sand particles together, which in turn improves soil strength in loose sandy soil formations[1–11]. This binding process is referred to as biocementation, which in turn increases the density of the soil. This helps in reducing the pore water pressure by resisting liquefaction, as well as reduction in post-shaking settlements during an earthquake[12]. Additionally, it is an effort made to achieve an economical and environmental-friendly method of soil treatment using fewer reagent concentrations.

The present study aims to improve the engineering properties of loose sandy soil by increasing calcite content through the biocementation process. The following parameters were considered for the two sets of experiments conducted: (a) Concentration of the bacterial solution and cementation reagent solution, (b) Mode of application of the solution, (c) Duration of treatment and (d) Effect of pH and reaction times.

The MICP-treated specimens were tested for unconfined compressive strength (UCS) in order to evaluate the strength gained. Scanning electron microscope (SEM) images of the treated specimens were obtained, and the distribution pattern and engineering behaviour of the treated soil with calcite formation were analysed.

Materials and methods

Materials

The sand used for preparation of the sand columns for MICP treatment was collected from the Yamuna river basin, New Delhi, India. The grain-size analysis was conducted according to IS: 2720-Part IV (ref. 13). The clean sand was sieved through sieve sizes that were grouped in three gradations as 0.425–1.18 mm (~3%), 0.150–0.425 mm (~77%) and ≤0.15 mm (~20%) respectively (Figure 1). Table 1 presents the precise description of soil gradation used in the present study compared to standard material. According to IS:383 (ref. 14), the sand used in the present study conforms to grading zone IV. Almost 97% of the sand is within the ideal size range 0.050–0.400 mm (ref. 15) and is preferred for free bacterial movement as reported by Maier *et al.*[16].

As shown in Table 1, the observed values of coefficient of uniformity (C_u) = 1.33 and coefficient of curvature

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Table 1. Physical indices of various river sands used for microbially induced calcite precipitation (MICP) treatment

River sand	D_{60} (mm)	D_{50} (mm)	D_{30} (mm)	D_{10} (mm)	C_u	C_c	G_s	Reference
Ottawa river (Canada)	–	0.12	–	–	1.6	0.8	2.65	5
Snake river (United States)	0.26	–	0.18	0.11	2.4	–	–	12
Atlas river (Canada)	0.37	–	0.28	0.20	1.9	–	–	12
Ottawa	–	0.22	–	–	1.4	0.9	2.65	38
Ottawa	–	0.46	–	0.30	–	–	–	29
Mississippi river (United States)	–	0.33	–	0.20	–	–	–	29
Yamuna river (India)	0.28	0.26	0.21	0.15	1.3	1.05	2.65	Present work

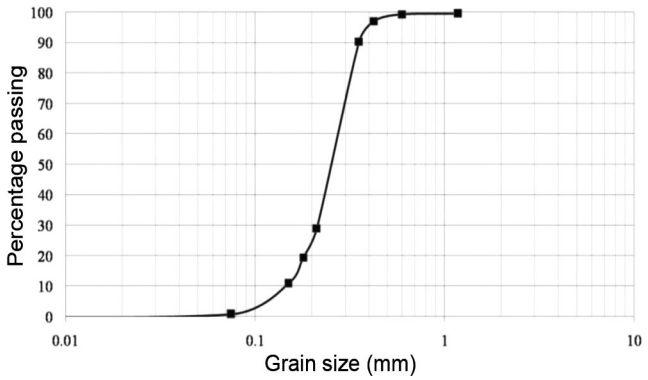

Figure 1. Grain-size distribution curve of the Yamuna sand specimen.

(C_c) = 1.05 for the selected sand, and hence, it is classified as poorly graded sand (SP) according to Unified Soil Classification System (USCS)[17]. Hence, such sand was selected as a desirable target for MICP treatment.

Sand-column preparation

Two experimental set-ups consisting of five sand columns were used, each packed with 400 g of sterilized sand in polyvinyl chloride (PVC) columns with an internal diameter of 55 mm and height 150 mm. These were used as moulds to prepare treated columns of the soil for the MICP process. The sand grains were sterilized by autoclaving at 121°C for 20 min, and then stored in an oven at 100° ± 5°C for 48 h. The initial column height and average density of the specimens were 120 mm and 14.04 kN m^{-3} respectively. Seating weights were successively applied on top of each specimen to give incremental pressure of 7, 14, 28 and 56 kPa. After application of initial pressure, the average sand column height reduced to 110 mm and the density increased to 15.30 kN m^{-3}. After three days of treatment, the final average height of the specimens was 100 mm and the measured average density was 16.84 kN m^{-3} with the added weight of the calcium carbonate. From thereon, subsequently there was no significant change in height.

Bacteria and bacterial solution

For experimental analysis, microbial strain *Sporosarcina pasteurii* (MTCC 1761) collected from the microbial type culture collection and gene bank (MTCC), Chandigarh, India was used[18]. It was formerly known as *Bacillus pasteurii*[19]. It is a common alkalophilic, gram-positive, nonpathogenic, aerobic soil bacterium found abundantly in natural soil. These bacteria have rod-shaped cells (approximately 1 μm in diameter and 0.5–3.0 μm in length)[1]. They are geometrically compatible in size and allow bacterially induced calcite formations. This bacterium has the capacity to produce the enzyme urease (urea amidohydrolase: EC:3.5.1.5) which hydrolyses urea during the MICP treatment producing calcite formations, as reported by Ferris *et al.*[20]. With the formation of extracellular calcite precipitation, the size of the microbe increases. This hinders the mobilization of calcite material if it is detached from the particle matrix[6]. A microenvironment may prevail with different pH concentration, dissolved inorganic carbon (DIC) and Ca^{2+}. All bacterial cells are electronegative in nature and capable of inducing carbonate precipitation by adsorbing the Ca^{2+} ions present in the soil to produce $CaCO_3$ (calcite)[21]. This acts as a crystal nucleation site and encapsulates the precipitate surrounding the cell wall on microscale[22]. The production of calcium carbonate crystals by soil bacteria is a natural phenomenon as in formation of caves[20]. The encapsulation of bacteria results in their death and rapid reduction of active bacteria having 'active' precipitation linked to ion transport (specifically Ca^{2+}) across cellular membranes[23–25].

Preparation of bacterial solution

The *S. pasteurii* (MTCC 1761) cells obtained in dried form were grown in nutrient broth (NB), which is an enrichment food for bacterial growth. Table 2 gives the composition of the bacterial solution (BS) used for MICP treatment in various studies.

To obtain sufficient cell concentration, the bacterial strain was grown in 3 g of NB, 10 g of NH_4Cl and 2.12 g of $NaHCO_3$ per litre of deionized water. The test liquid was sterilized by autoclaving at 121°C for 20 min after

Table 2. Composition of the bacterial solution used for MICP treatment

Chemical doses (g l^{-1})					
Urea	$CaCl_2$	Nutrient broth	NH_4Cl	$NaHCO_3$	Reference
11.2	27.6	4.5	3.8	1.60	9
20	3.7	3.0	10	2.12	45
20	3.3	3.0	10	2.12	34
20	1.4, 2.8, 5.6	3.0	10	2.12	46
20	11.25	3.0	10	2.12	Present work

Table 3. Composition of cementation reagent solution (CRS; urea and $CaCl_2$; g l^{-1}) used in the MICP treatment process

Urea	$CaCl_2$	Biochemical reaction of CRS with bacteria
30	73.50	At 0.5 M CRS during initial 8–24 h (stage II) in set I
45	110.20	At 0.75 M CRS during initial 8–24 h (stage II) in set II
15	37	At 0.25 M CRS from 24 h (stage III) over reaction times of 3, 7, 14, 21 and 28 days treated specimens in each set

adjusting the pH at 6.5 using 1 N HCl. After being autoclaved, the test liquid was cooled to 35°C. The liquid medium was mixed with bacterial cells for growth and then 20 g l^{-1} urea was added to initiate urea hydrolysis by urease enzyme released by the bacteria. The resulting test liquid was transferred to 250 ml Erlenmeyer flasks for incubation at 35°C on horizontal shakers at 120 rpm for 48 h. The test liquid without cells was stored in a test tube and incubated in parallel to avoid contamination. The test liquid medium containing incubated *S. pasteurii* cells is referred to as the bacterial solution. The BS was harvested to a cell concentration in the range 0.7–1.0 at a wavelength of 600 nm, referred to as OD_{600} (approximately estimated as 1×10^7 cells ml^{-1}). The BS was stored at 4°C. The urease activity of the culture was observed in the range 10–15 μM urea min^{-1}. Immediately before injection, 11.25 g l^{-1} of $CaCl_2$ was mixed thoroughly with BS to enhance the aggregation of bacterial cells and allow percolation through the soil matrix.

Preparation of cementation reagent solution

The urea–Ca^{2+} molar ratio of the cementation reagent solution (CRS) was fixed at 1 : 1. The reagents were added by 0.22 filter sterilization. Table 3 summarizes the MICP treatment process using CRS from a higher concentration (0.5 M in set I and 0.75 M in set II specimens during 8–24 h) to a lower concentration (0.25 M in all specimens after 24 h) based on the efficacy of the biochemical reaction.

Microbially induced calcite precipitation

During the reaction, urease enzymes released by the bacteria hydrolyse urea into ammonium and carbonate ions in the presence of water. The carbonate ions, in turn, react with Ca^{2+} from $CaCl_2$ in the solution to form $CaCO_3$ (refs 26 and 27) and the bacterial cells serve as nucleation sites in growing carbonate crystals at the cell surface[23]

$$\mathrm{Ca}^{2+} + \mathrm{cell} \rightarrow \mathrm{cell} - \mathrm{Ca}^{2+}, \quad (1)$$

$$\mathrm{Cell} - \mathrm{Ca}^{2+} + \mathrm{CO}_3^{2-} \rightarrow \mathrm{cell} - \mathrm{CaCO}_3. \quad (2)$$

The negatively charged bacteria, which are carbonaceous in nature pull Ca^{2+} ions present in the pore fluid to deposit on their cell surfaces (eq. (1)). The bacterial cells, in turn, serve as the nucleation sites to precipitate $CaCO_3$ (eq. (2))[6].

Soil treatment procedures

The specimens were treated in three stages at room temperature (25° ± 2°C). During stage I, 50 ml of the BS (1×10^7 cells ml^{-1}) was added and retained for 8 h to adsorb the cells in the sand particles. During stage II (8–24 h) and stage III (after 24 h), 150 ml of CRS was added at an interval of 4 h to allow the bacterial cells to promote calcium carbonate precipitation. During stage II, 0.50 M CRS in set I and 0.75 M in set II were added to saturate the specimens. After 24 h of treatment (stage III), 0.25 M CRS was added and one specimen each was kept for 3, 7, 14, 21 and 28 days respectively. The specimens were kept submerged in 0.25 M CRS. Figure 2 shows the flow chart of the MICP treatment process.

CRS was applied by gravity flow to percolate through the sand pores with Ca^{2+} ions that allow reaction with the carbonates to induce $CaCO_3$ precipitation by urease activity. The pH of the effluent solutions was measured at the interval of 4 h. The evidence of active hydrolysis is shown by pH value ≥7.

After 14 days, pH began to drop, and the environment became unsuitable for bacterial survival and hence the

reaction slowed down. It continued to drop in the 21 and 28 days treated specimens. To check continuation of the reaction, a fresh dose of BS (50 ml) was applied followed by CRS of 0.25 M in the 21 and 28 days treated specimens, but pH continued to drop below 7, thus showing discontinuity of the reaction. The MICP-treated specimens were oven-dried for compressive strength and accumulated calcite content test.

Determination of unconfined compressive strength

The unconfined compressive strength (UCS) test was carried out in the specimens treated over 14, 21 and 28 days according to IS:4332 (Part V)[28], under strain-controlled conditions at a uniform loading rate of 1.0 mm min^{-1}.

Determination of change in grain size due to biocementation

The increase in grain size of treated sand due to biocementation was determined by wet sieving according to IS:2720 (Part IV)[13]. The grain-size distribution curves of the specimens before and after treatment were used to examine the increase in grain size due to biocementation.

Determination of calcium carbonate content

To determine calcium carbonate content, 100 g each of the treated specimen was washed in HCl solution using 0.1 N. The calcium carbonate dissolved in acid wash solution was rinsed and drained from the soil through a #200 sieve (75 μm sieve) and oven-dried. The difference in mass of the oven-dried specimen before and after the acid wash was determined to estimate the mass of calcite[2,15,29].

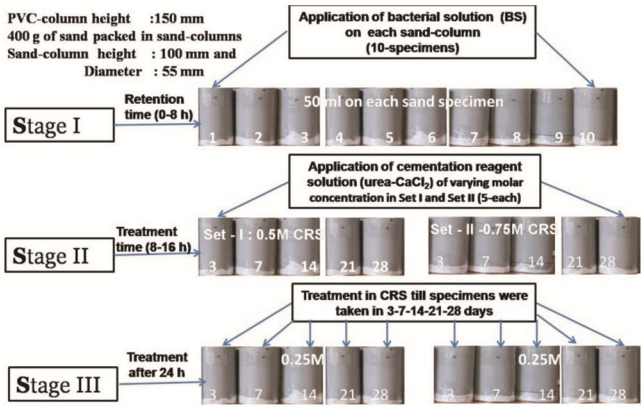

Figure 2. The microbially induced calcite precipitation treatment process using bacterial solution and cementation reagent solution (CRS) and specimens incubated for 3, 7, 14, 21 and 28 days.

Scanning electron microscopy

The selected specimens were oven-dried at 60°C for 1 h followed by sputter-coating with silver (Agar Sputter Coater). Images were obtained for the prepared specimens using SEM Zeiss, EVO 18 Research) to obtain the distribution pattern and behaviour of the calcite crystals.

Results and discussion

Effect of pH on MICP

During the MICP process, *S. pasteurii* produce a urease enzyme that hydrolyses urea into ammonium and hydroxide ion. The formation of hydroxide ion increases the pH and can shift the bicarbonate equilibrium and form carbonate ions. Moreover, increase in pH is due to the breakdown of complex proteins in the stationary growth phase[30]. The carbonate ions, in turn, react with free calcium ions present in the soil from $CaCl_2$ and precipitate as calcium carbonate crystals (calcite) in the soil. On the other hand, bicarbonates from urea hydrolysis and microbial respiration (release of CO_2) and lack of oxygen inhibit the increase in pH, thereby, the ureolytic activity of the bacteria decreases in the MICP process[30,31]. The carbonates react with Ca^{2+} from $CaCl_2$ to induce $CaCO_3$ as a binding material.

In stage II (8–24 h), pH value was fluctuated between 7.6 and 8.0, and remained at 7.8 in set II (0.75 M CRS) specimens. The pH level indicated that higher urease activity occurred at lower concentrations, i.e. 0.5 M CRS. Higher concentration does not favour the reaction, therefore, addition of chemicals would be a waste. From the initial stage to day-14, the pH was found to be varied between 7.0 and 8.0. For *S. pasteurii*, optimum pH was in the range 7.5–8 (ref. 30). From day-15 onwards, the pH dropped between 6.6 and 6.9 in the 21 and 28 days treated specimens. Urease activity reached its peak at pH 8.0 and began to decline at higher pH. During MICP treatment, the measured pH levels in all the specimens were in the optimum range (6.0–8.0)[31,32] required for microbial growth[16]. Rebata-Landa[15] also obtained a similar result of drop the bacterial activity between 16 and 32 days. This inhibited the urease activity after 120 h because of the release of bacterial protease enzymes with increase in treatment time[33]. Table 4 shows the pH levels measured during MICP treatment by various studies.

Table 4. pH levels during specimen treatment

Microorganism	pH	Reference
Sporosarcina pasteurii	9.5	46
S. pasteurii	8.7–9.5	47
S. pasteurii	9.3	48
S. pasteurii	9.1	49
S. pasteurii	7–8	Present work

Table 5. Percentage change in grain sizes of the sand after treatment

		Percentage change in grain sizes of the sand after treatment									
		Treated sand specimens (%)									
Gradation	Untreated sand (%)	S_3^1	S_7^1	S_{14}^1	S_{21}^1	S_{28}^1	S_3^2	S_7^2	S_{14}^2	S_{21}^2	S_{28}^2
First	3	9.7	13.2	12.8	10.8	9.4	10.7	12.2	13.0	9.5	9.9
Second	77	89	85.4	78.9	78.6	79.2	88	84.5	79.8	80.3	79.3
Third	20	1.3	1.4	8.3	10.6	11.4	1.3	3.3	7.2	10.2	10.8

The 3-, 7-, 14-, 21- and 28-day specimens are denoted by S_3^1, S_7^1, S_{14}^1, S_{21}^1 and S_{28}^1 for the set I and S_3^2, S_7^2, S_{14}^2, S_{21}^2 and S_{28}^2 for set II specimens respectively.

Effect of cementation solution on MICP

Treatment solutions were applied in three stages. In stage I (0–8 h), 50 ml BS was added and retained for 8 h in all specimens to percolate the bacterial cells within the sand grains. In stage II (8–24 h), 150 ml of 0.5 M CRS was added in set I and 0.75 M CRS in set II specimens for every 4 h to enhance bacterial activity. In stage III (after 24 h), a lower CRS of 0.25 M was added and the specimens were incubated for 3, 7, 14, 21 and 28 days to allow continuation of biochemical reaction to promote carbonate precipitation. It is most effective to use higher reagent concentration during 8–24 h of treatment[34]. The present study aimed to achieve significant soil strength using higher reagent concentration (0.5 M and 0.75 M CRS) during the initial 8–24 h, followed by lower concentration (0.25 M CRS) in subsequent treatments. Thus, efforts were made to reduce the cost of urea–$CaCl_2$, minimize wastage of chemicals, reduce harmful effects of chlorine in concrete or soil using this process. High reagent concentration between 0.5 and 1.0 M of CRS can precipitate higher calcite content, but the efficiency may be low[35,36]. Further, the efficiency of calcite formation in 0.5 M cementation solution was almost half of that in 0.25 M solution[37]. In this study, in stage I only BS was added, while higher CRS was used in stage II (0.5 M in set I and 0.75 M CRS in set II) and lower CRS in stage III (0.25 M CRS). The pH levels remained high in stage III till day-14 and thereafter began to decline. It can be concluded that sufficient urease activity can be achieved in lower concentrations.

Percentage change in grain size due to in situ biocementation

The oven-dried sand grains after wet sieving were sieved for grain-size analysis according to IS:2720-Part IV (ref. 13). The percentage fractions retained on each sieve were grouped in three gradations as was done for untreated sands. From Table 5, it can be observed that there is an increased in the percentage fractions retained in the first (0.425–1.180 mm) and second (0.150–0.425 mm) grain-size gradations. However, for the third gradation (≤ 0.150 mm), there is a decrease in the percentage of finer fractions. This is due to growth in grain size through biocementation. It is observed that the sand grains are coated with calcium carbonate, which also binds them together by bridging across several sand grains, thus forming coarser sand aggregates. These binding effects contribute towards the increase in compressive strength. The increase in grain size can be observed from Figure 3 *a* and *b* showing the grain-size distribution curves of untreated and treated sand specimens of set I and set II respectively.

Unconfined compressive strength

In soil treatment using cement grouting, sufficient strength is achieved after 28 days of curing, but in bacterially induced calcite precipitation formations it takes lesser time. Figure 4 shows the stress–strain plots of the 14, 21 and 28 days treated specimens. Feng and Montoya[38] found that UCS of the treatment duration of 3–5 days was more than that of 5–7 days. The curves show a gradual rise in axial stress–strain before reaching sharp peaks ranging from 1.1 to 1.44 MPa in set I and 1.1 to 2.18 MPa in set II specimens at a relatively low axial strains ranging from 1.5% to 2.5%, which were followed by sharp declines[6]. This range of peak strength may be classified as soft rock. The axial stress drops quickly after attaining the peak stress due to failure of the specimen on axial loading resulting from the degradation of cementation[44]. The mechanism of cementation and damage amid the grain formations may be captured by a numerical hardening–softening process active among the rock masses, as proposed by Trivedi[39–41].

From Table 6, it can be observed that UCS of the specimens treated at different reaction times varies from 1.1 to 2.18 MPa for 4.0%–8.0% of calcite content (~3 MPa for normal application, according to Mitchell and Santamarina[10]). From Table 6, it can also be observed that the compressive strength and calcite content do not necessarily continue to increase beyond median curing of 14 days[42,43]. The gain at higher concentrations (0.75 M in stage II–0.25 M in stage III) is almost two times that of lower concentrations (0.5 M in stage II–0.25 M in stage III) having calcite content 8% and 6% respectively. The

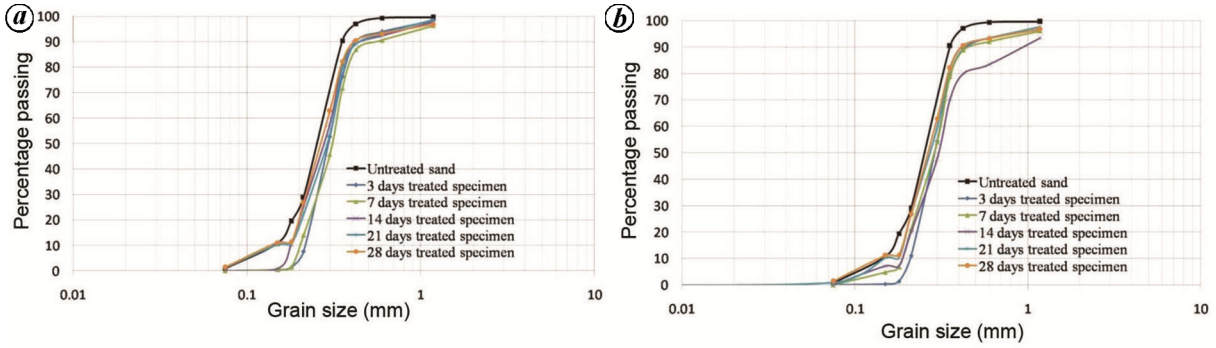

Figure 3. Percentage change in grain size from the untreated to treated specimens over 3, 7, 14, 21 and 28 days in (***a***) set I, 0.5–0.25 M and (***b***) set II, 0.75–0.25 M.

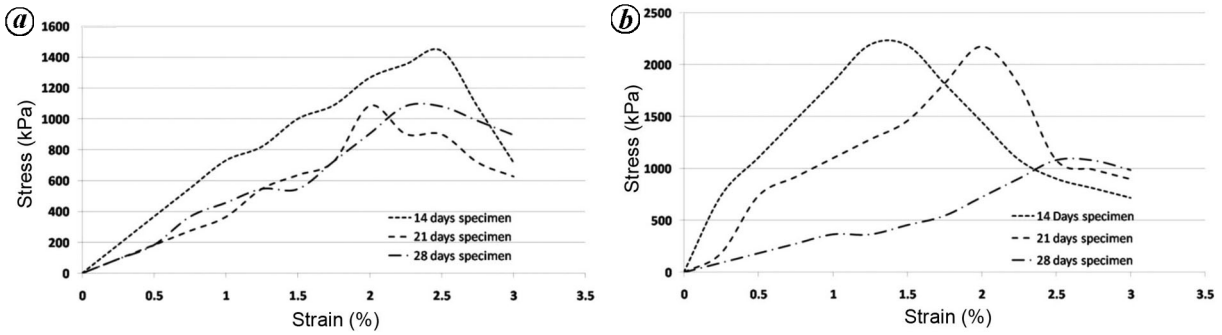

Figure 4. Stress–strain curves of 14, 21 and 28 days treated specimens in (***a***) 0.5–0.25 M, and (***b***) 0.75–0.25 M of CRS, i.e. from stage II (0.5 M in set I and 0.75 M in set II) to stage III (0.25 M).

Table 6. Compressive strength and $CaCO_3$ (%) content of the treated specimens

Molarity of CRS	Reaction (days)	Axial strain (%)	UCS (MPa)	$CaCO_3$ (%)
0.5–0.25 M	3	–	–	2.0
	7	–	–	3.2
	14	2.5	1.44	6.0
	21	2.0	1.10	4.0
	28	2.3	1.10	4.5
0.75–0.25 M	3	–	–	2.3
	7	–	–	3.3
	14	1.5	2.18	8.0
	21	2.0	2.17	7.5
	28	2.5	1.10	4.3

calcite content and strength in 21 and 28 days treated specimens decrease due to washing away of detached calcite particles as treatment duration increases. Hence, treatment beyond 14 days does not provide further additive strength or calcite content.

Figure 5 *a–c* shows the 14 days treated specimens before and during UCS tests. The heavily cemented parts are seen in the upper and lower portions of the specimens and lesser in the middle. The brittleness and decementation of MICP treated specimens lie approximately between 30 and 70 mm depth[29]. Decementation of the specimen initiates radially inwards and around the middle portion. The gained compressive strength was 1.1 MPa at approximately 4.5% calcite content in 21 days (set I) and 28 days (set II) treated specimens. It implies that an equal calcite formation is induced in the biocementation process of the specimens. In the case of 21 days treated specimen (set II), the compressive strength (2.17 MPa) and calcite content (7.5%) were almost twice that of the 28 days treated specimens. Thus strength is a function of the bacterially induced calcite precipitation formations (Table 6).

Analysis of scanning electron microscope images

SEM images for three selected specimens after drying at 60°C validated the presence of calcite formations. Furthermore, they showed the pattern and distribution of calcite on the surface and between the sand grains. The

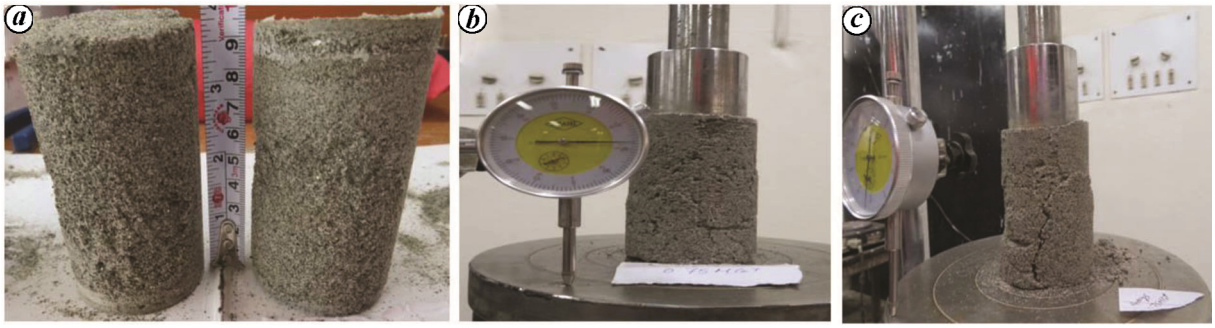

Figure 5. ***a***, Treated sand columns. ***b***, Axial loading on the specimen. ***c***, Specimen after failure.

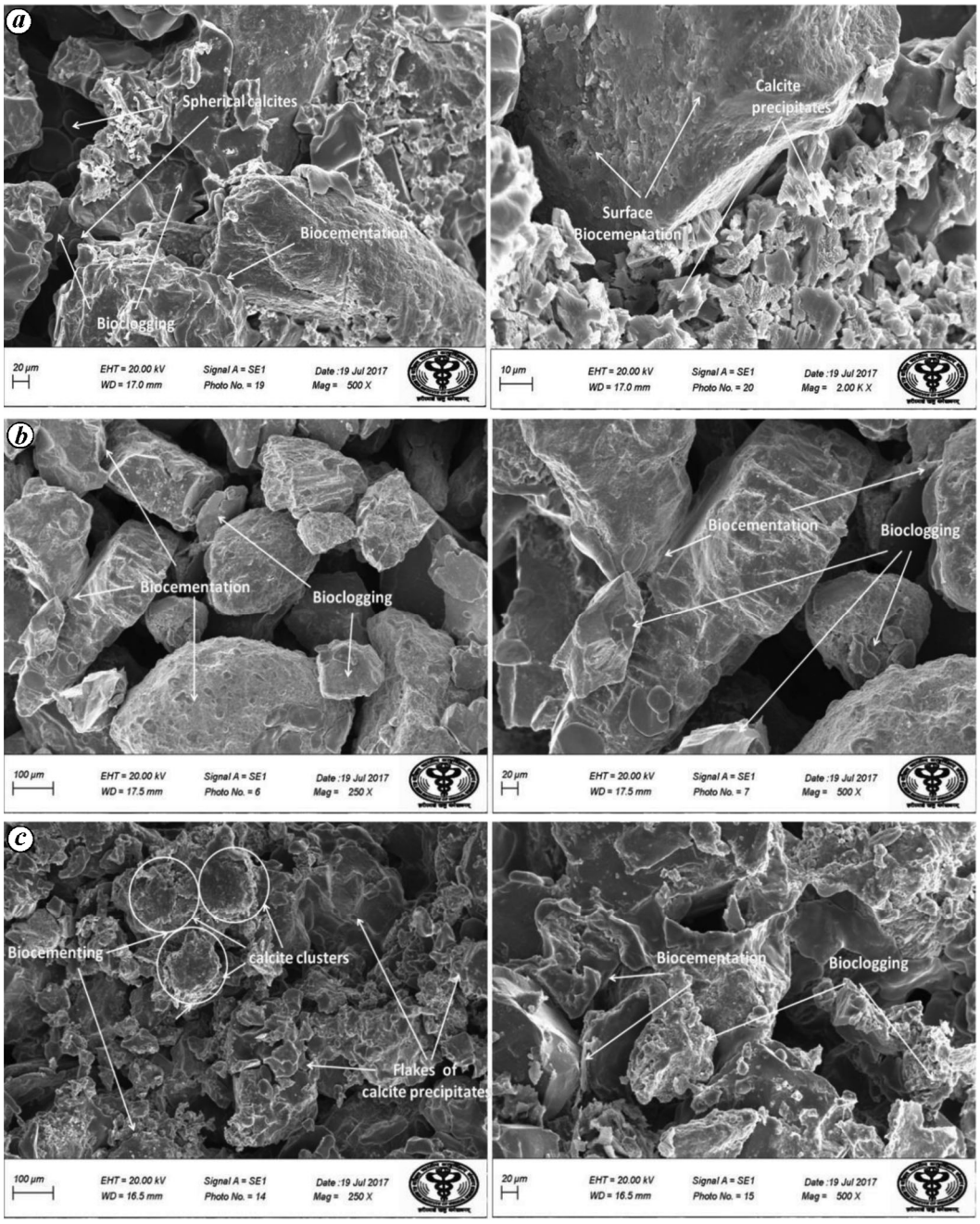

Figure 6. Scanning electron microscope images of treated specimens: (***a***) 28 days of set I, 0.5–0.25 M, (***b***) 28 days of set II and (***c***) 21 days of set II, 0.75–0.25 M.

calcite formation showed lighter grey shades with no regular structure. Figure 6 *a–c* shows SEM images of 28 days treated specimens of set I (0.5–0.25 M), 21 days and 28 days treated specimens of set II (0.75–0.25 M) respectively. The calcite formations show flaky structures scattered in layers and clusters with rough grain structure viewed at different magnifications. The calcite precipitate bridges the sand grains together and stiffens the joints during the biocementation process, in-filling the pores of the sand matrix during the bio-clogging process. Thus, biocementation increases soil strength and bio-clogging reduces the pore pressure.

Conclusion

The following conclusions can be made from the present study:

- Biocementation has been induced in loose and collapsible soil using urease enzymes produced by ureolytic bacteria (*S. pasteurii*). Urease enzymes enhance the formation of calcites that accumulate in clusters on the bacteria and bring about biocementation and bio-clogging of the pores of the sand matrix.
- Higher compressive strength and calcite content have been obtained in the specimens cured for 14 days. A higher concentration of reagents (0.5 M in set I and 0.75 M in set II) in the first 8–24 h followed by lower concentration (0.25 M) in the following days has been optimized for maximum calcite formation. The judicious use of reagent concentration has been found economical and to reduce the hazardous effects of chemicals on the environment.
- The cementation level has been achieved up to 8%. The cementation level of above 1.0% (15 kg m^{-3}) can provide measurable improvements in shear strength and hydraulic conductivity of residual soil[34].
- The calcite grain formation has been found to reduce the pore throat size, causing a reduction in pore pressure and hence hydraulic conductivity of the soil. This method would reduce liquefaction and settlement problems of structures constructed in loose sandy soils.

There have been many challenges in the field application of MICP, as it may be difficult to control the *in situ* microbial and chemical interactions to form calcite precipitation at the desirable levels. The soil structure depends on the interaction between minerals and organic matter present in it. The soil also provides a spatially heterogeneous habitat for microorganisms with variable needs of the substrate, nutrients, oxygen concentration and pH levels, thus affecting bacterial diversity and structure[44]. Thus, the achievable cementation level, uniformity, durability and other engineering properties in the sand formations are difficult but not unpredictable. In future, this technique would be helpful in solving geotechnical problems such as, to reduce the liquefaction potential in sand formations, reduce the swelling potential of clayey silt, mitigate wind erosion potential of loose sand deposits, pre-treatment of the subsurface to reduce settlement of highway structures, and in slope stabilization.

Despite the challenges faced in field applications at present, this method has tremendous potential in the future. With further studies, this soil treatment technique may be a solution to many geotechnical problems. This method would serve as a cost-effective and environmental-friendly approach to improving the engineering properties of silty soils.

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