

IMPROVING THE STRENGTH OF SAND BY MICCP PROCESS USING SEASHELL

Sanjoli Gupta^{1*}, Bindhu Lal², and Shashwati Ghosh Sachan³

ABSTRACT

Microbially Induced Calcium Carbonate Precipitation (MICCP) is a ground improvement technique in which bio-cement is formed using ureolytic bacteria. The MICCP process improves the shear strength and stiffness of sand. Calcium chloride or other calcium salts are used as a calcium source to carry out this process. In the present study, instead of calcium chloride, seashell was used as a calcium source for biocementation. The seashell is naturally available and consists of up to 98% calcium carbonate. Soluble calcium was obtained from the seashell by dissolving the seashells in diluted glacial acetic acid (5% acidity). As the concentration of acetic acid is low, it does not cause any environmental effect as it degrades rapidly into a harmless substance in the environment. Unconfined compressive strength test and permeability test were performed on MICCP treated sand using *Bacillus subtilis* as a urease-producing bacteria. The comparison was made between the sand treated with soluble calcium obtained from seashell and sand treated with calcium chloride, keeping the calcium concentration the same in both cases. Results show that seashell can also be used instead of calcium chloride as a calcium source, as it gave good results. The calcium carbonate content measurement test was also performed on the treated sand. The micro-level investigation was done by Scanning Electron Microscope analysis.

Key words: Seashell, microbially induced calcium carbonate precipitation (MICCP), unconfined compressive strength (UCS), permeability, scanning electron microscope.

1. INTRODUCTION

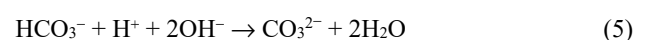
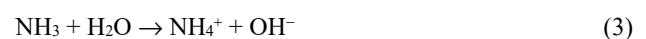
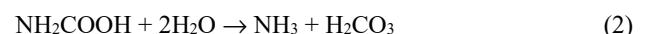
A sustainable and green soil improvement technique, termed as microbially induced calcium carbonate precipitation (MICCP) process, has been developed in recent years. This technique is based on the bio-geochemical process to improve the engineering properties of soil (Whiffin *et al.* 2007; Chu *et al.* 2012; Feng *et al.* 2015; Ivanov *et al.* 2015; Li *et al.* 2015; Cheng *et al.* 2016; Liu *et al.* 2017). Researchers have envisioned a wide range of applications for the microbial cementation process. For example, as a protective treatment for ornamental stone to self-healing cementitious materials, strength enhancement in soil strata, geo-environmental remediation mechanisms for contaminated soil and groundwater, earthquake-induced liquefaction hazard mitigation of soils, optimized oil recovery, industrial wastewater treatment, bearing capacity improvement of high plasticity clays, prevention of desertification by surface stabilization of erodible soils.

There are many biological processes that can lead to MICCP, like cementation by urease-producing bacteria (UPB), cementation by iron hydroxides using iron-reducing bacteria (IRB), hypothetical cementation by ferrous sulfide using sulfate-reducing bacteria (SRB), and cementation by denitrifying bacteria. How-

ever, out of all these processes, cementation by urease-producing bacteria is gaining more interest. Van Paassen *et al.* (2010) suggested that the urea hydrolysis process possesses the highest calcium carbonate conversion compared to other processes.

In this technique, the chemicals (urea and calcium source) and microbes (bacteria) are injected into the in-situ soil. MICCP process forms calcium carbonate precipitate in sandy soil and provides a bridge between the sand grains, restricting their movement and improving the soil's strength and stiffness (Harkes *et al.* 2010).

Al Qabany *et al.* (2012) reported four processes involved in MICCP: urea hydrolysis, increase in pH of the environment, surface absorption of calcium ions, and crystal formation of calcium carbonate. Urea hydrolysis is an essential process in which urease-producing bacteria (UPB) break down 1 mole of urea [CO(NH₂)₂] into 2 moles of ammonium ion (NH₄⁺) and 1 mole of carbonate ion (CO₃²⁻) [Eq. (1)].



The release of ammonium ion (NH₄⁺) causes an increase in pH, and in the presence of calcium ions, from a supplied calcium source, the precipitate of calcium carbonate (CaCO₃) is formed [Eq. (6)]. The microbially induced calcium carbonate precipitation starts at a pH level of 8.3 and increases up to pH 9 (Stocks-

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Fischer *et al.* 1999). As the precipitation starts, the pH level tends to reduce and goes back to neutral (DeJong *et al.* 2006).



A by-product of urea hydrolysis is ammonia. The geotechnical engineering properties of bio-treated soils were improved due to the bonding of soil particles by calcium carbonate precipitation (DeJong *et al.* 2013). This process is highly complex, and calcium carbonate precipitation depends on various factors like the type of bacteria, pH, temperature, the concentration of calcium and urea, nutrients for bacteria growth, and injection method.

In almost all the studies, calcium chloride is used as a calcium source for the MICCP process (Chu *et al.* 2013; Soon *et al.* 2013; Krishnapriya *et al.* 2015; Sharma *et al.* 2016). However, if calcium chloride is present in an excessive amount in the soil or concrete, it can be harmful and corrosive (Pruckner *et al.* 2004; Shi *et al.* 2012). On the other hand, calcium can be extracted from waste materials such as a seashell, oyster shell, or eggshell (Chung *et al.* 2014; Koteswara Rao *et al.* 2016). India has an extensive coastline, and the procurement of seashells in large numbers from beaches is not tricky. Since it does not disintegrate when in contact with air, it can be stored for a long time. A method to obtain soluble calcium from seashell and use it for the MICCP process for biocementation of sand is proposed in this paper.

A comparative study of unconfined compressive strength and permeability of MICCP treated sand using soluble calcium produced from seashell and calcium chloride with the same concentration of calcium was carried out. The results are presented in this paper.

2. MATERIALS AND METHODS

2.1 Properties of Sand

Poorly graded sand with grain size between 0.075 mm to 1 mm was selected for the current study. The maximum dry unit weight of the sand was 16.08 kN/m³, and the minimum unit weight of sand was 13.82 kN/m³. The grain size distribution is done as per IS:2720 (Part-IV)-1985, and the curve is presented in Fig. 1. The physical properties of sand are presented in Table 1. The sand was washed with distilled water to remove all salts and dried at 105°C for two days.

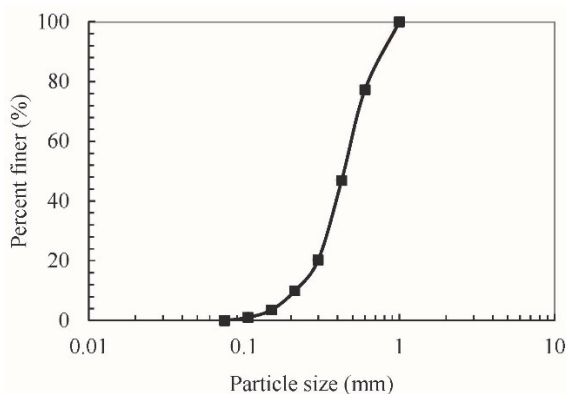


Fig. 1 Grain size distribution curve of sand

Table 1 Properties of sand

Properties	Value
Percentage of medium sand (0.425 ~ 2 mm)	46.87%
Percentage of fine sand (0.075 ~ 0.425 mm)	53.13%
D_{10}	0.21 mm
D_{30}	0.34 mm
D_{60}	0.50 mm
Coefficient of uniformity, C_u	2.35
Coefficient of curvature, C_c	1.12
Coefficient of permeability, k	3.94×10^{-5} m/sec
Specific gravity, G_s	2.6
e_{\min}	0.58
e_{\max}	0.84

2.2 Bacterial Media

The urease-producing bacteria used in the current experiments was *Bacillus subtilis* (a culture procured from Department of Bioengineering and Biotechnology, BIT Mesra, Ranchi). Christensen's Urea Agar was used to check the ability of bacteria to synthesize urea. The bacteria were grown on ATCC specified NH₄-YE media plate (Al Qabany *et al.* 2013) made of 10 gm/l ammonium sulfate (NH₄)₂SO₄, 20 gm/l of yeast extract and 20 gm/l of agar in 0.13 M Tris Buffer in pH 9. The NH₄-YE media plate was then incubated for 48 ~ 72 hours at 30°C. After the growth of bacteria on the plate, bacteria were inoculated in NH₄-YE liquid media consisting of 10 gm/l ammonium sulfate (NH₄)₂SO₄ and 20 gm/l of yeast extract in 0.13 M Tris Buffer in pH 9. All the chemicals used in preparing media and plate were autoclaved separately at 121°C for 25 minutes and mixed.

After inoculating, this bacterial liquid media culture was placed in an orbital shaker at 200 revolution per minute (rpm) at 30°C for 24 to 36 hours until the growth of bacteria was observed. The optical density measured at 600 nm wavelength (OD₆₀₀) of the culture varies from 1.0 to 1.5. The bacterial liquid media culture was then centrifuged at 4,000 rpm for 20 minutes in 25 ml volume. The bacterial cells collected down at the bottom of the centrifuge tube were washed with 0.9% NaCl and centrifuge again at 4,000 rpm for 20 minutes.

The harvested bacterial cells were then mixed with liquid media containing 10 gm/l of NH₄Cl, 2.12 gm/l of NaHCO₃, and 3 gm/l of nutrient broth as a nutrient source. This liquid media was also autoclaved at 121°C for 25 minutes before mixing with harvested bacterial cells. The UPB solution thus obtained was used in the bio-cementation of sand.

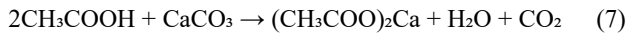
2.3 Seashell as Soluble Calcium

Seashell is mainly calcium carbonate (Hamester *et al.* 2012; Fombuena *et al.* 2014) and can only be dissolved using some acid. To dissolve seashells, glacial acetic acid diluted to 5% acidity was used. Seashells were initially washed with distilled water and placed in the oven at 105°C temperature for 24 hours. After that, seashells were crushed into powder and mixed with acid and placed in an orbital shaker for several days.

To check the effect of seashells and glacial acetic acid, they were mixed in different ratios of 1:4, 1:6, 1:8, and 1:10 by weight. The soluble calcium concentration obtained from the seashell and the glacial acetic acid solution was determined for each ratio. The concentration of soluble calcium was measured in terms of molarity. The results are shown in Fig. 2.

At different ratios of seashell-to-glacial acetic acid, different concentrations of calcium were obtained. When seashell reacts

with diluted glacial acetic acid, it forms calcium acetate, and carbon-di-oxide is evolved in the form of bubbles, which is a sign of reaction taking place (Eq. (7)).



The calcium concentrations of the soluble calcium solution made from seashell and diluted glacial acetic acid were measured by titration.

The data shown in Fig. 2 indicates that the seashell-to-glacial acetic acid ratio of 1:4 appears to produce the lowest concentration, and the ratio of 1:8 appears to produce the highest calcium concentration. From the results, it is clear that too much acid may not help in producing more soluble calcium, as the ratio of 1:10 is having less concentration of calcium than the 1:8 ratio of seashell-to-glacial acetic acid.

The highest concentration of 0.3 M was obtained on the 5th day for the ratio 1:4 of the seashell-to-glacial acetic acid. Similarly, for the ratio 1:6 of seashell-to-glacial acetic acid, the highest concentration of 0.5 M was obtained on the 7th day, and for the ratio 1:8 of the seashell-to-glacial acetic acid, the highest concentration of 1 M was obtained on the 6th day. It was also seen that the highest concentration of 0.83 M was obtained on the 4th day for the ratio 1:10 of the seashell-to-glacial acetic acid. Thus 5 days were taken as the duration for the production of calcium for the ratio of 1:4 of the seashell-to-glacial acetic acid. Similarly, the number of days is taken for other ratios.

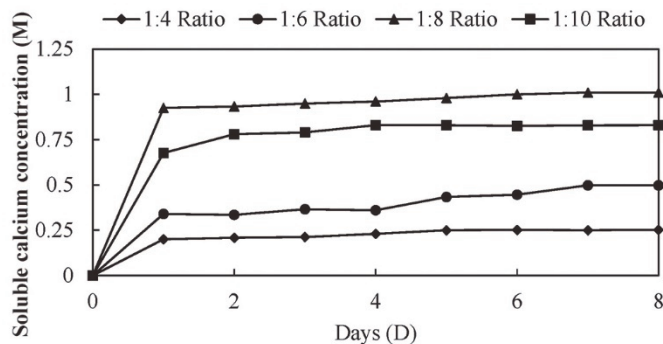


Fig. 2 Change in molarity with the ratio of seashell-to-glacial acetic acid

2.4 Sample Preparation

To prepare a sample for unconfined compressive strength (UCS) and permeability test, a PVC pipe of diameter 38 mm and height 200 mm was used. The bottom of the tube was fixed to a U-type tube, and the experimental setup is shown in Fig. 3(a), 3(b), and 3(c). The pipe was initially filled with gravels up to 20 mm height which acts as a drainage layer above which a scouring pad was placed, which acts as a filter between sand and gravel. Above this filter, dry sand was filled to a relative density of 40% up to a height of 76 mm, and then a scouring pad was again placed at the top of the sand. All the solutions were added from the top and collected at the bottom. The sand samples were flushed with water to remove any air bubbles within the sand, so that the precipitate of calcium carbonate is formed in the voids between soil grains. Then the bacterial media solution of 80 ml was poured into the sample and was left into the sample for 12 hours, so that the bacteria will attach to the sand particles.

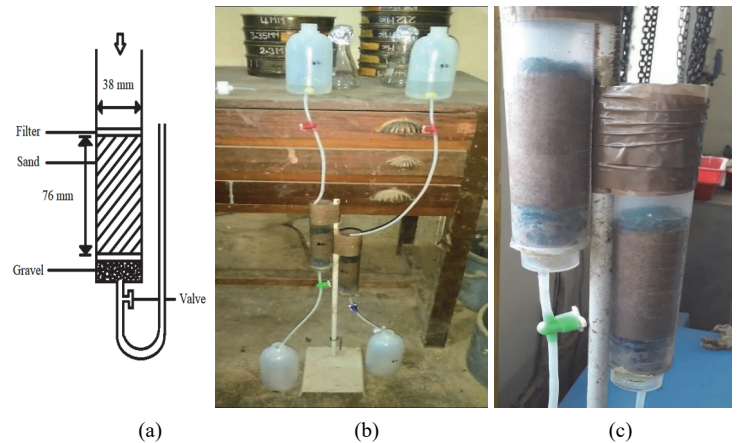


Fig. 3 Test devices: (a) systematic representation of the setup; (b) experimental setup for bio-cementation in the laboratory; (c) soil specimen mould

After adding bacterial media solution, 80 ml of cementation solution was poured into the sample, and the valve was adjusted so the solution would take 4 ~ 5 hours to drain from the bottom. The drained-out solution was collected and reapplied to the sample two times, after which the solution was left in the sample for 24 hours. For the samples to be treated using CaCl_2 , the solution of CaCl_2 and urea was added at different concentrations. Similarly, for the samples to be treated using the seashell, the solution of soluble calcium (obtained from dissolving seashell in glacial acetic acid) and urea was added at different concentrations. The concentration of urea was kept twice the concentration of soluble calcium because a significant portion of urea must be hydrolyzed to increase the pH for effective crystallization of calcium carbonate (Choi *et al.* 2016; Ivanov *et al.* 2017). The pH of the CaCl_2 -urea solutions was between 9 to 11, and the pH of the soluble calcium from seashell-urea solutions was between 8 to 10.5 before introducing them into the samples. Summary of the solutions with different concentrations for the preparation of samples using CaCl_2 and seashell is given in Table 2.

After 24 hours, the solution was drained out, and new bacterial media and the urea-calcium solution were added. To make sure the sample gets the solution uniformly, after 10 ~ 12 days of this process top scouring pad was removed because it was getting attached to the sample, and precipitate started to form on the scouring pad. After this, top of the sample was scratched in every two days so that the top of the sample was not closed by the CaCO_3 precipitate layer. The process was continued for 30 days so that the sample would gain maximum strength. In 30 days, the

Table 2 Cementation solution required for MICCP process

Method	Sample	CaCl_2 (M)	Urea (M)	Calcium from Seashell (M)	Urea (M)
Using CaCl_2	C-1	0.3	0.6	–	–
	C-2	0.5	1	–	–
	C-3	1	2	–	–
	C-4	0.83	1.66	–	–
Using Seashell	S-1	–	–	0.3	0.6
	S-2	–	–	0.5	1
	S-3	–	–	1	2
	S-4	–	–	0.83	1.66

complete cementation is done. After 30 days of treatment, the sample was cured in the natural environment for two weeks so added solution completely stopped flowing out from the bottom of the sample due to a decrease in permeability, ensuring that solution is completely dried out from the samples, and then it could be used for the permeability test. The sample was then taken out of the pipe and dried in an oven at 50°C for 24 hours to remove any left-over solution inside the sample. The MICCP treated samples are shown in Fig. 4. The diameter of the sample was 38 mm, and the height of the sample was 76 mm. The samples were irregular in shape at the top and bottom due to calcium carbonate precipitation, so all the samples were trimmed for a smooth surface to 76 mm length before the UCS test.



Fig. 4 Sand after MICCP treatment with different concentrations of soluble calcium and urea

3. TEST RESULTS

3.1 Permeability Test

The samples after biocementation were tested for permeability using the falling head method as per IS:2720 (Part XVII) - 1986 before the UCS test. Permeability test was carried out on the sand before and after treatment. The lowest permeability of calcium chloride treated samples was 1.13×10^{-6} m/sec, which is 97.13% less than the permeability of untreated sand; and the samples treated using seashell gives the lowest permeability of 1.63×10^{-6} m/sec, which is 82.15% less than the permeability of untreated sand. The reduction in permeability ranges from 76.29% to 97.13% for all the samples, and it is due to the filling of the voids of the sand particles with calcium carbonate precipitate. During an earthquake, soil liquefaction in cohesionless soils causes an increase in pore water pressure which causes settlement of buildings and structures above liquifiable soil. The MICCP would lead to a permeability of treated sand around 10 times smaller than that of untreated sand, which can significantly mitigate liquefaction in liquifiable soils. The difference in the space of voids is clear from SEM images (Fig. 10).

3.2 Unconfined Compressive Strength Test

The UCS test was performed on all the samples as per IS:2720 (Part X) (1991), as shown in Fig. 5. The UCS test results using CaCl₂ and seashell are shown in Fig. 6 and Fig. 7, respectively. The unconfined compressive strength obtained from this study varied from 794.34 kPa to 2237.87 kPa for sand treated using CaCl₂, and varied from 1292.42 kPa to 1917.20 kPa for sand treated using the seashell, which shows that the results are comparable. The maximum UCS of 2237.87 kPa was obtained when 0.5 M calcium chloride was used, and the maximum UCS of 1917.20 kPa was obtained when 0.5 M calcium from seashell was used. It indicates that 0.5 M is the concentration at which the MICCP process gives the maximum increase in strength. As the concentration of calcium is increased by more than 0.5 M, the strength of the soil is reduced. The increase in compressive strength of sand with the addition of seashell shows that seashell can bind the sand particles effectively.



Fig. 5 Unconfined compressive strength test of biocemented sand

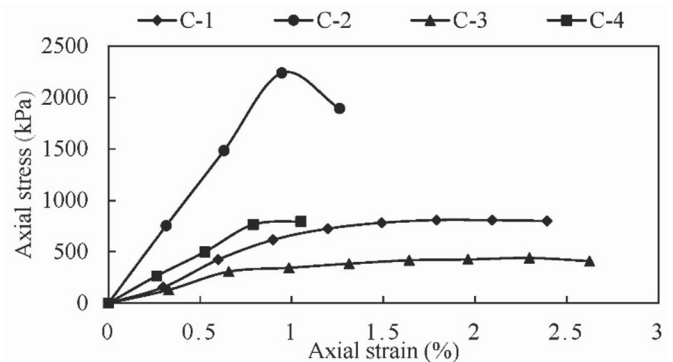


Fig. 6 Unconfined compressive stress-strain behavior of biocemented sand using calcium chloride as a calcium source

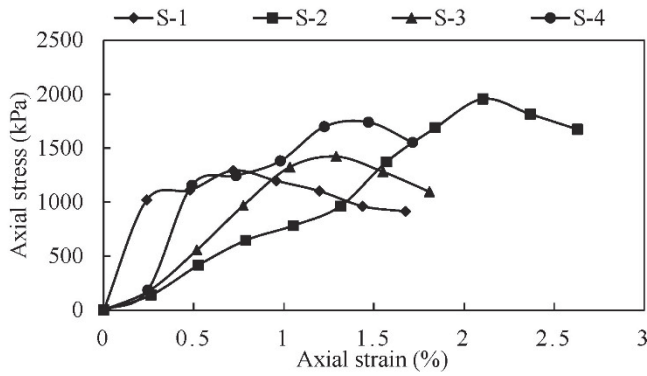


Fig. 7 Unconfined compressive stress-strain behavior of bio-cemented sand using seashell as a calcium source

3.3 CaCO₃ Content Measurement

Sand samples of 5 g were mixed with 20 ml of 1M HCl acid to dissolve calcium carbonate. Then all the solution and insoluble solids were washed by distilled water on filter paper for 10 minutes. This washing process removes all the soluble calcium from the sand particles. All the solid particles remaining on the filter paper were oven-dried and weighed. The weight difference between the original sand sample and the post washing sand sample was the mass of calcium carbonate. The calcium carbonate content was measured at the top, middle, and bottom for each sample. Calcium carbonate precipitate was detected over the entire length of the column, indicating that bacteria and cementation solution were present at all locations of the samples.

In order to determine the effect of calcium carbonate precipitation on the mechanical properties of the treated material, graphs were plotted to correlate the permeability and strength results with the calcium carbonate content of each of the tested samples (Figs. 8 and 9). Table 3 summarizes the results of all the tests.

At higher calcium carbonate contents, there was a significant improvement in the strength of sand relative to untreated sand. The results show that as calcium carbonate content increases, permeability decreases while unconfined compressive strength increases. Calcium carbonate binds the sand particles together, reducing the permeability and making particles more resistant to compressive forces. In both cases, from calcium chloride and seashell sources, it was also seen that 0.5 M of calcium gives maximum compressive strength. The increase in strength can be attributed to the calcium carbonate precipitate, strengthening the bond between the sand particles by filling the voids between them. The calcium carbonate precipitate crystals were small, more uniform, and homogeneously distributed for the sample treated with 0.5 M calcium concentration. It has also been reported by Al Qabany *et al.* (2012) that low cementation concentration (up to 0.5 M concentration of calcium) results in the calcium carbonate crystals of similar size, were very well distributed, and covered the contact area uniformly. For high cementation concentration (more than 0.5 M concentration of calcium), the precipitation pattern was less uniform with larger crystal sizes of calcium carbonate. They suggested that using lower chemical concentrations over a larger number of injections resulted in more homogeneous cementation.

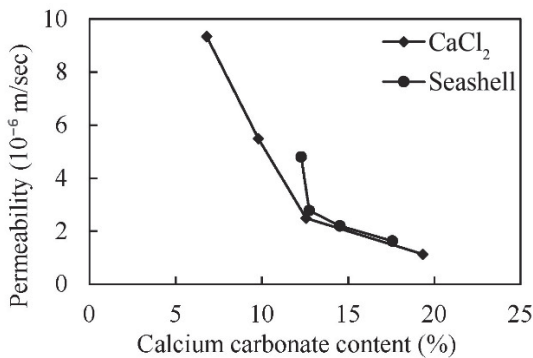


Fig. 8 Variation of permeability with different percentages of calcium carbonate content

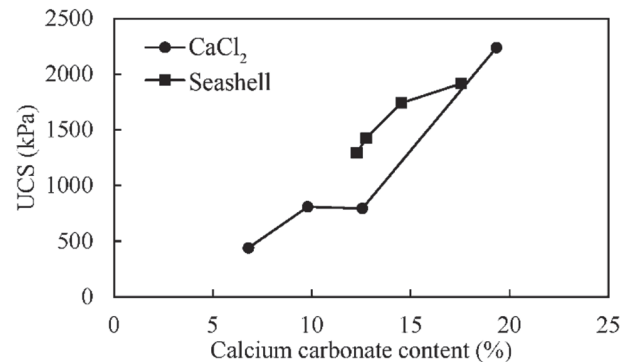


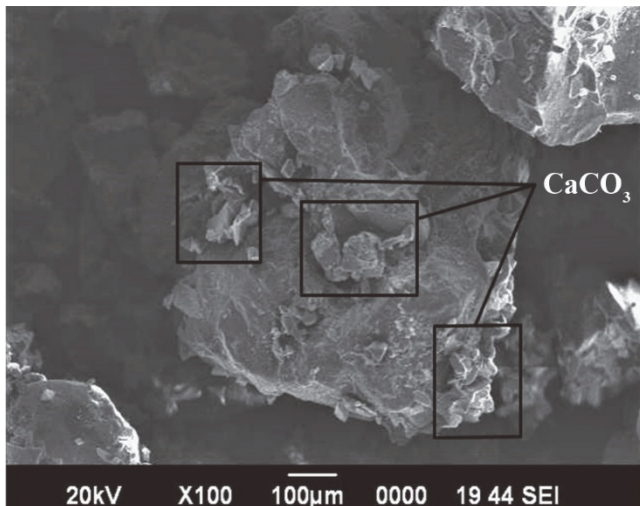
Fig. 9 Variation of unconfined compressive strength (UCS) with different percentages of calcium carbonate content

Table 3 Comparison of results of samples treated by MICCP process using calcium chloride and seashell

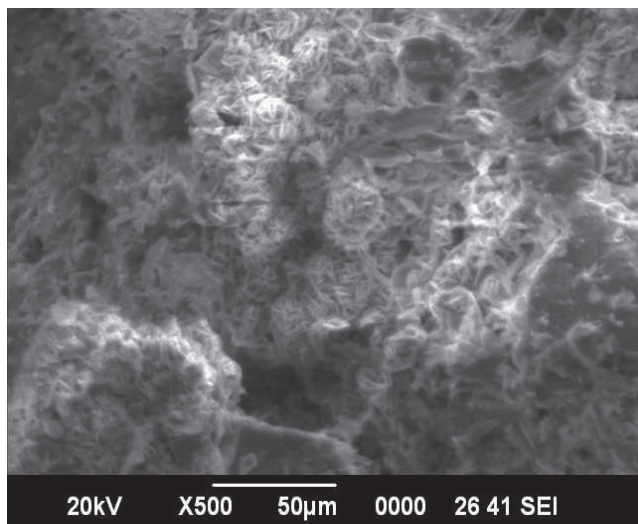
Calcium source	Test samples	Permeability (10 ⁻⁶ m/sec)	UCS (kPa)	Calcium carbonate content (%)			
				Top	Middle	Bottom	Average
Calcium chloride	C-1	5.49	808.46	11.20	7.34	10.86	9.80
	C-2	1.13	2237.87	21.40	17.86	18.76	19.34
	C-3	9.34	439.33	7.94	6.11	6.35	6.80
	C-4	2.49	794.33	14.36	11.08	12.24	12.56
Seashell	S-1	4.80	1292.42	13.38	10.44	13.02	12.28
	S-2	1.63	1917.20	18.72	16.54	17.46	17.57
	S-3	2.78	1422.74	14.62	10.88	12.76	12.75
	S-4	2.20	1740.68	15.50	13.78	14.32	14.53

3.4 SEM Analysis

SEM images of the selected sample were taken to visualize the calcium carbonate precipitation within the sample. A sample with a 0.5 M equimolar concentration of calcium from seashell was viewed under an SEM. The viewing of the sample was at 100 μm and 50 μm , as shown in Fig. 10, clearly show the presence of cohesive bonds connecting the sand grains, which validates the congestion of pores with calcium carbonate that imparts compressive strength to the sand sample. SEM observations of treated sand support the experimental findings.



(a) 100 μm



(b) 50 μm (showing heavy precipitation)

Fig. 10 Scanning Electron Microscope (SEM) images showing CaCO_3 precipitation around the sand surface for 0.5 M calcium from seashell

4. CONCLUSIONS

Microbially induced calcium carbonate precipitation has shown to be an effective method to increase the strength of sandy soil. MICCP process can be applied for biocementation of sand to improve the bearing capacity, mitigate liquefaction, control the

groundwater flow, control soil erosion, and soil remediation. Seashell is a naturally available waste material made up of calcium carbonate, so it can be used as a calcium source for biocementation. A method to use naturally available seashell as a calcium source in place of costly calcium salts for the MICCP process is presented in this paper. The following conclusions can be derived from this study:

1. Soluble calcium obtained by dissolving seashells into glacial acetic acid can be used as a calcium source for the MICCP process. The seashell-to-glacial acetic acid ratio of 1:6, having a concentration of 0.5 M, gives the highest value of unconfined compressive strength. It was also seen that in both cases, from calcium chloride and seashell sources, 0.5 M of calcium gives maximum compressive strength. Results of UCS and permeability using calcium chloride and seashell were comparable.
2. For biocementation using seashell, glacial acetic acid diluted to 5% is used to dissolve seashells. The rest of the process is the same as used in the biocementation using CaCl_2 . Seashell is the waste material, and the amount of glacial acid used is also significantly less as it is diluted to 5% only. Also, the cost of glacial acetic acid is significantly less than the cost of CaCl_2 , so the use of seashell is much more economical than CaCl_2 . It reduces the overall cost of this process as it can be produced in excess quantity at a very low price.
3. Concentration of cementing solution used in the MICCP treatment was found to affect the strength of cemented samples. It was found that the use of a 0.5 M concentration solution resulted in stronger samples and is helping in more calcium carbonate precipitation. When the calcium in the cementation solution increases above 0.5 M concentration, the strength of the sample starts reducing, and less precipitate of calcium carbonate is formed in sand, which means that increasing the concentration of calcium in cementation solution will not increase strength. With a 0.5 M concentration of calcium, the crystals of calcium carbonate precipitate were more uniform and homogeneously distributed.
4. As the calcium carbonate content increases, UCS increases while permeability is reduced. Maximum calcium carbonate content of 17.57% was obtained at a 0.5 M concentration of soluble calcium using seashell. UCS and permeability values at this calcium carbonate content were 1917.20 kPa and 1.63×10^{-6} m/sec, respectively.

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DATA AVAILABILITY

The data presented in this study are available from the corresponding author on reasonable request.

CONFLICT OF INTEREST STATEMENT

The authors declare that there is no conflict of interest.

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