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Strengthening sandy soils by microbial methods

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Abstract Some contemporary methods and materials are available in geotechnical engineering to improve the engineering performance of soils. Bacterial calcium carbonate cementation could be an innovative application by bonding at the particle contacts and increasing the shear strength. Bacteria type is the most effective factor on the calcium carbonate precipitation while incubation time, concentration, and pH were minor effects. The increasing urea concentration reduces the amorphous phase and increases the crystalline phase with vaterite. Meanwhile, the increasing incubation time is very effective transforming the vaterite crystals to calcite. *Paenibacillus favisporus* U3 forms the maximum calcium carbonate precipitation among the tested ones, and it is utilized for the geotechnical studies. The unit weights of the samples are not changed noticeably by the bacterial procedure. The uniaxial compressive strength of the tested core samples are varying in a quite large range from 66.8 to 204.1 kPa. The young modulus of the treated samples reaches up to 89.4 MPa. The Vp and Vs sonic velocity values rise up to 985 and 443 m/s, respectively. The images prove the homogeneous distribution of bacterial carbonate cement material among the soil grains, and it fills the gaps up to 0.5 mm size. The improvement provided by the bacterial treatment is sufficient to support the project loads in very large areas like transportation projects and factory sites. The method can be employed in the associated geotechnical engineering applications.

Keywords Bacteria · Biological mineralization · Soil strengthening

Introduction

In recent years, a large number of contemporary methods and materials are available in geotechnical engineering to improve the engineering performance of soils. They are miscellaneous in the matter of environmental impact, penetration depth, treatment uniformity, cost, etc. Naturally, they have benefits and drawbacks and researches are required on soil improvement to find out new materials and methods.

Microorganisms play substantial roles in geological processes. A role of bacteria on calcium carbonate precipitation was learned by Nadson (1903). Since then, a large number of researchers have studied interactions between microorganisms and minerals (Douglas and Beveridge 1998; Dong et al. 2000; Lower et al. 2001; Frankel and Bazylinski 2003; Edwards and Bach 2005; Braissant et al. 2007; Achal and Pan 2011; Biswas et al. 2015; Mueller 2015; Kuznetsov 2015). The method is widely employed in many applications like wastewater treatment (Hammes et al. 2003), bioremediation (Fujita et al. 2000; Warren et al. 2001), restoration of historical stones (Tiano 1995; Rodriguez-Navarro et al. 2003), oil recovery (Gollapudi et al. 1995; Nemati and Voordouw 2005), and concrete strengthening (Ramachandran et al. 2001).

In recent decays, geotechnical engineering is facing stupendous developments which lead to employ new techniques from other disciplines (Kumari and Xiang 2017). Bacteria produce the enzyme urease and the biological calcium carbonate cement forms. Microbiological carbonate precipitation can

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dramatically increase the physico-mechanical properties of uncemented soils. This ability has led the development and the implementation of microbiological processes in geotechnical engineering which was one of the innovative applications. It is an environmentally friendly, sustainable, and nondestructive method. Biological calcium carbonate precipitation was employed as a plugging agent in highly permeable water channels (Ferris et al. 1996). The following studies were mainly focused on the improvement of physical properties of the soils (Mitchell and Santamarina 2005). However, the engineering parameters such as shear strength and elastic modulus are more essential for a safer design. DeJong et al. (2006) have examined the undrained shear characteristics of microbial cemented sand. They have proved that the process biological calcite has bonded at the particle contacts and increased the shear strength. Karakas (2008) has concluded that the method can be used to improve shear strength of granular soils and has the potential to become a sustainable and cost-effective ground improvement technology in granular soils. Ivanov and Chu (2008) and Soon et al. (2013) studied the effects of the microbial calcite precipitation on the physico-mechanical properties of the soils and declared that the process effectively improved shear strength and reduced hydraulic conductivity for both residual soil and sand. Anbu et al. (2016) have highlighted the promising solutions of microbial calcite precipitation on the environmental problems.

A successful bacterial soil improvement application is dependent on some parameters including soil and bacteria types, media diversity, and environmental and geological parameters. This study is targeted to improve the engineering properties of the sandy soils using bacterial carbonate precipitation. The effective factors on the bacterial activity are examined to get maximum carbonate precipitation. Some geotechnical tests are performed on the treated soil samples to clarify the improvement. Mineralogical visualization has engaged to verify the phenomena both on polarized and SE microscopy. The study has put forth an effective application of a local strain to employ it for geotechnical engineering practices.

Material and Method

Microbiology

The microbiological study aimed to explore the bacteria with the highest carbonate precipitation. In order to achieve it, initial urea concentration, pH, and incubation time and temperature effects on the bacterial carbonate precipitations were investigated. Tested bacteria (*Bacillus aerius* U2, *Paenibacillus favisporus* U3, and *Lysinibacillus fusiformis* U5) were isolated from Israfil River in Denizli (Turkey). The samples were inoculated on urea agar containing phenol red. After 2–3 days of incubation, a color change from orange to pink in the medium indicates urea hydrolysis. Gram and endospore staining was performed to confirm the rod-shaped bacteria and stock. Bacterial strains were identified by 16S rDNA analysis at the Life Sciences Research and Application Center, Gazi University, Ankara (Turkey). These bacteria are tested under different urea concentrations (100, 200, 250, 300, 333, and 350 mM), pH (5.0 to 7.5), temperatures (20 to 42 °C), and incubation times (5, 7, 10, 14, and 20th days). For screening precipitation, calcium precipitation medium (CPM) was used. CPM contained 3.0 g/L nutrient broth (Difco), 25 mM $CaCl_2$, 25 mM $NaHCO_3$, and 333 mM urea (Ferris et al. 1996; Whiffin et al. 2007). The growth parameters such as initial pH (5.0–7.5), temperature (20–42 °C), and urea (100–350 mM) concentration on calcium carbonate mineralization by *B. aerius* U2, *P. favisporus* U3, and *L. fusiformis* U5 were tested. The EDTA titrimetric method was used (APHA 1989). The amount of calcium carbonate was calculated by the formula $CaCO_3 = (V1.M.1000) / V2)$ (V1: consumed EDTA, M: 1 mL EDTA = 0.96 mg $CaCO_3$, V2: sample amount (mL)). The medium in maximum mineralization of *B. aerius* U2 included the following materials: 3.0 g/L nutrient broth, 25 mM $CaCl_2$, 25 mM $NaHCO_3$, 300 mM urea, pH 5.5, and temperature 20 °C. For *P. favisporus* U3, the mineralization medium was composed of 3.0 g/L nutrient broth, 25 mM $CaCl_2$, 25 mM $NaHCO_3$, 100 mM urea, pH 6.5, and temperature 37 °C. The growth medium of *L. fusiformis* U5 was as follows: 3 g/L nutrient broth, 25 mM $CaCl_2$, 25 mM $NaHCO_3$, 350 mM urea, pH 6.5, and temperature 37 °C. The tests indicate that the optimum conditions for the precipitation are constant temperature 37 °C, pH 6.5, incubation time 10 days, and urea concentration 100 mM. The maximum amount of carbonate is 2805.12 mg/L in these conditions for *P. favisporus* U3.

Geotechnics

The geotechnical test samples are terrestrial sands in Denizli Province which originated from metamorphic rocks. The samples were sieved on no. 40 (0.42 mm) and no. 200 (0.074 mm) meshes, the retained materials between which were used. The sieved materials were sterilized at 121 °C for 15 min before experiments against undesired microorganism contamination. For soil improving tests, plexiglass tubes are used. They have 5.5-cm inner diameters and 12-cm length. Both ends of the tubes are covered by metal caps with only one input and output pipe. The filters with 0.074 mm opening size are also placed at the end of the tubes to prevent soil drift related to fluid flow (Fig. 1). Stress at 25 kPa is applied on the soil after filling the tubes to get standardized firmness. Then 5 L fluid containing the bacteria had been passed through the tubes, and the passing was repeated three times with 48-h intervals. A Watson Marlow 120S/DV peristaltic pump is employed to get different flow rates of the fluid. The rates were 21 mL/min (25 rpm

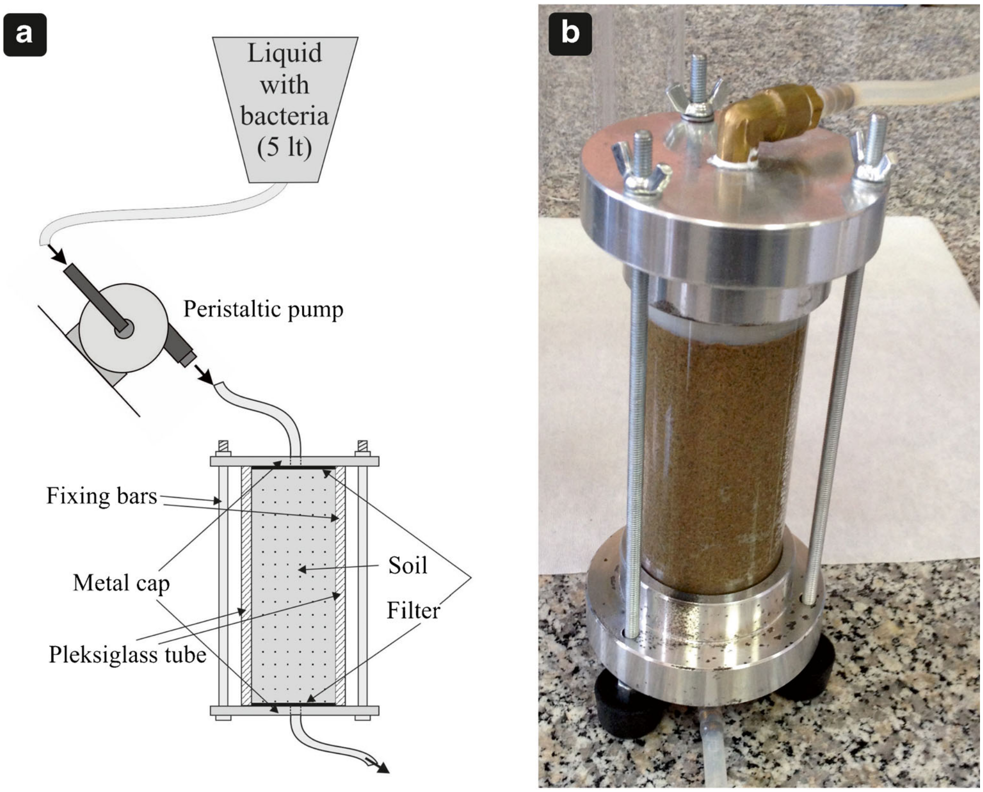

Fig. 1 Experimental assembly. **a** Schematic view. **b** Tube with sand

rotary per minute), 42 mL/min (50 rpm), and 84 mL/min (100 rpm) and free run with 60 cm falling head. At the end of the tests, the loose sand (natural) (Fig. 2a) has transformed to core samples (Fig. 2b) and they were subjected to unconfined compressive strength (UCS) and sonic velocity tests (Fig. 3a, b). The sonic velocities were measured by Proceq Pundit. The loose sand samples filled the tubes and 25 kPa stress is applied, and then the unit weights were calculated. All the tests were repeated three times, and average values were used.

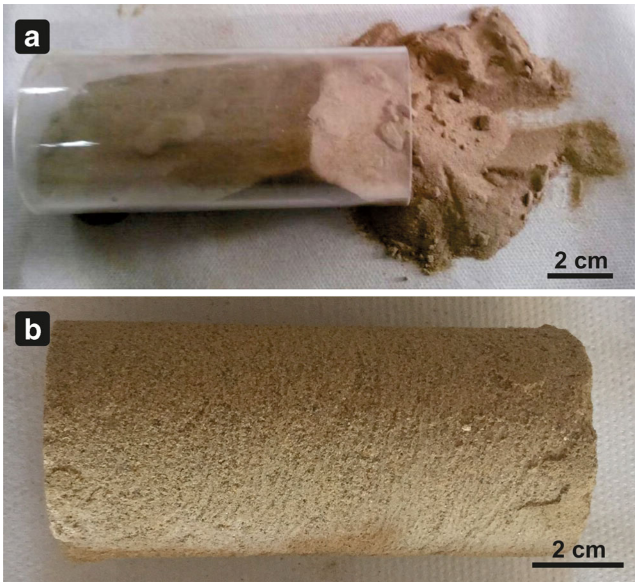

Fig. 2 The samples before (**a**) and after treatment (**b**)

Mineralogy

XRD (X-ray diffraction) was employed to delineate the crystal structure (crystalline, semi-crystalline, and amorphous) and mineralogical content of the calcium carbonate precipitations. SEM (scanning electron microscopy) analyses were used to determine the textural and chemical structure of the calcium carbonate precipitations and to visualize the grain-cement interaction. Polarized microscopy was utilized to picture the bio-carbonate cement among the sand grains. Epoxy adhesive was applied on thin sections to protect the soil-cement structure which formed after the bacterial improvement.

Results and discussion

The microbiological studies demonstrated that the amount of amorphous phase (i.e., extracellular polymeric substance) decreases with increasing incubation time and urea concentration. In addition to species *Bacillus*, incubation time has a major role on the mineral types (calcite and vaterite), which causes to increase the calcite ratio, whereas urea concentration does not have a clear effect on the mineral type. The temperature and pH effects are negligible to the increase in carbonate precipitation. pH modification is important for different

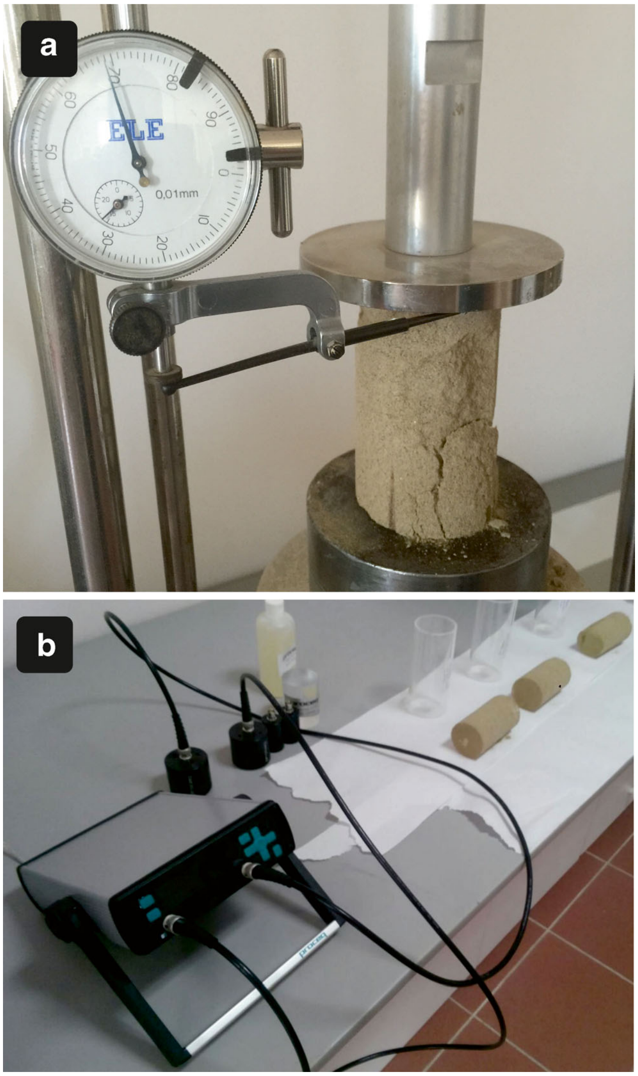

Fig. 3 UCS (**a**) and sonic velocity (**b**) measurements

cultures to achieve the maximum calcium carbonate rate in the mineralization. The results suggest that the optimum initial pH value was 6.5. While the pH was increased, the amount of carbonate was reduced. Temperature is another important factor affecting bacterial carbonate precipitation. The U2 and U5 strains were 100% identical to *B. aerius* (GenBank: KF861583.1) and *L. fusiformis* (GenBank: JQ900517.1), respectively. Strain U3 was 99% identical to *P. favisporus* (GenBank: NR_029071.1 and JN867753.1) (Life Sciences Research and Application Center, Gazi University). *P. favisporus* U3 is evaluated under five different temperatures: 20, 25, 30, 37, and 42 °C. Maximum bacterial mineralization is done effectively at 37 after 10 days. As a conclusion, optimization conditions such as urea concentration, pH, temperature, and incubation time increased the bacterial carbonate precipitation. The optimum conditions for the selected bacteria are urea concentration = 100 mM, temperature = 37°C,

Table 1 Minimum, maximum, and average values of the test data

	Unit weight (kN/m^3)					
	Min	Max	Mean			
Natural	13.8	14.1	14.0			
Free flow	14.3	14.6	14.5			
25 rpm	14.4	15.1	14.6			
50 rpm	14.5	14.7	14.6			
100 rpm	13.6	15.2	14.6			
			UCS (kPa)	E (MPa)		
	Min	Max	Mean	Min	Max	Mean
Free flow	51.3	81.4	66.8	11.7	49.7	23.9
25 rpm	85.4	267.7	204.1	40.0	113.0	89.4
50 rpm	134.4	149.8	142.9	34.9	49.7	40.7
100 rpm	76.9	94.0	85.2	29.7	36.6	33.6
	Vp (m/s)			Vs (m/s)		
	Min	Max	Mean	Min	Max	Mean
Natural	590	611	601	158	195	167
Free flow	786	849	820	296	320	312
25 rpm	905	1063	985	418	465	443
50 rpm	870	1030	954	344	398	370
100 rpm	820	915	893	341	387	352

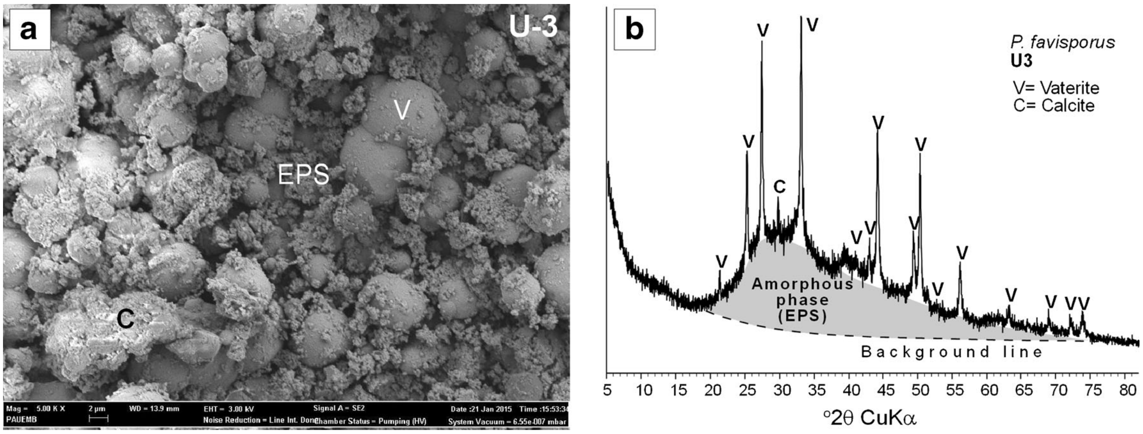

Fig. 4 SEM image (**a**) and XRD pattern (**b**) of bacterial carbonate precipitations under optimal conditions

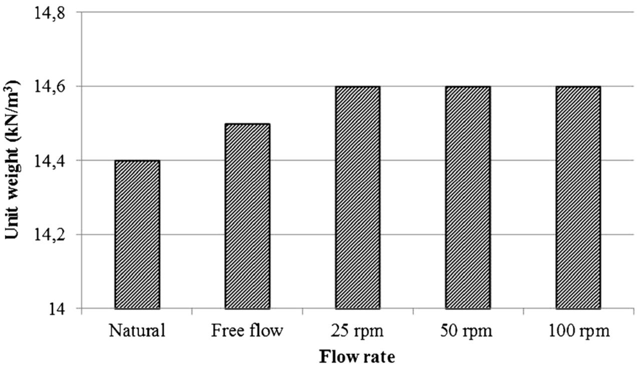

Fig. 5 Unit weights of the samples

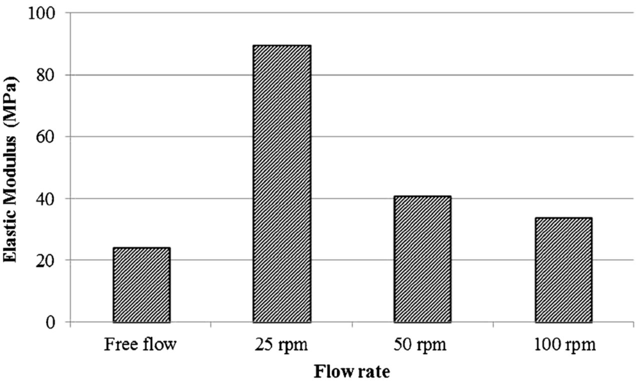

Fig. 7 Young modulus of the samples

initial pH = 6.5, incubation time = 10 days, and maximum calcium carbonate concentration = 2805 mg/mL. The obtained microbial carbonate product has been represented by vaterite, amorphous phase (EPS), and scarce calcite (Fig. 4).

Some physico-mechanical properties of the samples, namely, unit weight, UCS, Young modulus, and Vp and Vs sonic velocities, have been measured as in Table 1. The unit weights of the core samples were determined, and the values are very close to each other. The lowest value was for the natural sand samples, and the average is 14.4 kN/m^3. The unit weight of the free flow is slightly higher than the natural soil, and it is 14.5 kN/m^3. The unit weight of the rest is the same which has the highest value at 14.6 kN/m^3 when the flow rate is increased (Fig. 5). The data demonstrate that the increasing flow rates help to leave more material into the soil. However, the differences between them are negligible.

UCS of the tested core samples vary in a quite large range from 66.8 to 204.1 kPa. The UCS of the free flow has the lowest value at 66.8 kPa while that at 25 rpm has the highest at 204.1 kPa. Increasing the speed of the pump has not helped to obtain higher values. The strength is 142.9 and 85.2 kPa for 50 and 100 rpm, respectively (Fig. 6). Apparently, 21 mL/m flow rate (25 rpm) is the optimum to get the best results in the employed tests. It seems that higher rotary speeds of the pump are increasing pressure in the test cell. The bacteria activity is getting slower under higher pressures, and one atmosphere pressure is being used for bacteria sterilization.

Understandably, young modulus data of the samples are parallel to the UCS values. The extreme values are 23.9 and 89.4 MPa for free flow and 25 rpm, respectively. Young modulus has decreased to 40.7 MPa for 50 rpm and 33.6 MPa for 100 rpm (Fig. 7).

The sonic wave and mechanical test data are parallel to each other. Likewise, the lowest Vp was measured on natural soil sample as 601 m/s while the highest one was that of 25 rpm, at 985 m/s. The other values are close and range from 820 to 954 m/s. (Fig. 8). Similarly Vs has reached up to 443 m/s for 25 rpm. The others lie between 167 m/s and 370 m/s (Fig. 9).

Conventional soil improvement methods like cement and chemical grouting may be more applicable for some buildings and structures. High cost may be acceptable for this type of applications because of heavy loads and/or relatively small areas. Microbial improvement seems to be more convenient in large areas like transportation projects and factory sites. The expected shear load on a motorway is around 24 kPa (Wanatowski et al. 2008). UCS of the 25 rpm samples was more than 200 kPa, and its expected shear strength is around

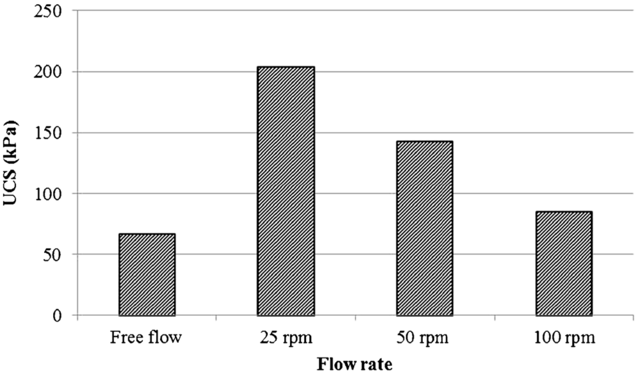

Fig. 6 UCS of the samples

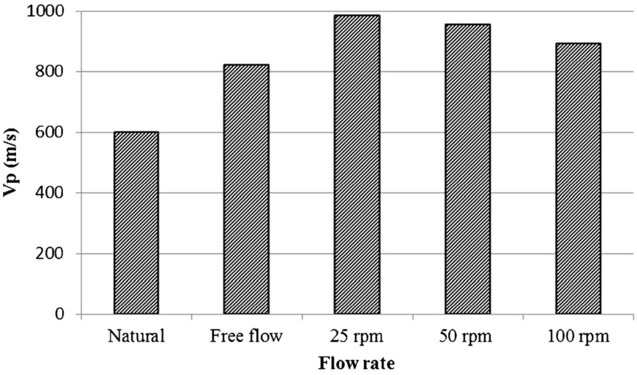

Fig. 8 Vp of the samples

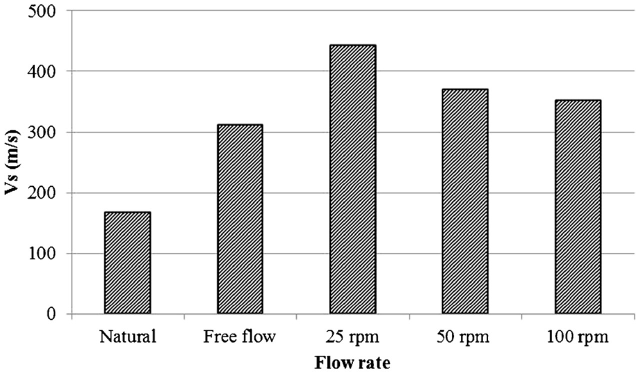

Fig. 9 Vs of the samples

100 kPa. This is much higher than the applied shear stress in the subgrade of a motorway. In other words, bacterial improvement will be satisfactory on the engineering properties of the sandy soils in many projects. As a final word, a proper microbial improvement application is a reliable and economic strengthening method to be employed in large areas.

The treated soil samples were also examined under both optical (Fig. 10a) and scanning electron microscopy (SEM) (Fig. 10b) to inspect soil grain and microbiological mineral precipitation and effectiveness of the process. The analysis of the SEM images on the polished surface shows that many of the soil grains were surrounded by biological calcium carbonate as confirmed by X-ray elementary map data (Fig. 11). The results suggest that the method can be employed to improve engineering properties of the sandy soil.

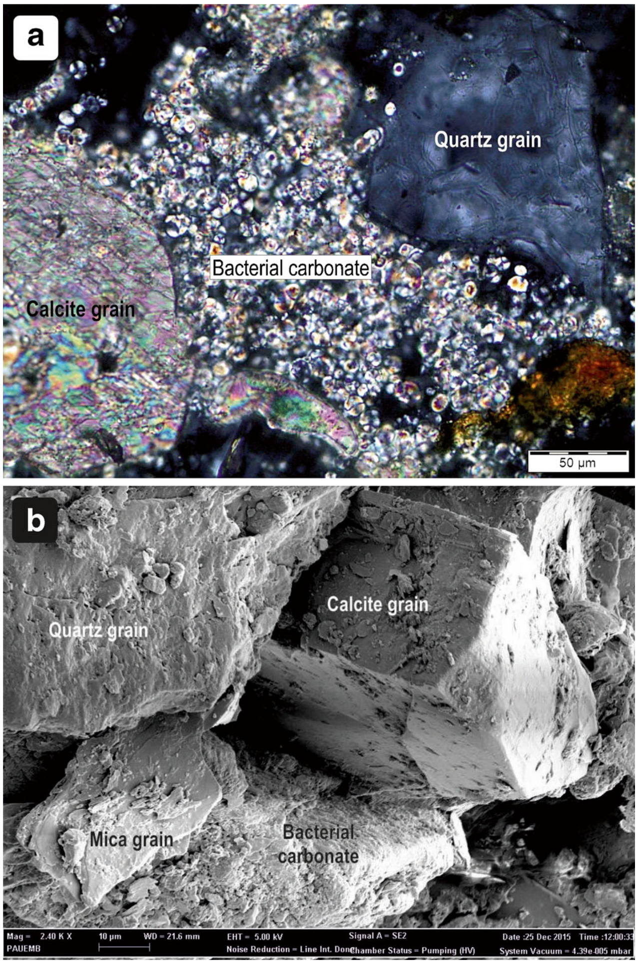

Fig. 10 The images of the 25 rpm-treated samples under **a** polarized microscope (crossed nicols), and **b** SEM secondary electron image (SE)

EDS analyses suggest that calcium carbonate is dominant in all forms, namely, vaterite, calcite, and EPS. The carbonated bacteria and EPS include a small amount of phosphate. The polarized microscopy images prove the homogeneous distribution of bio-carbonate cement material among the soil grains, and it fills the gaps up to 0.5 mm size. XRD data do not show any vaterite mineral in the treated soil.

Conclusions

The following conclusions can be drawn by this study:

Bacteria type was the most effective factor on calcium carbonate concentration while incubation time, concentration, and pH were minor effects. The urea concentration was the second important factor for the calcium carbonate precipitation. The increasing urea concentration reduced the amorphous phase and increased the crystalline phase with vaterite. Meanwhile, the increasing incubation time was very effective, transforming the vaterite crystals to calcite. The sample, coded U3, formed the maximum $CaCO_3$ among the tested ones, and it was utilized for the geotechnical studies. The optimum conditions for it were urea concentration = 100 mM, temperature = 37°C, initial pH = 6.5, incubation time = 10 days, and maximum calcium carbonate concentration = 2805 mg/mL.

The unit weight of the natural sand was 14.4 kN/m^3. Bacterial treatment has not made a noticeable change on the core samples, and the values lie between 14.5 and 14.6 kN/m^3. UCS of the tested core samples vary in a quite large range from 66.8 to 204.1 kPa. The UCS of the 25 rpm sample has the highest strength at 204.1 kPa. Increasing the speed of the pump has not helped to obtain higher values. The strength is 142.9 and 85.2 kPa for 50 and 100 rpm, respectively. The findings suggested that higher rotary speeds increase the pressure in the test cell which causes slower bacterium activity. The young modulus data of the samples were parallel to the UCS values. The extreme values are 23.9 and 89.4 MPa for free flow and 25 rpm, respectively. Young modulus has decreased to 40.7 MPa for 50 rpm and 33.6 MPa for 100 rpm (Fig. 6). In the same way, the sonic wave and mechanical test data are analogous. The lowest Vp was measured on the natural soil sample as 601 m/s while the highest one was that of 25 rpm, at 985 m/s. The Vs value has bounced from 167 m/s (natural soil) to a remarkable 443 m/s (25 rpm).

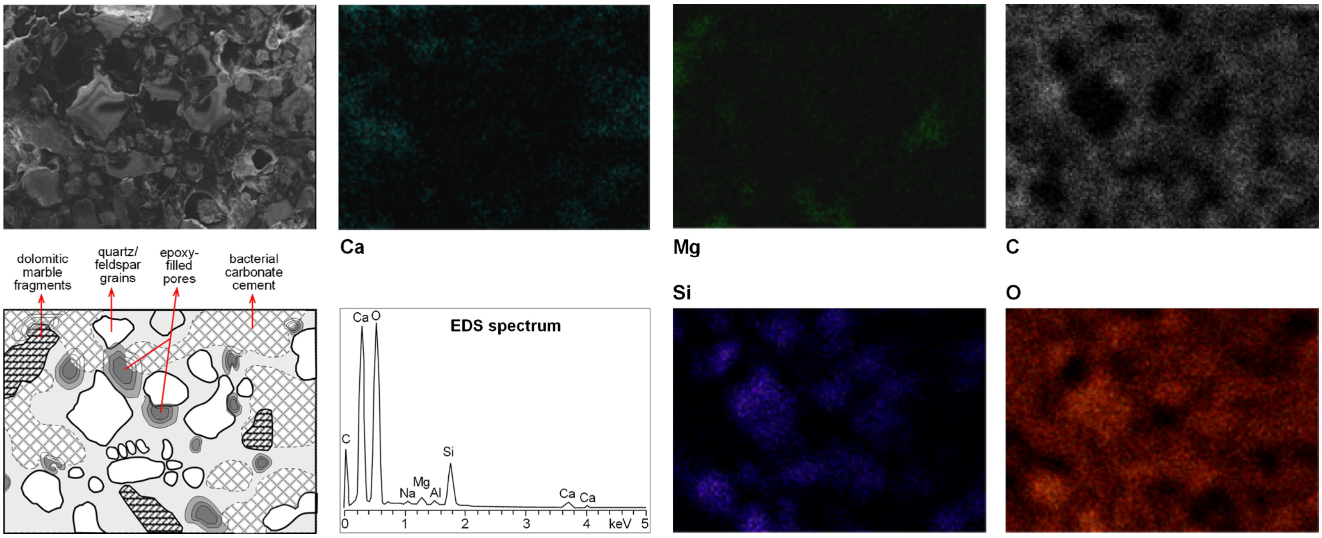

Fig. 11 The SEM-BSE image and schematic view of the 25 rpm-treated samples on the polished surface, EDS spectrum, and X-ray elementary maps of Ca, Mg, C, Si, and O

The calcium carbonate is dominant in all forms, namely, vaterite, calcite, and EPS. The polarized microscopy images proved the homogeneous distribution of bio-carbonate cement material among the soil grains, and it filled the gaps up to 0.5 mm size. XRD data do not show any vaterite mineral in the treated soil.

Microbial improvement seems to be more convenient in large areas like transportation projects and factory sites although the conventional soil improvement methods like cement and chemical grouting may be more common in relatively small areas. The improvement provided by the bacterial treatment is sufficient to support such project loads. The method can be employed in the associated geotechnical engineering applications.

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