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Effect of bacterial calcium carbonate precipitation on compressibility and shear strength of organic soil

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Abstract

In the past few years, the use of bacterial calcium carbonate precipitation has become popular as a ground-improvement technique for sandy soil. However, this technique has not been applied to organic soil. This study focused on bacterial calcium carbonate precipitation and its effect on the compressibility and strength of organic soil. A special injection system was prepared for inducing a bacterial solution into several samples. The bacterial solution was supplied to the samples by gravity for 4 days in specific molds designed for this work. Calcite precipitation was observed by monitoring the changes in the pH value and by measuring the amount of calcium carbonate in the organic soil. The changes in compressibility and strength were measured before and after the bacterial treatment. The test results showed that the pH values in the treatment medium reached the ideal values that are appropriate for calcite precipitation. It was found that the amount of precipitated calcium carbonate in organic soil increased by about 20% in the treated samples compared to that in the untreated samples. Moreover, the test results indicated that the bacterial treatment influenced the compressibility and shear strength of the organic soil. The results were supported by an energy-dispersive x-ray (EDX) analysis.

Keywords: *Sporosarcina pasteurii*; organic soil; Bacterial calcium carbonate precipitation; Compressibility; Strength

1. Introduction

Organic soils, which are found in many places around the world, are a mixture of finely divided particles of organic matter. In some instances, the soil may contain visible fragments of partly decayed vegetable matter and shells. The amount of organic matter in a soil significantly affects its geotechnical properties, including specific gravity, water content, liquid limit, plastic limit, density, hydraulic conductivity, compressibility, and strength. Constructing structures on an organic ground involves the risk of bearing capacity failure and excessive settlement. In order to prevent these risks, the geotechnical properties of organic soil are improved by ordinary improvement techniques, such as deep mixing with cement or lime, vertical drains, sand columns, and dry jet mixing (Hampton and Edil, 1998; Edil, 2003; Çelik and Çanakcı, 2011; Jelisic and Leppanen, 2003; Koda et al., 1989; Furstenberg et al., 1983; Yang et al., 1998).

In the past few years, the use of bacterial calcium carbonate precipitation (BCCP) has become popular as a ground-

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improvement technique. It is presented as a new and environmentally friendly method (Dejong et al., 2006). This new method has an advantage over conventional chemical treatments, which can be toxic and environmentally harmful and have a limited injection distance (Karol, 2003). The method is also cost-effective in comparison to chemical treatments (Ivanov and Chu, 2008). Many studies in the literature have reported that the microbial-induced calcite precipitation technique is very effective in increasing the shear strength and in decreasing the permeability of sandy and gravelly soil (Van Paassen et al., 2010b; Van Wijngaarden et al., 2010; Van der Star et al., 2011). Scaled-up studies were also performed on sandy soil and successful results were obtained (Van Paassen et al., 2010b).

The main role of bacteria in the calcite precipitation process has been to generate an alkaline environment through different physiological actions (Douglas and Beveridge, 1998; Dejong et al., 2010; Benini et al., 1999; Ciurli et al., 1999; Karatas et al., 2008; Van Paassen et al., 2010a; Hamdan et al., 2011b; Warthmann et al., 2000; Roden et al., 2002; Weaver et al., 2011). *Bacillus pasteurii*, a highly urease active bacteria, plays an important role in $CaCO_3$ precipitation (Bang et al., 2001; Dejong et al., 2006; Dejong et al., 2010). It should be noted that *B. pasteurii* has been reclassified as *Sporosarcina pasteurii* (Mitchell and Ferris, 2006). As can be seen in Table 1, it exhibits a high urease production. Hence, it has been used for microbial calcite cementation in many studies (Bachmeier et al., 2002; Sarda et al., 2009).

The main nutrient solution that is commonly used to provide the necessary nutrition for the bacteria, as well as the chemical compounds that are needed for soil cementation, contain $NaHCO_3$, NH_4Cl, $CaCl_2$, urea, and a nutrient broth (mixture of peptone, yeast extract, beef extract, and NaCl). Under favorable environmental conditions, *S. pasteurii* uses urea as an energy source, producing ammonia (NH_3) and carbon dioxide (CO_2), which tend to increase the pH in the proximal environment. The net increase in pH causes the calcium ions to react with the carbonic acid and hydroxide ions to form calcium carbonate bonds (Dejong et al., 2010). The chemical reaction is given in Eq. (1). The calcite bonds tend to form at the particle-to-particle contacts in the soil matrix, which has an overall cementing effect between soil particles.

$$Ca^{2+} + HCO_3^- + OH^- \rightarrow CaCO_3 + H_2O \quad (1)$$

Bacterial calcium carbonate precipitation or cementation has been applied to a variety of civil engineering applications, such as to repair cracks in rock and concrete, to improve the bearing capacity, to reduce permeability, to increase dilative tendencies, and to increase the strain stiffness in sand (Gollapudi et al., 1995; Ramachandran et al., 2001; Bang et al., 2001; Ramakrishnan, 2007; Jonkers et al., 2009; Lo Bianco and Madonia, 2007; Dejong et al., 2006; Whiffin et al., 2007; Ivanov and Chu, 2008; Al Qabany, 2011; Rusu et al., 2011; Chou et al., 2011; Mortensen and Dejong (2011); Al Qabany and Soga, 2013; Martinez et al., 2013; DeJong, 2013).

Table 1
Urease production by different bacterial cultures (Sarda et al., 2009).

Bacteria culture	Urease activity (urea/ml)
Bacillus pasteurii	17.5
Bacillus lentus	10
Brevibacterium ammoniagenes	12.5

Gollapudi et al. (1995) used microbial mineral plugging to reduce the porosity of rock fractures. A series of column tests was conducted in order to evaluate the effectiveness of the proposed microbial technique. The flow rate into the fractures via the column was measured during the tests, and the absence of the flow signified the completion of plugging. Their results showed that microbial plugging was highly related to bacterial concentration, pH, the flow rate in the fractures, and the presence of contaminants.

Dejong et al. (2006) demonstrated that microbially cemented specimens exhibited increases in the strength of sandy soil. Their results showed that the behavior of the bacterially cemented specimens was similar to that of gypsum-cemented soil.

Whiffin et al. (2007) examined the effect of microbial carbonate precipitation on the permeability and shear strength of sandy soil. In their study, a 5-meter sand column was used to simulate field conditions. The results from triaxial tests conducted on specimens from the treated sand column showed that the soil porosity, strength, and stiffness were all significantly affected by the calcium carbonate content.

Chou et al. (2011) conducted a laboratory study to evaluate the effect of growing, dead, and resting cells on the geomechanical properties of microbially cemented sand. They performed direct shear and California Bearing Ratio (CBR) tests on sand specimens and found that the bacterial cells effectively improved the geomechanical properties of the sand. An analysis of the sand from CBR specimens treated with growing cells demonstrated that the microbial processes contributed to the clogging of the porous medium.

Martinez et al. (2013) worked on optimizing MICP treatment by varying the procedural parameters, including the flow rates, the flow direction, and the formulations of the biological and chemical amendments. They monitored the physical, chemical, and biological properties essential to the performance of MICP, including shear wave velocity, permeability, calcium carbonate content, aqueous calcium, aqueous ammonium, aqueous urea, and bacterial density. Their experiments showed that the shear wave velocity of treated soil increased from 140 m/s to an average of 600 m/s.

Al Qabany and Soga (2013) conducted unconfined compressive strength and permeability tests on sand samples treated with $0 \cdot 1$, $0 \cdot 25$, $0 \cdot 5$, and 1 M urea–calcium chloride solutions. The MICP treatment increased the strength of the treated samples. The magnitude of this increase depended on the concentration used in the treatment; the use of a low-urea calcium-chloride solution resulted in stronger samples. The use of a high-urea calcium-chloride-concentration solution resulted in a rapid drop in permeability at the early stage of the calcite precipitation, whereas the use of a low-chemical-concentration solution was found to result in a more gradual and uniform decrease in permeability.

In all previous studies, the BCCP technique was used in an attempt to improve the geotechnical properties of granular soil and successful results were obtained. The information in the literature on the application of BCCP to organic soil and its effect on compressibility and strength is very limited. This study focuses on the investigation of the feasibility of calcite precipitation and its effect on the strength and compressibility of organic soil. *S. pasteurii*, a type of non-pathogenic organism found naturally in soil, was used throughout the study.

2. Materials and methods

2.1. Materials

An isolated bacterial culture of S. pasteurii NCIMB 8221 was used in this study. The organic soil was obtained from the Sakarya region of Turkey. This soil is classified as OH "sandy organic silt" according to the Unified Soil Classification System (USCS), and as peat according to Wüst et al. (2003). Chemical and physical analyzes were carried out on organic soil and the results are presented in Table 2. In all the tests, organic soil samples that passed through sieve No. 2 mm and remained in sieve No. 100 (0.15 mm) were used. This range was chosen in order to provide enough space for the bacteria to have a cell diameter usually in the range of 0.5–3 μm (Madigan and Martinko, 2003). Moreover, the permeability of the organic soil was measured and found to be 5.2×10^{-3} cm/s. The soil was placed in a firing oven at 440°C for 4 h, and the organic content was estimated according to the ASTM D 2974 standard. According to the ASTM D 2974 standard, the ash content of the soil was 40%. A wet sieve analysis of the ash showed that the soil contained 15% silt and clay, 25% sand, and 60% organic materials. The liquid limit of the organic soil was estimated by fall cone tests and found to be 125%.

2.2. Methods

2.2.1. Preparing of urea nutrient agar, bacteria culturing, and counting

Urea nutrient agar was generally used to cultivate all the Bacillus spp. that had the ability to hydrolyze the urea. Table 3 shows the solid and liquid contents of the media. To prepare this media, the solid components were added to distilled water to bring the volume to 1 L and mixed well. The mixture was then gently heated, and brought to a boiling temperature. Later, it was autoclaved for 15 min at 15 psi pressure at 121 °C. After autoclaving, it was cooled to 50–55 °C. A total of 50 ml of sterile urea solution was added to the mixture and mixed well. The urea nutrient agar medium was then immediately poured into approximately 20 culture plates under a laminar flow hood. The laminar flow hood provided filtered air to reduce the risk of contaminating the culture growth media prior to the introduction of the *S. pasteurii* cultures.

One loopful of pure colony was taken from the stock bacteria and streaked onto each culture plate. All the plates were then inverted and incubated for 48–72 h at 30 °C in an incubator. After incubation, the colony growth of *S. pasteurii* had occurred for each of the single resulting colonies transferred from the plate to a 250 ml culture flask containing 200 ml urea nutrient broth. The culture flask was then wrapped in cheesecloth to filter the atmospheric biocontaminants, while providing oxygen to the bacteria, and incubated for 48 hours at 30 °C (Lo Bianco and Madonia, 2007). After incubation, 40-ml batches of the bacteria culturing solution were transferred from the culture flask to a series of 50-ml culture tubes. Each culture tube was centrifuged at 3000 rpm for 20 min to separate the suspension from the supernatant. The supernatant was removed by pouring it into a separate flask, and the remaining bacterial pellet was then used for the bacterial treatment process that was applied to each soil specimen.

The instrument DensiCHEK Plus 21255-P1ML1 was used to measure the optical density of the microorganism suspension. The instrument provides values in McFarland units, proportional to the microorganism concentrations. The device was indicated for use with polystyrene and glass test tubes and the reading range was 0.0–4.0 McFarland (approx. cell density of $(1.5 \times 10^8$–12×10^8 CFU/ml)) at a wavelength of 600 nm. It generated a McFarland value using basic colourimetry, which is a method of measurement that relates the amount of color in a transparent medium (liquid) to the amount of a particular substance in the liquid. In general, the concentration of the substance being measured is proportional to the intensity of the color of the solution. The darker the color is, the higher the concentration.

Table 2
Engineering properties of the organic soil used in the study.

Properties of organic soil	Content (%)
Organic content (%)	60
pH	6.5
Natural water content (%)	256
Liquid limit (%)	125
Plasticity index (%)	NP
Specific gravity (g/cm^3)	1.97
$CaCO_3$	0
NO_3–N (ppm)	50–150
NH_4–N (ppm)	40–140
K (ppm)	0–20
P (ppm)	0.2–0.7

Table 3
Details of solid and liquid contents of the media.

Composition	Quantity
Agar	15 g
Peptone	5 g
NaCl	5 g
Yeast extract	2 g
Beef extract	1 g
Urea solution[a]	50 mL

[a]Urea solution (add 20 g of urea to distilled water and bring the volume to 100 mL. Mix well. Filter and sterilize).

Table 4
Details of the treatment solution.

Agar components	Constituents	Amount
Urea medium	Nutrient broth powder	3 g
	Urea ($NH_2(CO)NH_2$)	20 g
	NH_4Cl	10 g
	$NaHCO_3$	2.12 g
	Distilled water	1 L
Calcium chloride solution	$CaCl_2 \cdot 2H_2O$	18.5 g $CaCl_2$/100 mL distilled water

2.2.2. Bacterial treatment solution preparation

To prepare the bacterial treatment solution, urea medium was first created. Nutrient broth (3 g), urea (20 g), NH_4Cl (10 g), $NaHCO_3$ (2.12 g), and 500 ml of distilled water, as shown in Table 4, were mixed to create the urea medium solution. Each of the solid ingredients was mixed thoroughly in 500 mL of distilled water until it had dissolved, and the pH of the resulting urea medium solution was adjusted to 6.0 with the use of 5 N HCL prior to autoclaving. Distilled water was then added to reach the final required volume (1 L). After autoclaving, the pH of the urea medium was measured to be 7.0. The pH value of the resulting 1 L of solution was adjusted by aerating the solution to increase the initial pH value from 7.0 to 8.0. In this way, the solution became suitable for biological activity for bacteria. This 1 L of aerated solution was divided into two approximate portions of 800 ml and 200 ml. Spun *S. pasteurii* cells were added to the 800-ml portion of the aerated urea medium and the flask was gently agitated to re-suspend the cells. A 20-ml volume of calcium chloride solution (18.5 g $CaCl_2$/100 ml distilled water) was then added to the aerated urea (200 ml), which lowered the pH of the solution slightly. The re-suspended bacterial solution was then added to the urea–calcium chloride solution. Five hundred milli litre of the combined solution consisting of urea, calcium chloride, and *S. pasteurii* cells was injected quickly into the specimens by gravity at a flow rate of approximately 20 ml/min and a hydraulic head of 1 m to prevent the calcium carbonate from precipitating in the pore fluid due to the simultaneous introduction of bacteria and calcium. This initial "biological treatment" was then allowed to set for a minimum of 12 hours to allow the microbes to attach to the particles of the soil samples. After 6 hours, 500-ml nutrient treatments of urea and $CaCl_2$ were injected through the specimens at the same flow rate. This nutrient treatment process was then periodically repeated (every 6 hours) for 4 days.

2.2.3. Sample preparation

One control sample (untreated 1) and three bio-treated samples (bio-treated 1, bio-treated 2, and bio-treated 3) were prepared for consolidation tests in cylindrical molds having a diameter of 75 mm and a height of 20 mm, as shown in Fig. 1. In addition, one control sample (untreated 2) and three bio-treated samples (bio-treated 4, bio-treated 5, and bio-treated 6) were prepared for direct shear tests in box molds having dimensions of $60 \times 60 \times 20$ mm^3, as shown in Fig. 2. All the

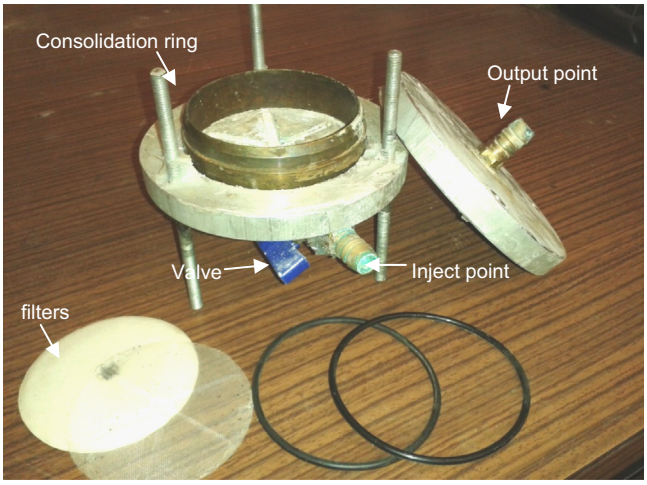

Fig. 1. Sample preparation for consolidation measurement.

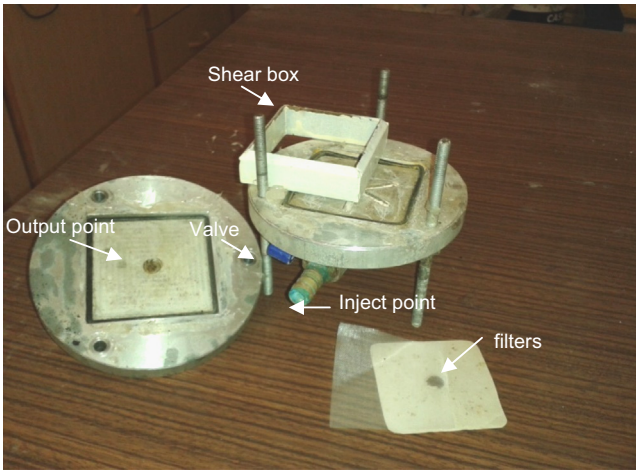

Fig. 2. Sample preparation for direct shear measurement.

soil samples were placed into the oven for 30 min. at 80 °C to destroy any micro-organisms that may affect the calcium carbonate precipitation process. After that, the organic soil was filled into the molds in a loose state having a dry density equal to 0.6 and 0.69 g/cm^3 for the consolidation tests and the direct shear tests, respectively. Each end of the samples was fitted with a filter paper, as shown in Fig. 1. The samples were initially treated by Bacterial media (*S. pasteurii*, urea, and $CaCl_2$) and then allowed to set for a minimum of 12 hours to allow the microbes to attach to the particles of the soil samples. Then, every 6 hours, nutrient treatments consisting of urea and $CaCl_2$ were injected through the specimens at the same flow rate of approximately 20 ml/min at a hydraulic head of 1 m. This nutrient treatment process was then periodically repeated for 4 days. During the treatment periods, the samples were incubated at 28 °C in a room prepared for this study. After each treatment period, the samples were cured at a room temperature of 25 °C for 5 days, and then consolidation and direct shear tests were performed on the samples. Throughout the treatment periods, the changes in the pH values were monitored at different time intervals. Also, at the end of all the

tests, the amounts of calcium carbonate in the samples were determined by a calcimeter instrument.

2.2.4. PH measurement

Changes in the pH values were monitored to determine the presence of biological activity (8.3–9.3) in the soil samples. The effluents of the samples were measured using a pH meter as a reassurance of calcite precipitating in the soil samples.

2.2.5. Strength and compressibility tests

The effect of BCCP on the compressibility and shear strength was determined by performing direct shear tests and one-dimensional consolidation tests. The consolidation tests were done according to ASTM D2435 for determining the magnitude and the rate of consolidation of the soil when it was restrained laterally and drained axially while being subjected to incrementally applied controlled-stress loading (11, 22, 45, 90, 180, and 360 kPa) and then unloading (180, 90, 45, 5, and 0 kPa). After setting the soil sample in the consolidation cell and applying each controlled-stress loading, the dial readings were taken for a period of 24 h at a range of times (0, 0.25, 0.5, 1, 2, 4, 8, 16, 30, 60, 120, 240, 480, 960, and 1440 min).

Direct shear tests were carried out according to ASTM D3080. This test method covers the determination of the consolidated drained (CD) shear strength of one specimen of a soil material under direct shear boundary conditions. CD means that shear was not started until after the settlement resulting from the applied normal load had stopped, and the shearing force was applied so slowly that no pore pressure developed in the sample. The specimen was deformed at a constant rate of displacement (0.02 mm/min) to make sure that the excess pore water pressure dissipated during the shearing process (Morgensters and Tchalenko, 1967; Dyvik et al., 1987). Direct shear tests were performed under various normal stresses (13.6, 20.4, and 27.2 kPa).

2.2.6. Calcium carbonate content measurement

In order to measure the amount of precipitated calcium carbonate, a calcimeter was used (Calcimeter Bernard). For this purpose, 1 g of a soil sample was treated with hydrochloric acid (HCL) in an enclosed reactor vessel. The basic principle for determining the amount of $CaCO_3$(%) is based on the volumetric analysis of the carbon dioxide CO_2 gas, which evolves during the reaction between the acid solution (HCl-4N) and the carbonate fraction of the soil specimen, as follows:

$$CaCO_3 + 2HCl_2 \rightarrow CaCl_2 + H_2O + CO_2\uparrow \quad (2)$$

The resulting pressure generated in the closed reactor is directly proportional to the carbonate content of the specimen. This pressure was measured with a bourdon tube pressure gauge, which was pre-calibrated with reagent grade $CaCO_3$ as

$$CaCO_3(\%) = [(1000 \times m_2 \times (V_1 - V_3))/(m_1 \times (V_2 - V_3))] \times [(100 + W)/100] \quad (3)$$

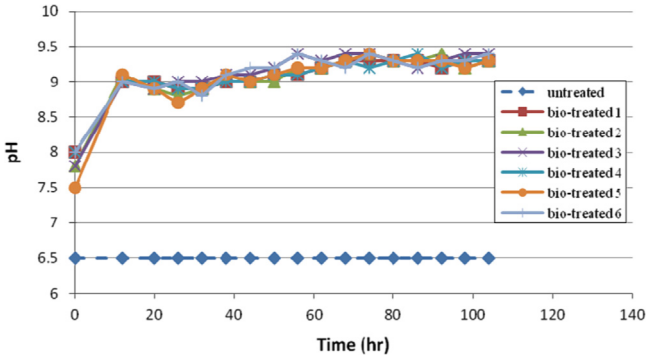

Fig. 3. Values of PH for treated and untreated organic soil samples.

where,

m_1: mass of soil sample (g)
m_2: standard mass of $CaCO_3$ (0.3 g)
V_1: volume reading of CO_2 in soil sample tube (ml)
V_2: average volume reading of CO_2 in standard tubes (ml)
V_3: average volume reading of CO_2 in reference tubes (ml)
W: water content (%)

3. Results and discussion

3.1. pH values in treated organic soils

When the organic soil samples were treated with the bacterial solution (bacteria, urea, and $CaCl_2$), the pH values were monitored to determine the presence of biological activity within the soil samples. Fig. 3 depicts the changes in the pH values over time. As can be seen in the figure, the pH values reached around 9.3 for organic soil samples after 12 h. According to Hammes and Verstraete (2002), the pH value was a key parameter in the $CaCO_3$ precipitation. A local rise in pH often causes the bacteria to serve as nucleation sites for crystallization. The decomposition of urea provides a high pH environment (Dejong et al., 2006). In the literature, the ideal range in pH values for the bacteria to precipitate calcite was reported to be between 8.3 and 9.3 (Stocks et al., 1999; Dejong et al. 2010). In the present study, the experimental results showed that the BCCP technique was applicable to organic soil.

3.2. Effect of BCCP on consolidation

Consolidation tests were undertaken using a conventional odometer device. The study focused on the primary consolidation behavior of untreated and treated organic soil samples. Four samples (untreated 1, bio-treated 1, bio-treated 2, and bio-treated 3) were prepared in a loose state having a dry density of 0.6 g/cm^3. The loose state was selected to show the effect of calcium carbonate precipitation on the compressibility behavior. The void ratios versus the log pressure curves of the samples are shown in Fig. 4. It is clear from these curves that the variations in void ratios with respect to entire stress levels are similar among the three bio-treated organic soil samples. This indicates that the treatment process used in this study is

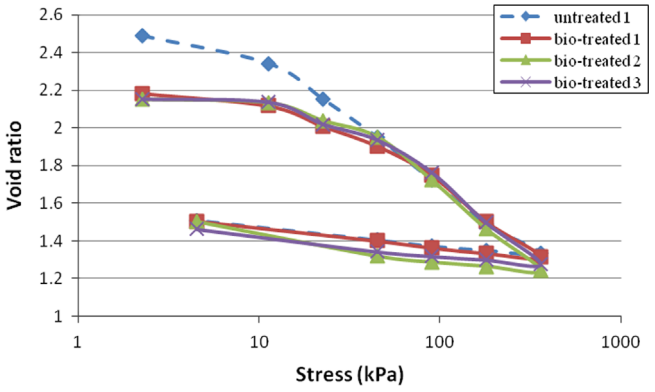

Fig. 4. Void ratio versus stress of treated and untreated organic soil samples.

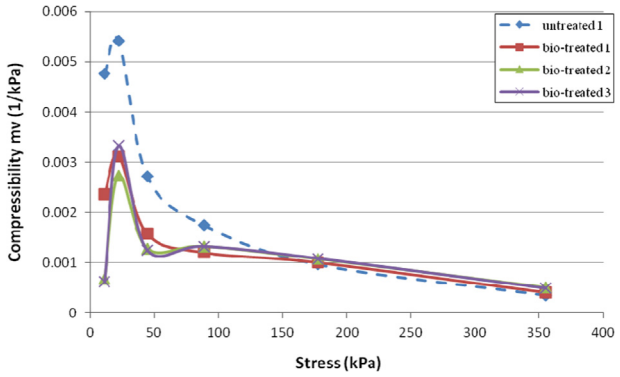

Fig. 5. Coefficient of compressibility of treated and untreated organic soil samples.

repeatable and consistent. Fig. 4 also shows the apparent difference in compressibility behavior between the treated and untreated samples. For instance, the change in void ratio for the treated samples was less than that for the untreated sample. Fig. 4 shows that the BCCP-treated samples have two consolidation yield stresses. On the other hand, the control sample has unique consolidation yield stress. It is believed that the BCCP-treated sample reaches its first consolidation yield point after breakage of the bond between the calcite crystals and the organic soil particles beyond 11 kPa. After this stress level, the solid calcite particles that filled the voids in the organic soil continue to take more stress with very little deformation until 45 kPa that seems to be the second consolidation yield stress. The untreated soil reaches its consolidation yield stress at nearly 11 kPa.

The coefficient of compressibility (mv) is generally used to calculate the vertical settlement of a soil layer having an initial thickness that is subjected to an increase in vertical stress. Fig. 5 shows the variation in mv under different levels of consolidation stress. The figure indicates that the mv values were higher for the untreated sample than for the treated samples. This indicates that the compressibility of the treated samples decreased when the voids in the organic soil filled with solid calcite particles that also caused cementation between the organic soil particles. After high stress levels, the coefficient of compressibility values for the untreated and treated soil samples became the same.

Table 5
Values of compression index and compression ratio under stress levels (11–45 kPa).

Sample	Cc	$Cc/(1+e_0)$
Untreated 1	0.6863	0.1966
Bio-treated 1	0.4482	0.1284
Bio-treated 2	0.5047	0.1446
Bio-treated 3	0.4567	0.1308

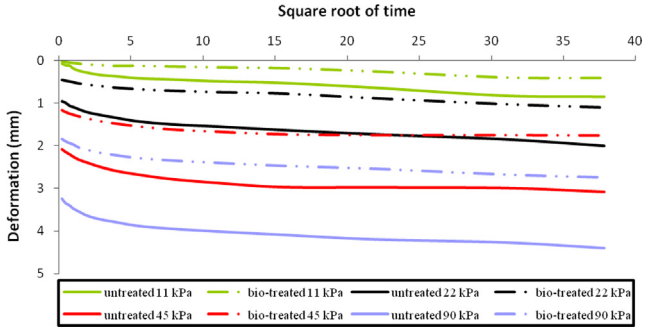

Fig. 6. Square root of time versus deformation for treated and untreated samples.

The compression index (Cc) and compression ratio $Cc/(1+e_0)$ of the treated and untreated samples were calculated. Table 5 shows the change in these values between stress levels of 11 kPa and 45 kPa. It is seen from the table that, at this range in stress levels, the compression index and the compression ratio of the treated samples were less than those of the untreated sample. On the other hand, beyond this range in stress levels, Cc and $Cc/(1+e_0)$ were similar for both the treated and untreated samples. The reduction in Cc and $Cc/(1+e_0)$ indicates that BCCP improves the compressibility behavior of the organic soil.

Fig. 6 and Table 6 show the square root of time vs the deformation graph and time for reaching the end of primary consolidation (t_{90}) for the treated and untreated samples for different stress levels. It can be noted from the graph that the end of the primary consolidation of the treated samples is longer than that of the untreated one. This increase can be attributed to the clogging effect of the BCCP process (Whiffin et al., 2007). The clogging of the pore spaces with the calcite particles reduced the dissipation of the water from the soil. This was supported by the permeability values of the treated and untreated samples derived from the odeometer tests. The permeability of the untreated sample was 5.2×10^{-3} cm/s and that of the treated samples was 4.5×10^{-4} cm/s. Fig. 7 shows the changes in the coefficient of consolidation (cv) with pressure. The effect of the calcite precipitation is clearly seen between the treated and untreated soil samples. For the same stress levels, the cv values for the treated samples are always less than those for the untreated one. This indicates that the BCCP process reduces the primary consolidation, but that the time for the completion of the primary consolidation increases.

Table 6
Values of square root of t_{90} under stress levels (11–180 kPa).

Pressure (kPa)	Square root of time (t_{90})	
	Untreated sample	Treated sample
11	2.1	3.2
22	1.9	2.5
45	1.8	2.1
90	1.5	1.9
180	1.35	1.9

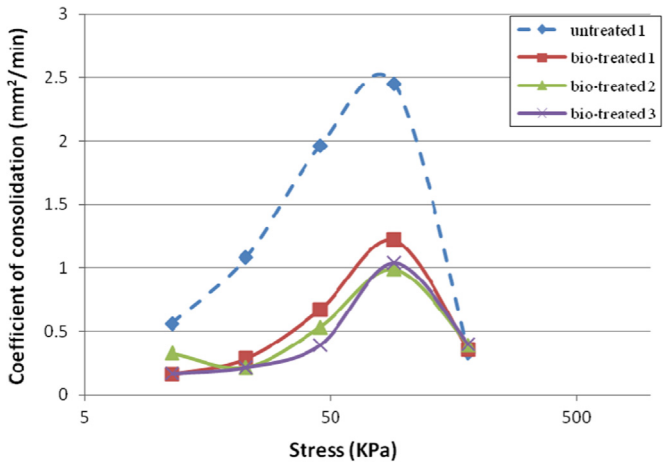

Fig. 7. Change in coefficient of consolidation (cv) with pressure for treated and untreated samples.

3.3. Effect of BCCP on shear strength

The shear strength of a soil is defined as the ultimate or the maximum shear stress that a soil can sustain. Cohesion and the angle of internal friction are the two inherent shear strength parameters of a soil. In order to compare these inherent properties of the treated and untreated soils, four samples (untreated 2, bio-treated 4, bio-treated 5, and bio-treated 6) were tested in the direct shear apparatus. Fig. 8 shows the shear stress versus horizontal displacement under different normal stress levels. It can be seen from the figure that the shear stress levels of the treated samples are higher than those of the untreated soil samples. Although the soil samples were prepared in their loose state, it was observed that the treated soil sample behaves like dense granular soils under low normal stress, as shown in Fig. 9. This may be attributed to the cementation of the soil particles by the BCCP process. Under high normal stress, the calcite bonds between the soil particles were broken; therefore, peak shear stress was not observed. The same behavior was also seen in the triaxial tests performed on the bio-cemented sand samples under high back pressure (Ayse, 2010).

Cohesion and the angle of internal friction were estimated at a 2-mm deformation where peak shear stress was observed in the treated soil sample, and 6-mm horizontal displacement was the maximum horizontal displacement attained in the tests. It can be seen from Table 7 that the cohesion values of the untreated sample are 8.4 kPa and 5.8 kPa at horizontal

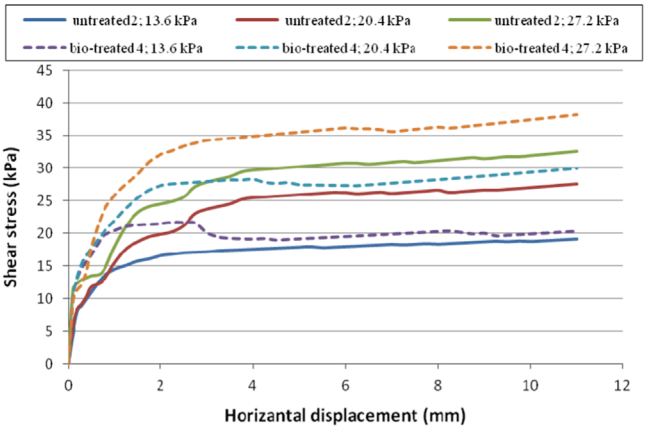

Fig. 8. Shear stress distribution versus horizontal displacement under different normal stresses.

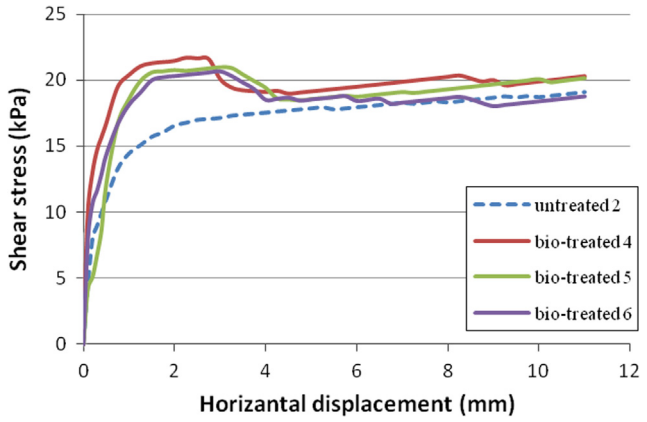

Fig. 9. Shear stress versus horizontal displacement under (13.6 kPa) normal stress.

Table 7
Shear strength parameters before and after BCCP at different horizontal displacements.

Horizontal displacement (mm)	Cohesion (kPa)		Angle of internal friction (degree)	
	Untreated	Treated	Untreated	Treated
2	8.4	11	30	38
6	5.8	2.7	43	50

displacements of 2 mm and 6 mm, respectively. These values are consistent with the literature (Edil and Wang, 2000; Huat et al., 2009). The internal friction angles of the untreated soil sample are 30° and 43° for horizontal displacements of 2 mm and 6 mm, respectively. These values are within the range reported by Mesri and Ajlouni (2007) and Landva and La Rochelle (1983). The wide range in internal friction angles of organic soil is attributed to the fiber content and its orientation (Mesri and Ajlouni, 2007). The test results showed that BCCP treatment changed the shear strength parameters of the organic soil. It is seen from Table 7 that the cohesion of the treated soil increased at a low displacement, but decreased at a high

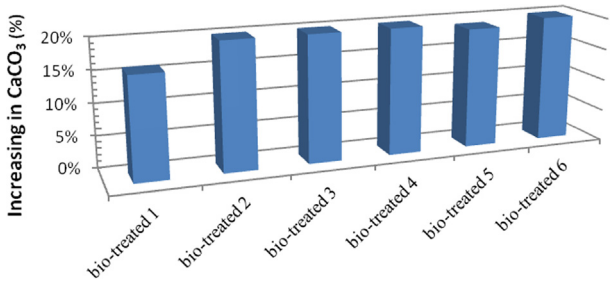

Fig. 10. Increase in $CaCO_3$ content in the treated organic soil samples.

horizontal displacement. The increase in cohesion at a low displacement can be attributed to cementation between the organic soil particles. While low cohesion values at a high horizontal displacement can be explained by the breakage of the cementation bonds. The BCCP treatment increased the internal friction angle of the organic soil for both low and high horizontal displacements. This relative increase can be explained by additional friction between the solid calcite particles precipitated in the organic soil. The change in shear strength seems to be limited. This may be improved with further work.

Although calcite was precipitated in the organic soil, its strength was very different than that of the sand reported by Van Paassen et al. (2009). The cementation with calcium carbonate in sandy soil binds the solid sand particles. However, the same calcium carbonate binds soft organic soil particles. When stress is applied to the sand sample, failure occurs either through strong calcite or sand particles. Therefore, high compressive strength is obtained in MICP-treated sandy soil (Van Paassen et al., 2009). On the other hand, failure occurs through weak organic particles or bonds between strong calcite and weak organic soil particles. Therefore, low strength is obtained in treated organic soil. Hence, it can be concluded that the failure of organic particles governs the strength of bio-cemented organic soil. For this reason, the strength obtained from organic soil is less than that obtained from sandy soil with same amount of calcite. This is also true for concrete. The compressive strength is controlled by both the strength of the aggregate and the strength of the paste, and it depends on which of these two fails first. Studies reported in the literature have shown that concrete containing organic content has lower strength. This is attributed to the failure of the weak points of the concrete matrix that are organic particles (Basri, et al., 1999; Alengaram et al., 2008; Mannan and Ganapathy, 2004).

3.4. Amount of $CaCO_3$ in organic soil

At the end of consolidation and the direct shear tests, the samples were used to measure the amount of precipitated $CaCO_3$ in the organic soil. Fig. 10 shows the changes in the amount of $CaCO_3$ content in all the treated organic soil samples. It can be seen from this figure that the amount of $CaCO_3$ increased by around 20% in the organic soil. This demonstrates that $CaCO_3$ precipitation occurs in organic soil. However, the amount of $CaCO_3$ was comparatively less in the organic soil than in the sandy soil (Sidik et al., 2013). This difference in calcite precipitation in organic soil and sandy soil can be attributed to soluble organic ligands and other organic matter that are well-known inhibitors of $CaCO_3$ precipitation and crystal growth (Lebron and Suarez, 1998). Researchers have related this inhibition mechanism to a number of factors. According to Lin and Singer (2005a), when the organic molecules are absorbed onto a mineral surface, they can either induce dissolution or impair crystal growth, depending on the saturation conditions. Other studies have proposed that the organic matter content prevents $CaCO_3$ precipitation by coating the existing $CaCO_3$ crystal surfaces, thus blocking their nucleation sites and preventing homogeneous crystal growth (Inskeep and Bloom, 1986a; Lebron and Suarez, 1996, 1998; Lin and Singer, 2005, 2006).

SEM images of the organic soil before and after treatment are shown in Fig. 11(a–b). The images revealed $CaCO_3$ crystals on the surface and pores of the organic soil particles. Figs. 12 and 13 show the EDX spectrum before and after

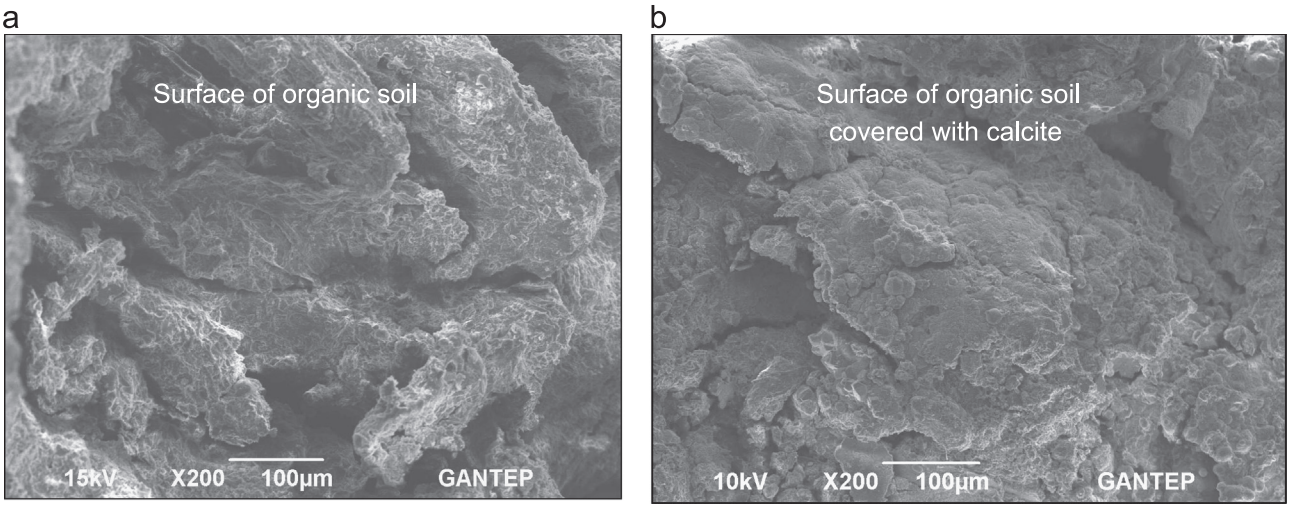

Fig. 11. SEM images of organic soil. (a) before BCCP treatment and (b) after BCCP treatment.

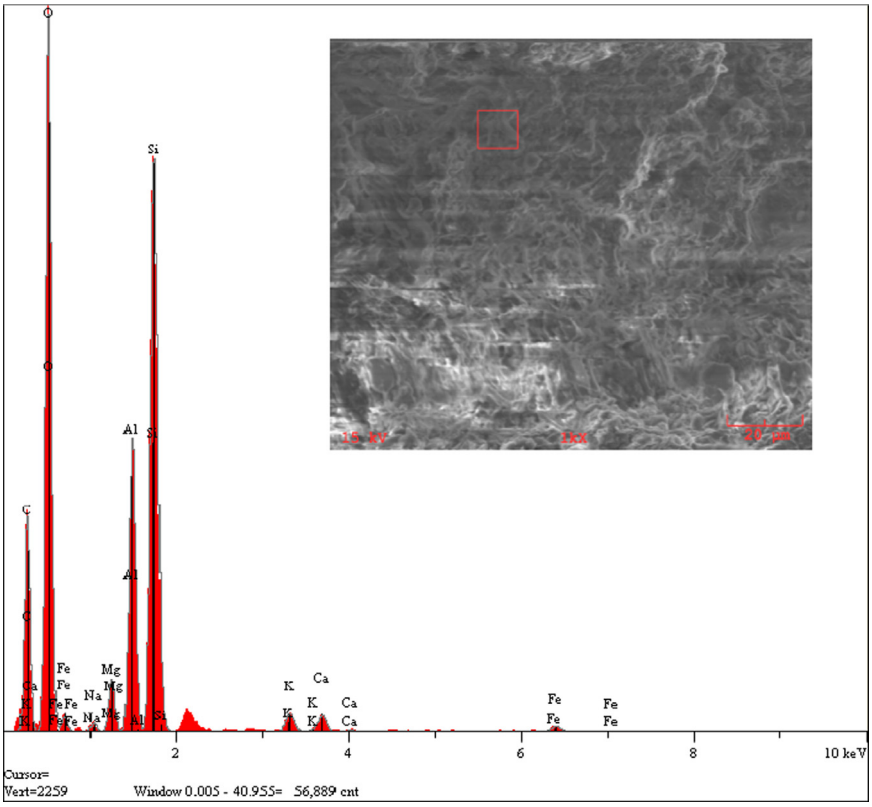

Fig. 12. EDX analysis of organic soil before BCCP treatment.

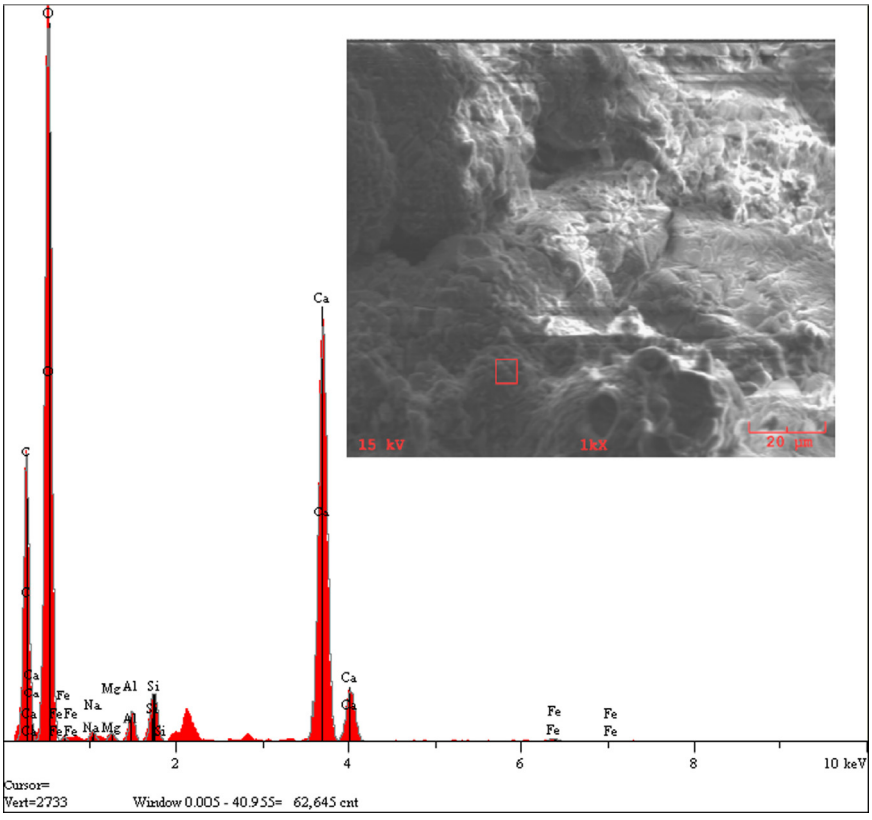

Fig. 13. EDX analysis of organic soil after BCCP treatment.

treatment of the organic soil. Fig. 12 reveals that the soil sample before treatment consisted mainly of Si, K, Al, Fe, and Mg that are the main minerals in any soil. An EDX spectrum taken after treatment clearly shows that the main component in the soil is Ca compared to the other minerals, as shown Fig. 13. This indicates that $CaCO_3$ particles formed in the organic soil.

4. Conclusions

The laboratory-scale experimental results in this study proved that bacterial calcite precipitation is feasible for organic soil. The test results showed that the shear strength and the compressibility behavior of organic soil can be improved by this new technique. The preliminary attempt made in this work revealed that the BCCP method increased the shear strength and reduced the compressibility of the organic soil. The findings of the study should encourage geotechnical engineers to use the BCCP technique as a new and alternative improvement method for organic soil. However, the use of this technique with this soil remains to be quantified in future studies.

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References

Alengaram, U.J., Jumaat, M.Z., Mahmud, H., 2008. Influence of cementitious material and aggregates content on compressive strength of Palm Kernel Shell concrete. J. Appl. Sci. 8 (18), 3207–3213.

Al Qabany, A., 2011. Microbial carbonate precipitation in soils (Doctoral dissertation). University of Cambridge, UK.

Al Qabany, A., Soga, K., 2013. Effect of chemical treatment used in MICP on engineering properties of cemented soils. Geotechnique 63 (4), 331–339.

Ayse, O., 2010. A Study on the Triaxial Shear Behavior and Microstructure of Biologically Treated Sand Specimens (A master thesis in Civil Engineering). University of Delaware.

Bachmeier, K.L., Williams, A.E., Warmington, J.R., Bang, S.S., 2002. Urease activity in microbiologically-induced calcite precipitation. J. Biotechnol. 93, 171–181.

Basri, H.B., Mannan, M.A., Zain, M.F.M., 1999. Concrete using waste oil palm shells as aggregate. Cem. Concr. Res. 29, 619–622.

Bang, S.S., Galinat, J.K., Ramakrishnan, V., 2001. Calcite precipitation induced by polyurethane-immobilized *Sporosarcina pasteurii*. Enzym. Microb. Technol. 28, 404–409.

Benini, S., Rypniewski, W.R., Wilson, K.S., Miletti, S., Ciurli, S., Mangani, S., 1999. A new proposal for urease mechanism based on the crystal structures of the native and inhibited enzyme from *Bacillus pasteurii*: why urea hydrolysis costs two nickels. Structure 7 (2), 205–216.

Çelik, F. Çanakcı, H., 2011. Shear strength properties of organic soil with sand column. In: Proceedings of the International Balkan Conference on Challenges of Civil Engineering, EPOKA University Tirane, Albania.

Chou, C.-W., Seagren, E.A., Aydilek, A.H., Lai, M., 2011. Biocalcification of sand through ureolysis. ASCE J. Geotech. Geoenviron. Eng. 137 (12), 1179–1189.

Ciurli, S., Benini, S., Rypniewski, W.R., Wilson, K.S., Miletti, S., Mangani, S., 1999. Structural properties of the nickel ions in urease: novel insights into the catalytic and inhibition mechanisms. Coordination. Chem. Rev. 190–192, 331–355.

Dejong, J.T., Fritzges, M.B., Nusslein, K., 2006. Microbially induced cementation to control sand response to undrained shear. J. Geotech. Geoenviron. Eng. 132, 1381–1392ASCE 1090-0241.

Dejong, J.T., Mortensen, B.M., Martinez, B.C., Nelson, D.C., 2010. Biomediated soil improvement. Ecol. Eng. 36 (2), 197–210.

DeJong, J.T., 2013. Biogeochemical processes and geotechnical applications: progress, opportunities and challenges. Geotechnique 63 (4), 287–301.

Douglas, S., Beveridge, T.J., 1998. Mineral formation by bacteria in natural Communities. FEMS Microb. Ecol. 26, 79–88.

Dyvik, R., Berm, T., Lacasse, S., Raadim, B., 1987. Comparison of truly undrained and constant volume direct simple shear tests. Geotechnique 37 (1), 3–10.

Edil, T.B., 2003. Recent advances in geotechnical characterization and construction over peats and organic soils. In: Proceedings of the 2nd International Conference in Soft Soil Engineering and Technology, Putrajaya (Malaysia), pp. 1–16.

Edil, T.B., Wang, X., 2000. Shear strength and Ko of peats and organic soils. In: Edil, T.B., Fox, P.J. (Eds.), Geotechnics of Highwater Content Materials, 1374. ASTM Special Technical Publication, USA, pp. 209–225.

Furstenberg, A., Lechowicz, Z., Szymanski, A., Wolski, W., 1983. Effectiveness of Vertical drains in organic soils. Improv. Ground 2, 611–616.

Gollapudi, U.K., Knutson, C.L., Bang, S.S., Islam, M.R., 1995. A new method for controlling leaching through permeable channels. Chemosphere 30, 695–705.

Huat, A.A., Bujang, B.K., Sina, K., 2009. Experimental investigation on geomechanical properties of tropical organic soils and peat. Am. J. Eng. Appl. Sci. 2 (1), 184–188.

Hamdan, N., Kavazanjian, E. Jr., Rittmann, B.E., Karatas, I., 2011b. Carbonate mineral precipitation for soil improvement through microbial denitrification. In: Proceedings of the GeoFrontiers 2011: Advances in Geotechnical Engineering, Dallas, TX, ASCE Geotechnical Special Publication 211, pp. 3925–3934.

Hammes, F., Verstraete, W., 2002. Key roles of pH and calcium metabolism in microbial carbonate precipitation. Rev. Environ. Sci. Biotechnol. 1, 3–7.

Hampton, M.B., Edil, T.B., 1998. Strength gain of organic ground with cement-type Binders. Soil Improv. Big Digs 81, 135–148.

Inskeep, Bloom, P.R., 1986a. Calcium carbonate supersaturation in soil solutions of Calciaquolls. Soil Sci. Soc. Am. J. 50 (6), 1431–1437.

Ivanov, V., Chu, J., 2008. Applications of microorganisms to geotechnical engineering for bioclogging and biocementation of soil in situ. Rev. Environ. Sci. Biotechnol. 7, 139–153.

Jelisic, N., Leppanen, M., 2003. Mass stabilization of peat in road and railway Construction. Geotech. Special publ. 1201, 552–561.

Jonkers, H.M., Thijssen, A., Muyzer, G., Copuroglu, O., Schlangen, E., 2009. Application of bacteria as self-healing agent for the development of sustainable concrete. Ecol. Eng. 12 (36), 3–6. http://dx.doi.org/10.1016/j.ecoleng.2008.

Karatas, I., Kavazanjian, E. Jr., Rittmann, B.E., 2008. Microbially induced precipitation of calcite using Pseudomonas denitrificans. In: Proceedings of the 1st International Conference on Biogeotechnical Engineering, Delft (CDROM).

Karol, R.H., 2003. Chemical Grouting and Soil Stabilization. Marcel Dekker, New York, 558.

Koda, E., Szymanski, A., Wolski, W., 1989. Behavior of geodrains in organic subsoil. Proceedings, 2, pp. 1377–1380.

Landva, A.O., La Rochelle, 1983. Compressibility and shear characteristics of radforth Peats. In: Jarett, P.M. (Ed.), Testing of Peat and Organic Soils (ed.) ASTM STP 820. American Society for Testing and MaterialsAnn Arbor, Michigan USA, Philadelphia, PA, pp. 157–191.

Lebron, Suarez, 1996. Calcite nucleation and precipitation kinetics as affected by dissolved organic matter at 25. and pH > 7.5. Geochim. Cosmochim. Acta 60 (15), 2765–2776.

Lebron, Suarez, 1998. Kinetics and mechanisms of precipitation of calcite as affected by PCO_2 and organic ligands at 25 °C. Geochim. Cosmochim. Acta 62 (3), 405–416.

Lin, Singer, 2005a. Inhibition of calcite crystal growth by polyphosphates. Water Res. 39 (19), 4835–4843.

Lin, Singer, 2006. Inhibition of calcite precipitation by orthophosphate: speciation and thermodynamic considerations. Geochim. Cosmochim. Acta 70 (10), 2530–2539.

Lo Bianco, A., Madonia, G., 2007. B.U.L.M. technique for increase of the bearing capacity in the pavement layers subjected to biological treatment. University of Palermo, 4th International Siiv Congress, Palermo (Italy).

Madigan, M.T., Martinko, J.M., 2003. Brock Biology of Microorganisms, 11th ed. Prentice-Hall, Upper Saddle River, N.J.

Martinez, B.C., DeJong, J.T., Ginn, T.R., Mortensen, B.M., Barkouki, T.H., Hunt, C., Tanyu, B., Major, D., 2013. Experimental optimization of microbial induced carbonate precipitation for soil improvement. ASCE J. Geotech. Geoenviron. Eng. 139 (4), 587–598.

Mannan, M.A., Ganapathy, C., 2004. Concrete from an agricultural waste-oil palm shell (OPS). Build. Environ. 39, 441–448.

Mesri, G., Ajlouni, M., 2007. Engineering properties of fibrous peats. J. Geotech. Geoenviron. Eng. 133 (7), 850–866.

Mitchell, A.C., Ferris, F.G., 2006. The Influence of *Sporosarcina pasteurii* on the Nucleation and Growth of Calcium Carbonate. Geomicrobiol. J. 23, 213–226.

Morgensters, N.R., Tchalenko, J.S., 1967. Microscopic structures in kaolin subjected to direct shear. Geotechnique 17, 309–328.

Mortensen, B.M., DeJong, J.T., 2011. Strength and stiffness of MICP treated and subjected to various stress paths. In: Proceedings of the GeoFrontiers 2011: Advances in Geotechnical Engineering, Dallas, TX, ASCE Geotechnical Special Publication 211, pp. 4012– 4020.

Ramachandran, S.K., Ramakrishnan, V., Bang, S.S., 2001. Remediation of concrete using micro-organisms. ACI Mater. J. 98, 3–9.

Ramakrishnan, V., 2007. Performance characteristics of bacterial concrete-a smart biomaterial. In: Proceedings of the First International Conference on Recent Advances in Concrete Technology, Washington, DC, pp. 67–78.

Roden, E.E., Leonardo, M.R., Ferris, F.G., 2002. Immobilization of strontium during iron biomineralization coupled to dissimilatory hydrous ferric oxide reduction. Geochim. Cosmochim. Acta. 66 (16), 2823–2839.

Rusu, C., Cheng, X., Li, M., 2011. Biological clogging in Tangshan sand columns under salt water intrusion by *Sporosarcina pasteurii*. Adv. Mater. Res. 250–253, 2040–2046.

Sarda, D., Choonia, S., Sarode, D.D., Lele, S.S., 2009. Biocalcification by B*acillus pasteurii* urease: a novel application. J. Ind. Microbiol. Biotechnol. 36, 1111–1115.

Sidik, W., Canakci, H., Kilic, H., Celik, F., Bakteriyel Kalsiyum Karbonat Olusumu Uzerinde Kumlu Zeminlerin Dane Capinin Etkisi. 5. Geoteknik Sempozyumu 5–7 Aralik 2013 2013, Cukurova Universitesi Adana, Turkey.

Stocks, F.S., Galinat, J.K., Bang, S.S., 1999. Microbiological precipitation of $CaCO_3$. Soil Biol. Biochem. 31 (11), 1563–1571.

Van der Star, W.R.L., Van Wijngaarden, W.K., Van Paassen, L.A., Van Baalen, L.R., Van Zwieten, G., 2011. Stabilization of Gravel Deposits using Microorganisms85–90.

Van Paassen, L.A., Harkes, M.P., van Zwieten, G.A., van der Zon, W.H., van der Star, W.R.L., van Loosdrecht, M.C.M., 2009. Scale up of BioGrout: a biological ground reinforcement method. In: Proceedings of the 17th International Conference on Soil Mechanics and Geotechnical Engineering, Alexandria, Egypt, pp. 2328–2333.

Van Paassen, L.A., Daza, M.C., Sorokin, D.Y., Van der Zon, W., Van Loosdrecht, M.C., 2010a. Potential soil reinforcement by biological denitrification. Ecol. Eng. 36, 168–175.

Van Paassen, L.A., Ghose, Ranajit, Van der Linden, T.J.M., Van der Star, W. R.L., Van Loosdrecht, M.C.M., 2010b. Quantifying biomediated ground improvement by ureolysis: large-scale biogrout experiment. J. Geotech. Geoenviron. Eng. 136 (12), 1721–1728.

Van Wijngaarden, W.K., Vermolen, F.J., Van Meurs, G.A.M., Vuik, C., 2010. Modelling biogrout: a new ground improvement method based on microbial-induced carbonate precipitation. Trans. Porous Med.http://dxdoi.org/10.1007/s11242-010-9691-8.

Warthmann, R., van Lith, Y., Vasconcelos, C., McKenzie, J.A., Karpoff, A.M., 2000. Bacterially induced dolomite precipitation in anoxic culture experiments. Geology 28 (No. 12), 1091–1094.

Weaver, T., Burbank, M., Lewis, R., Lewis, A., Crawford, R., Williams, B., 2011. Bio-induced calcite, iron, and manganese precipitation for geotechnical engineering applications. In: Proceedings of the GeoFrontiers 2011: Advances in Geotechnical Engineering, Dallas, TX, ASCE Geotechnical Special Publication 211, pp. 3975– 3983.

Wüst, R.A.J., Bustin, R.M., Lavkulich, L.M., 2003. New classification systems for tropical organic-rich deposits based on studies of the tasek Bera Basin, Malaysia. Catena 53, 133–163.

Whiffin, V.S., van Paassen, L.A., Harkes, M.P., 2007. Microbial carbonate precipitation as a soil improvement technique. Geomicrobiol. J. 25 (5), 417–423.

Yang, D.S., Yagihashi, J.N., Yoshizawa, S.S., 1998. Stabilization of very soft soils and organic soils. Soil Improv. Big Digs 81, 96–110.