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Applicability of biocementation for organic soil and its effect on permeability

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Abstract. In past few years, the use of bacterial calcium carbonate precipitation (*biocementation*) has become popular as a ground improvement technique for sandy soil. However, this technique was not applied to organic soil. This study focused on bacterial calcium carbonate precipitation and its effect on permeability in organic soil. A special injection system was prepared for inducing bacterial solution to the samples. The bacterial solution supplied to the samples by gravity for 4 days in specific molds designed for this work. Calcite precipitation was observed by monitoring pH value and measuring amount of calcium carbonate. Change in the permeability was measured before and after *biocementation*. The test results showed that the pH values indicates that the treatment medium is appropriate for calcite precipitation, and amount of precipitated calcium carbonate in organic soil increased about 20% from untreated one. It was also found that the *biocementation* can be considered as an effective method for reducing permeability of organic soil. The results were supported by Scanning electron microscopy (SEM) analysis and energy-dispersive x-ray (EDX) analysis.

Keywords: bacillus pasteurii; organic soil; biocementation; pH distribution; calcimeter test; permeability of organic soil; scanning electron microscope; -energy-dispersive x-ray-

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 soil significantly affects its geotechnical properties including specific gravity, water content, liquid limit, plastic limit, density, hydraulic conductivity, compressibility, and strength. In order to improve geotechnical properties of organic soils different improvement techniques were used (Hampton and Edil 1998, Edil 2003, Çelik and Çanakcı 2010, 2011). Hampton and Edil (1998), Jelisic and Leppanen (2003), and Hebib and Farrell (2003) used deep mixing, cementations materials such as lime or cement blended into soils used to improve geotechnical properties of organic soil. Koda *et al*. (1989) and Furstenberg *et al*. (1983) were used vertical drains to improve compressibility and strength of organic soil. Dry jet mixing and sand columns methods developed by Yang *et al*. (1998) as improvement technique

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to reduce compressibility, increase the strength and reduce the permeability of organic soil.

In past few years, the use of bacterial calcium carbonate ($CaCO_3$) precipitation (*biocementation*) has become popular as a ground improvement technique, with *biocementation* presented as a new and environmentally friendly method (Dejong *et al*. 2006). The main role of bacteria in the precipitation process has been attributed to their ability to generate an alkaline environment throughout different physiological actions (Douglas and Beveridge 1998). Considerable research on carbonate precipitation by bacteria has been done using ureolytic bacteria (Stocks *et al*. 1999). These bacteria are able to influence the precipitation of $CaCO_3$ by the production of the urease enzyme. This enzyme catalyzes the hydrolysis of urea to CO_2 and ammonia, resulting in an increase in the pH and carbonate concentration in the bacterial environment (Stocks *et al*. 1999).

The application of bacterial calcium carbonate precipitation or cementation has been used in variety of geotechnical engineering applications, such as: cracks repair in granite and concrete (Gollapudi *et al*. 1995, Ramachandran *et al*. 2001, Bang *et al*. 2001, Ramakrishnan 2007, Jonkers *et al*. 2009), improving the bearing capacity of soil (Lo Bianco and Madonia 2007, Dejong *et al*. 2006), pore filling and particle binding (Ivanov and Chu 2008) filling pores and reducing permeability (Whiffin *et al*. 2007). The most of earlier *biocementation* applications, bacteria called *Bacillus pasteurii* played an important role in $CaCO_3$ precipitation (Bang *et al*. 2001, Dejong *et al*. 2006, 2010). It exhibits high urease production. Hence, it has been used for microbial calcite cementation in many studies (Bachmeier *et al*. 2002, Sarda *et al*. 2009).

Permeability is an important soil parameter for any project where flow of water through soil is a matter of concern for example, seepage through or under a dam and drainage from sub grades or backfills. Previous research involving *biocementation* treatments have primarily been focused on reducing the permeability of porous media. *Biocementation* coats or bridges individual soil particles, gradually reduce the pore size within the soil fabric and thereby reduces the hydraulic conductivity. Whiffin *et al*. (2007) observed that biocementation in sandy soil reduced permeability significantly. Ferris *et al*. (1996) also observed nearly 20% reduction in permeability of sandy soil after calcite precipitation. Nemati and Voordouw (2003) created calcite cementation within a Berea sandstone core enzymatically and reduced the permeability by 98%. Nelson and Launt (1991) suggested a Microbially enhanced oil recovery process that uses bacteria to plug highly permeable soil deposits in an effort to seal off water-bearing zones and improve the yield of reservoir oil.

In all previous studies, the *biocementation* technique was used in attempt to improve geotechnical properties of sandy soil. The information in the literature on the application of *biocementation* in organic soil is very limited. This study focuses on investigation of applicability of calcite precipitation, and its effect primarily on permeability properties of organic soil. *B. pasteurii* a non-pathogenic organisms that is found naturally in soil, was used throughout the study.

2. Materials and methods

2.1 Microorganism

An isolated bacterial culture of *B. pasteurii NCIMB 8221* was used in this study. The bacteria were purchased from NCIMB Ltd, UK., a microbiology and chemical analysis company that houses the biggest reference collection of industrially and environmentally valuable micro-

organisms in the country.

2.2 Organic soil

The organic soil used in this study was obtained from the Sakarya region, Turkey. This organic soil is classified as peat (Wüst *et al*. 2003). Some chemical and physical properties of the organic soil used in the tests are given in Table 1. In all the tests, fibrous peat samples that remain on #100 (0.15 mm) sieve size were used. The soil was placed in a firing oven at 440°C for 4 h, and the organic content was estimated according to ASTM D 2974 standard. According to the ASTM D 2974 standard, the ash content of the soil was % 40. Wet sieve analysis of the ash showed that the soil contained 15% silt and clay, 25% sand, and 60% organic materials. Liquid limit of the organic soil was estimated by fall cone test and found to be % 125.

2.3 Preparation of urea nutrient agar

Urea nutrient agar was used for the cultivation of B. pantothenticus. Table 2 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 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

Table 1 Engineering properties of the organic soil used in the study

Properties of organic soil	Content (%)
Organic content (%)	50-70
pH	4.5-6.5
Organic carbon (%)	20-30
Water keeping capacity, (in volume, %)	85-95
Natural water content (%)	256
Liquid limit (%)	125
Plastic limit (%)	None plastic
Specific gravity (g/cm^3)	1.97

Table 2 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*	50 mL

*urea solution (Add 20 g urea to distilled water and bring the volume to 100 mL. Mix well. Filter sterilize)

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 *B. pasteurii* cultures.

2.4 Making the bacteria culture

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 *B. pasteurii* had occurred, each of the single resulting colonies was transferred from the plate to a 250 ml culture flask containing 200 ml urea nutrient broth. The culture flask was then wrapped using cheesecloth, to filter 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 minutes 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.

2.5 Bacteria counting

DensiCHEK Plus 21255-P1ML1 instrument was used to measure the optical density of a microorganism suspension Fig. 1. The instrument provides values in McFarland units, proportional to microorganism concentrations. The device indicated for use with polystyrene and glass test tubes and the reading range is 0.0-4.0 McFarland. It generates a McFarland value using basic colorimetry, 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.

2.6 The preparing for bacterial treatment solution

To prepare the bacterial treatment solution, urea medium was first created. Nutrient broth (3 g),

Fig. 1 DensiCHEK Plus instrument used to measure the optical density of a microorganism suspension

urea (20 g), NH4Cl (10 g), NaHCO3 (2.12 g), 500 mL distilled water Table 3, were mixed to create the urea medium solution. Each of the solid ingredients were mixed thoroughly in 500 mL of distilled water until they dissolved, and the pH of the resulting urea medium solution was adjusted to 6.0 using with 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 resulting 1 L of solution was adjusted by stirring the solution to aerate it, until the pH was increased from an initial value of approximately 7.0 to approximately 8.0, as measured using a pH meter. This 1 L of aerated solution was divided into two approximate portions, one 800 mL and one 200 mL. Spun *B*. *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. 500 mL of the combination solution consisting of urea, calcium chloride, and *B*. *pasteurii* cells was injected into the base of the specimens by gravity at a flow rate of approximately 20 mL/min at a hydraulic head 1 m. 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 consisting of Urea and $CaCl_2$ were

Table 3 Details of the treatment solution

Agar components	Constituents	Amount
Urea medium	nutrient broth powder	3 g
	Urea ($NH2(CO)NH_2$	20 g
	NH_4Cl	10 g
	$NaHCO_3$	2.12 g
	Distilled water	1 L
calcium chloride solution	$CaCl_2.2H_2O$	18.5 g $CaCl_2$ / 100 mL distilled water

Fig. 2 Special system created for bacterial treatment

injected through the specimens at the same flow rate. This nutrient treatment process was then periodically repeated (every 6 hours) for 4 days. Fig. 2 shows the special system created for bacterial treatment.

2.7 Sample preparation

The samples were prepared according to the Permeability test. Samples (control, bio 1, bio 2, bio 3) prepared in cylindrical molds have a diameter of 75 mm and height of 150 mm Fig. 3. All soil samples were placed into the oven for 30 min. at 80°C to execute any micro organisms that may affect calcium carbonate precipitation process. After that the organic soil filled in to molds in loose state having dry density equal to 0.68 gm/cm^3. Each ends of the samples were fitted with filter paper shown in Fig. 3. These soil samples then injected by bacterial medium (*B. pasteurii*, urea, and $CaCl_2$) and left in solution for 12 hours. The samples were incubated at 28°C. Then the samples were treated by urea medium and $CaCl_2$ every 6 hours period. After treatment period the samples cured for five days and then tested by permeability apparatus. Throughout the treatment time change in pH values were monitored at different time intervals. Also at the end of tests the amount of calcium carbonate in the samples were determined by using calcimeter instrument. The results were supported by Scanning electron microscopy (SEM) analysis and energy-dispersive x-ray (EDX) analysis.

2.8 Changes in pH values

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

2.9 Permeability test

Permeability refers to the porosity of a material to allow fluid to move through its pores. Permeability is an important soil parameter for any project where flow of water through soil is a

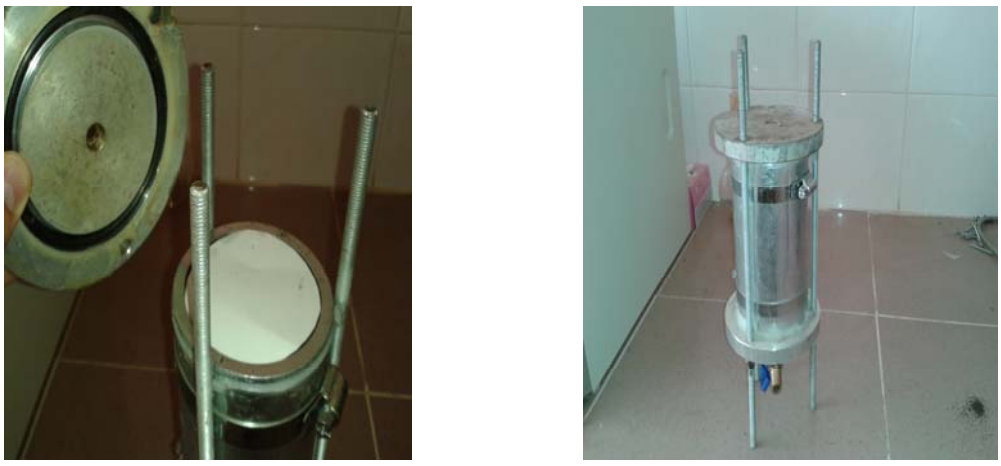

Fig. 3 Sample preparation for permeability measurement

Fig. 4 The calcimeter instrument

matter of concern for example, seepage through or under a dam and drainage from sub grades or backfills. In this study falling-head method, according to ASTM D 2435 was used to test the effect of *biocementation* on the permeability of organic soil.

2.10 Calcium carbonate content

The soil (1 g) was treated with hydrochloric acid (HCl) in an enclosed reactor vessel. Carbon dioxide gas is evolved during the reaction between the acid and carbonate fraction of the specimen. The resulting pressure generated in the closed reactor is directly proportional to the carbonate content of the specimen Fig. 4. This pressure was measured with a bourdon tube pressure gauge, which pre-calibrated with reagent grade $CaCO_3$. After 4 days of the treatment, a calcimeter was used to determine the amount of $CaCO_3$ in the soil.

2.11 SEM and EDX analysis

The scanning electron microscope used for analyze the crystals precipitated in organic soil samples. At room temperature the samples dried, and then tested at accelerating voltages 15 kV by SEM. The samples were gold coated with a sputter coating Emitech K575 prior to the SEM analysis. For further characterizing the mineral constituents of the crystals tested by EDX analysis.

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, $CaCl_2$), the pH values were monitored to determine the presence of biological activity within the soil

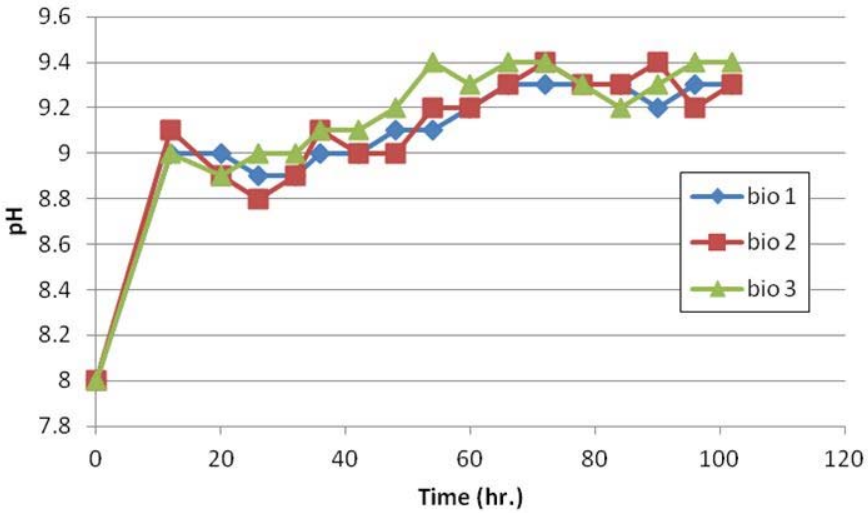

Fig. 5 Values of PH for organic soil samples

samples. Fig. 5 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 $CaCO_3$ 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 of pH values for 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 *biocementation* technique was applicable in organic soil.

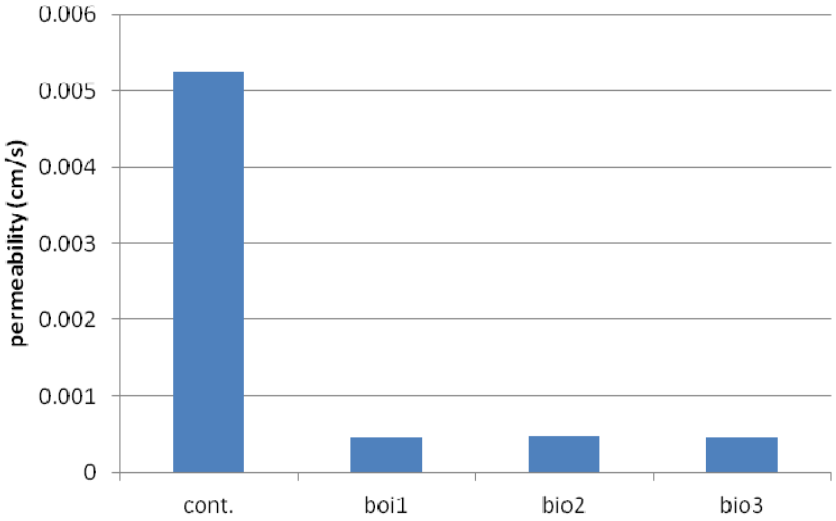

Fig. 6 Decreasing in the permeability of treated organic soil samples

3.2 Effect of biocementation on permeability

Treatment techniques can result in the improvement of a variety of soil properties including permeability. Fig. 6 shows change in permeability of treated organic soil samples (bio1, bio2 and bio3) and control sample. As it is seen from the graph calcite precipitation caused nearly 10-fold reduction in permeability (from 5.2×10^{-3} cm/s to 4.5×10^{-4} cm/s). Table 4 shows permeability and drainage characteristics of soils according to Casagrade and Fadum (1940). The test results indicates that the coefficient of permeability category of treated organic soil samples changed from good to poor. *Biocementation* occurs in pores within soil particles, reducing the pore throats and subsequently preventing water flow (Whiffin *et al*. 2007). According to Gollapudi *et al*. (1995) the microbial process was more productive in the presence of pores, which provided more nucleation sites for the bacteria. The formation of calcites in the pores reduced the flow rate in the voids. Test results also showed that calcite precipitation was not uniform throughout the soil sample. This was confirmed by measuring amount of calcite at different interval in the soil samples. This was discussed in the following section.

3.3 Amount of $CaCo_3$ in organic

At the end of 4 days of treatment, the samples were tested with a calcimeter to observe changes in amount of $CaCO_3$ in the organic soil. Fig. 7 shows the change in $CaCO_3$ content in the organic soil samples. It can be seen from the Fig. 7 that the amount of $CaCO_3$ increased 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 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 (Lebrón and Suárez 1998). Researchers have related this inhibition mechanism to a number of factors. According to Lin and Singer (2005), when the organic molecules are absorbed onto a mineral surface, depending on the saturation conditions, they can either induce dissolution or impair crystal growth. Other studies proposed that the organic matter content prevents $CaCO_3$

Table 4 Permeability and drainage characteristics of soils by Casagrade and Fadum (1940)

<table>
<tr><td></td><td colspan="12">Coefficient of permeability k (cm/s) (log scale)</td></tr>
<tr><td></td><td colspan="12">10^{2} 10^{1} 1.0 10^{-1} 10^{-2} 10^{-3} 10^{-4} 10^{-5} 10^{-6} 10^{-7} 10^{-8} 10^{-9}</td></tr>
<tr><td>Drainage</td><td colspan="6">Good</td><td colspan="2">Poor</td><td colspan="4">Practically Impervious</td></tr>
<tr><td rowspan="2">Soil types</td><td colspan="3" rowspan="2">Clean gravel
Clean sands, clean sand and gravel mixures</td><td colspan="3">Very fine sands, organic and inorganic silts, mixtures of sand silt and clay, glacial till, stratified clay deposits, etc.</td><td colspan="4" rowspan="2">“Impervious” soils, e.g., homogeneous clays below zone of weathering</td></tr>
<tr><td colspan="3">“Impervious” soils modified by effects of vegetation and weathering</td></tr>
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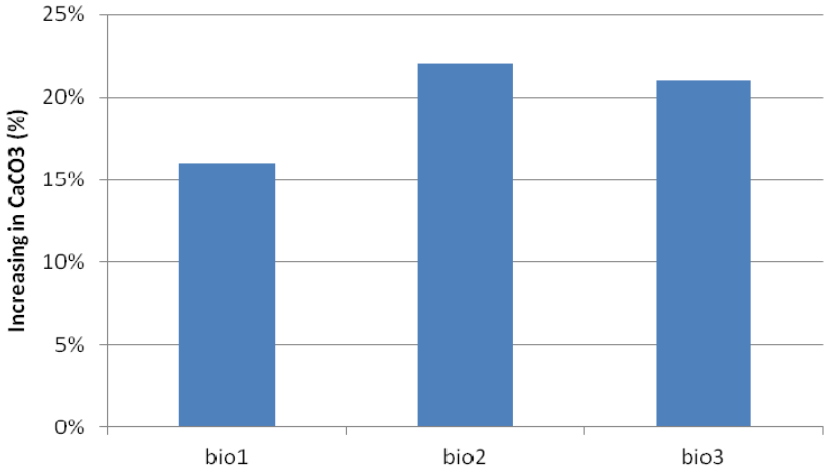

Fig. 7 Increase in $CaCO_3$ content in the treated organic soil**s**

precipitation by coating existing CaCO3 crystal surfaces, thus blocking their nucleation sites and preventing homogeneous crystal growth (Inskeep and Bloom 1986, Lebrón and Suárez 1996, 1998, Lin and Singer 2005, 2006).

Chemical reactions during *biocementation* in soils are modulated by the soil structure. The soil pore network resulting from the soil structure influences the kinetics of a reaction principally by regulating the diffusion of reactants to reaction sites and by providing reaction surfaces (Pinner and Nye 1982). The cell diameter of bacteria is usually in the range of 0.5 to 3 μm, and bacterial spores, stress resistant resting stages of some species, may be as small as 0.2 μm (Madigan and Martinko 2003). Microorganisms are capable of moving freely in the pore spaces of coarse-grained materials, either by self-propelled movement or by passive diffusion. However, the smaller pore spaces of finer grained soils prohibit the entry and free movement of microorganisms. Therefore, bacteria are not expected to enter through pores smaller than approximately 0.4 μm (Mitchell and Santamarina 2005). The pore network in organic soil is complex (Stevenson 1994). It may affect the passage of microorganisms in the soil and reaction surfaces. As a result, the amount of $CaCO_3$ precipitation in organic soils, however 19% increasing in $CaCO_3$ reduced permeability of organic soil.

In previous studies researchers also investigated variation in precipitated $CaCo_3$ along the treated soil specimen. Same investigated was also carried out in this study. For this purpose amount of $CaCo_3$ was measured at five different points after permeability measurement was completed. The first measurement was made from a sample taken directly from the injection point. The second measurement was made 25 mm from injection point, and the rest three measurements were made 50 mm intervals. Fig. 8 Shows change in calcite precipitation along the specimen.

As can be seen from the figure that the profile of calcium carbonate was not homogeneous over the specimen length. The highest amounts of calcium carbonate precipitation were obtained at the injection point. The amount of $CaCo_3$ reduces with distance from injection point. This can be attributed to the slow flow rates of injection. Due to this slow rate of treatment the bottom of the soil specimen closest to the injection point was exposed to significantly bacterial treatment solution than the top of the soil specimen. In order to produce a more homogeneous result, the

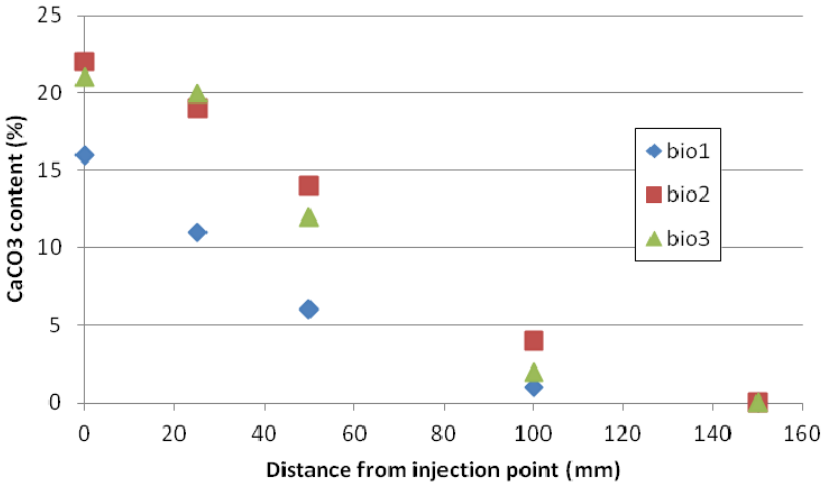

Fig. 8 increase in $CaCO_3$ content in the treated organic soils

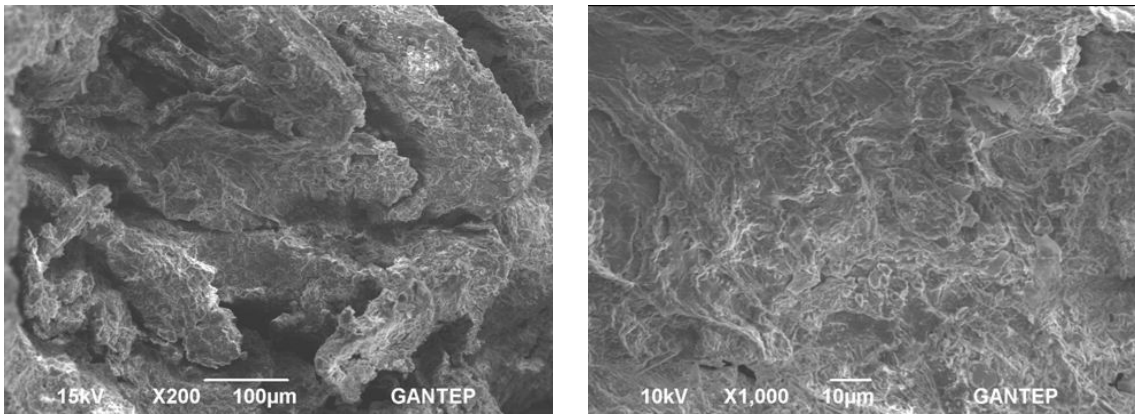

Fig. 9 SEM images of organic soil before *biocementation* treatment

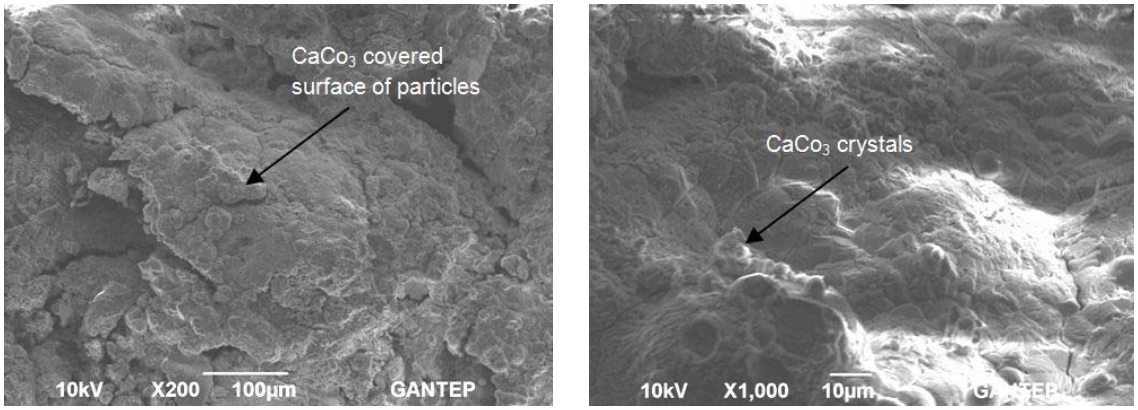

Fig. 10 SEM images of organic soil before *biocementation* treatment

balance between supply and conversion needs be shifted (Whiffin *et al*. 2007).

3.4 SEM images for calcium carbonate crystals

SEM images of the organic soil before and after the treatment are given in Figs. 9-10. These images reveals that $CaCO_3$ crystals precipitated on the surface and pores of the organic soil particles. SEM pictures Fig. 10 shows that the bacteria served as the nucleation sites for the mineralization process (Achal *et al*. 2009).

3.5 Energy-dispersive X-ray (EDX) analysis

The organic soil samples were analyzed by EDX before and after *biocementation* treatment. Figs.11-12 display the elemental spectral analysis for organic soil samples before and after

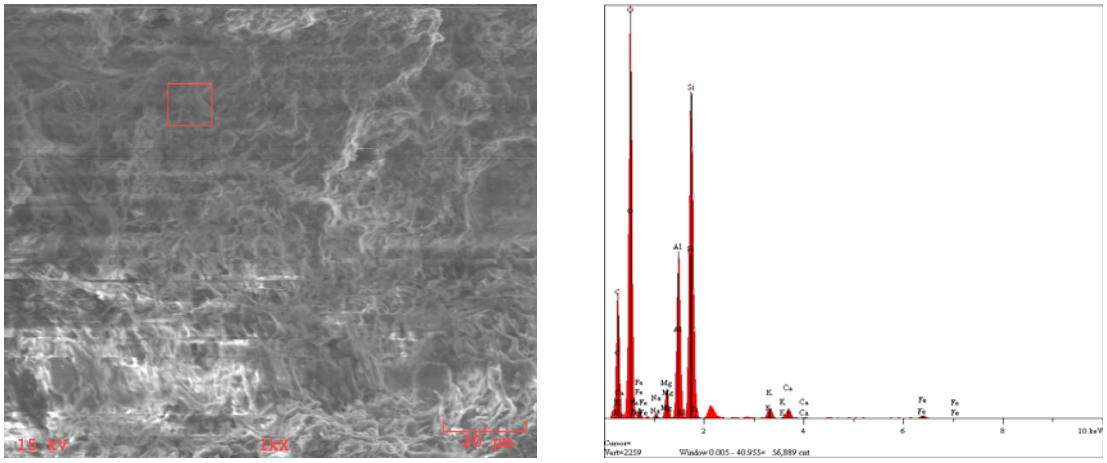

Fig. 11 EDX analysis of organic soil after *biocementation* treatment

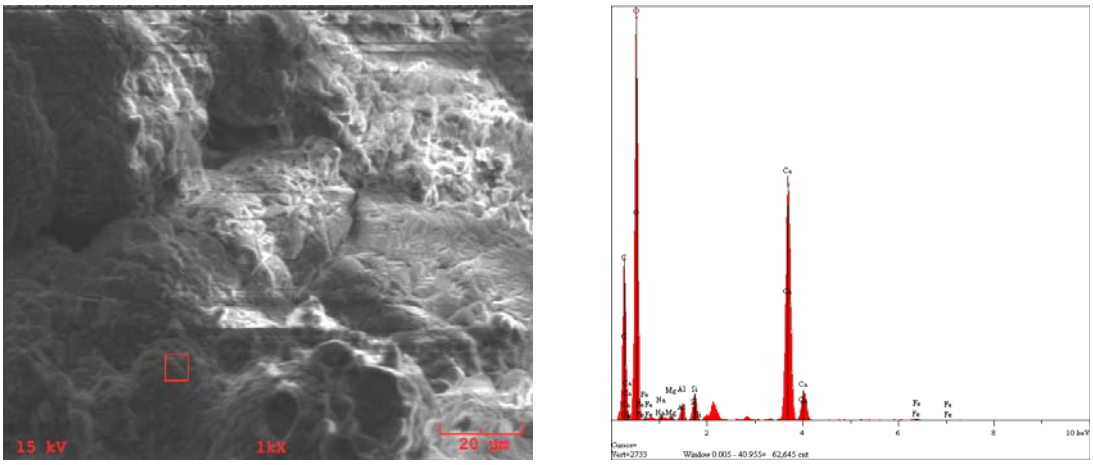

Fig. 12 EDX analysis of organic soil after *biocementation* treatment

treatment. As it is shown in Fig. 11 before treatment of organic soil spectra shows Si and Al that are related with organic soil and soil particles mixed with it. It is interesting to note that Ca peaks were not observed at all in organic soil before treatment. In Fig. 12, the spectra indicate Ca peaks associated with the calcite crystals that were covered all soil particles due to this reason Si and Al peaks were not observed.

4. Conclusions

This study described a successful *biocementation* application in organic soil using natural microbial processes. In this study *B. pasturii* was used as microorganism in calcite precipitation. The pH of the surrounding medium is one of the important factors successful *biocementation*. The pH value of the surrounding medium in the present study for organic soil reached 9.3. According to the literature, this is an ideal value for calcite precipitation. This study demonstrates that bacterial $CaCO_3$ precipitation (*biocementation*) in organic soil is possible. However, the amount of $CaCO_3$ precipitated in the organic soil was lower than that in the sand. The reduced level of $CaCO_3$ precipitated in the organic soil is mainly attributed to the amount of soluble organic ligands and the soil's complex pore network. Also, calcium carbonate precipitation was not uniform over the specimen length. The highest amounts of calcium carbonate precipitated at the injection point. One of the most important findings of this study is reduction in permeability. Test study showed that the coefficient of permeability of the organic soil reduced in 10 fold after *biocementation*. The findings of the study encourages the geotechnical engineers to use *biocementation* as an improving technique to modify compressibility and shear strength of organic soil.

Acknowledgments

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