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"Research Note"

AN INVESTIGATION OF BACTERIAL CALCIUM CARBONATE PRECIPITATION IN ORGANIC SOIL FOR GEOTECHNICAL APPLICATIONS*

W. SIDIK[1], H. CANAKCI[2**] AND I. H. KILIC[3]
[1,2]Dept. of Civil Engineering, University of Gaziantep, Gaziantep-Turkey
Email: canakci@gantep.edu.tr
[3]Dept. of Biology, Gaziantep University, Gaziantep-Turkey
[1]Dept. of Civil Engineering, Kerkuk University, Kerkuk-Iraq

Abstract– This study focused on the process of bacterial calcium carbonate ($CaCO_3$) precipitation (BCCP) in organic soil. Two samples, organic soil and sand, in glass boxes having dimensions 6×6×2 cm were immersed in bacterial medium (*Bacillus pasteurii*, urea, and $CaCl_2$) for 4 days. During the treatment period, the samples were treated with urea medium and $CaCl_2$ every 6 h. Changes in pH values were monitored at different time intervals. At the end of the treatment period, the amount of $CaCO_3$ was determined with a calcimeter test. The test results showed that the pH values fluctuated between 9 and 9.4 during the treatment period. This range of pH values indicates that the treatment medium is appropriate for BCCP. The amount of precipitated $CaCO_3$ in the organic soil sample increased about 8% compared with the untreated sample. Calcium carbonate precipitation in sand is found to be higher than the organic soil. The results were supported by Scanning Electron Microscopy (SEM) analysis and Energy-Dispersive X-ray (EDX) analysis.

Keywords– Bacillus pasteurii, organic soil, bacterial calcium carbonate precipitation, calcimeter test

1. INTRODUCTION

Organic soil, which is found in many places around the world, is a mixture of finely divided particles of organic matter. 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 [1]. Different improvement techniques have been employed to improve geotechnical properties of organic soil [1-4]. In the past few years, the use of bacterial calcium carbonate ($CaCO_3$) precipitation (*BCCP*) has become popular [5-7], *BCCP* has been presented as a new and environmentally friendly method. An advantage of microbial bacterial over chemical treatment is that the first may be non-toxic, whereas many chemical grouts, especially those based on acrylamides, lignosulfonates, and polyurethane, are toxic and environmentally harmful [8]. *BCCP* or cementation has been applied in a variety of civil and geotechnical engineering applications [5-7, 9-12]. *B. pasteurii* played an important role in $CaCO_3$ precipitation [7]. It exhibits high urease production. Hence, it has been used for microbial calcite cementation in many studies.

In all previous studies, the *BCCP* technique was used to improve geotechnical properties of sandy soil. The information in the literature on the application of *BCCP* in organic soil is very limited. Therefore, this study investigated the applicability of *BCCP* in organic soils using *B. pasteurii*, non-pathogenic organisms found naturally in soil.

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**Corresponding author

2. MATERIALS AND METHODS

a) Materials

An isolated bacterial culture of *B. pasteurii NCIMB 8221* was used in this study. The organic soil used in this study was obtained from the Sakarya region, Turkey. This organic soil is classified as peat [13]. Some chemical and physical properties of the organic soil used in the tests are given in Table 1.

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
Natural water content (%)	256
Liquid limit (%)	125
Plastic limit (%)	No plastic
Specific gravity	1.97

b) Methods

1. Sample preparation and measurements: Two samples, loose organic soil and sand, were prepared in glass boxes having dimensions of 6×6×2 cm, and ends of the samples were fitted with filter material. The soil samples were placed in an oven for 30 min. at 80 ^{o}C to eliminate any micro organisms that might affect $CaCO_3$ precipitation process. The organic soil and the sand samples were treated by immersing them in a bacterial medium *(B. pasteurii*, urea, and CaCl2) and left in the medium for 12 h. All the samples were then incubated at 28^{o} C. After the incubation process was completed, urea medium and $CaCl_2$ were added to the organic soil and the sand samples every 4 h on the first day. This was extended to every 6 h for the following 3 days to provide a suitable environment for $CaCO_3$ precipitation. The treatment solution was changed by pumping. Throughout the treatment period, changes in the pH values were monitored at different time intervals using a pH meter. At the end of 4 days the amount of $CaCO_3$ in both the organic soil and the sand was determined using a calcimeter instrument.

The morphology of the bacteria and the crystals in agar and soil samples were analyzed with SEM (Zeiss EVO50) under accelerating voltages ranging from 5 to 15 kV. The mineral constituents of the crystals were further characterized by EDX analysis.

3. RESULTS AND DISCUSSION

a) pH values in both organic and sandy soils

When the organic and sandy soils 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 samples. Figure 1 depicts the changes in the pH values over time. As can be seen in the figure, the pH values reached around 9.3 for both the organic soil and the sandy soil after 12h. In the literature, the ideal range of pH values for bacteria to precipitate calcite was reported to be between 8.3 and 9.3 [14]. In the present study, the experimental results showed that $CaCO_3$ precipitation in the organic soil by bacteria is possible.

b) Amount of precipitated $CaCo_3$

At the end of 4 days of treatment, the samples were tested with a calcimeter to observe changes in amount of $CaCO_3$ in both the organic soil and the sandy soil. Figure 2 shows the change in $CaCO_3$ content in the two soil types. It can be seen from the figure that the amount of $CaCO_3$ increased 8 % in the organic soil and 35 % in the sandy soil. This clearly shows that $CaCO_3$ precipitated in the organic soil. However, the amount of precipitated $CaCO_3$ in the organic soil was comparatively less than the sandy soil. This can be attributed to the amount of soluble organic ligands and the complex pore network of the organic soil.

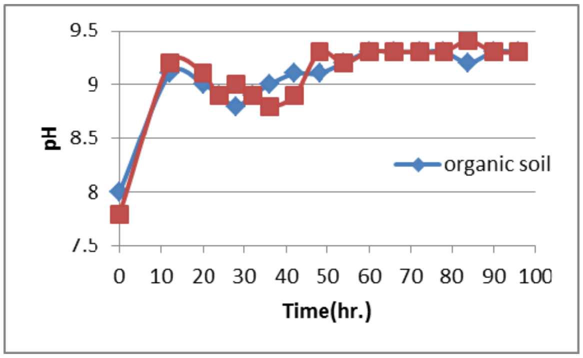


Fig. 1. The values of pH for both organic and sandy soils samples

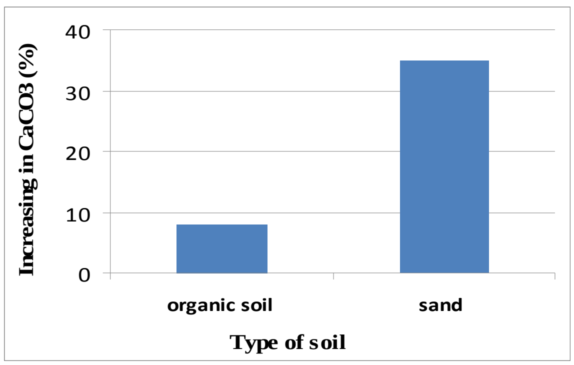


Fig. 2. An increase in the amount of calcium carbonate in each soil samples

c) SEM and EDX images for $CaCO_3$ crystals

The SEM images of the sandy soil before and after the treatment are shown in Fig. 3a and b. The images clearly show the formation of $CaCO_3$ crystals on the surface and voids between the particles of sand. SEM images of the organic soil before and after BCCP are given in Fig. 4a and b. The images reveal $CaCO_3$ crystals on the surface of the organic soil particles. The organic soil and sand samples were analyzed by EDX after the treatment. Figures 5 and 6 display the elemental spectral analysis for the sand and organic soil samples, respectively. The spectra for both soil types indicate Ca peaks associated with the calcite crystals. The peaks for other elements originated from the treatment solution and the culture medium.

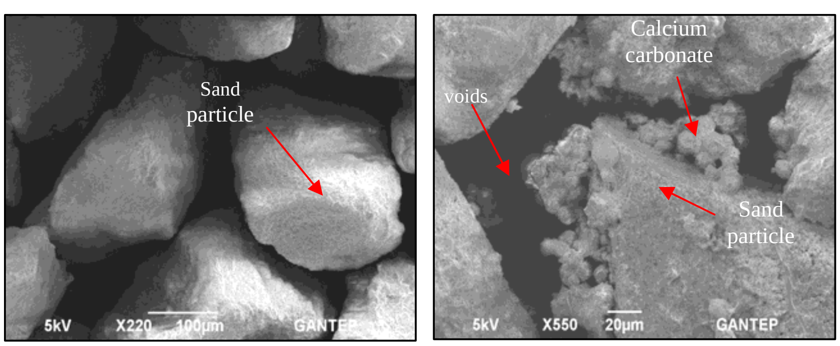
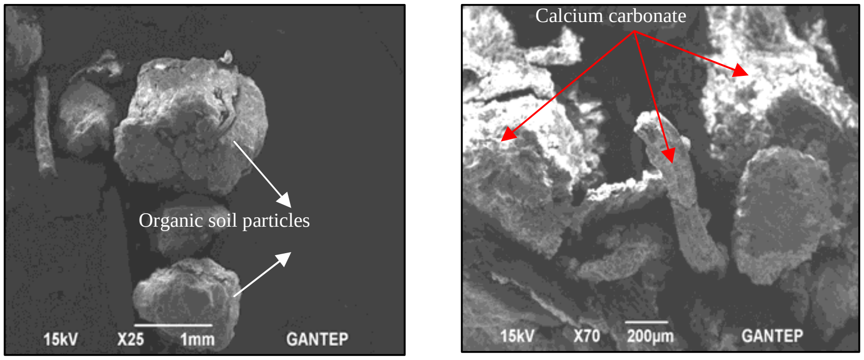


Fig. 3. Sand particles a) before treatment b) after treatment



(a) (b)

Fig. 4. Organic soil particles a) before treatment b) after treatment

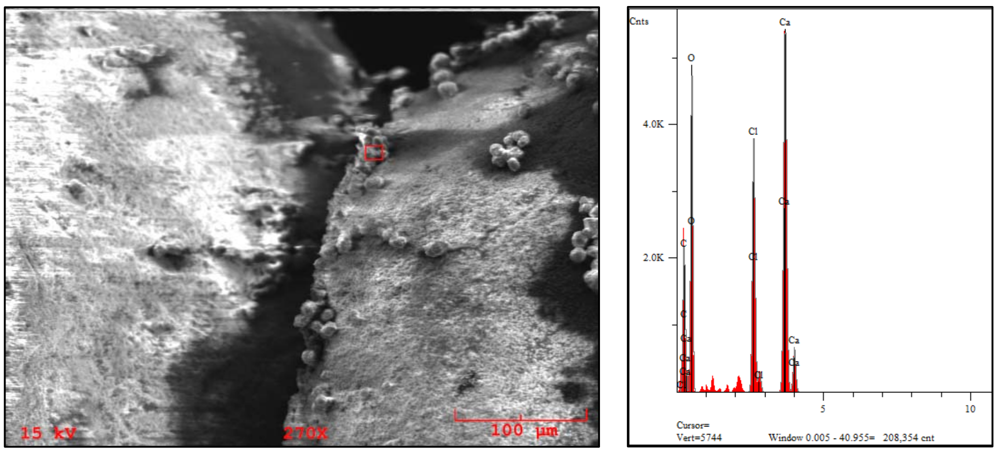


Fig. 5. EDX image and analysis of sand sample

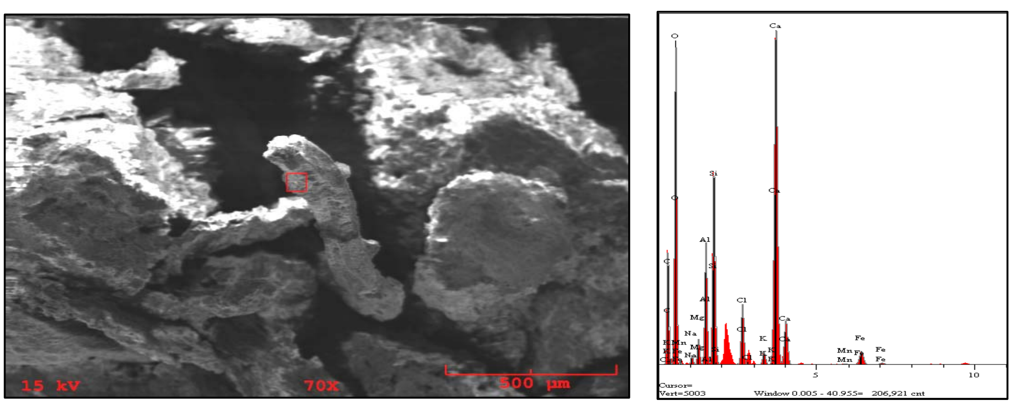


Fig. 6. EDX image and analysis of organic soil sample

4. CONCLUSION

The study described a treatment for successful *BCCP* in organic soil using natural microbial processes. Calcite precipitation was achieved using a non-pathogenic organism *B. pasteurii,* which is found naturally in soil. This work demonstrated that bacterial $CaCO_3$ precipitation in organic soil is possible. However, the amount of $CaCO_3$ precipitated in the organic soil was less compared to 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. The findings of the present study may encourage the use of *BCCP* in improving geotechnical properties of organic soil.

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