Effect of curing method on unconfined compressive strength of silty sand treated with Bacillus *Megaterium*

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Abstract. Laboratory tests were carried out to evaluate the effect of curing method on the unconfined compressive strength (UCS) of silty sand treated with Bacillus *megaterium*. (B. *megaterium*) using the Microbial Induced Calcite Precipitation (MICP) technique. Compaction test was conducted using the British Standard light (BSL) energy. UCS tests were performed on two sets of specimens treated with varying B. *megaterium* suspension densities and cementation reagent using trial mix ratio of 50% B. *megaterium* (B) and 50 % cementation reagent (C) (i.e., 50B-50C) for the treated specimen and 100 % cementation reagent (C) (i.e., 100C) for the control specimen. One set of specimens extruded from compaction mould was air-cured at room temperature for 24 hours, while the second set of specimens were placed in sealed polythene bags immediately after extrusion from the moulds. The results obtained show that the peak UCS values of the air-cured specimens and the sealed specimens are 448.8 kN/m² and 19.8 kN/m², respectively, for treatment with B. *megaterium* suspension density of 6.0 x 10⁸ cells/ml. The study shows that the UCS of silty sand treated with B. *megaterium* is sensitive to curing method.

Keywords: Bacillus megaterium, Cementation reagent, Silty sand, Unconfined compressive strength

1. Introduction

The dynamics of the world we live in change day in, day out with the constant need to make improvements on the way we do things as well as the preservation of the environment becomes paramount. Conventional soil improvement technology causes damage to the environment and as such, greater assiduity/attention is being given to environmentally friendly soil modification techniques [1].

The concept of employing microorganisms in bio-geotechnical engineering field was first pioneered in 1992 [2]. Thereafter, more studies have been carried out with respect to the utilization of microbes as alternative to the various eco-friendly soil improvement technologies considered in the engineering field [3-6]. The motivation for this technique is basically the ability of the microbes to catalyse chemical reactions that generates precipitates that has the ability to coat or/bind soil grains. This method of inducing calcite precipitate is identified as Microbial induced calcite precipitation (MICP). The MICP technique generally increases soil strength, improve surface erosion control, reduces permeability and seismic remediation [6,7]. Microbial-induced calcite precipitation (MICP) method is regarded as a current and an innovative technique of soil improvement [8-13]. Current investigations have suggested that employing special bio-mediated soil
Improvement techniques have justified the effective performance and environmental sustainability that can prevent collapse of loose soil sand that results in distortion or failure in foundation on the one hand, and liquefaction on the other [8, 14-18].

In a study using Bacillus pasteurii and sandy soil, Soon [19] reported that the treated soil specimen showed a behaviour of low failure of strain softening shear with improvement in shear stiffness and maximum shear capacity as compared to a soil specimen that hasn’t undergone treatment. DeJong [8], reported that MICP treated sand is projected to have a life span of 50 years as long as alkaline conditions remain, which is in alignment with expected life span for most engineering structures. The longevity can be extended through occasional retreatment. Soon [20] also stated that there was sufficient bettering in the shear strength and hydraulic conductivity of sandy and residual soil samples, however, the residual soil samples recorded higher shear strength values than the sandy soil, while the reverse was the case in the hydraulic conductivity results. He further stated that MICP treatment was dependent on density of soil, condition of treatment and soil type. Esringu [21] utilized Bacillus megaterium on contaminated sandy soil but the study evolved around the remediation of Boron, Cadmium and Lead, polluted soils. Muthukumaran [22] studied the effect of MICP on the strength of cohesionless soils under dissimilar environmental circumstances. The author reported an exponential rate of strength gain in the treated samples and stated that the level of improvement was dependent on the calcium carbonate precipitated, which was in turn dependent on a favourable environment for urease production from the microbes and sufficient reaction time for the reagents. However, most investigations on MICP have focused on the utilization of sand and very little work has been done on silty soils, which underscores the need for this study.

Silty soils are usually found in semi-arid environments and are referred to as a collection of macroscopic particles of a size between sand and clay, which originated from the minerals quartz and feldspar [23]. Silty soils act differently from clay and sand. Silt may exist as a soil (frequently combined with clay or sand) or as sediment combined in suspension with water (also called suspended load) and in a water body such as a river. In addition it may exist as soil located at the base of water bodies, like mudflows from landslides. Silty soil is usually associated with a floury feel when free from moisture, and a slippery feel when moist. During shear, Silty Sand due to increasing strains has a propensity to dilate and decrease in pore pressure [24, 25]. Silty sand soils usually do not have cohesion amidst their particles and most of their shear strength values are based on their internal friction.

This research was aimed at the comparative assessment of the effect of curing method on the unconfined compressive strength (UCS) of a B. megaterium-induced calcite precipitate treated silty sand soil using MICP technique. The specific objective was to ascertain the alterations in the UCS properties of silty sand soil when treated with stepped B. megaterium suspension density.

2. Materials and Methods

2.1 Materials

2.1.1 Silty sand soil. The silty sand sample used for this research were obtained from Wudil Local Government Area (latitude 11° 786N and longitude 8° 840E) in Kano State, Nigeria.

2.1.2 Microorganism. The bacterium used as the soil improvement agent in this investigation is Bacillus megaterium. It is Gram-positive rod-shaped bacteria classified as ATCC 14581 in the American Type Culture Collection (ATCC). Five separate bacteria suspension density of distinct turbidities on the McFarland turbidity scale, MFS (i.e., 0.5, 2.0, 4.0, 6.0 and 8.0 with equivalents $1.5 \times 10^8$ cells/ml, $6 \times 10^8$ cells/ml, $1.2 \times 10^9$ cells/ml, $1.8 \times 10^9$ cells/ml and $2.4 \times 10^9$ cells/ml, respectively) were used in this study. The control soil was referenced as 0 cells/ml (0 MFS).
2.1.3 Cementation Solution. Cementation solution was prepared using a mixture of chemicals at varying proportions. Chemicals that were used for the cementation solution include the following per litre of water purified by distillation: 3 g of nutrient broth (Bacto); 20 g of urea; 10 g of NH₄Cl; and 2.12 g (the same as 25.2 mM) of NaHCO₃. The pH of the medium was adjusted to 6.0 with 6 M HCL preceding in time to autoclaving. 10 ml of filter, completely cleaned or uncontaminated solution containing 2.80 g CaCl₂ were added afterward as reported by Stocks-Fischer et al. [26].

2.2 Methods

2.2.1 Unconfined compressive strength. The test for the unconfined compressive strength (UCS) of the soil samples were performed in agreement with specification outlined in BS 1377-7: 1990 [28] as well as [29], utilizing the British Standard light (BSL) compactive effort. Compaction of the soil samples was done in a 1000 cm³ mould at each of their OMC. With the use of an extruder, each of the compacted soil specimen was then gently removed from the mould. Thereafter, three cylindrical specimens with 38.1 mm diameter and 76.2 mm length each were cored from the extruded specimen. The cored specimens were cured for 7 days and each specimen was placed on the bottom platen of a compression machine before applying a compressive force at a strain controlled rate of 0.10 %/min. Results of the axial deformation and force were recorded simultaneously at fixed intervals until the specimen failed. The compressive stress was computed using equation (1):

$$\sigma \text{(kN/m}^2\text{)} = \left[\frac{R \times Cr \times (100 - E \%) \times 100}{(100 \times Ao)}\right]$$

Where:
- E % = l / Io
- E % = Strain Percent
- l = Amount of compression at any stage
- R = Load ring reading at Strain E
- Cr = Mean calibration of load ring
- Io = Initial Length of the specimen.
- Ao = Initial cross sectional area
- $\sigma$ (kN/m²) = Compressive stress at strain E.

2.2.2 Soil sample preparation and treatment. Two sets of specimen were utilized. 3000 g of soil sample passing BS No. 4 sieve (4.76 mm aperture) was used for each set. For the control specimen, only cementation reagent which volume was obtained from the total volume determined from equation (2) was mixed with the soil sample. For the treated specimens, the soil was mixed with each bacterial suspension density and cementation reagent in a pan. A trial mix ratio of 50% B. megaterium suspension density (B) and 50% cementation reagent (C) (i.e., 50B-50C) was adopted for the two sets of specimens. The calculation of the volume of each of the bacterial suspension density ($1.5 \times 10^8$, $6 \times 10^8$, $1.2 \times 10^9$, $1.8 \times 10^9$ and $2.4 \times 10^9$ cells/ml) and cementation reagent added to the soil were determined using equations (2) and (3), respectively:

1) For 50B-50C mix ratio: In this mix, the soil was mixed with 50% bacterial suspension (50B) and 50% cementation reagent (50C) of the total volume which was obtained from equations (2) – (4):

$$Total \, Volume = \frac{Optimum \, Moisture \, Content}{100} \times Weight \, of \, soil \, sample$$

(2)
After treating the soil sample with the various mix ratios, the samples were sealed with polythene sheet for 24 hours for proper hydration, before being subjected to the UCS test procedure. However, the curing process was different for each of the two sets of specimen with one set air-cured (i.e, Specimen 1) at room temperature for 24 hours, while the second set was sealed immediately after extrusion from the moulds (i.e, Specimen 2).

3. Results and Discussion

3.1 Natural soil
The natural moisture content of the soil was 20 %. The high moisture content was as a result of the period of the year in which the soil samples were collected. The index properties of the natural soil are shown in Table 1. The sample is brown in colour and has 6.65 % fines passing No. 200 sieve. The soil was classified as A-3(0) in agreement with American Association of State Highway and Transportation Officials [30] and as a poorly graded sand with silt (SP-SM) according to the Unified Soil Classification System [31].

| Properties                              | Quantity |
|-----------------------------------------|----------|
| Percentage passing 0.075 mm sieve       | 6.7      |
| Natural moisture content, %             | 20.0     |
| Specific gravity                        | 2.63     |
| Liquid limit, %                         | -        |
| Plastic limit, %                        | Non plastic |
| Plasticity index, %                     | Non Plastic |
| Linear shrinkage, %                     | -        |
| AASHTO classification                   | A-3(0)   |
| USCS classification                     | SP-SM    |
| Maximum dry density, Mg/m³              | 1.67     |
| Optimum moisture content, %             | 8.5      |
| Colour                                  | Brown    |

3.2 Mechanism of Microbial Induced Calcite Precipitation (MICP) Process
In order to stimulate calcite mineralization, which is an after-effect of microbial metabolic activity [32], an aerobic urease producing bacteria is used to induce the calcite precipitate. This MICP process is known as Urea Hydrolysis. Although there are other processes (aerobic oxidation, sulphate reduction, denitrification, etc.), Van Paassen [33] suggested the adoption of Urea Hydrolysis for MICP process because of their high calcite conversion rate in contrast with other processes [26].
3.3 Influence of B. megaterium Induced Calcite Precipitate on Unconfined Compressive Strength of Silty Sand Soil

3.3.1 Unconfined compressive strength.

The variation of the unconfined compressive strength of silty sand with B. megaterium suspension density is shown in Figure 1. The UCS values for the control specimen (i.e., treatment with cementation reagent only) are 75.10 and 26.08 kN/m² for Specimen 1 and Specimen 2 respectively. For Specimen 1, the UCS initially increased from the value recorded for the control specimen to a peak value of 448.84 kN/m² at B. megaterium suspension density of $6.0 \times 10^8$ cells/ml and thereafter decreased to 261.56 kN/m² at B. megaterium suspension density of $2.4 \times 10^9$ cells/ml. For Specimen 2, the value recorded for the control specimen generally decreased to 13.35 kN/m² at B. megaterium suspension density of $2.4 \times 10^9$ cells/ml.

The higher UCS value obtained for the Specimen 1 in comparison with Specimen 2 is due to a higher calcite precipitated within air-dried Specimen 1 mainly as a result of greater oxygen available for reaction. B. megaterium is an aerobic microbe which thrives better in the presence of air to promote the growth of the microbes and increase the conversion rate that resulted in the production of more calcium carbonates than in sealed Specimen 2 which was not exposed [34]. Bacteria cell density has been reported as one of the factors that influence the calcite formation, as urease hydrolysis has a direct relationship with density of microbes in a given cell, since microbes act as nucleation sites for the precipitation of calcite during the MICP method. Hence, a higher concentration of microbes which means more available nucleation sites, often results in higher urease activity and consequent increase in calcite precipitated [19,26]. This resulted in the deposition of a greater amount of CaCO₃ within the pore spaces of Specimen 1, than Specimen 2. Similar findings were reported by Lian [35] in their study of Bacillus megaterium induced carbonate mineralization, that the process of crystallization was affected by the existence of bacterial cell surfaces and metabolic products.

The decrease in the UCS values recorded in the two specimens could be ascribed to the low volume of cementation reagent when compared to the bacteria cell population in those suspension densities. As
reported by Anbu [36], urea hydrolysis is influenced greatly by the volume of cementation reagent and concentration of cells in a given bacteria suspension density. Hence, the volume of cementation reagent supplied to those suspension densities with higher cell concentrations was not sufficient to generate sufficient calcite that would enhance the bond and strength of the treated soil mass [19, 37 and 38].

4. Conclusion

The silty sand classified as A-3 (0) and a poorly graded sand with silt (SP-SM) in AASHTO and USCS, respectively, was treated with five B. megaterium suspension density (B) of 0, 1.5 x 10^8, 6.0 x 10^8, 1.2 x 10^9, 1.8 x 10^9 and 2.4 x 10^9 cells/ml and cementation reagent (C). A mix ratio of 50B-50C was adopted for the treatment of samples, while the control specimen was treated with cementation reagent only. Results obtained indicate that the UCS values recorded for Specimen 1 (i.e., air-cured) were higher than those of Specimen 2 (i.e., sealed) due to a higher urease activity and calcite formation resulting from the exposure to oxygen. It can be deduced that the curing method influences the strength gain of B. megaterium treated silty sand.

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