

PAPER 43 – UNCONFINED COMPRESSIVE STRENGTH OF BACILLUS *SPHAERICUS* INDUCED PRECIPITATE TREATED LATERITIC SOIL

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ABSTRACT

This study evaluated the potential of *Bacillus sphaericus* (*B. sphaericus*) in microbial-induced calcite precipitation improvement of the unconfined compressive strength (UCS) of lateritic soil. The soil, classified as A-2-6 (1) in the American Association of State Highway and Transportation Officials (AASHTO) system and SC in the Unified Soil Classification System (USCS), was treated with stepped *B. sphaericus* suspension of various densities of 0 , 1.5×10^8 , 6.0×10^8 , 1.2×10^9 , 1.8×10^9 , 2.4×10^9 cells/ml at varying concentration of cementation reagents of 0.25, 0.5, 0.75 and 1 M. The cementation reagent used contained 3 g of Nutrient broth, 20 g of urea, 10 g of NH_4Cl , 2.12 g of NaHCO_3 and 2.8 g CaCl_2 per litre of distilled water. Soil specimens were prepared using bacteria to cementation reagent mix ratio of 25:75 with stepped *B. sphaericus* suspension density and compacted using three energy levels, namely, British Standard light (BSL), West African Standard (WAS) and British Standard heavy (BSH). The treated soil specimens were air-cured at an ambient laboratory temperature of $25 \pm 2^\circ\text{C}$ for 7, 14 and 28 days, respectively. Peak UCS values of 947.40, 989.28 and 1,240.82 kN/m^2 for BSL; 1,387.6, 1,481.72 and 1,743.43 kN/m^2 for WAS as well as 1,432.24, 1,598.34 and 1,835.98 kN/m^2 for BSH compaction were recorded at 7-, 14- and 28- days curing periods, respectively, for treatment with mix ratio *B. sphaericus* suspension density of (1.2×10^9 cells/ml): Cementation reagent (0.5 M). The UCS values of 1,743.43 and 1,835.98 kN/m^2 recorded for the specimens compacted with WAS and BSH energy at 28 days curing period meet the requirement for base material in the construction of low-volume roads.

Keywords: *Bacillus sphaericus*, Cementation solution, Lateritic soil, Unconfined compressive Strength

1. INTRODUCTION

Micro-organisms found in soil that are unicellular consist primarily of bacteria and archaea having a diameter range of 0.5 to 3 μm (Dejong *et al.*, 2013; Sayed *et al.*, 2019). They are present in the soil by entrapment during deposition (Sayed *et al.*, 2019) or migration utilising the available pore spaces (Dejong *et al.*, 2013).

Currently, soil improvement techniques such as chemical grouting or mixing with cement have over time been successful (Mahawish *et al.*, 2016). Grouting can be described as an artificial injection of chemical formulas that most times alter the soil pH level, and cause soil and groundwater contamination, which is not unconnected to their characteristics of being hazardous and toxic (Chittoori *et al.*, 2019).

Microbial-induced calcite precipitation (MICP) is a bio-chemical process of soil strengthening that seeks to improve soil properties by enhancing its engineering properties, particularly strength and permeability. The MICP process is carried out by injecting micro-organisms which produce urease and also adding reagents for aiding cementation thereby producing a cementation compound generated to alter the engineering soil properties (Yang *et al.*, 2019). These micro-organisms are mostly not harmful and, as a result, will have little or no negative effect on the environment especially on humans (Umar *et al.*, 2019). According to Dejong *et al.* (2010), MICP studies and application cuts across many disciplines from geotechnical engineering to sciences such as microbiology and geochemistry working together to achieve an environmentally friendly way of stabilizing soils.

Laterites are highly weathered soils formed by the concentration of hydrated iron and aluminium oxides and have no reasonable constant properties (Osinubi 2005). Laterites were first studied in humid tropics and mostly found where leached soils are predominant. They are formed in weathering systems by the process of laterisation, which involves the breakdown of ferro-alumino silicate minerals and the permanent deposition of sesquioxides (Al_2O_3 and Fe_2O_3) within the profile to form the laterite horizon of material known as laterite.

Studies recently conducted on the application of the MICP technique which is still considered an innovation for the improvement of soils (Osinubi *et al.*, 2019) have shown improvement in strength and hydraulic conductivity properties of the soils when used in the design of waste containment barrier systems. Other successful studies conducted using the MICP approach include; increasing liquefaction resistance (Montoya *et al.*, 2013), improving the stability of slopes and foundation-bearing capacity (Whiffin *et al.*, 2007), creating a water-impermeable crust on a sand surface (Stabnikov *et al.*, 2011), crack healing in concrete and masonry (Amidi and Wang, 2015), treatment of waste (Chu *et al.*, 2009), heavy metal immobilization (Fujita *et al.*, 2010), shallow carbon sequestration (Washbourne *et al.*, 2012), fine tailings consolidation (Liang *et al.*, 2015), as well as shear strength and compressibility of organic soil (Canakci *et al.*, 2015).

Previous research on soil improvement considered the use of conventional additives such as bitumen, lime, cement, pozzolanic material, agro-industrial waste, etc., which are either expensive or harmful to the environment and hence not sustainable. Therefore, this study is targeted at the assessment of UCS of lateritic soil treated with *B. sphaericus* (which is a more sustainable and environment-friendly approach to soil improvement) as the base material in the construction of low-volume roads. This involved treating the soil with *B. sphaericus* suspension density (i.e., 0, 1.5×10^8 , 6.0×10^8 , 1.2×10^9 , 1.8×10^9 , 2.4×10^9 cells/ml) at varying concentrations of cementation reagent (0.25, 0.5, 0.75 and 1 M). The soil specimens were prepared using bacteria to cementation reagent mix ratio of 25%:75% and compacted using three energy levels. The specific objectives were to assess the viability of *B. sphaericus* as an agent for lateritic soil strength development for use as the base material in the construction of low-volume roads as well as the morphological and microstructural arrangement of the natural and treated soil samples.

2. MATERIALS AND METHODS

2.1 Materials

2.1.1 Soil

The soil for the study was collected using the disturbed sampling method from the Abagana area of Anambra state, Njikoka Local Government Area. (Latitude $6^{\circ}12'15''\text{N}$ and Longitude $7^{\circ}0'40''\text{E}$) sourced at depths between 0.5 and 3.0 m.

2.1.2 Bacteria

The Gram-positive micro-organism used in this research is *Bacillus sphaericus* rod-shaped bacterium with a 2 - 5 μm diameter size. The bacteria were isolated from the soil of which six (6) different samples were collected and used for the isolation, identification and characterisation of the *Bacillus sphaericus*. They were inoculated on Nutrient Broth, Yeast Extract Nutrient Agar, Nutrient Agar and MICP agar, respectively.

2.1.3 Cementation reagent

The cementation reagents were varied by using an equimolar concentration of urea and calcium chloride to produce cementation solutions of different molar concentrations (0.25M, 0.5M, 0.75M and 1M)

2.2 Methods

2.2.1 Index properties

The index property test was conducted according to BS 1377 (1990) and BS 1924 (1990) for natural and treated soils.

2.2.2 Sample preparation

The soil sample to be used was passed through a sieve of 425 μm aperture and mixed for the bacteria-cementation mix ratios of 25%:75% which gave the highest calcite value based on the calcite content test carried out. The composition ratio is based on the liquid limit of the natural soil. *B. sphaericus* was applied at suspension densities of 0, 0.5, 2.0, 4.0, 6.0 and 8.0 McFarland standards which is equivalent to 0, 1.5×10^8 , 6.0×10^8 , 12×10^8 , 18×10^8 and 24×10^8 cells/ml, respectively, at a varying concentration of cementation reagent of 0.25, 0.5, 0.75 and 1M.

2.2.3 Atterberg limits

Atterberg limits (liquid limit, plastic limit, plasticity index test) are essential for soil classification and identification. The treated soil-bacteria-cementation reagent was air-dried, after which it was pulverised and passed through a sieve 425 μm aperture before testing.

2.2.4 Calcite content

For the calcite content determination, the acid-washing method was adopted as reported by (Osinubi et al., 2019). 5g of the treated soil was measured and mixed with 20 ml of 2M hydrochloric acid to dissolve CaCl_2 . After that, the mixture was filtered with filter paper of pore size 200mm. The filtrate was rinsed and washed for 10 min using distilled water to remove any dissolved calcium from the soil. The particles on the filter were oven-

dried for 24 hours at a temperature of 105°C and weighed. The difference in mass between the original sample A and the washed sample B was recorded as calcium carbonate (Calcite) mass. The percentage calcite content CC was computed using equation (1):

$$CC=100-\frac{B}{A}\times 100 \quad (1)$$

2.2.3 Unconfined compressive strength

Air-dried soil sample of 3 kg was mixed with optimum moisture content determined by dynamic compaction of the soil sample. The standard BS compaction mould was utilized. In the mould, the sample was placed and compacted in layers and blows corresponding to the energy level considered. The sample was then removed from the mould with the aid of a hydraulic lift and three samples of 38 by 76 mm were cored out and placed in a polythene bag for at least 48 hours before testing to allow for complete saturation. For the test, the axial load was gradually applied until shear failure occurred. For the test, failure is said to have occurred when two or three successive readings are equal or decreasing in descending sequence. The experiment was conducted with the addition of *B. sphaericus* suspension density (cells/ml) of 0, 1.5x10⁸, 6.0x10⁸, 12x10⁸, 18x10⁸ and 24x10⁸ at different concentrations of cementation reagent (0.25, 0.5, 0.75 and 1 M), respectively and compacted using three energy levels of BSL, WAS and BSH compaction loads all carried out in accordance with BS 1377 (1990) and BS 1924 (1990) for the natural and treated soils. The curing times for the samples were 7 days, 14 days and 28 days.

2.2.6 Microstructural analysis

A microanalysis test was carried out on the lateritic soil (natural and treated) to determine the change in morphology due to the formation of calcite bonds on the interparticle surface of the bio-treated soil. It was carried out using Phenom world pro desktop SEM (Phenom World, 2017).

3. RESULTS AND DISCUSSION

3.1.1 Index properties

The properties of the natural lateritic soil are classified as A-2-6 (1) in the AASHTO system and SC (clayey sand) in the USCS (AASHTO 1996 and ASTM 1992) are summarised in Table 1. The natural lateritic soil is reddish-brown, with 34.95 % passing through the BS sieve no. 200 size, with kaolinite being the dominant clay mineral.

Table 1. Natural properties of the soil

S/No.	Property	Quantity
1	Natural moisture content (%)	19.6
2	Percentage passing No. 200 sieve	34.95
3	Liquid limit (%)	36.5
4	Plastic limit (%)	20.06
5	Plasticity index (%)	16.44
6	Linear shrinkage (%)	8.37
7	Specific gravity	2.67
8	AASHTO classification	A – 2 – 6 [1]
9	USCS	SC
10	Colour	Reddish-brown
11	Dominant clay mineral	Kaolinite

3.1.2 Calcite content

The variation of the calcite content for the lateritic soil specimens prepared with stepped *B. sphaericus* suspension density at varying cementation reagents is presented in Figure 1. Generally, the calcite content of all the specimens increased as the cementation reagent concentration increased to peak values before decreasing. It was observed that the optimum calcium carbonate content values were 9.0%, for bacteria - cementation mix ratio of 25%:75% at *B. sphaericus* suspension density of 1.2 x 10⁹ cells/ml and 0.5 M cementation reagent. This is not unconnected to the fact that the cementing reagent used in the MICP process plays a vital role in the precipitation of calcium carbonate, and excess usage of this calcium chloride could reduce urease activity (Soundara et al., 2020).

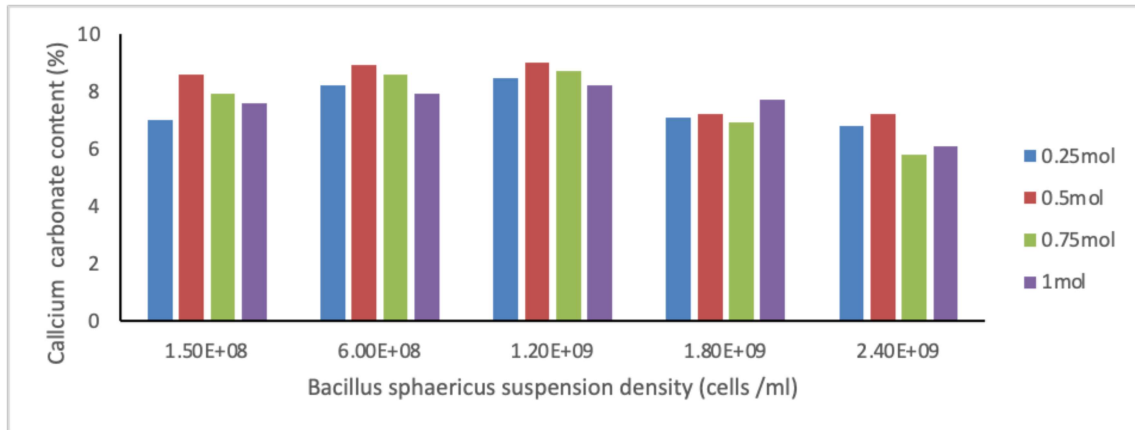


Figure 1. Calcite content of soil bacteria with varying cementation solutions mix (25%BS:75% cementation)

3.1.3 Atterberg limits

The variation of Atterberg limits (LL, PL, PI) of the lateritic soil treated with stepped suspension density of *B. sphaericus* of 0, 1.5×10^8 , 6.0×10^8 , 1.2×10^9 , 1.8×10^9 , 2.4×10^9 cells/ml using bacteria to cementation mix ratios of 25 %:75 % at the stepped concentration of cementation reagent of 0.25, 0.5, 0.75 and 1 M. Generally, the increases in LL might result from the increase in water content needed for urea hydrolysis. Urease-positive enzymes were produced from the biochemical reaction, thereby hydrolysing urea into carbonate ions (CO_3^{2-}) and ammonium ions (NH_4^+). Generally, the increase in PL to peak values before decreasing could probably be due to an adequate amount of Ca^{2+} not being available on the calcite surface, thereby causing a reduction in the deposition of ion pairs (Dhami et al., 2016). The decrease in the PI values could probably result from calcite precipitation in the soil matrix voids. Generally, a decrease in PI values indicates soil improvement for any engineering use. The lateritic soil treated with *B. sphaericus* suspension density of 1.2×10^9 cells/ml and 0.5 M cementation reagent gave the best PI value indicating a better potential for soil improvement as presented in Figure 2.

The LL decreased from the natural value of 36.5 % to 34.2 % at 1.2×10^9 cells/ml before increasing to 37.20% at the highest treatment of 2.4×10^9 cells/ml. The PL increased from the natural value of 20.06% to 24.70% at the maximum treatment of 2.4×10^9 cells/ml. The PI value decreased from the natural value of 16.44% to 11.60% at 1.2×10^9 cells/ml before increasing to 12.50% at the treatment of 2.4×10^9 cells/ml.

The Atterberg limits findings in this study are consistent with the results reported by Moravej et al., 2018.

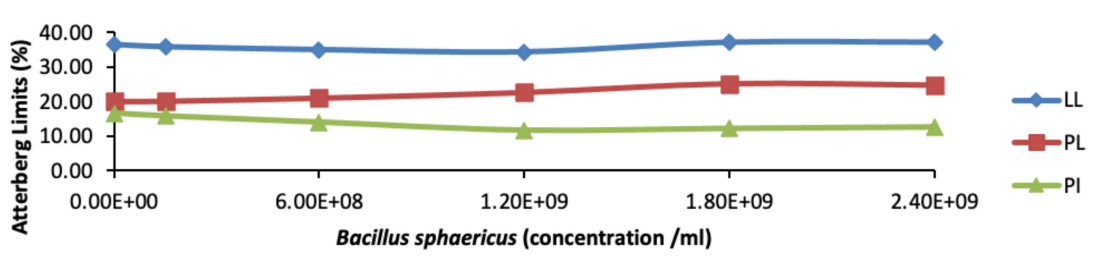


Figure 2. Variation of Atterberg limits of lateritic soil treated with *B. Sphaericus* suspension density at 0.5 M cementation reagent concentration

3.1.4 Unconfined compressive strength

The results of the UCS for BSL are presented in figures 3-5, the WAS are presented in figures 6-8 while that of the BSH are presented in figures 9-11, respectively, for the *B. sphaericus* suspension densities (i.e., 0, 1.5×10^8 , 6.0×10^8 , 1.2×10^9 , 1.8×10^9 , 2.4×10^9 cells/ml) at a varying concentration of cementation reagent (0.25, 0.5, 0.75 and 1 M) using bacteria to cementation reagent mix ratio of 25%:75%, and compacted using three energy levels, namely, British Standard light, (BSL), West African Standard (WAS) and British Standard heavy (BSH). The treated soil specimens were air-cured at an ambient laboratory temperature of $25 \pm 2^\circ \text{C}$ for 7, 14 and 28 days,

respectively. Peak UCS values of 947.40, 989.28 and 1,240.82 kN/m² from natural values of 789.38, 803.20 and 900.28 kN/m² respectively, for BSL, 1,387.6, 1,481.72 and 1,743.43 kN/m² from natural values of 832.45, 848.64 and 999.89 kN/m² respectively, for WAS as well as 1,432.24, 1,598.34 and 1,835.98 kN/m² from the natural values of 928.03, 1030.08 and 1130.43 kN/m² respectively, for BSH compaction were recorded at 7, 14 and 28 days curing periods, respectively, for treatment with 1.2×10^9 cells/ml of *B. sphaericus* suspension density and 0.5M of cementation reagent. The values of the UCS obtained generally showed an increasing trend with an increase in compaction energy with BSH compaction energy showing the highest UCS values for all treatments evaluated. The results showed that the greater UCS value obtained is also a function of compaction load as well as the precipitations of calcites that were formed during the MICP process. This result is consistent with the studies by Osinubi et al. (2017) and Osinubi et al. (2020).

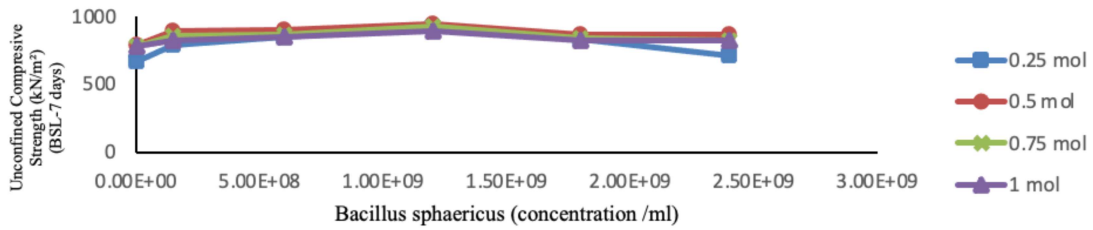


Figure 3. Graph of UCS of Lateritic soil treated with *B. sphaericus* suspension at varying cementation (BSL-7days curing)

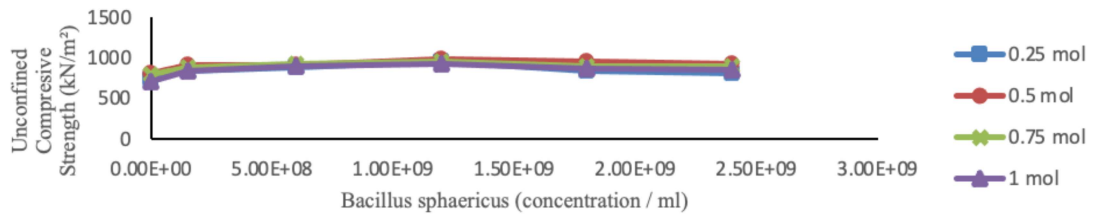


Figure 4. Graph of UCS of Lateritic soil treated with *B. sphaericus* suspension at varying cementation (BSL-14days curing)

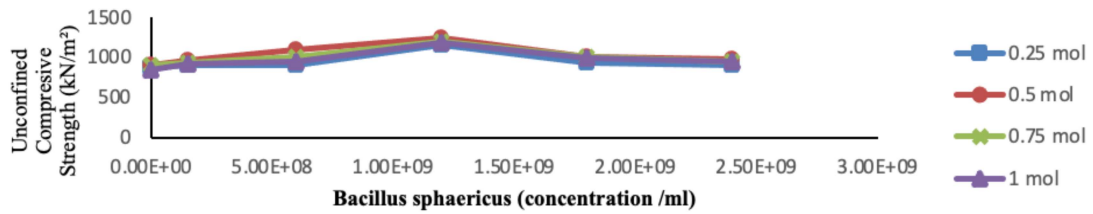


Figure 5. Graph of UCS of Lateritic soil treated with *B. sphaericus* suspension at varying cementation (BSL-28 days curing)

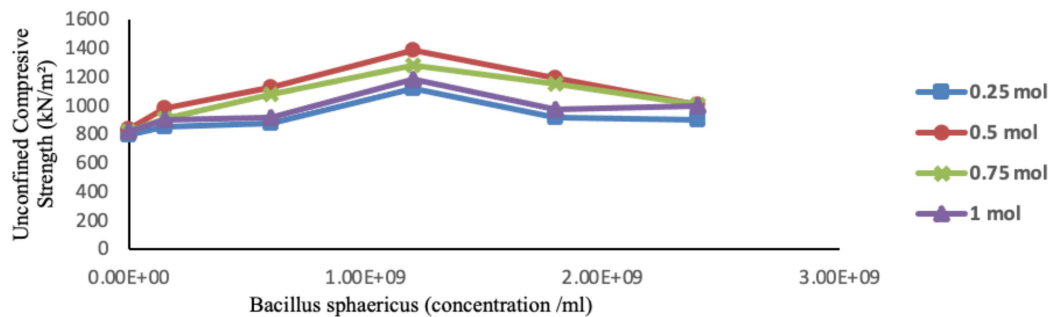


Figure 6. Graph of UCS of Lateritic soil treated with *B. sphaericus* suspension at varying cementation (WAS-7 days curing)

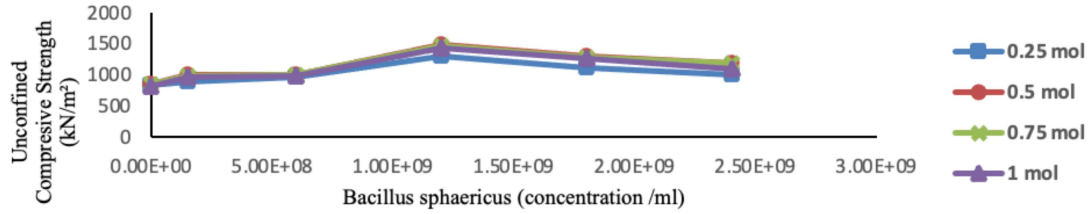


Figure 7. Graph of UCS of Lateritic soil treated with *B. sphaericus* suspension at varying cementation (WAS-14 days curing)

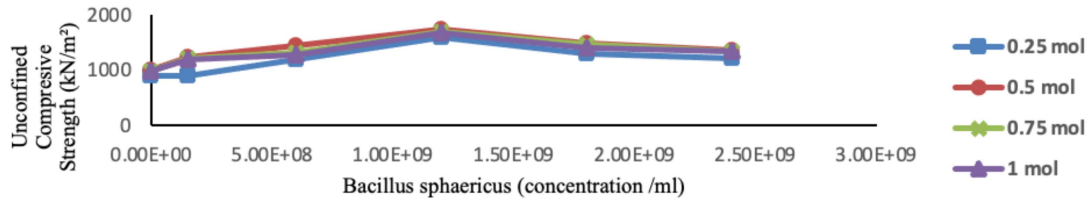


Figure 8. Graph of UCS of Lateritic soil treated with *B. sphaericus* suspension at varying cementation (WAS-28 days curing)

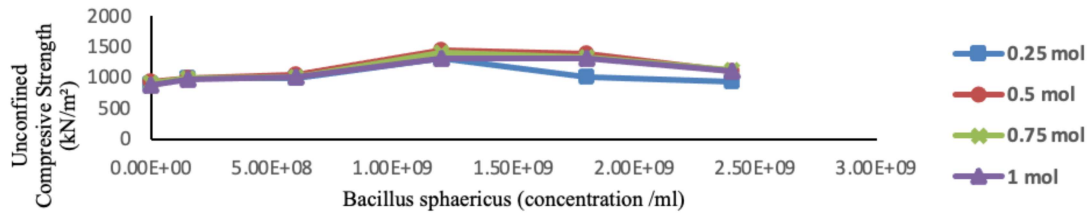


Figure 9. Graph of UCS of Lateritic soil treated with *B. sphaericus* suspension at varying cementation (BSH-7 days curing)

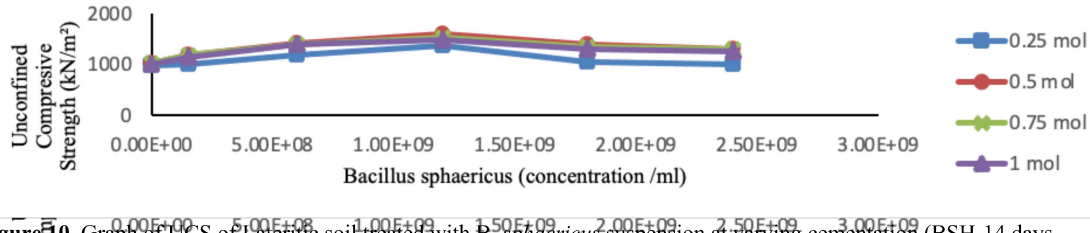
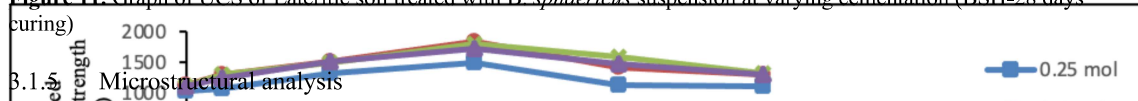


Figure 10. Graph of UCS of Lateritic soil treated with *B. sphaericus* suspension at varying cementation (BSH-14 days curing)

Figure 11. Graph of UCS of Lateritic soil treated with *B. sphaericus* suspension at varying cementation (BSH-28 days curing)



B.1.1. Microstructural analysis

The calcite crystals precipitation and growth on a micro-scale were examined by SEM micrographs of the soil (natural and treated soil specimens). Plates 1 and 2 (at x300 magnification) show a micrograph of the soil sample used for the UCS test for the 25% Bacteria and 75% cementation reagent mix at 0.5M concentration at a bacteria suspension density of 1.2×10^9 cells/ml. For the untreated UCS soil (Plate 1a), the thick lump particle could be attributed to the cohesive structure of the soil indicating that a thick diffuse double layer formed by the interaction of clay minerals and multivalent cations was still exerting a dominant impact on the soil-water combination. For the treated soil's microstructure (Plate 1b), the soil appears more uniform and consolidated resulting in a more bonded solid structure which possibly is a result of the calcite precipitate after the MICP process. This finding is in agreement with the findings by Cheng et al. (2013) and Al Qabany et al. (2012)

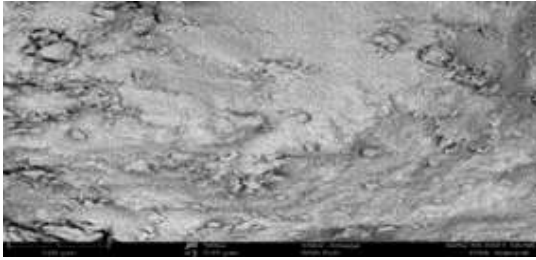
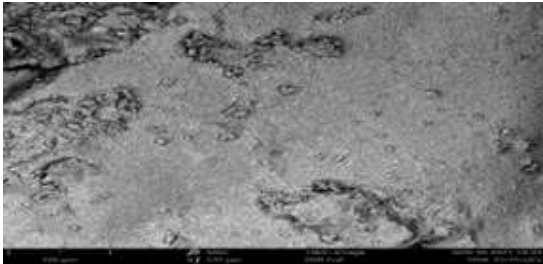


Plate 1a: Micrograph of Natural soil



Plates 1b: Micrograph of treated soil

4. CONCLUSION

An evaluation of the influence of *B. sphaericus* in MICP technique improvement of the UCS of lateritic soil was carried out. The lateritic soil classified as A-2-6 (1) in the AASHTO and SC in the USCS was treated with stepped *B. sphaericus* suspension density (i.e., 0, 1.5×10^8 , 6.0×10^8 , 1.2×10^9 , 1.8×10^9 , 2.4×10^9 cells/ml) at a varying concentration of cementation reagent (0.25, 0.5, 0.75 and 1 M) using bacteria to cementation reagent mix ratio of 25% to 75% and compacted using three energy levels. MICP technique improved the Atterberg limits properties of the natural lateritic soil at *B. sphaericus* suspension density of 1.2×10^9 cells/ml and 0.5 M cementation reagent. Peak UCS values of 947.40, 989.28 and 1,240.82 kN/m² for BSL, 1,387.6, 1,481.72 and 1,743.43 kN/m² for WAS as well as 1,432.24, 1,598.34 and 1,835.98 kN/m² for BSH compaction were recorded at 7-, 14- and 28- days curing periods, respectively, for treatment with mix ratio *B. sphaericus* suspension density of (1.2×10^9 cells/ml): Cementation reagent (0.5 M). The UCS values of 1,743.43 and 1,835.98 kN/m² recorded for the specimens compacted with WAS and BSH energy at 28 days curing period did not meet the 7 days curing period criterion for cement treated soil of 1730 kN/m² requirement specified by TRRL (1977), However, the values of the UCS from the study were significantly improved.

RECOMMENDATION

Based on the findings in this study, it is recommended that to improve the UCS characteristics of an A-2-6(1) or SC soil, a bacteria-cementation mix ratio of 25 % and 75 % *B. sphaericus* suspension density of 1.2×10^9 cells/ml and 0.5 M cementation reagent, respectively can be used.

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