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Effect of Varying Cementation Reagent Concentration on the Index and Physico-Chemical Properties of Lateritic Soil Treated with Bacillus *sphaericus*

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Abstract: This study evaluated the index and physico-chemical characteristics of lateritic 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) treated with stepped Bacillus *sphaericus* (B*s*) 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) for varying cementation reagent (C_r) concentration (i.e., 0.25, 0.5, 0.75 and 1.0 M) using 25 B*s*:75 C_r, 50 B*s*:50 C_r, and 75 B*s*:25 C_r mix ratios, respectively. Results obtained show that the optimum calcium carbonate contents were 9.0 %, 8.7 %, and 6.5 % for the mix ratios stated above, respectively, for Bs (1.2×10^9 cells/ml) and C_r (0.5 M) with urease activity of 80.8 ms/cm and optimum pH of 8.99. Bio-treatment of soil with 25 % bacteria (1.2×10^9 cells/ml) and 75% C_r (0.5 M) mix ratio reduced the liquid limit (LL) of the natural soil from 36.5 to 34.2 %, and the plasticity index (PI) from 16.4 to 11.6 %. Microanalysis of specimens showed that the treated soil appears more uniform and aggregated. The findings of the study show that bio-treatment with 25 Bs (1.2×10^9 cells/ml) : 75 C_r (0.5 M) mix ratio improved the index and physico-chemical properties of the lateritic soil considered in the study.

Keywords: Bacillus *sphaericus*; cementation reagent; index properties; lateritic soil; physico-chemical properties

1. Introduction

Soil stabilisation or improvement of soil is employed when it is more economical to overcome a deficiency in a readily available material than to bring in one that fully complies with the requirements of specification for the soil [1]. Stabilisers and modifiers could be organic or inorganic chemical compounds, organic compounds being resinous and bituminous materials acting as water-proofers and sometimes behaving similarly to glue to add cohesive strength. Inorganic chemical compounds include Portland cement, lime, slag, sodium silicate, phosphorus compounds and sometimes a combination of various inorganic salts, such as sodium chloride and calcium chloride that have been long used in stabilisation. Their main function is to reduce plasticity and facilitate densification [2].

Previous research on soil improvement considered using 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. According to [3], soil improvement techniques like chemical grouting or mixing with cement have shown positive outcomes. These can be described as artificial injection of chemical formulas that, most times, alter the soil pH level and cause soil and groundwater contamination; this is not unconnected to hazardous / toxic nature of the additives [4, 5].

Too much dependence on industrially manufactured soil improving additives (e.g., cement, lime, and bitumen) has kept the cost of stabilisation high. Consequently, underdeveloped and poor nations are unable to provide accessible roads for their rural dwellers that constitute a higher percentage of their agrarian population. Also, a large quantity of carbon dioxide is released during the production of cement, which is a major construction material worldwide.

Based on the foregoing, a better, environmentally friendly, efficient, and effective remedial technique suitable for soil stabilisation might be the biogenic/microbial technique of soil improvement. This trending microbial geotechnology has proven to be highly effective and efficient in soil improvement works with ease and reduced cost, and it enhances environmental sustainability [6].

Microbial-induced calcite precipitation (MICP) is a bio-chemical process of soil strengthening that utilises urea hydrolysis, sulphate reduction, denitrification, aerobic oxidation, and other processes to produce calcite [7]. When compared to other investigation procedures, urea hydrolysis yields the highest rate of calcite precipitation [1]. During urea hydrolysis, the urease enzyme, which is either externally supplied [8] or produced by micro-organisms *in situ* [1] facilitates a chemical reaction in which urea ($CO(NH_2)_2$) is broken down. This microbial bio-cementation process has very little or no harmful effect on the environment. Microorganisms, in particular bacteria, can alter the arrangement of the soil particle sizes, influence the arrangement of the soil matrix by enhancing crystallisation within soil matrix. Subsequently, after these activities, the soil may behave differently (e.g., there may be an increase in hydrodynamic dispersion, chemical retardation, or the migration of fine particles) [1].

Laterites are formed by the process of laterisation, which takes place in a weathering system, resulting in the permanent deposition of sesquioxides (i.e., Al_2O_3 and Fe_2O_3) by the breakdown of ferro-aluminosilicate minerals [9]. Most laterites in their natural states are deficient for use in construction works and require some improvement, especially in areas where erosion is a problem. Researchers, over the years have been looking for less expensive and more environmentally friendly strategies to enhance the properties of these deficient soils [4]. The MICP technique of soil improvement modifies the arrangement of the soil particle sizes and influences the arrangement of the soil matrix by enhancing crystallisation within the soil matrix. Therefore, this study was aimed at the assessment of the impact of different cementation reagent concentrations on the index and physico-chemical properties of the lateritic soil bio-treated with Bacillus *sphaericus.* The objectives include culturing of micro-organism from the lateritic soil in large quantities required for the soil improvement process, characterisation of the natural soil and B. *sphaericus* from the soil, evaluation of the plasticity properties of the natural and bio-treated soil, and micro-analysis of specimens of the natural and bio-treated soil using scanning electron microscope (SEM).

2. Materials and Methods

2.1. Materials

2.1.1. Soil

The method of disturbed sampling was used to collect the soil from a site prone to erosion, located in the Abagana district (Latitude 6°12′15″N and Longitude 7°0′40″E), Njikoka Local Government Area, Anambra state at depths in the range 0.5 - 3.0 m.

2.1.2. Bacteria

The Gram-positive micro-organism used in the study is *Bacillus sphaericus* which is a rod-shaped bacterium with 2 - 5 μm diameter.

2.1.3. Cementation Reagent

The reagents were varied by using an equal molar concentration of calcium chloride and urea to produce cementation solutions of different molar quantities (i.e., 0.25 M, 0.5 M, 0.75 M, and 1 M)

2.2. Methods

2.2.1. Isolation and Characterisation of Bacteria

Bacillus sphearicus bacterial type was used in the study. The bacteria was isolated from the soil of which six (6) different samples were collected and used for the isolation, identification, and characterisation of the *Bacillus sphearicus*. They were inoculated on Nutrient Broth, Yeast Extract, Nutrient Agar, and MICP agar, respectively.

2.2.3. Biochemical and Confirmatory Test

Urease Production

The enzymatic capacity of the test organism to degrade urea agar slant for ammonia and carbon dioxide production through hydrolysis that enables the culture medium to turn alkaline thereby resulting in change of colour from orange to pink, indicated the presence of positive urease test organism. This process was carried out by inoculating the test organism on a urea agar slant that was incubated at 37° C for 24 hours.

2.2.4. Index Properties

The index tests for the untreated and bio-treated soils were conducted in accordance to relevant specifications [10, 11].

Sample preparation

The soil sample used was passed through BS No. 40 sieve (425 μm aperture) and treated using bacteria-cementation mix ratios of 25 % : 75 %, 50 % : 50 %, and 75 % : 25 %, respectively (adapted from [2]). The liquid limit (LL) of the natural soil determined this mix ratio. Bacillus *sphaericus* was administered at suspension densities of 0, 0.5, 2.0, 4.0, 6.0 and 8.0 McFarland standards corresponding to 0, 1.5×10^8, 6.0×10^8, 12×10^8, 18×10^8 and 24×10^8 cells/ml, respectively, at varying concentrations of cementation reagent of 0.25, 0.5, 0.75 and 1 M.

2.2.5. Atterberg Limits

Atterberg limits tests are essential for soil classification and identification. The air-dried and pulverized natural soil was sieved using BS No. 40 sieve prior to its mixture with the bacteria specimen.

2.2.6. Calcite Content

The acid-washing procedure was adopted as reported by [12] for the calcite content determination.

2.2.7. Measurement of Urease Activity Using Electrical Conductivity Test

The test method was carried out as proposed in the literature [11] by measuring the solution’s electrical conductivity every minute using an electrical conductivity meter to determine the level of the activity of the urease.

2.2.8. pH

The growth of urease producing bacteria, the crystallization of $CaCO_3$, and the enhancement of soil engineering properties are influenced by the pH value.

2.2.9. Microstructural Analysis

Micro-analysis of lateritic soil (natural and bio-treated) specimens was carried out using scanning electron microscope (SEM) to determine the change in morphology brought on by the growth of calcite precipitates on the interparticle surface of the biochemically treated soil.

3. Results and Discussion

3.1. Index Properties

The natural lateritic soil is classified as A-2-6 (1) in the AASHTO system [13] and SC (clayey sand) in the USCS [14].

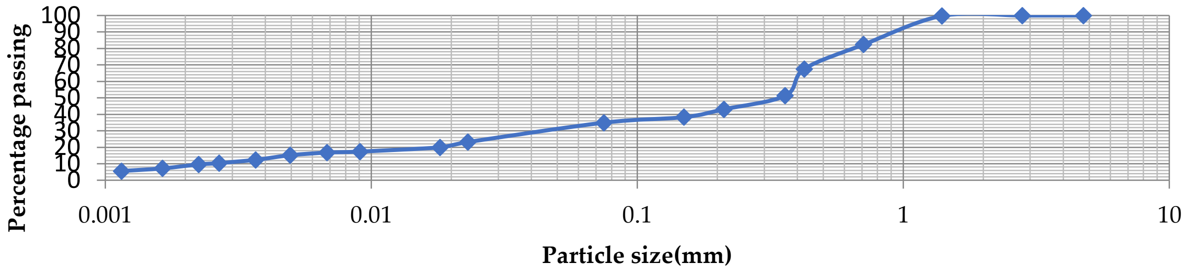


Figure 1. Particle size distribution curve of the natural lateritic soil.

3.2. Physico-Chemical Properties

3.2.1. Urease Activity

The electrical conductivity (EC) test was employed to investigate the bacteria urease activity in MICP [15]. The EC test result is presented in Figure 2. The peak urease activity value recorded was 80.8 ms/cm at cementation reagent concentration of 0.5 M and bacteria suspension density of 1.2 x 10^9 cells/ml.

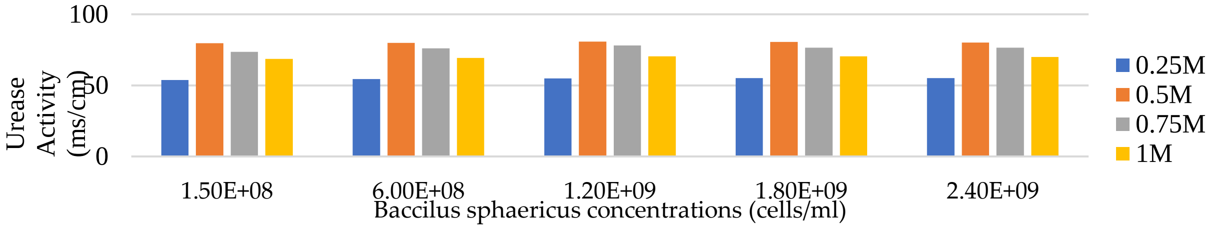


Figure 2. Variation of urease activity of lateritic soil - cementation solution mixtures with Bacillus *sphaericus* suspension density.

3.2.2. pH

The pH has a significant impact on the MICP technique because it affects the quantity of calcite precipitated at the end of the process as well as the number and performance of the microbes. The urea activity can also be reflected through the pH [16]. The optimum pH value of 8.99 was recorded at cementation reagent concentration of 0.5 M and bacteria suspension density of 1.2 x 10^9 cells/ml, as presented in Figure 3.

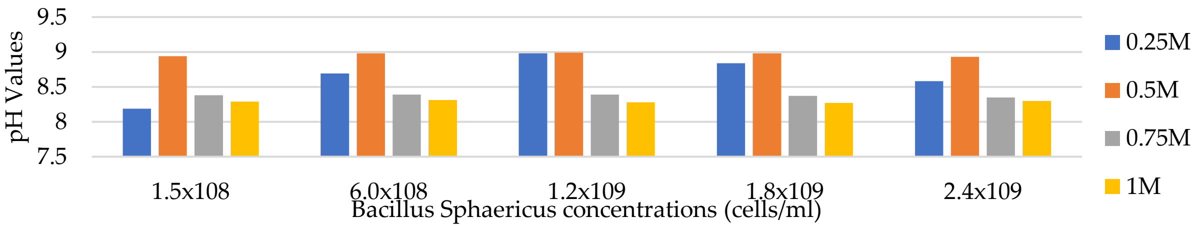


Figure 3. Variation of pH values of lateritic soil-cementation solution mixtures with Bacillus *sphaericus* suspension density.

3.2.3. Calcite Content

The lateritic soil was treated using stepped B. *sphaericus* suspension density to cementation reagent concentration mix ratios of 25 %: 75 %, 50 % : 50 %, 75 % : 25 %, respectively. A typical variation of calcium carbonate formed in the lateritic soil with B. *sphaericus* suspension density for mix ratio of 25 %: 75 % for different cementation reagent concentration is presented in Figure 4. Generally, the calcite content of all the treated specimens increased as the concentration of the cementation reagent increased to peak values before decreasing. The optimum calcium carbonate content (CCC) values were 9.0 %, 8.7 %, and 6.5 % for bacteria-cementation mix ratios of 25 % : 75 %, 50 % : 50 %, and 75 % : 25 %, respectively, at B. *sphaericus* suspension density of 1.2 x 10^9 cells/ml and cementation reagent concentration of 0.5 M.

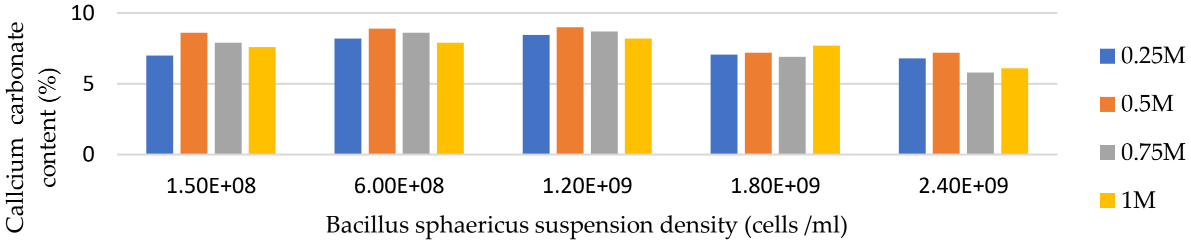


Figure 4. Variation of calcite content of lateritic soil - cementation solution mixtures with Bacillus *sphaericus* suspension density (25 % BS : 75 % cementation reagent mix ratio).

3.2.4. Atterberg Limits

The changes in Atterberg limits (LL, PL, and PI) and linear shrinkage (LS) of the lateritic soil bio-treated with stepped B. *sphaericus* suspension densities of 0, 1.5 x 10^8, 6.0 x 10^8, 1.2 x 10^9, 1.8 x 10^9, 2.4 x 10^9 cells/ml using bacteria to cementation mix ratios of 25 % : 75 %, 50 % : 50 %, 75 % : 25 %, respectively, at stepped cementation reagent concentration of 0.25, 0.5, 0.75 and 1 M were considered. A typical result for the 0.25 M cementation reagent is presented in Figure 5.

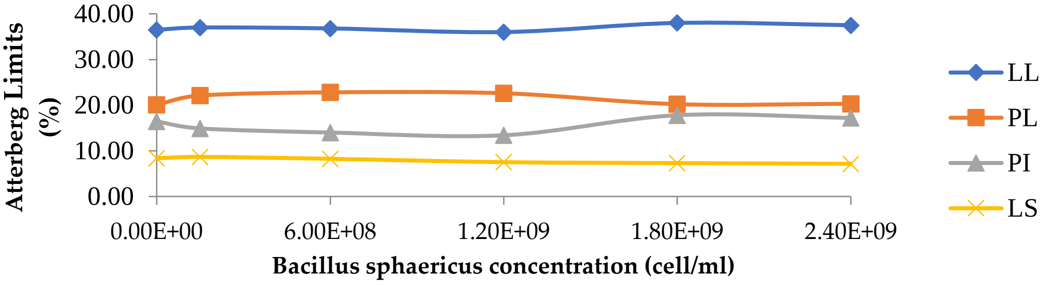


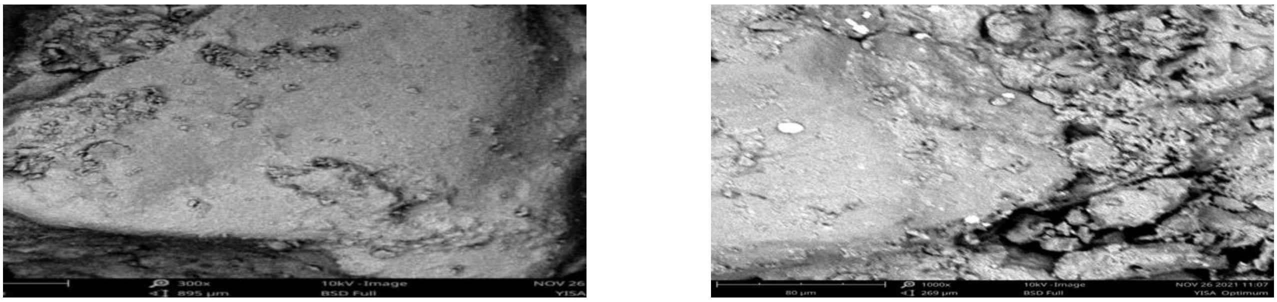
Figure 5. Variation of moisture content of lateritic soil - 0.25 M cementation reagent with B. *sphaericus* suspension density.

The LL increased from 36.5 % for the natural soil reaching its peak value at 38.0 % upon treatment with 1.8 x 10^9 cells/ml of B. *sphaericus* and with a further increase in the microbial density to 2.4 x 10^9 cells/ml, the LL value reduced to a value of 37.5 %. Similarly, the PL increased from 20.1 % for the untreated soil to a peak value of 22.8 % when treated with B. *sphaericus* suspension density of 1.2 x 10^9 cells/ml however, at 2.4 x 10^9 cells/ml the value decreased to 20.3 %. On the other hand, PI value decreased from 16.4 % for the untreated natural soil to 13.4 % *B. sphaericus* suspension density of 1.2 x 10^9 cells/ml. The LS value decreased from 8.4 % for the untreated soil to a minimum of 7.2 % at 2.4 x 10^9 cells/ml. Similar results were obtained for samples treated with higher bacterial suspension densities and cementation reagent concentrations.

The study showed that lateritic soil bio-treated with 25 % B. *sphaericus* (1.2 x 10^9 cells/ml) : 75 % cementation reagent (0.5 M) mix ratio gave the best plasticity index value indicating a better potential for soil improvement. The Atterberg limits results obtained in the study are consistent with the findings documented in the literature such as [17].

3.3. Microstructural Analysis

The calcite crystals precipitation and growth on a micro-scale were examined by scanning electron microscope (SEM). The micrographs for the untreated natural and the bio-treated lateritic soil specimens on Plates I(a) (at x300 magnification) and I(b) (at x1000 magnification) for Atterberg limits specimens prepared with 25 % Bacteria (1.2 x 10^9 cells/ml) : 75 % cementation reagent (0.5 M) mix ratio. Calcite precipitated, as confirmed using X-ray diffraction (XRD) analysis, on and between the soil grains is depicted on Plate I(b).



(**a**) (**b**)

Plate I. Micrograph of the natural lateritic soil at 300x magnification(a) and micrograph of the bio-treated lateritic soil (b) with 25 % Bacteria (1.2 x 10^9 cells/ml) : 75 % cementation reagent (0.5 M) mix ratio.

4. Conclusions

From the laboratory test results of the physico-chemical and index characteristics of the lateritic soil treated with stepped Bacillus *sphaericus* (B. *sphaericus*) suspension density at varying cementation reagent concentration, the following can be deduced:

1. Gram-positive, rod-shaped Bacillus *sphearicus* bacterial type isolated from each of the six (6) separate soil samples collected had total bacteria count not less than 3.65 x 10^4 cfu/ml
2. For the three mix ratios considered at varying cementation reagent and suspension densities, the PI decreased from 16.4 % to minimum values of 11.6 %, 12.2 %, and 16.2 % for the 25 % : 75 %, 50 % : 50 % and 75 % : 25% bacteria-cementation reagent mix ratios, respectively, for bio-treatments with B. *sphaericus* suspension density of 1.2 x 10^9 cells/ml and cementation reagent concentration of 0.5 M, 1.8 x 10^9 cells/ml and 0.5 M, as well as 6.2 x 10^8 cells/ml and 0.25 M, respectively.
3. The micrograph of the bio-treated soil specimen is more uniform and aggregated than that of the natural soil.

5. Recommendation

Based on the results obtained in the study, the physico-chemical and index properties of the A-2-6 or SC soil can be improved using 25 % B. *sphaericus* (1.2 x 10^9 cells/ml) : 75 % and 0.5 M cementation reagent (0.5 M) mix ratio.

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Conflicts of Interest: The authors have no clash of interests.

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