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Effect of Reagent Concentration on Strength of Lateritic Soil Bio-Treated with *Bacillus Thuringiensis*-Induced Calcite Precipitate Tested with Pocket Penetrometer †

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Abstract: The strength of lateritic soil bio-treated with *Bacillus thuringiensis* (Bt)-induced calcite precipitate was investigated using a pocket penetrometer (PPT). The effect of bacterial (Bt) and cementation solution concentration (C_s) on the strength of the microbial-induced calcite precipitate (MICP) worked soil was also evaluated. Soil samples were treated with Bt and C_s using three mix ratios (i.e., 25% Bt: 75% C_s, 50% Bt: 50% C_s and 75% Bt: 25% C_s) based on natural soil liquid limit (LL = 36.0%). Bt suspension densities of 0, 1.5 × 10^8, 6.0 × 10^8, 12 × 10^8, 18 × 10^8 and 24 × 10^8 cells/ml were applied to the soil with four varying C_s concentrations (i.e., 0.25, 0.5, 0.75 and 1 M). The prepared specimens were allowed to homogenise and equilibrate at laboratory conditions. A pocket penetrometer-PPT was used to test the unconfined compressive strength (UCS) of the prepared specimens at 3, 5, and 7 days after bio-treatment to evaluate the strength of the MICP worked soil at different moisture contents. The results obtained show that UCS values increased with higher Bt and C_s as well as with reduction in moisture content as the bio-treated soil equilibrated with the environment. The recorded UCS values for the mix ratios considered are in the order: 50% Bt: 50% C_s > 25% Bt: 75% C_s > 75% Bt: 25% C_s. Therefore, PPT can be used to determine the approximate unconfined compressive strength of treated soil.

Keywords: *bacillus thuringiensis;* pocket penetrometer; strength; lateritic soil; MICP

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1. Introduction

The success of any construction project is hinged on the comprehensive knowledge of the physico-mechanical dynamics of the bearing soil. The resistance of soil to penetration under loading condition during construction remains one of the key aspects to evaluate the soil bearing strength [1]. The factors that impact the soil strength include clay size, moisture content and the proportion of the clay mineralogy. The soil strength and its water holding ability is therefore a function of the soil texture, mineralogy as well as distribution and composition of the particle sizes [2].

Conventionally, triaxial test is used in the laboratory to determine the unconfined compressive strength (UCS) of soils especially those with significant amount of clay content. However, the test is time consuming and at a high cost. Also, cored samples for the test become so difficult to maintain the desired intactness hence the variability that exist between laboratory and in-situ test results [3]. A fast and easy means of assessing the UCS of soil is the use of hand pocket penetrometer (PPT) at a low cost in the laboratory and on the field [4,5].

Current soil improvement trends suggest a widely utilised technique bordering on intentional inducement of desirable minerals (calcite) into the soil using different bio-inspired or bio-mediated approaches to improve the soil bearing capabilities [5,6]. Soil microbes have also been used liquefaction mitigation through a process known as microbial induced desaturation (MID) [7–10]. However, the commonly used bio-inspired or bio-mediated methods are the microbial induced calcite precipitation (MICP) and enzymatic induced calcite precipitation (EICP) [11]. The former requires soil microbes to hydrolyse urea for the desired calcite formation. The latter method does not require microbes, however, the urease enzymes is extracted from plants and introduced into the soil when other favourable conditions are put in place for calcite precipitation occurs [5,11].

In this study, the MICP bio-treated soil moisture variations and strength development was evaluated using PPT.

2. Materials and Methods

Soil: The lateritic soil was sourced at Abagana (Latitude 6° 12′ 15″ N and Longitude 7° 0′ 40″ E) in Anambra State, Nigeria. Samples were taken at depths between 0.5 m and 3 m. The properties of the natural soil determined using British Standard [12] procedures for testing natural soils are summarised in Table 1.

Table 1. Parameters of the natural lateritic soil.

Property	Quantity
Natural moisture content (%)	19.6
Percentage passing No. 200 sieve	33.2
AASHTO [13] classification	A – 2 – 6 (2)
USCS	SC
Specific gravity	2.67
Liquid limit (%)	36.0
Plastic limit (%)	17.1
Plasticity index (%)	18.9
Linear shrinkage (%)	7.7
Dominant clay mineral	Kaolinite
Colour	Reddish-brown

Cementation reagent: The cementation solution (C_s) is composed of equi-molar of calcium chloride and urea as well as other nutrients such as sodium bicarbonate, ammonium chloride and nutrient broth. The molar concentrations were varied to produce four different C_s (i.e., 0.25, 0.5, 0.75 and 1 M). The mass concentration of the various components that make up the C_s is presented in Table 2.

Table 2. Mass per litre of cementation reagents.

Concentrations (M)	0.25	0.5	0.75	1.0
Calcium chloride ($CaCl_2$) (g/L)	27.75	55.49	83.24	110.98
Nutrient broth (g/L)	3	3	3	3
Sodium bicarbonate ($NaHCO_3$) (g/L)	2.12	2.12	2.12	2.12
Ammonium chloride (NH_4Cl) (g/L)	10	10	10	10
Urea ($CO(NH_2)_2$) (g/L)	15.05	30.03	45.05	60.06

Microorganism: The soil microorganism (*Bacillus thuringiensis* (Bt)) was isolated and identified from the soil to be treated in the laboratory using culture and biochemical confirmatory test kits for identifying and characterising Bt.

Preparation of sample: 300 g of soil with maximum particle size of 425 μm (i.e., soil that passed through BS No. 40 sieve) were treated with bacterial and cementation solutions in three separate mix ratios of 25% Bt: 75% Cs, 50% Bt: 50% Cs and 75% Bt: 25% C_s based on liquid limit (LL = 36%) of the natural soil. The prepared soil samples were allowed to equilibrate at the prevailing laboratory conditions (i.e., temperature of $25^o \pm 2^o$ C and relative humidity of 100%). A pocket penetrometer (PPT) was utilised to test the UCS of the prepared samples at 3, 5 and 7 days after treatment to measure the strength of the MICP treated soil at different moisture contents. Stepped bacterial suspension density of 0, 1.5×10^8, 6.0×10^8, 12×10^8, 18×10^8 and 24×10^8 cells/ml equivalent to 0, 0.5, 2.0, 4.0, 6.0 and 8.0 McFarland standards, respectively, were used in the study. The varying bacterial suspension densities were applied to the soil with four varying cementation reagent concentrations (i.e., 0.25, 0.5, 0.75 and 1 M) consisting of equal molars of $CaCl_2$ and $CO(NH_2)_2$ and other ingredients that make up the cementation solution as described by [14].

Measurement of strength with PPT: The PPT was used as a quick check to estimate the strength development of the bio-treated soil for the three mix ratios stated earlier. The PPT held perpendicular to the surface of the prepared soil is gradually pushed into the soil at a constant pressure until the calibrated grove machined at the tip of the piston becomes even with prepared soil sample surface. The estimated strength (UCS) is read directly on the lower end of the ring closest to the handle, away from the end of the piston. The indicator ring remains in position after releasing the piston (see Figure 1). The average of three similar values were taken as the estimated strength of the bio-treated soil.

(**A**) (**B**) (**C**)

Figure 1. (A, B) Pocket penetrometer testing. (C) Pocket penetrometer.

3. Results and Discussion

3.1. Effect of Cementation Solution Concentration and Bacterial Population on Strength Development

The strength values of the prepared samples recorded after three days were 98.06 kPa each for the three mix ratios used (i.e., 25% Bt: 75% Cs, 50% Bt: 50% Cs and 75% Bt: 25% C_s). However, the strength increased from 98.06 kPa recorded for 0.25 M concentration to 147.1 kPa when treated with 1.0 M concentration. Similarly, there were observed increment in strength values as the Bt population in the mixture increased. The results obtained further revealed that the resistance to penetration (strength) also increased as the exposure period (3–7 days) increased regardless of the mix ratio considered (see Figures 2 and 3).

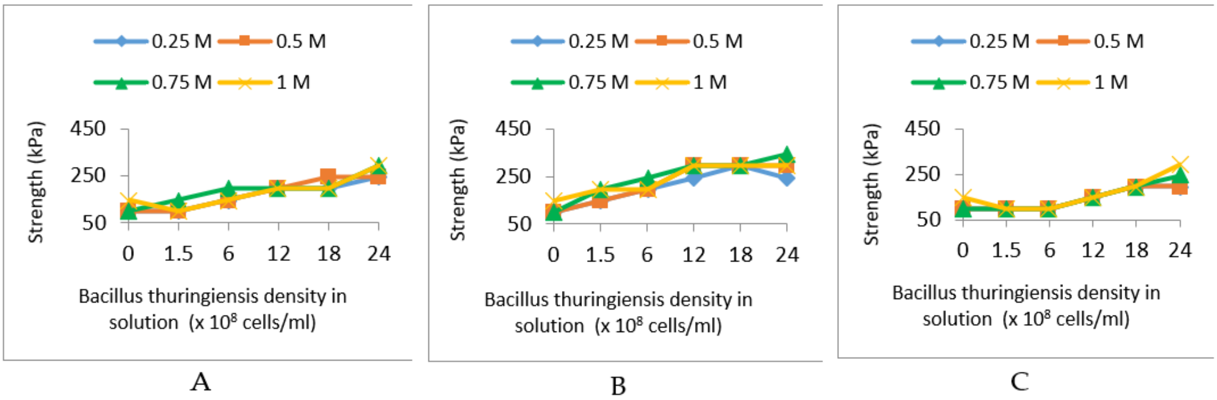

Figure 2. Variation of PPT strength (3 days) of lateritic soil with Bacillus thuringiensis suspension density for different mix ratios: (**A**) 25% Bt: 75% C_s (**B**) 50% Bt: 50% C_s (**C**) 75% Bt: 25% C_s.

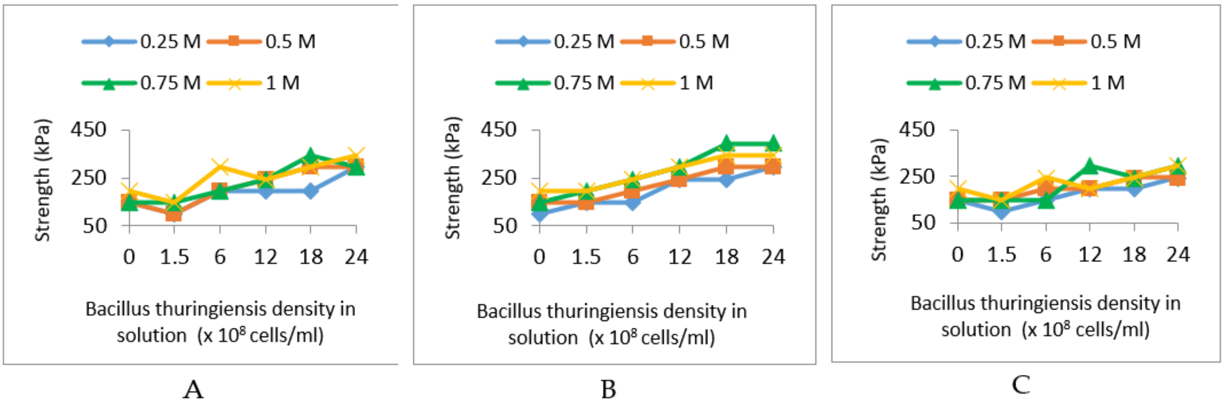

Figure 3. Variation of PPT strength (7 days) of lateritic soil with Bacillus thuringiensis suspension density for different mix ratios: (**A**) 25% Bt: 75% C_s (**B**) 50% Bt: 50% C_s (**C**) 75% Bt: 25% C_s.

The increase in PPT values at higher concentration of Cs and Bt population density in mixture may be due to high urease activity during the hydrolysis process as there were enough microbes in the mixtures to degrade urea and consequently calcite was precipitated in the soil matrix resulting in increased strength [5,6,11,15,16]. Similar reports are bound in literature including those of [1,4] among others.

3.2. Effect of Cementation Solution Concentration and Bacterial Population on Moisture Content

The variations of moisture content of lateritic soil with Bacillus *thuringiensis* suspension density for the different mix ratios (25% Bt: 75% C_s, 50% Bt: 50% C_s, 75% Bt: 25% C_s) are presented in Figures 4 and 5.

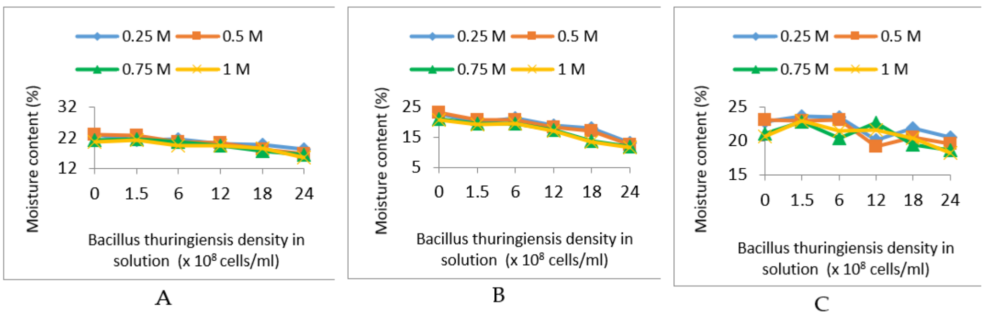

Figure 4. Variation of moisture content (5 days) of lateritic soil with Bacillus *thuringiensis* suspension density for different mix ratios: (**A**) 25% Bt: 75% C_s (**B**) 50% Bt: 50% C_s (**C**) 75% Bt: 25% C_s.

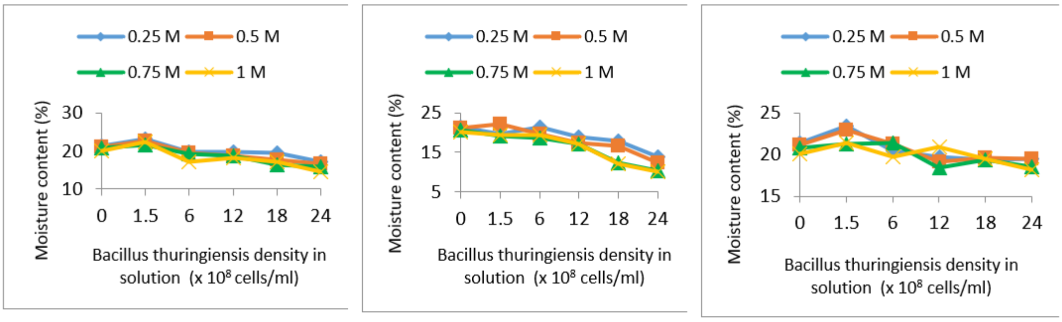

Figure 5. Variation of moisture content (7 days) of lateritic soil with Bacillus *thuringiensis* suspension density for different mix ratios: (**A**) 25% Bt: 75% C_s (**B**) 50% Bt: 50% C_s (**C**) 75% Bt: 25% C_s.

The moisture content of the bio-treated soil decreased with increase in exposure period (3–7 days) as well as Bt population density in the mixture regardless of the mix ratio used. Higher strength values recorded on the 7th day after treatment recorded the lowest moisture content values. It was observed that the strength of the bio-treated soil reduced with higher moisture content probably due cation exchange reaction involving the negatively charged electrons on the surface of the clay particles held together by electrostatic forces. Addition of moisture resulted in development of the diffused double layer of water surrounding the clay particles which weakened the electrostatic bonds resulting in strength decrease of the soil matrix [1,17,18].

3.3. Effect of Mix Ratio on the Strength of Treated Lateritic Soil

The distributions of the recorded strength and moisture content values against the three mix ratios are shown in Figures 5 and 6. The recorded strength and moisture values indicated higher dispersion of data at 50% Bt: 50% C_s suggesting a higher and wider range of strength values were achieved after bio-treatment of the lateritic soil (see Figure 5). Similarly, lower range of moisture content values were recorded at 50% Bt: 50% C_s (see Figure 6). The bio-treatment mix ratio performance is in the order of 50% Bt: 50% C_s > 25% Bt: 75% C_s > 75% Bt: 25% C_s (see Figures 6 and 7).

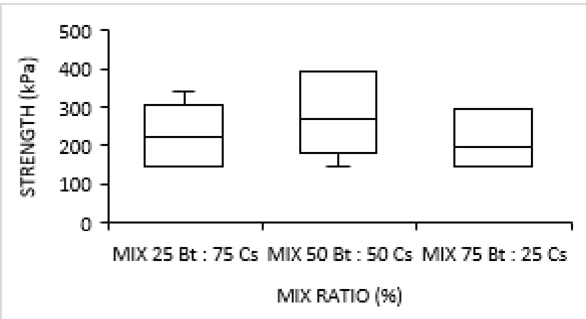

Figure 6. Distribution of pocket penetrometer data for the three mix ratios (after 7 days).

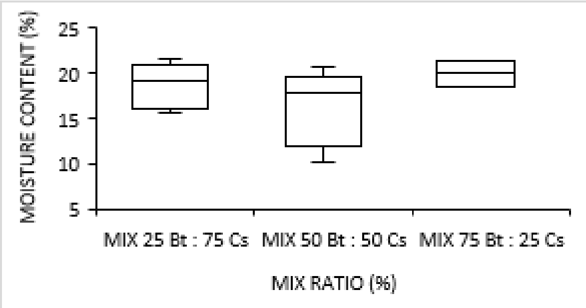

Figure 7. Distribution of moisture content data for the three mix ratios (after 7 days).

4. Conclusion

The following deductions can be made from the results of the study:

1. The PPT measured UCS values increased with higher Cs concentration and Bt suspension density in the soil matrix.
2. The moisture content of the bio-treated soil decreased as the soil attained equilibrium with the prevailing conditions of the test environment.
3. The 50% Bt: 50% C_s mix ratio recorded the highest strength values followed by 25% Bt: 75% C_s with intermediate values and the lowest values recorded for 75% Bt: 25% C_s mix ratio.
4. PPT can be used for quick assessment of strength development of bio-treated lateritic soil.

5. Recommendation

Based on the results of the study, it is recommended that lateritic soil treated with 50% Bt: 50% C_s mix ratio be further evaluated for use as an embankment material.

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