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Evaluation of the consistency and microstructural properties of lateritic soil treated using microbial induced calcite precipitation technique

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Abstract

The consistency of lateritic soil amended using Bacilus thuringiensis (Bt) via microbial-induced calcite precipitation (MICP) technique was evaluated. Three mix ratios of Bacilus thuringiensis suspension and cementation reagent (C_s) (i.e., 25% Bt : 75 % C_s, 50% Bt : 50 % C_s and 75% Bt : 25% C_s) were prepared on the basis of the natural soil liquid limit. Bacilus thuringiensis density in solution of 0, 1.5 × 10^8, 6.0 × 10^8, 12 × 10^8, 18 × 10^8 and 24 × 10^8 cells/mL were treated with four (4) varying cementation reagent levels of 0.25, 0.5, 0.75 and 1 M, correspondingly. Specimens were subjected to consistency limits, calcium carbonate content (CCC) and scanning electron microscopy (SEM) tests. The consistency limit findings indicated that lateritic soil treated using a 50:50 Bt–Cs mix ratio recorded the best improvement with the lowest plasticity index value of 14.3% for 6 × 10^8 cells/ml at 0.5 M compared to the 18.9% plasticity index for the natural soil. SEM results depicted calcites formation and microstructure modification of the treated lateritic soil. It is therefore suggested that lateritic soil amended with 50% Bt (6 ×10^8 cells/ml): 50% C_s (0.25 M) and 50% Bt (24 ×10^8 cells/ml): 50% C_s (0.5 M) with PI values of 16.9% and 15.7% respectively could be used to enhancee the consistency and other microstructural characteristics of lateritic soil for use as fill or embankment material.

Nomenclature and units

M	Molar concentration
Bt	*Bacilus thuringiensis*
C_s	Cementation reagent
CCC	Calcium carbonate content

1.0 Introduction

Lateritic soils are widely used in embankment construction because they are readily available in many regions, particularly across the tropics, including Nigeria, which helps lower overall project costs. Despite this advantage, their engineering properties present several difficulties that must be resolved to achieve durable and stable embankment performance. Enhancing lateritic soils typically requires overcoming issues such as moisture susceptibility, high plasticity, inconsistent composition, compaction challenges, and the risk of instability (Osinubi *et al*., 2021; Kanyi *et al*., 2025a). As a result, improvement strategies usually combine chemical stabilization, mechanical reinforcement, adequate drainage measures, and stringent construction practices.

In recent years, the use of soil microorganisms to improve the geotechnical characteristics of soils for different engineering purposes has grown steadily and is now widely accepted within the field of geo-environmental engineering. The method relied on the ability of soil microorganisms, especially the bacillus species, to precipitate calcite under favourable conditions (DeJong, 2006; Jiang *et al.*, 2020). The precipitated calcite is known to have some interesting properties of clogging or binding (DeJong *et al.,* 2010; Devrani *et al*., 2021; Etim *et al*., 2023) the soil particles together desirable for enhancing soil overall performance when loaded. The production of this desirable mineral (calcite) within the soil matrix for the purpose of enhancing the soil properties for engineering use is achieved through a technique termed microbial induced calcite precipitation (MICP) (DeJong, 2006; Osinubi *et al*., 2020a; Yohanna *et al*., 2022; Dubey *et al*., 2022).

The mechanisms driving the MICP technology usually starts with the production of urease enzymes through bacterial metabolism. This is closely followed by urea hydrolysation by the microbial urease enzymes yielding NH_4^+ (ammonium) and CO_3^{2-} (carbonate) ions. Finally, the generated CO_3^{2-} ions combine with Ca^{2+} (calcium ions) in a given environment abundant with calcium resulting in calcite formation (Stocks-Fischer *et al.*, 1999; Whiffin *et al.*, 2007; Ruyt and Zon, 2009; Jiang *et al.*, 2020; Liu *et al.*, 2021; Kanyi *et al*., 2023).

Soil enhancement via bio-mediation is therefore achieved by biochemical reactions involving the soil microbes and the cementing agent in the soil matrix, which change the coefficient of permeability, stiffness and strength behaviour of the soil for overall engineering performance. Urea hydrolysis remains the most utilised soil bio-mediation strategy primarily due to its convenience, controllability and capability of producing high amount of calcite in a short period (Nemati and Voordouw, 2003; Cheng *et al*., 2014; Animesh and Ramkrishnan, 2016; Wang *et al*., 2022).

The choice of soil improvement technique for a given site is not a single outcome hypothesis but rather dependent on several variables such as the type of soil to be modified, equipment and its availability, skills, cost, construction period as well as past experiences (Sadek and Khoury, 2000). Chemical stabilisation and grouting have been reportedly used for improving low-strength and highly plastic soil for adequate engineering applications. The success is however compromised with well reported issues of environmental concerns, particularly global warming (Karol, 2003; Peethamparann *et al*., 2009). The search for soil improvement techniques that are not only sustainable but also eco-friendly birthed the MICP technology. This is achieved by the production of fragment-binding minerals via microbial mechanisms. The process has been applied in geo-environmental engineering in plugging, strengthening and improving soils, cyclic resistance as well as dilative tendency of granular soils (Druckenmiller *et al.*, 2006; van Paassen *et al.*, 2010; Burbank *et al.*, 2012; Montoya *et al.*, 2013; Murtala *et al.*, 2016; Zamani and Montoya 2018; Osinubi *et al.,* 2017; 2019a, b, c, d; 2021; Montoya, 2023). Microbial induced calcite precipitation has also been found to cause a decrease in hydraulic conductivity as well as reduction in internal and surface erosion caused by wave action, wind and water jet (Salifu *et al.*, 2016; Amin *et al.*, 2017; Ning-Jun *et al.*, 2017; Adam, 2018).

The MICP technique is a fairly recent research field utilised in solving civil and environmental drawbacks however, several studies (e.g., Mitchell and Santamarina, 2005; Dejong *et al*., 2006; Okwadha and Li, 2010; DeJong *et al*., 2010; Gat *et al*., 2014; Murtala *et al*., 2016; Dhami *et al*., 2018; Delgado-Baquerizo *et al*., 2018; Osinubi *et al*., 2020a, b; Yohanna *et al*., 2022; Dubey *et al*., 2022; Etim *et al*., 2023) have been conducted. The findings showed significant enhancement of the properties of treated soils compared with their natural states.

The overwhelming part of bacilli strains are able to precipitate calcium carbonate by converting urea into ammonia and carbon dioxide (Hammes *et al.*, 2003). A great number of researches reported in scholarly domain (e.g., Hata *et al.*, 2013; Ning-Jun *et al.*, 2017; Amin *et al.*, 2017; Osinubi *et al*., 2020b) adopted *Bacillus pasteurii* as urease positive microorganism for the purpose of stimulating calcite precipitate in consolidating sand columns. Little has been done with regard to the use of other bacilli as well as other soil types. The potency of a different bacillus strain (i.e., *Bacillus thuringiensis*) in improving soil properties was investigated.

This study evaluated the plasticity behaviour of lateritic soil treated with varying bacterial (*Bacillus thuringiensis*) populations in solution as well as varying the cementation solution by using equimolar urea and calcium chloride to obtain different molar concentrations (0.25, 0.5, 0.75 and 1 M) of the resulting cementation solutions.

The success of this research findings would mean new and improved alternative ways to address soils with consistency limits concerns as it affects their performance when used as embankment material.

2.0 Materials and Methods

2.1 Materials

2.1.1 Soil

A representative specimen was sourced at Abagana, specifically on the coordinates of 6° 12' 15" N and 7° 0' 40" E on latitude and longitude, respectively, in Anambra State, Nigeria. Disturbed specimens were taken at depths ranging from 0.5 m to 3 m.

2.1.2 Microorganism

The soil bacterium utilised for the research experimentation was *Bacillus thuringiensis* (*Bt*). The organism was extracted from the soil intended for treatment and cultured through biochemical confirmatory assays for characterising and identifying *Bt* under laboratory conditions.

2.2.3 Cementation solution

The constituents that formed the cementation solution (C_s) are as shown in Table 1.

Table 1. Cementation reagents

Chemical name	Chemical formula
Nutrient broth	-
Ammonium chloride	NH_4Cl
Urea	$CO(NH_2)_2$
Sodium bicarbonate	$NaHCO_3$
Calcium chloride	$CaCl_2$

The cementation solution comprised of equimolar of $CaCl_2$ and $CO(NH_2)_2$, as reported by Murtala *et al.* (2016), in addition to 10 g ammonium chloride (provided to boost the growth of the bacteria), 2.12 g of sodium bicarbonate (to maintain an alkaline pH of the solution) and 3 g nutrient broth dissolved per dm^3 of de-ionised water. These quantities are reported to be the optimum amounts in cementation solution for an efficient MICP process (Stocks-Fisher *et al.*, 1999; DeJong *et al.*, 2006; Osinubi *et al.*, 2019d). On the strength of preventing urea decomposition by heat during autoclaving, it was introduced in the solution after other reagents were autoclaved under sterile conditions (Osinubi *et al.*, 2021). Four (4) varying cementation solutions (0.25, 0.5, 0.75 and 1 M) used in the study (see Table 2) were achieved using equations 1 and 2.

$$\text{Molar concentration (M)} = \frac{Mass\ concentration\ (g)}{Molar\ mass\ (^{g}/_{mol})} \quad (1)$$

$$\text{Mass concentration} = Molar\ concentation\ \left(^{mol}/_{dm^3}\right) \times Molar\ mass\ \left(^{g}/_{mol}\right) \quad (2)$$

Table 2. Mass concentration of cementation reagents per litre

Chemical	Solution reagents mass concentration (g/l)			
	0.25 (M)	**0.5 (M)**	**0.75 (M)**	**1 (M)**
Nutrient broth	3	3	3	3
Ammonium chloride (NH_4Cl)	10	10	10	10
Urea ($CO(NH_2)_2$)	15.05	30.03	45.05	60.06
Sodium bicarbonate ($NaHCO_3$)	2.12	2.12	2.12	2.12
Calcium chloride ($CaCl_2$)	27.75	55.49	83.24	110.98

2.2 Methods

2.2.1 Index properties

Index properties (i.e., consistency limits, specific gravity, natural moisture content and sieve analysis) were determined in accordance with British Standard (BS) 1377 (1990) for natural soils and BS 1924 (1990) for stabilised soils. Soil classification was carried out using both the AASHTO – American Association of State Highway and Transportation Officials (1986) system and the USCS – Unified Soil Classification System procedures (ASTM D2487, 2006).

2.2.2 Bacterial isolation, purification and preservation

The isolation of *Bacillus thuringiensis* (*Bt*) utilised the selective method described by Travers *et al.* (1987) using sodium acetate with minor modifications as reported by Eberemu *et. al*.,2023.

2.2.3 Characterisation of Bt

The suspected *Bt* isolates collected were characterised using cultural and biochemical characteristics typical of *Bt* strains in line with Gram/spore staining routines (Bergey 2004; Willey *et al.*, 2008). The biochemical properties tested include: motility, citrate utilisation, catalase, oxidase, urea hydrolysis, Methyl red, Voges Proskaeur, casin hydrolysis and arginine hydrolysis.

2.2.4 Viscosity

RV DV-1 Brookfield digital viscometer set at 50 rpm was used to determine the viscosity (a measure of fluid's resistance to flow) of the prepared various bacterial density in solution as well as the varying cementation solutions following steps as presented by Hassan *et al*., 2006.

2.2.5 Preparation of MICP sample for consistency limits

The specimen preparation for the MICP treatment followed the procedure described by Osinubi *et al.* (2019d). Samples were primed in three (3) distinct mix ratios (that is, Bt and C_s combined in proportions of 25% to 75%, 50% to 50%, and 75% to 25%) representing bacterial (*Bt*) to cementation reagent (C_s) ratio on the basis of the natural soil liquid limit (36%). The proportioned mixes above translated to 9%:27%, 18%:18% and 27%:9% bacterial and cementation reagents, respectively, used during experimentation. 300 g of soil specimens screened using a BS No. 40 sieve (425 µm aperture) were mixed with the proportioned mixes listed above and allowed to dry under ambient conditions in the laboratory before carrying out consistency limit tests. The outcomes of the three mix ratios were compared to establish the MICP treatment with the best improvement output. Varying bacterial solutions were prepared in terms of the *Bt* population in the solution. These were prepared using McFarland standards (Chapin and Lauderdale, 2003). Stepped bacterial density in solution of 0, 1.5×10^8, 6.0×10^8, 1.2×10^9, 1.8×10^9 and 2.4×10^9 cells/mL were applied in the experiments. The various bacterial density in solution were treated with four (4) varying cementation reagents (i.e., $CaCl_2$ and $CO(NH_2)_2$) with concentrations of 0.25, 0.5, 0.75 and 1 M. The bacterial solution and the prepared samples used for the consistency limits test are shown on Figure 1a -c.

A B C

Figure 1. (a) *Bacillus thuringiensis* solution (b and c) prepared samples with bacterial and cementation solutions for consistency limits.

2.2.6 Determination of calcite content

The procedure for determination of calcium carbonate (calcite) content (CCC) represented as a percent by mass of the total sample following documented steps reported by Choi *et al.* (2017). In the washing with acid method, 5 g of the primed sample (treated soil) was stirred in 20 ml of HCl (1 M) to evanesce the calcite formed during treatment. The resulting solution and the undissolved solids were then filtered on a filter paper in 0.75 mm sieve (sieve No. 200) and washed with distilled water for 10 minutes to get rid of dissolvable calcium available in the soil particles. The residual soil grains on the filter paper were subjected to oven regulated temperature (105° C) to completely dry off moisture for 24 hours before being weighed. The change in the specimen's mass prior to washing (A_1) and that after washing (A_2) was determined as the mass of CCC formed. This is expressed as a percentage using equation (3):

$$CCC\ (\%) = 100 - \frac{A_2}{A_1} \times 100 \qquad (3)$$

2.2.7 Microanalysis

The inter particle and precipitated calcite bond formed during MICP treatment were assessed using Phenom-world analyser for the evaluation of particle morphology at the microscopic level using scanning electron microscopy (SEM).

3.0 Results and Discussions

3.1 Index properties

The soil intrinsic and laboratory experimental results indicate that it is an A – 2 – 6 (2) or SC soil when classified using AASHTO (1986) and USCS (ASTM D 2487, 2006) soil classification systems, respectively. According to Osinubi *et al*. (2020b), the predominant clay mineral in the soil from the same study site as determined from Xray diffraction (XRD) analysis is Kaolinite, and the silica to sesqueoxide {i.e., $SiO_2/(Al_2O_3 + Fe_2O_3)$} ratio value of 1.64 falls within the range of 1.33 and 2.00 thus, qualifying soil of the study location as lateritic soil. The particle size characteristics of the untreated lateritic soil is shown in Figure 2. The size fractions (Craig 2004) and other properties of the natural soil are listed in Tables 3 and 4, respectively.

Table 3. Summary of soil fraction contents

Particle size fraction	(%)
Gravel component (> 2.0 mm)	0.1
Sand component (0.06 - 2.0 mm)	76.9
Silt component (0.002 - 0.06 mm)	15.9
Clay component (< 0.002 mm)	7.1

Table 4. Index and physical characteristics of the natural lateritic soil

Property	Quantity
Natural water content (%)	19.6
Specific gravity of solids	2.67
Fraction passing No.200 sieve (%)	33.2
Liquid limit (%)	36.0
Plastic limit (%)	17.1
Plasticity index (%)	18.9
Linear shrinkage (%)	7.7
Classification by USCS	SC
Classification by AASHTO	A – 2 – 6 (2)
Major clay mineral	Kaolinite
Colour	Reddish-brown

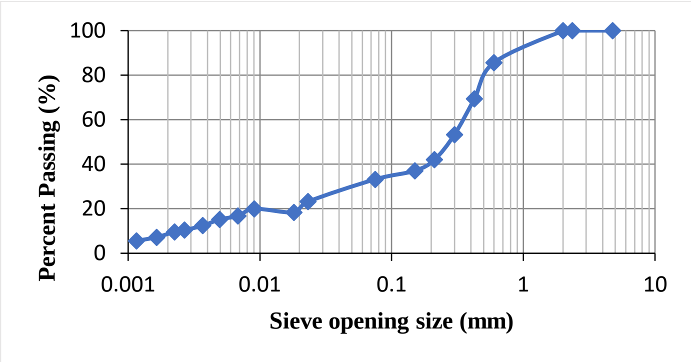


Figure 2. Distribution of particle sizes in the untreated soil

3.2 Bacterial isolates characterisation and viscosity of solutions used

The colonial morphology of isolated organisms having microscopic as well as biochemical properties typical of *Bt* is presented in Table 5. On LB agar, colonies that appear circular, dry, and creamy white, with wavy margins and flat elevation (see Figure 3a) were putatively regarded as Bt. Urease test was conducted to investigate the ability of *Bt* isolates to hydrolyse urea. This is because the organism utilised during the MICP process must be urease positive for effective calcite conversion from the chemical reaction (Mujah *et al*., 2019). Therefore, the test result indicates that the *Bt* isolates can secrete urease enzymes, as was observed in the pink colouration after the introduction of *Bt* isolates into the urea source when placed in an incubator for 24 hours (see Figure 3b). The viscosity test (see Figure 3c) results of microbial and cementation solutions are presented in Table 6.

Table 5. Biochemical test for characterisation of bacteria

Test	Reaction
*Colonial morphology	+
Shape of cell/ Gram reaction	+/R
Endospore stain	+
Chains of cell	+
Motility	+
Oxidase	+
Catalase	+
Voges Proskauer	+
Methyl Red	-
Urea hydrolysis	+
Arginine hydrolysis	-
Casein hydrolysis	+
Utilisation of citrate	+
Comment	*Bacillus thuringiensis*

*Colonies that appear circular, dry, and creamy white, with wavy margins and flat elevation on Luria Bertani (LB) agar. R = rod, + = positive result, - = negative result.

The bacterial solution becomes relatively more viscous as the *Bt* population density increases (see Table 6). The viscosity of the cementing solution increased marginally as the cementing concentration increased. The viscosity of 9.175 mPa.s recorded for 0.25 M solution increased to 9.427, 9.439 and 9.662 mPa.s for 0.5, 0.75 and 1 M solutions, respectively. The increase in the viscosity values could possibly be attributed to the increased *Bt* population (biomass) and cementation ingredients that constitute the mixtures. The results align with MICP process requirements described by Achal and Pan, 2011 as well as Osinubi *et al.*, 2019d.

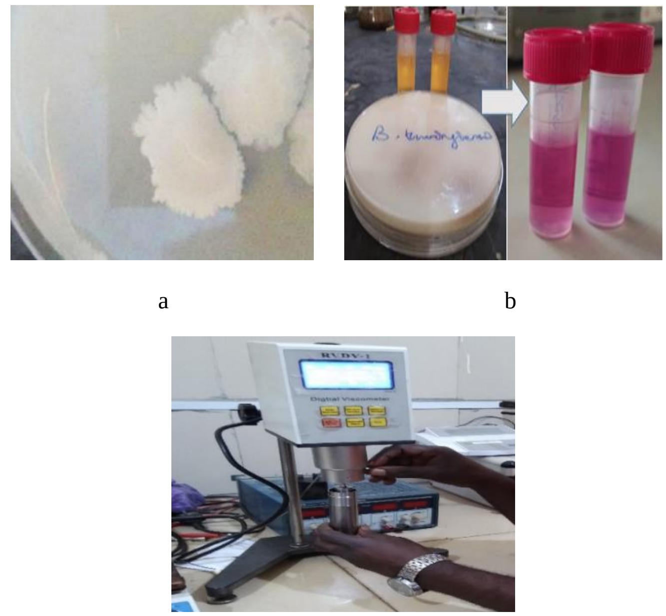


Figure 3. (a) *Bacillus thuringiensis* on Luria-Bertani (LB) agar plate (b) Urea hydrolysis test (c) Viscosity test

Table 6. Viscosity characteristics of bacterial and cementation solutions used during experimentation.

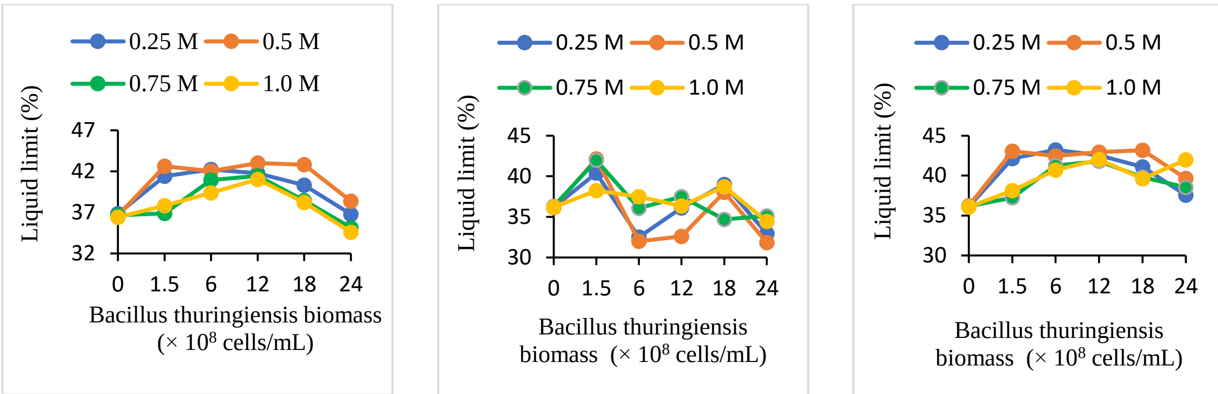
Bacterial/cementation solution	Viscosity (mPa.s)	Temperature ^{0}C
0 cell/ml (distilled water)	1.024	28
0 cell/ml (Tap water)	0.946	29.3
1.5×10^8 cell/mL	4.446	29.1
6.0×10^8 cell/mL	5.329	29.1
12×10^8 cell/mL	6.921	29.1
18×10^8 cell/mL	8.265	29.1
24×10^8 cell/mL	8.082	29.1
Cementation solution 1 (0.25 M)	9.175	29.1
Cementation solution 2 (0.50 M)	9.427	29.1
Cementation solution 3 (0.75 M)	9.439	29.3
Cementation solution 4 (1 M)	9.662	29.3

3.3 Consistency limits

The results of consistency limits for the *Bacillus thuringiensis* density in solution - cementation solutions – lateritic soil mixtures are presented in Figures 4-6. These limits- which include; liquid limits (LL), plastic limits (PL) as well as plasticity index (PI), were explored to investigate the natural and treated soil behaviour as it changes from one consistency level to another at different moisture content (solid phase to liquid phase) for the three mix ratios of bacterial and cementation solution application (i.e., 25% *Bt* : 75% C_s, 50% *Bt* : 50% C_s and 75% *Bt* : 25% C_s) considered.

3.3.1 Liquid limit

The LL results for the varying lateritic soil - *Bt* - C_s mixtures are illustrated in Figures 4a - c. The outcomes depicted an initial rise in the LL values as the bacterial population densities increased in the resulting soil mixtures for the three mix ratios considered.



a b c

Figure 4. Liquid limit variation of lateritic soil with *Bacillus thuringiensis* density in solution when prepared under varying mix proportions: (a) 25% *Bt*: 75% C_s (b) 50% *Bt*: 50% C_s (c) 75% *Bt*: 25% C_s

The LL values initially raised to peak values of 43, 42.1 and 43.2% at 12 × 10^8, 1.5 × 10^8 and 18 × 10^8 cells/mL at 0.5 M for mix ratios 25% *Bt* : 75% C_s, 50% *Bt* : 50% C_s and 75% *Bt* : 25% C_s, respectively. Thereafter, the LL values decreased with higher *Bt* suspension densities. The effect of cementation reagent concentration did not produce a definite trend of LL, especially when 50% *Bt* : 50% C_s mix ratio was considered (see Figure 4b). However, there was a noticeable reduction in the LL values at higher concentrations of C_s (i.e., 0.75 and 1 M) when 25% *Bt* : 75% C_s and 75% *Bt* : 25% C_s mix ratios were considered (see Figure 4a and c).

The initial rise in LL values may be connected to the high amount of water required for urea hydrolysis. The enzymatic decomposition of urea in the presence of water produces $CO3^{2-}$ (carbonate ions) and NH_4^+ (ammonium ions) in the resulting soil matrix, which also increases the pH of the mixture (Wang *et al.*, 2020). This, in turn, led to strong attraction for water by the clay minerals present in the soil, thus increasing the diffuse double layer, which resulted in the initial rise in the LL of the soil matrix. The decrease in the LL values at elevated Bt populations could be linked to the non-plastic nature of calcium carbonate (calcite) precipitated inside the pore network of the soil mixture. In addition, the cation exchange reaction involving the calcite formed, multivalent cations such as Fe^{3+}, Ca^{2+} and Al^{3+} available and the clay minerals within the soil may also be implicated in the reduction of LL limit values (Murtala *et al.*, 2016; Ning-Jun *et al.*, 2017; Osinubi *et al.*, 2019d, 2021).

3.3.2 Plastic limit

The change in the PL of lateritic soil – *Bt* – C_s mixtures for the three mix ratios considered is presented in Figure 5a - c. The PL values generally increased to peak values with higher bacterial density in solution and thereafter decreased regardless of the mix ratio and cementation reagent concentration considered. Peak PL values of 22.8%, 26.2%, and 21.7% were achieved at cell densities of 1.2 × 10^8 cells/mL for 1 M, 1.5 × 10^8 cells/mL for 0.75 M, and 1.2 × 10^8 cells/mL for 1 M, corresponding to *Bt*:C_s mixing ratios of 25:75, 50:50, and 75:25, respectively (see Figure 5a – c). The increase in PL value was probably due to the formation of vaterite (anhydrous calcium carbonate, $CaCO_3.H_2O$) because of inadequate calcium ions (Ca^{2+}) in the mixture. Since insufficient amounts of calcium ions (Ca^{2+}) were neither absorbed nor available on the surface of calcium carbonate (calcite), the reduction in depositions of ionic pairs ($CaCO_3$) as well as alteration of vaterite to calcium carbonate (calcite) decreased the moisture content (Li *et al.*, 2013; Osinubi *et al.*, 2019d; 2021). The water of crystallisation in the vaterite may have initiated the inherent increase in moisture that resulted in increased PL value with higher bacterial population in the mixture. The results agree with the outcomes reported by Jiang *et al.* (2020) and Osinubi *et al.* (2017; 2021).

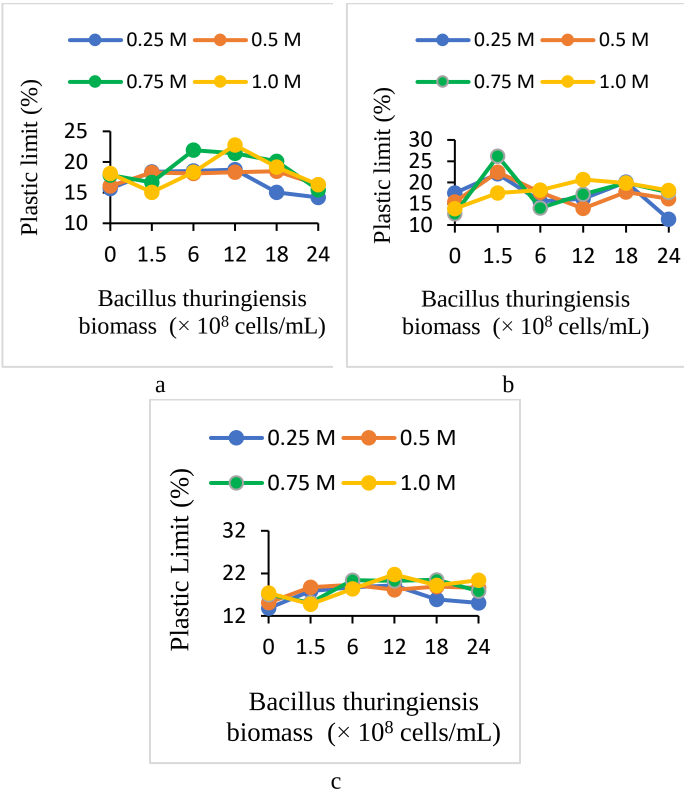


Figure 5. Plastic limit variation of lateritic soil with Bacillus *thuringiensis* density in solution when prepared under varying mix proportions: (a) 25 % *Bt*: 75 % C_s (b) 50 % *Bt*: 50 % C_s (c) 75 % *Bt*: 25 % C_s

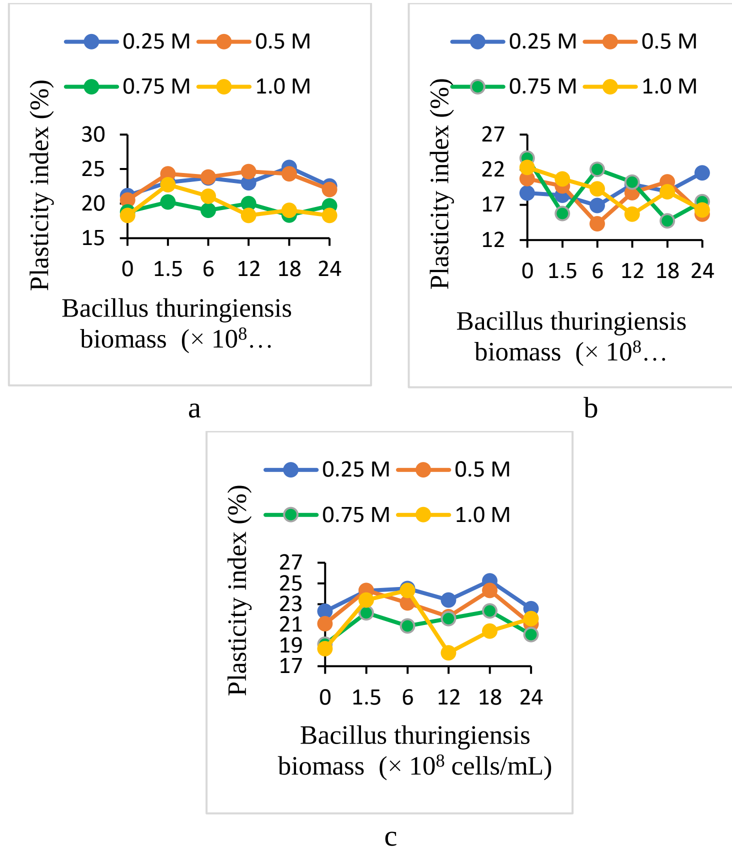


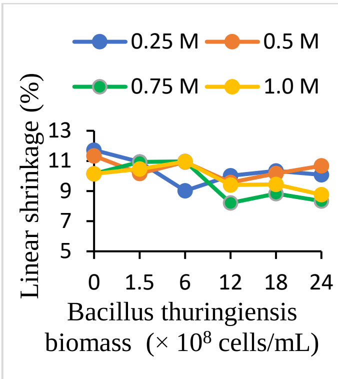
Figure 6. Plasticity index variation of lateritic soil with Bacillus *thuringiensis* density in solution when prepared under varying mix proportions: (a) 25% *Bt*: 75% C_s (b) 50% *Bt*: 50% C_s (c) 75% *Bt*: 25% C_s

3.3.3 Plasticity index

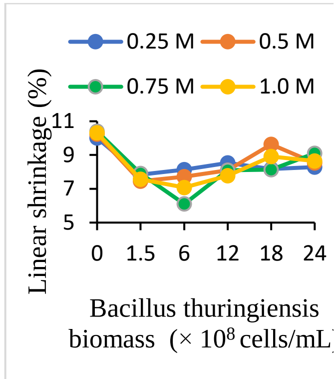
The change in plasticity index of lateritic soil – *Bt* – C_s mixtures for the three mix ratios considered is presented in Figure 6a - c. The PI values increased to peak values before decreasing when 25% *Bt* : 75% C_s and 75% *Bt* : 25% C_s mix ratios were considered. On the other hand, a marginal decreasing trend was observed for 50% *Bt* : 50% C_s mix ratio. The minimum PI values obtained were 18.3 % (2.4 ×10^8 cells/mL - 1 M) (see Figure 4a), 14.3 % (6 x 10^8 cells/ml - 0.5 M) (see Figure 4b) and 18.3 % (12 x 10^8 cells/mL - 1 M) (see Figure 6c) for 25% *Bt* : 75% C_s, 50% *Bt* : 50% C_s and 75% *Bt* : 25% Cs mix ratios, respectively. It can be deduced from the PI values recorded that bio-treatment with 50% *Bt* : 50% C_s mix ratio generally produced lower values in comparison to the values produced by 25% *Bt* : 75% C_s and 75% *Bt* : 25% C_s mix ratios. This may be connected to an adequate quantity of *Bt* population and cementation reagent concentration that precipitated calcite during the ureolytic enzyme decomposition of urea with the available water within the media. This resulted in the optimum production of calcite in the treated soil and, hence, the overall improvement in terms of lower plasticity index values as indicated in the best improved PI values recorded for 50% *Bt* : 50% C_s mix ratio. Lower PI values are indicative of soil improvement (Nigeria General Specification, 1997). The obtained results are consistent with the findings reported by Neupane *et al.* (2016), Liu *et al.* (2021) and Osinubi *et al.* (2021).

3.4 Linear shrinkage

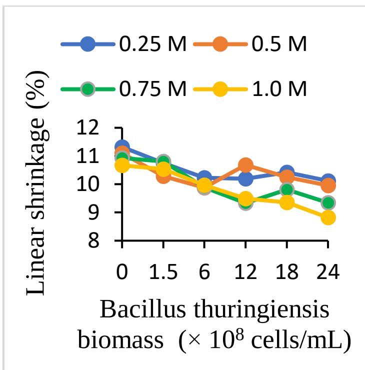
The change in linear shrinkage (LS) of lateritic soil – *Bt* – C_s mixtures for the three mix ratios is presented in Figure 7a - c. The LS showed a general decreasing trend as the *Bt* population density in the bacterial – C_s – soil mixtures increased. When lateritic soil was treated with C_s only (i.e., 0.25, 0.5, 0.75, and 1 M), the LS values of 11.7, 11.3, 10.2, and 10.1% recorded decreased to minimum values of 9.0, 9.4, 8.2, and 8.8 % at 6 × 10^8, 1.2 × 10^9, 1.8 × 10^9, and 2.4 × 10^9 cells/mL, respectively, when 25% *Bt*: 75% C_s bio-treatment mix ratio was considered (see Figure 7a). Similar trends were observed for 50% *Bt*: 50% C_s and 75% *Bt*: 25% C_s bio-treatment mix ratios (see Figure 7b and c). The decrease in LS values with increase in *Bt* density in solution of the bio-treated soil matrix could be linked to cationic exchange reaction involving the available multivalent cations (Fe^{3+}, Ca^{2+}, Al^{3+}) in the soil with the soil clay minerals and the calcite formed in addition to the non-plastic nature of the bio-treated soil matrix (Li *et al*., 2013; Osinubi *et al.*, 2019d; 2021; Kanyi *et al.*, 2025b).



a



b

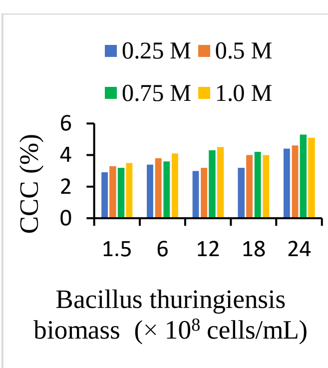


c

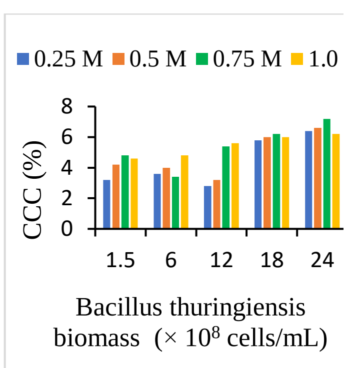
Figure 7. Linear shrinkage variation of lateritic soil with Bacillus *thuringiensis* suspension density when prepared under varying mix proportions: (a) 25% *Bt*: 75% C_s (b) 50% *Bt*: 50% C_s (c) 75% *Bt*: 25% C_s

3.5 Calcite content

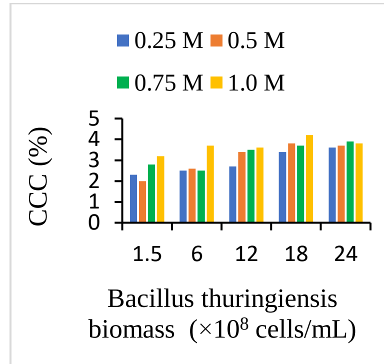
The change in calcium carbonate (calcite) content (CCC) of lateritic soil – *Bt* – C_s mixtures for 25 % *Bt* : 75 % C_s, 50 % *Bt* : 50 % C_s and 75 % *Bt* : 25 % C_s mix ratios is presented in Figure 8a – c, respectively.



a



b



c

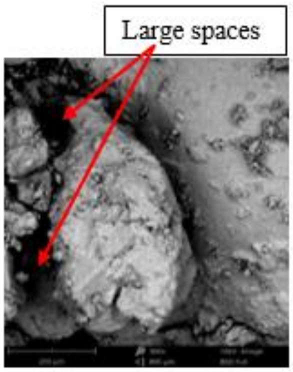
Figure 8. Calcium carbonate content variation of lateritic soil with Bacillus *thuringiensis* density in solution when prepared under varying mix proportions: (a) 25% *Bt*: 75% C_s (b) 50% *Bt:* 50% C_s (c) 75% *Bt*: 25% C_s

CCC values increased with higher *Bt* cell density and C_s. The baseline CCC of 1.8% rose to 5.3%, 7.2%, and 4.4% under 2.4 × 10^9 cells/mL–0.75 M Cs (25:75 *Bt*:C_s), 2.4 × 10^9 cells/mL–0.75 M C_s (50:50), and 1.8 × 10^9 cells/mL–1 M C_s (75:25), respectively. It was also observed that *Bt* density in solution between 1.8 × 10^9 cells/mL and 2.4 × 10^9 cells/mL for 0.5, 0.75, and 1 M cementation reagent concentrations recorded CCC values in the range 3.7 - 7.2% for the three bio-treatment mix ratios considered. The 50 % *Bt*: 50 % C_s mix ratio recorded the highest CCC value of 7.2% using 2.4 × 10^9 cells/mL - 0.75 M C_s mixture. The increase in CCC value with higher bacterial density in solution and cementation reagent concentration could be due to the large population of microorganisms in the bio-treated lateritic soil as well as adequate amount of calcium source in the cementation reagent that facilitated complete hydrolysis of urea and subsequent crystallisation of calcite due to the existence of Ca^{2+} ions in the soil matrix. Comparable outcomes were noted by Murtala *et al.* (2016) and Osinubi *et al.* (2019d; 2021).

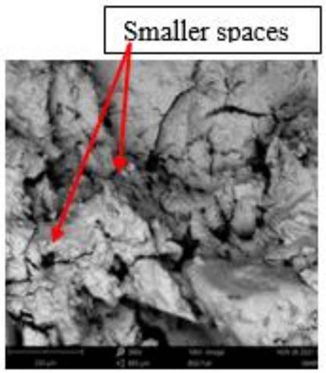
3.6 Microanalysis

Microanalyses of specimens of the naturally occurring and bio-treated lateritic soil were conducted to examine the microstructure characteristics, such as particle shapes, texture as well as verify the precipitation of calcite as desired in the treated lateritic soil. The micrographs for the naturally occurring lateritic soil, lateritic soil - cementation reagent mixture alone (0.75 M) and the optimum lateritic soil - 2.4 × 10^9 cells/mL - 0.75 M mixture prepared using 50% *Bt* : 50% C_s mix ratio that recorded the best improved PI and CCC values are shown on Figure 9a – c, respectively.

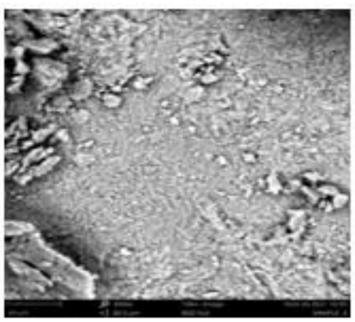
Macro cracks within the untreated lateritic soil are shown on Plate IIIA, while such cracks were mostly sealed in the bio-treated specimen in addition to aggregation of smaller particle sizes that formed more compact material with smoother morphology (see Figure c). The sealing of the relatively larger cracks in the treated soil could be attributed to the precipitated calcite crystals that bridged the soil particles together. When lateritic soil was treated exclusively with the Cs, a similar microstructure was noted (see Figure 9b). This may be due to inherent natural ureolytic bacteria available in the natural soil before the addition of cementation solution (calcium source), thus triggering the MICP process. The observed microscopic structural morphology supports the observations documented by Ning-Jun *et al*. (2017) and Osinubi *et al*. (2019d; 2021).



a



b



c

Figure 9. Microstructure of lateritic soil specimens observed at 300× magnification: (a) Natural soil (b) Treatment with 0.75 M cementation solution only (c) Optimal bio-treatment with 2.4×10^9 cells/ml - 0.75 M cementation solution mixture.

5.0 Conclusions

The study assessed the consistency limits of lateritic soil identified as A – 2 – 6 (2) and SC using the AASHTO and USCS system, respectively, when treated using microbial-induced calcite precipitation technique. The study experimental programme evaluated the availability and viable quantity of *Bt* in the soil, and the morphological and biochemical characterisation of the *Bt* isolates. The CCC % precipitated in the soil matrix, microanalysis using SEM and the consistency of lateritic soil treated with *Bacilus thuringiensis* (*Bt)* induced calcite precipitate was also evaluated. The inferences made from the results of the research are:

1. The microorganism isolated from the natural lateritic soil, which was sub-cultured for microbial-induced calcite precipitation (MICP) application, exhibited biochemical characteristics typical of *Bt.*
2. The 50 % *Bt* : 50 % C_s mix ratio bio-treatment produced the best improved plasticity index (PI) values of 16.9 % (6×10^8 cells/mL - 0.25 M), 14.3 % (6×10^8 cells/mL - 0.5 M), 14.7 % (1.8×10^9 cells/mL - 0.75 M), and 15.7 % (1.2×10^9 cells/mL - 1 M).
3. Bio-treatment of lateritic soil with *Bt* (in the range 1.8 – 2.4×10^9 cells/mL) - C_s (0.5, 0.75, and 1 M) recorded CCC values in the range 3.7 - 7.2 % for the three mix ratios considered. Bio-treatment with 2.4×10^9 cells/mL - 0.75 M using the 50 % *Bt* : 50 % C_s mix ratio produced the peak CCC value of 7.2 %.
4. The SEM results indicate the formation of calcite and microstructure alteration due to MICP treatment of the lateritic soil.

RECOMMENDATION

Drawing on the results of the study, the lateritic soil bio-treated with 50 % *Bt* (6×10^8 cell/ml): 50 % C_s (0.25 M) and 50 % *Bt* (24×10^8 cell/ml): 50 % Cs (0.5 M) mix ratios can be used to improve the consistency properties of lateritic soil for the construction of embankments.

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Declaration of conflict of interest

The authors jointly contributed to the conception, design, and implementation of this study. They were involved in drafting the manuscript and revising it critically to ensure substantial intellectual content. This work has not been submitted to or considered by any other journal or publication outlet. Furthermore, the authors declare no affiliations or relationships with any organization that has a direct or indirect financial interest in the subject of this manuscript.

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