Microbial-Induced Calcite Precipitation Study on the Plasticity and Compaction Characteristics of Lateritic Soil Treated with Bacillus *Megaterium* in Urea-CaCl\(_2\) Culture Medium

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Abstract. An ecofriendly method of soil improvement known as Microbial Induced Calcite Precipitation (MICP) has received significant recognition in the past decade. This study presents a report on the capability of MICP in modifying the plasticity and compaction properties of lateritic soil bio-treated with different suspension densities of a ureolytic microorganisms - Bacillus *megaterium* (*B. megaterium*). Specimens were prepared with liquid limit moisture of the natural soil in three mix ratios of *B. megaterium* (*B*) and cementation reagent (*C*) (urea medium): 25 % *B* : 75 % *C*, 50 % *B* : 50 % *C* and 75 % *B* : 25 % *C*. Another set of specimens were prepared at optimum moisture content (OMC) equivalent covering 1/3 pore volume of *B. megaterium* and 2/3 pore volume of urea medium introduced in three sequences at 6-hour interval to steadily initiate MICP mechanisms. The results of the various mix ratios indicated improvement in plasticity index (PI) with higher *B. megaterium* suspension density. The best improved PI value was obtained for specimen treated with 75 % *B*: 25 % *C* mix ratio at *B. megaterium* suspension density of 2.40 \(\times 10^9\) cells/ml with an equivalent maximum 5.3 % CaCO\(_3\) content. The maximum dry density (MDD) and OMC values marginally increased with higher *B. megaterium* suspension density as well as calcite content. The micrographs obtained from scan electron microscope (SEM) showed changes from untreated to bio-treated state, which resulted in non-uniform precipitation of calcite in the soil. The variation in elemental quantifications displayed by the X-ray energy dispersive spectroscopy (EDS) validate the formation of calcite within voids and on surface of the soil particles.

1. Introduction
Concerns over use of chemical stabilizers for soil strengthening and indeed improvement have been raised, especially as most chemical additives have some debilitating effects on the environment [1]. The increasing awareness on environmental issues caused by the use of chemical stabilizers has brought about an innovative approach which adopts green and eco-friendly technologies. A unique soil improvement practice involving biologic alteration in properties of soil can be achieved by means of microbiologically induced calcite precipitation (MICP). MICP is a relatively novel technique that utilizes microorganism, nutrients and biological processes to strengthen bearing strength, mitigate liquefaction, reduce permeability and shrinkage cracks in soils [1-8]. The eco-friendly features of ureolytic-microorganisms are most often than not the basis for its use. MICP has presented tremendous ground-breaking researches ranging from laboratory scale investigation to potential full-scale field implementation [9-14]. Although not without challenges...
consistent with conditions that favour precipitation as well as deposition of calcite, a case study approach to upscale MICP into field or large scale application based on specific applications/objectives was used and reported by [15]. Studies have shown that MICP techniques have been used in biocementation of leaky media, environmental remediation of contaminated sites, increased soil strength, reduction of desiccation cracks, improved plasticity properties, reduction in permeability of fine-grained soil, etc. [1, 7-8, 15-23].

The reaction mechanisms for the formation and spread of calcites products in MICP have been reported in several studies [1, 24]. Detailed reactions are given in eqs. (1) - (7). Urease enzymes released by the microorganism is used in the spontaneous hydrolysis of urea - calcium source. The bacteria cell offers itself as nucleation site from where calcium ions (Ca^{2+}) concentration and calcite in the form of Ca^{2+} and CO_{3}^{2-} in solution (see eqs. (6) and (7)) is formed.

\begin{align}
CO(NH_{2})_{2} + H_{2}O & \xrightarrow{\text{Microbial urease}} NH_{2}COOH + NH_{3} \quad (1) \\
NH_{2}COOH + H_{2}O & \xrightarrow{\text{Spontaneous}} NH_{3} + H_{2}CO_{3} \quad (2) \\
H_{2}CO_{3} & \leftrightarrow HCO_{3}^{-} + H^{+} \quad (3) \\
NH_{3} + 2H_{2}O & \leftrightarrow 2NH_{4}^{+} + 2OH^{-} \quad (4) \\
HCO_{3}^{-} + H^{+} + 2OH^{-} & \leftrightarrow CO_{3}^{2-} + 2H_{2}O \quad (5) \\
Ca^{2+} + \text{Bacteria cell} & \rightarrow \text{Cell} - Ca^{2+} \quad (6) \\
Cell - Ca^{2+}CO_{3}^{2-} & \rightarrow \text{Cell} - CaCO_{3} \quad (7)
\end{align}

Most studies in the field of MICP have mainly focused on engineering properties of coarse grained soils. However, few systematic studies have been carried out on the effect of MICP process on the plasticity behaviour of fine-grained soils [6, 18-19, 25]. Furthermore, limited literature exists which compare evaluation from different methods of sample preparation prior to consistency limit test. This study focused on the growing area of research on microbiological interactions in biogeochemistry exclusive to soil plasticity in MICP mechanisms. The potential of B. megaterium in the swamp of spore forming and urease producing enzymatic bacteria is explored in studying the plasticity and compaction behaviour of fine-grained soil. The specific objectives include, but are not limited to the determination of liquid limit, plasticity index and calcite content of lateritic soil bio-treated with B. megaterium in urea-calcium medium as well as the most effective bacteria - cementation reagent mix ratio.

2. Materials and Methods

2.1. Materials

2.1.1. Soil

Lateritic soil sample was collected from Abagana, Anambra state, Nigeria, using the method of disturbed sampling.

2.1.2. Bacteria

The microorganism Bacillus species (B. megaterium) bacteria was used. The microorganism was cultured and grown from the soil sample used in this study. B. megaterium is characteristically a gram-positive, rod-like, endospore forming and urease positive.

2.1.3. Cementation reagent

The cementation reagent is composed of 20 g(NH_{2})_{2}CO (urea), 10 g NH_{4}Cl (Ammonium chloride), 3 g nutrient broth, 2.8 g CaCl_{2} (Calcium chloride) and 2.12 g NaHCO_{3} (Sodium hydrogen trioxocarbonate.
3 (iv) per 1 dm$^3$ (de-ionized water) as reported by researchers [25-29]. After autoclaving the other solutions in the cementation reagent, urea was aseptically added to avoid the decomposition of urea by heat [7, 8, 20, 21, 25, 30].

2.2. Methods
2.2.1. Index properties

Tests on the natural and treated soil were performed based on procedures outlined in [31] and [32], respectively.

2.2.2. Sample preparation

Two methods were adopted for the preparation of samples for Atterberg limits tests. The first method adopted is the procedure reported by [18, 19, 33]. The method involved the introduction of 1/3 and 2/3 pore volume of bacterial and urea medium, respectively, to activate MICP process. In this study, air-dried sample of lateritic soil was pulverized and sieved through 4.76 mm openings. The pore volume was obtained using the OMC of natural soil compacted with British Standard light (or standard Proctor) energy. The soil - bacteria mixture for each test was obtained by mixing each 250 g of the soil with 1/3 pore volume of B. megaterium at suspension densities 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, respectively. The MICP process was initiated by the introduction of 2/3 pore volume of urea medium (cementation reagent) into the soil-bacteria mixtures in three cycles at 6 hours’ intervals. After the third cycle, the lateritic soil - B. megaterium - cementation reagent mixtures were sealed in polythene bags for 24 hours to enhance saturation and hydration before drying under laboratory condition.

The second method of sample preparation adopted is procedure reported by Osinubi et al. (2019c). Three specimens were prepared for different mix ratios of B. megaterium + urea medium. The composition ratio was considered based on the liquid limit moisture of the natural soil. Each 250 g of soil that was passed through sieve with 425 μm openings was mixed with the pre-determined liquid limit of the natural soil of 42.0 % prepared in three ratios of 14 %: 28 % (i.e., 25 % B: 75 % C), 21 %: 21 % (i.e., 50 % B: 50 % C) and 28 %: 14 % (i.e., 75 % B: 25 % C). The treated specimens were dried under laboratory condition to enable MICP modification of the soil structure. The stepped B. megaterium suspension density of, 0, 1.50 x 10$^8$, 6.0 x 10$^8$, 1.20 x 10$^9$, 1.80 x 10$^9$ and 2.40 x 10$^9$ cells/ml (i.e., equivalent of 0, 0.5, 2.0, 4.0, 6.0 and 8.0 McFarland standards) used in this study is based on McFarland standards [34].

2.2.3. Atterberg limits

The treated air-dried lateritic soil - B. megaterium - cementation reagent specimens were pulverized and passed through sieve with 425 μm aperture before testing using the procedure outlined in [31]. The average of three consistent results was used in the computations.

2.2.4 Calcite content

The calcite content was determined using the washing method reported by [35]. 5 g each of natural soil and treated soil were dissolved in 20 ml of 1-M HCl (gravimetric acid washing) to remove CaCl$_2$ residue. The solvent was then filtered using filter paper with openings corresponding to No. 200 sieve. The filtrate on the filter paper was then rinsed and washed for about 10 minutes with distilled water to completely remove dissolved calcium from the soil. Thereafter, particles of solid which were left on the filter paper were oven-dried at 105°C for 24 hours and weighed. The change in mass between the original sample (B$_1$) and washed sample (B$_2$) was determined as the mass of CaCO$_3$. The test was
repeated in triplicate and the average taken for the mixtures considered. The calcite content (CC) was determined using eq. (8):

$$CC(\%) = 100 - \frac{B_1}{B_2} \times 100$$  

2.2.5 Compaction

The procedure outlined in [31] for BSL compaction was used.

2.2.6 Microanalysis

Scanning electron microscopy, SEM (Phenom-world analyser) was used to assess the morphological variations due to the precipitation and spread of calcite imprints that bonded the particle contact points of the bio-treated specimens. The results were complimented with x-ray Energy dispersive spectroscopy (EDS) and automated statistical analyser that aided the display of semi quantitative elemental analysis of the basic component of untreated and bio-treated soil.

3. Results and Discussion

3.1. Index property

The tested soil was classified as A-4(3) soil using AASHTO [36] or SC soil using USCS [37]. The particle size distribution and summary of the properties of the natural lateritic soil are provided in Figure 1 and Table 1, respectively.

| Property                           | Quantity   |
|------------------------------------|------------|
| Percentage passing 0.075 mm sieve  | 35.3       |
| Natural moisture content (%)       | 11.6       |
| Liquid limit (%)                   | 41.5       |
| Plastic limit (%)                  | 15.9       |
| Plasticity index (%)               | 25.6       |
| Specific gravity                   | 2.65       |
| AASHTO classification              | A-4(3)     |
| USCS classification                | SC         |
| Colour                             | Reddish-brown |
| Dominant clay mineral Kaolinite    | Kaolinite  |

**Table 1. Properties of natural lateritic soil**

![Figure 1. Particle size distribution curve for natural soil](image-url)
3.2. Effect of B. megaterium on liquid limit of lateritic soil

The effect of the B. megaterium suspension density on liquid limit (LL) of lateritic soil for the two methods of treatment considered is presented in Fig. 2. The LL of specimen bio-treated with 1/3 pore volume bacteria (PVB) and 2/3 pore volume urea medium (cementation reagent) (PVC) i.e., 13 PVB: 2/3 PVC, increased from 41.5 % for the untreated to a peak value of 45.7 % at B. megaterium suspension density of 2.4 × 10⁹ cells/ml. The steady increase in liquid limit of 1/3PVB: 2/3VPVC specimen could probably be due to inadequate calcium (Ca²⁺) from urea-CaCl₂ medium/cementation reagent required to precipitate the CO₃²⁻ into CaCO₃ or inadequate nucleation site (from bacteria); required to absorbed/attract mobile positive end of ions of CaCO₃ ions and free divalent Ca²⁺ onto its surface [1].

![Figure 2. Variation of liquid limit of lateritic soil with B. megaterium suspension density](image)

For the second method of treatment, the liquid limit values of specimens treated with 25 % B: 75 % C and 50 % B: 50 % C mix ratios initially increased from 41.5 % for the untreated soil to peak values of 46 % and 46.5 %, respectively, at B. megaterium suspension density of 12 × 10⁹ cell/ml. Thereafter, LL values decreased marginally to 44 % and 45 %, respectively, with increase in B. megaterium suspension density up to 2.4 × 10⁹ cell/ml. Also, the LL of specimen treated with 75 % B: 25 % C mix proportion marginally increased from 41.5 % for the untreated to 42.5 % at 2.4 × 10⁹ cell/ml. The initial increase in LL value could be attributed to increase in water content required for the continuous hydrolysis of urea. The biochemical reaction produced urease positive enzymes required to actively hydrolyse urea into ammonium ions (NH₄⁺) and carbonate ions (CO₃²⁻) in solution which increased the pH of the mixtures [25, 38]. The process probably increased the affinity of the soil clay mineral for water that increased its diffuse double layer thereby increased the liquid limit of the mixtures [39, 40]. The recorded decrease in LL value with higher B. megaterium suspension density could be due to increasing cell forming unit, which provided greater surface sites for nucleation of CaCO₃ and free Ca²⁺ [23, 41]. The non-precipitated calcium carbonate that was formed in the pore of the soil contributed to the decrease in LL values of the treated soil specimens [20, 42]. Furthermore, the decrease in LL value could also be due to the presence of multivalent cations (Ca²⁺, Al³⁺, Fe³⁺, etc.); present in the soil which may have led to cation exchange between the soil clay minerals and precipitated calcite. This stimulates flocculation of clay minerals as reported in [25, 43-45]. The results of this study show that, higher B. megaterium population in colony forming unit/ml (cfu/m) or suspension density/ml is required to drastically reduce the LL value of lateritic soil [25].

3.3. Effect of B. megaterium suspension density on plasticity limits

The plastic limit (PL) generally increased with increasing B. megaterium suspension density to peak values at 2.4x10⁹ cell/ml for the two methods of treatment considered (see Fig. 3). A probable explanation for the trend could be due to insufficient Ca²⁺ in solution thus leading to the possible
formation of vaterite (anhydrous calcite CaCO₃·H₂O) [17, 46]. Adequate amount of Ca²⁺ was not available and neither adsorbed on surface of the calcite causing a reduced deposition of ion pairs (CaCO₃) and conversion of vaterite to calcite which decreased the water content (i.e., plastic limit) [17, 47]. It implies that the crystallized water in vaterite could have initiated an intrinsic rise in moisture of the mixture that resulted in the increasing PL values that increased with higher B. megaterium suspension density. Similar findings were reported by [6, 25, 42].

![Figure 3. Variation of plastic limit of lateritic soil with B. megaterium suspension density](image-url)

### 3.4. Effect of B. megaterium suspension density on plasticity index

The changes observed in plasticity index (PI) with increasing B. megaterium suspension density recorded for the two methods of bio-treatment is shown in Fig. 4. The trends were affected by the LL and PL values because PI is the mathematical difference between the former and latter. In the method of treatment of soil with 1/3 PVB: 2/3 PVC mix ratio, the PI value initially increased to a peak value of 28.0 % at 6.0×10⁸ cell/ml and thereafter decreased to a minimum value of 21.7 % at B. megaterium suspension density of 2.4 × 10⁹ cell/ml. On the other hand, for the second method, specimen treated with 25 % B: 75 % C mix ratio, the PI value increased from 25.6 % (untreated soil) to 30.19 % at B. megaterium suspension density of 1.2×10⁹ cell/ml and thereafter decreased to a minimum value of 24.3 % at 2.4 × 10⁹ cell/ml. For specimen treated with 50 % B: 50 % C mix ratio, PI value initially increased from 25.6 % (untreated soil) to a peak value of 30.7 % at B. megaterium suspension density of 6.0×10⁸ cell/ml and thereafter decreased to a minimum value of 22.8 % at 2.4 × 10⁹ cell/ml. The PI of specimen treated with 75 % B: 50 % C mix ratio progressively decreased with increased B. megaterium suspension density from 25.6 % (untreated soil) to 16.2 % at 2.4 × 10⁹ cell/ml.

Overall, the treatment of lateritic soil using the two methods considered indicated improvement in plasticity of the natural soil, which can be attributed to the formation of calcite during the MICP process. It is pertinent to reiterate that, the methods adopted in this study are based on the findings reported by Osinubi et al. [18, 19, 25]. Since plasticity index is a soil improvement criterion, the performance of the methods of treatment considered can be ranked as 75 % B: 25 % C > 1/3 PVB%: 2/3 PVC% > 50 % B: 50 % C > 25 % B: 75 % C. The results recorded in this study show that compositional variables (Bacteria: Cementation reagent mix ratio) could affect the plasticity characteristics of fine-grained soils during the MICP process in agreement with Osinubi et al. [25].
3.5. Calcite content
The calcite content increased as B. megaterium suspension density increased for the two methods of treatment considered as shown in Fig 5. The highest calcite content of 5.3 % was recorded for the treatment of soil with 75 % B: 25 % C mix ratio that produced the best PI value of 16.2 % at B. megaterium suspension density of $2.4 \times 10^9$ cells/ml. The result is not unconnected with the increased microorganism due to higher bacteria cell concentration which produced more urease enzyme to commence the hydrolysis of urea present in the cementation reagent [48] as well as offering more nucleation site which facilitated precipitation of calcite [23, 26].

3.6. Compaction characteristics
The maximum dry density (MDD) of lateritic soil marginally increased from 1.72 Mg/m$^3$ for the untreated soil to 1.79 Mg/m$^3$ at $24 \times 10^8$ cells/ml B. megaterium suspension density (Fig. 6). The observed increase in MDD was probably due to the 65.0 % sand fraction (i.e., 0.075 - 4.73 mm) which is higher than 35.0% silt content (i.e., passing 0.075 mm) recorded for the natural soil that enhanced the geometric compatibility and pore throat requirement relative to the bacteria size that is essential for effective MICP process. Another reason for the increased MDD could be due to supposed higher specific gravity of the precipitated calcite that filled the pores of the soil with lower specific value of 2.65. Findings reported by researchers [18, 19, 49-51] suggest that calcites precipitated are aggregated as one in both weight and specific density.
The optimum moisture content (OMC) of lateritic soil marginally increased from 17.2% for the untreated soil to 17.7% at B. megaterium suspension density of $24 \times 10^8$ cells/ml. The increase could be due to insufficient supply of Ca$^{2+}$ from urea-CaCl$_2$ medium with increased B. megaterium cells/ml within the suspension that possibly facilitated the deposition of both calcite and vaterite (anhydrous calcite CaCO$_3$,H$_2$O) [17, 46, 47]. The crystallized water in the vaterite went into solution thereby increasing the moisture content of the soil. This reaction suggests that greater quantity of water would be required to obtain the optimum value.

3.7. Scanning electron microscopy

The scanning electron microscope (SEM) micrographs of untreated soil (0 cell/ml) and soil treated with 75% B: 25% C mix ratio that produced the best PI value at B. megaterium suspension density of $24 \times 10^8$ cell/ml is shown on Plate I. The micrographs of untreated soil (see Plate Ia) depict larger and smaller particle sizes with characteristically smooth-to-rough surface structure. The central thick lump particle could be due to the cohesive structure of the soil due to addition and mixing with water. This possibly suggests that a thick diffuse double layer due to reaction of clay minerals and multivalent cations was still holding or still held a dominant influence on the soil-water mixture. The microstructure of the treated soil (see Plate Ib) show imprints of calcites precipitated and bonded to the inter particle surface that bridged contact points. However, the precipitated calcite was not uniformly distributed. The additional irregular calcite deposition configuration might be attributed to the non-uniform circulation/percolation of the cementation reagent relative to the B. megaterium cells/ml [49]. The magnifications reported in this study was based on the most probable clarity offered during testing.
Plate I. Micrographs of lateritic soil at (a) x1000 magnifications untreated soil (b) x300 magnifications soil treated with 75 % B: 25 % C mix proportion at B. megaterium suspension density of $24 \times 10^8$ cells/ml

The results of energy dispersive spectroscopy (EDS) obtained from the SEM of the untreated and bio-treated specimen are shown in Fig 7a and Fig 7b, respectively. The presence of Si, Al and Fe in the soil indicated the predominant presence in the soil. However, there were some observed slight variation in other elements. Basically, the higher Ca in treated soil compared to untreated could be associated with formation of calcite and free Ca$^{2+}$ ions in solution available at the nucleation site of bacterial cells [26, 27, 52]

**Figure 7.** EDS with quantitative atomic and weight concentration; (a) Untreated soil (b) Soil treated with 75 % B: 25 % C mix proportion at B. megaterium suspension density of $24 \times 10^8$ cells/ml

4. **Conclusion**

The study investigated the plasticity and compaction characteristics as well as calcite content of bio-treated lateritic soil using different compositional variables (i.e., B. megaterium -cementation reagent mix ratios). Two methods of specimen preparations were adopted for the evaluation of Atterberg limits, compaction and calcite content. SEM complimented with EDS was used to study the variation in
microstructural morphology and elemental quantification analysis of the untreated and bio-treated specimen. Treatment of lateritic soil with 75% B. megaterium: 25% urea medium at B. megaterium suspension density of $2.40 \times 10^9$ cells/ml can be effectively utilized to reduce the plasticity index of A-4(3) or SC to a more desirable value using the MICP technique. The compaction characteristics of the treated soil significantly improved. The microanalysis carried out using SEM and EDS support the improved properties of the treated soil. In order to obtain significantly higher values for the properties considered in the study, it is recommended that higher B. megaterium suspension density be used.

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