

Effect of Mode of Treatment of *Brevibacillus Brevis* on Plasticity Characteristics of Bio-Treated Lateritic Soil in Microbial-Induced Calcite Precipitation Application

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ABSTRACT

An economical and sustainable method of soil improvement known as Microbial Induced Calcite Precipitation (MICP) has received significant attention in the past decade. The plasticity of lateritic soil bio-treated at stepped *Brevibacillus brevis* (*B. brevis*) suspension density and cementation reagent concentration using three mode of treatment (i.e. mixing, injection and spraying method). The *B. brevis* suspension densities and cementation reagents used to trigger the MICP process are 0, 0.5, 2.0, 4.0, 6.0 and 8.0 McFarland Standards (i.e., 0, 1.50×10^8 , 6.0×10^8 , 1.20×10^9 , 1.80×10^9 and 2.40×10^9 cells/ml and 0.25, 0.5, 0.75 and 1.0 M, respectively. The liquid limit value used to prepare the samples with three mix proportions of the bacteria and cementation reagent (viz: 25% bacteria-75% cementation reagent, 50% bacteria-50% cementation reagent and 75% bacteria-25% cementation reagent) is the one obtained from natural lateritic soil for all the three modes outlined. Atterberg limits and calcite content using the acid wash method tests were conducted on the treated specimens for all the three treatment modes. Results obtained show a general decrease in the Atterberg limits with higher *B. brevis* suspension density and cementation reagent concentration for mixing, injection and spraying method respectively. The best enhancement of plasticity index was obtained for lateritic soil sample treated with a mix ratio of 75 % *B. brevis* (2.40×10^9 cells/ml for mixing an injection as well as 1.8×10^9 cells/ml for spraying method of treatment): 25 % cementation reagent (0.50M, 0.25 M and 0.75M for mixing, injection and spraying mode) with a corresponding peak calcite content of 12.0 (2.40×10^9 cells/ml and 1.0M), 5.38 (2.40×10^9 cells/ml and 1.0M) and 3.50% (1.8×10^9 cells/ml and 1.0M) for mixing, injection and spraying treatment mode respectively.

Keywords: Atterberg limits, *Brevibacillus brevis*, Lateritic soil, Plasticity, Microbial-induced calcite precipitation (MICP).

INTRODUCTION

The improvement in the engineering properties of soil from the addition of additives is known as soil stabilization. These engineering properties include soil stiffness, shear strength, durability and reduction in hydraulic conductivity etc. Mechanical, chemical, biological or both biological and chemical additives be able to use to improve the engineering behaviour of soils. Current research involves a novel, green, economical and sustainable system known as the Microbial Induced Calcite Precipitate (MICP) process, which utilises chemicals and micro-organisms to improve soil properties. This method is promising and studies conducted earlier recorded successful results (e.g., DeJong, Fritzges, and Klaus, 2006; Lee, Wei, Chew, and Siew, 2012; Chu, Ivanov, and Stabnikov, 2014; Sani, Moses, and Oriola, 2020; Sani and Bala, 2021; Abubakar, 2023) indicative of soil improvement.

The use of microbes for the purpose of stabilisation of soil is a novel option for enhancement of the engineering properties of sand. The microbiological method is more ecologically friendly than other ordinary treatment techniques in practice (Wath and Pusadkar, 2016; Sani et al., 2020; Sani and Bala, 2021). Bio-cementation using (MICP) technique involves the use of microbes in urea hydrolysis to produce carbonate ions that re-join with solution that is rich in calcium (i.e., CaCl_2) to produce calcium carbonate that fixes ground specimens in position; this results in improving the soil engineering properties (Cheng, Shahin, Cord-Ruwisch, Addis, Hartanto, and Elms, 2014a; Rajabi, Kiani, Khavazi, Rouhipour, and Khormali, 2019; Osinubi, Eberemu, Ijimdiya, Yakubu, Gadzama, Sani, and Yohanna, 2020; Garba et al., 2024; Garba et al., 2025). However, regardless of the perfections associated with MICP urease positive microbes, it should be dispersed consistently and stable in places where they were introduced into the sand. Inadequate mode of treatment lead the microbes to be situated only in some portions of sand or be blushed out of the sand being treated. the available approaches to distribute microbes and relax them over an 18 cm long soil Harkes, van Paassen, Booster, Whiffin, and van Loosdrecht, (2010) reported cradle; the authors originated that introduction of unmixed bacteria solution, trailed by the 1-pore volume of high salinity obsession watery (50 mM of calcium chloride) can effectively grip nearly all microorganisms suspension present in the soil bed. It is pertinent to state that, presently there is no preferred mode of treatment; only the most commonly used method (i.e., Injection method) is well documented in the literature (David, 2019).

Currently there is no preferred mode of treatment available, only the most commonly used method is revealed (i.e., injection method).

MATERIALS AND METHODS

Materials

Soil sample: The laterite soil specimens used in this research, was obtained from a burrow pit in Shika, Sabon Gari Local Government Area of Kaduna State, Nigeria.

The urease positive bacterium is rod-shaped, spore-forming and Gram-positive was used.

The cementation reagent: constituted of 20 g Urea, 10 g NH_4Cl , 3 g Nutrient broth, 2.8 g CaCl_4 and 2.12 g NaHCO_3 per litre of distilled water, which has been used as reported (Stocks-Fischer et al., 1999; DeJong et al., 2006; Al Qabany et al., 2011; Park et al., 2014; Tirkolaei and Bilsel, 2016).

Methods

Isolation of the bacterium species from lateritic soil. Isolation of the bacterium species from lateritic soil was done by serial dilution

Index properties: The experimental procedures was conducted in accordance with procedures outlined in BS 1377 (1990) and BS 1924 (1990) for control and treated soil samples.

Calcium carbonate content: The determination method used is in accordance with that reported by Mortensen et al. (2011) and Choi et al. (2017) known as the acid wash method. In this approach, 5 g of natural and B. brevis treated soil were mixed with 20 mL 1-M hydrochloric acid (HCl) acid to dissolve calcium carbonate. Then all the solution and insoluble solids were wash away by distilled water using filter paper with a coarse aperture size in a No. 200 sieve for 10 minutes. All soluble calcium was removed from the soil atoms. At that moment all residue on the sieve were oven-dried and weighed. The difference in weight between the novel soil samples (A) and after washing sample (B) was the weight of calcium carbonate content (CCC) present. The CCC was evaluated as

$$CCC = (100 - (B/A)) \times 100 \dots\dots\dots 1$$

Methods of treatment

Numerous researchers have used various methods in introducing bacteria to soil specimens. The most common methods used to introduce bacteria solution into the soil are grouting, spraying and mixing method (Abubakar, 2023; Abubakar et al., 2026).

Mixing method

In this method, the lateritic soil samples were mixed using spatula with a volume of each *B. brevis* suspension and cementation reagent in a bowl (plate Ia). Three trial mixes were adopted for the computation of the volume of each of the bacterial suspension density (0 , 1.5×10^8 , 6.0×10^8 , 12×10^8 , 18×10^8 and 24×10^8 cell/ml respectively) and cementation reagent was added to the soil.

After mixing the soil sample with the three trial mixes for the various bacterial suspensions; the treated samples were left air-dried before being pulverized and passed through BS No. 40 sieve ($425 \mu\text{m}$ aperture) for calcite contents test procedures.

Injection method

In this method 60ml the syringe was used to inject the required bacteria (0 , 1.5×10^8 , 6.0×10^8 , 12×10^8 , 18×10^8 and 24×10^8 cell/ml respectively) and cementation reagent to the soil samples (plate Ib). The lateritic soil sample was treated in this method, both bacteria and cementation solution was grouted alternately by specify volume, from top to bottom, following the vertical flow path regulated by a peristaltic pump. Primarily, this is attained by allowing a retention period (normally 3 hours after introducing bacteria culture to the sand column) (Whiffin, et al., 2007).

After injecting the soil sample with the three trial mixes for the various bacterial suspensions, the treated samples were left to air-dry, before being pulverized and passed through BS No.40 sieve ($425 \mu\text{m}$ aperture) for calcite contents test procedures.

Spraying method

In this method ordinary pump (air freshener spraying can) is used to spray the required bacterial solution into the lateritic soil sample in a bowl (plate Ic). After spraying the bacterial solution (0 , 1.5×10^8 , 6.0×10^8 , 12×10^8 , 18×10^8 and 24×10^8 cell/ml respectively), the cementation reagent was added to the soil. The soil samples were left undisturbed for a minimum of 4 hours, after which the nutrient cycles was supplied to feed on the microorganisms. After spraying, the soil sample with the three trial mixes for the various bacterial suspensions, were treated and left to air-dry before being pulverized and passed through BS No. 40 sieve ($425 \mu\text{m}$ aperture) for calcite contents test procedures.

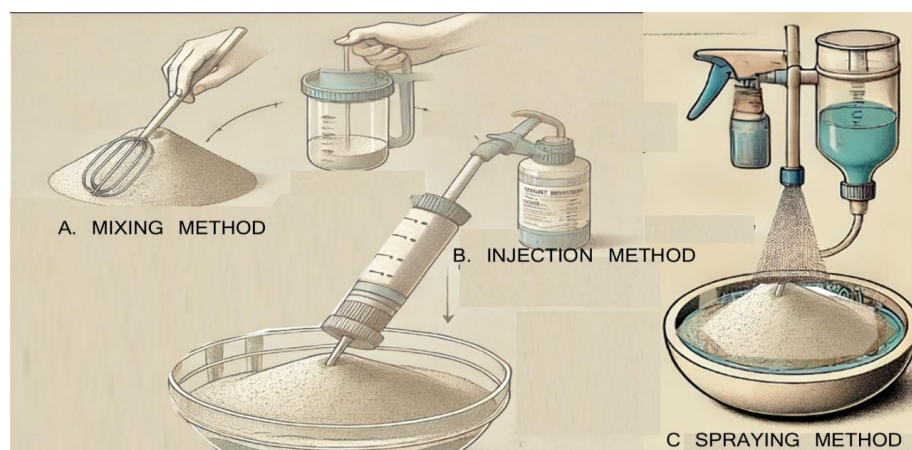


Plate I: schematic diagram for Modes of treatments considered: (a) mixing method (b) grouting method (c) spraying method (Source: Abubakar et al., 2026)

RESULTS AND DISCUSSION

The summary of these properties are shown in Table 1. The particle size distribution curve is shown in Figure 1. The soil is reddish brown in colour with liquid limit of 46.27 %, plastic limit of 27.37 % and plasticity index of 18.90 % respectively. The soil is classified as CL soil in the Unified Soil Classification System USCS (ASTM, 1992), while A-7-6(4) according to AASHTO soil classification system (AASHTO, 1986). The natural soil also has linear shrinkage of 11.3 % and specific gravity of 2.55 respectively.

Table 1: Properties of the natural lateritic soil

Property	Quantity
Percentage passing No. 200 sieve	58.05
Natural moisture content (%)	16.30
Liquid limit (%)	46.27
Plastic limit (%)	27.37
Plasticity index (%)	18.90
Linear shrinkage (%)	11.30
Specific gravity	2.55
AASHTO classification	A-7-6(4)
USCS classification	CL

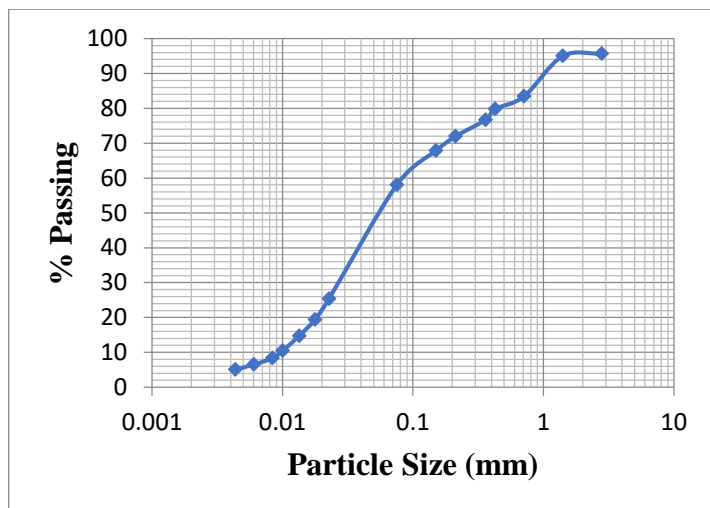


Fig. 1: Particle size distribution curve of the natural silty sand used in the study

Atterberg limit

Consistency tests (i.e., liquid limit (LL), plastic limit (PL) and plasticity index (PI)) were used to appraise the plastic performance of soils in relation to the water content of the soil with respect to its change from -solid to liquid state (Kanyi, 2017, Yohanna, Osinubi, Eberemu, Ijimdiya, and Sani, 2022). The effects of different mix ratios of *B. brevis* (B) and cementation reagent (C) for different *B. brevis* nucleation sites on consistency of lateritic soil treated with different mix ratios (i.e., 25 % B : 75 % C, 50 % B : 50 % C and 75 % B : 25 % C) are shown in Figs. 2 - 4.

Liquid limit

The effects of *B. brevis* nucleation site and cementation concentration on liquid limit (LL) of lateritic soil for the three modes of treatment are shown in Fig. 2. In all the mixing modes considered, the LL values initially increased to peak values and thereafter decreased with increase in *B. brevis* nucleation site and cementation reagent concentration.

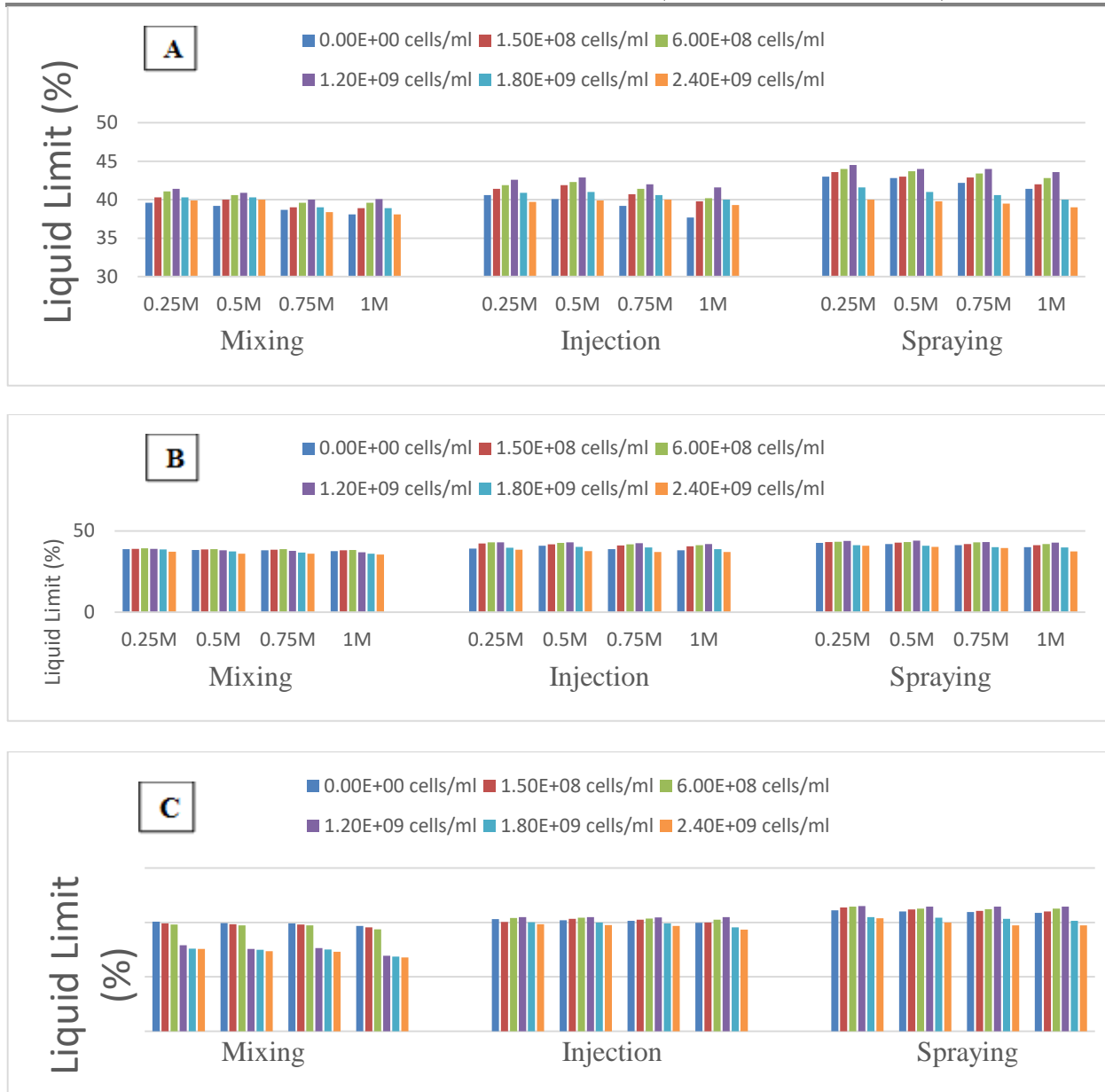


Figure 2: Variation of liquid limit of lateritic soil - *B. brevis* (B) mixtures with cementation reagent concentration (C) for the three modes of application when prepared with different mix ratios: (a) 25 % B : 75 % C (b) 50 % B : 50 % C (c) 75 % B : 25 % C

In the mixing method of treatment of lateritic soil peak LL values of 41.4 % (12×10^8 cells/ml and 0.25 M), 39.3 % (6×10^8 cells/ml and 0.25 M) and 40.3 % (0 cells/ml and 0.25 M) for specimens prepared with 25 % B : 75 % C, 50 % B : 50 % C and 75 % B : 25 % C, respectively. For the injection method of treatment of lateritic soil peak LL values of 42.9 % (12×10^8 cells/ml and 0.25 M), 43.0 % (12×10^8 cells/ml and 0.5 M) and 42 % (12×10^8 cells/ml and 0.25, 0.5 & 1 M) for specimens prepared with 25 % B : 75 % C, 50 % B : 50 % C and 75 % B : 25 % C, respectively. The spraying method of treatment of lateritic soil recorded peak LL values of 44.5 % (12×10^8 cells/ml and 0.25 M), 43.9 % (12×10^8 cells/ml and 0.25 M) and 46.0 % (12×10^8 cells/ml and 0.25 M) for specimens prepared with 25 % B : 75 % C, 50 % B : 50 % C and 75 % B : 25 % C, respectively. For all the mix ratios (i.e., 25 % B : 75 % C, 50 % B : 50 % C and 75 % B : 25 % C) and modes of treatment (i.e., mixing, injection and spraying) considered, the primary reason for the increase in LL values recorded could probably be connected to the increase in water content necessary for the hydrolysis of urea. The urease positive enzymes formed through biological reaction was required to energetically hydrolyze urea into ammonium ions (NH_4^+) and carbonate ions (CO_3^{2-}) in solution which increased the pH of the mixtures (Osinubi, Yohanna, Eberemu, and Ijimdiya, 2019a; Wangvan, Paassen, and Edward, 2020). This process possibly increased the attraction of the

soil clay crystal for water that improved its diffuse double layer and thus increased the liquid limit of the mixtures in consideration (Neumann, David, and Zuo, 2011; Sani, Etim, and, Joseph, 2019).

The decrease in LL values with higher *B. brevis* nucleation site could probably be due to increased cell developing units that provided larger surface area for calcium carbonate (CaCO_3) and free Ca^{2+} (Osinubi et al., 2020; Muynck, Belie, and Verstraete, 2020). The non-plastic CaCO_3 that was precipitated in the pore space of the soil also reduced the LL values of the treated soil sample (Neupane, 2016; Osinubi, Gadzama, Eberemu, Ijimdiya, and Yakubu, 2019b). The study showed that higher *B. brevis* nucleation site is vital for the significant reduction in the LL value of lateritic soil (Osinubi, Gadzama, Eberemu, and Ijimdiya, 2019c).

Plastic limit

The effects of *B. brevis* suspension density and cementation reagent concentration on plastic limit (PL) of lateritic soil for the three modes of treatment are shown in Fig. 3.

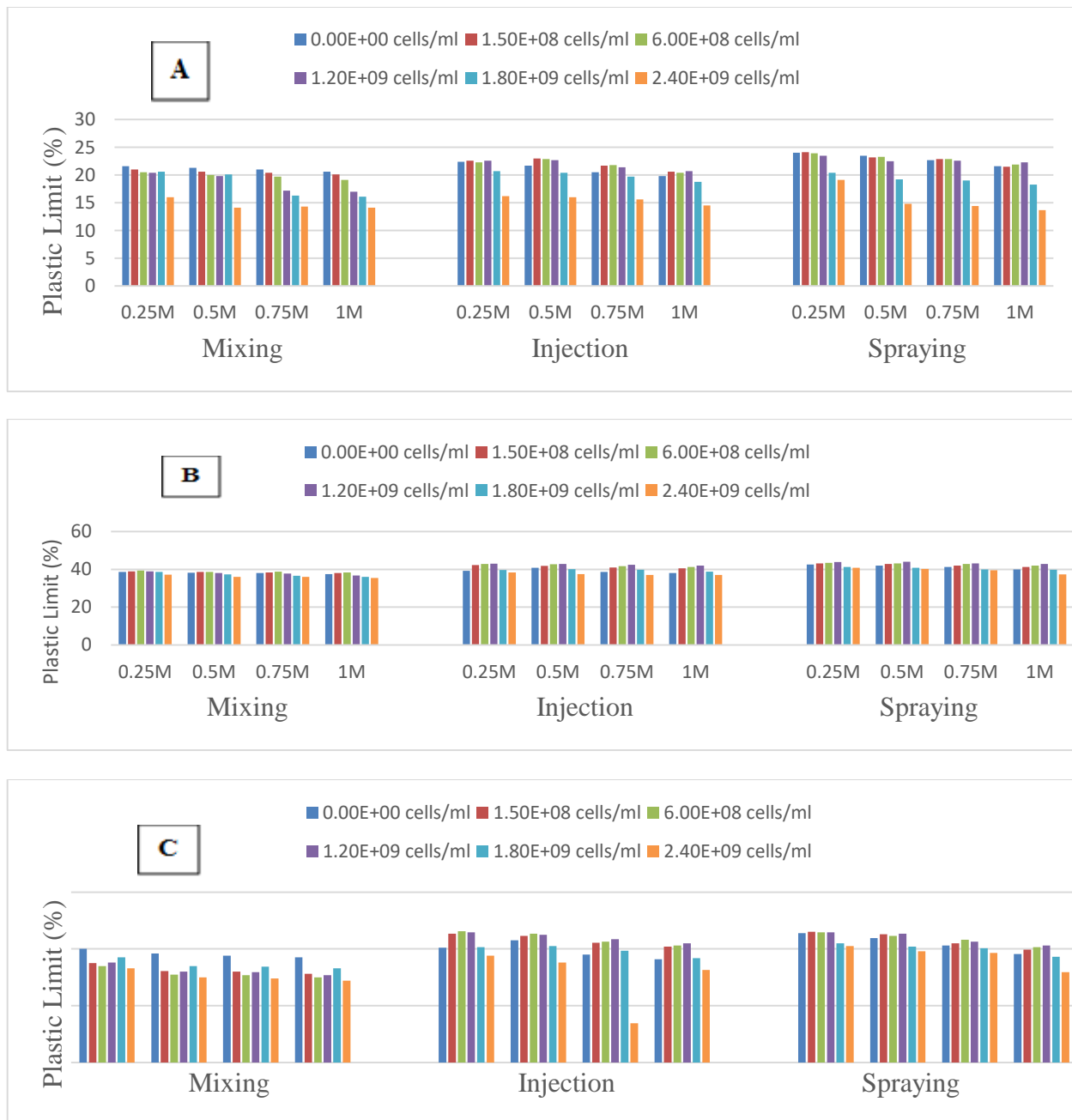
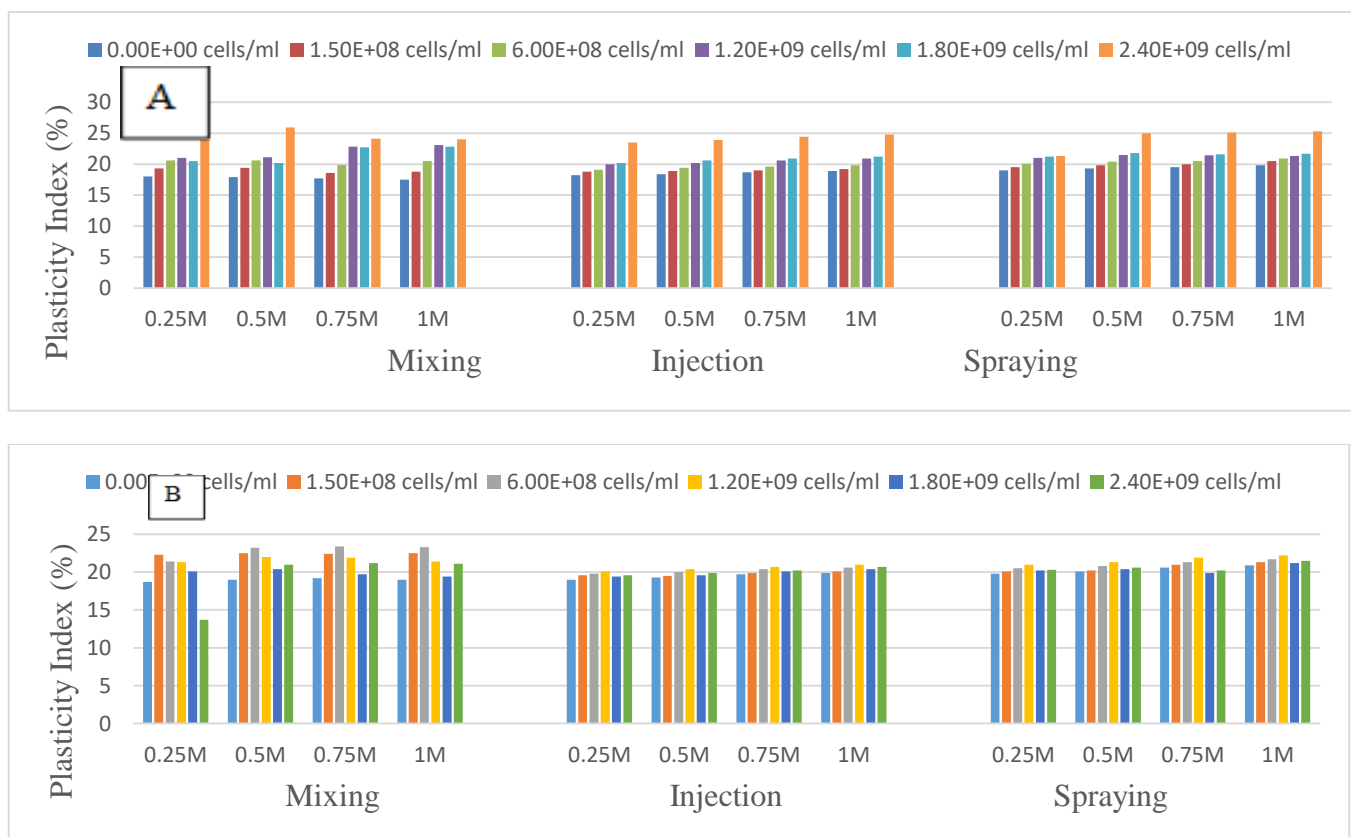


Fig. 3: Variation of plastic limit of lateritic soil - *B. brevis* (B) mixtures with cementation reagent concentration (C) for the three modes of application when prepared with different mix ratios: (a) 25 % B : 75 % (b) 50 % B : 50 % C (c) 75 % B : 25 % C

In the mixing method of treatment of lateritic soil peak PL values of 21.6 % (0 cells/ml and 0.25 M), 20 % (0 cells/ml and 0.25 M) and 22.8 % (1.5 & 6 x 10⁸ cells/ml and 0.75 M) for specimens prepared with 25 % B : 75 %, 50 % B : 50 % C and 75 % B : 25 % C, respectively, this might be connected to the hydrolysed urea thus led to the development of calcite thus support in hardening the soil (Yohanna et., al, 2022). For the injection method of treatment of lateritic soil peak PL values of 23.0 % (6 x 10⁸ cells/ml and 0.25 M), 23.1 % (1.50 x 10⁸ cells/ml and 0.5 M) and 24.5 % (6 x 10⁸ cells/ml and 0.25 & 0.5 M) for specimens prepared with 25 % B : 75 %, 50 % B : 50 % C and 75 % B : 25 % C, respectively. The spraying method of treatment of lateritic soil recorded peak PL values of 24.1 % (1.50 x 10⁸ cells/ml and 0.25 M), 23.0 % (1.50 x 10⁸ cells/ml and 0.25 M) and 28.4 % (12 x 10⁸ cells/ml and 1 M) for specimens prepared with 25 % B : 75 %, 50 % B : 50 % C and 75 % B : 25 % C, respectively. For all the mix ratios and modes of treatment considered, the probable reason for the increase in PL values might not be unconnected to the decreased quantity of Ca²⁺ in solution that facilitated the formation of anhydrous calcite (CaCO₃.H₂O) (Li et al., 2013; Dhami et al., 2016; Yohanna et., al, 2022). Adequate quantity of Ca²⁺ was neither accessible nor adsorbed on calcite surface triggering reduced deposition of ion couples (CaCO₃) and alteration of anhydrous calcite (vaterite) to calcium carbonate which reduced the plastic limit (PL) (Dhami., Sundhakara, and Mukherjee, 2013; Li, Cheng, Zhou, Zhu, and Yu, 2013; Yohanna et., al, 2022). This indicates that the conserved water in vaterite could have started a vital increase in moisture of the mixture that led to increased PL values that increased with higher *B. brevis* nucleation site and cementation reagent concentration. Similar findings were reported by researchers (e.g., Neupane, 2016; Osinubi et al., 2017; 2019b). This underscores the significance of the treatment constituents on the plasticity of lateritic soil treated with *B. brevis* suspension density. Consequently, appropriate consideration of treatment constituents is essential for field application in order to attain the anticipated workability (Yohanna et al., 2022).

Plasticity index

The variation of plasticity index of lateritic soil - *B. brevis* mixtures with cementation reagent concentration for the various modes of treatment when different mix ratios were used is shown in Fig.4.



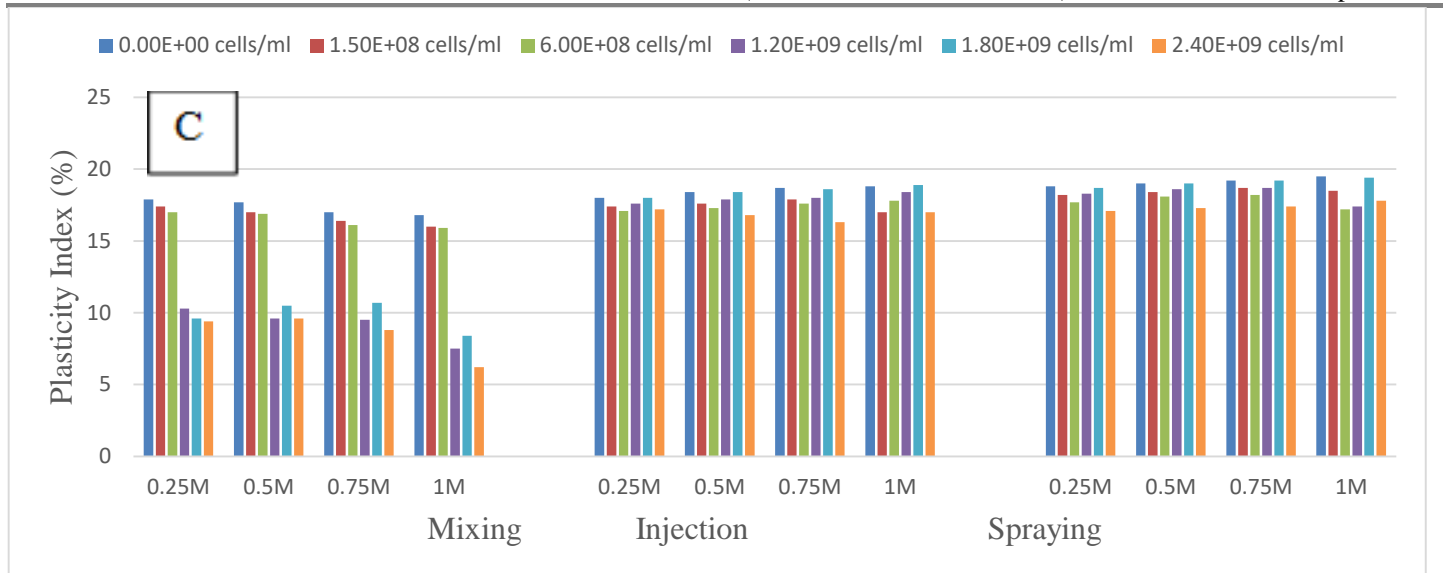


Fig. 4: Variation of plasticity index of lateritic soil - *B. brevis* (B) mixtures with cementation reagent concentration (C) for the three modes of application when prepared with different mix ratios: (a) 25 % B : 75 % C (b) 50 % B : 50 % C (c) 75 % B : 25 % C

In the mixing method of treatment of lateritic soil peak PI values of 25.9 % (24×10^8 cells/ml and 0.5 M), 23.4 % (6×10^8 cells/ml and 0.75 M) and 17.9 % (0 cells/ml and 0.25 M) for specimens prepared with 25 % B : 75 % C, 50 % B : 50 % C and 75 % B : 25 % C, respectively. For the injection method of treatment of lateritic soil peak PI values of 24.8 % (24×10^8 cells/ml and 1 M), 20.7 % (24×10^8 cells/ml and 1 M) and 18.9 % (18×10^8 cells/ml and 1 M) for specimens prepared with 25 % B : 75 % C, 50 % B : 50 % C and 75 % B : 25 % C, respectively. The spraying method of treatment of lateritic soil recorded peak PI values of 25.3 % (24×10^8 cells/ml and 1 M), 21.9 % (12×10^8 cells/ml and 0.75 M) and 19.4 % (18×10^8 cells/ml and 1 M) for specimens prepared with 25 % B : 75 % C, 50 % B : 50 % C and 75 % B : 25 % C, respectively.

For the plasticity index (PI), all the mix ratios and modes of treatment considered the decrease in values could probably be due to increase in the quantity of urease positive enzyme released within the soil matrix for possible calcite formation by *B. brevis* that caused the decrease in the plasticity of the specimens. Similar findings were reported by Osinubi et al. (2017); Osinubi, Eberemu, Yohanna, Ijimdiya, Adjei, and Sada, (2018a) who used *B. pumilus* and *Sporosarcina pasteurii* in their studies. Also, Sheelah and Yosadian (2012) reported decrease in PI with increase in *B. pasteurii* in the improvement of cohesive soils. Other factors that could be responsible for the decrease in PI are the geochemical processes that usually occur within the soil structure such as biofilm formation, biogas generation and the formation of biopolymers and other extracellular polymeric substances (EPS) (Kavazanjian, Iglesias, Karatas, 2009; DeJong, Soga, Kavazanjian, Burns, van Paassen, 2013; Osinubi et al., 2019c). The results of this study show that constituent parameters (i.e., Bacteria : Cementation reagent mix ratio) could significantly affect the plasticity behaviour of fine-grained soils through the MICP process in agreement with the findings reported by Osinubi et al. (2019c). This was also as a result of the precipitation of calcites produced during the MICP process.

Generally, the reduction in PI values recorded for all the three modes of treatment considered showed signified improvement in soil property with mode of treatment in the order mixing mode > injection mode > spraying mode. The probable explanation for this trend of reduction in PI value is not unconnected to the retention capacity and the mobilisation of the urease enzyme when ureolytic bacteria was introduced into the soil matrix (Bernard, 2019). Detailed tests results are given in Table A4.6 (g - i) of the Appendix.

CONCLUSION

The lateritic soil categorized as A-7-6 (4) and CL in AASHTO and USCS, respectively, was treated with *B. brevis*, nucleation sites 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. Samples were

subjected to pH, electrical conductivity, index properties, calcite contents and linear shrinkage tests using the British Standard light (BSL) energy level. For the consistency properties and linear shrinkage characteristics, only cementation reagent was used to the control samples, while three trial mixes were adopted for the *B. brevis* treated samples, to study the effect of the bacteria (B)-cementation (C) reagent blend treatment on the soil sample. The trial mixes for the bacteria (B)-cementation (C) reagent combination treatment include, 50B-50C, 25B-75C and 75B-25C. Established on the recorded results in this research the below deductions can be made:

Consistency properties (LL, PL and PI) enhanced with greater *B. brevis* nucleation site with the best outcomes documented for the 75B-72C mix. However, the results fell below the Nigerian General Specifications requirements of less than or equal to 35 % Liquid limit, 12 % Plasticity index and 35 % passing No. 200 sieve, for use as a sub-base material for all treatment modes and mixes ratio considered except for 75 % B: 25 % C where by the Liquid limit and Plastic limit from 12×10^8 up to 24×10^8 cells/ml conformed with Nigerian General Specifications requirements.

RECOMMENDATION

From the results obtained in this research work, it is recommended that 75 % *B. brevis* – 25 % Cementation reagent mix ratio be used for the upgrading of the index properties and calcite contents of lateritic soil as a subgrade material in the erection of low-volume roads.

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