

# Microbial Induced Calcite Precipitate Improvement of Aeolian Soil for Use as a Construction Material

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## ABSTRACT

An environmentally friendly and successful technique of soil improvement known as Microbial Induced Calcite Precipitation (MICP) has attracted the attention of geotechnical engineers. This study was conducted to modify the engineering behaviour of an aeolian soil bio-treated with *Bacillus brevis* (*B. brevis*) to evaluate its suitability for use in construction works. Stepped *B. brevis* suspension density (i.e., 0.5, 2.0, 4.0, 6.0 and 8.0 McFarland standards equivalent to  $0$ ,  $1.50 \times 10^8$ ,  $6.0 \times 10^8$ ,  $1.20 \times 10^9$ ,  $1.80 \times 10^9$  and  $2.40 \times 10^9$  cells/ml, respectively) and cementation reagent (i.e., 0.25, 0.5, 0.75 and 1.0 M), respectively, were utilized to activate the MICP process. Compaction was conducted using British Standard light (BSL) (or standard Proctor) energy. Tests results indicate that the engineering behaviour of the bio-treated non-plastic aeolian silt soil improved. The maximum dry density (MDD) and the angle of internal friction values marginally increased, while cohesion and optimum moisture content (OMC) decreased with higher *B. brevis* suspension density and cementation reagent concentration. Peak angle of internal friction and minimum cohesion were recorded at  $1.5 \times 10^8$  cells/ml. Highest unconfined compressive strength (UCS) value of  $215.58 \text{ kN/m}^2$  was obtained for specimen treated with 25 % *B. brevis* ( $6.0 \times 10^9$  cells/ml): 75 % cementation reagent (0.75 M) mix ratio. The results recorded show that the improved aeolian soil could be used in engineering works.

**Keywords:** Aeolian soil, *Bacillus brevis*, Microbial-induced calcite precipitate, unconfined compressive strength

## INTRODUCTION

Recently, a new soil treatment technique to control erosion was developed. The technique is sustainable and environmentally friendly and is used to improve soil engineering behaviour such as strength, durability and porousness (DeJong *et al.*, 2010; Osinubi *et al* 2018; Osinubi *et al* 2019; Osinubi *et al* 2020). The method is called Microbial Induced Calcium Carbonate Precipitate or Microbial Induced Calcite Precipitation (MICP) and it is a biologically natural method. Urea hydrolyzing bacteria are mostly used in most applications of MICP. The technique is very simple, long-lived, low cost as well as efficient and there is no excess proton production (Whiffin *et al.*, 2007; Armstrong *et. al.*, 2021)

Soil improvement methods need to be sustainable and environmentally friendly. Industrial soil improvement additives such as Portland cement, lime, asphalt and sodium silicate have been successfully used in soil enhancement (Karol, 2003; Basha *et al.*, 2005). However, the injection of industrially produced chemicals often alters the pH level of soil, contaminates soil and groundwater due to their toxic and hazardous nature

(Karol 2003; DeJong *et al.*, 2006). Therefore this study was focused on the use of MICP technique to improve the engineering properties of an aeolian soil with low unconfined compressive strength (UCS).

## MATERIALS AND METHODS

### Materials

#### Soil

The aeolian silty soil used in this study was collected from Wudil Local Government Area (11°47'39.2712" N and 8°50'20.5152" E), Kano state, Nigeria. The soil sample were collected by method of disturbed sampling at a depth of 15 cm so as to minimise organic materials. The soil samples were placed in a clean polythene bag and transported to Ahmadu Bello University, Zaria, Kaduna State, where they were stored at laboratory room temperature of  $25 \pm 2^\circ \text{C}$ .

#### Microorganism

The microorganisms used in this study is *B. brevis*, which was isolated from the natural soil. The urease positive microbe is rod-shaped, spore-forming and Gram-positive.

#### Cementation reagent

The cementation mixture consisted of 20 g Urea, 10 g  $\text{NH}_4\text{Cl}$ , 3 g Nutrient broth, 2.8 g  $\text{CaCl}_4$  and 2.12 g  $\text{NaHCO}_3$  per litre of disinfected water, which has been used in numerous studies (e.g., Stocks-Fischer *et al.*, 1999; Dejong *et al.*, 2006; Al Qabany *et al.*, 2011; Park *et al.*, 2014; Tirkolaei and Bilsel, 2017). In all the cited studies, 3 g/l of nutrient broth was extra to the cementation mixture since it is the most practical amount for survival of the microbes (Sharma and Ramkrishnan, 2016). However, the constituent was varied to obtain diverse concentrations such as 0.25, 0.5, 0.75 and 1.0M.

### Methods

#### Microbes isolation, culture/growth medium Isolation of *B. brevis*

The culture of *B. brevis* from soil was done by the tyrosine selection method as described by Edwards and Seddon (2000). For all samples, 10 g aeolian soil was extracted to 90 mL of disinfected water and raised for 45 minutes at  $28^\circ \text{C}$  on a rotatory shaker (B. Bran scientific & instrument company, England) at 250 rpm. Thereafter, 2 mL of the broth culture was added to 20 mL of sterile Tyrosine broth that consisted (g/l) of disinfected water, 6.5 g/l nutrient broth and 5.0 g/l tyrosine; they were then autoclaved at  $121^\circ \text{C}$  for 15 min in 50 mL Erlenmeyer flask. The solution was rinsed for 4 hours at  $28^\circ \text{C}$  on a rotatory shaker at 250 rpm. After incubation, 5 mL aliquots from all culture were placed in hot water bath operating at  $80^\circ \text{C}$  for 3 minutes. And then 0.1 mL was spread on Tyrosine agar (Nutrient broth 6.5 g/L, tyrosine 5 g/L and Agar 15g/L) and incubated at  $28^\circ \text{C}$  for 24 hours. After 24 hours of incubation, bacterial clusters on the dishes were visually examined. Colonies showing morphological features such as being light brown, non-spreading, serrated edges and clear halos typical for the *B. brevis*, were sub-cultured on Tyrosine agar.

#### Identification of *B. brevis*

The cultural and biochemical characteristics of the isolates were used for a presumptive characterization of the *B. brevis* (Cowan and Steel, 2003; Bergey, 2004) and then confirmed based on the retention factor (RF) on the thin layer chromatography plate as compared to the standard (Gramicidin).

## Compaction

The air-dried soil sample passing British Standard (BS) sieve with 4.76 mm opening was used. British Standard light, BSL (or standard Proctor) energy was derived from 2.5 kg rammer dropping through 30 cm onto three layers in a 1000 cm<sup>3</sup> mould, each getting 27 uniformly spread blows.

## MICP Treatment

The soil sample was treated by mixing it with *B. brevis* (B) suspension densities of 0, 1.5 x 10<sup>8</sup>, 6.0 x 10<sup>8</sup>, 12 x 10<sup>8</sup>, 18 x 10<sup>8</sup> and 24 x 10<sup>8</sup> cells/ml, respectively, at 1/3 pore volume consistent with reported by Rowshanbakhta *et al.* (2016) and the cementation solution (C). The treated samples were then used in preparing specimens for the strength test.

## Unconfined compressive strength

The compacted bio-treated specimens were extruded from the 1,000 cm<sup>3</sup> compaction moulds using a cylinder-shaped core cutter with a height of 76.2 mm and internal diameter of 38.1 mm. The specimens were cured for 24 h at a laboratory temperature of 25 ± 2 °C to improve calcite development within the soil before being wrapped in polythene bags for 7 days being the curing period. Subsequently, the specimens were positioned in a load frame driven at a constant strain rate of 0.10 %/min until failure occurred. Three different samples were used for each test and the average results was recorded.

# RESULTS AND DISCUSSION

## Index Properties of the Natural Soil

The natural soil had a moisture content of 27.0 % as at the time of sampling due to the rainy season. The physical properties of the soil sample are summarized in Table 1. The particle size distribution curve is presented in Figure 1. The soil is brownish in colour; it is non-plastic in nature and has 3.0 % fines passing through sieve No. 200. It is classified as A-3(0) soil group using the AASHTO system of soil grouping (AASHTO, 1986) and sandy silt (SM) by the Unified Soil Classification System (ASTM, 1992). In their natural state, aeolian soils are loose materials and lack cohesiveness which tends to hamper their strength and makes them unsuitable for most engineering projects such as pavement construction and building foundations. The aeolian soil used in this study was adjudged as poorly graded sand having 38 % of its particles finer than No. 200 sieve aperture. Therefore, it requires improvement in order to be used in engineering works.

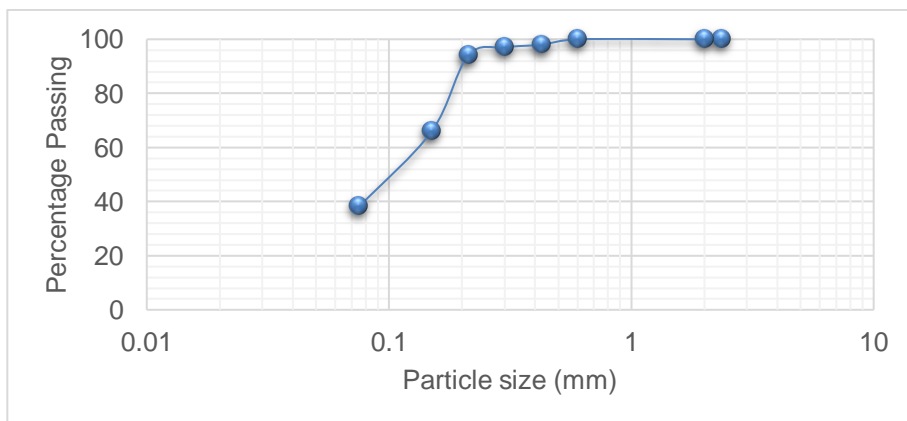


Figure 1. Particle size distribution curve of the natural aeolian soil.

Table 1. Properties of the natural aeolian soil used in the study

Property	Quantity
Percentage passing No. 200 sieve	38.0
Natural Moisture Content, %	27.0
Liquid Limit, %	–
Plastic Limit, %	Non-plastic
Plasticity Index, %	Non-plastic
Linear Shrinkage, %	–
Specific Gravity	2.63
AASHTO Classification	A-3(0)
USCS Maximum Dry Density, Mg/m <sup>3</sup>	SP-SM 1.62
Optimum Moisture Content, %	2.0
Colour	Brown

### Compaction Characteristics

#### Maximum dry density

The variation of maximum dry density (MDD) of aeolian silty sand with *B. brevis* suspension density for varying cementation solution concentration when BSL compaction energy was used is shown in Figure 2. Generally the MDD values initially increased to peak values at *B. brevis* suspension density of  $6 \times 10^8$  cells/ml, before reducing with higher *B. brevis* suspension density and cementation reagent concentration. The highest MDD value of  $1.22 \text{ Mg/m}^3$  was recorded for soil treated with B ( $6.0 \times 10^8$  cells/ml) – C (0.75 M) mix ratio.

The comparatively high MDD values recorded at *B. brevis* nucleation site of  $6 \times 10^8$  cells/ml are symbolic of the development of denser soil mass due to the precipitated calcite that filled the soil pore spaces present in the aeolian soil (De Muynck *et al.*, 2010; Bu *et al.*, 2018; Seifan *et al.*, 2019 and Seifan *et al.*, 2020). Furthermore, the higher MDD values recorded could probably be due to the stable combination between the bacteria and cementation reagent, which triggered the precipitation of more calcite in the specimens.

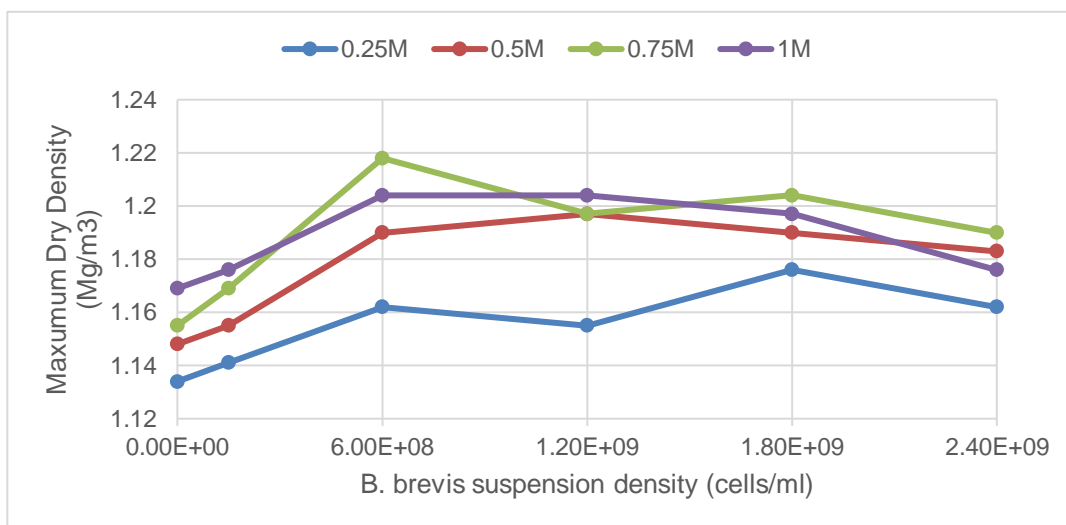


Figure 2. Variation of maximum dry density of aeolian soil with *B. brevis* suspension density

### Optimum moisture content

The variation of optimum moisture content (OMC) of aeolian soil with *B. brevis* suspension density is shown in Figure 3. The OMC values recorded initially decreased with increase in *B. brevis* nucleation site and cementation solution, with the least values recorded at  $1.2 \times 10^9$  cells/ml before increasing with higher *brevis* suspension density and cementation solution concentration. The OMC values decreased from a minimum value of 8.0 % (0.75M) for the control (natural soil) to the least values of 5.7 % (1 M) at  $12 \times 10^8$  cells/ml, before increasing at  $24 \times 10^8$  cells/ml.

The initial decreasing trend of OMC values might be attributed to the void spaces in the soil being occupied by calcite precipitated through biogeochemical response among the bacteria and the cementation solution that resulted in a decreasing tendency for water absorption (Anbu *et al.*, 2016; Hoang, 2018).

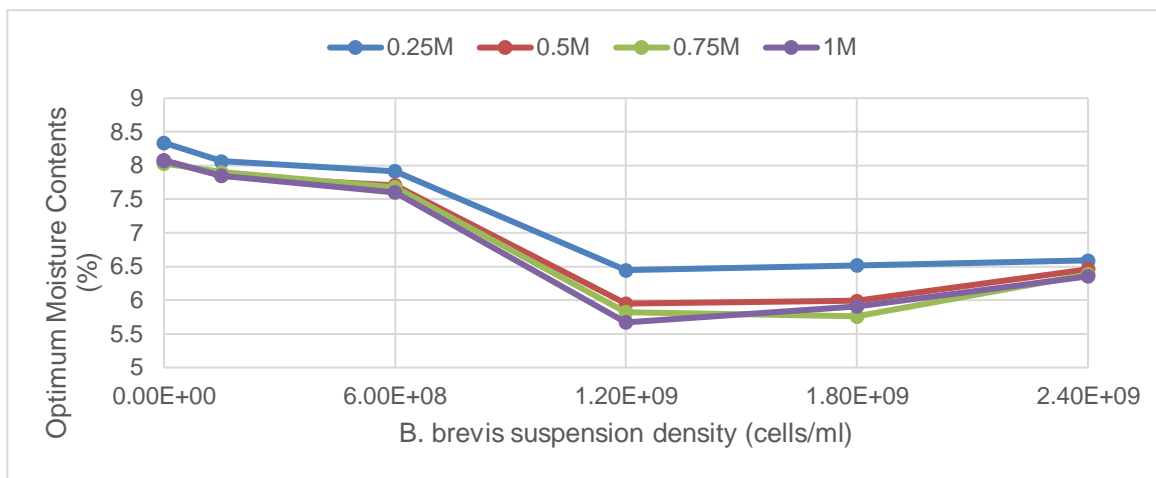


Figure 3. Variation of optimum moisture content of aeolian soil with *B. brevis* suspension density

### Unconfined Compressive Strength

The variation of unconfined compression strength (UCS) of the aeolian silty sand prepared at OMC with *Bacillus brevis* suspension density is presented in Figure 4. Generally, the UCS values increased with increase in *Bacillus brevis* nucleation site and cementation reagent concentration. The highest UCS values were achieved at *Bacillus brevis* suspension density of  $6.0 \times 10^8$  cells/ml for all cementation solution concentrations considered. The peak UCS value of  $150.91 \text{ kN/m}^2$  was obtained for treatment with B ( $6.0 \times 10^8$  cells/ml) – C (0.75 M).

The lower UCS value recorded for the control sample in comparison to the bio-treated specimens could be due to the formation of more calcite, resulting from the introduction of more microbial cells into the treated specimens during the MICP process. Therefore, a larger population of microorganisms, which implies more accessible nucleation sites, frequently leads to increased urease activity and, as a result, an increase in calcite precipitation (Soon *et al.*, 2012; Osinubi *et al.*, 2019 b and b; Osinubi *et al.*, 2020; Sani *et al.*, 2020)

The observed reduction in UCS values after the peak values were attained for the treated specimens could be attributed to the low volume of cementation reagent when associated to the microbial population in those nucleation sites. As reported by Anbu *et al.* (2016), the capacity of cementation solution and cell absorption in a particular microbial solution density has a significant impact on urea hydrolysis. It implies that, the volume of cementation reagent given to the nucleation sites with greater cell concentrations was insufficient to produce enough calcium carbonate to improve the connection and strength of the bio-treated soil mass (Soon *et al.*, 2012; Osinubi *et al.*, 2019a).

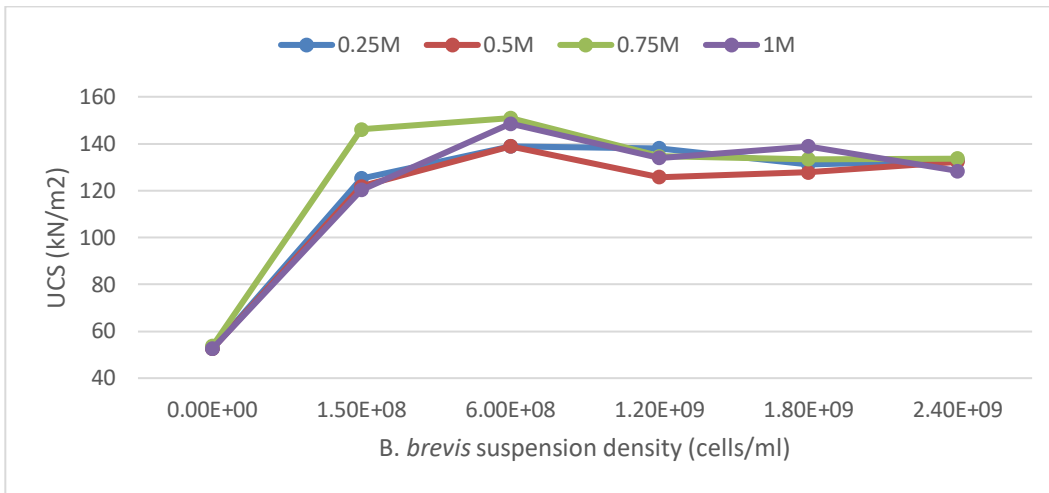


Figure 4. Variation of unconfined compressive strength (7 days curing) of aeolian soil with *B. brevis* suspension density

**Cohesion**

The variation of cohesive of Aeolian soil with *B. brevis* suspension density is shown in Fig. 4. The cohesive values initially decreased to minimum values at *B. brevis* suspension density of  $6 \times 10^8$  cells/ml and thereafter increased at  $12 \times 10^8$  cells/ml and decreased thereafter with higher bio-treatment. The results recorded showed a marginal improvement in the cohesion of the Aeolian soil which reduced from 25.27 kN/m<sup>2</sup> to 18.34 kN/m<sup>2</sup> although the pseudo-coarse particles or transition particles from clay to silt fraction of the Aeolian soil properties were improved by the precipitated calcite. (Sani and Bala, 2021).

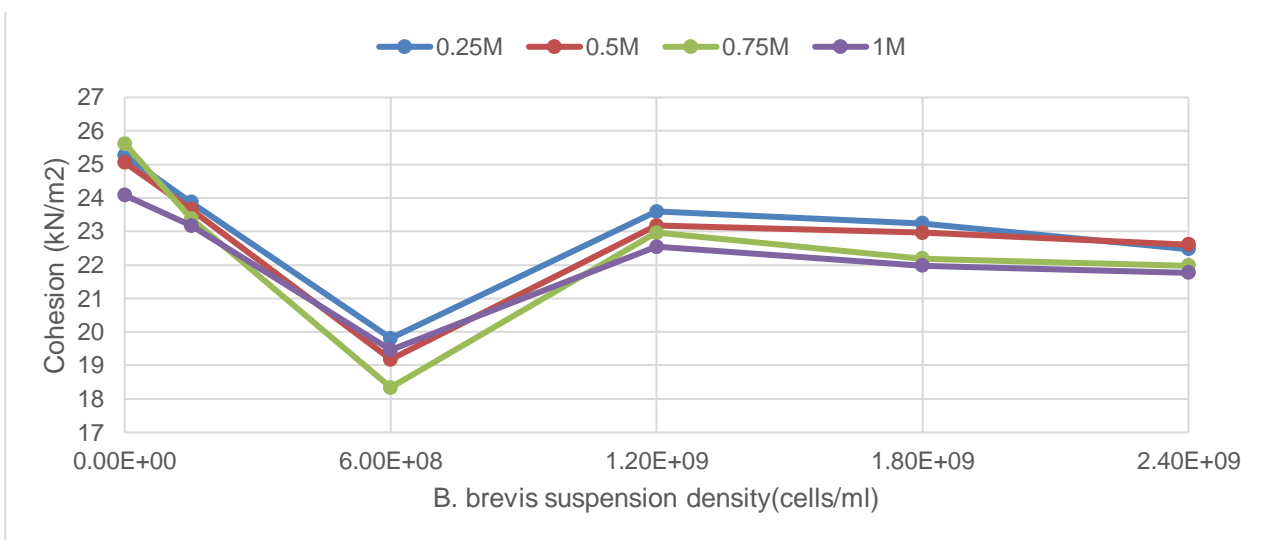


Figure 4: Variation of cohesion of aeolian soil with *B. brevis* suspension density

**Angle of Internal Friction**

The variation of angle of internal friction of Aeolian soil with *B. brevis* suspension density is presented in Fig. 5. Particularly the angle of internal friction values increased for cementation reagent concentrations 0.25, 0.5 and 0.75 M with increase in *B. brevis* suspension density, but decreased with higher *B. brevis* suspension density and for cementation solution concentration of 1.0 M. The peak angle of internal friction of 25.6° was recorded at  $6.0 \times 10^8$  cells/ml.



The increase in angle of internal friction value recorded can be attributed to the role of the precipitated calcite within the soil during MICP process. The ureaze enzyme generated the MICP biochemical response, by hydrolyzing urea and the ammonium ( $\text{NH}_4^+$ ) formed increased the pH which triggered the bicarbonate ( $\text{HCO}_3^-$ ) to precipitate calcium ion ( $\text{Ca}^{2+}$ ) from the calcium chloride solution. The calcite formed bridged the aeolian soil particles and filled the pores. The calcite crystals were responsible for the bonding between the soil elements and restricted the movement of the soil grains and thereby increased angle of internal friction. (Harkes *et al.*, 2010 ; Mujah *et al.*, 2017 ; Osinubi *et al.*, 2019b and Sani *et al.*, 2020a and b).

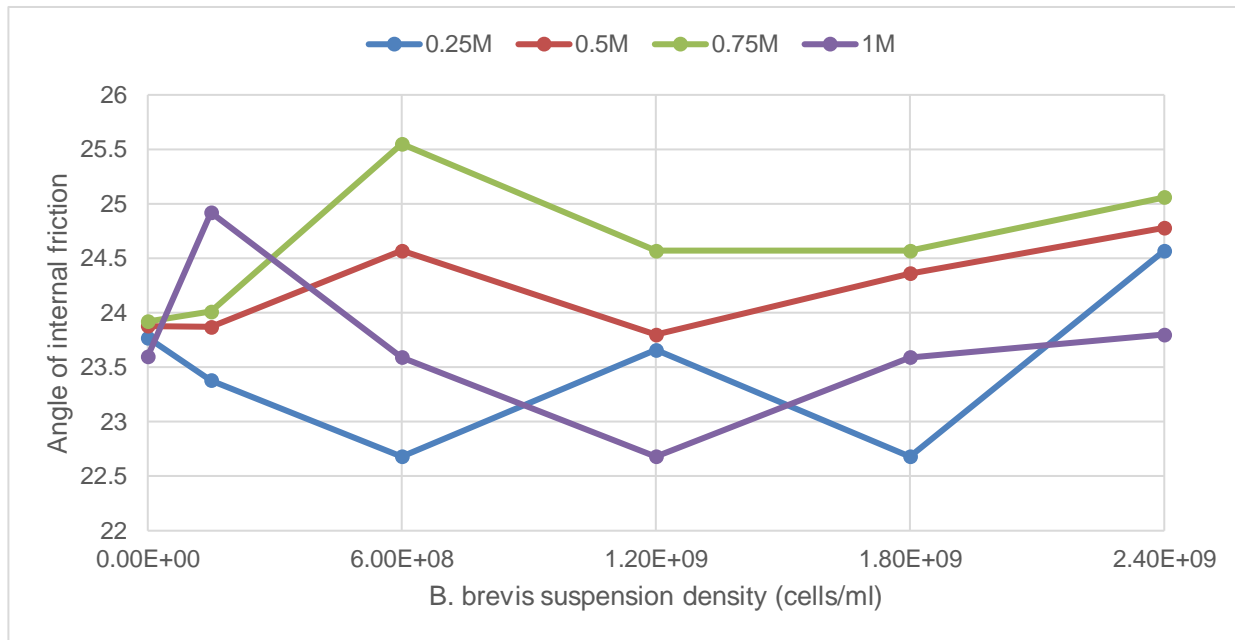


Figure 5: Variation of the angle of internal friction of Aeolian soil with *B. brevis* suspension density

## CONCLUSION

The natural aeolian soil classified as A-3 (0) and SP-SM in AASHTO and USCS systems, respectively, was subjected to treatment with cementation reagent only as the control. Specimens bio-treated with *B. brevis* suspension densities of  $1.5 \times 10^8$ ,  $6.0 \times 10^8$ ,  $1.2 \times 10^9$ ,  $1.8 \times 10^9$  and  $2.4 \times 10^9$  cells/ml and cementation solution concentration of 0.25, 0.50, 0.75 and 1.0 M, respectively, and compacted with BSL energy were used in the study. Based on the results obtained the following conclusions can be made:

1. The Atterberg limits (i.e., liquid limit, plastic limit and plasticity index) indicated that the soil is non-plastic.
2. Compaction characteristics (MDD and OMC) showed that peak MDD value of  $1.22 \text{ Mg/m}^3$ , was recorded for *B. brevis* ( $6.0 \times 10^8$  cells/ml) and cementation reagent (0.75 M) while the least OMC value of 5.7 % was recorded for  $12 \times 10^8$  cells/ml and 1.0 M treatment.
3. Peak UCS value of  $150.91 \text{ kN/m}^2$  was recorded for treatment with *B. brevis* ( $6.0 \times 10^8$  cells/ml) and cementation solution (0.75 M).

The least cohesion value of  $18.34 \text{ kN/m}^2$  and peak angle of internal friction value of  $25.5^\circ$  were recorded for treatment with *B. brevis* ( $6.0 \times 10^8$  cells/ml) and cementation solution (0.75 M), while

Based on the results of the study, it is recommended that in order to improve strength, the lateritic soil be bio-treated with *B. brevis* ( $6.0 \times 10^8$  cells/ml) and cementation solution (0.75 M) be used to improve the aeolian soil considered for use in engineering works.

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