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A hydrogeological assessment of groundwater flow direction and aquifer potential in Ufuma and Environs, Anambra State

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***ABSTRACT** : This study evaluates the strength enhancement of silty sand using Microbial-Induced Calcite Precipitation (MICP) with Bacillus megaterium. The soil, classified as A-3 (0) under AASHTO and SP-SM under USCS, was treated with varying bacterial suspension densities ranging from 1.5×108 cells/ml, 6 × 108 cells/ml, 1.2 × 109cells/ml, 1.8 × 109 cells/ml and 2.4 × 109 cells/ml and compacted using British Standard Light (BSL), West African Standard (WAS), and British Standard Heavy (BSH) energies. Laboratory investigations including unconfined compressive strength (UCS), California bearing ratio (CBR), durability assessment, and statistical analysis using ANOVA were conducted. Results showed significant improvement in strength characteristics of treated soils compared with control specimens. Peak UCS values of 448.84 kN/m^2, 689.98 kN/m^2, and 749.82 kN/m^2 were recorded for BSL, WAS, and BSH compactive efforts, respectively, at optimal bacterial density. Similarly, treated soils exhibited improved CBR values under both soaked and unsoaked conditions, with maximum performance observed at 6.0 × 10^8 cells/ml suspension density. Durability assessment indicated increasing resistance to loss in strength with higher bacterial density and compactive effort, although values remained below recommended durability thresholds. Statistical analysis confirmed that microbial treatment had a significant influence on the engineering properties of silty sand. The findings demonstrate the potential of MICP using Bacillus megaterium as a sustainable soil stabilization technique for improving the strength and load-bearing capacity of silty sand for geotechnical engineering applications.*

***KEYWORDS:** Hydrmicrobial soil stabilization, UCS, CBR, Bacillus megaterium, durability, compaction energy*

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I. INTRODUCTION

Soil stabilization is essential in geotechnical engineering, particularly for fine-grained soils prone to failure. Conventional stabilizers such as cement and lime, though effective, contribute significantly to carbon emissions and environmental degradation. This has driven interest in sustainable alternatives such as microbial-induced calcite precipitation (MICP), which leverages microbial activity to precipitate calcium carbonate within soil pores, thereby improving strength and stiffness (Mitchell &Santamarina, 2005; DeJong et al., 2010).

Recent advances confirm MICP's promise. Liu et al. (2024) demonstrated that MICP significantly improves the strength and stability of low-cohesion soils, positioning it as a cost-effective alternative for sustainable construction. Wang et al. (2024) optimized injection methods for MICP at field scale, showing that treatment efficiency depends on reagent distribution and microbial density. Zhang et al. (2023) reviewed MICP applications, noting its interdisciplinary relevance and effectiveness in mitigating geotechnical failures caused by human activities. Sazzad et al. (2025) applied Bacillus megaterium in sand stabilization, confirming its role as a viable strain alongside Bacillus subtilis.

Despite these advances, durability under soaked conditions remains a major limitation. Studies (Medvey&Dobszay, 2020; Liu et al., 2024) highlight that water ingress reduces calcite bonding, leading to strength loss. This gap underscores the need for further research into cementation reagent optimization and hybrid stabilization techniques.

This paper evaluates the strength and durability of silty sand treated with Bacillus megaterium via MICP, focusing on unconfined compressive strength (UCS), California bearing ratio (CBR), and durability under soaked conditions. The study contributes to sustainable infrastructure development in Nigeria and beyond.

II. MATERIALS AND METHODOLOGY

2.1 Materials

2.1.1 Soil

Silty sandy soil sample utilized for this study was collected from Wudil Local Government Area (latitude 11° 786N and longitude 8° 840E) in Kano State, Nigeria.

2.1.2 Microorganism

The microorganism used in this study was Bacillus megaterium (ATCC 14581), obtained from the American Type Culture Collection (ATCC), USA. This Gram-positive rod-shaped bacterium was cultured in a liquid medium containing 10 g NH_4Cl, 20 g urea, 3 g nutrient broth, and 2.12 g $NaHCO_3$ per litre of distilled water, sterilized in an autoclave at 121 °C for 20 minutes. On the McFarland turbidity scale; five distinct bacteria suspension densities, (i.e., 0.5, 2.0, 4.0, 6.0 and 8.0 with equivalents 1.5×10^8 cells/ml, 6×10^8 cells/ml, 1.2×10^9cells/ml, 1.8×10^9 cells/ml and 2.4×10^9 cells/ml, respectively) MFS were utilized in this study. The soil used as a control was referred to as 0 cells/ml (0 MFS).

2.1.3 Cementation reagent

The urea hydrolysis process was activated using cementation reagent. This study's cementation reagent comprised 3g of nutritional broth, 2.8 g of calcium chloride ($CaCl_2$), 10 g of ammonium chloride (NH_4Cl), 2.12 g of sodium bicarbonate (NaHCO3) and 20 g of urea ($CO(NH_2)_2$), per litre of de-ionized water (Stocks-Fischer et al., 1999; Stoner *et al.*, 2005; DeJong*et al.,* 2006; Al Qabany*et al.,* 2011).

2.2 Methods

Three test procedures were conducted to ascertain the optimum amount of B. *megaterium* suspension density that gave the required improvement when the silty sandy soil was stabilized using 1.5×10^8 cells/ml, 6×10^8 cells/ml, 1.2×10^9cells/ml, 1.8×10^9 cells/ml and 2.4×10^9 cells/ml B. *megaterium* suspension density and cementation reagent only. The following are the tests which were carried out: unconfined compression, durability, and California bearing ratio (CBR).

Initially, tests were carried out to assess the natural soil's strength qualities. After this initial test, similar test procedures were adopted to establish these properties for the control soil samples, in which only a volume of cementation reagent which was obtained from Equation (1) was utilized.

$$Total\ Volume = \frac{Optimum\ Moisture\ Content}{100} \times Weight\ of\ soil\ sample \qquad (1)$$

For the treated specimens, a volume of cementation reagent and a volume of each bacterial suspension were utilized, with the bacterial suspension partially replacing an amount of the total volume of cementation reagent. The volume for each bacterial suspension and cementation reagent was obtained from Equations (2) and (3), respectively.

$$Volume\ of\ Bacteria = \frac{50}{100} \times Total\ Volume \qquad (2)$$

$$Volume\ of\ Cementation\ Reagent = \frac{50}{100} \times Total\ Volume \qquad (3)$$

This was done for specimens prepared using the BSL, WAS, and BSH compactive efforts. Samples were then placed in polythene bags and sealed for 24 hours, to allow for proper hydration. The sample was then compacted in accordance with the methods described in BS 1924 (1990), Part 7 of BS 1377 (1990), as well as Nigerian General Specifications (1997) for the UCS, durability and CBR tests, respectively, for each of the compactive efforts but not extruded from the moulds for 24 hours to ensure proper saturation. Thereafter, the specimens were exposed to UCS, durability and CBR tests were carried out in line with the procedures in BS 1377 (1990): Part 7, BS 1924 (1990) and Nigerian General Specifications (1997) for the compactive efforts considered.

III. RESULTS AND DISCUSSION

3.1 Properties of the Natural Silty Soil

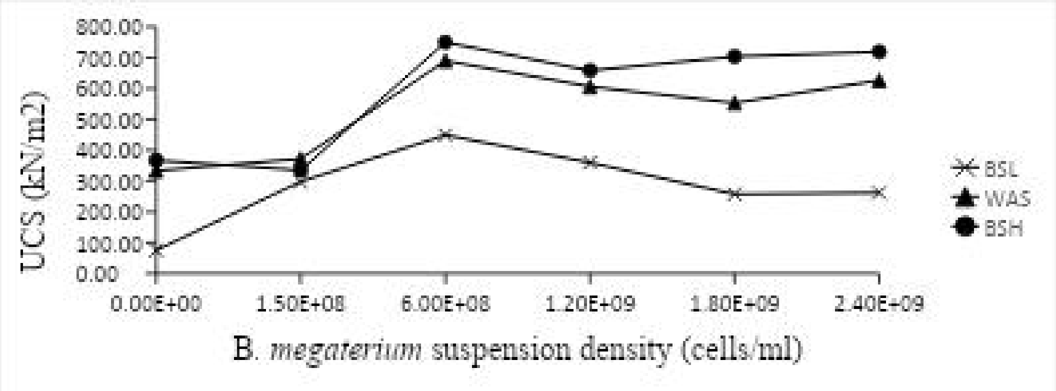
Some geotechnical properties of the natural soil classified as A-3(0) (AASHTO, 1986) and poorly graded (SP) to silty sand (SM) (ASTM, 1992) are summarised in Table 1. The soil sample is unsuitable for use as a subbase material as stipulated in the Nigerian General Specifications (1997).

3.2 Unconfined compressive strength

The results of the unconfined compressive strength (UCS) tests performed for the control and treated soil specimens utilizing BSL, WAS and BSH compactive efforts and cured for 7, 14 and 28 days, are shown in Figures 1a - 1c. The variation of the UCS of the control with *B. megaterium* suspension density treated specimens, compacted with BSL, WAS and BSH efforts for various curing periods are shown in Fig. 1a - 1c.

Table 1: Engineering Properties of the Natural Silty Sand

Property	**Condition/Compactive Effort**	**Value**
Percentage Passing No. 200 Sieve (%)	–	6.7
Optimum Moisture Content (%)	British Standard Light (BSL)	6.6
	West African Standard (WAS)	4.7
	British Standard Heavy (BSH)	3.2
Unconfined Compressive Strength (kN/m²)	7 Days (BSL)	195.2
	7 Days (WAS)	417.2
	7 Days (BSH)	429.7
	14 Days (BSL)	577.8
	14 Days (WAS)	615.5
	14 Days (BSH)	714.2
	28 Days (BSL)	731.1
	28 Days (WAS)	748.1
	28 Days (BSH)	911.9
California Bearing Ratio (Unsoaked, %)	BSL	17.9
	WAS	24.1
	BSH	31.3
Colour	–	Brown



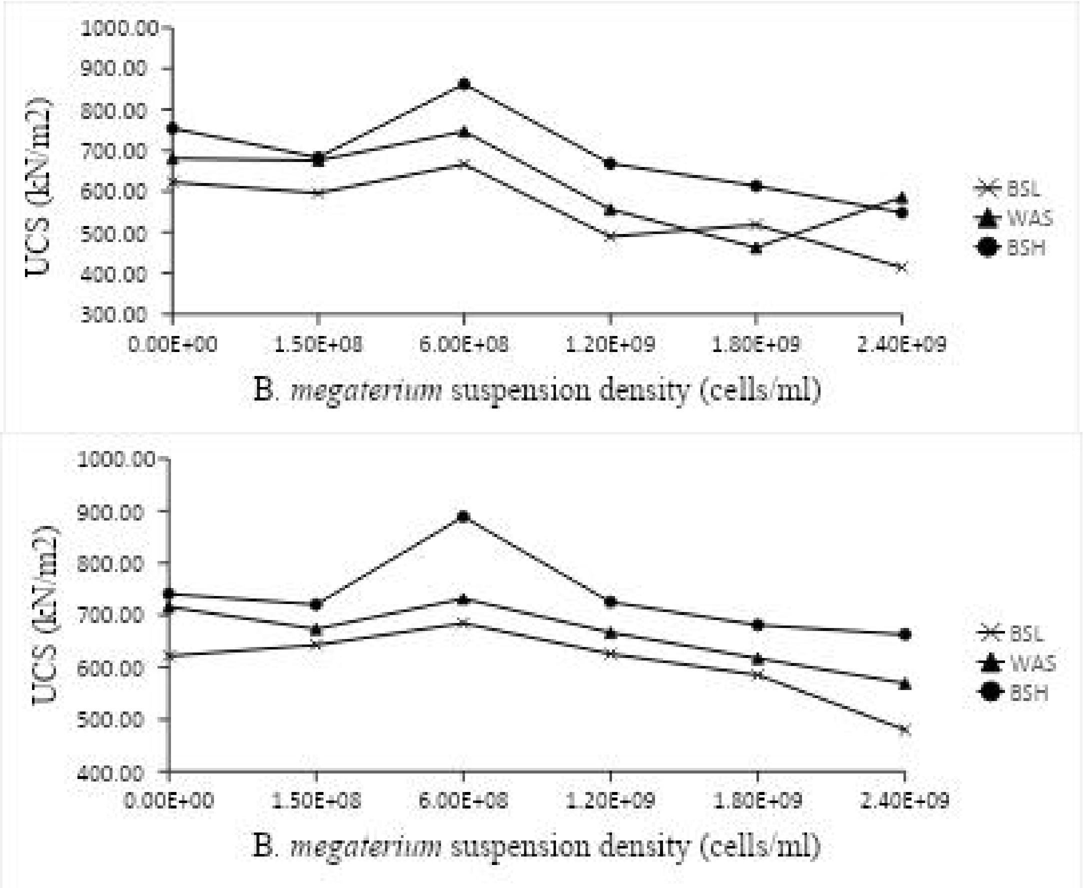


Fig 1: Variation of unconfined compressive strength of silty sand with B. *megaterium* suspension density for varying curing period: (A) 7 days (B) 14 days (C) 28 days.

From the plot (Fig. 1a), the UCS values for the control specimen after 7 days curing period was 621.67, 715.96 and 740.67 kN/m^2 for BSL, WAS and BSH compactive effort respectively. For the specimens treated with B. *megaterium suspension* density, the recorded peak UCS values for the same period was obtained at B. *megaterium* suspension density of 6.0 x 10^8 cells/ml as 684.38 kN/m^2, 731.57 kN/m^2 and 888.80 kN/m^2 for BSL, WAS and BSH compactive effort respectively before steady decrease were recorded for subsequent B. *megaterium suspension* densities. Similar patterns were recorded for the 14days and 28 days curing periods (see Fig. 1b and 1c).

The lower value generated for the control specimen in comparison to the treated specimen, could be because of the generation of more calcite, resulting from the introduction of more microbial cells into the treated specimens. Bacteria cell density has been reported as one of the factors that influence the calcite formation, as urease hydrolysis has a direct relationship with density of microbes in a given cell, since microbes act as nucleation sites for the precipitation of calcite during the MICP process. Hence, a higher concentration of microbes which means more available nucleation sites; often results in higher urease activity and consequent increase in calcite precipitated (Stocks-Fischer *et al.,* 1999; Soon *et al.,* 2012; Cheng *et al*., 2014, Osinuni *et al*., 2020; Rajasekar*et al.,* 2021). This resulted in the deposition of a greater amount of $CaCO_3$ within the pore spaces of the treated specimen, than the control specimen. Lian*et al.* (2006) reported similar findings in their study of B.*megaterium* induced carbonate mineralization, that the process of crystallization was affected by the existence of bacterial cell surfaces and metabolic products.

The decrease in the UCS values recorded after the peak values obtained for the treated specimens can be attributed to the low volume of cementation reagent when compared to the bacteria cell population in those suspension densities. As reported by Anbu *et al.* (2016), urea hydrolysis is influenced greatly by the volume of cementation reagent and concentration of cells in a given bacteria suspension density. Hence, the volume of cementation reagent supplied to those suspension densities with higher cell concentrations was not sufficient to generate sufficient calcite that would improve the bond and strength of the treated soil mass (Soon *et al.,* 2012; De Muynck *et al.,* 2010; Osinubi *et al.,* 2019).

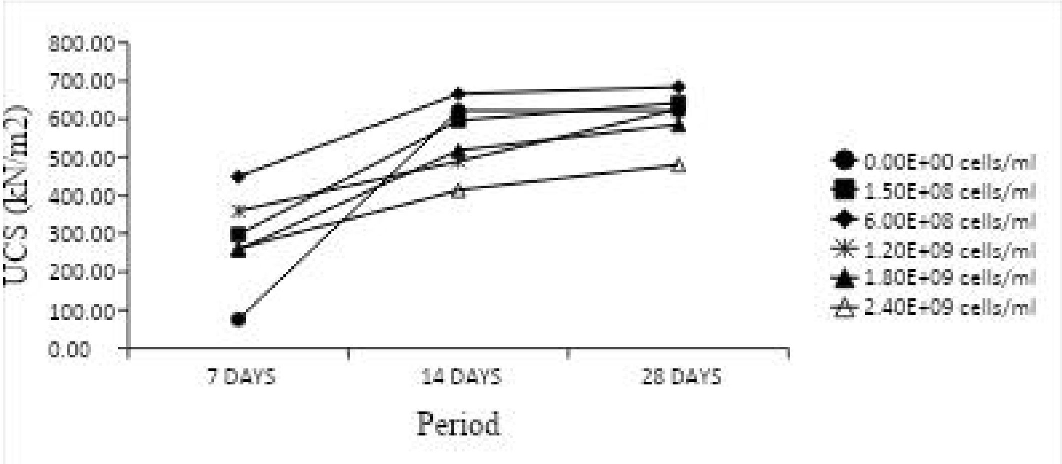
The two–way analysis of variance (ANOVA) test on the UCS results for BSL, WAS and BSH compaction energies is provided in Table 2. The result for the 7 days curing period showed that the effects of the B. *megaterium* suspension density on silty sand soil was statistically significant ($F_{CAL} = 9.863 > F_{CRIT} = 3.326$). For the 14 days curing period, it showed that the effects of the B. *megaterium* suspension density on silty sand soil was statistically significant ($F_{CAL} = 13.275 > F_{CRIT} = 3.326$) and for the 28 days curing duration, the result indicated that the effects of the B. *megaterium* suspension density on silty sand soil was also statistically significant ($F_{CAL} = 13.981 > F_{CRIT} = 3.326$).

Table 2: Two-way analysis of variance for unconfined compressive strength UCS Test results of silty and sand - cementation reagent (i.e., the control)

Property	Source of Variation	Degree of Freedom	FCAL	P-Value	FCRIT	Remark
7 days curing	Compactive effort	2	26.147	1.07E-04	4.103	F_{CAL}> F_{CRIT},Significant Effect
	B. *megaterium* suspension density	5	9.863	1.27E-03	3.326	F_{CAL}> F_{CRIT},Significant Effect
14 days curing	Compactive effort	2	13.703	1.37E-03	4.103	F_{CAL}> F_{CRIT},Significant Effect
	B. *megaterium* suspension density	5	13.275	3.80E-04	3.326	F_{CAL}> F_{CRIT},Significant Effect
28 days curing	Compactive effort	2	27.126	9.13E-05	4.103	F_{CAL}> F_{CRIT},Significant Effect
	B. *megaterium* suspension density	5	13.981	3.05E-04	3.326	F_{CAL}> F_{CRIT},Significant Effect

3.2.1 Effect of curing period on unconfined compressive strength

The variations of UCS values of the control and treated soil specimens with curing periods, using BSL, WAS and BSH compactive efforts, respectively, are shown in Fig. 2a - 2c.



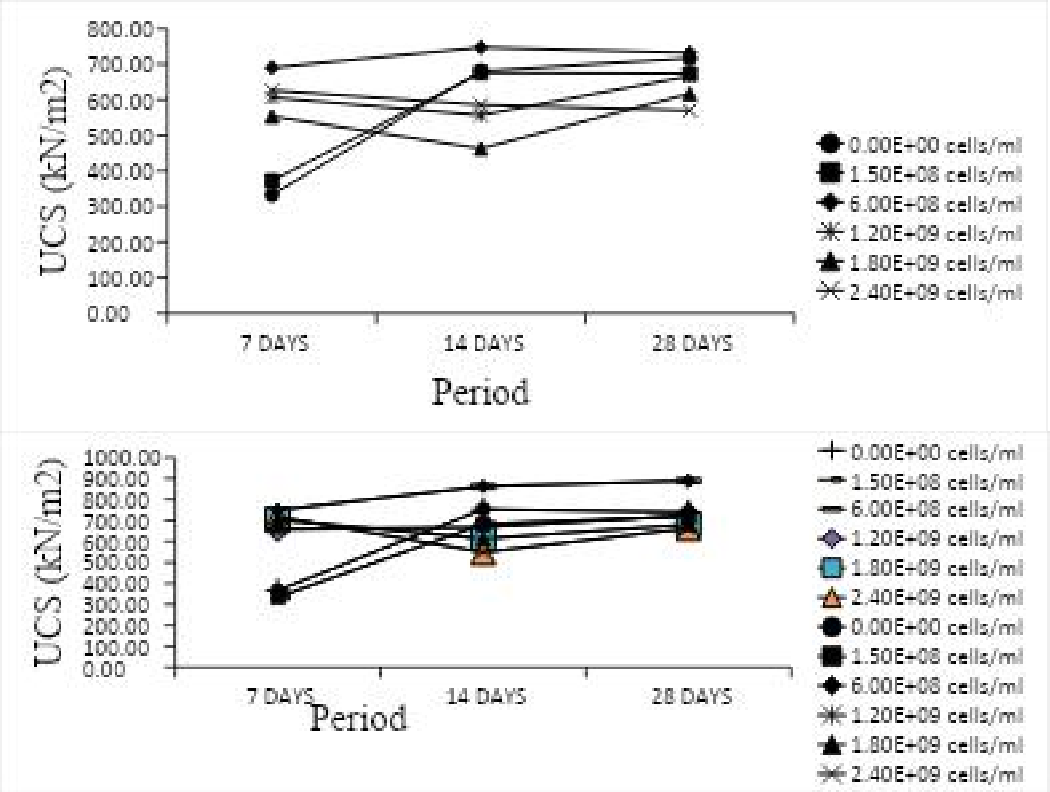


Fig. 2: Variation of unconfined compressive strength of silty sand soil with B. *megaterium suspension* density for varying compactive effort: (a) BSL (b) WAS (c) BSH.

Maximum UCS values for the treated specimens obtained are 448.84 kN/m^2, 666.04 kN/m^2, and 684.38 kN/m^2 for the BSL compaction energy after 7, 14 and 28 days curing periods, respectively. The recorded peak UCS values using WAS compactive effort, for the treated samples were 689.98 kN/m^2, 746.59 kN/m^2 and 731.57 kN/m^2, after curing periods of 7 days, 14 days and 28 days, respectively (see Fig. 3.2b). For the BSH compactive effort, the recorded peak UCS values for the treated samples after 7 days, 14 days and 28 days curing periods were 749.82 kN/m^2, 862.14 kN/m^2 and 888.80 kN/m^2, respectively (see Fig. 2c).

From the plots in Fig. 2, it was observed that generally, the recorded UCS values increased with increase in curing period for the various compactive efforts considered. This could be attributed to strength gain with time, as result of the increased bond between the soil particles and the precipitated calcite (Chittoori*et al.,* 2018).

The two–way analysis of variance (ANOVA) test on the UCS results for 7 days, 14 days and 28 days curing periods (see Table. 2), for BSL compactive effort showed that the effects of the B. *megaterium* suspension density on silty sand soil was not statistically significant (F_{CAL} = 2.445 > F_{CRIT} = 3.326). For the WAS compaction effort, it showed that the effects of the B. *megaterium* suspension density on silty sand was not statistically significant (F_{CAL} = 0.993 > F_{CRIT} = 3.326) and for the BSH compaction effort, the result also indicated that the effects of the B. *megaterium* suspension density on silty sand was statistically significant (F_{CAL} = 1.515 > F_{CRIT} = 3.326).

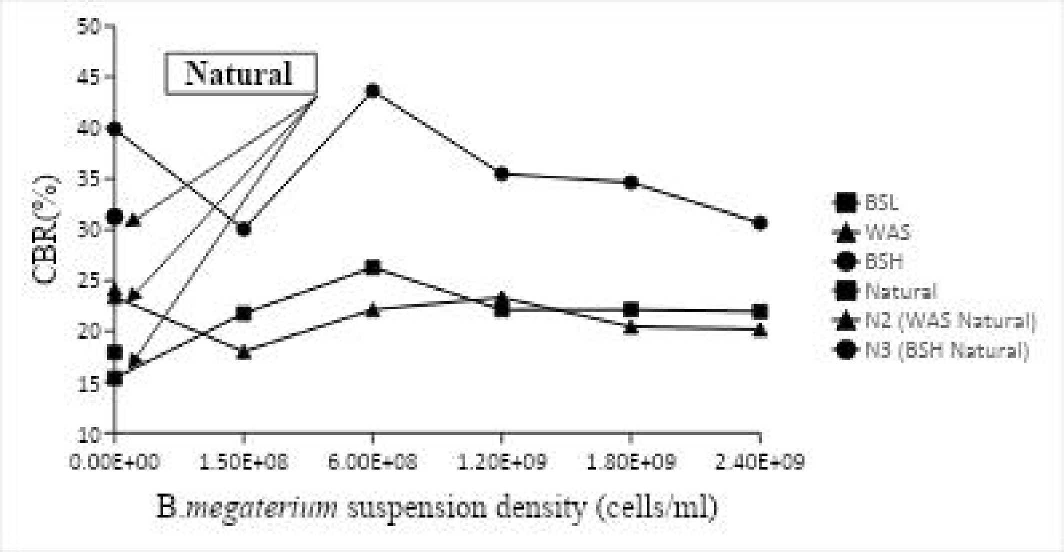
Table 3: Two-way analysis of variance for effect of curing period on unconfined compressive strength of silty sand – cementation reagent mixtures (i.e., the control)

Property	Source of Variation	Degree of Freedom	FCAL	P-Value	FCRIT	Remark
BSL	B. *megaterium* Suspension Density	5	2.445	1.07E-01	3.326	FCAL< FCRIT, No Significant Effect
	Curing Period	2	27.209	9.01E-05	4.103	FCAL> FCRIT, Significant Effect
WAS	B. *megaterium* Suspension Density	5	0.993	4.69E-01	3.326	FCAL< FCRIT, No Significant Effect
	Curing Period	2	2.309	1.50E-01	4.103	FCAL< FCRIT, No Significant Effect
BSH	B. *megaterium* Suspension Density	5	1.515	2.69E-01	3.326	FCAL< FCRIT, No Significant Effect
	Curing Period	2	2.246	1.56E-01	4.103	FCAL< FCRIT, No Significant Effect

3.3 California bearing ratio

The California bearing ratio (CBR) test is conducted to ascertain the strength of a soil. It is a penetration test that evaluates the strength of a soil for use as a road pavement layer material. The pavement thickness is often determined with the aid of empirical curves from the obtained results.

The plots of the un-soaked and soaked CBR values with B. *megaterium* suspension density is shown in Fig. 3 for the control and treated soil samples, using the various compaction efforts.



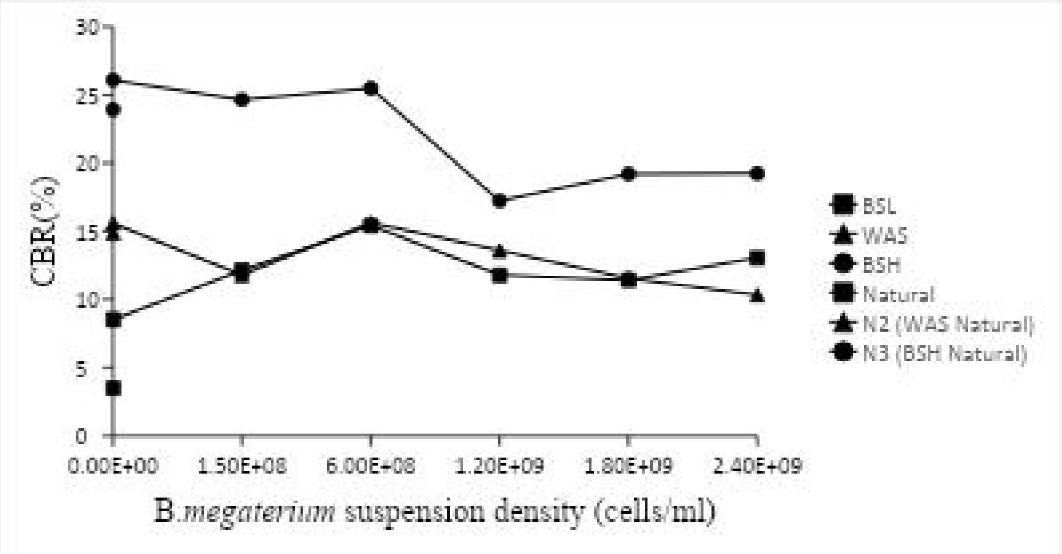


Fig. 3: Variation of California bearing ratio of silty sand with B. *megaterium* suspension density for varying compactive effort: (A) Un-soaked (B) Soaked.

For the un-soaked CBR specimens (see Fig. 3.3a), the CBR values recorded for the control specimen were 15 %, 23 % and 40 % for BSL, WAS and BSH compactive efforts, respectively, while for the treated specimens, peak CBR values of 22 %, 26 % and 40 % for BSL, WAS and BSH compactive effort, respectively, were obtained at the B. *megaterium* suspension density of 6.0 x 10^8 cells/ml.

Generally, the CBR values for the treated specimens were higher than those of the natural and control specimens. The high CBR values obtained for the treated specimens was due to the biogeochemical reaction between the B. *megaterium*microbes and the calcium ions in the cementation fluid, which resulted in the generation of sufficient calcite. The newly formed calcite was able to fill the available pore spaces within the soil mass, hence improving the bond between the individual soil grains (Anbu *et al.,* 2016; Rajasekar *et al.,* 2021).

The plots of the soaked CBR values for the control specimens using BSL, WAS and BSH compactive effort recorded peak values of 9 %, 16 % and 26 %, respectively. For the treated specimens, peak CBR values of 15 %, 16 % and 26 % were recorded for BSL, WAS and BSH compactive effort, respectively, at 6.0 x 10^8 cells/ml B. megaterium suspension density. The lower soaked CBR values in comparison with the values for the un-soaked specimens could be as a result of the absorption of water during the saturation of the specimens in the curing bath, which reduced the strength of the specimens (Kanyi, 2017; Ahmad *et al*, 2021).

The two–way analysis of variance (ANOVA) test on the CBR results for BSL, WAS and BSH compaction efforts (see Table 3.3) showed that the effects of the B. *megaterium* suspension on silty sand soil was statistically significant for the unsoaked samples ($F_{CAL} = 5.866 > F_{CRIT} = 3.326$) and for the soaked samples ($F_{CAL} = 5.008 > F_{CRIT} = 3.326$).

Table 4: Two-way analysis of variance for California bearing ratio results of silty sand – cementation reagent mixtures (i.e., the control)

Property	**Source of Variation**	**Degree of Freedom**	**F_{CAL}**	**P-Value**	**F_{CRIT}**	**Remark**
Un-soaked condition	Compactive Effort	2	1.347	3.03E-01	4.103	$F_{CAL} < F_{CRIT}$, No Significant Effect
	B. *megaterium* Suspension Density	5	5.866	8.73E-03	3.326	$F_{CAL} > F_{CRIT}$, Significant Effect
Soaked condition	Compactive Effort	2	1.975	1.89E-01	4.103	$F_{CAL} < F_{CRIT}$, No Significant Effect
	B. *megaterium* Suspension Density	5	5.008	1.48E-02	3.326	$F_{CAL} > F_{CRIT}$, Significant Effect

3.4 Durability assessment

Durability assessment of soil specimens was carried out by estimating the resistance to loss in strength of the specimens when immersed in water, which simulates the worse conditions that could be experienced in the field (Ola, 1974). A permissible 20 % loss in strength (i.e., 80 % resistance to loss in strength) is recommended for a specimen cured for 7 days and immersed in water for 4 days (Ola, 1974; Osinubi *et al*, 2020; Medvey and Dobszay, 2020).

The plot of the resistance to loss in strength values for the control and B.*megaterium*treated specimens is shown in figures 4. For the control specimens, the recorded resistance to loss in strength values were 1.43 % (i.e., 98.57 % loss in strength), 2.62 % (i.e., 97.38 % loss in strength) and 2.75 % (i.e., 97.25 % loss in strength) for BSL, WAS and BSH compaction, respectively.

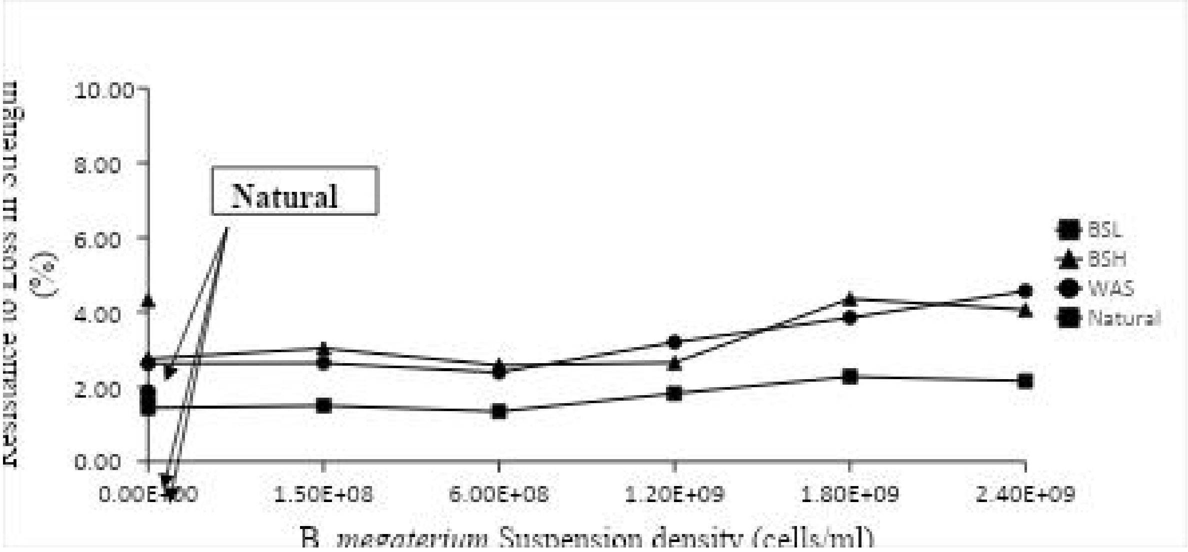


Fig.4: Variation of the resistance to loss in strength of silty sand with B. *megaterium suspension* density

The resistance to loss in strength values in treated samples increased with higher B. *megaterium* suspension and compactive effort. The resistance to loss in strength values recorded increased from 1.5 % (i.e., 98.5 % loss in strength), 2.64 % (i.e., 97.36 % loss in strength) and 3.03 % (i.e., 96.97 % loss in strength) for BSL, WAS, and BSH compaction, respectively, at B. *megaterium*suspension density of 1.5 x 10^8 cells/ml to 2.16 % (i.e., 97.84 % loss in strength), 4.56 % (i.e., 95.44 % loss in strength) and 4.06% (i.e., 95.94 % loss in strength) for of BSL, WAS, and BSH compactive effort, respectively, at B. *megaterium*suspension density of 2.4 x 10^9 cells/ml.

The durability results obtained fall short of the recommended loss in strength value of 20 % (i.e., 80 % resistance loss in strength). The higher durability values obtained with increased compactive effort could be the result of bond loss with ingress of water, produced by variations in the grain size fractions of the specimen due to the higher calcite crystal precipitated at these compactive efforts; these may have affected the bond in the specimens compacted at the various compactive effort. This led to the lower resistance to strength loss value obtained with increased compactive effort (Kanyi, 2017; Medvey and Dobszay, 2020).

The durability results for BSL, WAS, and BSH compactive efforts subjected to a two–way analysis of variance (ANOVA) test (see Table 3.4) showed that the effects of the B. *megaterium*suspension density on silty sand soil was statistically insignificant ($F_{CAL} = 2.9375 < F_{CRIT} = 3.326$

Table 5: Analysis of Variance in Two Ways for Durability Assessment Results of soil – cementation reagent mixtures (i.e., the control) soil samples.

Property	**Source of Variation**	**Degree of Freedom**	**FCAL**	**P-Value**	**FCRIT**	**Remark**
Durability Assessment	Compactive Effort	2	11.4108	2.63E-01	4.103	$F_{CAL} > F_{CRIT}$, Significant Effect
	B. *megaterium* Suspension Density	5	2.9375	6.92E-02	3.326	$F_{CAL} < F_{CRIT}$, No Significant Effect

IV. CONCLUSION

This study demonstrates that microbial-induced calcite precipitation (MICP) using Bacillus megaterium significantly enhances the strength and bearing capacity of silty sand. The treated soils achieved UCS and CBR values that satisfy Nigerian General Specifications for subgrade materials, confirming the potential of MICP as an eco-friendly stabilization technique. However, durability performance under soaked conditions fell short of recommended thresholds, indicating that water ingress remains a critical challenge.

Future research should focus on optimizing cementation reagent volumes, exploring alternative microbial strains, and integrating MICP with conventional stabilizers to improve long-term durability. By addressing these limitations, MICP can be advanced as a sustainable solution for geotechnical engineering applications in Nigeria and globally.

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