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Effect of *Bacillus megaterium* on the Physio-Chemical and Compaction Characteristics of Silty Sand

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Abstract

Silty soil obtained from Wudil Local Government Area, Kano State—classified as A-3(0) under the AASHTO system and SP-SM under the Unified Soil Classification System (USCS)—was treated using the Microbial-Induced Calcite Precipitation (MICP) technique to enhance its geotechnical properties. The study investigated the effects of varying concentrations of *Bacillus megaterium* (0, 1.5×10^8, 6.0×10^8, 1.2×10^9, 1.8×10^9, and 2.4×10^9 cells/ml) on the compaction and index properties of the soil. A premixing method was employed, and treated samples were prepared using bacterial suspension-to-cementation reagent ratios of 25:75, 50:50, and 75:25, with the control sample comprising 100% cementation solution. Results showed that the maximum dry density (MDD) was achieved at a bacterial concentration of 6.0×10^8 cells/ml, corresponding with an optimum moisture content (OMC), indicating improved soil densification. The findings suggest that MICP treatment, particularly at optimal bacterial concentrations and reagent ratios, can enhance the compaction characteristics of silty sand, with implications for sustainable ground improvement techniques in civil engineering applications.

Keywords: *Bacillus megaterium*; Cementation Reagent; Silty Sand; Calcium Carbonate Content; Cation Exchange Capacity; pH; OMC; MDD

INTRODUCTION

With the growing demand for environmentally responsible engineering solutions, researchers are seeking alternatives to conventional soil stabilization methods, which often pose ecological challenges. Biological techniques have emerged as promising, eco-friendly approaches to soil improvement (Eberemu et al., 2021).

The application of microorganisms in geotechnical engineering was first reported in 1992 by Ferris and Stehmeier. Since then, microbial processes have gained recognition as viable and sustainable options for enhancing soil behavior, leading to extensive experimental and field studies (DeJong et al., 2006; Chu et al., 2014; Kim & Youn, 2016; Eberemu et al., 2021).

The underlying mechanism involves microbial activity that induces calcite formation, which bonds soil particles. This technique, commonly referred to as Microbial-Induced Calcite Precipitation (MICP), has been shown to enhance geotechnical properties such as soil strength, erosion resistance, permeability, and even seismic performance (Kim & Youn, 2016).

Several recent studies affirm that microbial approaches are effective in stabilizing soils while offering environmental advantages. Such methods are particularly beneficial for loose or unstable sands that are prone to settlement, liquefaction, or foundation failure (Alvarado, 2009; Kim & Youn, 2016; Jiang et al., 2017). Beyond geotechnical applications, bio-based treatments have been reported to enhance the compressive strength and long-term durability of construction materials, thereby reducing structural deficiencies (Achal et al., 2013).

Improvements in shear resistance and reduced permeability have also been associated with MICP-treated soils, demonstrating the method's potential in addressing common geotechnical challenges (Whiffin et al., 2007; Lo Bianco & Madonia, 2007; van Paassen, 2011; Osinubi et al., 2017; Osinubi et al., 2018). In addition, bio-cementation has been applied successfully in sandy soils, contributing to ground improvement strategies and providing sustainable alternatives to chemical stabilizers (Achal et al., 2009; DeJong et al., 2013; Gat et al., 2014; Wei et al., 2015; Murtala et al., 2016; Dhami et al., 2018).

This study therefore investigates the role of *Bacillus megaterium* in inducing calcite precipitation and its subsequent effect on the physio-chemical and compaction properties of silty sand. Emphasis is placed on evaluating how different bacterial suspension densities influence soil behavior, with a view to potential application in road construction and other geotechnical works.

MATERIALS AND METHODS

Materials

Silty sand soil. The silty sand used in this study was collected from Wudil Local Government Area, Kano State, Nigeria (Latitude 11°786N, Longitude 8°840E).

Microorganism Bacillus megaterium, a rod-shaped, Gram-positive bacterium listed under ATCC 14581, was selected as the microbial agent for soil treatment. Bacterial suspensions of varying turbidity, based on the McFarland turbidity standard (MFS), were prepared with cell densities of 0.5, 2.0, 4.0, 6.0, and 8.0 MFS units, corresponding to 1.5×10^8, 6×10^8, 1.2×10^9, 1.8×10^9, and 2.4×10^9 cells/ml. The untreated sample (control) contained no bacterial cells (0 MFS).

Cementation Solution The cementation solution was created by dissolving specific chemicals in distilled water per liter as follows: 3 g of nutrient broth, 20 g of urea, 10 g of ammonium chloride (NH_4Cl), and 2.12 g (equivalent to 25.2 mM) of sodium bicarbonate ($NaHCO_3$). The solution's pH was adjusted to 6.0 using 6 M HCl before sterilization. Afterwards, 10 ml of a filtered solution containing 2.80 g of calcium chloride ($CaCl_2$) was added, based on the procedure by Stocks-Fischer et al. (1999).

Methods

Physio-chemical and compaction characteristics.

To determine the most effective bacterial density for soil stabilization, five bacterial concentrations (1.5×10^8, 6×10^8, 1.2×10^9, 1.8×10^9, and 2.4×10^9 cells/ml) were tested, alongside a control sample using only the cementation reagent. The following tests were conducted:

i. Cation Exchange Capacity (CEC)
ii. pH Level
iii. Calcium Carbonate Content (CCC)
iv. Compaction tests using three energy levels: British Standard Light (BSL), West African Standard (WAS), and British Standard Heavy (BSH)

Soil sample preparation and treatment.

Initial tests established the index and compaction properties of the untreated soil. The same procedures were applied to the control sample, which was mixed solely with cementation solution, in accordance with BS 1377 (1990) and BS 1924 (1990).

For treated samples, soil was thoroughly blended with a measured volume of B. megaterium suspension (B) and cementation solution (C) using a mixing bowl. A 50:50 ratio of bacterial suspension to cementation solution was used for calculating the volumes. The equations used to determine these volumes are outlined as:

1) 50B - 50C: In this mix ratio, the soil was mixed with 50 % B. *megateriums* suspension (50B) and 50 % cementation reagent (50C) of the total volume which was obtained from the equations;

$$Total\ Volume = \frac{Liquid\ Limit\ of\ Natural\ Sample}{100} \times$$

$$Weight\ of\ soil\ sample\ Total\ Volume = \frac{Optimum\ moisture\ content}{100} \times$$

$$Weight\ of\ soil\ sample \qquad (1)$$

$$Volume\ of\ Bacteria = \frac{50}{100} \times Total\ Volume$$

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

$$Volume\ of\ Cementation\ Reagent = \frac{50}{100} \times Total\ Volume$$

$$Volume\ of\ Cementation\ Reagent = \frac{50}{100} \times Total\ Volume$$

(3) After mixing, the samples were air-dried, then crushed and sieved through a BS No. 4 (4.75 mm) sieve to prepare them for compaction testing.

RESULTS AND DISCUSION

Index Properties of the Natural Silty Sand

The native silty sand exhibited a relatively high natural moisture content of 20%, likely due to its collection during the rainy season. Table 1 presents the fundamental properties of the soil, while Table 2 shows its oxide composition. The particle size distribution curve is depicted in Figure 1. The sample appeared brown and was classified as non-plastic. It contained 6.7% fines passing through the No. 200 sieve. Based on

AASHTO (1986), it falls under the A-3(0) classification, while the USCS (ASTM, 1992) categorizes it as a poorly graded to silty sand (SP-SM). In its natural form, the soil is loose and lacks cohesion, which diminishes its strength and limits its suitability for structural applications like pavement and foundation work. Hence, soil improvement was necessary.

Table 1. Basic Properties of the Silty Sand Used in the Study.

Properties	Quantity
Percentage passing 0.075 mm sieve	6.7
Natural moisture content, %	20.0
Specific gravity	2.63
Liquid limit, %	-
Plastic limit %	Non plastic
Plasticity index, %	Non Plastic
Linear shrinkage, %	-
AASHTO classification	A-3(0)
USCS classification	SP-SM
Maximum dry density, Mg/m^3	
British Standard Light	1.53
West African Standard	1.58
British Standard Heavy	1.60
Optimum moisture content, %	
British Standard Light	6.6
West African Standard	4.7
British Standard Heavy	3.2
Colour	Brown

Table 2: Oxide composition of the silty sand used in the study

Oxide	Concentration (%)
MgO	2.62
Al_2O_3	6.698
SiO_2	90.56
P_2O_5	0.284
SO_3	0.153
Cl	0.073
K_2O	1.186
CaO	0.165
TiO_2	0.571
MnO	0.021
CeO_2	0.007

Oxide	Concentration (%)
Fe_2O_3	0.639
ZnO	0.008
SrO	0.079
ZrO_2	0.3797

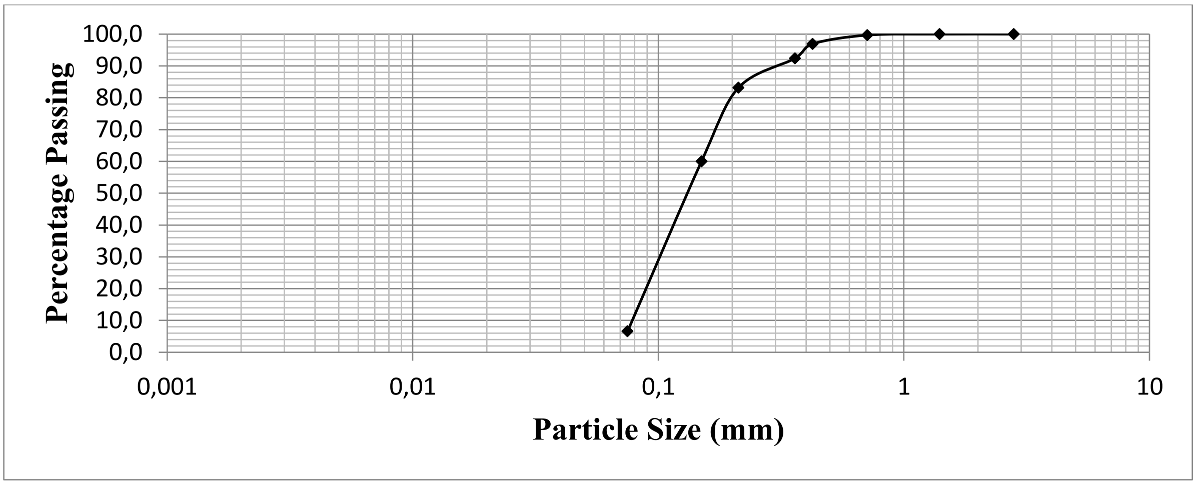

Fig. 1: Particle size distribution curve of the natural silty sand used in the study

Effect of B. *megaterium* on the Index Properties of Silty Sand

Cation exchange capacity

Figure 2 illustrates how CEC values changed with varying bacterial densities. The CEC rose from 4.3 Cmol/kg in the control sample to 5.4 Cmol/kg at the highest bacterial density of 2.4×10^9 cells/ml. However, a noticeable dip in CEC at 6.0×10^8 cells/ml might be due to a strong biochemical reaction between the bacteria and the cementation solution. This could have caused calcite formation, changing the soil's composition and reducing fine particles (Soon et al., 2012; Anbu et al., 2016).

Fig. 2: Variation of cation exchange capacity of silty sand with B. megaterium suspension density

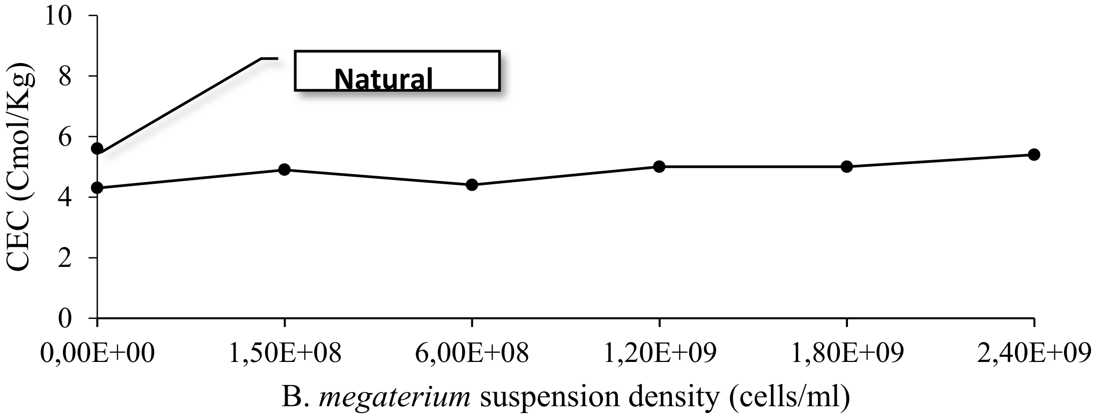

Fig. 2: Variation of cation exchange capacity of silty sand with B. *megaterium* suspension density

pH

Urease-positive bacteria like B. megaterium can increase the surrounding pH by breaking down urea into ammonium and carbonate ions. These carbonates react with calcium to form calcium carbonate, which binds soil grains and improves strength (Anbu et al., 2016; Neupane, 2016).

The variation of pH of silty sand with B. megaterium suspension density is presented in Figure 3. It can

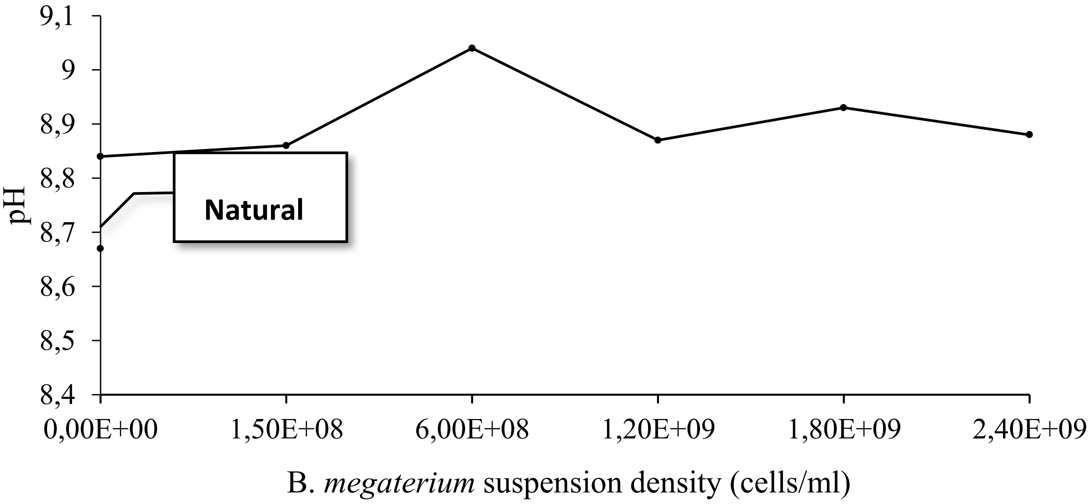

Fig. 3: Variation of pH of silty sand with B. megaterium suspension density

As shown in Figure 3, pH values initially rose from 8.84 (control) to a peak of 9.04 at a bacterial density of 6.0 × 10^8 cells/ml, before declining to 8.88 at 2.4 × 10^9 cells/ml.

The peak value suggests increased urease activity and calcite formation due to the breakdown of urea and release of ammonia (Soon et al., 2012).

Calcium carbonate content

The wash method (Sun-Gyu et al., 2017) was used to measure calcium carbonate content, and results are displayed in Figure 4. The untreated sample had a CCC of 0.8%, which increased to a maximum of 4% at 6.0 × 10^8 cells/ml, then decreased to 1.4% at 2.4 × 10^9 cells/ml. This variation likely results from calcite forming on bacterial surfaces (Mayur & Jayes, 2013; Choi et al., 2016; Sun-Gyu et al., 2017).

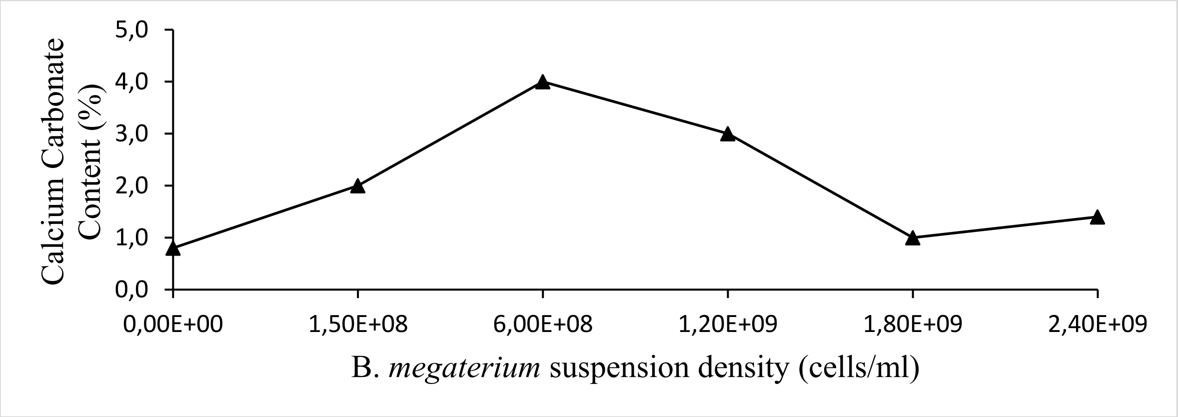

Fig. 4: Variation of calcium carbonate content of silty sand with B. megaterium suspension density

Compaction Characteristics

Maximum dry density

Figure 5 shows how MDD values varied with bacterial concentration under different compaction efforts (BSL, WAS, and BSH). The MDD peaked at 6.0 × 10^8 cells/ml for all efforts, then declined at higher concentrations. The control sample recorded MDDs of 1.62, 1.66, and 1.70 Mg/m³ for BSL, WAS, and BSH, respectively. At the optimal bacterial density, MDDs rose to 1.66, 1.70, and 1.74 Mg/m³, respectively.

This increase suggests that more calcite filled the voids within the soil, resulting in denser samples. The balance between bacterial activity and cementation solution at this density likely encouraged greater calcite deposition (De Muynck et al., 2010; Bu et al., 2018; Seifan et al., 2019, 2020).

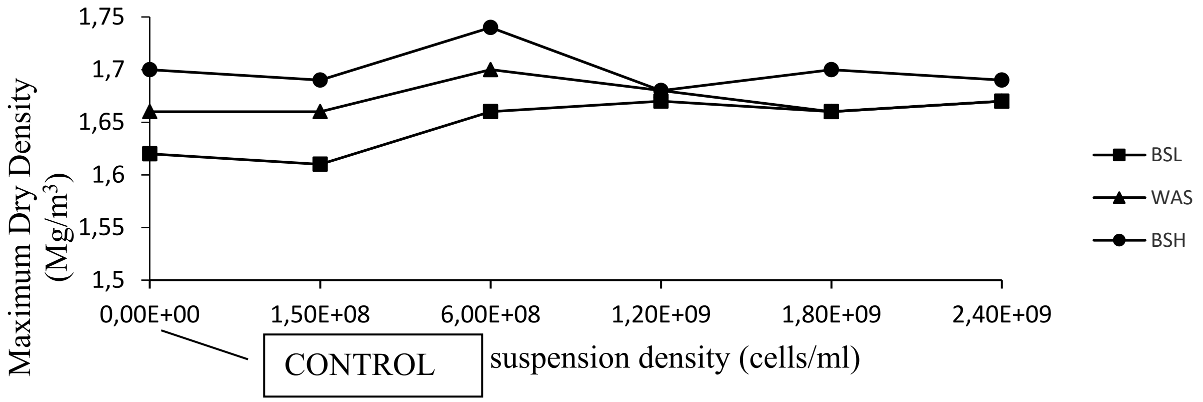

Fig. 5: Variation of maximum dry density of silty sand soil with B. megaterium suspension density.

Optimum moisture content

Figure 6 presents OMC values across various bacterial concentrations and compactive efforts. The lowest OMC values—7.5%, 6.5%, and 5% for BSL, WAS, and BSH—were observed at 1.2×10^9 cells/ml. At the highest density (2.4×10^9 cells/ml), values rose to 6.8%, 7.5%, and 8.5%, respectively.

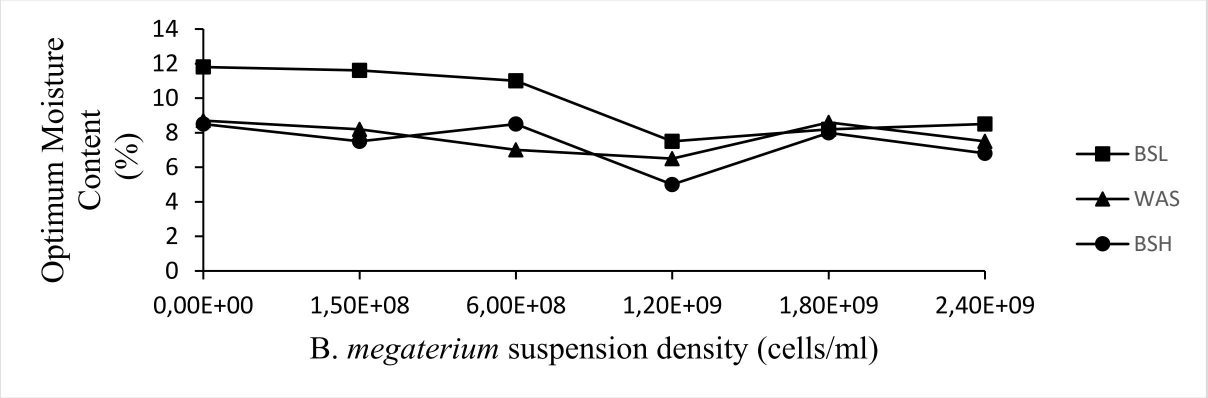

Fig. 6: Variation of optimum moisture content of silty sand with B. megaterium suspension density.

The control sample had OMC values of 11.8%, 8.7%, and 8.5%. The drop in moisture content for treated samples is attributed to calcite filling pore spaces, reducing the soil's capacity to absorb water (Anbu et al., 2016; Hoang, 2018).

CONCLUSION

The silty sand used in this study was classified as A–3(0) under AASHTO and SP-SM according to the Unified Soil Classification System (USCS). It was treated with different concentrations of *Bacillus megaterium* suspensions, while the control samples received only the cementation solution. Physio-chemical analyses and compaction tests were conducted under three compactive efforts: British Standard Light (BSL), West African Standard (WAS), and British Standard Heavy (BSH).

The findings revealed the following key outcomes:

The Cation Exchange Capacity (CEC) increased from 4.3 Cmol/kg in the control to a peak of 5.4 Cmol/kg at the highest bacterial density of 2.4×10^9 cells/ml.

The pH level reached a maximum value of 9.04 at a bacterial density of 6.0×10^8 cells/ml, indicating optimum urease activity and calcite precipitation.

The Maximum Dry Density (MDD) also peaked at this bacterial density, attaining values of 1.66, 1.70, and 1.74 Mg/m^3 for BSL, WAS, and BSH, respectively.

Corresponding reductions in Optimum Moisture Content (OMC) were observed, with values as low as 7.0% under WAS compaction at the optimal density.

These results demonstrate that *Bacillus megaterium* significantly enhances the engineering properties of silty sand through microbial-induced calcite precipitation. The most effective bacterial concentration was found to be 6.0×10^8 cells/ml, which consistently improved both chemical reactivity and compaction behavior.

The study confirms the potential of MICP as a sustainable alternative to conventional chemical stabilizers in geotechnical engineering. Specifically, the technique offers an environmentally friendly means of improving soil density, reducing water affinity, and strengthening weak silty soils for road construction and foundation applications.

Future research should focus on evaluating the durability of treated soils under cyclic wetting-drying and freeze-thaw conditions, as well as scaling up the method for field applications in different soil environments.

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