

POTENTIALS OF *BACILLUS AMYLOLIQUEFACIENS* ON IMPROVEMENT IN THE GEOTECHNICAL CHARACTERISTICS OF POOR LATERITIC SOIL

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ABSTRACT

Microbial Induced Calcite Precipitation (MICP) is a green technology which is being employed in the stabilisation of poor soil. Microorganisms that are capable of producing urea are introduced into the soil alongside chemical solution consisting of urea and calcium salt. This thus forms calcite within the soil matrix, thereby leading to reduction in permeability and increase in the soil strength. A new urease producing Bacterium-*Bacillus Amyloliquefaciens* was locally isolated from a highly plastic lateritic soil characterised as A-2-4(7) soil and was used to stabilize the same soil. Engineering and biochemical tests were performed on the treated specimen to assess the performance of the microbe. Unconfined Compression Strength (UCS) of the treated soil increased from 285.63 to 335.13 kPa within 60 days of curing age while the permeability (K) decreased from 6.10×10^{-6} to 4.36×10^{-7} cm/s within the same curing age. The calcite content has the peak precipitation on the sixtieth day with a value of 31.89g. Cation Exchange Capacity (CEC) of the treated specimen had the highest (39.908 meq/100g) on day 60 of curing. SEM images of the treated samples show fully packed crystal structure of calcite clogging and cementing the soil particles. The regression statistical models for both UCS and K show that coefficients of determination (R^2) for both models exceed 96%, indicating that the models capture the majority of the variability in the experimental dataset and remain consistently high, confirming that the five included predictors contribute meaningfully to each of the models without overfitting. Thus, use of MICP in lieu of conventional materials is recommended, being eco-friendly and effective in soil stabilisation.

Keywords: *Bacillus Amyloliquefaciens*; Geotechnical; Lateritic soil; MICP; Stabilisation.

1. | Introduction

Lateritic soil is an exceedingly weathered tropical or subtropical residual earth material consisting of varying distributions of particle sizes ranging from clayey to gravelly grains, and is characterized by concentration of iron and aluminum oxides Nnochiri and Aderinlewo (2016). Lateritic soils have numerous applications and uses, in road construction they are used as subgrade materials and as pavements base. This is because they can easily be manipulated on the road surface, and has stable grading with suitable proportion to act as binders (Layade and Ogunkoya, 2018). Odinkemere with others in 2024 evaluated the engineering problems associated with lateritic soil Odinkemere *et al.*, (2024).

The effectiveness of lateritic soils as foundations for structures varies and hinged on the soil nature, weathering, topography, the condition of drainage and the foundation type, and the magnitude of imposed load Layade and Ogunkoya (2018)

Poor lateritic soils had posed a threat to engineering structures from time immemorial, and had been stabilised using conventional materials like cement, lime, fly-ash and agricultural residues (Durojaye *et al.*, 2025). All these may results into leachates which contaminate groundwater Bagriacik *et al.*, (2021). As a result, the use of green technology such as biocementation technique surfaced and had been employed for soil treatment (Durojaye *et al.*, 2022).

This method employs microbial and biochemical processes which take place within soil matrix to precipitate calcium carbonate cementing the soil grains or clogging the soil pore (Osinubi *et al.*, 2018). Stabilization by MICP is a nobel technique used in the stabilisation of soil, in which environment-friendly microbes are isolated to enhance the index and engineering characteristics of soil (Durojaye *et al.*, 2025). According to Wath and Pusadkar (2016), the microbial treatment of soil is a non-toxic and eco-friendly alternative to conventional improvement of soil properties The technique had been employed to decrease hydraulic conductivity as well as improve the unconfined compressive strength of lateritic soil using named organism (Durojaye *et al.*, 2022).

Chiet *et al.*, (2015) explored the capability of *Bacillus subtilis* for the tropical residual soil treatment in which unconfined compression strength was tremendously increased. Also, Anthony *et al.*, (2017) used *sporosarcina pasteurii* inoculated in urea and calcium chloride solution inoculum to treat a lateritic soil whose coefficient of permeability was reduced by 83%.

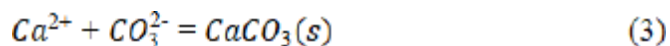
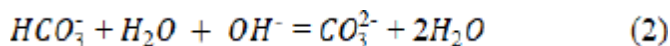
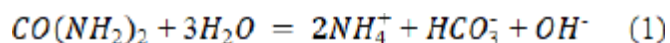
This green technique has also been utilised to remove heavy metals of wastewater. Rajasekar *et al.*,

(2021) reported that ureolytic Microbial Produced Calcite Precipitation is an efficient approach for the treatment of wastewater via removal of heavy metals, with adjustment in pH, ammonium ion is released and calcium/urea source being the only constraints that need to be overcome. MICP technique had also been employed in bioremediation of contaminated soils. Zahraa and Khitam. (2022) reported that *Bacillus Subtilis* improved the strength characteristics of lead-contaminated soil with appreciable UCS values.

Microorganisms with this potential could be extracted from numerous sources like calcareous sand as well as limestone cave soils (Gunjo and Heejung, 2016). Durojaye *et al.* (2025) screened urease-producing bacteria from various geologic sources for microbial-induced calcite precipitation. There are various factors that influence MICP, these include bacterial cytology, bacterial concentration, temperature, pH, cementation medium/solution, and some other influencing factors that affect the crystallisation and morphology of the cementing phase (Tiantian *et al.*, 2025).

MICP technique, when compared with the use of conventional stabilisation materials, has proven to be relatively less costly alternative for soil treatment, and has shown very good prospects in respect of strength enhancement and permeability reduction (Durojaye *et al.*, 2022). It is also applicable in the cementation of mining site tailings. According to Changyu *et al.* (2022), a greater strength of biocemented tailings was achievable with the use of MICP. It was proven that biocementation process is effective by the use either aerobic or facultative-anaerobic bacteria in the cementation of tailings.

The MICP method is based on urea hydrolysis by ureolytic organisms, followed by introducing calcium ions (Ca^{2+}) to the unsuitable soil, thereby forming calcium carbonate (CaCO_3). The chemical equations below explains the process of MICP:



(Rajasekar *et al.*, 2021)

This research work aimed at improving the geo-technical properties of poor laterite with *Bacillus Amyloliquefaciens*.

2. | Materials and Methods

2.1 | Sample and Sampling

Laterite sample was collected at a laterite deposit located at Soka, Oluyole local Government area, Ibadan, Oyo State along Lagos –Ibadan Expressway, Nigeria with geographical coordinates: 7° 19' 54" North and 3° 53' 37" East. The location and description of soil deposit site is shown in Figure 1

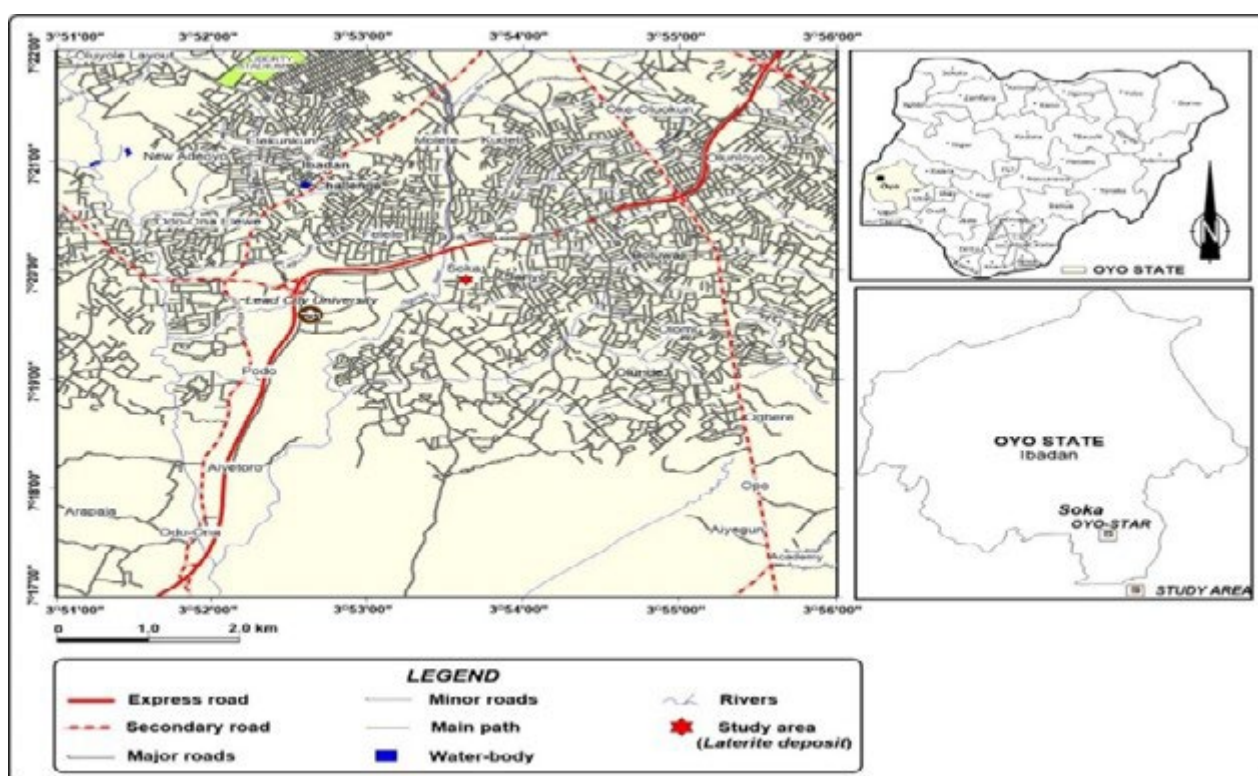


Figure 1 | Geographical Location of the sampling site

2.2 | Culture Enrichment

The collected soil samples were enriched, as to isolate urease-producing *Bacillus*; one gram of the collected soil sample was introduced into 50 mL of nutrient broth (NB) augmented with 6% urea. This was incubated under aerobic conditions (130 rpm) for 120 h at 30°C.

2.2.1 | Isolation of microorganisms from the samples

One millilitre of enriched culture samples were diluted out serially, 0.1 mL of the diluent was plated out on nutrient agar fortified with 6% urea. The plates were incubated under aerobic conditions at room temperature for about 42 h. the plates were observed for visible growth and subsequent sub-culturing was done until pure culture of the isolates were obtained.

2.3 | Culture Preservation

Preservation of selected urease-producing *Bacillus* strains, glycerol stocks were prepared, and pure bacterial cultures will allow growing in Nutrient broth at 37 °C for 16 h. Then 0.5 mL of culture broth was stored in a vial containing sterilized 500µl glycerol.

2.4 | Molecular Characterisation

Molecular characterisation of the selected urease-producing organisms was done using Molecular Evolutionary Genetic Analysis (MEGA) version 6 according to the method of Tamura *et al.*, (2013) and Mahidi, (2015).

2.4.1 | Inoculum preparation

Bacillus strains high potential for production of urease enzymes were cultivated aerobically in a medium containing 30 g/l of Tryptic Soy Broth and 20 g or 40 g of urea per litre of deionised water pH of 7.5) and then sterilised at 121°C and 15 psi for 15 min. incubation was allowed for 24 -36 h on a rotary shaker at 150 rpm and 30°C until it reached approximately OD600 (Optical Density at 600nm wavelength) according to Al Qabany *et al.* (2011). Afterward the bacterial suspension was centrifuged at 8000 rpm for 10 min. The supernatant was then decanted and the pellet was used for treating the lateritic soil samples.

2.5 | Total Viable Count (TVC)

TVC was estimated by the serial dilution method, for recovery of the inoculated *Bacillus* strain used in treatment of the soil specimens. This was carried out according to the method of Avishai *et al.*, (2014). The serial dilution was performed with the crushed and hydrolysed treated specimens into 10⁷-fold dilution factor (Wali *et al.*, 2023).

Unconfined Compression Strength: This was carried out in conformity with BS 1377-7:1990. Cylindrical specimens (50mm diameter and 100mm height) were trimmed to 38 mm diameter and 76 mm height so as to conform to the standard dimension of the compression machine. An axial load at constant strain rate (usually 1-2 per revolutions per minute) was applied until failure. Thus, unconfined compression strength was calculated as the ratio of load at failure per cross-sectional area of the sample.

$$\sigma_{\text{UCS}} = \frac{P_{\text{max}}}{A_0} \quad (4)$$

Where:

σ_{UCS} is Unconfined Compressive Strength (in units of stress, e.g., kPa or psi)

P_{max} is Maximum load applied (in units of force, e.g., Newtons or pounds)

A_0 is Initial cross-sectional area of the specimen (in square units, e.g., m² or in²)

Permeability: The water flow rate via the treated samples was measured in accordance with BS 1377-5:1990 using falling head method, with a permeameter mould with capacity of 1000 litres ,12.73 millimeter height and diameter of 100 millimeter.

Cation Exchange Capacity: This was determined in accordance with BS7755-312:1996. For classification, cation exchange capacity (CEC) of soils is usually measured at a standard pH value of 7, which implies neutrality. CEC of soils is the varying chemical characteristic whose values depend on clay minerals content, organic matters content as well as pH value for the soil matrix (Vladimir, 2023).

2.5.1 | Calcite Content Determination

The values of calcite formed during MICP process in the stabilized samples were determined by using acid-washing technique described in ASTM D4373.

The principle is hinged on the reaction between calcite (CaCO_3) and a strong acid (HCl) which results in the formation of CaCl_2 , CO_2 and H_2O . The treated sample was pulverised, sieved through sieve of less than 2 mm aperture and weighed as the initial weight, W_i . 1M of HCl was introduced (dropwise) into 1-2 g of the sample in a beaker until bubbling stopped i.e. evolution of CO_2 . The mixture was then rinsed with distilled water, dried at 105°C . The residue was then cooled in a desiccator and reweighed, W_f . Weight loss due to the evolution of CO_2 is used to calculate the amount of calcite. This is the final weight of the sample minus the initial weight

Calcite Content (Weight loss) =

$$W_i - W_f \qquad (5)$$

2.6 | X-ray Diffraction

The mineralogical test was performed on the natural lateritic soil using X-ray powder diffraction so as to identify the soil mineral constituents. This was done in conformity with the procedure of Jozanikohan and Moshiri (2016).

2.7 | Scanning Electron Microscopy

Two dimensional images of the treated lateritic soil were observed with Scanning Electron Microscope. A 12mm steel disk with a carbon tape cover was used to collect samples. A gold coating was also applied on the disk by a gold sputter system (Denton, Vacuum Desk IV) with argon air. The images were then viewed on the disk and produced under the Scanning electron microscopic instrument.

3. | Results and Discussion

3.1 | Physical Characteristics of Soil Sample

The soil sample was characterised and classified, following the British Standard Code (BS 1377-

1990) and AASHTO system of soil classification, as an A-2-4(7) laterite. Natural moisture content and organic content of the soil are 11.63% and 2.59% respectively. The soil properties are summarised in Table 1.

Table 1 | Basic Properties of the Natural Soil

Basic Property	Value/Index
Moisture Content (%)	11.63
Organic Content (%)	2.59
Silt Content (%)	4.00
Liquid Limit (%)	48.00
Plastic Limit (%)	25.00
Plasticity Index (%)	23.00
Specific Gravity	2.61
Optimum Moisture Content (%)	13.0
Maximum Dry Density (kg/m^3)	1914.0
Average pH	4.89
Percentage Passing Sieve No.200	28.20

The physical characteristics of the natural sample were listed in Table 1. The laterite sample was characterised, in accordance with the American Association of State Highway and Transportation Officials AASHTO, and Unified Soil Classification Systems USCS, as A-2-7(3) and Clayey Sand SC soil respectively. The soil has plasticity index which is greater than 17, which suggests a highly plastic soil (Ramamurthy and Sitharam, 2014). The coefficient of curvature (C_c) of the sample was 1.07, which falls between 1 and 3 and a percentage fine of 28.20%; these characterise the soil as well-graded (Figure 2).

3.2 | Molecular Phylogenetic Analysis

Molecular Evolution Genetics Analysis (MEGA) VERSION 6.0 was used to construct the phylogenetic relationship between the isolates Figure 3. The phylogenetic analysis of the isolate UC10 was joined to *Bacillus Amyloliquefaciens* (KM014570.2:249-

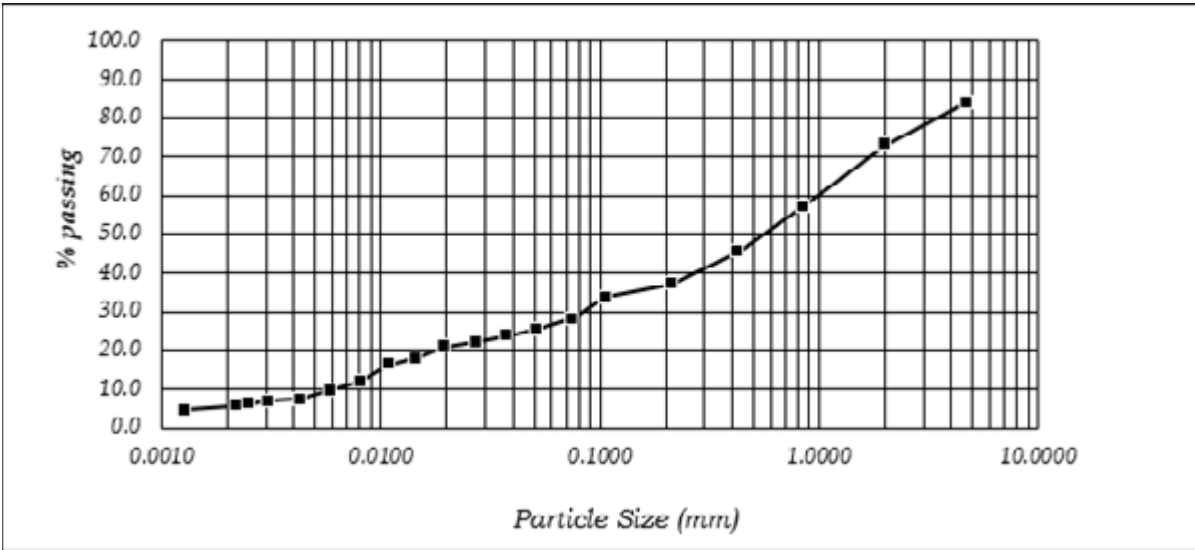


Figure 2 | Sieve Analysis Curve of the natural soil

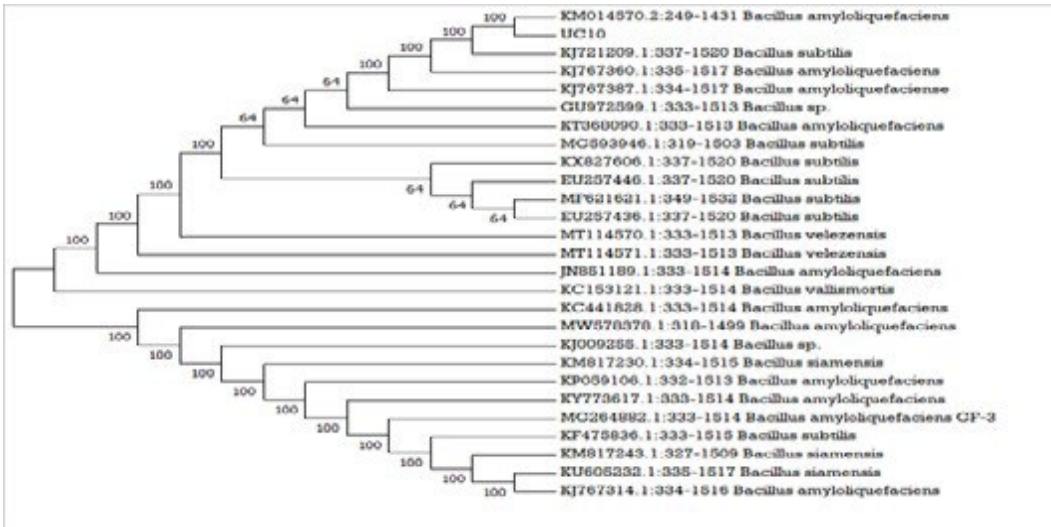


Figure 3 | Phylogenetic relationship of urease-producing Bacillus strains isolated from various sources

1431) by using the neighbor-joining method that is based on maximum composite likelihood.

3.3 | Total Viable Bacterial Counts

Figure 4 shows a continuously increasing bacterial growth occurring in the first consecutive 40 days of curing age after which a constant growth was observed between 40- and 60-days curing age. This is consonance with the findings of (Montville *et al.*, 2003).

3.4 | Average Values of Unconfined Compression Strength

A substantial boost strength development in the treated soil specimen was recorded in the first ten days of bacterial treatment from 285.63 kPa to 315.03 kPa (Figure 5). Also, there was a steady increase over the next fifty days of treatment. A 335.15 kPa peak strenght was achieved at a curing age of 60 days. Thus, UCS values increase with curing age. This agrees with the study outcome of (Ignatius *et al.*, 2022). This depicts an appreciable percentage increase in strength of 17.33%.

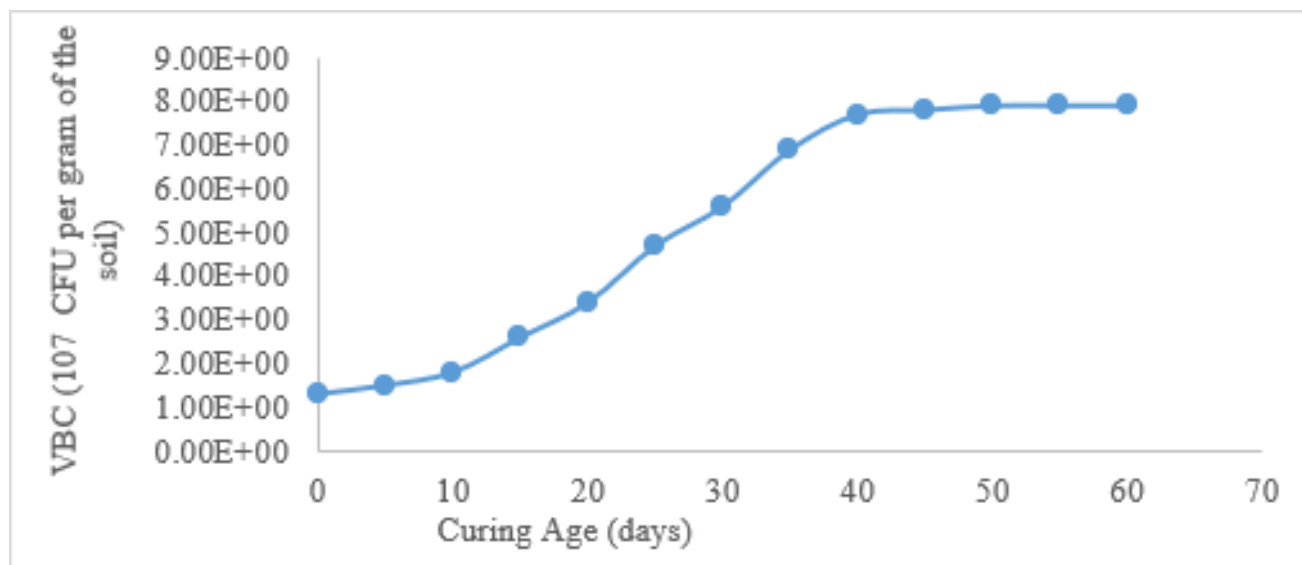


Figure 4 | Variation of Total Viable Bacteria Counts with the curing ages of treated samples

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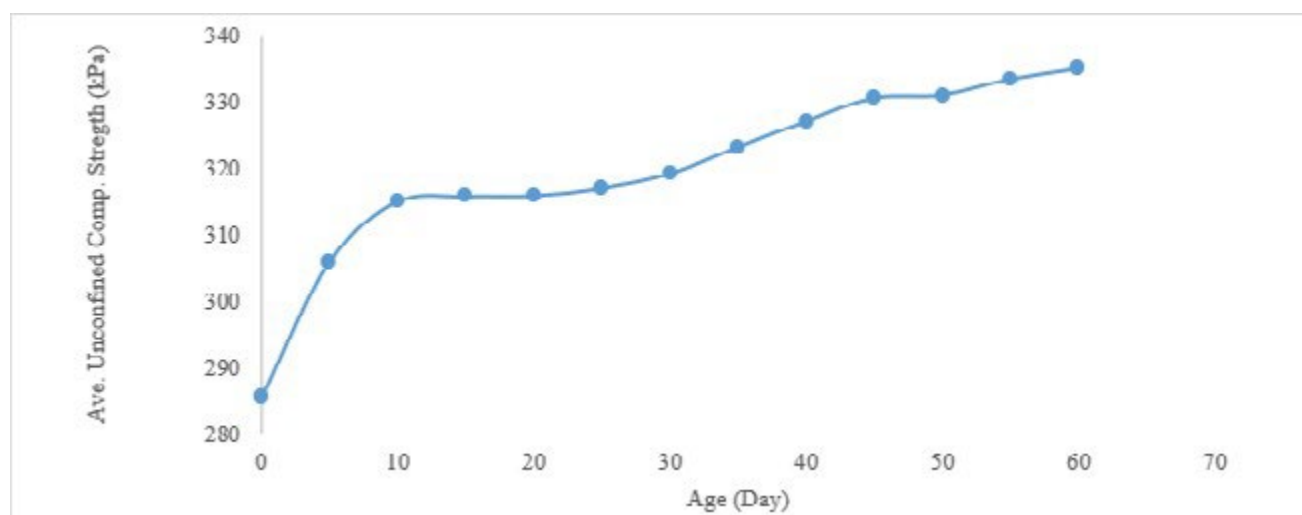


Figure 5 | Average Unconfined Compression strength against Curing Age of treated sample

3.6 | Average Coefficients of Permeability

There was a steep decrease in the coefficients of permeability over the first five curing days from 0.0000061 cm/s to 0.00000297 cm/s. This is

51.31% reduction in the permeability coefficient of the bio-treated soil. Meanwhile, a gentle increase was observed between the ages 5 and 10 days of treatment from 2.97×10^{-6} cm/s to 2.93×10^{-6} cm/s. The highest reduction in the coefficient of permeability

4.36×10^{-7} cm/s was recorded at the curing age of 60 days Figure 6. Maximum percentage reduction in the permeability coefficient, when compared with

that of the control specimen was 92.85%. This result conforms with the research outcomes of (Anthony *et al.*, 2017)

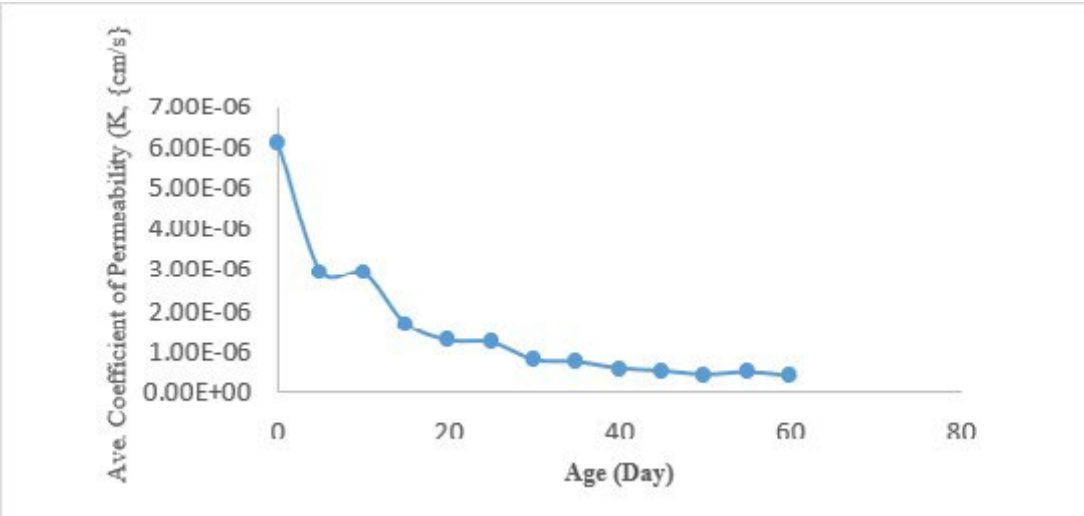


Figure 6 | Variation of Average Coefficients of permeability with Curing Ages of treated Samples

3.7 | Average Values of Cation Exchange Capacity CEC

The average cation exchange capacity of the untreated natural lateritic soil was found to be 16.77 milliequivalent/100g (meq/100g). The cation exchange capacity CEC of the treated specimen steadily increased over the first ten days from 16.77

meq/100g to 33.84 meq/100g. Thereafter, a marginal progression in the value of CEC was observed after the age of 10 days through curing age 60 days Figure 7. Thus the CEC of the treated soil increases with the curing age. Besides, both UCS increased with reduction in coefficient of permeability of the treated soil Figure 5 and Figure 6, as CEC increased with age.

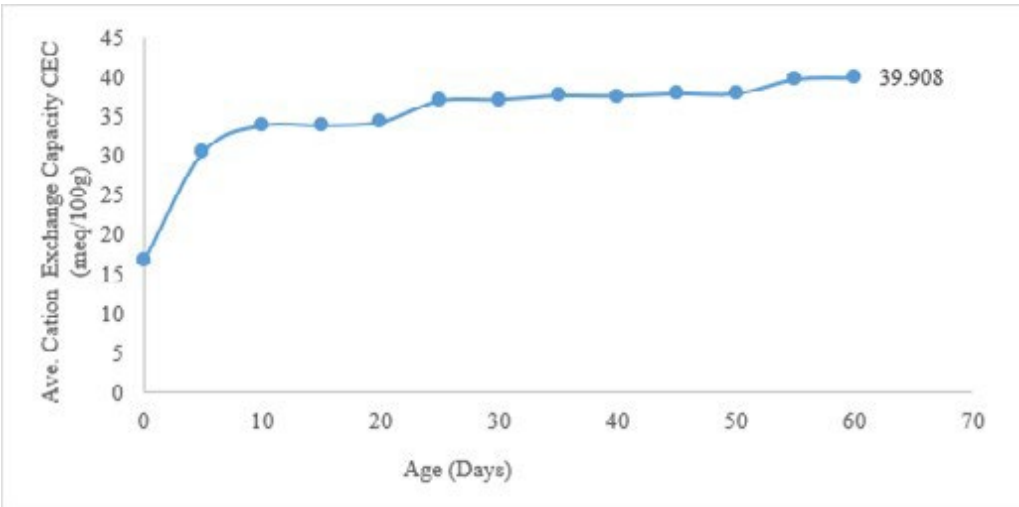


Figure 7 | Variation of Average Cation Exchange Capacity with Curing Age of treated Samples

3.8 | Average Calcite Content

The content of calcium carbonate produced within soil pores continuously increased from the curing age of 5days through 60days.The peak amount of

calcite 31.89 g was formed at age 60 days as shown in Figure 8. The calcite content in the treated samples kept rising with the curing age. This is a similar research outcome with (Gunjo *et at.*, 2016)

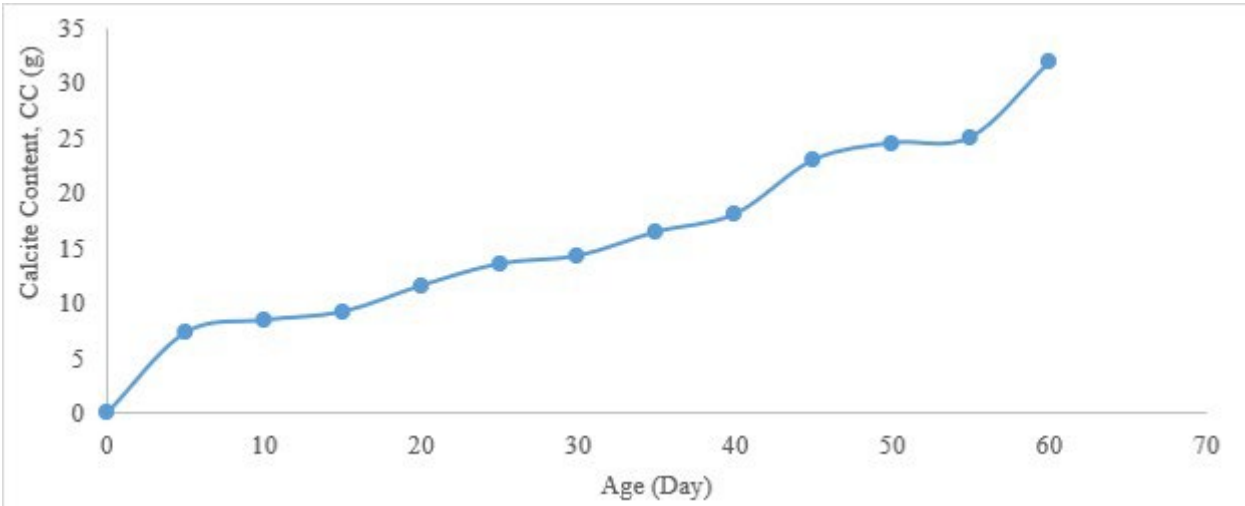


Figure 8 | Variation of Calcite Content with Curing Age of Treated Samples

3.9 | X-ray Diffraction

The mineralogical analysis conducted on the natural lateritic soil indicated that the dominant minerals in the soil are Kaoline, Quartz, Chlorite, Orthoclave,

and Muscovite Figure 9. The highest peak intensity count was obtained in Quartz at Bragg’s angle (2 Theta) of 26.705⁰ and interplanar spacing d-value of 3.3354 angstorms. This is in consonance with the findings of Durojaye *et al.* (2022).

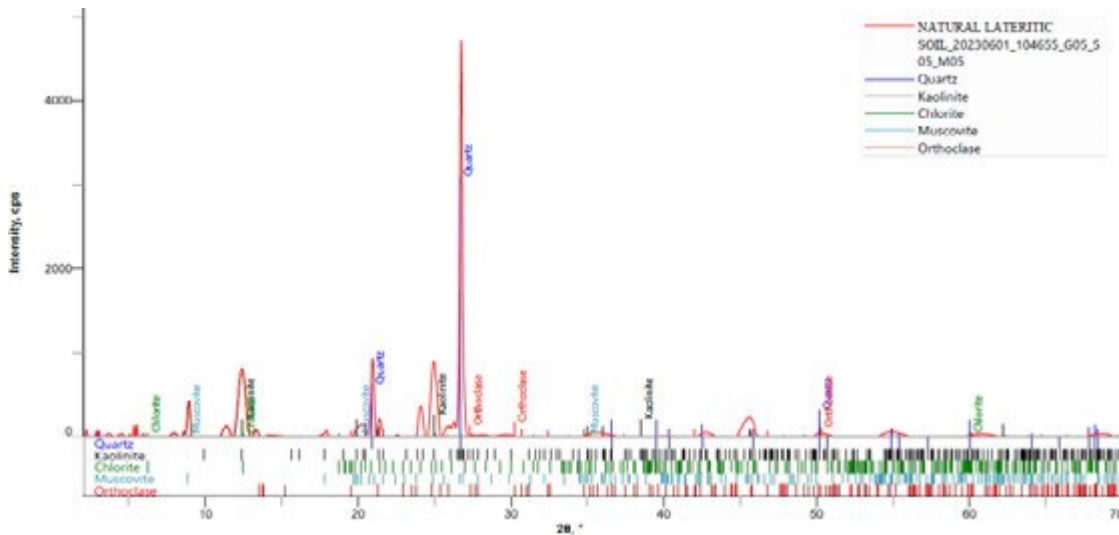


Figure 9 | X-ray Diffractograph of Natural Lateritic Soil

Scanning Electron Microscopy: The Micrograph of both the untreated and treated soil sample with *Bacillus Amyloliquefaciens* are shown as plate 1a-d. The photomicrographs of the treated specimens show fully packed the crystal structure of calcium

carbonate clogging and cementing pores of the soil mass. This is in tandem with the findings of (Osinubi *et al.*, 2018). This is responsible for increased soil strength and reduction in permeability.

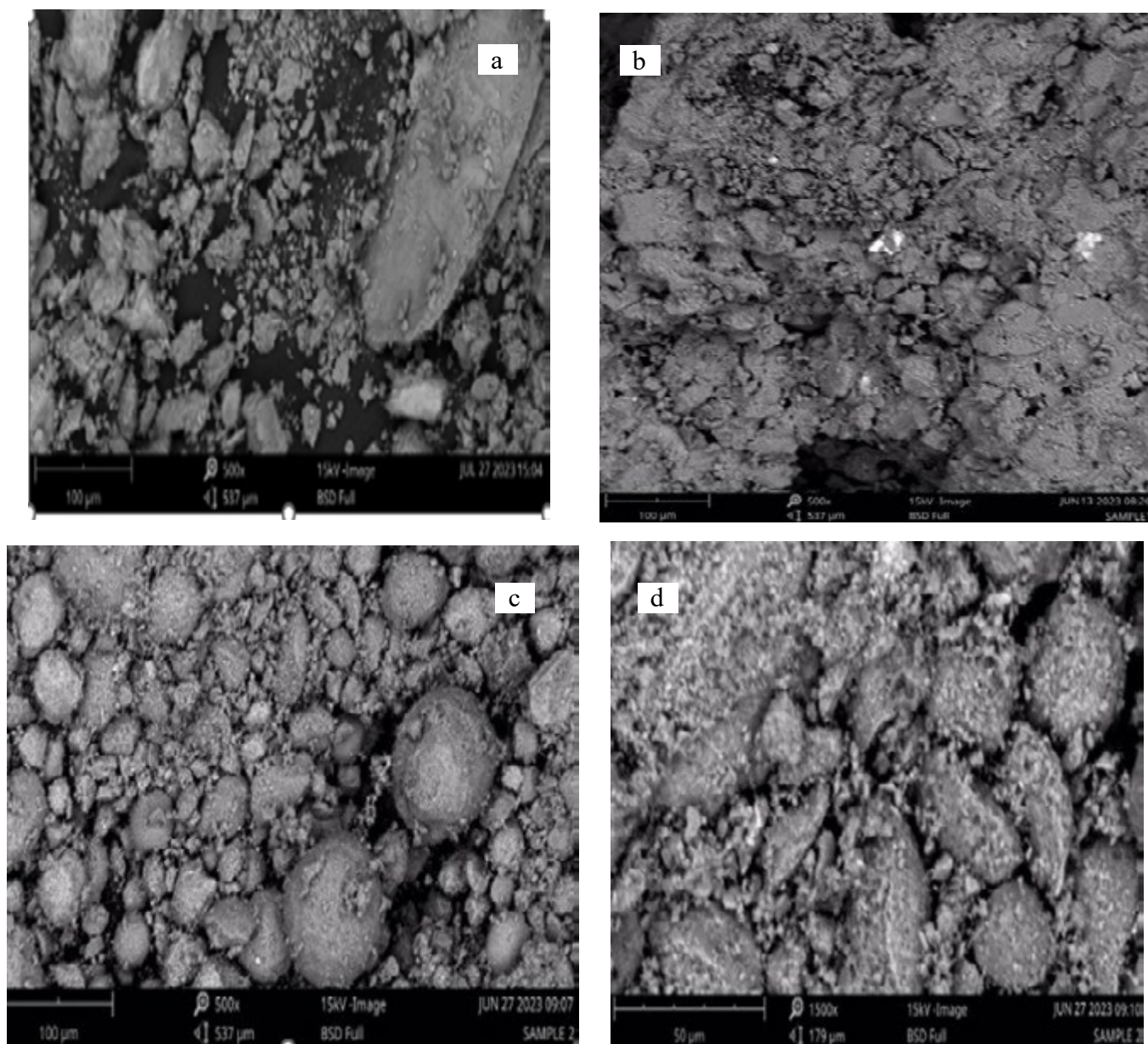


Plate 1(a)-(d) | SEM Images of the control(untreated) natural lateritic soil sample(a), treated soil samples at 20,40- and 60-days curing age;(b), (c), and (d) respectively.

3.10 | Statistical Models Development and Validation

To quantitatively evaluate the relationships between biological, chemical and engineering parameters governing the behaviour of the microbial-treated soil,

multiple linear regression analysis was employed. Two separate regression models were developed in order to independently examine the determinants of both mechanical and hydraulic performance of the lateritic soil. The experimental dataset consisted

of 13 observations obtained from laboratory tests on the biologically treated soil specimens. The predictor variables used in the regression analysis were: Curing Age A (days), Viable Bacterial Counts VBC ($\times 10^7$ CFU/g), Cation Exchange Capacity CEC (meq/100g), Calcite Content CC (g), Permeability K (cm/s) and Unconfined Compression Strength U (kPa). Model estimation was carried out using the Ordinary Least Squares (OLS) Method.

Model 1: Strength Prediction Model

In the first model, Unconfined Compression Strength (UCS) was considered as the dependent variable, while Curing Age(A), Total Viable Count (TVC), Cation Exchange Capacity (CEC) and Permeability (K) were included as predictors. The estimated regression equation obtained from the OLS analysis is:

$$U = 261.957 + 0.1754A - 0.0560TBC + 1.3349CEC + 0.3569CC + 3.052 \times 10^5 K \quad (6)$$

The model captures the combined influence of curing age, bacterial activity, cation exchange capacity, calcite precipitation, and hydraulic conductivity on the UCS of the biologically treated soil sample. The model explains approximately 97.85% (being R^2 value) of the variability in compressive strength, indicating excellent predictive capability. The high adjusted R^2 of 96.31% confirms that the included predictors contribute meaningfully to the model without overfitting to the small experimental dataset.

Model 2: Permeability Prediction Model

The second regression model was developed to evaluate the determinants of Permeability (K). Permeability was treated as the dependent variable, while curing Age A, Total Viable Counts TVC, Cation Exchange Capacity CEC, Calcite Content CC and compressive strength U were used as predictors. The estimated regression equation is:

$$\begin{aligned} &= 8.044 \times 10^{-9} + 1.333 \times 10^{-9} A - 1.944 \times 10^{-7} T1 \\ &- 2.428 \times 10^{-7} CEC + 2.548 \times 10^{-8} CC \\ &+ 8.051 \times 10^{-5} U \end{aligned} \quad (7)$$

The permeability model explains 96.10% (being R^2 value for model 2) of the variation in hydraulic conductivity, demonstrating strong predictive reliability. The adjusted R^2 of 93.31% confirms that the model is not over-fitted and that the five predictors collectively provide a robust description of permeability behaviour.

3.11 | Model Validation and Reliability Assessment

Model adequacy was evaluated using several statistical diagnostics commonly recommended for regression validation in engineering applications. First, the coefficients of determination (R^2) for both models exceed 96%, indicating that the models capture the overwhelming majority of variability in the experimental dataset. The adjusted R^2 values (96.31% and 93.31%, respectively) remain consistently high, confirming that the five predictors contribute meaningfully to each model without overfitting. Also, the F-test for overall regression significance yields very small probability values in both models ($p < 0.001$), demonstrating that the predictor variables collectively explain a statistically significant portion of the response variation. Besides, the Durbin-Watson statistic was used to assess residual autocorrelation. Model 2 (permeability) produces a value of 2.32, indicating no significant serial correlation. Model 1 (strength) produces a value of 1.05, suggesting mild positive autocorrelation, which is common in small sequential experiment datasets and does not substantially compromise inference given the high overall F-significance. Moreover, the signs and physical magnitudes of the regression are broadly consistent with accepted soil mechanics principles. CEC consistently shows a strong positive influence

on strength and a strong negative influence on permeability, which aligns with the known role of surface charge interactions in controlling both cementation and pore structure in stabilised soils.

4. | Conclusion

The outcome of this study depicts that *Bacillus Amyloliquefaciens* has the ability to enhance the strength of poor laterite soil as well as reduce its permeability. Also, bio-treatment of soil using the microbe isolated from such soil (a phenomenon called bio-stimulation) is achievable. *Bacillus Amyloliquefaciens* was isolated from the poor lateritic soil was eventually employed to stabilise

it. It was deduced from the regression analysis models that Cation Exchange Capacity CEC of the biotreated soil aided calcite precipitation, and had a strong positive influence on the strength and a negative influence on its permeability; increase in the CEC results into increased strength and decrease in permeability of the soil matrix. The effect of CEC aligns with the known role of surface charge interaction in controlling both cementation and pore structure in stabilised soil. However, the strength development and reduced coefficients of permeability in the treated soil slightly depend on total viable bacterial count TVC, and largely on CEC, Calcite content and curing age.

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