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Effect of curing procedure on strength development of microbial induced calcite precipitate treated lateritic soil

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ABSTRACT

The study investigated the effect of different curing procedures (i.e., wax-cured, oven-cured at 30^o C and air-cured at ambient temperature of 25 ± 2^o C) on the strength development of compacted lateritic soil treated with *Bacillus thuringiensis* (Bt). Five different Bt suspension densities (i.e., 0, 1.5 x 10^8, 6.0 x 10^8, 12 x 10^8, 18 x 10^8 and 24 x 10^8 cells/ml) were pre-mixed in the soil with four stepwise concentrations of cementation solutions (C_s) (i.e., 0.25, 0.5, 0.75 and 1 M) in the ratio of 50 % Bt : 50 % C_s of the soil optimum moisture content (OMC) and compacted using British Standard light (BSL), West African Standard (WAS) and British Standard heavy (BSH) energies. The experimental results indicate that the strength of specimens are in the order: curing at ambient condition > oven-curing > wax-curing. Peak strength value of 1,239.38 kN/m^2 for specimens compacted with BSL energy and air-cured at ambient condition for treatment with mix ratio of 24 x 10^8 cells/ml (Bt) : 1 M (C_s). Also, peak values of 524.61 kN/m^2 for mix ratios 24 x 10^8 cells/ml : 1 M and 1,208.75 kN/m^2 for 24 x 10^8 cells/ml : 0.75 M were recorded for specimens wax-cured and oven-cured, respectively. Higher strength values were recorded at higher compactive effort regardless of the curing method used. Microanalysis results showed that calcite crystals precipitated bridged the soil particles. Based on the findings recorded, the lateritic soil treated with *Bacillus thuringiensis* (24 x10^8 cells/ml): 0.75 M (C_s) mix ratio and exposed to ambient conditions could be used to improve the strength of lateritic soil.

Keywords: Bacillus thuringiensis, Curing method, Lateritic soil, Microbial induced calcite precipitate, Strength

1 INTRODUCTION

Investigations into wider utilization of the millions and millions of available soil microbes capable of inducing important minerals of interest (e.g., calcite) in the soil to upgrade the soil engineering behaviour suitable for various geo-environmental engineering applications have been on for more than a decade (Mitchell and Santamarina 2005; Osinubi *et al*., 2019a, b). The many reported studies in this area have established a process (microbial induced calcite precipitation- MICP) through which excellent and reliable outcomes were achieved (Murtala and Khairul, 2017; Jiang *et al.,* 2020; Liu *et al.,* 2021; Sharma *et al*., 2021; Yohanna *et al*., 2022). The process often occurs naturally when favourable conditions exist in binding sand aggregate into sandstone, however, the MICP technique could be utilised and adopted as soil improvement process without serious environmental concerns (Konhauser, 2009; Murtala and Khairul, 2017). Soil improvement through bio-mediation is therefore actualised by biochemical reaction between the microorganisms and the cementing agent in the soil matrix which changes soil properties such as hydraulic conductivity, stiffness and strength for the better. Urea hydrolysis (an irreversible reaction between urea and water which results to the formation of carbonate and ammonium ions thereby increasing the alkalinity of the soil pore fluids thus inducing calcite precipitation) remains the most popular adopted soil bio-mediation method primarily due to its ease of relative controllability and

capability of producing high amount of calcite in short time frame (Animesh and Ramkrishnan, 2016; Cheng *et al.,* 2014; Wang *et al*., 2022).

Although this emerging soil improvement technique is gradually gaining ground in terms of acceptability, it does come with few challenges which ought to be overcome for optimal applications in real life engineering situations. One key challenge is the complexity of the MICP biogeochemical processes involved, making it difficult to control and predict accurately the performance of soil treated using MICP technique at the macro scale (Mahawish *et al*., 2018). However, the technique has some edge over conventional cement in terms of environment friendliness. Also, the bio-fluids used in MICP are less viscos than cement slurry and can easily penetrate larger distances and also enter into smaller fissures (Mahawish *et al*., 2018; Wang *et al*., 2022).

In this study, the strength behaviour of compacted lateritic soil treated with varying mix ratios of *Bacillus thuringiensis* suspension density (i.e., 0, 1.5 x 10^8, 6.0 x 10^8, 12 x 10^8, 18 x 10^8 and 24 x 10^8 cells/ml) and cementation solution (i.e., 0.25, 0.5, 0.75 and 1 M) was evaluated. The treated soil was subjected to different curing conditions to assess their strength development over a period of seven days for possible mitigation of soil erosion.

2 MATERIALS AND METHODS

2.1 Materials

2.1.1 Soil sample

The lateritic soil was obtained from Abagana (6^o 12’ 15” N latitude and 7^o 0’ 40” E longitude), in Anambra State, Nigeria. Disturbed method of sampling was used to collect samples at depths between 0.5 m and 3 m.

2.1.2 Microorganism

Bacillus thuringiensis (Bt) isolated from the soil sample was cultured in the laboratory through biochemical confirmatory tests consistent with standard methods for identifying and characterising Bt.

2.1.3 Cementation solution

Four different concentrations (i.e., 0.25, 0.5, 0.75 and 1 M) of cementation solution (C_s) were prepared and used during experimentation. The chemical components that make up the cementation solution include: Nutrient broth, urea, ammonium chloride, sodium bicarbonate and calcium chloride. Fixed quantities of nutrient broth (3 g/l), ammonium chloride (10 g/l) and sodium bicarbonate (2.12 g/l) and equimolar of urea and calcium chloride based on previous studies (Stocks-Fisher *et al.,* 1999; Animesh and Ramkrishnan, 2016; Murtala and Khairul*,* 2017) were combined to form the different concentrations of solution used (see Table 1).

Table 1. *Mass concentration of cementation reagents per litre*

Concentrations (M)	**0.25**	**0.5**	**0.75**	**1**
Nutrient broth (g/l)	3	3	3	3
Ammonium chloride (g/l) (NH_4Cl)	10	10	10	10
Urea ($CO(NH_2)_2$) (g/l)	15.05	30.03	45.05	60.06
Sodium bicarbonate (g/l) ($NaHCO_3$)	2.12	2.12	2.12	2.12
Calcium chloride (g/l) ($CaCl_2$)	27.75	55.49	83.24	110.98

2.2 Methods

2.2.1 Bacterial culture and preservation

Bacillus thuringiensis (Bt) was isolated and cultured using the selective method reported by Travers *et al.* (1987) using sodium acetate with minor modifications. 10 g of the sourced soil was mixed with distilled water (90 ml) and the mixture was then incubated on a 250 rpm rotary shaker at 28^{o} C for a period of 45 minutes. Broth culture (2 ml) was mixed with 20 ml sterilized nutrient broth (Luria-Bertani) which contains yeast extract (5 g/l), Tryptone (10 g/l) and NaCl (5 g/l). Then, sodium acetate (0.25 molar concentration and pH value of 6.8) was added to buffer the resulting culture in Erlenmeyer flask (50 ml) and incubated at 28^{o} C on a 250 rpm rotary shaker for 4 hours. 5 ml aliquots were taken from each culture after incubation and placed in hot water bath set at temperature of 80^{o} C for 3 minutes. Finally, 0.1 ml was dispersed on nutrient agar (Luria-Bertani) (15 g/l agar, 10 g/l Tryptone, 5 g/l NaCl and 5 g/l yeast extract). This was then placed in the incubator for 24 hours at 28^{o} C.

Typical Bt colonies in terms of morphology (i.e., flat elevation, wavy margin, creamy white, dry and circular appearance on LB agar) were collected, sterilized and sub-cultured on T3 agar composed of 15 g/l agar, 2 g/l Peptone, 0.05 M Sodium phosphate, 1.5 g/l yeast extract, 0.005 g/l $MnCl_2$ and 3 g/l Tryptone. The pure cultures were then stored at 4^{o} C for future use.

2.2.2 Index properties

Common soil tests such as specific gravity, sieve analysis, natural moisture content, consistency limits and soil classification were carried out following British Standard (BS) 1377 (1990) protocols for evaluating natural soils and BS 1924 (1990) protocols for evaluating stabilised soils.

2.2.3 Compaction

Compaction of specimens was done using three energies (i.e., British Standard light, BSL, West African Standard, WAS and British Standard heavy, BSH) as per procedures presented in British Standard (BS) 1377 (1990) to establish moisture – density relationships.

2.2.4 Preparation of specimens

Bacterial solution was initially added to 3000 g of soil which passed through BS No. 4 sieve (4.76 mm opening) and thoroughly mixed. Each of the Bt suspension densities considered (i.e., 0, 1.5 x 10^8, 6.0 x 10^8, 12 x 10^8, 18 x 10^8 and 24 x 10^8 cells/ml equivalent to 0, 0.5, 2.0, 4.0, 6.0 and 8.0 McFarland Standards (MFS), respectively) were added independently to the soil at 50 % of optimum moisture content (OMC) and placed in sealed polythene bags for a period of six (6) hours for proper hydration and attachment of microorganisms to soil particles. At the end of each bacterial application and retention period, four varying concentrations of cementation solutions (C_s) (i.e., 0.25, 0.5, 0.75 and 1 M) were also independently added at 50 % relative of optimum and thoroughly mixed before compaction.

2.2.5 Unconfined compressive strength

The compacted soil specimens were extruded and trimmed to height to diameter ratio of 2:1 before being cured using three different procedures (i.e., wax-cured, oven-cured at 30^{o} C and direct exposure to ambient temperature) for a period of seven days. Thereafter it was subjected to unconfined compression strength test until failure on a machine operating at controlled rate of 0.02 %/min. strain (Head, 1994). The average value of three tested specimens of each mixture was recorded as the unconfined compressive strength (UCS) value.

2.2.6 Microstructural analysis

The microstructure of the optimal bio-treated specimen was studied using scanning electron microscopy (SEM).

3 RESULTS AND DISCUSSION

3.1 Index properties

The index properties of the natural lateritic soil are summarised in Table 2. The laboratory results show that the natural soil is an A – 2 – 6 (2) or SC soil in AASHTO (1986) and USCS (ASTM D 2487-06) soil classification systems, respectively. The distribution of the natural soil particle sizes is as presented in Figure 1.

Table 2. *Physical properties of the natural lateritic soil*

Property	Quantity
Natural moisture content (%)	19.6
Specific gravity	2.67
Percentage passing No. 200 sieve	33.2
Liquid limit (%)	36.0
Plastic limit (%)	17.1
Plasticity index (%)	18.9
Linear shrinkage (%)	7.7
USCS	SC
AASHTO classification	A – 2 – 6 (2)
Dominant clay mineral	Kaolinite
Colour	Reddish-brown

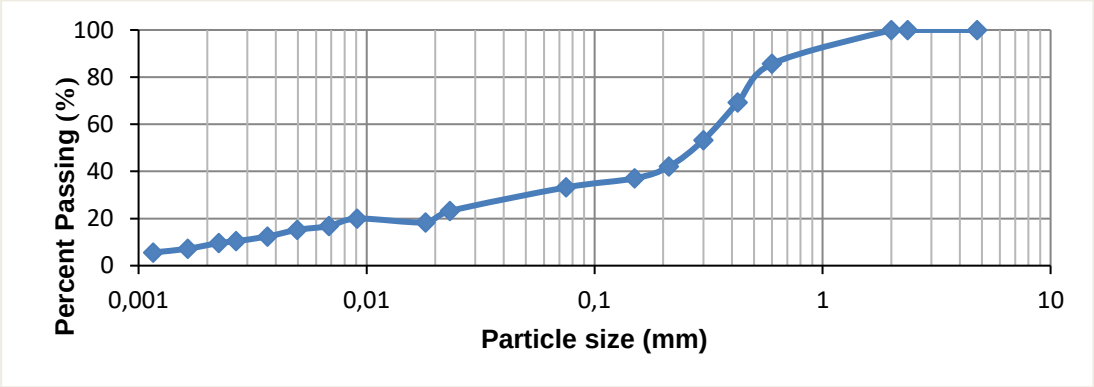


Figure 1. Particle size distribution curve of the natural lateritic soil

3.2 Unconfined compressive strength

The unconfined compressive strength (UCS) values were determined for specimens compacted with three different energies (i.e., BSL, WAS and BSH) and subjected to three different curing procedures (i.e., wax-cured, oven-cured at 30^{o} C and air-cured at ambient temperature) (see Plates I and II).

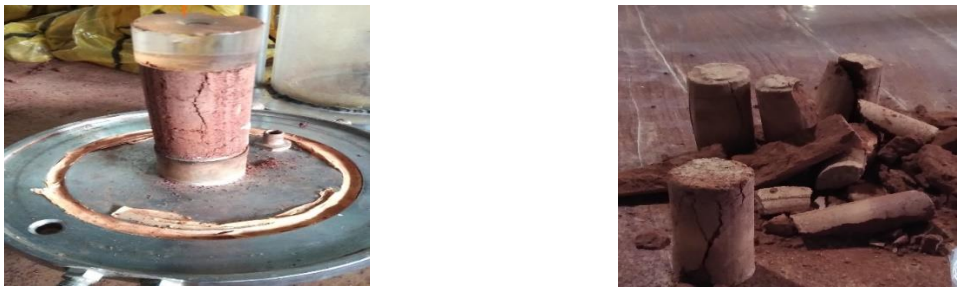


Wax-cured and air-cured specimens



Oven-curing at 30^{o} C

Plate I. *Different curing methods*



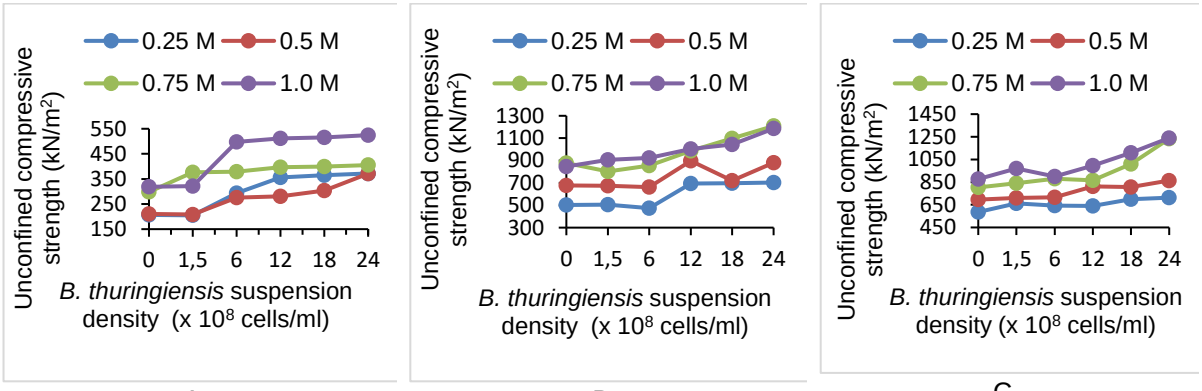
Failed specimen Crushed specimens

Plate II. *Unconfined compressive strength test specimens*

3.2.1 Effect of curing method and bacterial population on UCS

The UCS values of the compacted virgin soil using BSL energy were 185.16, 463.58 and 477.09 kN/m^2 for specimen wax-cured, oven cured at 30^o C and air-cured at ambient temperature, respectively. For WAS and BSH compaction energies, the UCS values of the virgin soil were 484.96, 712.45 and 737.69 kN/m^2 as well as 762.51, 803.08 and 839.69 kN/m^2, respectively, when specimens were wax-cured, oven-cured at 30^o C and air-cured at ambient temperature, respectively, for seven (7) days.

The 7-day UCS values increased with higher bio-treatment using different mix ratios regardless of the curing method adopted (see Figures 2-4). Peak UCS value of 524.61 kN/m^2 was obtained for 24 x 10^8 cells/ml : 1 M mix ratio when specimens were wax-cured, 1208.75 kN/m^2 for 24 x 10^8 cells/ml : 0.75 M mix ratio when specimens were oven-cured at 30^o C and 1239.38 kN/m^2 for 24 x 10^8 cells/ml : 1 M mix ratio when specimens were air-cured at ambient temperature. The results also revealed that the bio-treated specimens gained more strength when air-cured at ambient temperature and this was closely followed by specimens oven-cured at 30^o C, while wax-cured specimens recorded the least gain in strength. The observed trend was consistent for the three energies used in the study (see Figures 2-4).



A B C

Figure 2: *Variation of unconfined compressive strength (BSL compaction) of lateritic soil with Bacillus thuringiensis suspension density for the different curing methods: (A) Wax-cured (B) Oven-cured at 30^o C (C) Air-cured at ambient temperature.*

The increase in UCS values at higher Bt suspension density may be traced to adequate amount of microorganism in the mixture which hydrolysed the urea component of the C_s thereby precipitating calcite crystals in the mixture that enhanced the crushing strength (Osinubi *et al*., 2019a; Wang *et al*., 2022). It implies that increase in UCS values signified increased bacterial activity when bacterial population in the mixture increased (Murtala and Khairul, 2017). The higher UCS values for the air-cured specimens could be due to the ability of the microorganism to operate optimally at the natural prevailing environmental conditions. When the natural environment is altered, the microbes tend to adjust to the newly created environment, thus reducing their hydrolysing capability which results in lesser quantities of calcite formed in the mixture and consequently recording lesser UCS values. These results are consistent with the findings reported by Tsukamoto *et al*. (2013), Cheng *et al*. (2014), Sharma *et al*. (2021) and Wang *et al*. (2022).

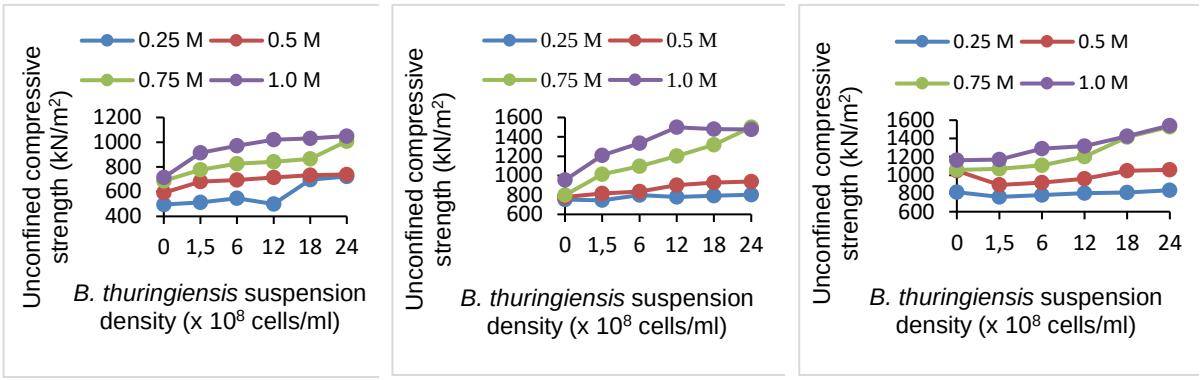


Figure 3: *Variation of unconfined compressive strength (WAS compaction) of lateritic soil with Bacillus thuringiensis suspension density for the different curing methods: (A) Wax-cured (B) Oven-cured at 30^{o} C (C) Air-cured at ambient temperature*.

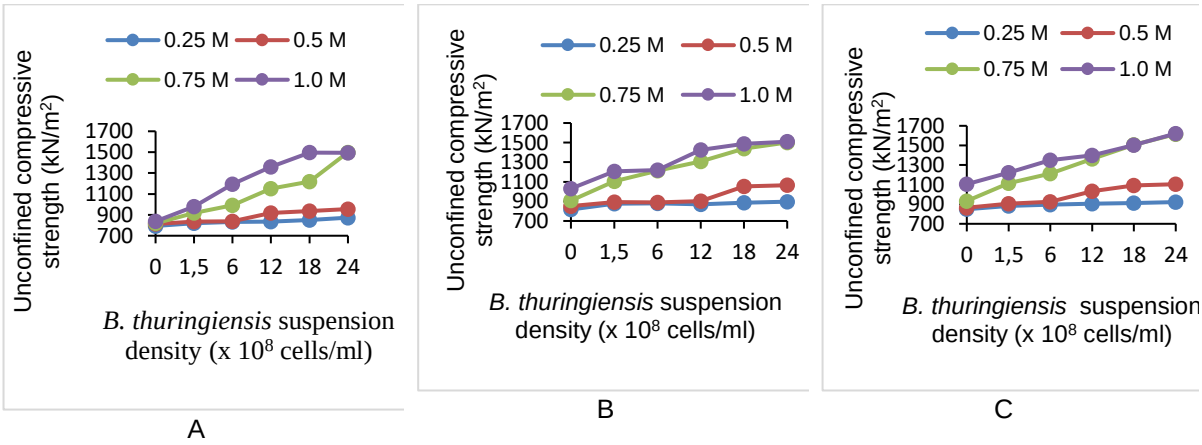


Figure 4: *Variation of unconfined compressive strength (BSH compaction) of lateritic soil with Bacillus thuringiensis suspension density for the different curing methods: (A) Wax-cured (B) Oven-cured at 30^{O} C (C) Air-cured at ambient temperature.*

3.2.2 Effect of compaction energy and cementation solution concentrations on UCS

The change in UCS of lateritic soil - *Bt* - C_s mixtures with compactive efforts considered is presented in Figures 5 - 10. The results revealed increased UCS values as the compaction energy increased. The observed trend was not affected by the curing methods considered thus underscoring the fact that compaction energy affects the strength values. Therefore, it is desirable to use highest compactive effort to achieve more reliable strength in the field (Yohanna *et al*., 2022). The UCS values recorded for the compactive efforts considered were ranked in decreasing order as BSH > WAS > BSL. Also, it was observed that UCS values increased with increase in C_s molar concentration due to increase in urease activity in the mixture that resulted in the formation of calcite crystals responsible for binding or clogging soil particles thereby enhancing strength (Murtala *et al*., 2016).

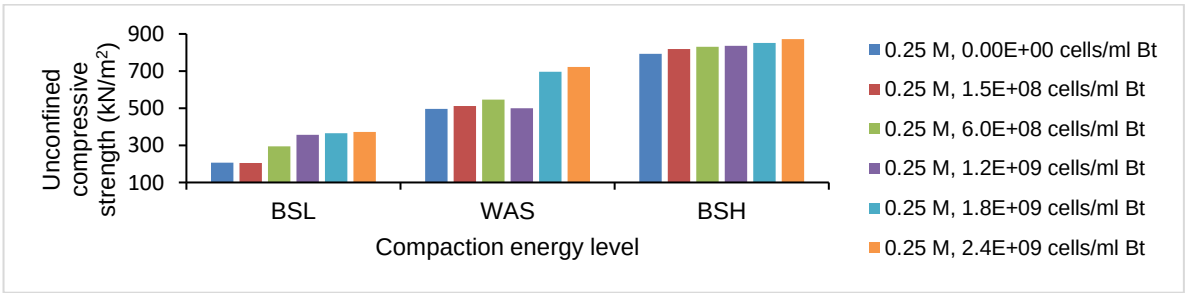


Figure 5: *Variation of unconfined compressive strength of lateritic soil – 0.25 M C_s – Bacillus thuringiensis mixtures with compaction energy (Wax-cured).*

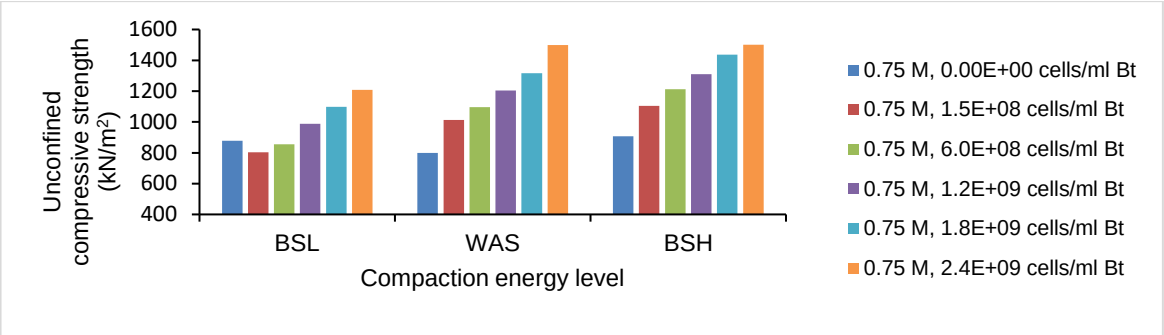


Figure 6: *Variation of unconfined compressive strength of lateritic soil – 0.75 M C_s – Bacillus thuringiensis mixtures with compaction energy (Oven-cured at 30° C).*

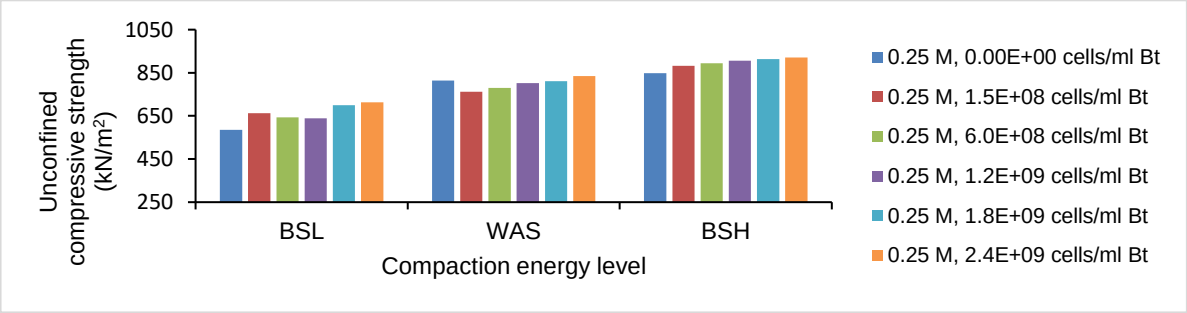


Figure 7: *Variation of unconfined compressive strength of lateritic soil – 0.25 M C_s – Bacillus thuringiensis mixtures with compaction energy (Air-cured at ambient temperature).*

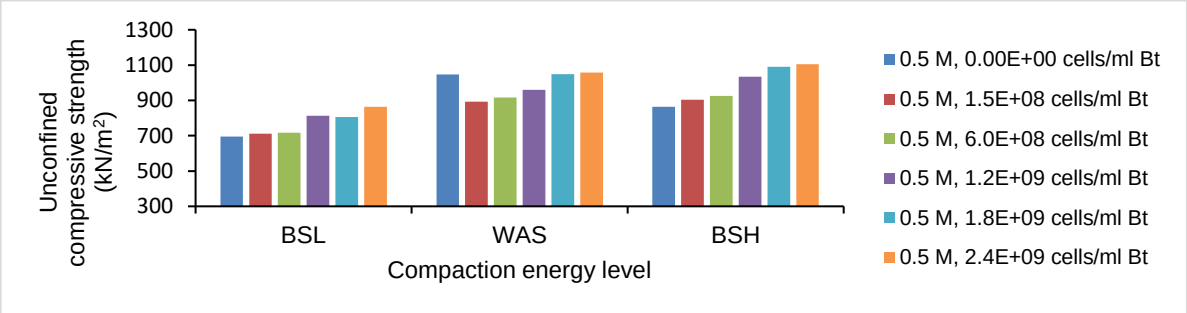


Figure 8: *Variation of unconfined compressive strength of lateritic soil – 0.5 M C_s – Bacillus thuringiensis mixtures with compaction energy (Air-cured at ambient temperature).*

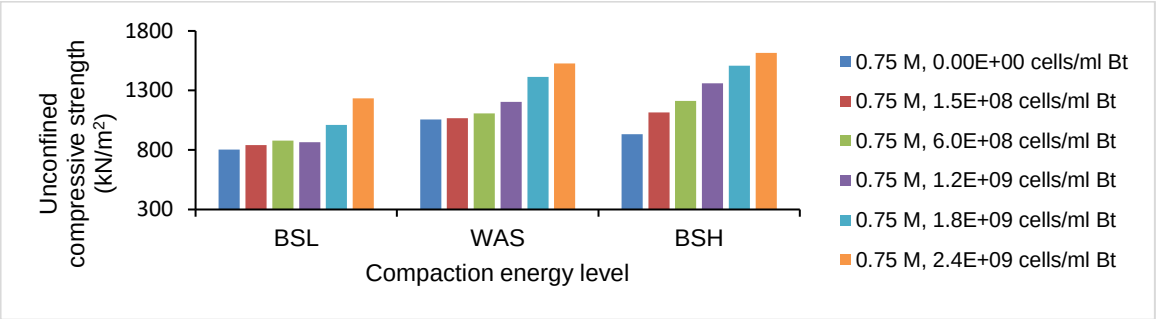


Figure 9: *Variation of unconfined compressive strength of lateritic soil – 0.75 M C_s – Bacillus thuringiensis mixtures with compaction energy (Air-cured at ambient temperature).*

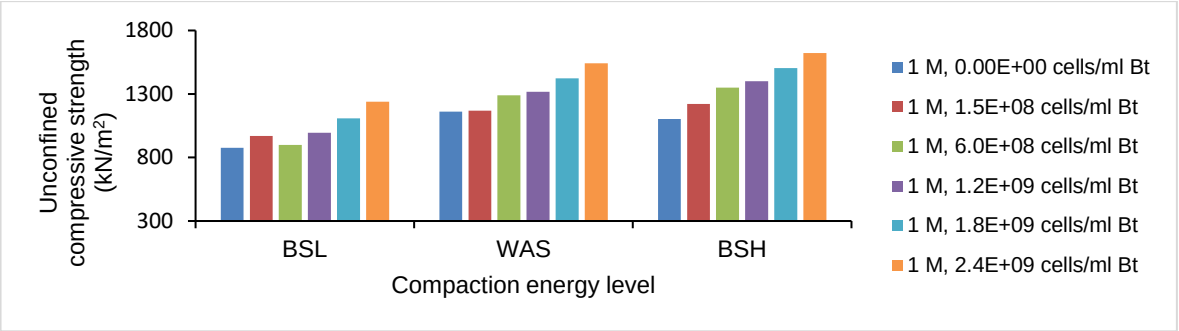
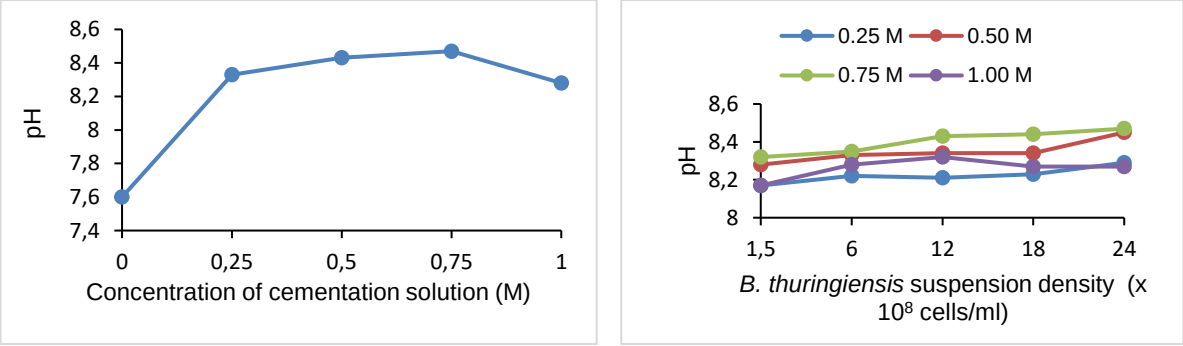


Figure 10: *Variation of unconfined compressive strength of lateritic soil – 1M C_s – Bacillus thuringiensis mixtures with compaction energy (Air-cured at ambient temperature).*

3.3 pH of cementation solution concentrations and bacterial mixtures

The variations of pH with cementation solution concentration and Bt suspension density are shown in Figure 11. The pH values increased as the molar concentrations of the C_s increased. The initial pH value of 7.6 increased to 8.47 (peak) at 0.75 M and thereafter decreased to 8.28 at 1 M (see Figure 11A). Similarly, the pH values increased with increase in Bt population density as could be seen in Figure 11B. The decrease in pH beyond 0.75 M may be explained in terms of probable decrease in bacterial urease activity (Murtala and Khairul, 2017). The recorded pH values fall within the reported range of 6 - 9 suitable for MICP process (Sun *et al.,* 2018).



A B

Figure 11: *Variation of pH with the materials used for bio-treatment: (A) Cementation solution (B) Bacillus thuringiensis*

3.4 Microstructural analysis

The microstructural analysis of the natural and optimal bio-treated specimens was carried out using SEM. Soil compacted with BSL energy and air-cured at ambient temperature which recorded greater resistance to crushing relative to the specimens wax-cured and oven-cured at 30° C were used for the SEM analysis. The micrographs for the natural lateritic soil, lateritic soil – C_s mixture alone (0.75 M) and the optimum blend based on UCS results (i.e., lateritic soil - Bt (24 x 10^8 cells/ml) at 0.75 M are shown on Plates III – V, respectively.

The micrograph of natural soil at 500x magnification with wide cracks in the untreated lateritic soil is shown on Plate III. However, the observed voids were filled after bio-treatment thereby depicting a smoother morphology as shown on Plate V. The elimination of relatively lager voids in the treated soil could be explained in terms of calcite crystals that were precipitated after treatment which bonded the soil particles. Similar microstructure was observed when lateritic soil was treated with cementation reagent alone (see Plate IV). This is consistent with reports made by Ning-Jun *et al.* (2017) and Osinubi *et al.* (2019b; 2021).

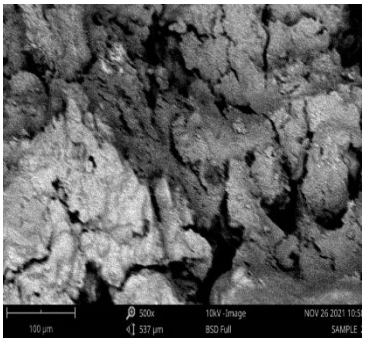


Plate III: *Micrograph of natural lateritic soil at 500x magnification*.



Plate IV: *Micrograph lateritic soil treated with cementation solution alone (0.75 M) at 1500x magnification*.



Plate V: *Micrograph of the optimum lateritic soil- Bt (24 x 10^8 cells/ml) Cs (0.75 M) mixture at 1500x magnification*.

4 CONCLUSIONS

The study involved laboratory tests on the natural lateritic soil classified as A – 2 – 6 (2) or SC soil in AASHTO and USCS soil classification systems, respectively bio-treated with *Bacillus thuringiensis* (Bt) and cementation solution (Cs) using the MICP technique and cured using different procedures. Based on the results obtained, the following conclusions can be made:

1. Bio-treated specimens air-cured at ambient temperature recorded the highest resistance to crushing followed by specimens oven-cured at 30^o C while specimens wax-cured recorded the least resistance to crushing.

2. Peak UCS value of 524.61 kN/m^2 was recorded for specimens treated with Bt (24 x 10^8 cells/ml): C_s (1 M) mix ratio when wax-cured, 1,208.75 kN/m^2 for Bt (24 x 10^8 cells/ml) : C_s (0.75 M) mix ratio when specimens were oven-cured at 30^o C and 1,239.38 kN/m^2 for Bt (24 x 10^8 cells/ml): C_s (1 M) mix ratio when specimens air-cured at ambient temperature.

3. Higher compaction energy produced specimens with higher UCS values in decreasing order BSH > WAS > BSL regardless of the curing procedure adopted.

4. The pH values increased with higher molar concentration of the cementation solution up to 0.75 M and thereafter decreased, while the values increased with increase in Bt suspension density.

5. SEM results showed that calcite crystals precipitated after bio-treatment filled the voids and bonded the soil particles.

Based on the findings of the study, the lateritic soil treated with *Bacillus thuringiensis* (24 x10^8 cells/ml): 0.75 M (C_s) mix ratio and air-cured at ambient temperature could be used for mitigation of soil erosion.

REFERENCES

AASHTO (1986). Standard Specification for Transportation, Material and Methods of Sampling and Testing. 14th Edition. American Association of State Highway and transportation official Washington D.C.

Animesh S., Ramkrishnan R. (2016). Study on effect of microbial induced calcite precipitates on strength of fine grained soils. *Perspectives in science*-Elsevier 8, 198—202.

ASTM D 2487 (2006). Standard practice for classification of soils for engineering purposes (unified soil classification system), American Society for Testing and Materials. Philadelphia.

BS 1377 (1990). *Method of Testing Soils for Civil Engineering Purpose*. British Standard Institute, BSI, London.

BS 1924 (1990). *Method of Test for Stabilized Soils.* British Standard Institute BSI London.

Cheng L., M. A. Shahin, R. Cord-Ruwisch, M. Addis, T. Hartanto, and C. Elms, (2014). “Soil stabilization by microbial induced calcium carbonate precipitation: Investigation of some important physical and environmental aspects,” in *7th International Congress on Environmental Geotechnics*, 10-14 November, Melbourne, Australia, 2014.

Head KH (1994) Manual of soil laboratory testing. Permeability, Shear Strength, and Compressibility Tests, vol 2. Pentech Press, London.

Jiang, N., Tang, C., Hata, T., Courcelles, B., Dawoud, and Singh, D.N. (2020). Bio- mediated Soil Improvement: the Way Forward. *Soil Use Manag*. 36 (2), 185–188.

Konhauser, K. O. (2009). *Introduction to geomicrobiology*. John Wiley & Sons.

Liu, B., Xie, Y. H., Tang, C. S., Pan, X. H., Jiang, N. J., Singh, D. N., Cheng, Y. J., Shi, B. (2021). Bio-mediated method for improving surface erosion resistance of clayey soils. *Engineering geology* 106295, 1-10.

Mahawish A, Bouazza A, Gates WP (2018). Improvement of coarse sand engineering properties by microbially induced calcite precipitation. *Geomicrobiol J* 35(10):887–897.

Mitchell, J. K., Santamarina, J. C. (2005).Biological considerations in geotechnical engineering.*Journal of Geotechnical and Geoenvironmental Engineering*-ASCE, 131(10): 1222e33.

Murtala U., Khairul A. K., Kenny T. P. C., (2016). Biological process of soil improvement in civil engineering: A review *Journal of Rock Mechanics and Geotechnical Engineering*-Elsevier, 8: 767-774.

Murtala U., Khairul A. K., (2017). Effect of cementation reagents concentrations on microbial calcite precipitation in residual soil. *Malaysian Journal of Civil Engineering* 29(1):79-90

Ning-Jun, J., Soga, K. and Kuo, M. (2017).Microbially induced carbonate precipitation for seepage-induced internal erosion control in sand-clay mixtures. *Journal of Geotechnical and Geoenvironmental Engineering*-ASCE, 143(3): 04016100 (04016114).

Osinubi, K. J., Sani, J. E., Eberemu, A. O., Ijimdiya, T. S., Yakubu, S. E. (2019a). Unconfined Compressive Strength of *Bacillus Pumilus* Treated Lateritic Soil. *Proceedings of the 8th International Congress on Environmental Geotechnics Volume 3,*pp 410-418.

Osinubi, K. J., Gadzama E. W., J. E., Eberemu, A. O., Ijimdiya, T. S., Yakubu, S. E. (2019b). Evaluation of the Strength of Compacted Lateritic Soil Treated with *Sporosarcina Pasteurii. Proceedings of the 8th International Congress on Environmental Geotechnics Volume 3,*pp 419-428.

Osinubi, K. J., Eberemu, A. O., Ijimdiya, T. S., Etim, R. K. (2021). Microbial-Induced Calcite Precipitation Study on the Plasticity and Compaction Characteristics of Lateritic soil treated with Bacillus megaterium in Urea-CaCl2 Culture Medium. 2nd International Conference on Sustainable Infrastructural Development (ICSID), Theme: Sustainable Infrastructural Development (Virtual), Track 5: Geotechnics and Transportation Infrastructure, Covenant University, Ota, Ogun state, Nigeria, 27–28 July. *IOP Conference Series: Materials Science and Engineering* 1036 012031, pp 1-13. doi:10.1088/1757-899X/1036/1/012031.

Sharma Meghna, Neelima Satyam & Krishna R. Reddy (2021): Effect of freeze-thaw cycles on engineering properties of biocemented sand under different treatment conditions. *Engineering Geology* (284) 1-15.

Stocks-Fischer, S., Galinat, J.K. and Bang, S.S. (1999). Microbiological precipitation of $CaCO_3$. *Soil Biologyand Biochemistry*-Elsevier, 31 (11): 1563–1571.

Sun, X., Miao, L., Tong, T. and Wang, C. (2018). Improvement of Microbial-Induced Calcium Carbonate Precipitation Technology for Sand Solidification. *Journal of Materials in Civil Engineering*, ASCE, 30(11): 04018301, 1-8.

Travers, R.S., Martin, P. A. W., & Reichelderfer, C. F. (1987). Selective process for efficient isolation of soil *Bacillus spp. Applied & Environmental Microbiology* 53: 1263-1266.

Tsukamoto M., T. Inagaki, Y. Sasaki, and K. Oda, “Influence of relative density on microbial carbonate precipitation and mechanical properties of sand,” in *Proceedings of the 18th International Conference on Soil Mechanics and Geotechnical Engineering*, 2013, pp. 2613-2616.

Wang Yuze; Charalampos Konstantinou; Kenichi Soga; Jason T. DeJong; Giovanna Biscontin; Alexandre J. Kabla (2022). Use of microfluidic experiments to optimize MICP treatment protocols for effective strength enhancement of MICP-treated sandy soils. *Acta Geotechnica*, 1-22. https://doi.org/10.1007/s11440-022-01478-9(0123456789.

Yohanna, P., Eberemu, A. O., Ijimdiya, T. S., Osinubi, K. J. (2022). The effect of *Bacillus coagulans* and moulding water content on the unconfined compressive strength of lateritic soil. *Engineering Science & Technology* 3(1) 69-83.