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ORIGINAL RESEARCH ARTICLE

EFFECT OF BIO-CEMENTATION ON THE STRENGTH CHARACTERISTICS OF COMPACTED LATERITIC SOIL UNDER STERILIZED AND UNSTERILIZED CONDITIONS

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ABSTRACT

Naturally, not all soils readily meet specifications for engineering use. Therefore, improvement of soil engineering properties is one of the main concerns of a civil engineer. This paper evaluates the strength characteristics of a deficient lateritic soil classified as an A-4(3) using the American Association of State Highway and Transportation Officials (AASHTO) classification system and CL using the Unified Soil Classification system (USCS) after improvement, using microbial induced calcite precipitation (MICP) technique. The soil sample was obtained from a site in Abagana, in Anambra State. It was treated with varying suspension densities up to 2.40 x 10^9 cells/ml of *Sporosarcina pasteurii* (*S. pasteurii)* a non-pathogenic microorganism cultured from the soil, under sterilized and unsterilized condition. The treated soil was compacted using the British Standard Light (Standard Proctor) compactive effort at the moulding water content range of -2 to +4% optimum moisture content (OMC). The compacted soil specimens were then permeated a cementitious reagent containing 20 g of Urea, 10 g of NH_4Cl, 3 g of Nutrient broth, 2.8 g of $CaCl_2$ and 2.12 g of $NaHCO_3$ per litre of de-ionized water to aid the microbial induced calcite precipitation (MICP) process in three circles with 2/3rd pore volume. The Atterberg limits results showed a better improvement in the sterilized than the unsterilized specimens. Similarly, the unconfined compressive strength generally recorded higher results in the unsterilized specimens. Highest unconfined compressive strength (UCS) results were recorded at *S. pasteurii* suspension densities of 1.20 x 10^9 /ml and 1.80 x 10^9 /ml for the unsterilized and sterilized specimens respectively. Microstructural analysis using X-ray Diffraction performed on precipitates derived through the interaction of *S. pasteurii* and the cementitious reagent confirmed calcite as the dominant composition of the precipitate, analysis using the scanning electron microscopy (SEM) on the MICP treated soil depicts presence of calcite precipitate and bio-cementation on the surface morphology of the treated soil grains. It was concluded that there are other species of microorganisms that augmented the MICP processes in the unsterilized specimens resulting to higher UCS values in the study. 1.20 x 10^9 /ml *S. pasteurii* suspension density was recommended as the optimal mix for the treatment of the unsterilized soil.

1.0 Introduction

The improvement of soil engineering properties is one of the major tasks of a civil engineer (Sotoudehfar *et al*., 2016; Tang *et al*., 2020). It is noteworthy that not all soils formed will readily meet specification for engineering use (Jayanthi and Singh, 2016). For over a century, several procedures of soil improvement such as application of lime, cement, bitumen, chemical additives such as sodium silicate, lignin and chemical grouting etc. have been established and being used in several engineering projects. Although most of these methods of soil/ground improvement currently in use satisfy their engineering requirements, they have been reported to be environmentally unfriendly. The demand for, innovative and sustainable techniques of ground improvement have generally compelled researchers to look for novel and more sustainable ways of satisfying society's demand. This has led to the discovery of a multi-disciplinary study between geotechnical engineers and microbiologist known as Microbially Induced Calcite Precipitate (MICP). This discovery has provided engineers with the idea to consider soil not only as engineering materials but also as an ecosystem (Mujah *et al*., 2017). Dejong *et al.* (2013) reported that, MICP technique has shown some superior results of applications in geotechnical engineering. This could be done beneath existing structures without being an obstruction to the structures, enabling the improvement over longer distances. Therefore, the discovery of MICP processes of improving the engineering properties of soil in the last two decades should not be seen to have only disproved the long-term misconception of the conventional geotechnical engineering, but also as one of the manifestations of interdisciplinary approaches to fit in ideas and techniques from other disciplines to advance innovative solutions to complex problems to geotechnical engineering problems (Kavazanjian and Karatas, 2008; Dejong *et al.,* 2013). MICP method of soil improvement has attracted an intensive research interest globally, which is novel as well as promising and sustainable, it is cost effective, non-disruptive in-situ ground improvement to a lot of geotechnical problems (Mujah *et al*., 2017; 2021; Burdalski and Gomez, 2020; Choi *et al*., 2020; Wani and Mir, 2020; Cui *et al*., 2021; Gowthaman *et al*., 2021 and Osinubi *et al.,* 2021a). For effective MICP process using ureolysis, the presence of a urease enzyme secreting bacteria is vital (Bachmeier *et al*., 2002). During MICP process, there are two major reactions that may occur individually or simultaneously: when strength improvement is the target, bio-cementation should be considered. According to Mujah *et al*. (2017), this discovery of MICP has provided engineers with the idea to consider soil not only as a construction material but also as an ecosystem. Calcite ($CaCO_3$) is essential for effective MICP, it is also product of a two-stage chemical reaction resulting from MICP process, (see equations. (1) and (2) which is responsible for improving the soil properties through biocementation and bioclogging.

$$CO(NH_2)_2 + 2H_2O \rightarrow 2NH_{4^+} + CO_3^{2-} \quad (1)$$

$$Ca^{2+} + CO_3^{2-} \rightarrow CaCO_{3\downarrow} \quad (2)$$

In equation (1), urea is hydrolyzed by the urease positive bacteria breaking it into ammonium and carbonate, while in equation (2) the product of the hydrolyzed urea, in the presence of calcium source yields to calcite as a precipitate.

Dejong *et al.* (2013) considered soil as a living ecosystem which offers the potential for innovative and sustainable solutions to several geotechnical problems which was further

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buttressed by Osinubi *et al.* (2019); Osinubi et al. (2021b); Mohammadizadeh *et al.* (2020); Nafisi *et al.* (2020); Hoang *et al.* (2020); Lai *et al.* (2021) and Wang *et al.* (2021).

The objective of this study was to evaluate the strength characteristics of compacted lateritic soil under sterilized and unsterilized conditions, treated with an ureolytic microorganism and cementation reagent. The organism was locally isolated from the lateritic soil.

2. Materials and Methods

2.1 Materials

2.1.1 Soil sample type

Lateritic soil with pore throat that allowed the free mobility of the microorganisms when in compacted form was used in the study. It was obtained from Abagana (Latitude 6°10'15" N and Longitude 6°58'10" E), in Njikoka Local Government Area, Anambra State, Nigeria. The method of disturbed sampling was adopted during the sample collection at depths between 0.3 m and 3.0 m.

2.1.2 Bacteria

The species of bacteria used in this research is *S. pasteurii* commonly found in soil, the urease positive bacteria is rod-shaped, spore-forming and Gram-positive, was cultured from the lateritic soil sample in the Department of Microbiology, Modibbo Adama University, Adamawa State.

2.1.3 Cementitious reagent

The cementitious reagent reported in several studies (e.g., Stocks-Fischer *et al.,* 1999; Venkata *et al*., 2016; Tirkolaei and Bilsel, 2017; Osinubi *et al.,* 2019; 2021a&b) was used. It is composed of 20 g Urea, 10 g NH_4Cl, 3 g Nutrient broth, 2.8 g $CaCl_2$ and 2.12 g $NaHCO_3$ per litre of de-ionized water. 3 g/l of nutrient broth was added to the cementation reagent as the most viable amount for survival of bacteria (Sharma and Ramkrishnan, 2016).

2.2 Methods

2.2.1 Bacteria culture/growth medium

The identification of the bacteria was carried out using the biochemical method (conventional procedure) according to Cheesbrough, (2006). Bacteria were cultured in an Ammonium-Yeast Extract media described in Mortensen *et al.* (2011) and Feng *et al.* (2014). The bacteria *S. pasteurii* was isolated from soil, inoculated on media containing the following: 20g yeast extract, 10g ammonium chloride, 2g urea 0.1g nickel per litre of de-ionized water, NaOH was used to adjust the pH of the media to 9.0. The media was poured in an aliquot amount of 10ml per culture bottle corked with foil paper, autoclaved at 121°C per 1.1kg pressure for a period of 15 min. 1g each of the soil samples were inoculated on each of the culture bottle and incubated at 37^0C for 24-48hours to aid proper isolation, identification and characterization of *S. pasteurii* as an isolated urease producing organisms.

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2.2.2 Determination of Bacteria suspension density

The density of bacteria cell was varied in stepped *S. pasteurii* suspension of McFarland standard 0.5, 2, 4, 6 and 8, which is equivalent to 0, 1.50×10^8, 6.0×10^8, 1.20×10^9, 1.80×10^9 /ml and 2.40×10^9 cells/ml, respectively. The maximum volume of microorganisms mixed with the soil was one-third (1/3) of the pore volume as recommended by Rowshanbakht *et al.* (2016).

2.2.3 Test tube experiments to characterize calcite precipitate

3ml of *S. pasteurii* for suspension densities of 0, 1.50×10^8, 6.0×10^8, 1.20×10^9, 1.80×10^9 and 2.40×10^9 cells/ml, were mixed with 7ml of the cementation reagent in three cycles at six hours interval, this was allowed to remain in the laboratory for 24 hours at a temperature range of 24 ± 2^0 C, the precipitate resulting from their reactions was filtered through a filter paper, the solids retained on the filter paper was oven dried at a temperature of 105^0C and was kept for characterization using XRD test. The composition of the precipitate was determined using X-Ray Diffraction (XRD) Empyrean model (manufactured by Malvern Panalytical Netherlands). The precipitate was grounded into a fine powder using the sample preparation block and compressed in the flat sample holder to create a flat, smooth surface that was later fixed on the sample stage in the XRD cabinet which determined the average bulk composition.

***2.2.4 Sample preparation*:** 3000g of the pulverized air-dried soil sample passing through BS No.4 sieve (4.76 mm) was thoroughly mixed at moulding water contents between -2 % and +4 % relative to OMC each containing 1/3 of its pore volume representing each *S. pasteurii* suspension densities. Specimens were cured for 12 hours in sealed polythene bags in the laboratory at 24 ± 2^0C temperature for hydration and distribution of the microorganisms within the soil before compaction. This was followed with the permeation of the compacted specimens with the cementation reagent in three circles with 2/3 of their pore volume each under gravity within 24 hours to initiate the MICP process.

2.2.5 Index properties

The determination of the natural moisture content, specific gravity, and particle size distribution were in accordance with procedures outlined in BS 1377: 1990.

2.2.5.1 Atterberg limits determination

Soil samples passing through BS No. 40 sieve (425 µm) was used for Atterberg limits tests which consist of liquid limit, plastic limit and plasticity index. The samples were treated with varying densities of *S. pasteurii* at the optimum moisture content (OMC) of the soil for British Standard Light (BSL) compactive effort and then treated with the cementation reagent in three cycles each with 2/3 pore volume within a 24-hour period. The treated soil samples were air-dried at room temperature before conducting the tests in accordance with the procedure outlined in BS 1377: 1990.

2.2.5.2 Compaction experiments

Compaction was carried out in accordance with the method outlined in BS 1377, (1990) to obtain the OMC. Compaction was done using the BSL compactive effort (Standard Proctor).

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2.2.5.3 Sterilization of soil

10,000 g of the air dried soil sample was measured into a stainless steel bucket with cover, which was put into an oven set at a temperature of 120°C. The sample was allowed to remain in the oven for three hours, after which it was allowed to cool down to room temperature, the above procedure was repeated three times to ensure that spore forming organisms are properly sterilized. Similar procedures are discribed in Trevors, (1996); Mahmood *et al.* (2014) and Yan *et al.* (2019). This was repeated until the required quantity of soil sample needed for the test was realized.

2.2.5.4 Unconfined compressive strength (UCS)

The treated specimens were cured in the moulds for 24 hours before they were extruded. The extruded specimens were further cured for 24 hours in the laboratory at a temperature of 24 ± 2°C and then sealed in polyethene bags for another 24 hours. It was then crushed to failure using the UCS machine Model number **24-9150/01 ELE International, United Kingdom** at a regulated strain of 0.02 %/min. Specimens tested had an average height to diameter ratio of 2:1. Crushing of specimens was carried out in triplicates with the average result obtained used in accordance with guidelines specified in BS 1377 (1990).

2.2.5.5 Calcite content (Acid Washing Method)

5 g of the treated soil was mixed with 20 mL of 2-M Hydrochloric acid (HCl) to dissolve calcium carbonate on the treated specimens. All the solution and insoluble soil solid were washed with distilled water on filter paper having a coarse pore size in a No. 200 sieve for 10 minutes in order to remove all soluble calcium from the soil particles. The solid particles retained on the sieve were oven-dried at a temperature of 105° C for 24 hours and weighed. The difference between the mass of the originally treated soil sample (A) and the washed soil sample (B) was the mass of calcite. The calcite content (CC) was computed using Equation (3) as reported in Soon, *et al.* (2014) and Choi, *et al.* (2017).

$$CC = 100 - \frac{B}{A}x100\,\% \qquad (3)$$

2.2.6 Microanalysis

Microanalysis using scanning electron microscopy (SEM) Phenom World Pro G6 desktop Thermo Fisher scientific, Netherland, was conducted on both natural and *S. pasteurii* treated lateritic soil to investigate the changes in morphological features due to the formation and distribution of calcite bonds on the inter particle surface in the micro-structure of the soil. Specimens of lateritic soil optimally treated with *S. pasteurii* were used in the study. The test was performed using Phenom World Pro desktop SEM equipped with a software tool that could automate data collection and duplicate analysis. The specimen was positioned in an electrically powered tilt and rotating specimen holder which was regulated by a dedicated motion regulator which initiates an infinite 360° rotation with a pseudo-eucentric tilting adjusted focus oscillating from 10° to 45°, which allows the creation of exact size and information from micro and nano fibre samples (Phenom World, 2017). Similar procedure was reported by Osinubi *et al.* (2020).

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3.0 Results and Discussion

3.1 Test tube experiments to characterize calcite precipitate

Figure 1 shows the microstructural analysis using X-ray Diffraction on the precipitates derived through the test tube experiment described in section 2.2.3, i.e. the interaction between *S. pasteurii* and the cementitious reagent. The result confirmed calcite as the dominant composition of the precipitate with few impurities of Quartz and Actinolite, which was earlier reported by Konhauser, (2006) to be among the most cations frequently associated with biogenic minerals. The XRD spectra presented in this study agrees with the report by Carmona *et al.* (2016).

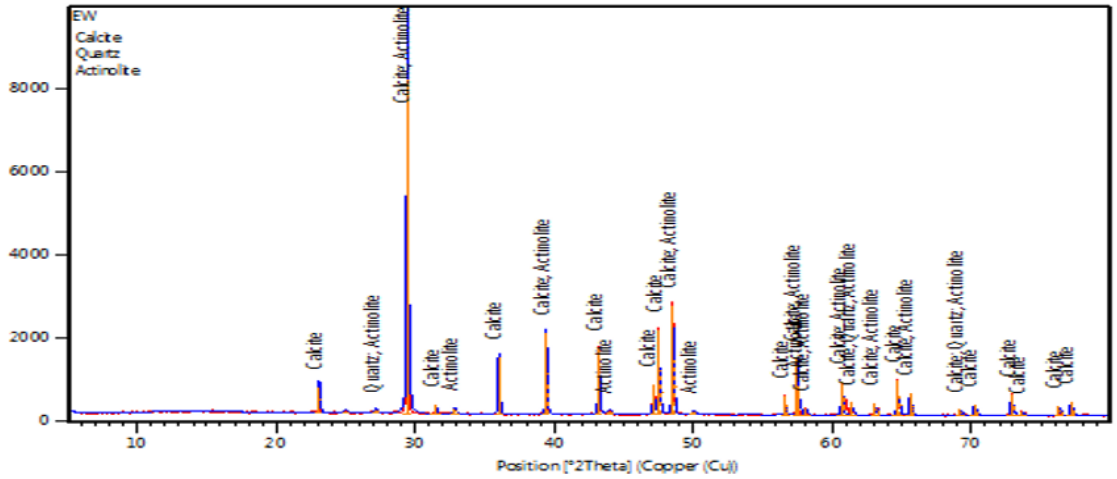

Figure 1: X-ray Diffraction of precipitate obtained from test tube experiment

3.2 Index properties

Table 1 summarizes the index properties of the natural lateritic soil. The particle passing BS sieve No. 200 is 36.0%, with silt and clay contents of approximately 22 % and 14 %, respectively. The liquid and plastic limits recorded were 44.0 and 21.6%, respectively; while the plasticity index was 22.5%. Based on the results, the soil is classified as A-4(3) using the American Association of States Highways and Transportation Officials (AASHTO, 1986) classification system and CL using the ASTM-Unified Soil Classification System (USCS-ASTM, 1992). The OMC and the maximum dry density are 15.3 % and 1.83 Mg/m^3 respectively. The X-Ray Diffraction test showed kaolinite as the dominant clay mineral.

Table 1: Physical properties of the natural soil

Property	
Natural moisture content (%)	11.3
Percentage Passing No. 200 sieve (wet sieve)	36.0
Liquid Limit (%)	44
Plastic Limit (%)	21.6
Plasticity Index (%)	22.5
CEC(meq/100 g)	5.50
Specific Gravity	2.62
AASHTO classification	A – 4(3)
USCS	SC
Colour	Reddish brown
Dominant clay mineral	Kaolinite

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3.3 X-Ray Diffraction of natural soil.

Table 2 shows the oxide composition of the natural soil. It could be observed that the highest oxide composition recorded by the soil is silicon, followed by Aluminium and Iron respectively. The silica- sesquioxide ratio {$SiO_2/(Al_2O_3 + Fe_2O_3)$} recorded is 1.64, according to Okeke *et al.* (2016), a soil is said to be lateritic, if it has a silica-sesquioxide ratio value between 1.33 and 2.0, while any value higher than 2.0 is indicative of non-lateritic soil.

Table 2: Oxide composition of the natural lateritic soil

Oxide	SiO_2	Al_2O_3	CaO	TiO_2	V_2O_5	Cr_2O_3	Fe_2O_3	MnO	CuO	ZrO_2	LOI	Total
Concentration (%)	56.5	19.00	0.33	2.89	0.061	0.051	15.41	0.075	0.056	0.290	4.54	99.20

3.4 Atterberg Limits

3.4.1 Liquid Limit

The variation of liquid limit (LL) with *S. pasteurii* suspension densities for sterilized and unsterilized samples is shown in Figure 2. It was observed that the LL for the unsterilized specimen (LLUS) recorded higher values compared to the natural LL, the values recorded are: 44%, 49.5%, 49.0%, 50.5%, 49.5% and 49.5% for 0, 1.50 x 10^8, 6.0 x 10^8, 1.20 x 10^9, 1.80 x 10^9 and 2.40 x 10^9 cells/ml of *S. pasteurii* suspension densities, unlike the unsterilized specimens, there was a steady decrease in the liquid limit for sterilized specimens (LLS) values with increase in *S. pasteurii* suspension densities, the values recorded are 45.5%, 41.6%, 39.3%, 40.1%, 40.1% and 40.4% for 0, 1.50 x 10^8, 6.0 x 10^8, 1.20 x 10^9, 1.80 x 10^9 and 2.40 x 10^9 cells/ml of *S. pasteurii* suspension densities with the lowest value of 39.3% recorded at 6.0 x 10^8 cells/ml *S. pasteurii* suspension density. It was further observed that the sterilized specimen recorded a lower LL values than the unsterilized specimen, which suggest that the existence of organisms in the specimens may have played a vital role for observed trend of results. Similar results were reported by Yosodian *et al.* (2012) and Liu *et al.* (2020).

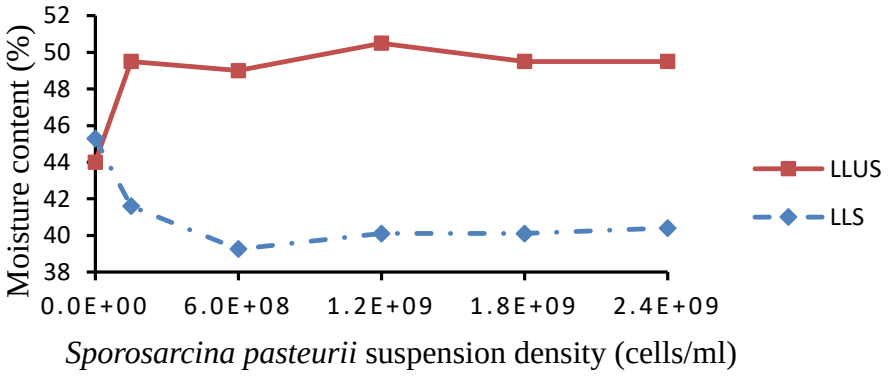

Figure 2: Variation of liquid limits with S. *pasteurii* suspension density for Lateritic soil under sterilized and unsterilized condition

3.4.2 Plastic Limit

Figure 3 shows the effect of different MICP treatment for different *S. pasteurii* suspension densities on the plastic limit (PL) for sterilized and unsterilized specimens, it was observed

that there was a drastic decrease in the PL values from 21.55% for natural to 14.82% at 1.50 x 10^8 cells/ml *S. pasteurii* suspension density, which could entirely be due to decrease in CO_2 in the sterilized soil specimen which is a major ingredient for microbial reaction in MICP processes. Another reason could be due to the participatory role by other bacterial species, which later continued to increase with not only *S. pasteurii* suspension densities all through the reactions 15.29%, 15.65%, 16.27% and 17.37% at 6.0 x 10^8, 1.20 x 10^9, 1.80 x 10^9 and 2.40 x 10^9 cells/ml, *S. pasteurii* suspension densities for unsterilized specimen. On the other hand, the sterilized specimens recorded a continuous decrease in the PL values from 22.75% for natural to 20.61% at 6.0 x 10^8 cells/ml, after which it increased to 21.02% and then decreased steadily to 19.30% at 2.40 x 10^9 cells/ml *S. pasteurii* suspension density. When improvement in engineering properties is the target, an increase in the PL of the treatment is desirable since it will result to decrease in the plasticity index of the soil, which is an indication of improvement in the engineering properties of the soil, this have been observed in the sterilized compared to unsterilized specimens elsewhere (Osinubi *et al.,* 2019; Hafez *et al.,* 2019).

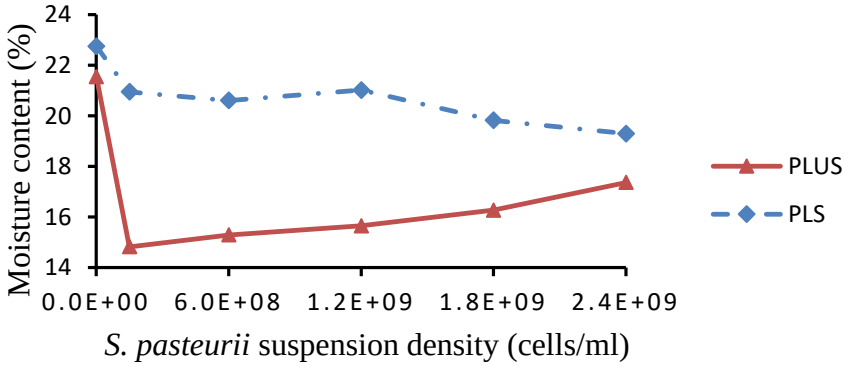

Figure 3: Variation of plastic limits with *S. pasteurii* suspension density for Lateritic soil under sterilized and unsterilized condition

3.4.3 Plasticity Index

Figure 4 shows the effect of *S. pasteurii* suspension densities on the plasticity indices (PI) for the sterilized and unsterilized specimens. It was observed that the unsterilized specimens recorded higher PI compared to sterilized specimen, generally there was an increase in the PI values in the unsterilized specimens upon MICP treatment. The following values were recorded for unsterilized specimens: 22.45%, 34.68%, 33.71%, 34.85%, 33.23% and 32.13% for 0, 1.50 x 10^8, 6.0 x 10^8, 1.20 x 10^9, 1.80 x 10^9 and 2.40 x 10^9 cells/ml, *S. pasteurii* suspension densities, while for sterilized specimens, there was a steady decrease in the PI values from 22.45% for the natural to 18.65% which is the lowest PI value at 6.0 x 10^8 cells/ml *S. pasteurii* suspension density, thereafter it steadily increased to 19.08%, 20.28% and 21.11% at 1.20 x 10^9, 1.80 x 10^9 and 2.40 x 10^9 cells/ml, *S. pasteurii* suspension densities for sterilized specimens. The increase in PI values for both unsterilized and the sterilized specimens could be linked to the secretion of Exopolymeric substance as a result of the interaction between the microbes and the soil during the MICP processes. These findings are consistent with those reported by Moravej *et al.* (2018); Osinubi, *et al.* (2019), but did not agree with the findings reported by Neupane (2016). The results presented suggest that the sterilized specimens recorded more improvement in the engineering properties of the lateritic soil through the decrease PI.

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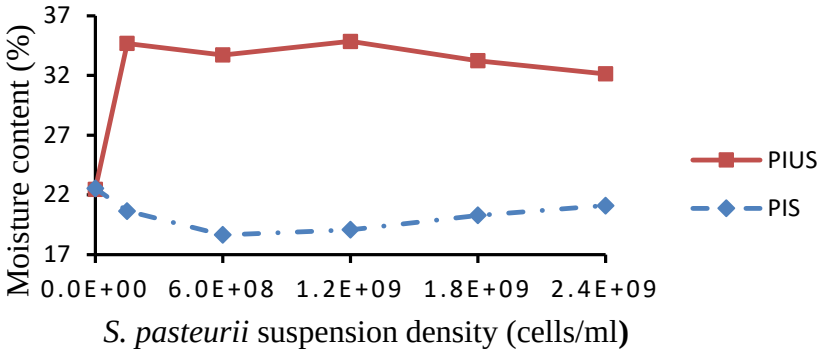


Figure 4: Variation of plasticity index with *S. pasteurii* suspension density for Lateritic soil under sterilized and unsterilized condition

3.5 Distribution of calcite content

Figure 5 shows the precipitation characteristics of calcite content with compacted soil column depth. It could be observed that the amount of calcite precipitate recorded in the unsterilized specimen were generally higher than those recorded in the sterilized specimens with the highest calcite content of 10% and 7% recorded respectively at the top of both compacted soil columns, this could be linked with the roles played by other urease positive bacteria that may be present in the unsterilized specimens (Hafez *et al.,* 2019). It was also observed that the calcite precipitate distribution pattern was not uniform in both soil columns, this could be due to the urease activities of the microorganisms did not equal or balance the flow of the cementation, which may have resulted into the non-uniform formation of calcite precipitate similar to findings by Shahin (2017) who reported that it is difficult to obtain a homogenous cementation in MICP due to imbalance in the flow of microorganisms and the cementation reagent within the pore space of soils. This The results presented suggest that there could be other species of microorganisms present in the unsterilized specimen which were destroyed in the sterilized specimen which would have contributed to additional ureolytic activities of *S. pasteurii* used in this study, this may have given an edge to the unsterilized over the sterilized specimens in the formation of more calcite specimen. The calcite precipitate distribution pattern for the sterilized specimen is similar to those obtained by Shahin (2017) in a sand column containing 10% of fines.

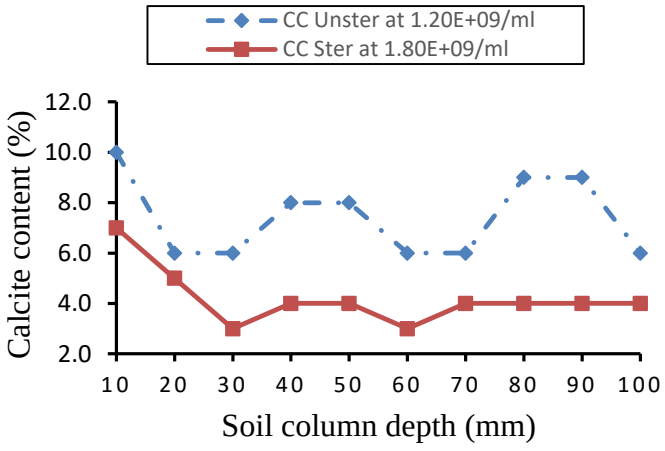
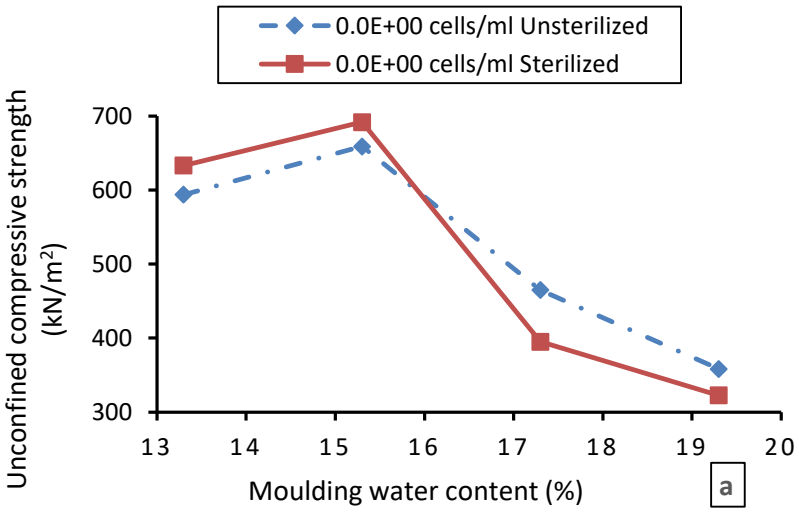
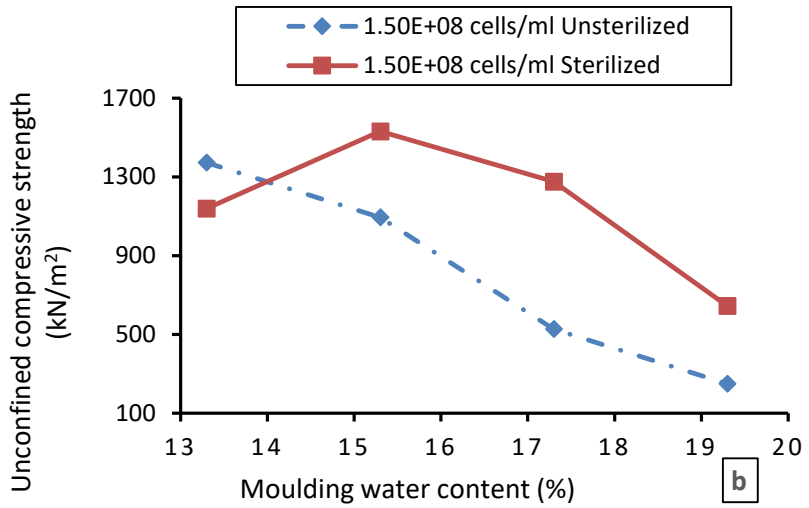


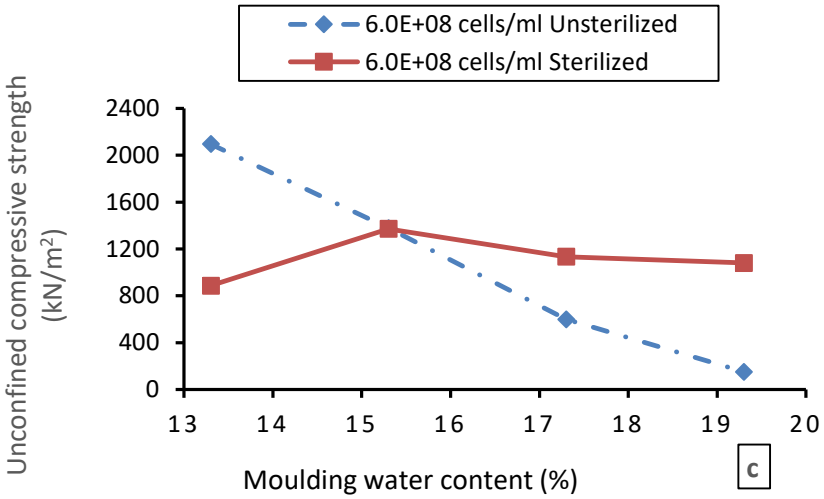
Figure 5: Distribution of calcite content with soil column depth for Lateritic soil under sterilized and unsterilized condition

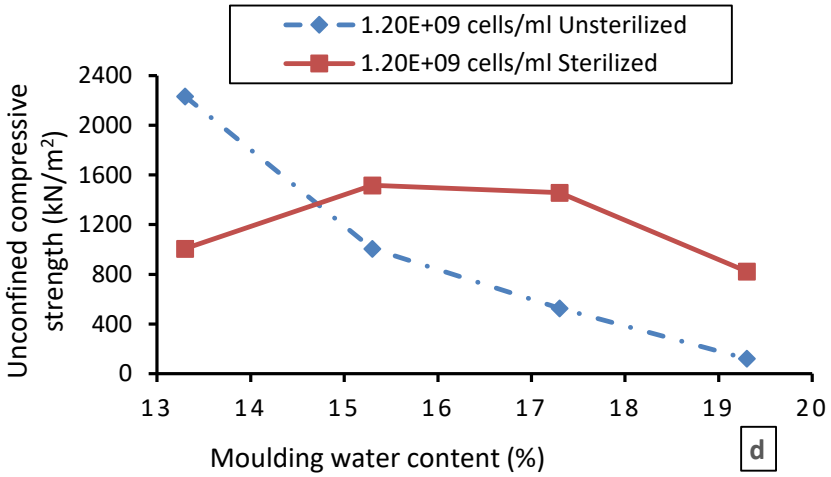
3.6 Effect of moulding water content on the unconfined compressive strength of Lateritic soil under sterilized and unsterilized condition.

Figures 6a-f illustrates the variation of unconfined compressive strength (UCS) of lateritic soil under sterilized and unsterilized condition with moulding water content. Peak UCS values were observed in the unsterilized specimens at moulding content of 13.3%, which is equivalent to 2 % dry of OMC the peak UCS values recorded were: 659.00kN/m^2, 1372.69 kN/m^2, 2095.49 kN/m^2, 2232.28 kN/m^2, 1869.54 kN/m^2 and 1796.44 kN/m^2, for 0, 1.50 x 10^8, 6.0 x 10^8, 1.20 x 10^9, 1.80 x 10^9 and 2.40 x 10^9 cells /ml of S. *pasteurii* suspension densities. Comparing the above results with those recorded for sterilized specimens, peak UCS values are: 691.95 kN/m^2, 1530.53 kN/m^2, 1371.33 kN/m^2, 1516.35 kN/m^2, 1881.09 kN/m^2 and 1644.54 kN/m^2, for 0, 1.50 x 10^8, 6.0 x 10^8, 1.20 x 10^9, 1.80 x 10^9 and 2.40 x 10^9 cells/ml of S. *pasteurii* suspension densities. It was observed that the highest UCS value of 2232.28 kN/m^2 was recorded at 1.20 x 10^9 cells/ml S. *pasteurii* suspension density for unsterilized specimens while lesser UCS value of 1881.09 kN/m^2 was recorded as the peak value for the sterilized specimens although higher population of microorganisms were used (1.80 x 10^9 cells/ml S. *pasteurii* suspension density). The higher results recorded for the unsterilized as against the sterilized specimens might be due to the presence of other species of urease positive organisms in the unsterilized soil, another reason is that in unsterilized specimens, the CO2 which is an important ingredient in calcite formation was affected, as it will have been decreased through sterilization. These may have contributed positively in the MICP process that eventually added to the ureolytic activities of S. *pasteurii* used in the study, causing more calcite precipitation thereby increasing the UCS values more than the sterilized specimens. Although it is not a standard practice in engineering to sterilize soils before use, the UCS results recorded in this study has demonstrated that other species of microorganisms might have played a vital role in the improvement of soil properties during MICP processes. The results obtained for unsterilized specimens in this study have established that higher soil strength can be attained at lower moulding water content which agrees with the findings earlier reported by Cheng *et al.* (2013; 2017); Mujah *et al.* (2017; 2021) and Shahin (2017).









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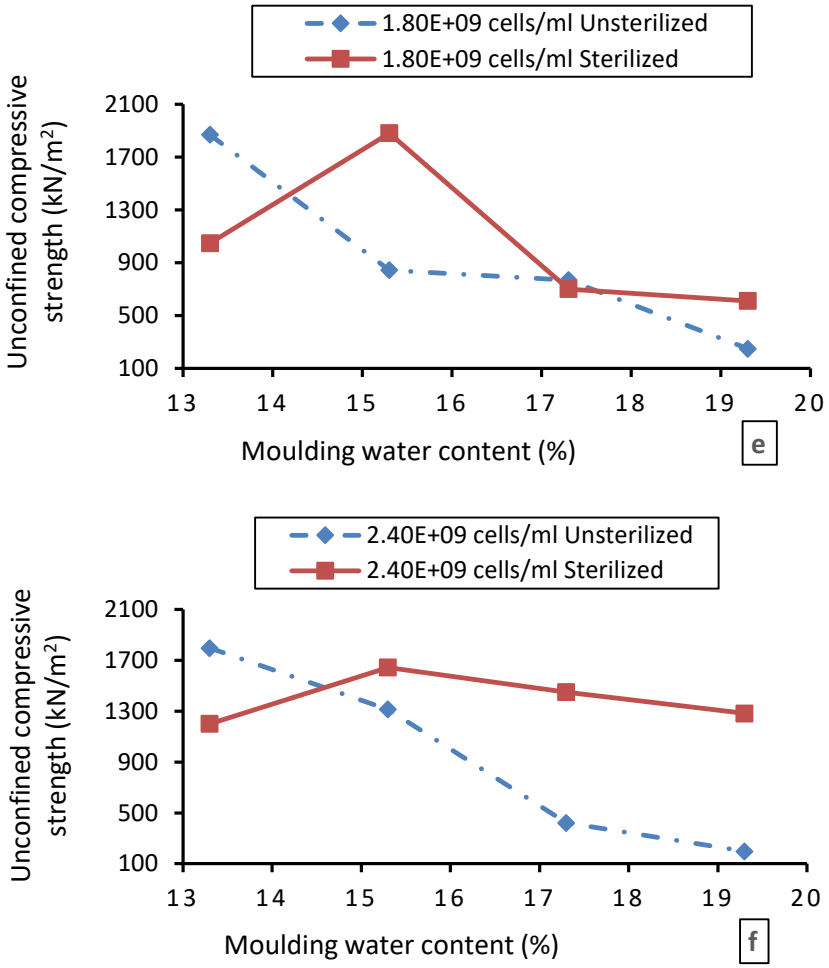

Figure 6: Variation of Unconfined compressive strength with moulding water content for Lateritic soil under sterilized and unsterilized condition, (a) natural soil, (b) $1.50x10^8$, (c) $6.0 x10^8$, (d) $1.20x10^9$, (e) $1.80x10^9$ and (f) $2.40x10^9$/ml *S. pasteurii* suspension densities

3.7 Scanning Electron Microscopy

Figures 7 a-c shows the scanning electron micrograph of the natural, the unsterilized and the sterilized treated soils, in the natural soil (Figure 7a), there are pores/voids that are observed with no calcite precipitate, which eventually decreased as observed in Figure 7b unsterilized treated soil due to calcite precipitates around the soil particles, comparing the micrographs of the sterilized with the unsterilized soils, the quantities of calcite precipitate in the unsterilized specimen was more pronounced than those in the sterilized soil as could be observed in Figures 7b and c. This suggests that there are other species of ureolytic microorganisms that have immensely contributed in the precipitation of more calcite in the unsterilized. From the SEM results presented, it is clear that the MICP treatment of an A-4(3) lateritic soil using ureolysis resulted into formation of higher quantity of calcite precipitates in the unsterilized than the sterilized specimen.

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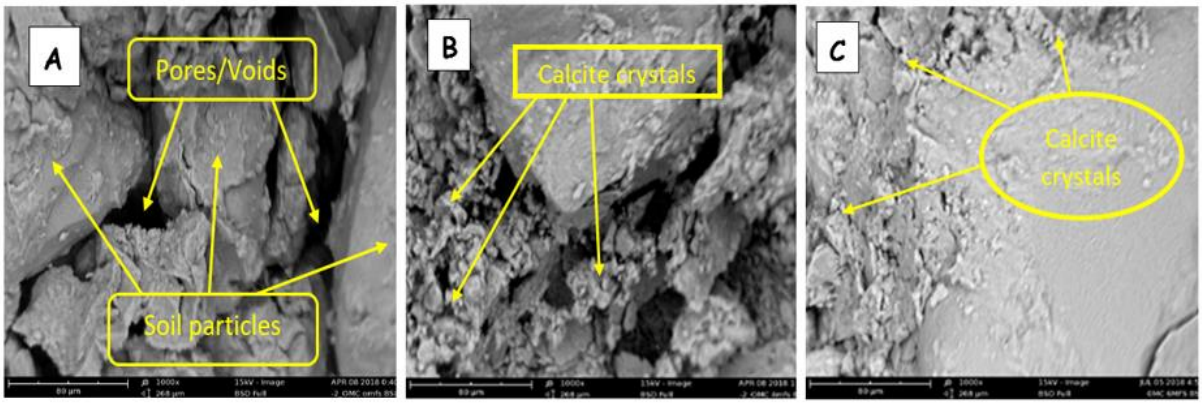

Figure 7: Micrograph at x1000 Magnification: (A) natural soil, (B) Unsterilized soil treated with 1.20 x 10^9cells/ml suspension density at -2% OMC, (C) Sterilized soil treated with 1.80 x 10^9 cells/ml suspension density at OMC

Figure 8 shows the scanning electron micrograph of the natural and the treated soil for Atterberg limits tests. Figure 8a is for the natural soil, hair cracks and voids could be observed as indicated by the arrows, which disappeared mainly due to bio-cementation and bio clogging after the treatment with *S. pastuerri* and the cementitious reagent (Figure 8b). The result presented has demonstrated the effectiveness of bio-cementation and bio clogging in MICP process as reported in the literature.

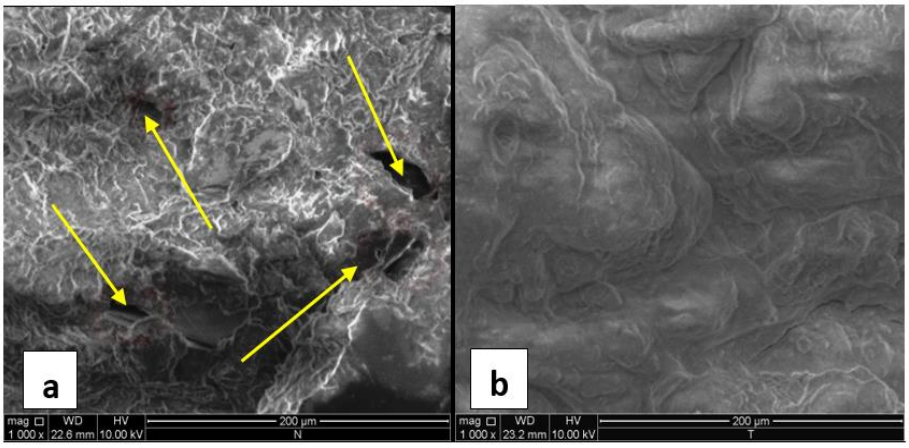

Figure 8: Micrograph at x1000 Magnification of samples for Atterberg limits: (a) natural soil, (b) Unsterilized soil treated with 1.20 x 10^9cells/ml suspension density at -2% OMC

4. Conclusions

In this study, the sterilized specimens recorded more improvement in the engineering properties of the lateritic soil through the decrease in PI values of the treated soil. This work has demonstrated that higher unconfined compressive strength for the treated soil can be obtained at lower degree of saturation, this finding is challenging the widely belief that bio-cemented soils need to be treated under full saturated conditions. Although soils are not normally sterilized before use in engineering practice, the UCS values recorded were favorably higher in unsterilized specimens compared to the sterilized, it has also been established that bio-cementation is the method to adopt for strength gain in MICP. It has also been demonstrated that other species of microorganisms present in the soil contribute in the MICP improvement of the engineering properties of soil. The SEM analysis presented, have also

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demonstrated that the MICP treatment of an A-4(3) lateritic soil using ureolysis resulted into formation of higher quantity of calcite precipitates in the unsterilized than the sterilized specimen.

The optimum mix of 1.20 x 10^9 /ml *S. pasteurii* suspension density is recommended for the treatment of an A-4(3) Lateritic soil which can be used in lightly trafficked road.

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