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Investigation of the Effects of Calcite Precipitate from Locally Sourced Microbes on some Engineering Properties of a Silty Clayey Soil

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Abstract
Microbially Induced Calcite Precipitation (MICP) is a bio-geochemical process that employs ureolytic bacteria to precipitate calcium carbonate within soil matrices, thereby enhancing engineering properties. This study examined the effect of locally sourced bacterial strains processed with cementation solutions on the plasticity, strength, and permeability properties of a silty clayey soil. The MICP treatment of the soil involved the use two bacterial concentrations (10^7 and 10^9 cfu/ml) with cementation solutions (0.25M, 0.45M and 0.50M concentrations). Bacillus anthracis was identified as a viable bacterial strain based on its high urease activity and high amounts of $CaCO_3$ precipitates. The soil was classified as A-2-6 under the AASHTO classification system. The untreated soil exhibited moderate plasticity (LL = 31.18%, PL = 19.13%, PI = 12%), low unsoaked CBR and UCS values of 24.36% and 0.74 kN/m² respectively, and high permeability coefficient (k) of 1.1×10^{-5}m/s, indicating poor subgrade quality. Microbial treatment significantly improved the properties of the soil: PI was reduced to 4% with bacteria and cementation of 10^9 cfu/ml and 0.50M respectively. The CBR improved from 24.36% to a maximum value of 36.05% with no curing, and a maximum value of 74.44% at 10^9 cfu/ml, 0.50M cementation, and 96 days curing. A maximum UCS value of 1.25 kN/m² at at 10^9 cfu/ml, 0.50M cementation, and 60 days curing, over the 0.74 kN/m² of the untreated soil was obtained. There was a reduction in the *permeability of the treated soil, with gradual increase of parameter as the bacterial and cementation concentrations increased. Based on the CBR values, the treated soil can be adopted for subgrade or sub-base course applications. This study showed that the calcite precipitate from Bacillus anthracis (a local bacteria strains) and 0.50M cementation solution effectively improved the engineering properties of silty/clayey soil for subgrade and sub-based applications, with adequate protection from water intrusion.*

Keywords: *Microbially Induced Calcite Precipitation (MICP), Indigenous microbial strains, Soil engineering properties, Subgrade, Sub-base.*

1.0 Introduction

Microbially Induced Calcium Carbonate Precipitation, also known as Microbially Induced Calcite Precipitation (MICP), refers to the same process of using microbes to form calcium carbonate in soils. MICP is a bio-geochemically driven calcium carbonate ($CaCO_3$) precipitation technology, that makes use of naturally occurring bacteria to bind soil particles together through the precipitation of calcite within the soil strata (Rimantas *et al*., 2017). Since the early 2000s, research on MICP has progressed through several key stages, including understanding its underlying mechanisms, selecting effective microbial strains, optimising chemical formulations, analysing microstructural changes, assessing environmental impacts, and developing predictive models. The type of mineral produced through microbial activity is governed more by the environmental conditions than by the biological mechanisms alone. Consequently, a single bacterial species may precipitate different minerals depending on the specific conditions in which it functions. In natural environments, $CaCO_3$ precipitation is best understood as the result of a complex interplay between chemical and biochemical processes (Ercole *et al*., 2001).

$CaCO_3$ exists in three polymorphic forms: calcite, aragonite, and vaterite (Al-Thawadi, 2013; Dhami *et al*., 2013). Calcite is the most common and most stable polymorph of calcium carbonate (see Figure 1), and is predominantly found in sedimentary rocks. Its crystals are trigonal–rhombohedral (Rimantas *et al*., 2017). Among the minerals formed through microbial mineralization, calcite is particularly valuable due to its stability and prevalence in natural settings. Although many microbial processes occur in nature, such as

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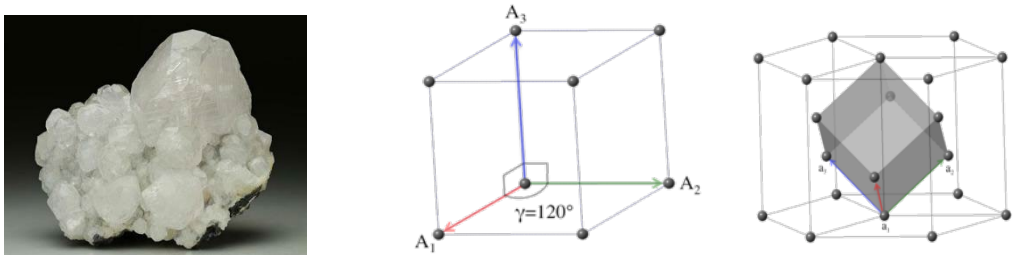
reduction, oxidation, and dissolution, only mineralization of inorganic substances can produce stable and strong binding material within the soil matrix (Ivanov and Chu, 2008).



(a) (b) (c)

Figure 1: Visual representation of calcite crystal morphology and unit cell geometry.
(a) Calcite (b) Trigonal Crystal System (c) Rhombohedral Crystal
Source: *(Google images; source unknown).*

Dhami *et al.* (2013) found that mineralization through urea hydrolysis is simple and easy to control, making it a better option than other microbial pathways. Efforts have been made to use denitrifying bacteria for precipitation (Van Paassen *et al.*, 2010; Martin *et al.*, 2013; Hamdan *et al.*, 2016). While these bacteria managed to cause precipitation throughout the soil, Van Paassen *et al.* (2010a) observed that the amount of $CaCO_3$ produced was much lower than the total converted, resulting in a non-homogeneous precipitation inside the soil. Additionally, Pham *et al.* (2016) pointed out that high levels of inhibitory intermediates like nitrite and nitrous oxide, along with the slower reaction rate of denitrification compared to urea hydrolysis, make denitrification less practical for soil stabilisation techniques.

Ureolytic bacteria can be found predominantly in nature. Nevertheless, only bacteria that possess high urease activity and produce high amounts of $CaCO_3$ precipitates within a short period of time are desired for MICP (Wei *et al.*, 2015; Kalkan, 2020). Numerous bacteria are known to produce urea, namely, *Proteus mirabilis, Proteus Vulgaris, Ureaplasma urealyticum, Corynebacterium urealyticum, Brucella spp., Helicobacter Pylori, Sporosarcina pasteurii, Bacillus megaterium, Bacillus sphaericus, Bacillus subtilis, Alcaligenes spp., etc.* (Bang *et al.*, 2001; Ercole, 2001; Ivanov and Chu, 2008; Achal *et al.*, 2012; Cheng *et al.*, 2014; Sikha, 2016; Rimantas *et al.*, 2017; Balu *et al.*, 2020; Kalkan, 2020; Muhammad *et al.*, 2020, 2021; Smith and Lee, 2022; Garcia and Patel, 2023).

The most commonly used bacteria for MICP are *Sporosarcina pasteurii* and the *Bacillus spp.* Ivanov and Chu (2008); Md Mizanur *et al.* (2020) described *S. pasteurii* as a type of aerobic bacterium more reliable in hydrolyzing urea into ammonia (moderate alkaline condition) by generating adenosine triphosphate (ATP) through its high secretion of urease enzyme. Figure 2 below shows the physical difference between the bacteria commonly utilised in the MICP process.

(a) Sporosarcina paste

Ivanov and Chu (2008) considered facultative anaerobic bacteria as the most suitable agent for in-situ MICP soil stabilisation due to their unique characteristics. Facultative anaerobic bacteria stay active in both oxygen-rich and oxygen-poor environments. They have Gram-positive cell walls that are very resistant to changes in osmotic conditions, showcasing their resilience pressure. Besides, these types of bacteria can be found effortlessly in the environment. Al-Thawadi (2013) highlighted that only pure ureolytic bacteria strains, which are cultivated under sterile conditions and without contamination, are compatible with MICP due to the presence of the high urease enzyme that mediates the urea hydrolysis catalysis. Burbank *et al.* (2012) further stated that only pure ureolytic Bacteria Cultures (BC) can produce highly active urease enzyme metabolic activity. The authors claimed that the desired urease activity of pure ureolytic BC for soil stabilisation is 4 – 50 mm urea/min. Burbank *et al.* (2011) proposed the use of enrichment mediums containing

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molasses, urea, sodium acetate trihydrate, ammonium chloride and yeast extract to enrich the production of indigenous, highly active ureolytic bacteria in-situ and to successfully induce soil bio-cementation. Cheng *et al.* (2013) produced highly active ureolytic bacteria by cultivating non-sterile chemostat cultures in a medium with a pH of 10 and a urea concentration of 0.17M. These conditions helped the growth of urease-active bacteria and facilitated the continuous reproduction of enriched bacteria on-site.

Precipitation of $CaCO_3$ by urea hydrolysis has been explained in detail in several studies (Hillgartner *et al*., 2001; Hammes *et al*., 2003; Burbank, 2010; Waller, 2011; Cheng, 2012; Martinez, 2012; Montoya, 2012; Cheng *et al*., 2014; Md Mizanur *et al*., 2020). In general, the process can be categorised into two main stages: (i) urea hydrolysis stage; and (ii) $CaCO_3$ precipitation. The following general chemical reactions of the urea hydrolysis process for the two stages were presented by Cheng *et al.* (2013):

$$CO(NH_2)_{2[aq]} + 2H_2O_{[l]} \xrightarrow{\text{Urease}} 2NH_{4\,[aq]}^{+} + CO_{3\,[aq]}^{2-} \quad (1)$$

$$Ca^{2+}{}_{[aq]} + CO_{3\,[aq]}^{2-} \xrightarrow{\text{Precipitation}} CaCO_{3[s]} \quad (2)$$

During the urea hydrolysis stage, 1 mole of urea $[CO(HN_2)_2]$ is hydrolysed to produce 2 moles of ammonium ions $[NH_4^+]$ and 1 mole of carbonate ion $[CO_3^{2-}]$. In the next stage, the calcium ion $[Ca^{2+}]$ derived from the calcium chloride $[CaCl_2]$ of the Cementation Solution (CS) reacts with the carbonate ion $[CO_3^{2-}]$ to form 1 mole of calcium carbonate $[CaCO_3]$ crystals.

Microbiological surveys and applied studies in Nigeria have further documented locally cultured ureolytic bacteria capable of urease activity and calcium carbonate precipitation. For example, isolates identified as *Bacillus spp*. were recovered from contaminated soils in Abeokuta and shown to possess metabolic capabilities relevant to bioremediation, demonstrating that culturable, robust Gram-positive strains occur in that locality (Adeosun *et al.*, 2015). Broader screening efforts in Nigeria and neighbouring regions have explicitly targeted soil ureolytic communities, isolating and characterizing strains from Nigerian sites and testing their urease activity, calcite precipitation in vitro (i.e., calcite precipitation under controlled laboratory conditions), and preliminary soil stabilization performance; these studies report promising ureolytic isolates and recommend local strain selection for MICP applications (Aliyu *et al*., 2023). Together, these works indicate that indigenous Nigerian bacteria with the biochemical capacity for MICP are present and can be cultured using standard enrichment and isolation protocols (e.g., urea-amended media, urease assays, and molecular identification). This research investigated the effect of calcite precipitate from locally sourced microbes on the atterberg limits, strength parameters and the permeability of a silty clayey soil.

2.0 Materials and Methods

The soil sample and the waste used in this study were obtained at the Federal University of Agriculture, Abeokuta (FUNAAB), located along Alabata Road in Odeda Local Government, Ogun State, Nigeria, with geographical coordinates Latitude 7°13′47.86″N and Longitude 3°26'21.14″E. The engineering properties of the untreated soil were determined following ASTM D698, ASTM D2166, ASTM D2434, ASTM D4318, and AASHTO T193 test methods. Subsequently, the soil was treated with cultured bacteria and the effects on the properties were also determined.

For the microbial analysis, some waste samples were collected from cow pens at the Farm Area, FUNAAB. They were extracted from the site topsoil at depths of 10, 20, and 30cm. Sampling was conducted using a sterile spatula, and the samples were placed in sterile sample bottles for laboratory analysis. The enrichment process involved inoculating 0.5g of the waste sample into 50ml of urea broth containing 6% (w/w) urea (HiMedia, sterile filtered 0.45μm, added after autoclaving). The mixture was incubated under aerobic batch conditions at 30^0C for 120 hours with shaking at 130 rpm. An aliquot of 1ml was then serially diluted, and 0.1ml of the enriched sample was inoculated onto urea nutrient agar plates, evenly spread using a sterilized L-shaped spreader, and incubated aerobically at 30°C for 24 hours. Representative colonies were subsequently subcultured onto Plate Count Agar, Eosin Methylene Blue (EMB) Agar, and Nutrient Agar, and incubated at 30°C for another 24 hours.

For the preparation of urea nutrient agar, the solid components were dissolved in distilled water to a final volume of one litre and mixed thoroughly. The mixture was gently heated to boiling, autoclaved for 15 minutes at 15 psi and 121°C, then cooled to 50 - 55°C. A sterile urea solution was added aseptically and mixed uniformly. The medium was immediately poured into culture plates under a laminar flow hood to minimize contamination.

Direct assays of urease activity were carried out by taking 1.0 ml of a 24 hours old culture, which was inoculated into bottles containing 9.0 ml of 1.11 M urea solution and monitored for 5 mins at $25 \pm 2C^0$. The respective conductivity values were measured and recorded by immersing the probe of the conductivity meter (EC800 Laboratory Benchtop Conductivity Meter, APERA) into the bacterial-urea solution (Harkes *et al*., 2010). At the end of the assay, a graph was plotted using the conductivity values (ms/cm) against time

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(min), the rate of conductivity change (ms/cm/min) was acquired from the slope of the plotted graph, which was then multiplied by the dilution factor and was taken as the ratio of the stock bacteria culture to the sampling bacteria culture before inoculation into the urea solution. The specific urease activity was derived by dividing the urease activity (mM urea hydrolysed/min) by the bacterial biomass OD600 (Whiffin, 2004) and OD was measured using a spectrophotometer (GENESYS™ 20, Thermo Fisher Scientific) at a wavelength of 600nm.

$$\text{Specific urease activity (mM urea hydrolysed/min/OD)} = \frac{(\text{mM urea hydrolysed.min}^{-1})}{\text{biomass (OD600)}} \quad (3)$$

Microbial inoculation was applied by infiltration at designated colony-forming units per millilitre (cfu/ml). Unlike the low-pressure percolation approach described by Van Paassen (2009), the cementation solution was incorporated using the premixing method, in which the bacterial suspension and cementation solution were uniformly mixed with the soil before specimen formation. This technique ensured even distribution of microbial activity and calcium sources throughout the soil matrix, thereby reducing the risk of near-surface clogging and enhancing homogeneity of treatment. The premixing method has been reported as an effective alternative for MICP in geotechnical applications, improving the strength and durability of treated soils (Muhammad *et al*., 2021; Liu *et al*., 2024).

By sourcing microorganisms locally, the need for long-distance transportation is eliminated, resulting in significant cost reductions and a reduced overall carbon footprint. Local microorganisms are already adapted to the region's environmental conditions, making them more resilient and effective. This approach supports the local ecosystem and avoids introducing potentially invasive species that could disrupt ecological balance. Additionally, using indigenous bacteria promotes ecological harmony and improves the efficiency of the MICP process.

3.0 Results and Discussion

Identified Bacteria

All isolates identified in this study were urease-positive and motile, indicating their ability to hydrolyze urea and actively migrate through soil pore spaces, traits that are particularly advantageous for MICP. The predominance of Gram-positive *Bacillus spp.* isolates was observed. These bacteria displayed rod-like forms and possess thick peptidoglycan cell walls, which confer resilience against environmental stresses such as drought, salinity, and pH fluctuations. This structural advantage allows them to persist longer in soil environments and maintain metabolic activity under challenging conditions (Dick, 2009). Gram-positive bacteria such as *Bacillus spp.* are frequently reported as efficient ureolytic organisms due to their robustness and ability to thrive in alkaline environments, which favour calcite precipitation (Achal and Pan, 2019).

Gram-negative bacteria, including *Alcaligenes spp.*, while less stress-tolerant, contribute significantly to urease activity and influence microbial community dynamics. Recent studies emphasize that both Gram-positive and Gram-negative groups play complementary roles: Gram-positives provide stability and persistence, while Gram-negatives enhance adaptability and functional diversity (Wang *et al.*, 2024; Loiko and Islam, 2024). Thus, the coexistence of both groups in the isolates highlights a balanced microbial system where resilience and adaptability jointly support effective calcite precipitation.

Urease activity was consistently positive across all identified isolates, confirming their ability to hydrolyze urea into ammonia and carbonate ions, which are essential precursors for calcium carbonate precipitation. However, high urease activity, was seen in *Bacillus anthracis*, and this directly correlates with stronger calcite formation.

Engineering Properties of Soil Sample

The untreated soil was classified as A-2-6 under the American Association of State Highway and Transportation Officials (AASHTO) soil classification system, indicating a silty or clayey gravel and sand with fair-to-poor subgrade quality. The liquid limit (LL) and plastic limit (PL) were 31.18% and 19.13% respectively, while the plasticity index (PI) was 12.05% (Table 1). These values suggest moderate plasticity, which can influence the soil's compressibility and susceptibility to volume changes under varying moisture conditions. The unsoaked and soaked CBR values were 24.36% and 9.16% respectively. The soaked value is about 62% reduction compared with the unsoaked value, showing the weak load-bearing tendency of the soil when in contact with water. The UCS value of 0.74 kN/m^2 reflected the limited shearing strength of the soils. These values are below the standard value typically required for pavement subgrades, which often demand CBR values above 30% (General Specifications for Road and Bridges, 1997). In addition, the soil exhibited a permeability coefficient (k) of 1.1 x 10^{-5} m/s, which is relatively high and indicates a material prone to seepage and reduced durability when exposed to water. Such permeability values further highlight the soil's vulnerability under field conditions, particularly in pavement or foundation applications.

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Table 1: Engineering properties of soil sample

Bacillus anthracis and A-2-6 soil		Untreated soil	Bacteria Concentration - 10^7			Bacteria Concentration - 10^9		
			0.25M	0.45M	0.50M	0.25M	0.45M	0.50M
Liquid limit (%)		31.18	40.87	33	23	31	16.4	11
Plastic limit (%)		19.13	16.43	14.74	10.92	19.59	11.24	6.99
Plasticity index		12.05	24.44	18.26	12.08	11.44	5.16	4.01
Optimum Moisture Content (%)		12.06	8.02	10.30	12.65	14.17	13.23	12.06
Maximum Dry Density (g/cm^3)		1.98	1.67	1.53	1.33	2.09	1.92	1.98
Permeability coefficient (m/s)		1.1×10^{-5}	1.36×10^{-6}	1.43×10^{-6}	1.48×10^{-6}	1.38×10^{-6}	1.45×10^{-6}	1.52×10^{-6}
	Curing days							
CBR (%)	0	24.36	26.11	27.87	30.22	31.90	32.54	36.05
	1		32.74	32.15	38.39	38.00	39.75	39.95
	3		38.00	38.58	47.94	46.77	52.61	56.51
	7		46.77	49.11	52.61	58.46	61.38	63.33
	28		53.59	54.56	57.10	68.20	69.18	71.13
	60		50.66	50.66	54.76	63.92	66.25	67.42
	96		61.38	61.77	65.28	64.31	74.44	68.20
Average CBR (%)			47.19	47.81	52.68	56.61	60.60	61.09
UCS (kN/m^2)	0	0.74	0.83	0.87	0.83	0.84	0.82	0.83
	1		0.55	0.58	0.77	0.59	0.62	0.65
	3		0.66	0.74	0.89	0.70	0.80	0.82
	7		0.78	0.81	0.99	0.82	0.96	0.99
	28		0.84	0.93	1.12	1.00	1.10	1.18
	60		0.95	1.08	1.23	1.12	1.25	1.25
	96		0.83	0.86	1.01	1.01	1.11	1.18
Average UCS (kN/m^2)			0.77	0.83	1.00	0.87	0.97	1.01

Effects of Bacteria on Soil Properties

Following microbial treatment, notable improvements in engineering properties were observed. To provide a clearer comparison of the untreated soil and the microbial treatments, the key engineering properties of the control and treated soils are summarized in Table 1.

Bacillus anthracis reduced the PI to 4% at bacterial concentration of 10^9 cfu/ml and cementation solution of 0.50M. The PI value was 67% reduction when compared with the untreated soil, indicating improvement in its plasticity. This further indicated that these strains can effectively stabilised the soil and reduced its susceptibility to shrink–swell behaviour. The bacteria generally improved the bearing capacity of the soil for both bacteria concentrations and all the cementation solutions, and curing days. The CBR was improved to a maximum value of 36.05% with no curing, and a maximum value of 74.44% at 10^9cfu/ml, 0.50M cementation, and 96 days curing. All the CBR values of the treated soil can be adopted for subgrade or sub-base course applications. Furthermore, a maximum UCS value of 1.25 kN/m^2 at 10^9 cfu/ml, 0.50M cementation, and 60 days curing, a 69% increase over the 0.74 kN/m^2 of the untreated soil. Higher bacterial and cementation concentrations, and longer curing times, confirmed the role of calcite precipitation in strengthening the soil matrix.

Bacillus anthracis caused a reduction in the permeability of the treated soil. However, gradual increase of permeability was observed as the bacterial and cementation concentrations increased. The bacteria was reported to be aerobic, spore forming, gram-positive, and non-motile bacillus. The spores form chains of rods that look like bamboo sticks and encapsulated (Cheng *et al.*, 2013). It can thus be hypothesized that the bacterial and cementation concentrations and non-motility caused lateral elongation of the bacteria without bridging, a phenomenon that increased the pores of the bacteria-soil blends. However, this can be validated or otherwise by microstructural analysis or other tests aside the ones conducted in this study.

4.0 Conclusion

1. The study revealed that microbial calcite precipitation (MICP) from *Bacillus anthracis* enhanced with cementation solution can effectively stabilized silty/clayey soil.
2. Substantial reduction in its' plasticity, and increase in load-bearing and shearing capacities were obtained which makes the soil adoptable as subgrade and sub-base course applications.

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3. Microbial calcite precipitation is thus a promising alternative to traditional chemical stabilisers, presenting a more environmentally friendly solution for soil stabilisation in Nigeria and comparable settings.

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