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RESEARCH ARTICLE

Bio-cementation of tropical lateritic soil via microbially induced calcite precipitation: Strength enhancement and microstructural mechanisms

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Abstract - This study investigates the effectiveness of Microbially Induced Calcite Precipitation (MICP) using *Bacillus subtilis* as a sustainable technique for biochemically stabilizing laterite soil. Lateritic soils, common in tropical regions, are problematic in construction due to high porosity, low strength, and brittle stress–strain responses. The main objectives were to characterize the physical properties of untreated laterite soil, evaluate the effect of MICP treatment on its stress–strain behaviour, and correlate mechanical improvements with microstructural changes using scanning electron microscopy (SEM) and quantitative image analysis. Results showed that untreated soil exhibited low peak stress (52 kN/m^2) and brittle failure dominated by splitting cracks. Following MICP treatment, peak stresses improved markedly to 235 kN/m^2 at 7 days, 296 kN/m^2 at 14 days, and 420 kN/m^2 at 30 days of curing. This shows that the strength exceeds commonly reported minimum requirements for stabilized subgrades in tropical pavement systems. Stress–strain curves showed higher stiffness, improved ductility, and more stable post-peak behaviour, while failure shifted toward cleaner shear cracking. The morphological analysis by SEM and image analyses confirmed progressive calcite precipitation, void filling, and stronger particle bonding over curing time. This study advances current knowledge by systematically integrating mechanical testing with microstructural quantification to elucidate the mechanics governing bio-cementation in laterite soils. These findings establish MICP as a low-carbon, environmentally friendly alternative to conventional chemical stabilizers, offering promising applications for subgrades and foundations in tropical geotechnical engineering.

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1. Introduction

Laterite soils, abundant in tropical regions such as Southeast Asia, Africa, and South America [1], are among the most challenging geomaterials in geotechnical engineering [2] due to their low shear strength [3], high compressibility [4], high porosity [5], and heterogeneous particle size distribution [6] Laterite soils are formed through intense and prolonged tropical weathering, which depletes silica ($SiO_2 < 30\%$) and enriches iron ($Fe_2O_3 \approx 40–50\%$) and aluminium oxides ($Al_2O_3 \approx 20–30\%$) [7], imparting a distinctive reddish coloration but resulting in poor bearing capacity and high plasticity, failing to meet subgrade and base course requirements in standards [8]. The most common method for lateritic soil improvement is chemical stabilisation. According to Wahab et al. [9], 6% cement with 7 days curing achieved 0.8 MPa UCS for low-volume roads, meeting Malaysian standards, with durability maintained over 15 wetting–drying cycles due to calcium aluminate hydrate, calcium silicate hydrate, and CASH gel formation and pore refinement, yet this approach is economically and environmentally costly, with cement production accounting for approximately 7–8% of global CO_2 emissions [10]. In addition, recycled gypsum waste such as magnesium-rich gypsum has been investigated for soil improvement, showing potential to reduce compressibility and enhance stability through cementitious reactions [11] - [12]. In addition to cement, other industrial solid waste materials have been explored for the stabilisation of lateritic soil. For example, cement kiln dust at dosages of 10–16 % has been shown to significantly increase the strength of lateritic soils due to its high alkalinity and the formation of cementitious gels such as calcium aluminate hydrate and calcium silicate hydrate through pozzolanic reactions [13] – [17]. Similarly, aluminium dross (AD) at 4–16% has been reported to enhance the California Bearing Ratio (CBR) and unconfined compressive strength (UCS) while reducing the plasticity index (PI), thereby improving workability and stability [18]. Fly ash (FA), when combined with synthetic fibre reinforcement, has also demonstrated promising results; mixtures containing 5–15 % FA and 0.25–1.0% fibre have achieved notable increases in UCS and CBR, with fibre contributing to tensile resistance and improved crack control [19]. Plastics and ceramics used at 10–20% content have been shown to reduce deformation and improve strength, attributed to pozzolanic activity between soil minerals and the aluminosilicate compounds in the waste materials [20]. Furthermore, incorporating shredded rubber tyre or polymer sack fibres has provided measurable gains in shear strength, durability, and resilience under repeated wetting–drying cycles [21] - [22]

Nonetheless, waste-based stabilisers are not without disadvantages. These materials can exhibit significant variation in chemical composition depending on the source and processing technique, resulting in varying performance outcomes [23]. Furthermore, these materials may contain trace elements or compounds that cause potential leaching to the supporting environment [24]. In addition, performance may be highly sensitive to local conditions that affect stability and efficiency, including soil mineralogy, moisture, and temperature; hence, it may be equally inconsistent across various environmental conditions [25]. These uncertainties may constrain their suitability for wider application or long-term use

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without detailed characterisation of the materials and field verification. Accordingly, a new stabilisation technique, MICP has emerged as a sustainable and environmentally friendly alternative to conventional methods [11], [26]- [27]. MICP employs ureolytic bacteria to biologically precipitate calcite [28], which binds soil particles [29], increases strength [26], reduces permeability [30] and improves soil quality by enhancing soil stiffness [31]. Unlike traditional approaches that rely on cement, lime, or waste-based additives, MICP offers a low-carbon solution by leveraging natural biochemical processes [32]. Although widely applied to sandy soils, its application to lateritic soils remains limited [33], with further research needed to understand the morphological and microstructural changes induced by calcite precipitation and to optimise treatment conditions for tropical, highly weathered soils.

Several recent studies have highlighted the capability of MICP to stabilise lateritic soil, including substantial increases in UCS, reductions in permeability, and enhanced structural integrity under optimised bacterial concentration, curing duration, and compaction energy [34] – [37]. Peak strengths exceeding 3000 kN/m^2 have been reported under controlled laboratory conditions by treating lateritic soil with *B. pumilus* suspensions and cementing reagents [35], while other studies confirm improved plasticity characteristics and compaction behaviour associated with increased $CaCO_3$ precipitation [2]. Despite these promising findings, most investigations primarily quantify bulk carbonate content or peak strength gain, with limited emphasis on the mechanistic relationship between calcite morphology, pore-scale fabric evolution, and macroscopic stress–strain response in highly weathered lateritic matrices. Furthermore, most reported studies focus on *Bacillus pumilus*, *Bacillus megaterium*, or *Sporosarcina pasteurii*, leaving other potentially viable ureolytic strains comparatively underexplored. Several strains of ureolytic bacteria have been used in MICP applications on lateritic soils, including *Bacillus pumilus*, *Bacillus megaterium*, and *Sporosarcina pasteurii*, which show varied urease activity, calcite precipitation capacity, and adaptation to environmental conditions. However, [38] claims that *Bacillus subtilis* not only improves soil structure but also enhances nutrient availability, which is crucial for plant growth in challenging soil conditions like laterite soil. Its ability to form durable biofilms enhances bacterial retention within the soil matrix, promoting uniform calcite nucleation and improved particle bonding [39]. Additionally, the metabolic versatility of *B. subtilis* supports sustained biochemical activity in iron- and aluminium–rich lateritic environments [40]

Building on recent evidence supporting MICP as a sustainable and self-healing stabilisation method for lateritic soils, this study evaluates the effectiveness of *Bacillus subtilis*–induced calcite precipitation in enhancing the mechanical strength and microstructural characteristics of tropical lateritic soil. Specifically, the study aims to (i) quantify the strength improvement through UCS testing, and (ii) characterise microstructural evolution using scanning electron microscopy (SEM) and ImageJ analysis to establish the relationship between calcite morphology, pore filling, and strength gain, with emphasis placed on linking fabric transformation and mechanical response rather than on bulk $CaCO_3$ quantification. This study presents the first systematic investigation of *Bacillus subtilis*–induced MICP for the stabilisation of tropical lateritic soil, addressing a clear gap in existing MICP research that has largely focused on *Bacillus pumilus, Bacillus megaterium*, and *Sporosarcina pasteurii*. The findings provide new insights into bacterial–soil compatibility in highly weathered, iron– aluminium–rich geomaterials. Unlike conventional MICP studies that correlate strength enhancement primarily with bulk $CaCO_3$ content, this work emphasises the linkage between microstructural fabric transformation and mechanical response, demonstrating how calcite morphology, pore-filling efficiency, and particle bonding govern UCS improvement in lateritic soils. A quantitative SEM–ImageJ–based microstructural analysis framework is introduced to evaluate pore refinement and bio-cementation patterns in MICP-treated lateritic soil, providing a reproducible, mechanistic approach to interpreting strength development beyond empirical carbonate measurements.

2. Methods and Materials

2.1 Lateritic Soil

Laterite soil samples were collected from Kuantan, Malaysia, air-dried, sieved, and stored to maintain their natural properties. The properties of laterite soil are summarised in Table 1. The studied laterite soil exhibits a specific gravity of 2.70, which falls within the typical range for Fe–Al oxide–rich tropical residual soils (2.60–2.73) [41]. Its low plasticity (LL = 22.9%, PI = 1.4%) is well below the common range for Malaysian laterites (LL ≈ 5–75%, PI ≈ 12–48%) [42], indicating a predominantly silty–sandy matrix with low clay activity. Accordingly, the soil is representative of low-plasticity lateritic materials rather than highly plastic, clay-rich laterites. [43] - [44] observed that low-plasticity soil facilitates better transport of bacteria and cementation solution migration compared to higher clay content; the pore connectivity is expected to restrict bacteria movement and ion transport. The maximum dry density (1.24 g/cm^3) is comparatively low relative to the ~1.39 g/cm^3 often reported for Malaysian laterites [45], suggesting a high-void fabric and loose particle arrangement. The untreated UCS of 52 kN/m^2 lies toward the lower end of the reported range for similar soils (18–200 kN/m^2) [9], highlighting its limited load-bearing capacity and confirming the need for stabilisation to satisfy Malaysian Public Works Department Road subgrade strength requirements (≥ 0.8 MPa) [46].

2.2 Bacteria

Ureolytic bacteria are widely employed in Microbially Induced Calcium Carbonate Precipitation (MICP) due to their ability to catalyse urea hydrolysis via the urease enzyme [47]. This enzymatic reaction hydrolyses urea into ammonium (NH_4^+) and carbonate (CO_3^{2-}) ions, which, in the presence of calcium ions, promote the precipitation of calcium carbonate ($CaCO_3$) [48]. In the present study, *Bacillus subtilis* was selected for its ureolytic activity, adaptability to varying environmental conditions, and documented effectiveness in bio-cementation processes [49] - [50]. The bacterial culture was procured from IM Bio Tech Aquatic, Pulau Pinang, Malaysia. Upon introduction into the soil matrix, *B. subtilis* facilitates the in-situ precipitation of calcium carbonate, which binds soil particles, reduces pore space, and consequently enhances the mechanical and geotechnical properties of the treated lateritic soil.

Table 1. Basic soil-engineering properties of laterite soil

Properties	Method	Value/measure
Specific gravity	BS 1377: Part 2: 1990	2.70
Liquid limit (%)	BS1377-2: 1990	22.90
Plasticity index	BS 1377-4: 1990	1.40
Maximum dry density (g/cm³)	BS 1377-4: 1990	1.24
Optimum moisture content (%)	BS 1377-4: 1990	19.40
UCS (kN/m^2)	BS1377-7: 1975	52.39

2.3 Chemical Reagents

Chemical reagents used in this experiment, shown in Table 2, were used to enable the MICP process by supplying nutrients, calcium sources, and an optimum pH at which *Bacillus subtilis* can precipitate $CaCO_3$. Analytical grade urea served as the primary nutrient for bacterial urease activity, while calcium chloride ($CaCl_2$) supplied calcium ions for calcite precipitation. The pH was adjusted with sodium hydroxide (NaOH) and sodium acetate ($C_2H_3NaO_2$) to create an appropriate biochemical environment, and nutrient broth was used to enrich the bacterial culture. All the reagents were assembled in accurate quantities with distilled water to ensure the purity and consistency of the solutions. The preparation of enrichment and cementation solutions was according to [51] – [53], with the cementation solution containing all reagents, whereas the enrichment solution excluded nutrient broth. All experimental procedures were carried out at the Cell and Molecular Laboratory, Centre for Research in Advanced Fluid and Processes (CARIFF), Universiti Malaysia Pahang Al-Sultan Abdullah (UMPSA), Gambang Campus.

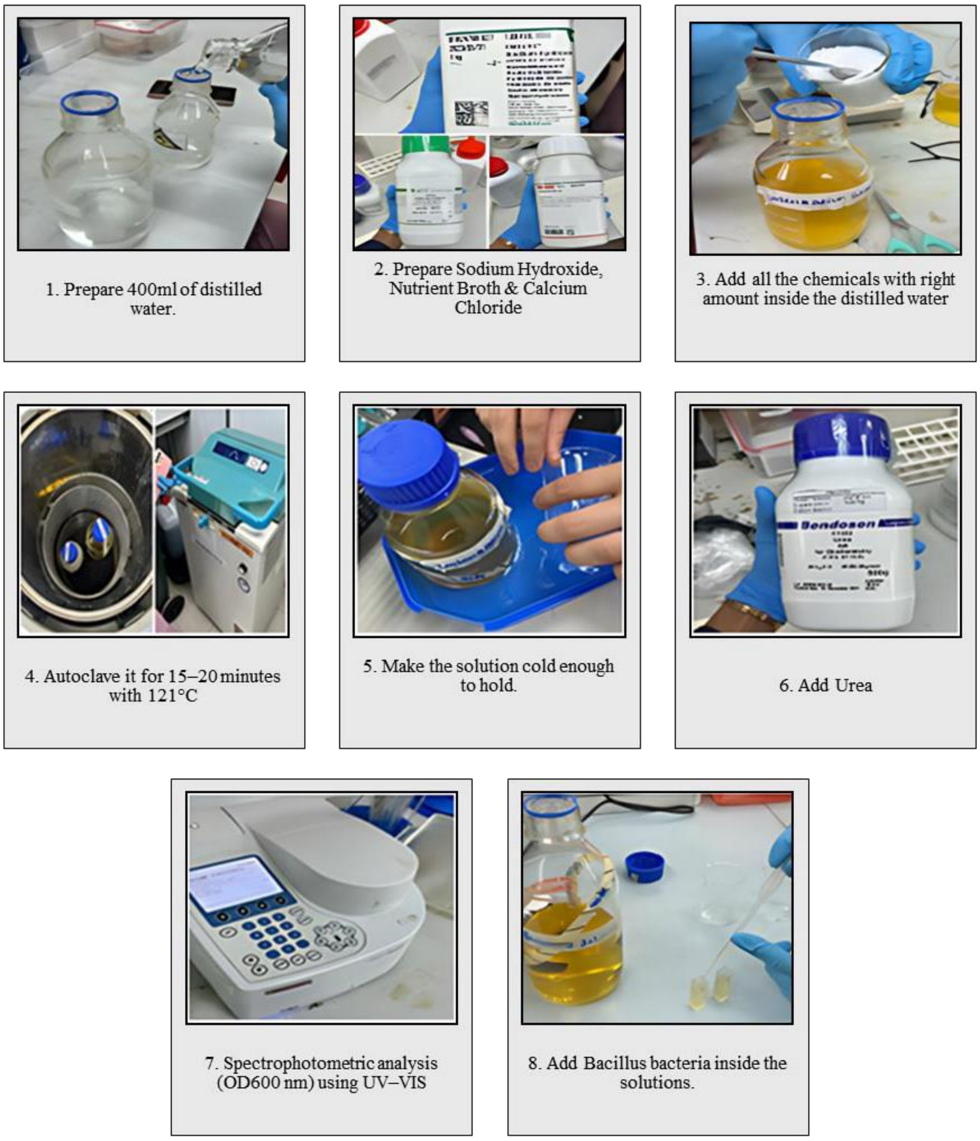

Figure 1. Preparation of enrichment solutions

Table 2. Chemical reagents

Chemical	Function
Urea ($CO(NH_2)_2$)	Nutrient source for bacteria
Calcium Chloride ($CaCl_2$)	Calcium source
Sodium Hydroxide (NaOH)	pH adjustment
Nutrient Broth	Base for Enrichment
Sodium acetate ($C_2H_3NaO_2$)	pH adjustment

2.4 Preparation of the Enrichment and Cementation Solution

The enrichment and cementation solutions were prepared following the procedures outlined in Tiwari and Satyam [51] and Rababah et al. [53], as illustrated in Figures 1 and 2. For both solutions, 400 mL of distilled water was measured into a sterile glass container, after which sodium hydroxide, nutrient broth, and calcium chloride ($CaCl_2$) were weighed in their specified amounts. For the enrichment solution, 4.1015 g of sodium acetate ($C_2H_3NaO_2$), 8.333 g of urea (CH_4N_2O), and 6.0 g/L of nutrient broth were added to the distilled water. For the cementation solution, the same formulation was used with the addition of 73.51 g of $CaCl_2$ to provide a calcium source for $CaCO_3$ precipitation. All chemicals were thoroughly mixed into the distilled water until fully dissolved, and the solution was sterilised using an autoclave at 121 °C for 15–20 minutes. Following sterilisation, the solutions were cooled to a safe handling temperature. For the enrichment solution, *Bacillus subtilis* was inoculated at a density of 1×10^6 CFU/mL. The bacterial concentration was confirmed by spectrophotometric analysis at 600 nm (OD_{600}) using a UV–Vis spectrophotometer, with an optical density of 0.08–0.13 corresponding to approximately 1×10^8 CFU/mL. Serial dilutions were performed as needed to achieve the target concentration for experimental use. The cementation solution, containing the same components as the enrichment medium plus $CaCl_2$, was prepared and stored for immediate application without bacterial inoculation. To ensure accurate bacterial dosing, the concentration of *Bacillus subtilis* was quantified by measuring optical density at 600 nm (OD_{600}). The supplier's calibration curve indicated that an OD_{600} range of 0.08–0.13 corresponds to approximately 1×10^8 CFU/mL. To achieve the target working concentration of 1×10^6 CFU/mL used in this study, two sequential 1:10 serial dilutions were performed using sterile distilled water. Fresh inoculum was prepared for each batch, and OD_{600} was verified immediately prior to mixing to minimise cell decay and ensure consistent viability across all treated specimens.

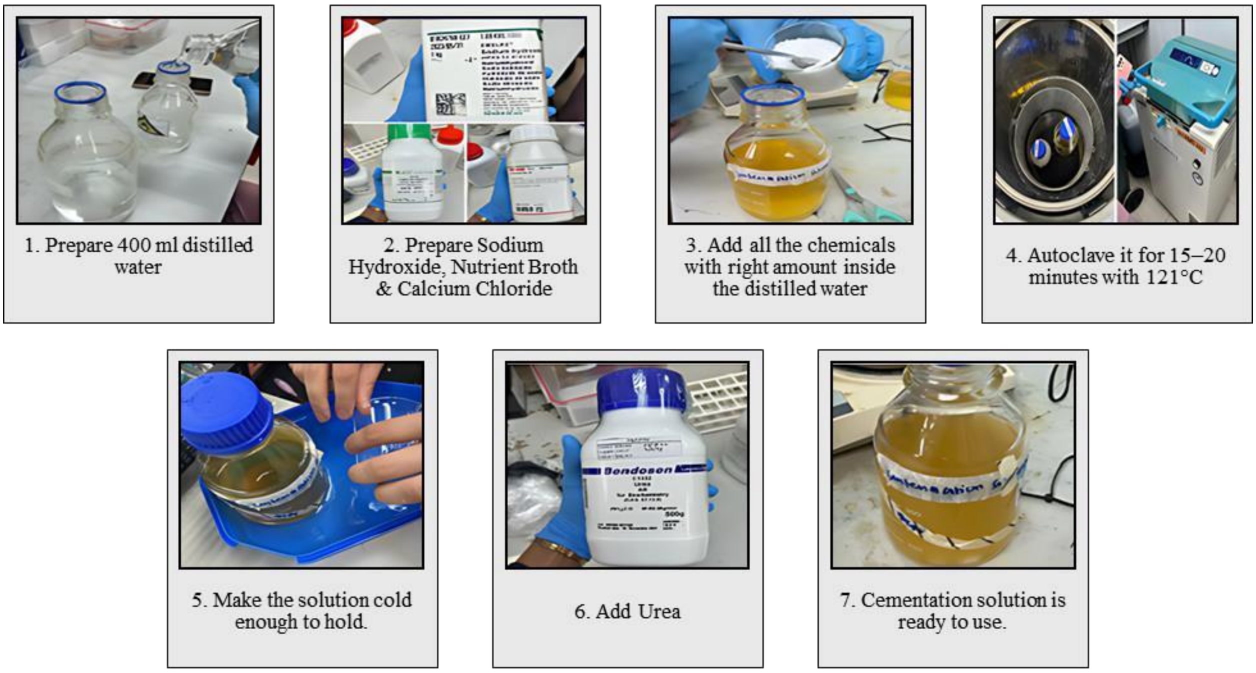

Figure 2. Preparation of cementation solutions

2.5 Sample Preparation

Lateritic soil samples were collected from KotaSAS, Kuantan (3°51'54.80"N, 103°17'11.67"E) at varying depths to capture differences in subsurface properties. After collection, the soil was air-dried at room temperature, stored in airtight containers to prevent moisture reabsorption, and kept under controlled laboratory conditions until testing. Prior to characterisation, representative portions were oven-dried at 105 °C for 24 hours to obtain a constant weight, ensuring uniformity in subsequent analyses. All geotechnical testing was carried out at the Soil Mechanics and Geotechnics Laboratory, Universiti Malaysia Pahang Al-Sultan Abdullah (UMPSA), Gambang, in accordance with British Standard BS 1377:2022. The characterisation program included the determination of moisture content, specific gravity, Atterberg limits and compaction properties (optimum moisture content and maximum dry density). These tests provided a comprehensive assessment of the soil's physical and mechanical properties, serving as the baseline for evaluating the performance of MICP treatment. The soil sample was sieved through a 2 mm mesh sieve and oven-dried at 105 °C to eliminate any existing microorganisms before adding the bacterial solution for all tests. Standard compaction tests were performed to obtain the maximum dry density (MDD) and optimum moisture content (OMC). The soil samples were mixed with an enrichment solution equal to 75% of the OMC and followed by a cementation solution for the remaining 25% of the OMC at a density of 1×10^6 CFU/mL, to maintain adequate moisture conditions for bacterial activity and uniform calcite precipitation while preserving the compaction characteristics of the soil matrix. In the mixing process, the enrichment solution was mixed first, followed by the cementation solution. The soil samples were thoroughly mixed to ensure a uniform distribution within the soil matrix. The soil specimens were then prepared for UCS and wrapped in plastic to maintain the optimum moisture content. To evaluate the effect of short-term and long-term improvements, the samples were allowed to cure for (7, 14, and 30) days (refer to Figure 3).

Figure 3. Sample preparation

2.6 Atterberg Limit Test

The liquid limit (LL) and plastic limit (PL) of the lateritic soil were determined in accordance with BS EN ISO 17892-12. Due to the fibrous nature of the laterite, coarse fibres were carefully removed using tweezers prior to testing, as permitted by the standard. The remaining soil fraction was thoroughly mixed with distilled water using a broad-bladed knife to form a homogeneous paste. The LL was determined using the Casagrande cup method, with moisture content measured at the closure point after 25 blows. The PL was determined by hand-rolling soil threads on a glass plate until they reached a diameter of 3 mm, at which point the moisture content was recorded. All tests were conducted in triplicate, and the plasticity index (PI) was calculated as the difference between LL and PL.

2.7 Standard Compaction Test

The compaction characteristics of the soil were determined using the Standard Proctor method in accordance with BS 1377-2:2022. Air-dried soil passing the 2 mm sieve was thoroughly mixed with varying moisture contents and compacted in a standard Proctor mould in three equal layers, each receiving 25 blows from a 2.5 kg rammer dropped from a height of 300 mm. The bulk density of each specimen was measured, and the corresponding dry density was calculated. The maximum dry density (MDD) and optimum moisture content (OMC) were obtained from the peak point of the dry density–moisture content curve

2.8. Curing Test and Unconfined Compressive Test

After thorough mixing of the soil, bacterial culture, and treatment solutions, the prepared specimens were compacted into cylindrical moulds for UCS testing and cured at 25 °C in the Environmental Laboratory, UMPSA, Gambang. The initial curing period lasted 7 days under controlled room temperature to promote bacterial activity, urea hydrolysis, and the precipitation of calcium carbonate within the soil matrix. During this stage, ureolytic bacteria metabolised the supplied urea, increasing pH and inducing $CaCO_3$ formation, which bonded the soil particles. Following the initial seven-day curing phase, specimens were maintained at 25 °C for a further 14 days to allow the precipitates to stabilise and mature, ensuring a more uniform distribution of $CaCO_3$ throughout the soil matrix. Curing durations of 7, 14, and 30 days were employed to assess short- and long-term strength development. The mixing process for incorporating the enrichment solution into the soil is illustrated in Figure 4. At the end of each curing period, UCS tests were performed in the Soil Mechanics and Geotechnics Laboratory at UMPSA using the unconfined compression method. A compressive load was applied axially to each specimen until failure, and the peak load was recorded for use in the stress-strain diagram. These results provided a quantitative evaluation of the improvement in soil strength and stability achieved through MICP treatment.

Figure 4. Sample preparation for UCS test

2.9 Scanning Electron Microscope

Following the curing process and UCS testing, representative specimens of the treated lateritic soil were prepared for microstructural analysis using a field emission scanning electron microscope (FEI Quanta 450 SEM) at the Centre for Research in Advanced Fluid and Processes, UMPSA, Gambang. To study the morphology and microstructure of the bio-treated soil, SEM imaging has been used to examine particle arrangement, the quantity and location of calcium carbonate precipitates, and changes in pore structure induced by the MICP treatment. These observations provided direct visual evidence of the cementation mechanisms contributing to the enhanced geotechnical properties.

3. Results and Discussion

3.1 Atterberg Limit Analysis

The Atterberg limits of the untreated lateritic soil were determined to assess its plasticity. Figure 5 illustrates the soil's position on the plasticity chart based on the LL and plasticity index (PI). The findings show that the soil has a liquid limit of 22.9% and plasticity index of 1.4%, which is much lower than the A-line of the Casagrande plot; thus, it is an ML (inorganic silt of low plasticity). According to the low PI, the soil tends to exhibit nearly non-plastic behaviour, usually attributed to laterites, which contain large proportions of silt and sand fractions with little clay activity [54]. This is consistent with the observed soil texture, which appeared friable and granular rather than cohesive [55]. The low clay fraction reduces the soil's capacity to retain water and decreases its potential for shrink–swell activity, making it more dimensionally stable but limiting its natural binding properties [56] - [57]. The plasticity of the current soil is substantially lower than that of lateritic soils in Malaysia. As an example, Saeed et al. [58] cited LL of 40-73% and PI of 19-30% for fine-grained lateritic soils in Pahang, whereas Yunusa et al. [59] reported LL of 57.5% and PI of 26.7%. In yet another Malaysian example, Uba Zango et al. [60] measured very high plasticity (LL = 79%, PI = 49%). These studies consistently highlight the highly plastic nature of many Malaysian laterites, in stark contrast to the present soil, which exhibits only marginal plasticity. Beyond Malaysia, soils from neighbouring regions also exhibit higher plasticity than those in the current study. As an example, lateritic soils in Thailand, as identified by Sudla et al. [61], had LL = 40.7% and PI = 19.8%, whereas in Indian lateritic soils [62], LL = 56% and PI = 27%. Both datasets indicate greater moisture sensitivity and clay activity than those of the soil tested in the present study. The low plasticity value observed in this study may be attributed to the high proportion of coarser particles and the relatively low clay mineral content [63]. It is the opposite of finely grained laterites high in kaolinite and iron-aluminium oxides that overall provide greater plasticity. The findings imply that although the current soil is actually lateritic in nature, its weathering level and resulting clay fraction are at the lower end of levels reported publicly in Malaysia and thus more similar in nature to the silty laterites of Brazil [64] and Nigeria, where LL values of 16-44% and PI values of non-plastic or 20% have been recorded. Overall, the Atterberg limits obtained in this study indicate that the tested lateritic soil is a low-plasticity silt (ML), in strong contrast to the higher-plasticity laterites widely reported in Malaysia and Southeast Asia. This low plasticity implies reduced volumetric instability but reinforces the need for stabilisation to enhance strength and durability for engineering applications.

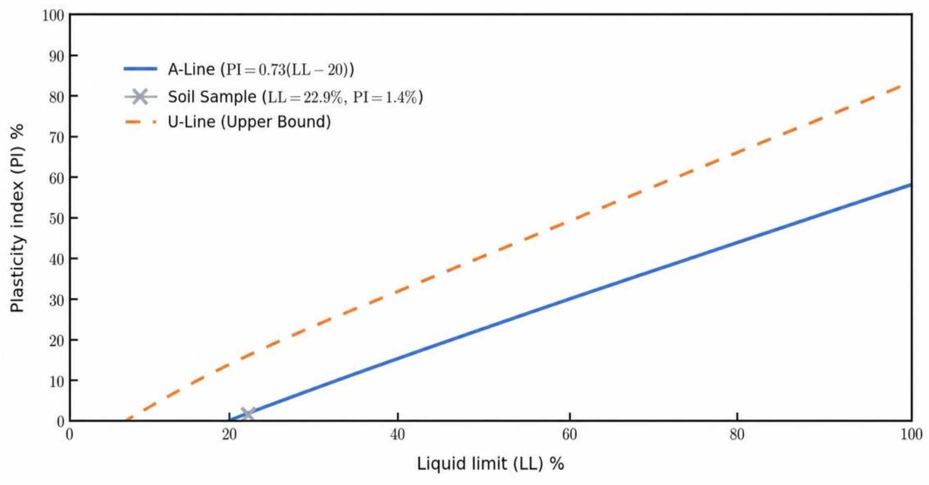

Figure 5. Plasticity chart

3.2 Specific Gravity

The specific gravity of the tested lateritic soil in this research was 2.70 Mg/m^3, which falls within the typical range for lateritic soils (2.60-2.86 Mg/m^3). The given SG yields results comparable to previous studies and thus consistent with reports on lateritic soils in Malaysia. For example, Saeed et al. [58] and Eisazadeh et al. [65] recorded values of 2.75 Mg/m^3, while [59] - [60] reported slightly lower values of 2.64–2.63 Mg/m^3. Similarly, Rashid et al. [66] documented 2.69 Mg/m^3, again close to the present finding. The stability of results across the Malaysian laterites indicates a strong preponderance of quartz, kaolinite, and iron-aluminium oxides in the soil mineralogy, resulting in an SG of around 2.65-2.75 Mg/m^3. The relatively moderate value observed in this study (2.70 Mg/m^3) indicates that the tested soil has a balanced proportion of siliceous and ferruginous forms and a low organic matter content.

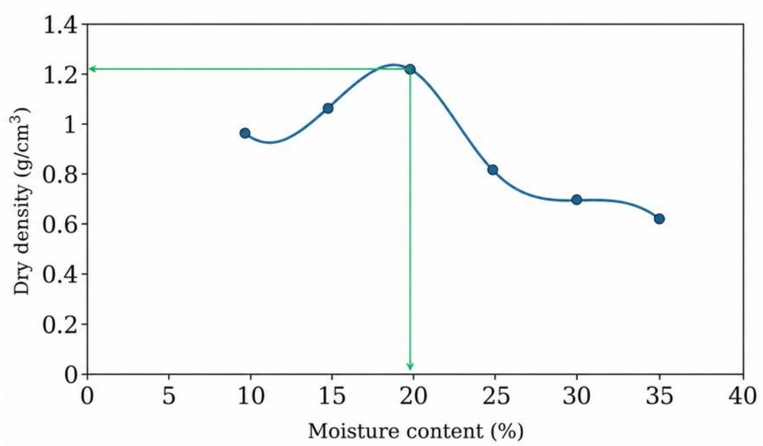

Figure 6. Compaction curve

3.3 Standard Compaction Test

The compaction behaviour of the tested laterite soil was evaluated in accordance with BS 1377-2:2022, using the standard Proctor method. Figure 6 illustrates the dry density–moisture content relationship, from which the maximum dry density (MDD) of 1.24 g/cm^3 and the optimum moisture content (OMC) of 19.40% were obtained. The studied laterite soil has a well-defined peak, with a maximum dry density of 1.24 g/cm^3 and an optimum moisture content (OMC) of 19.40%. This correlation reflects the typical compaction characteristic of fine-grained lateritic soils, whereby dry density increases with increasing moisture up to an optimum level, beyond which additional water decreases density [67] - [68]. At low moisture content, there is insufficient water to lubricate soil particles, leading to decreased density, as deformation in form cannot occur easily due to the water resistance of the soil [69]. As moisture content increases, water films form around particles, reducing interparticle friction and promoting closer packing, thereby increasing dry density [70] - [71]. Beyond the OMC, however, dry density drops rapidly along the compaction curve, reaching 0.841 g/cm^3 at approximately 25% moisture content. This occurs by replacing soil particle voids with excess water, an incompressible phase, thereby lowering the achievable dry density, even though compaction energy is still present. This is evident in the soil's fine structure, where excessive water creates vacuums that lead to water-filled voids and reduced stability of the soil framework.

3.4 Stress-Strain Behaviour

The stress–strain response of the untreated laterite soil under unconfined compressive strength is presented in Figure 7(a). The three replicate samples exhibited peak compressive stresses of 48.99 kN/m^2, 59.15 kN/m^2, and 49.02 kN/m^2, occurring at strain levels between 2.0% and 2.5%. The average peak stress is 52.39 kN/m^2, representing the maximum resistance of the natural soil under unconfined loading conditions. For clarity and statistical completeness, the untreated UCS values are reported as mean ± standard deviation: 52.39 ± 4.64 kN/m^2 (n = 3). The curves display typical stress-strain behaviour, where stress increases steadily with applied strain until reaching a peak, followed by a noticeable post-peak reduction. This behaviour is characteristic of lateritic soils with low plasticity [72], where the soil structure initially mobilises interparticle friction and weak cementation bonds before failure occurs [9]. The decline in stress after the peak indicates that once the soil fabric yields, it loses its ability to sustain higher loads and transitions into a residual strength stage. Sample 2 recorded the highest peak strength (59.15 kN/m^2) and a more extensive stress response over a slightly wider strain range, indicating a slightly denser or better interlocking of the particle structure. Conversely, Samples 1 and 3 had a lower peak (49 kN/m^2) and a steeper peak slope, indicating a brittle mode of failure. In terms of ductility, the soil demonstrated deformation up to 2.5% strain before significant strength loss. This relatively small strain capacity before peak stress suggests that the untreated laterite is brittle, with limited plastic deformation. The brittle response is also supported by the soil's low plasticity index (PI = 1.40%), which reflects minimal clay content and weak interparticle bonding. From an engineering perspective, the low peak strength and brittle stress–strain response indicate that untreated laterite in its natural condition is unsuitable for direct use in load-bearing applications [73]. Its behaviour under unconfined loading highlights the need for stabilisation methods to enhance both strength and ductility.

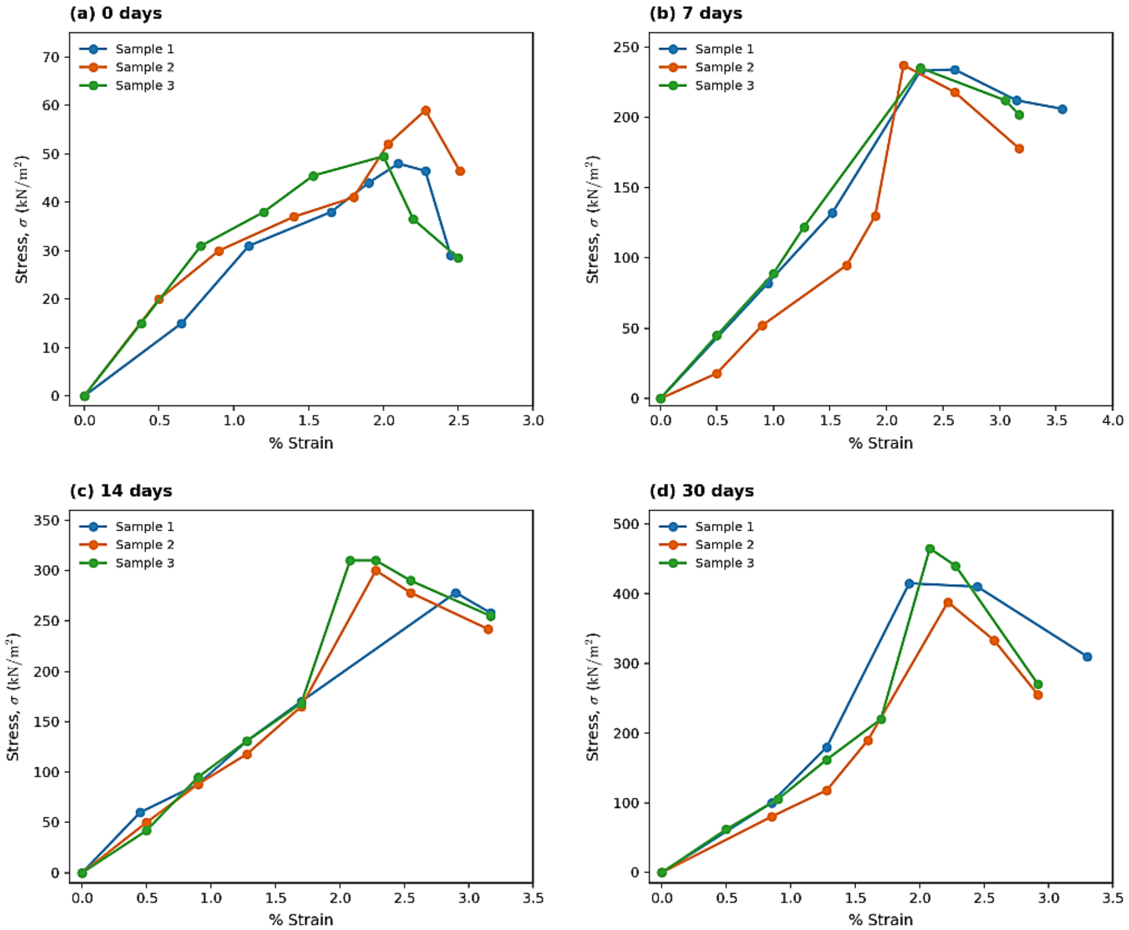

Figure 7. The stress–strain curves of treated lateritic soil samples for (a) 0 days, (b) 7 days, (c) 14 days, (d) 30 days of curing

The stress–strain response of the MICP-treated laterite soil after 7, 14, and 30 days of curing is illustrated in Figure 7(b–d). Each curing stage was represented by three replicate samples to ensure consistency and reliability of the experimental data. For the 7-day curing period (Figure 7(b)), the treated soil exhibited peak stresses in the range of 230–240 kN/m^2 at strains of around 2.0–2.5%. The relatively close spacing of the three replicates indicates good consistency, with only minor differences between samples. The post-peak stress reduction suggests a quasi-brittle failure, yet residual strength was still evident beyond the peak. At 14 days of curing (Figure 7(c)), the peak stress increased significantly, reaching approximately 300–310 kN/m^2. The initial stiffness of the curves was higher compared to the 7-day results, indicating that extended curing enhanced both bonding efficiency and soil rigidity. The shift of the peak strain to slightly higher values (~2.2–2.6%) suggests improved ductility in addition to strength. At 30 days of curing (Figure 7(d)), the stress–strain response shows a substantial improvement in strength, with peak stress values reaching approximately 420–460 kN/m^2 across the samples. Compared to earlier curing stages, the initial stiffness of the curves is significantly higher, reflecting stronger interparticle bonding due to extensive calcite precipitation. The peak strain values (~2.0–2.5%) indicate that, alongside increased strength, the soil retained moderate ductility, suggesting a balance between rigidity and deformation capacity. This confirms that prolonged curing promotes denser calcite networks, which enhance both load-bearing capacity and durability. Overall, the stress–strain behaviour clearly shows that curing time strongly influences the mechanical performance of MICP-treated laterite soils. Longer curing promotes increased calcite precipitation, resulting in higher peak stresses, improved stiffness, and enhanced energy absorption capacity. The consistency among replicates further validates the reliability of the MICP treatment process. Additionally, the average stress values for the three specimens at each curing stage were calculated and presented in Figure 8. The untreated laterite soil recorded an average stress of 52.39 kN/m^2, reflecting its weak structural resistance under loading. With MICP treatment, however, the stress values increased significantly with curing duration. After 7 days, the treated samples achieved an average stress of 234.79 kN/m^2, which further improved to 295.87 kN/m^2 at 14 days and reached 421.03 kN/m^2 after 30 days of curing. The corresponding statistical values for the treated samples are 234.79 ± 1.78 kN/m^2 at 7 days, 295.87 ± 13.72 kN/m^2 at 14 days, and 421.03 ± 32.26 kN/m^2 at 30 days (n = 3 for each curing duration), therefore surpassing these referenced UCS targets. This is because [74] claims that the range of UCS is normally around 100-300 kN/m^2 to stabilise subgrade for pavement applications, and AASHTO mentions for pavement design a minimum UCS of around 345 kN/m^2 as a benchmark.

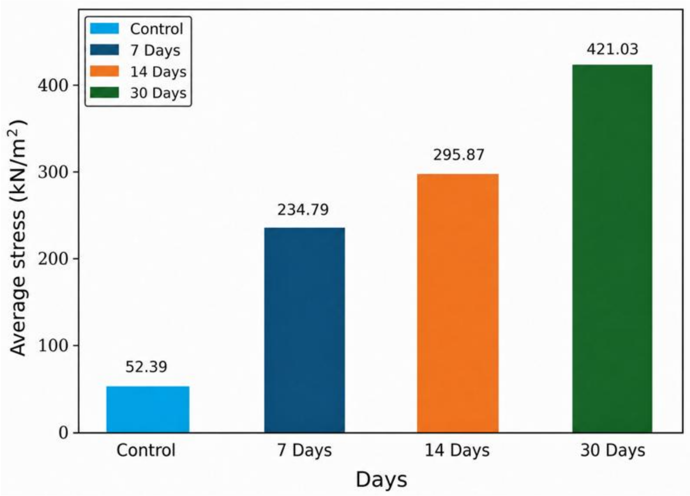

Figure 8. Average strength comparison of treated laterite soils

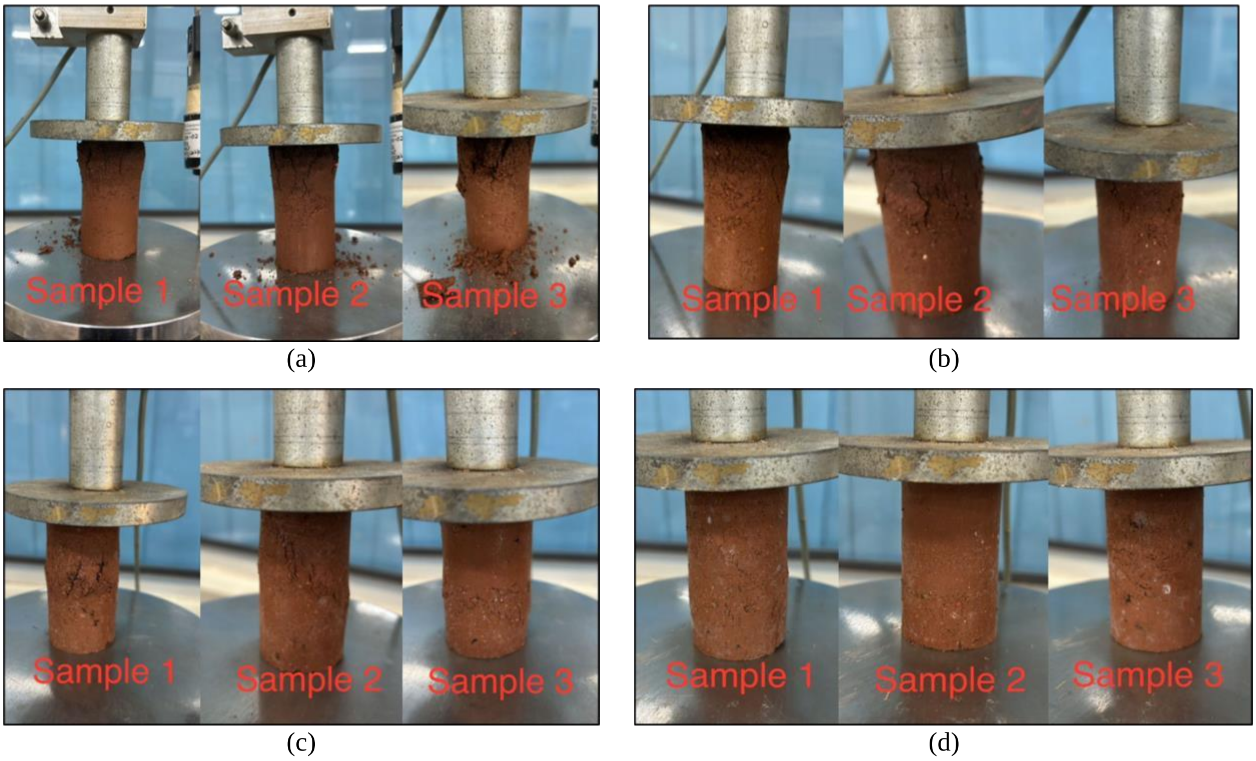

Figure 9. Post-failure condition soil samples for (a) 0 days, (b) 7 days, (c) 14 days, (d) 30 days of curing after UCS testing

This progressive increase indicates the effect of the MICP process on laterite soil. The steady increase in peak stress indicates that microbial activity has effectively promoted calcite precipitation over time, thereby densifying the soil matrix by bonding particles. The improved stress–strain behaviour, with higher load-bearing capacity and more stable post-peak responses, highlights MICP's ability to optimise both strength and ductility in lateritic soils. Furthermore, Figures 9(a–d) illustrate the post-failure condition of untreated and treated lateritic soils after unconfined compression testing. In the untreated laterite samples (Figure 9(a)), both splitting and diagonal cracks are evident. The presence of splitting cracks reflects tensile failure and specimen bulging, which is often associated with excessive compressibility and poor load-bearing capacity [75]. By contrast, diagonal cracks indicate shear failure, in which sections of the specimen slide relative to one another along defined shear planes [76]. For the treated specimens cured at 7, 14, and 30 days (Figure 9(b–d)), the predominant failure mode shifted to well-defined diagonal shear cracks, with minimal evidence of splitting or buckling-type fractures. This transition indicates that the MICP treatment enhanced interparticle bonding, allowing the soil to sustain higher axial stresses before failure and reducing the tendency for tensile splitting. In geotechnical terms, diagonal crack patterns after UCS indicate a lower risk of large settlements under structural loading [77]. The gradual shift in the mode of fracture in untreated laterite toward predominantly shear cracking in cured soils demonstrates the effectiveness of MICP in enhancing soil stability. The use of longer curing periods (14 and 30 days) resulted in the cleanest shear planes with the least fragmentation, indicating that longer microbial cementation increases structural integrity and compressive stress resistance.

3.5 Morphological Analysis

The microstructural changes induced by calcite precipitation were examined using SEM. This technique provides clear visualisation of the morphology, texture, and distribution of calcite crystals within the soil matrix [78]. For comparison, SEM images of untreated and treated laterite soils are presented in Figures 10–13. In the untreated laterite (Figure 10), the internal structure appears loosely packed, with visible pores and voids, and no evidence of calcite deposition is observed. It showed low initial strength and limited load-bearing capacity of the untreated soil.

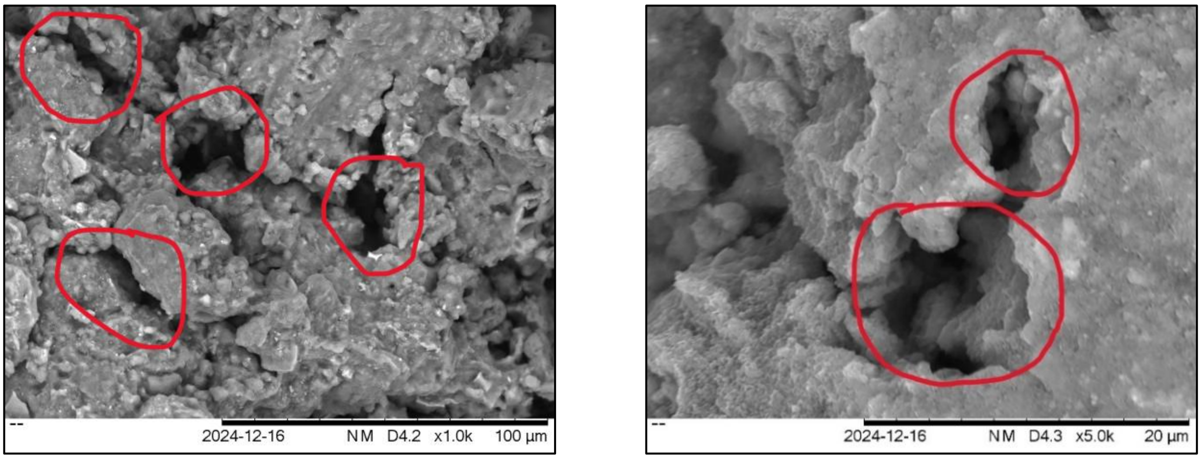

Figure 10. SEM image results for untreated soil under magnification 1000x and 5000x

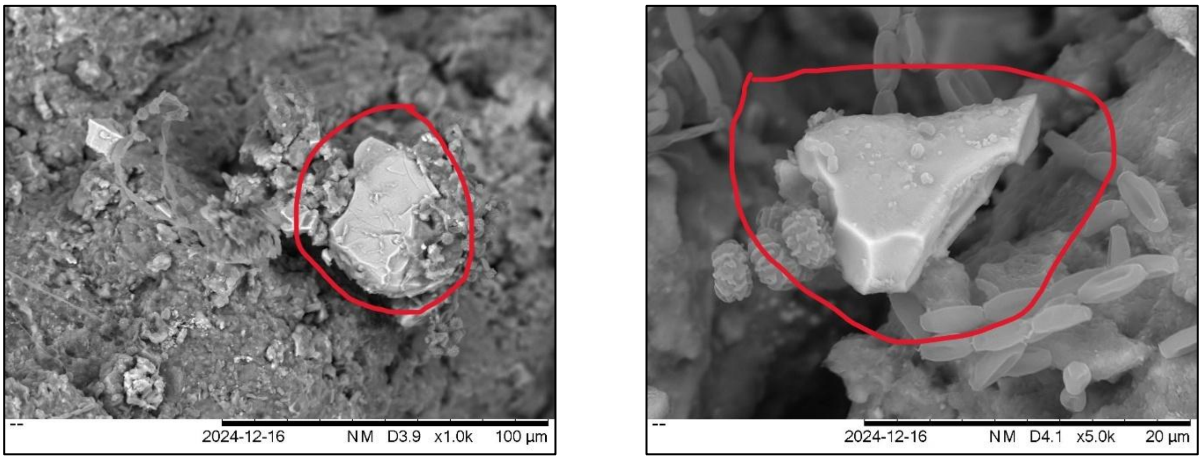

Figure 11. SEM image results of treated laterite 7 days of curing under magnification 1000x and 5000x

Figure 11 presents the SEM image of MICP-treated laterite soil after 7 days of curing, clearly illustrating the formation of calcite crystals within the soil matrix. The circled crystal exhibits a sub-rhomboidal morphology with smooth, layered surfaces, which is typical of biologically induced calcite precipitation [79]. The crystal is observed to bridge adjacent soil particles and partially fill pore spaces, thereby enhancing particle-to-particle contact and reducing porosity [80]. Such microstructural changes are critical in improving soil density and mechanical strength, as calcite acts as a cementing agent that binds grains together [70]. Similar observations were reported by DeJong et al. [81] and Soon et al. [82], in which calcite precipitation significantly reduced void ratios and increased bonding between soil particles in treated soils. The presence of a relatively large calcite crystal at this early curing stage indicates active microbial mineralisation and effective $CaCO_3$ deposition, which align with previous findings on MICP-treated soils [83] - [84]. These results provide visual confirmation that MICP treatment successfully transforms the microstructure of lateritic soils by reducing pore spaces, strengthening interparticle bonds, and thereby enhancing engineering performance. Figure 12 shows the SEM image illustrating the microstructural characteristics of MICP-treated laterite soil after 14 days of curing.

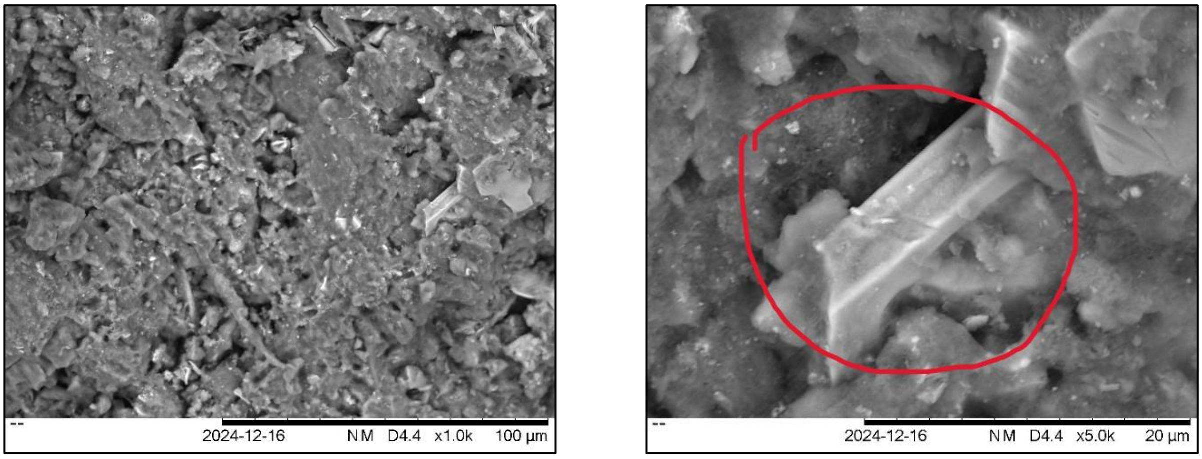

Figure 12. SEM image results of treated laterite 14 days of curing under magnification 1000x and 5000x

Figure 12 presents the SEM image of MICP-treated laterite soil after 14 days of curing, showing the progressive development of calcite crystals within the soil matrix. The circled elongated crystal displays a distinct, well-defined morphology, indicating advanced calcium carbonate precipitation. Compared to the 7-day curing stage, the crystal is larger and denser, with sharper edges and a more structured form, reflecting continuous microbial activity and mineral growth. The surrounding soil matrix appears more compact, with significantly reduced void spaces as calcite progressively fills pores and bridges particles. This cementation effect strengthens interparticle contacts and improves mechanical stability [85]. Similar elongated and crystalline calcite formations were reported in earlier studies on MICP-treated soils, where extended curing led to denser microstructures and stronger bonding between soil particles [70], [86]. The results confirm that prolonged curing enhances mineralisation, leading to reduced voids, greater cohesion, and improved durability of laterite soil. Thus, the 14-day SEM image highlights the cumulative effect of MICP in transforming the soil microstructure into a denser, more stable system through sustained calcite precipitation.

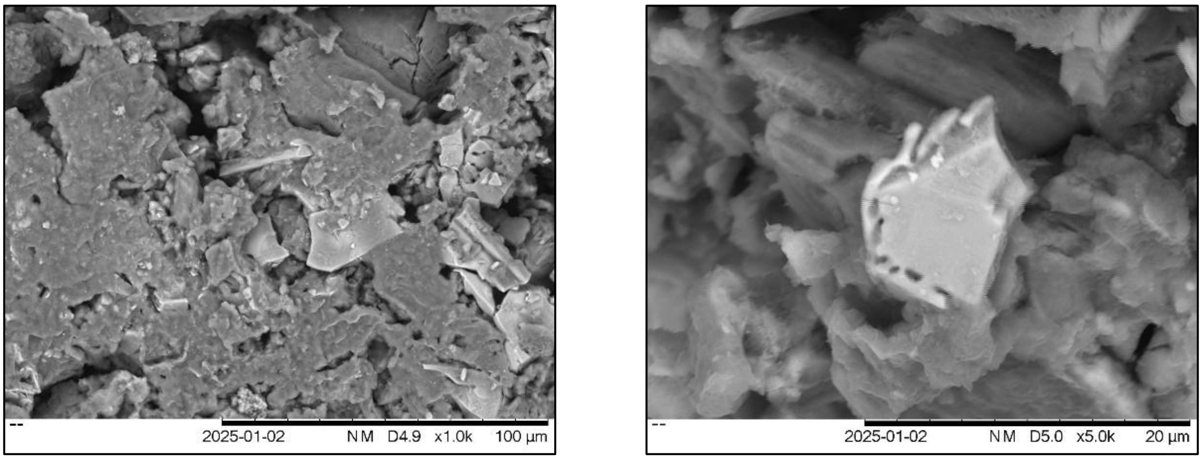

Figure 13. SEM image results of treated laterite after 30 days of curing under magnification 1000x and 5000x

Figure13 shows the SEM microstructure of MICP-treated laterite soil after 30 days of curing. At this stage, the soil matrix is significantly denser than at earlier curing periods, with most voids nearly filled by calcium carbonate crystals. The images reveal large, well-developed calcite crystals with angular and layered morphologies, typical of prolonged microbial precipitation. These crystals form continuous networks that bridge and bind soil particles, creating strong particle-to-particle contacts [87]. This extensive cementation effect reduces porosity, enhances soil compactness, and contributes to notable improvements in mechanical behaviour, including increased compressive strength and durability [88]. The observed calcite morphology is also consistent with findings by Abdul Wahab et al. [45], who documented angular, interlocking calcite crystals at advanced curing stages, further validating the effectiveness of MICP as a natural soil-stabilisation technique. The progression from 7 to 30 days of curing demonstrates the effectiveness of the MICP technique in transforming the microstructure of laterite soil. The incremental growth of calcite crystals and reduction in porosity over time highlight the importance of curing duration in achieving optimal soil stabilisation. By 30 days, the treated soil exhibits significant improvements in strength, durability, and resistance to environmental factors, making it suitable for long-term geotechnical applications

3.6 Quantitative Image Analysis

Binary thresholding was applied to segmented images to distinguish calcite precipitates from pore spaces, enabling estimation of area-based porosity. For each curing condition (0, 7, 14, and 30 days), multiple SEM images were taken from different regions of the specimens to ensure repeatability. Quantitative analysis confirmed a progressive reduction in estimated porosity as curing duration increased. The untreated laterite soil exhibited a high proportion of dark regions, corresponding to large and continuous void spaces, resulting in the highest estimated porosity. After 7 days of curing, initial calcite precipitation was observed as discrete white regions, leading to a modest reduction in porosity. At 14 days, the white regions became more extensive and interconnected, indicating progressive pore filling and enhanced particle bonding. After 30 days of curing, a substantial reduction in dark void spaces was observed, with calcite crystals forming a dense, continuous cemented matrix. As the curing time increased, the pore area fraction significantly decreased.

Specifically, compared to the untreated soil, the average pore area decreased to 83.37%, 48.98%, 41.14%, and 29.29% after 0, 7, 14, and 30 days of MICP treatment, respectively. The observed mechanical improvement, with UCS increasing from 52.39 kPa in untreated soil to 421.03 kPa after 30 days, is strongly correlated with these microstructural changes. The significant increase in load-bearing capacity was driven by progressive calcite precipitation, which reduced void spaces, improved interparticle bonding, and densified the soil matrix. This is confirmed by the inverse connection between pore area and strength. The averaged porosity values, obtained from multiple images, showed a clear decreasing trend, with associated standard deviations reflecting image-to-image variability. These results demonstrate good repeatability. The observed reduction in porosity and development of interconnected calcite networks is consistent with previous studies [81], [89], [90], which reported that prolonged curing enhances calcite content, strength, and durability in MICP-treated soils. The transition from isolated calcite deposits during early curing stages to a well-developed cemented structure at 30 days provides microstructural evidence of MICP's effectiveness in stabilising laterite soil.

4. Conclusions

This study demonstrates the potential of Microbially Induced Calcite Precipitation using *Bacillus subtilis* as a sustainable approach for stabilising tropical laterite soils. The untreated soil exhibited very low peak stress (52 kN/m^2) and a brittle stress–strain response, highlighting its poor load-bearing capacity and limited suitability for engineering use without treatment. MICP treatment significantly enhanced soil performance, with peak stresses rising to 235 kN/m^2 after 7 days, 296 kN/m^2 after 14 days, and exceeding 420 kN/m^2 at 30 days of curing, improved stiffness and ductility, and shifted failure modes from splitting to shear cracking. SEM and ImageJ analysis supported these mechanical trends, showing progressive calcite precipitation that filled voids, bridged particles, and developed dense crystal networks over time. This transformation of the soil fabric explains the observed improvements in stiffness, strength, and durability with extended curing. However, the microbially induced carbonate precipitation is effective in improving the microstructure and mechanical behaviour of laterite soil at the laboratory scale. Progressive calcite precipitation with increased curing time led to reduced porosity, enhanced particle bonding, and improved soil fabric, as confirmed through mechanical testing and microstructural observations. While the results indicate promising potential for the application of MICP-treated laterite soil in geotechnical engineering, the findings are based on controlled laboratory conditions only. Long-term durability, environmental exposure effects, and field-scale performance were not explicitly investigated in this study. Therefore, further research involving durability assessments, cyclic loading, wetting–drying conditions, and pilot-scale field trials is recommended before practical long-term implementation.

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Declaration of Competing Interests

The authors declare no conflicts of interest.

Credit Authorship Contribution Statement

M. S. D. E. Johar (Methodology; Validation; Data curation; Writing – original draft)
A. Azkil (Conceptualisation; Methodology; Investigation; Writing – review & editing)
M. A. Jaber (Methodology; Visualisation; Writing – original draft)
N. Muhammad (Conceptualisation; Investigation; Supervision, Writing-reviewing & editing)
A. Namdar (Analysis; Visualisation; Writing-reviewing & editing)

Availability of Data and Materials
The data supporting this study's findings are available on request from the corresponding author.

Ethics Declarations
This study did not involve human participants or animals. Ethical approval was therefore not required.

Generative Artificial Intelligence Declarations
The authors claim that artificially intelligent-assisted technologies, such as generative AI, were not used to generate content, ideas, or theories. We have just utilised AI to enhance readability and refine the language. This was used with extreme human control and oversight. The authors take full responsibility for reviewing and approving the content.

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