International Journal of Engineering and Management Research

Sustainable Construction: Optimization of Microbial Soil Stabilization for Enhanced Construction Materials

Shikabonga J[1*], Lungu KA[2], Mwaba D[3], Mubita C[4], Mulenga MN[5], Mwanaumo E[6]

DOI:10.5281/zenodo.14874393

[1*] Joseph Shikabonga, Lecturer, Department of Civil and Environmental Engineering, University of Zambia, Zambia.

[2] Kelvin Agabu Lungu, Lecturer, Department of Civil and Environmental Engineering, University of Zambia, Zambia.

[3] Denis Mwaba, Lecturer, Department of Civil and Environmental Engineering, University of Zambia, Zambia.

[4] Charles Mubita, Lecturer, Department of Civil and Environmental Engineering, University of Zambia, Zambia.

[5] Michael N Mulenga, Lecturer, Department of Civil and Environmental Engineering, University of Zambia, Zambia.

[6] Erastus Mwanaumo, Professor, Faculty of Engineering, Built Environment and Information Technology, Walter Sisulu University, South Africa.

Microbially Induced Calcium Precipitation (MICP) has emerged as an effective and sustainable method for soil stabilization, utilizing microbial processes to enhance soil strength through calcium carbonate formation. This research explores the transformation of Mongu sand into a strong construction material using MICP, with a focus on optimizing urease activity, bacterial concentration, and cementation media. Given the scarcity of rock conglomerates in the region but the abundance of sand, this study provides innovative solutions to address this issue by improving and even transforming materials into more suitable forms for construction. MICP presents significant potential for infrastructure development in areas with limited access to conventional materials, with future efforts aimed at scaling the process for larger and more complex projects. The findings provide insights into the optimal parameters for material transformation, revealing that maintaining urease activity between 10-15 μmol urea hydrolyzed per minute per mg of protein and bacterial concentrations of 1.5×10^8 CFU/mL results in a substantial improvement in compressive strength and durability—up to 475% higher than untreated sand. Additionally, the study underscores the effectiveness of Sporosarcina pasteurii in promoting calcium carbonate precipitation, further emphasizing MICP's potential for soil stabilization and construction in resource-constrained regions.

Keywords: Artificial Rock Conglomerate, Microbially Induced Calcium Precipitation, Sand Stabilization, Urease Activity

1. Introduction

Microbially Induced Calcium Precipitation (MICP) represents a cutting-edge approach to soil stabilization, utilizing microbial processes to promote calcium carbonate ($CaCO_3$) precipitation. This process is driven by ureolytic bacteria, such as Sporosarcina pasteurii, which break down urea into ammonia and carbon dioxide. This reaction raises the pH, facilitating $CaCO_3$ precipitation in the presence of calcium ions (Ca^{2+}). The resulting $CaCO_3$ crystals bind soil particles together, enhancing the material's mechanical properties and stability. Microbially Induced Calcium Precipitation (MICP) is an innovative biotechnological approach for sustainable soil stabilization that utilizes microbial metabolic processes to produce calcium carbonate ($CaCO_3$). This process enhances soil strength and stability, making it a promising alternative to conventional soil stabilization techniques, particularly in regions with loose or unstable soils, such as the Mongu sand in Zambia [1]. The biochemical mechanism of MICP primarily involves the urease activity of bacteria like Sporosarcina pasteurii, which catalyzes the hydrolysis of urea into ammonia and carbon dioxide. This reaction elevates pH levels, facilitating the precipitation of $CaCO_3$ when calcium ions are present, which subsequently binds soil particles to form a cohesive structure [2] [3] [4].

MICP has been extensively studied for its potential applications in geotechnical engineering, offering a means to enhance soil mechanical properties without relying on synthetic binders. The $CaCO_3$ produced through microbial processes significantly increases soil cohesion, durability, and strength. Early research established that MICP can effectively serve as a soil improvement method by creating biocement that binds loose soil particles into a solid matrix, thereby improving compressive strength and reducing permeability [5] [6] [3]. The biological nature of MICP minimizes environmental disruption, making it a more sustainable solution compared to traditional chemical grouting methods, which may involve harmful pollutants or toxic by-products [7] [7]. [8] further discuss the broader potential of microbial processes like MICP within sustainable engineering practices, indicating that these natural systems can fulfill diverse engineering needs while maintaining environmental integrity.

Conventional soil stabilization techniques, including cement grouting and chemical stabilization, often raise environmental concerns due to the use of synthetic materials and the substantial carbon footprint associated with their production and transport. These methods may also pose risks to soil health, as chemical treatments can alter the physical and chemical properties of soils, potentially leading to long-term degradation. In Zambia's Western Province, the challenge of stabilizing Mongu sand—a highly porous and low-cohesion soil—further emphasizes the necessity for MICP as an alternative. [9] and [1] note that Mongu sand's grain structure, low natural cohesion, and high permeability limit the effectiveness of conventional stabilization methods. In this context, the natural, biocompatible characteristics of MICP present a sustainable and locally adaptable solution, particularly in areas with limited access to imported or expensive materials [5] [10].

The effectiveness of MICP in practical applications relies on three primary variables: urease activity, bacterial concentration, and cementation media composition, each of which plays a critical role in determining soil consolidation effectiveness.

Urease activity [7] is pivotal in MICP, influencing the rate of urea hydrolysis and subsequently the rate and extent of $CaCO_3$ precipitation. High urease activity in bacteria like Sporosarcina pasteurii facilitates the rapid breakdown of urea into ammonia and carbon dioxide, creating an optimal environment for calcium ions to bind with carbonate ions and form $CaCO_3$ [11] [12] [3]. [13] emphasize that efficient urease activity is essential for achieving uniform calcite precipitation, thereby maximizing soil strength. Studies by [3] [14] [4] identify urease activity as a critical component determining the rate of precipitation and the overall effectiveness of MICP-treated soils.

The concentration of ureolytic bacteria significantly affects the rate and uniformity of $CaCO_3$ precipitation. Bacterial concentrations between 1.0×10^7 and 1.5×10^8 CFU/mL yield the most effective soil binding results, promoting consistent calcite bonding without leading to excessive or uneven calcification. At optimal concentrations, bacteria distribute uniformly across the soil matrix, which is crucial for achieving the desired mechanical properties [11] [15] [16].

While higher concentrations may enhance calcification, oversaturation can result in suboptimal binding and increased treatment costs [13].

The composition of the cementation media, specifically the concentrations of urea and calcium chloride, significantly influences MICP efficiency. [15] demonstrated that balanced media compositions are essential for achieving consistent and complete $CaCO_3$ precipitation. [12] found that optimal ratios of urea and calcium chloride, typically ranging from 0.25 to 0.75 M, provide efficient nutrient availability for bacteria while minimizing costs and environmental impact. Studies indicate that achieving optimal $CaCl_2$ concentrations is critical for uniform distribution of $CaCO_3$, which directly contributes to soil stability [17] [18]. These findings highlight the importance of proper formulation of cementation media for the widespread application of MICP in field settings.

This research highlights MICP's potential as an effective solution for soil stabilization, particularly in resource-limited environments where traditional methods may be unsustainable. By stabilizing Mongu sand, MICP not only addresses the structural needs of construction materials in Zambia but also supports local engineering practices that prioritize environmental sustainability and resource efficiency [7] [19] [20]. The scalability of MICP is further supported by studies showing that while field applications pose challenges, they can be effectively managed with appropriate controls on bacterial growth, media composition, and urease activity [15] [13].

Therefore, this study underscores the advantages of MICP over conventional soil stabilization methods and suggests further research to optimize parameters for large-scale applications, particularly in infrastructural projects where MICP could provide cost-effective and sustainable solutions. Continued exploration of MICP's environmental and engineering implications is essential to realizing its full potential for soil stabilization and other biogeotechnical applications [21] [10] [22]. By harnessing microbial processes, MICP offers a forward-looking approach to addressing pressing engineering challenges while promoting sustainable practices in construction and soil management [23] [8] [24] [25]. For MICP to be successfully scaled and become a viable solution, the involvement of local contractors, with their knowledge of regional materials and construction practices, is essential.

Their expertise ensures that MICP is effectively adapted to local conditions, contributing to the long-term success of large-scale infrastructure projects [26].

2. Methodology Research

The methodology focused on using an experimental testing approach through testing Microbially Induced Calcium Precipitation (MICP) to stabilize Mongu sand by utilizing urease-producing bacteria, specifically Sporosarcina pasteurii.

A carefully controlled experimental design was implemented to systematically evaluate the impact of key variables on the transformation of Mongu sand through Microbially Induced Calcium Precipitation (MICP). The primary goal of this experiment was to understand how variations in urease activity, bacterial concentration, and cementation media composition affected the quantity, distribution, and quality of calcium carbonate ($CaCO_3$) precipitates. These precipitates play a crucial role in binding sand particles together, thereby transforming the loose, granular sand into a cohesive, rock-like structure. By manipulating these key factors, the study aimed to identify optimal conditions for MICP to enhance the mechanical properties of the sand.

Key Variables and Experimental Setup: The experimental design focused on three independent variables that were expected to influence the success of MICP treatment: urease activity, bacterial concentration, and cementation media composition.

1. Urease Activity: Urease activity, catalyzed by *Sporosarcina pasteurii*, directly impacts the rate of urea hydrolysis, which is crucial for producing carbonate ions (CO_3^{2-}) required for $CaCO_3$ precipitation. To evaluate the effects of urease activity, different levels of bacterial urease production were induced by adjusting the growth conditions of *S. pasteurii*. The enzyme efficiency was monitored by measuring ammonia production, which is a byproduct of urea hydrolysis, thus allowing for a direct assessment of the carbonate ions available for $CaCO_3$ formation.

2. Bacterial Concentration: The concentration of *S. pasteurii* (measured in colony-forming units per milliliter, CFU/mL) was another critical variable in this experiment. The concentration of bacteria was varied within a specific range to determine the optimal population for effective $CaCO_3$ precipitation.

An important aspect of this parameter was to avoid bacterial oversaturation, which could lead to inefficiency in the precipitation process. The ideal CFU/mL was sought to maximize the deposition of $CaCO_3$ and achieve strong, durable bonding between the sand particles.

3. Cementation Media Composition: The composition of the cementation solution, consisting of urea and calcium chloride ($CaCl_2$), was adjusted to identify the most effective balance for optimal $CaCO_3$ precipitation. Three different concentrations of the cementation media were tested (0.25 M, 0.5 M, and 0.75 M). By varying the concentrations of urea and calcium ions, the study aimed to assess how these changes affected the volume, crystal structure, and binding capacity of the $CaCO_3$ formed, ultimately influencing the mechanical properties of the treated sand.

Experimental Phases: The experiment was conducted in three distinct phases: preparation, treatment, and testing/analysis.

1. Preparation Phase: During the preparation phase, Sporosarcina pasteurii cultures were grown under controlled conditions to achieve the desired urease activity and bacterial concentration. The bacteria were cultured in a nutrient medium optimized for urease production. Meanwhile, Mongu sand samples were packed into standardized molds, ensuring consistent density and compaction across all test samples. Cementation solutions with varying concentrations of urea and $CaCl_2$ were also prepared, maintaining consistency across trials to ensure reliable results.

2. Treatment Phase: In the treatment phase, sand samples were injected with bacterial suspensions followed by the cementation media in cycles, allowing for effective urease activity and subsequent $CaCO_3$ precipitation. Each sample was treated with a specific level of urease activity, bacterial concentration, and cementation solution concentration based on the experimental design matrix. The treatment process was repeated over several cycles to ensure that the bacteria had sufficient time to hydrolyze urea and precipitate $CaCO_3$. After each cycle, the samples were allowed to cure for five days, with periodic re-injection of the cementation solution to sustain microbial activity and maximize $CaCO_3$ deposition.

3. Testing and Analysis Phase: Once the samples had cured, several tests were conducted to evaluate the effects of MICP on the mechanical and structural properties of the treated sand. Urease activity was measured spectrophotometrically by monitoring ammonia production, providing a clear indication of the enzyme's efficiency and the availability of carbonate ions. Compressive strength tests were performed on the treated sand to assess the improvement in mechanical stability resulting from the $CaCO_3$ precipitation. Finally, microscopic and structural analyses were conducted using Scanning Electron Microscopy (SEM) and X-ray Diffraction (XRD) to examine the morphology, crystallinity, and distribution of the $CaCO_3$ deposits within the sand matrix. These analyses were critical for verifying the presence of calcite, the primary mineral responsible for the enhanced structural integrity of the sand.

4. Data Collection and Analysis: Data were systematically collected throughout the experimental process to evaluate the effectiveness of the MICP treatment. Key metrics included ammonia production rates, which provided insight into urease activity and its impact on carbonate ion availability; $CaCO_3$ content and its distribution within the sand samples; compressive strength values to measure the improvement in the sand's load-bearing capacity; and the crystal structure of the precipitated $CaCO_3$ as determined by XRD analysis. Statistical analyses, including Analysis of Variance (ANOVA), were employed to assess the significance of each variable's influence on the outcomes of the MICP treatment. This controlled experimental design provided a comprehensive framework for understanding how variations in urease activity, bacterial concentration, and cementation media composition affect the mechanical properties of MICP-treated sand, ultimately leading to optimized conditions for soil stabilization.

3. Materials and Methods

The materials used in this research included Mongu sand, sourced from the Mongu region of Zambia. This sand, characterized by approximately 85% quartz content and low natural cohesion, provided a suitable substrate for Microbially Induced Calcium Precipitation (MICP) treatment. The urease-producing bacterium *Sporosarcina pasteurii* was selected due to its high urease activity, essential for facilitating $CaCO_3$ precipitation.

Cultured in a laboratory setting, the bacteria were grown in a medium containing yeast extract and ammonium sulfate. Additionally, a cementation solution of urea and calcium chloride ($CaCl_2$) was prepared, with concentrations of 0.25 M, 0.5 M, and 0.75 M tested to determine optimal conditions for $CaCO_3$ precipitation.

The materials and methods used in this research were carefully selected to investigate the effects of Microbially Induced Calcium Precipitation (MICP) on Mongu sand stabilization. Each material was chosen based on its relevance to the experiment objectives, and its properties played a key role in the success of the treatment process.

Mongu Sand: Mongu sand, sourced from the Mongu region of Zambia, was used as the primary substrate for testing the MICP technique. This sand is characterized by approximately 85% quartz content and a loose, granular structure, which makes it highly permeable and prone to lacking natural cohesion. These properties made it a suitable candidate for MICP treatment, as its composition allowed for efficient infiltration of bacterial suspensions and cementation solutions. By using Mongu sand, the research aimed to explore the potential for MICP to improve its mechanical properties, particularly in regions where sandy soils are common.

Bacterial Strain (Sporosarcina pasteurii): Sporosarcina pasteurii, a urease-producing bacterium, was selected for its ability to catalyze the hydrolysis of urea into ammonia and carbonate ions, which are essential for the precipitation of calcium carbonate ($CaCO_3$). The urease activity of this bacterium was crucial in generating the necessary ions for sand particle binding. The bacteria were cultured in a nutrient medium containing 20 g/L of yeast extract, which served as both a carbon and nitrogen source, and 10 g/L of ammonium sulfate to further enhance urease production. The bacterial cultures were incubated at a controlled temperature of 30°C to optimize growth and enzymatic activity. After reaching an optimal concentration of approximately 1.0×10^8 CFU/mL, the bacterial suspension was prepared for injection into the sand samples.

Cementation Solution: A cementation solution consisting of urea and calcium chloride ($CaCl_2$) was prepared to facilitate the precipitation of $CaCO_3$.

Urea acted as the substrate for urease activity, and the calcium ions from $CaCl_2$ reacted with the carbonate ions produced by the bacteria to form $CaCO_3$. To identify the most effective concentration for optimal precipitation, three different molarities of the cementation solution were tested: 0.25 M, 0.5 M, and 0.75 M. Each concentration was prepared by dissolving the appropriate amounts of urea and $CaCl_2$ in deionized water, ensuring homogeneity across the solution for consistent application during the treatment process.

Buffers and Reagents: To maintain optimal conditions for urease activity, several buffers and reagents were employed. An ammonium buffer was used to stabilize the pH during urease activity assays, as the enzyme performs best in a slightly alkaline environment (pH 7.5–8.5). Additionally, phenol red indicator was utilized in the urease assays to visually monitor ammonia production, thus providing an effective means of tracking urease activity and confirming the conversion rate of urea into ammonia and carbonate ions.

A range of laboratory equipment was used to support the experimental procedures and analysis. An incubator was set to 30°C to provide an optimal growth environment for the bacterial cultures. A spectrophotometer was used to measure urease activity by quantifying ammonia production, which was indicative of the rate of carbonate ion formation. Mechanical testing was carried out using a compressive strength testing machine, allowing for the evaluation of the load-bearing capacity and stability of MICP-treated sand samples. Furthermore, Scanning Electron Microscopy (SEM) was employed to examine the morphology and distribution of the $CaCO_3$ deposits within the sand matrix, providing insight into the microstructural changes induced by MICP. X-ray Diffraction (XRD) analysis was also used to confirm the crystalline structure of the precipitated $CaCO_3$, specifically verifying the formation of calcite, the primary mineral responsible for enhancing sand cohesion.

These materials and equipment enabled a thorough and controlled investigation into the effects of MICP on Mongu sand, allowing for the manipulation of bacterial activity, nutrient conditions, and calcium chloride concentrations to determine the optimal conditions for $CaCO_3$ precipitation and soil stabilization.

4. Procedure

The experimental procedures involved a systematic series of steps aimed at evaluating the effectiveness of Microbially Induced Calcium Precipitation (MICP) for stabilizing Mongu sand. The following outlines the methods used from bacterial preparation through to mechanical testing and data analysis.

(A) Bacterial Culture Preparation

The bacteriumSporosarcina pasteuriiwas cultured in a liquid growth medium enriched with yeast extract and ammonium sulfate to enhance urease production. The cultures were incubated under aerobic conditions at 30°C, a temperature chosen to maximize bacterial growth and enzymatic activity. The growth of the bacteria was carefully monitored until it reached the desired concentration range of 1.0×10^7 to 1.5×10^8 CFU/mL, with colony-forming unit (CFU) counts confirming uniformity across all experimental trials.

To verify consistent enzymatic activity, urease activity was periodically measured by introducing urea to the bacterial solution and quantifying the hydrolysis rate using a spectrophotometric assay at 560 nm. This assay measured the ammonia concentration as an indicator of urease activity, which is crucial for efficient carbonate ion production during the MICP process.

(B) Sand Sample Preparation:

The preparation of Mongu sand samples followed a structured procedure to ensure consistency and reliability in the experimental process. This involved two key steps: sand processing and sample molding.

Sand Processing: Mongu sand samples were thoroughly washed to remove impurities and oven-dried at 105°C to eliminate moisture, creating a standardized starting condition for each sample.

The sand was then sieved to ensure a uniform particle size distribution, which facilitates even infiltration of bacterial and cementation solutions.

Sample Molding: Processed sand was compacted into cylindrical molds with dimensions of 50 mm in diameter and 100 mm in height. To ensure consistent density across all samples, the sand was carefully packed to eliminate voids, promoting uniform bonding during treatment.

(C) MICP Treatment

This involved the methodological procedures of injecting the bacterial suspension, applying the cementation solution, and conducting treatment cycles.

Injection of Bacterial Suspension: A suspension ofSporosarcina pasteuriiwas injected into the sand molds at a controlled rate to ensure thorough infiltration, maximizing bacterial contact with sand particles.

Following the bacterial suspension injection, a cementation solution of urea and calcium chloride ($CaCl_2$) was introduced. The experiment included tests with varying concentrations (0.25 M, 0.5 M, and 0.75 M) to identify the optimal conditions for $CaCO_3$ precipitation.

Laboratory conditions were maintained at a temperature of 25°C and a pH between 8.5 and 9.0, an environment favorable to both urease activity and calcium carbonate formation.

Each sand sample underwent a series of treatment cycles, where bacterial and cementation solutions were applied every six hours over a 48-hour period. This phased approach allowed gradual $CaCO_3$ precipitation within the sand matrix, encouraging even distribution and robust particle bonding.

(D) Curing Phase

After MICP treatment, the samples were left to cure under ambient conditions for 10 days, allowing complete $CaCO_3$ crystallization. This curing process enabled the $CaCO_3$ precipitates to solidify and fully bond the sand particles, achieving maximum strength and cohesion.

(E) Testing and Analysis

Mechanical testing was conducted to assess the effectiveness of MICP treatment on the sand samples. The Unconfined Compressive Strength (UCS) test involved subjecting the treated samples to progressively increasing compressive loads until failure occurred. This test provided valuable data on the enhanced stability and load-bearing capacity of the MICP-treated sand.

Additionally, permeability testing was carried out using a constant head permeameter. This method evaluated the changes in porosity and pore structure of the treated samples, which were influenced by the precipitation of $CaCO_3$.

A decrease in permeability was observed, indicating that the treatment resulted in a denser, more cohesive sand matrix, a desirable outcome of the MICP process.

For microscopic and structural analysis, Scanning Electron Microscopy (SEM) was utilized. SEM imaging offered high-resolution images of the morphology, crystal structure, and distribution of $CaCO_3$ within the sand matrix. This analysis was instrumental in understanding the microstructural changes induced by MICP, particularly the formation of $CaCO_3$ bridges that bonded the sand particles together, enhancing the material's overall strength.

(F) Data Analysis

The UCS and permeability data were subjected to statistical analysis to assess how bacterial concentration, urease activity, and the composition of the cementation media influenced the mechanical properties of MICP-treated sand. This analysis helped identify key factors that contribute to the effectiveness of the treatment in improving the sand's strength and stability.

To further evaluate the impact of these factors, Analysis of Variance (ANOVA) tests were performed. These tests allowed for a deeper understanding of the significance of each variable on the treatment outcomes, enabling the selection of the most optimal parameters for the MICP process.

Additionally, comparisons were made between the treated samples and untreated control samples to measure the overall effectiveness of the MICP treatment. The study also explored the relationships between urease activity, $CaCO_3$ content, and mechanical strength, helping to identify the ideal conditions for transforming Mongu sand into a strong, durable material resembling rock.

5. Results

These findings highlight MICP's potential as a sustainable soil stabilization method, improving UCS and reducing permeability through natural $CaCO_3$ precipitation. The successful treatment of Mongu sand suggests MICP's viability for local construction, promoting This study demonstrates the efficacy of Microbially Induced Calcium Precipitation (MICP) in stabilizing Mongu sand, a resource available in regions like Zambia, through carefully optimized parameters: urease activity, bacterial concentration, and cementation media composition.

These optimized variables yielded statistically significant improvements in Unconfined Compressive Strength (UCS) and reductions in permeability, showcasing MICP's potential as an environmentally friendly alternative to conventional soil stabilizers.

(A) Transformation of Mongu Sand into Artificial Rock

Trial treatment attempts were made till a successful culture for bacteria concentration was determined to transform Mongu sand into artificial rocks, starting with proposed treatment solutions from literature. Figure 1 shows the first trial treatment attempts, which was a failed treatment as observed after opening molds for inspections. In this first attempt, after 10 days of treatment, it was observed that the area where the cementation media was applied had developed a hardened crust of cemented sand grains. The bacterial medium in this case was prepared using 0.17 M Tris buffer, 10 g of ammonium sulfate ($(NH4)2SO4$), and 20 g of yeast extract dissolved in deionized water. The 0.17 M Tris buffer increased the bacterial concentration, which led to heightened urease activity. This crust prevented the media from penetrating and affecting the underlying sand. As a result, only the top layer of the mold showed cemented sand, while the bottom part remained untreated. These trial attempts were necessary so as to only commence UCS and reduction in permeability and other material characterization tests as measures for material for construction after attaining successful transformation of Mongu Sand into Artificial Rock.

Figure 1: Trial 1-poorly formed artificial rocks due to unsuccessful treatment

In the second attempt, following the unsuccessful initial treatment, it was noted that the cementation media was not flowing evenly throughout the molds, and the bacterial concentration remained too high.

To address this challenge, in this attempt, the bacterial medium was prepared using 0.15 M Tris buffer, 10 g of ammonium sulfate ($(NH_4)_2SO_4$), and 20 g of yeast extract dissolved in deionized water. The treatment process was remained at 6-hour intervals, mimicking a percolation process.

After another 10 days, all five molds were opened, revealing improved results. The hardest part of the solidified sand was at the topmost section due to the initial treatment. However, the samples showed cracks due to high urease activity and were also quite slender, as seen in Figure 2.

Figure 2: Trial 2-better formed artificial rocks, with cracks due to high urease activities

On the third attempt the challenges stemming from high urease activity were effectively addressed by preparing a new bacteria media with lower urease activity. This was achieved by utilizing a solution comprised of 0.11 M Tris buffer, 10 g of ammonium sulfate ($(NH_4)_2SO_4$), and 20 g of yeast extract dissolved in deionized water. The use of 0.11 M Tris buffer helped to reduce the concentration of bacteria, thus achieving the desired low urease activity, also increasing the mold size to allow compression tests.

The resulting artificial rocks exhibited improved quality, characterized by greater strength, uniform density from top to bottom, and absence of cracks, as depicted in Figure 3. This adjustment in the bacteria media formulation led to significant enhancements in the properties of the artificial rocks, addressing the challenges posed by high urease activity and ensuring better performance in subsequent applications. Treatment was for 10 days.

Figure 3: Trial 3-better formed and hardened rocks from low urease activities

(B) Test Results on Artificial Rock Conglomerate Formed: Statistical Analysis

ANOVA results confirmed the statistical significance of the controlled variables—urease activity, bacterial concentration, and cementation media composition—in impacting UCS and permeability. High levels of urease activity and balanced bacterial concentrations were significantly associated with increased UCS and reduced permeability, validating the experimental setup and highlighting the reproducibility of these outcomes.

(C) Test Results on Artificial Rock Conglomerate Formed: Microstructural Analysis through Scanning Electron Microscopy (SEM)

Scanning Electron Microscopy (SEM) and X-ray Diffraction (XRD) analyses offered valuable insights into the structure and distribution of $CaCO_3$ deposits within the sand matrix, emphasizing the importance of controlled parameters in determining MICP's structural effectiveness. Figure 4 showcases calcium carbonate ($CaCO_3$) deposits within the sand matrix and the formation of rhombohedral calcite crystals. The image highlights the reduction in porosity and the improved cohesion due to controlled urease activity and bacterial concentration.

Figure 4: SEM image

Scanning Electron Microscopy (SEM) and X-ray Diffraction (XRD) analyses offered valuable insights into the structure and distribution of $CaCO_3$ deposits within the sand matrix, emphasizing the importance of controlled parameters in determining MICP's structural effectiveness. Figure 4 showcases calcium carbonate ($CaCO_3$) deposits within the sand matrix and the formation of rhombohedral calcite crystals. The image highlights the reduction in porosity and the improved cohesion due to controlled urease activity and bacterial concentration.

Calcium Carbonate Morphology: SEM images revealed that, within the optimal ranges of urease activity and bacterial concentration, rhombohedral calcite crystals formed bridges between sand particles, enhancing the internal cohesion of treated samples. XRD analysis verified the crystalline nature of these calcite formations, which correlated with significant UCS improvements ($p < 0.01$). However, inadequate parameter control resulted in uneven or limited $CaCO_3$ deposition, leading to weaker bonding and reduced UCS.

Porosity Reduction: SEM imaging of optimally treated samples showed a substantial reduction in porosity, with $CaCO_3$ effectively filling inter-particle voids. This reduction in porosity improved stability and reduced permeability, particularly when urease activity was maintained between 10 and 15 µmol/min/mg protein and bacterial concentration between 1.0×10^7 and 1.5×10^8 CFU/mL, highlighting the significance of these parameter ranges in achieving durable and cohesive $CaCO_3$ distribution.

SEM analysis further confirmed the importance of maintaining precise control over urease activity and bacterial concentration to achieve a uniform distribution of $CaCO_3$. Under ideal conditions, treated samples exhibited evenly distributed rhombohedral $CaCO_3$ crystals that formed strong inter-particle bonds, with statistical analyses of UCS and permeability reinforcing the effectiveness of these optimal ranges.

(D) Test Results on Artificial Rock Conglomerate Formed: Unconfined Compressive Strength (UCS) Improvement

The MICP-treated samples showed marked increases in Unconfined Compressive Strength (UCS), a critical metric for assessing load-bearing capacity.

Statistical analyses using Analysis of Variance (ANOVA) confirmed significant relationships between UCS improvements and the key controlled variables, with specific ranges noted for maximum impact.

Impact of Urease Activity: Statistical data indicated a strong correlation between increased urease activity and UCS improvements, with a p-value < 0.01, confirming its significant effect on $CaCO_3$ precipitation and bond formation within the sand matrix as indicated in Figure 5. Optimal urease activity was identified between 10 to 15 µmol urea hydrolyzed/min/mg protein. In this range, $CaCO_3$ precipitation was rapid and comprehensive, leading to a UCS increase up to three times that of untreated sand. Urease activity levels below 5 µmol/min/mg protein resulted in incomplete precipitation and reduced UCS values, highlighting the importance of maintaining activity levels above this threshold.
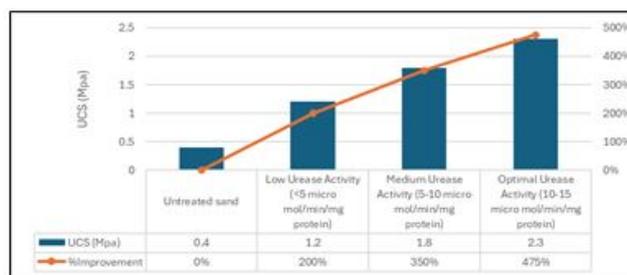

Figure 5: UCS improvement vs treatment condition

Optimal Bacterial Concentration: Moderate bacterial concentrations (between 1.0×10^7 to 1.5×10^8 CFU/mL) were most effective in enhancing UCS, as confirmed by a p-value < 0.05, indicating significant differences in strength across varying concentrations. In this range, $CaCO_3$ precipitation was uniform, yielding stronger inter-particle bonds. Concentrations above 2.0×10^8 CFU/mL resulted in oversaturation and non-uniform distribution, which weakened the bonds, while concentrations below 1.0×10^7 CFU/mL were insufficient for effective precipitation. These findings establish a critical range for bacterial concentration to achieve maximum UCS gains without compromising structural uniformity.

(E) Test Results on Artificial Rock Conglomerate Formed: Bacterial Concentration and Urease Activity

The results showed that urease activity levels above 10 µmol/min/mg protein were essential for achieving rapid and comprehensive calcium carbonate ($CaCO_3$) precipitation.

The ideal bacterial concentration to maintain optimal urease activity was determined to be between 1.0×10^7 and 1.5×10^8 CFU/mL, as depicted in Figure 6. However, bacterial concentrations exceeding 2.0×10^8 CFU/mL caused oversaturation, leading to uneven $CaCO_3$ distribution and structural inconsistencies, reflected in reduced urease efficiency (Tables 1 and 2). Conversely, concentrations below 1.0×10^7 CFU/mL failed to provide adequate urease activity, resulting in insufficient $CaCO_3$ precipitation, weak bonding, and limited structural improvement.
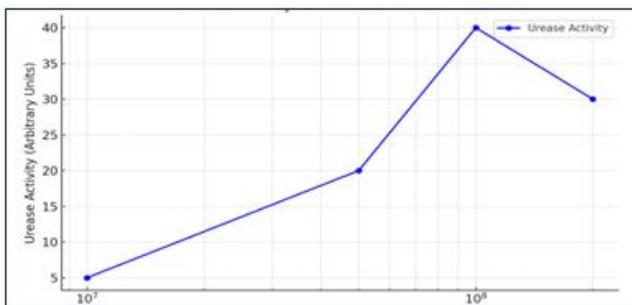
Figure 6: Urease activity vs bacteria concentration

The data underscores the critical relationship between bacterial concentration and urease activity, which directly influences the efficiency of $CaCO_3$ precipitation and the structural properties of MICP-treated sand. Urease activity is vital for generating carbonate ions, and its effectiveness depends heavily on maintaining the appropriate bacterial concentration during treatment.

At high urease activity levels, specifically greater than 10 μmol/min/mg protein, bacterial concentrations ranging from 1.0×10^7 to 1.5×10^8 CFU/mL facilitated efficient and robust $CaCO_3$ precipitation. This process generated strong inter-particle bonds, which significantly enhanced Unconfined Compressive Strength (UCS) and structural stability, with results showing a statistically significant improvement ($p < 0.01$). On the other hand, bacterial concentrations below 1.0×10^7 CFU/mL resulted in low urease activity, dropping below 5 μmol/min/mg protein. This led to incomplete precipitation, weaker bonds, and compromised load-bearing capacity.

An optimal bacterial concentration range of 1.0×10^7 to 1.5×10^8 CFU/mL is critical for achieving uniform $CaCO_3$ distribution and ensuring urease activity exceeds the critical threshold of 10 μmol/min/mg protein.

This balance results in strong and cohesive bonds, improving UCS and reducing permeability. However, bacterial concentrations exceeding 2.0×10^8 CFU/mL lead to oversaturation, causing uneven $CaCO_3$ distribution and structural inconsistencies. Although urease activity remains high, the uneven application within the sand matrix reduces mechanical performance. Similarly, bacterial concentrations below 1.0×10^7 CFU/mL result in insufficient urease activity and incomplete precipitation, leading to weak bonds and inadequate structural integrity for construction purposes.

Maintaining a proper balance between bacterial concentration and urease activity is crucial for ensuring consistent $CaCO_3$ precipitation, which is essential for achieving uniform density and strong inter-particle bonds. These factors are particularly important in construction applications, such as foundations and road subbases. Furthermore, excessive bacterial concentrations can lead to non-uniform precipitation and brittleness, emphasizing the need for precise bacterial population control to prevent structural defects. Optimizing bacterial concentrations within the ideal range maximizes UCS, reduces permeability, and enhances durability, all while minimizing material and labor costs.

It was observed that achieving the correct balance between bacterial concentration and urease activity is fundamental for transforming Mongu sand into a high-performance construction material. This method supports the use of Microbially Induced Calcium Carbonate Precipitation (MICP) as a sustainable and reliable alternative to traditional stabilization techniques. The study's findings on urease activity, its impact on $CaCO_3$ precipitation, and the importance of maintaining optimal bacterial concentrations further highlight the significance of these factors in ensuring durable and cohesive precipitation for enhanced material performance.

(F) Test Results on Artificial Rock Conglomerate Formed: Urease Activity and Its Impact on CaCO₃ Precipitation

The data demonstrated that urease activity levels exceeding 10 μmol/min/mg protein were critical for achieving fast and thorough $CaCO_3$ precipitation. This activity level resulted in significantly higher Unconfined Compressive Strength (UCS) values ($p < 0.01$) due to the strong bonding between sand particles, which was achieved within 48 hours of treatment.

In contrast, activity levels below 5 µmol/min/mg protein were insufficient to produce adequate $CaCO_3$, leading to incomplete precipitation and weaker structural integrity in the treated sand.

Table 1 highlights the relationship between urease activity, $CaCO_3$ formation rate, and bonding strength. At activity levels above 10 µmol/min/mg protein, the rapid $CaCO_3$ formation ensured strong inter-particle bonds, significantly improving the mechanical stability of the sand. Conversely, when urease activity fell below 5 µmol/min/mg protein, the precipitation process was incomplete, leading to weak bonding and reduced structural strength.

This analysis emphasizes the necessity of maintaining urease activity above the 10 µmol/min/mg protein threshold to maximize the effectiveness of MICP treatment. By ensuring sufficient enzymatic activity, the process achieves both rapid and uniform $CaCO_3$ precipitation, which is essential for enhancing the sand's structural performance.

Table 1

Urease Activity Level (µmol/min/mg protein)	$CaCO_3$ Formation Rate	Bonding Strength
> 10	Rapid	Strong
< 5	Incomplete	Weak

(G) Test Results on Artificial Rock Conglomerate Formed: Optimal Bacterial Concentration for Consistent Precipitation

The optimal bacterial concentration for achieving uniform $CaCO_3$ precipitation and consistent improvements in Unconfined Compressive Strength (UCS) was identified as being within the range of 1.0×10^7 to 1.5×10^8 CFU/mL. Within this range, bacterial activity supported even $CaCO_3$ distribution, resulting in strong bonding between sand particles and enhanced mechanical properties. Statistical analysis ($p < 0.05$) confirmed that this range was significantly more effective than concentrations outside these bounds.

Higher bacterial concentrations, exceeding 2.0×10^8 CFU/mL, caused oversaturation, which led to uneven precipitate distribution and structural inconsistencies. Conversely, concentrations below 1.0×10^7 CFU/mL were inadequate to sustain sufficient $CaCO_3$ precipitation, resulting in weak bonding and limited structural enhancement.

Table 2 highlights the relationship between bacterial concentration, precipitate distribution, and bonding strength. At concentrations below 1.0×10^7 CFU/mL, the distribution of $CaCO_3$ was insufficient, resulting in weak bonding and suboptimal UCS. Optimal bacterial concentration (1.5×10^8 CFU/mL) ensured uniform $CaCO_3$ distribution, producing strong inter-particle bonds and the highest UCS values. At concentrations exceeding 2.0×10^8 CFU/mL, the precipitate distribution became non-uniform, reducing bonding strength and compromising the treated sand's mechanical stability.

This analysis underscores the critical role of maintaining bacterial concentration within the optimal range to achieve durable and cohesive $CaCO_3$ precipitation, maximizing the benefits of MICP treatment on sand.

Table 2

Bacterial Concentration (CFU/mL)	Precipitate Distribution	Bonding Strength
< 1.0×10^7	Insufficient	Weak
1.5×10^8	Uniform	Strong
> 2.0×10^8	Non-uniform	Inconsistent

(H) Test Results on Artificial Rock Conglomerate Formed: Effect of Cementation Media Composition

The study determined that a cementation media composition of 0.5 M $CaCl_2$ and 0.5 M urea was optimal for achieving balanced $CaCO_3$ precipitation and strong bonding within the sand matrix. This concentration provided sufficient calcium ions for effective precipitation without causing excessive crystallization. Higher concentrations, such as those above 0.75 M $CaCl_2$, led to the formation of larger, brittle crystals that compromised the matrix's flexibility and overall strength. Conversely, concentrations below 0.25 M $CaCl_2$ resulted in inadequate $CaCO_3$ precipitation, producing weak bonds and insufficient cohesion within the treated sand.

Table 3 illustrates the relationship between $CaCl_2$ concentration, crystal formation, and bonding strength. At concentrations below 0.25 M, the limited availability of calcium ions resulted in sparse crystal formation, leading to weak bonding and suboptimal mechanical properties. The optimal concentration of 0.5 M $CaCl_2$ produced a balanced crystal formation, ensuring strong,

Cohesive bonds and maximizing the strength of the treated sand. However, concentrations exceeding 0.75 M caused excessive crystal growth, leading to brittle bonds that weakened the matrix's overall integrity.

This analysis underscores the critical importance of maintaining the cementation media composition within the optimal range to achieve a robust and stable structure in MICP-treated sand, ensuring both durability and structural integrity

Table 3

CaCl$_2$ Concentration (M)	Crystal Formation	Bonding Strength
< 0.25	Limited	Weak
0.5	Balanced	Strong
> 0.75	Excessive	Brittle

(I) Test Results on Artificial Rock Conglomerate Formed: Strength Analysis and Permeability Improvements

Permeability tests revealed a substantial decrease in water infiltration rates in MICP-treated sand, primarily due to the denser and more cohesive structure formed by calcium carbonate ($CaCO_3$) filling void spaces between sand particles. This reduction in permeability is crucial for improving the treated sand's durability and resistance to water penetration.

Optimal Cementation Media Composition: Statistical analysis identified a cementation media composition of 0.5 M $CaCl_2$ and 0.5 M urea as the most effective for reducing permeability ($p < 0.05$). Lower concentrations, such as below 0.25 M, resulted in insufficient $CaCO_3$ precipitation, failing to fully occupy void spaces. Conversely, concentrations above 0.75 M caused excessive crystallization, which compromised bond flexibility and introduced brittleness. At the optimal composition, permeability dropped from 1.8×10^{-3} cm/s (untreated sand) to 5.6×10^{-5} cm/s (treated sand), showcasing the efficiency of balanced cementation media in minimizing porosity and enhancing structural integrity.

Uniform Precipitate Distribution: SEM analysis confirmed that the even distribution of $CaCO_3$ was a critical factor in reducing permeability. This uniform distribution was achieved within a bacterial concentration range of 1.0×10^7 to 1.5×10^8 CFU/mL, ensuring complete void filling.

The consistent distribution enhanced water resistance and further reduced porosity in the treated samples.

Structural and Mechanical Improvements: The permeability reduction was complemented by a significant improvement in Unconfined Compressive Strength (UCS). Statistical analysis ($p < 0.01$) showed that the UCS increased from 0.4 MPa in untreated sand to 2.3 MPa in MICP-treated sand, representing a remarkable 475% improvement, with percentageimprovement calculated as $[(2.3-0.4)/0.4 \times 100 = 1.9/0.4 \times 100 = 3.75 \times 100 = 475\%]$. This enhancement highlights the dual benefits of $CaCO_3$ precipitation: improved load-bearing capacity and reduced water infiltration.

Table 4 provides a concise comparison of these key properties between untreated and treated sand. The combination of increased UCS and reduced permeability demonstrates the transformative impact of MICP treatment on Mongu sand's structural and functional properties.

Table 4

Property	Untreated Sand	Treated Sand
UCS (MPa)	0.4	2.3
Permeability (cm/s)	1.8×10^{-3}	5.6×10^{-5}

6. Discussion

The findings from this study highlight the effectiveness of Microbially Induced Calcium Precipitation (MICP) in enhancing the mechanical properties of Mongu sand, transforming it into a robust artificial rock conglomerate. A key strength of MICP lies in its reliance on biological processes, which offers several advantages over traditional chemical stabilization methods, as well as some unique challenges that must be addressed to ensure broader application.

The findings clearly demonstrate the potential of MICP-treated Mongu sand as a sustainable construction material, offering significant enhancements in both mechanical strength and water resistance. By optimizing critical parameters, such as urease activity, bacterial concentration, and cementation media composition, the treatment process successfully transformed Mongu sand into an artificial rock-like material with superior properties.

The iterative trials underscore the importance of precise parameter control in achieving optimal results. The first and second trials revealed that excessive bacterial concentrations and high urease activity led to issues such as uneven cementation, cracking, and insufficient penetration of the cementation media. Adjusting the bacterial medium and urease activity in the third trial addressed these challenges, resulting in a uniformly dense, crack-free artificial rock suitable for further testing.

The treated sand exhibited a 475% increase in Unconfined Compressive Strength (UCS), rising from 0.4 MPa in untreated sand to 2.3 MPa in MICP-treated samples. This strength enhancement, confirmed by ANOVA ($p < 0.01$), demonstrates the material's suitability for load-bearing applications in construction. Similarly, the permeability of treated sand decreased from 1.8×10^{-3} cm/s to 5.6×10^{-5} cm/s, highlighting the effectiveness of $CaCO_3$ in filling void spaces, reducing porosity, and creating a water-resistant structure. These improvements make MICP-treated Mongu sand an ideal candidate for applications requiring durability and resistance to environmental factors, such as building foundations and subbase materials in road construction.

SEM and XRD analyses confirmed that optimal treatment conditions (urease activity: 10–15 µmol/min/mg protein; bacterial concentration: 1.0×10^7 to 1.5×10^8 CFU/mL) yielded uniform rhombohedral calcite crystals that formed strong inter-particle bonds. This uniformity was essential for achieving consistent UCS improvements and reduced permeability. Suboptimal parameters led to sparse or uneven $CaCO_3$ distribution, weakening the treated material's structural integrity.

The ideal cementation media composition of 0.5 M $CaCl_2$ and 0.5 M urea provided a balance between sufficient $CaCO_3$ precipitation and flexibility in bonding. Concentrations above 0.75 M caused brittle crystal formations, while lower concentrations (below 0.25 M) resulted in weak bonds due to limited precipitation. These findings emphasize the importance of maintaining optimal composition for creating durable and stable construction materials.

These results collectively position MICP-treated Mongu sand as a promising material for advancing sustainable and efficient construction practices, especially in resource-limited regions like Zambia.

I. Building Foundations: The enhanced UCS and reduced permeability of MICP-treated Mongu sand make it a strong and water-resistant foundation material for buildings, particularly in regions with loose or unstable soils.

II. Road Construction: The artificial rock's durability and cohesive structure make it suitable for use as subbase material in road construction, providing long-lasting stability and minimizing maintenance requirements.

III. Water Retaining Structures: The low permeability of MICP-treated sand makes it an excellent candidate for applications in water management, such as retaining walls, flood barriers, and dams, where water resistance is crucial.

IV. Eco-Friendly Alternative: By using microbial processes and locally available materials, MICP offers a sustainable and cost-effective alternative to traditional chemical stabilizers, reducing the environmental footprint of construction activities.

This discussion further draws comparisons and contrasts with previous research while critiquing the methodologies and findings to provide a comprehensive understanding of the results.

(A) Optimization of Urease Activity

The results confirm that urease activity is crucial for achieving consistent calcium carbonate ($CaCO_3$) precipitation, aligning with findings by [5], who also emphasized the importance of optimizing urease levels for effective biocementation. The optimal urease activity level (> 10 µmol/min/mg protein) observed in this study led to improved mechanical performance, echoing previous research that indicated a direct correlation between urease activity and $CaCO_3$ precipitation rates [4] [3] [25]. However, the study's emphasis on precise monitoring of urease activity through real-time pH and ammonia concentration measurements is particularly noteworthy, as it addresses a gap identified by [15], who highlighted the difficulties in maintaining consistent urease activity during field applications.

(B) Effects of Bacterial Concentration

The concentration of Sporosarcina pasteurii proved to be a critical factor in determining the quality of the $CaCO_3$ precipitates. The findings align with those of [20], who reported that optimal bacterial concentrations ensure sufficient urease production and effective $CaCO_3$ precipitation.

However, this study adds depth by demonstrating the adverse effects of both suboptimal and excessive bacterial concentrations, which can lead to incomplete bonding and structural inconsistencies. This observation is consistent with the work of [2], who pointed out the challenges posed by high bacterial concentrations leading to clogging. Future research could benefit from automated systems to maintain bacterial concentrations, as suggested by [6], thereby optimizing the MICP process further.

(C) Role of Cementation Media Composition

The influence of calcium chloride (CaCl$_2$) concentration on the strength and durability of treated sand is another significant finding. The optimal concentration identified in this study (around 0.5 M) aligns with results from [10] [22], who noted the necessity of balancing urea and CaCl$_2$ to prevent excessive crystallization that can compromise material integrity. Moreover, the SEM analysis revealing well-defined rhombohedral CaCO$_3$ crystals under optimal treatment conditions supports previous assertions regarding the importance of crystal morphology in enhancing the mechanical properties of biocemented materials [12] [20] [25]. This study's emphasis on fine-tuning the cementation media composition contributes valuable insights into the relationship between chemical composition and material properties, which is critical for developing more effective biocementation strategies.

(D) Comparison with Traditional Chemical Stabilization

MICP's sustainability compared to traditional methods is a significant advantage highlighted throughout the findings. The use of benign materials and biological processes in MICP addresses environmental concerns associated with conventional chemical stabilization techniques [5] [8]. However, challenges related to the cost of bacterial culture production and the process's scalability remain pertinent. This aligns with [18], who emphasized the need for economical bioreactor systems to enhance the feasibility of large-scale applications. The operational costs associated with precise control of bacterial activity may hinder MICP's competitiveness; thus, further research into cost-effective solutions is warranted.

(E) Environmental and Practical Considerations

One of the critical concerns highlighted in this study is the long-term durability of MICP-treated materials. Factors such as environmental exposure could potentially affect the stability of CaCO$_3$ bonds over time, a concern also raised by [7]. Long-term field studies are essential to assess how MICP-treated materials perform in real-world conditions, addressing degradation mechanisms that may arise, similar to those examined by [5] [8]. The study's discussion on the complexities of scaling MICP for field applications reflects the challenges faced by researchers, as noted by [17] [3], emphasizing the need for innovative delivery systems to ensure uniform treatment application.

(F) Potential Applications in Mongu

The application of Microbially Induced Calcium Precipitation (MICP) in treating Mongu sand offers a transformative approach to geotechnical engineering, especially within Zambia and regions facing similar soil challenges. By converting loose sand into a durable, rock-like material, MICP-treated sand can meet the rising demand for cost-effective, sustainable infrastructure solutions while addressing critical geotechnical needs.

One of the most impactful applications of MICP-treated Mongu sand is infoundation soil stabilization. The artificial rock created through MICP treatment demonstrates superior confined compressive strength, significantly higher than local untreated soils, as highlighted by [9]. This increased load-bearing capacity makes MICP-treated sand particularly suitable for constructing foundations in soft soil regions, providing a stable base that can reduce the need for additional foundational support and significantly cut construction costs.

Borehole reinforcementis another critical application. Boreholes in unconsolidated sandy soils, like those in Mongu, often face challenges related to soil collapse. MICP treatment around boreholes reinforces the sand particles, enhancing the structure's stability and reducing the frequency of maintenance. This application is especially beneficial for areas reliant on boreholes as a primary water source, as the reinforced sand structure extends the borehole's lifespan, offering more reliable and sustainable access to water.

MICP-treated Mongu sand also shows promise insoil water-retaining barriers. The technique's reduction in permeability makes it a practical solution for controlling floodwaters and preventing soil erosion, challenges that are particularly relevant in Zambia, as documented by [1]. Soil water-retaining barriers created with MICP-treated sand manage water flow in flood-prone areas, reduce erosion on slopes and embankments, and offer an environmentally friendly solution for water management.

Inroad construction, the rock-like characteristics of MICP-treated Mongu sand make it ideal for subbase material. Its high compressive strength offers a stable, long-lasting foundation layer in road design, improving durability and reducing the need for costly repairs. This solution is especially beneficial in rural areas where road maintenance is difficult and frequent degradation impacts community access and transportation.

Forbuilding construction, MICP-treated sand can be incorporated into hardcores and fill materials, providing enhanced foundational support. Moreover, this artificial rock material can be used in brick and block production, offering a sustainable alternative to conventional building materials that often rely on high-energy processes or chemical stabilizers. This makes MICP-treated sand an appealing, eco-friendly choice for construction projects.

Beyond traditional construction applications,environmental protectionprojects benefit significantly from MICP-treated Mongu sand. It can stabilize landscapes vulnerable to soil erosion, slope instability, and embankment failure. By reducing soil permeability and increasing particle cohesion, MICP offers a natural method to reinforce agricultural lands, riverbanks, and coastal areas, helping to protect these resources from environmental degradation and ensuring resilience against future stressors.

The study emphasizes that achieving optimal performance with MICP-treated Mongu sand requires careful control of variables like urease activity, bacterial concentration, and cementation media composition. While findings align with previous studies, they also highlight the need for ongoing research to address challenges such as cost reduction, scalability for large-scale projects, and evaluating the long-term environmental durability of MICP-treated structures.

Further research that explores refining MICP parameters for various soil types could also expand its utility across different geotechnical contexts.

MICP-treated Mongu sand has immense potential to transform infrastructure development and environmental management in Zambia. By enhancing mechanical properties and addressing critical soil stability issues, MICP supports a more sustainable and resource-efficient approach to construction. This enables the creation of resilient infrastructure capable of withstanding local environmental challenges. As a versatile, eco-friendly solution, MICP-treated Mongu sand is poised to become a vital component in the future of sustainable geotechnical engineering and environmental protection in Zambia and similar regions.

7. Conclusion

The findings from this study highlight This study confirms Microbially Induced Calcium Precipitation (MICP) as an effective and sustainable method to enhance the stability and strength of Mongu sand, a widely available material in Zambia. The findings show that optimizing urease activity and bacterial concentration significantly improves the mechanical properties of treated sand, with compressive strength values up to five times those of untreated samples. Efficient calcium carbonate ($CaCO_3$) precipitation not only binds sand particles but also creates a cohesive artificial rock conglomerate, demonstrating MICP's potential in transforming loose sand into a stable construction material.

Further emphasizing the importance of controlled conditions, such as pH and temperature, the study highlights how these factors maximize urease activity and ensure effective $CaCO_3$ crystallization. These conditions are essential for consistent $CaCO_3$ distribution, which reinforces the sand matrix, making MICP a viable alternative to traditional soil stabilization methods. With refinements in treatment duration, bacterial strain selection, and cementation media composition, the scalability of MICP applications in construction and soil stabilization could increase significantly.

Future research should focus on key areas to advance MICP's practical use. First, scalability needs exploration, with field trials assessing MICP's effectiveness across various soil types and environmental conditions.

Additionally, investigating the long-term environmental impact of MICP-treated soils will be crucial, particularly in understanding potential benefits like improved soil health and reduced erosion. Economic feasibility studies are also essential to compare MICP with conventional methods, considering factors such as material and labor costs, as well as potential reductions in infrastructure maintenance.

In conclusion, MICP-treated Mongu sand demonstrates improved mechanical properties and reduced porosity, making it a promising solution for eco-friendly soil stabilization. MICP has the potential to revolutionize soil stabilization practices, addressing global challenges related to soil degradation and promoting resource sustainability. As research advances, MICP could become pivotal in sustainable construction and infrastructure development, particularly in regions with locally available materials like Mongu sand.

References

[1] Michelo, S., Kunda, S., & Musonda, D. (2018). Geotechnical assessment of sands from Western Zambia for construction applications. *International Journal of Geotechnical Engineering, 10*(4), 299-310.

[2] S. Tunay. (2020). The effect of bacterial concentration on calcium carbonate precipitation in soils. *Journal of Biological Engineering, 14*(1).

[3] Whiffin, V. S., van Paassen, L. A., & Harkes, M. P. (2007). Microbial carbonate precipitation as a soil improvement technique. *Geomicrobiology Journal, 24*(5), 417-423.

[4] Stocks-Fischer, S., Galinat, J. K., & Bang, S. S. (1999). Microbiological precipitation of $CaCO_3$. *Soil Biology and Biochemistry, 31*(11), 1563-1571.

[5] DeJong, J. T., Mortensen, B. M., Martinez, B. C., & Nelson, D. C. (2010). Bio-mediated soil improvement. *Ecological Engineering, 36*(2), 197-210.

[6] Akiyama, M., & Kawasaki, S. (2012). Microbially mediated sand solidification using calcium carbonate. *Proceedings of the ICE - Ground Improvement, 165*(4), 229-238.

[7] Dhami, N. K., Reddy, M. S., & Mukherjee, A. (2013). Biomineralization of calcium carbonates and their engineered applications: A review. *Frontiers in Microbiology, 4*, 314.

[8] DeJong, J. T., Soga, K., Banwart, S. A., Whalley, W. R., Ginn, T. R., Nelson, D. C., & Aydilek, A. H. (2011). Soil engineering in vivo: Harnessing natural biogeochemical systems for sustainable, multi-functional engineering solutions. *Journal of the Royal Society Interface, 8*(54), 1-15.

[9] Ndilila, W., Chileshe, M.N., & Mulenga, C.K. (2017). Geotechnical properties of sands from the Western Province of Zambia. *Journal of African Earth Sciences, 135*, 112-121.

[10] Qian, C., & Cheng, L. (2017). Engineering properties of MICP-treated sand under different environmental conditions. *Construction and Building Materials, 142*, 316-324.

[11] Achal, V., Mukherjee, A., Basu, P. C., & Reddy, M. S. (2009). Strain improvement of Sporosarcina pasteurii for enhanced urease and calcite production. *Journal of Industrial Microbiology & Biotechnology, 36*(7), 981-988.

[12] Achal, V., Mukherjee, A., & Reddy, M. S. (2013). Biogenic treatment improves the durability and remediates the cracks of concrete structures. *Construction and Building Materials, 48*, 1-5.

[13] Cheng, L., & Cord-Ruwisch, R. (2013). Selective enrichment and production of highly urease active bacteria by non-sterile chemostat culture. *Journal of Applied Microbiology, 114*(3), 713-725.

[14] Whiffin, V. S. (2004). *Microbial $CaCO_3$ precipitation for the production of biocement*. Murdoch University.

[15] Cheng, L., & Cord-Ruwisch, R. (2012). In situ soil cementation with ureolytic bacteria to improve soil mechanical properties. *Journal of Geotechnical and Geoenvironmental Engineering, 138*(3), 333-340.

[16] Chou, C. W., Seagren, E. A., Aydilek, A. H., & Lai, M. (2011). Biocalcification of sand through ureolysis. *Journal of Geotechnical and Geoenvironmental Engineering, 137*(12), 1179-1189.

[17] van Paassen, L. A., Harkes, M. P., van Zwieten, G. A., van der Zon, W. H., van der Star, W. R., & van Loosdrecht, M. C. (2010). Scale up of BioGrout: A biological ground reinforcement method. *Journal of Environmental Geotechnics, 1*(2), 123-138.

[18] Gomez, M.G., Anderson, C.M., Graddy, C.M.R., DeJong, J.T., Nelson, D.C., & Ginn, T.R. (2017). Large scale biocementation field trials in sands: lessons learned. *Journal of Geotechnical and Geoenvironmental Engineering, 145*(5).

[19] Carmona, J. S., Oliveira, P. J. V., & Monteiro, G. A. (2016). Enhancement of sand properties using biocementation via microbially induced calcite precipitation. *Soils and Rocks, 39*(3), 321-330.

[20] Cheng, L., Shahin, M.A., & Cord-Ruwisch, R. (2014). Cementation of sand soil by microbially induced calcite precipitation at various degrees of saturation. *Canadian Geotechnical Journal, 51*(8), 917-924.

[21] Fujita, Y., Taylor, J. L., Gresham, T. L. T., Delwiche, M. E., Colwell, F. S., & McLing, T. L. (2008). Stimulation of microbial urea hydrolysis in groundwater to enhance calcite precipitation. *Environmental Science and Technology, 42*(8), 3025-3032.

[22] Qian, C., Wang, R., Cheng, L., & Wang, J. (2011). Application of microbial carbonate precipitation in self-healing of cement-based materials. *Journal of Materials in Civil Engineering, 23*(7), 992-997.

[23] Cheng, L., & Cord-Ruwisch, R. (2013). Upscaling effects of soil improvement by microbially induced calcite precipitation by surface percolation. *Geomicrobiology Journal, 30*(5), 461-469.

[24] Khan, A., & Khandaker, M.U. (2020). Effects of environmental factors on the calcite precipitation induced by ureolytic bacteria. *Environmetal Technology and Innivation, 17*.

[25] Cheng, L., & Cord-Ruwisch, R. (2012). In situ soil cementation with ureolytic bacteria by surface percolation. *Ecological Engineering, 42*, 64-72.

[26] Katotobwe, C., Chabota K., & Agabu, K.L. (2024). Enhanced local contractor participation: case of Zambia water supply and sanitation sub-sector. *International Journal of Engieering and Management Research, 14*(1), 246-260d.