Available online at www.CivileJournal.org

Civil Engineering Journal

(E-ISSN: 2476-3055; ISSN: 2676-6957)

Vol. 10, No. 08, August, 2024

Enhancing Soil Stability through Innovative Microbial-Induced Calcium Carbonate Techniques with Sustainable Ingredient

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Received 21 April 2024; Revised 19 June 2024; Accepted 25 July 2024; Published 01 August 2024

Abstract

Expansive soil poses significant challenges for civil engineers, leading to structural damage, particularly in lightly loaded structures. This study employs an innovative and sustainable recipe to stabilize highly expansive soil using the Microbial-Induced Calcium Carbonate Precipitation (MICP) technique by substituting conventional ingredients with olive mill wastewater and hydrated lime. A series of laboratory tests were performed to evaluate the improvement in Atterberg's limits, Free Swell, Unconfined Compressive Strength (UCS), and pH, in addition to a series of qualitative measurements, including X-ray diffraction (XRD), Scanning Electron Microscopy (SEM), Optical Microscopic Images, and bacteria growth rate. Different mellowing periods and different cementation concentrations were used. The proposed recipe results showed a 50% reduction in the soil's free swell value. The UCS of the treated soil using the proposed recipe was eight times that of the untreated soil and twice that of the soil treated with the traditional recipe. The SEM images showed flocculation and aggregation in the soil particles, with the voids becoming smaller and filled with calcium carbonate ($CaCO_3$). The XRD results showed the formation of new $CaCO_3$ particles. The optimized recipe demonstrated remarkable enhancement improvement and significant changes in soil physical properties and microstructure.

Keywords: Expansive Soil; Microbial-Induced Calcium Carbonate Precipitation (MICP); Stabilization; Olive Mill Wastewater; Environmental Sustainability.

1. Introduction

Expansive soil is the soil that exhibits volume changes in response to variations in moisture content. The clay mineral montmorillonite, capable of absorbing a significant amount of water and increasing the thickness of the clay layer, is the primary factor contributing to the swelling of the expansive soil [1]. The expansive soil volume change resulted in a significant challenge in civil engineering due to its potential to cause damage to infrastructure, lightweight buildings, and pavement systems. The damage caused by the behavior of expansive soil resulted in billions of dollars in losses [2].

Several stabilization techniques improved expansive soil's physical and geotechnical properties. These techniques aim to transform the soil into a more stable condition, improving shear strength, load-bearing capacity, and swelling/shrinkage characteristics. The traditional stabilization agents depend on using different agents, such as cement,

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http://dx.doi.org/10.28991/CEJ-2024-010-08-08

lime, asphalt, etc., to improve soil properties. These techniques lead to increased energy consumption, depletion of nonrenewable resources, and significant adverse environmental impacts, including substantial CO_2 emissions [3]. In recent years, a new and eco-friendly soil stabilization technique has emerged in geotechnical engineering, known as the Microbial-Induced Calcium Carbonate Precipitation (MICP) technique. MICP is one of the biological technologies that has shown promising results in the cementation of soil particles due to the precipitation of calcium carbonate ($CaCO_3$) minerals [4–11].

In the MICP process, different microbial activities generate carbonate within an environment rich in calcium [12, 13]. The urea hydrolysis is recognized as the most extensively researched method in geotechnical engineering [14]. This process involves the hydrolysis of urea (producing CO_3^{2-}) by urease, and through biochemical activity, it forms calcium carbonate precipitation [15]. The precipitated $CaCO_3$ acts as a bonding agent and bridge between soil particles, improving soil strength, stiffness, stability, and permeability. Sporosarcina pasteurii is the most commonly used bacterial species in most MICP applications. Its widespread distribution in nature, ability to withstand harsh environments, and high resistance to physical and chemical agents make it usable in field environments [4].

The application of biological treatment in civil engineering in general and in geotechnical engineering, especially, has increased in the last few years. MICP is one of the biological technologies that has shown promising results from the cementation of soil particles due to the precipitation of calcium carbonate [5]. Generally, MICP technology is considered an efficient treatment technology for improving and strengthening the sandy soil by precipitating the calcite minerals. Since the sand is porous and permeable, it is considered a suitable environment for the bacteria to move freely and deposit calcium carbonate.

Although several researchers consider the MICP technology unsuitable for clayey soil because of the small size of clay particles and minimum porosity [2], several studies have shown promising results for treating fine-grained soil through MICP technology. Li et al. (2021) [16] used the MICP technology to improve the compression characteristics of the expansive soil through high-pressure consolidation tests. Results showed that the expansive soil's compression characteristics achieved optimal enhancement after six treatments with the microbial solution; additional treatments did not improve the soil's compression performance. Chittoori et al. (2021) [17] found that the MICP treatment of three soils with varying clay contents (30%, 40%, and 70%) indicated no improvement in mellowing beyond two days, and the optimal improvement occurred within two days of mellowing and seven days of curing. Tian et al. (2022) [18] reported that conducting CD triaxial tests on expansive soil treated with MICP resulted in an increase in cohesiveness from 29.52 kPa to 39.41 kPa, and an increase in internal friction angle from 20.13° to 29.58°. Tiwari et al. (2024) [19] conducted a study to evaluate the effectiveness of the MICP-bio stimulation technique in improving the long-term performance of expansive soil under cyclic loading. Results showed a 205% increase in calcite content in bio-treated samples, improving splitting tensile strength and UCS. Microstructure analysis revealed a uniform distribution of new calcite particles. Tian et al. (2024) [20] used triaxial consolidated-undrained (CU) creep tests to determine if MICP stabilization improved expansive soil creep and long-term strength. The long-term shear strength of MICP-stabilized expansive soil was found to increase significantly. However, the internal angle of friction and long-term cohesion were slightly reduced.

The MICP is an effective technique for stabilizing the soil matrix and making it stiff and firm [21]. Moreover, in terms of economics, it is viewed as a low-cost and long-term efficient treatment method [14, 22]. According to Naveed et al. (2020), the cost of synthetic grouting is around ($2 - $72/$m^3$ of soil), whereas the cost of microbial grouting in traditional MICP grouting is approximately around ($0.5 - $9/ m^3 of soil) [14]. To enhance the efficiency of the MICP technique, a new recipe has been proposed in this study. This new recipe substitutes the two most important ingredients in the enrichment and cementation solutions (urea and calcium chloride) with olive mill wastewater and hydrated lime (calcium hydroxide).

Olive mill wastewater is black-colored liquid wastewater produced while pressing olives into oil, and it significantly impacts the environment and the economy if it is not treated or used [23]. The uncontrolled discharge of OMWW resulted in severe environmental problems, such as deterioration of water resources quality, threats to aquatic life, and soil contamination by heavy metals in OMWW. The biological treatment of soil contaminated with wastewater is the best way to remove heavy metals and organic load. The microorganisms feed on the organic loads and degrade the heavy metals [24].

There are many reasons to improve the MICP recipe. Economically, replacing the traditional chemicals and ingredients in the well-known MICP recipe with OMWW, a wastewater material containing sufficient nutrients and materials to support bacterial growth, reduces costs. Therefore, the proposed MICP recipe’s cost is lower due to the reliance on the OMWW, and the hydrated lime is commercially available. In terms of the environmental aspect, the uncontrolled OMWW dumping into the environment is a big concern since it is considered harmful. In this work, the OMWW can be used as a nutrient medium for the bacteria with no negative effects on survival. In addition, using the traditional MICP recipe (urea recipe) has some risks and health hazards, such as ammonia releases and urea-induced soil swelling. Ammonium, a by-product of urea hydrolysis, has the potential to pollute the sub-surface environment. In the long term, ammonia will lower the soil's pH, which may dissolve the amount of the precipitated calcium carbonate [25]. Moreover, Narasimha Rao & Chittaranjan (2010) [26] stated that the soil contaminated with urea increases the swelling pressure.

Furthermore, several countries worldwide have suffered for a long time from the problem of water poverty and rising water demand; safe disposal of large amounts of OMWW and reducing freshwater use is environmentally beneficial. Finally, the proposed method was practical for applying the MICP treatment technique without needing special equipment or skillful workers. These explanations inform the use of the proposed MICP recipe since it does not contain urea and its product.

This study optimizes the MICP recipe and evaluates the effectiveness of using a proposed MICP recipe instead of the traditional recipe to stabilize expansive soil. In the proposed MICP recipe, S. pasteurii degrades the heavy metals in OMWW, consuming the organic matter and effectively precipitating the $CaCO_3$. Therefore, the study aims to overcome traditional stabilization techniques' drawbacks by using sustainable and green stabilization agents to reduce the negative environmental impact while cutting the cost of conventional stabilization techniques.

2. Experimental Works

2.1. Materials

2.1.1. Soil

A disturbed soil sample with high expansion potential was used in this study; the soil sample was obtained from a depth equal to 2-3 meters beneath the ground surface from Eastern Irbid City in Jordan. The Characteristics and geotechnical properties of the untreated soil sample are represented in Table 1. The location of the study area and the sampling process of the soil are represented in Figure 1.

Table 1. Untreated Soil Characteristics

Property	Value
Specific Gravity (G.S.)	2.69
Gravel%	0%
Sand%	3.26%
Silt%	35.24%
Clay%	61.5%
Maximum dry density (g/cm³)	1.437
Optimum moisture content	30%
UCS (kPa)	202
Free Swell %	15.5%
$CaCO_3$%	3.14%
LL (%)	73
PL (%)	38.4
PI (%)	34.6
USCS classification	CH

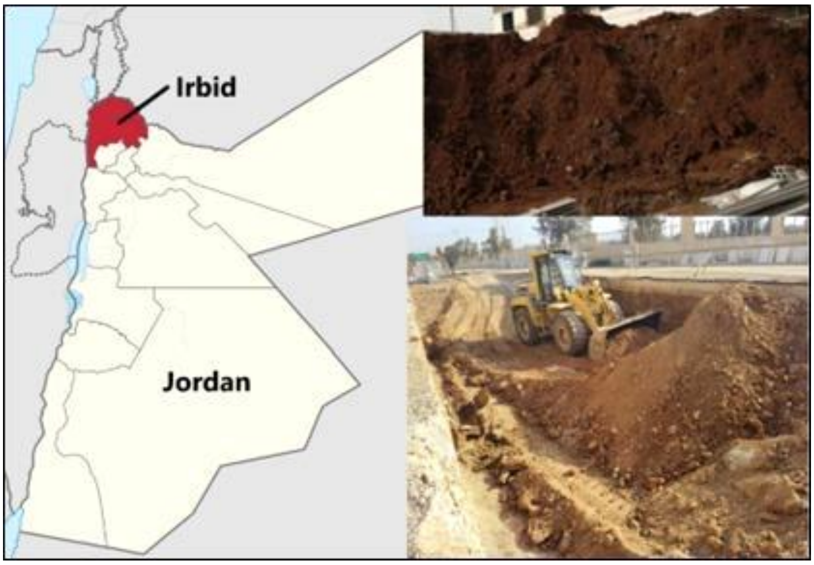


Figure 1. The location of the study area and the sampling process of the soil

2.1.2. Bacteria

Sporosarcina Pasteurii bacterium (ATCC 11859) was used in this research. It is a rod-shaped, gram-positive, and non-pathogenic bacterium with a length that varies between (1.3-4) and width between (0.5-1.2) microns, as shown in Figure 2.

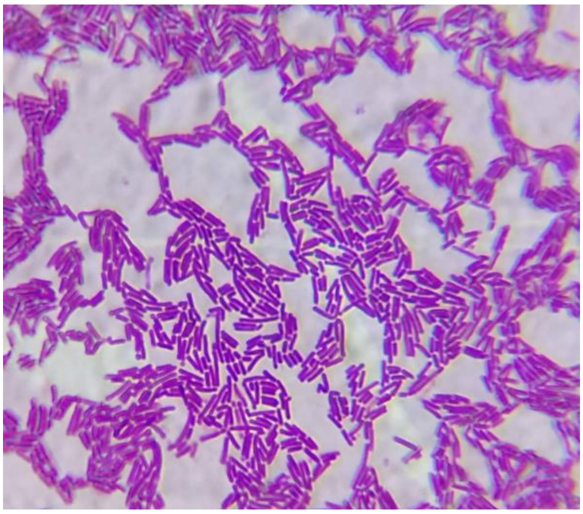


Figure 2. Bacillus pasteurii under a light microscope

2.1.3. Olive Mill Wastewater (OMWW)

OMWW is an aqueous waste containing some solid residue from olive production. The OMWW chemical composition is summarized in Table 2. The pH value for the OMWW used in this research was 4.5.

2.1.4. Hydrated Lime $Ca(OH)_2$

Hydrated lime is widely used as a chemical stabilizer in geotechnical applications. The hydrated lime was used to provide a calcium source $(Ca)^{+2}$ in the cementation solution instead of using calcium chloride and to raise the media's pH to enable the microorganisms' growth and precipitate the $CaCO_3$.

Table 2. The chemical composition of olive mill wastewater (OMWW)

Parameter	Unit	Avg.
Ca	(mg/L)	70.87
Na	(mg/L)	121.49
K	(mg/L)	4260
COD (*10^{-3})	(mg/L)	38.34
TP	(mg/L)	141.2
TN	(mg/L)	390.4
T-Phenols	(mg/L)	2419
TSS	(mg/L)	4430

2.1.4. Hydrated Lime Ca(OH)2

Hydrated lime is widely used as a chemical stabilizer in geotechnical applications. The hydrated lime was used to provide a calcium source $(Ca)^{+2}$ in the cementation solution instead of using calcium chloride and to raise the media's pH to enable the microorganisms' growth and precipitate the $CaCO_3$.

2.2. Methods

In the MICP process, the bacteria provide the urease enzyme; this enzyme is responsible for the urea hydrolysis process to decompose the urea into ammonium (NH_4^+) and carbonate ions CO_3^-. The released ammonium (NH_4^+) through the urea hydrolysis process is responsible for raising the environment's pH above 8.3 and initiating the precipitation of $CaCO_3$ by a chemical reaction that combines the carbonate with the available calcium ions in the cementation media.

In the proposed MICP recipe, the precipitation process of $CaCO_3$ in soil with the absence of urea in both the enrichment solution and cementation solution in the proposed MICP recipe can be explained by the respiration process of bacteria. Since *Sporosarcina Pasteurii* is an aerobic bacterium, it takes oxygen (O_2) and releases carbon dioxide (CO_2) *in* respiration. Then, a chemical reaction will occur; the (CO_2) with the existing H_2O in the OMWW combines and forms carbonic acid H_2CO_3, as represented in Equation 1.

$$CO_2 + H_2O \rightarrow H_2CO_3 \tag{1}$$

The carbonic acid H_2CO_3 with the existing H_2O molecules in the OMWW and the silicate minerals in the soil will form bicarbonate HCO_3^- as represented in Equation 2.

$$H_2CO_3 + H_2O + silicate\ minirals \rightarrow HCO_3^- + cations(Ca^{+2}, Fe^{+2},\ Na^+, etc.) + clays \tag{2}$$

The HCO_3^- can directly react with the Ca^{+2} to form the precipitated $CaCO_3$, as represented in Equation 3, or the HCO_3^- can decompose and generate $(CO_3)^{2-}$ which can be combined with the Ca^{+2}, to precipitating $CaCO_3$ as represented in Equation 4.

$$Ca^{+2} + 2HCO_3^- \rightarrow\ CaCO_3 \downarrow + H_2O \tag{3}$$

$$HCO_3^- +\ H_2O\ \leftrightarrow\ CO_2^{-3} +\ H_3O^+ \tag{4}$$

This suggestion confirmed what Tang et al. (2020) [27] stated: Bacillus and Sporosarcina are aerobic bacteria; they generate CO2 through respiration. The generated CO2 in an alkaline environment can be converted into $(CO_3)^{-2,}$ and this $(CO3)^{-2}$ metabolized and precipitate $CaCO_3$.

The Sporosarcina pasteurii bacterium (ATCC 11859) was used in this research. The bacterium was refreshed twice in tryptone soya yeast extract broth (TSYEB, HiMedia, Mumbai, India) fortified with 20 g/L urea at 37 °C for 20 h. For the experiment, 100 µL of the S. pasteurii culture was transferred into 50 mL TSYEB fortified with urea and incubated at 37 °C for several days to achieve a good sporulation ratio. Next, the bacterium was collected by centrifugation and resuspension of the culture in phosphate-buffered saline (PBS) to remove the impurities and metabolic by-products. Further, the cell suspension was pasteurized for 20 min at 80°C in a water bath and then rapidly cooled for 5 min to ensure all the cells were spores. Finally, the obtained spore suspension was kept at 4°C until use.

2.2.1. Preparation of the Enrichment and Cementation Solution in the Traditional MICP Recipe

The MICP recipe was made using the Tiwari et al. (2021) method [28]. For making an enrichment solution, 100 mM sodium acetate ($C_2H_3NaO_2$), 333 mM urea (CH_4N_2O), and 2.0 g/L tryptone soy broth were dissolved in distilled water. These substances have important minerals, vitamins, and amino acids, which make a suitable environment for the culture of native soil bacteria. After sterilization of the enrichment solution, bacteria were added with a density equal to 1×106 CFU/mL.

The bacterial count was determined by conventional spread plating on tryptone soy agar (TSA, Oxoid Ltd., Basingstoke, U.K.) and spectrophotometric analysis (OD600 nm) adjusting turbidity to 0.5 McFarland standard (OD= 0.13- 0.08) at a wavelength of 600 nm resulting in a suspension containing about approximately 1×108 CFU/ml followed by serial dilution.

The cementation solution consisted of the same materials as the enrichment solution and 250 mM calcium chloride ($CaCl_2$). The cementation solution was prepared with three different molarities of ($CaCl_2$): 0.25M, 0.5M, and 1.0M.

2.2.2. Preparation of the Enrichment and Cementation Solution in the Proposed MICP Recipe

Similar to the traditional MICP recipe, the enrichment solutions comprised only 100% OMWW. Then, the spore solution was added with a density of 1 × 106 CFU/mL. The cementation solution consisted of 1M hydrated lime Ca (OH)2 and was added to 100% concentration of OMWW. The cementation solution was prepared with three different Ca (OH)2 molarities: 0.25M, 0.5M, and 1.0M.

2.2.3. Preparation of Soil Sample

The soil sample was sieved on sieve No.40 and oven-dried at oven temperature (105 ℃) to kill 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 S. pasteurii density of 1×106 CFU/mL, to achieve the best improvement of plasticity characteristics as recommended by Osinubi et al. (2019) [29]. Three cementation solutions were prepared (0.25M, 0.5M, and 1.0M). In the mixing process, the enrichment solution was mixed first, then followed by the cementation solution. The soil samples were thoroughly mixed to ensure a uniform distribution of S. pasteurii within the soil matrix. The soil specimens were then 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 (1, 7, 28, and 90) days. Figure 3 shows the practical methodology for dealing with the MICP technique.

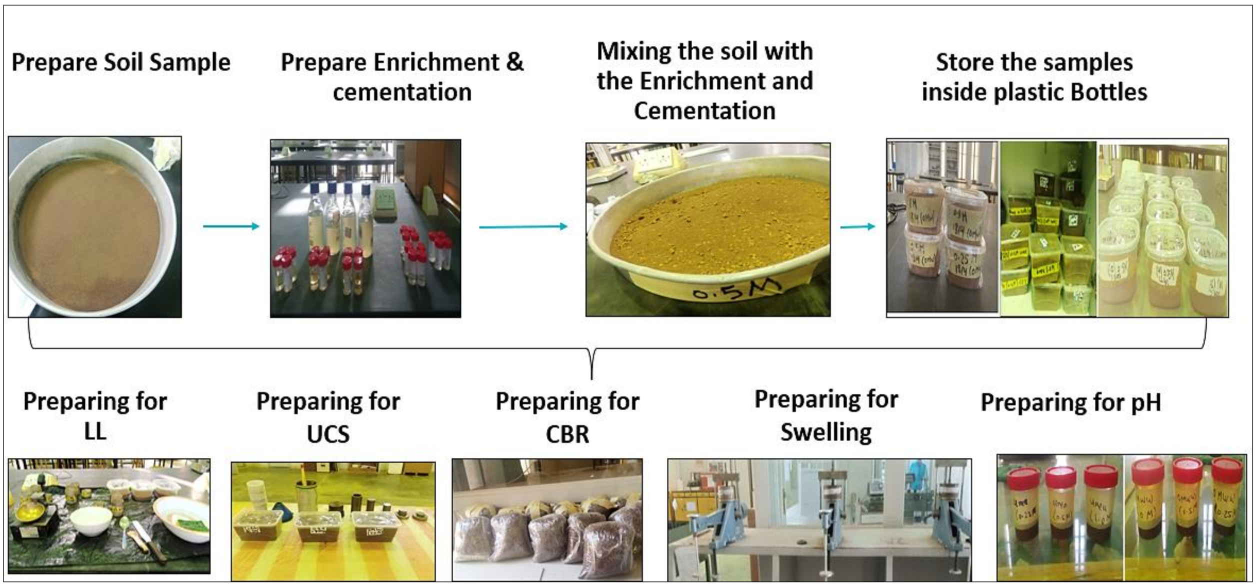


Figure 3. A practical methodology of the MICP process

3. Results and Discussion

Various geotechnical properties were evaluated before and after implementing the stabilization process to quantify and qualitatively assess the extent of improvement achieved. The analysis focused on comparing the geotechnical properties of the soil samples before and after treatment, considering factors such as the mellowing period and the concentration of the cementation solution.

3.1. pH Results

Figure 4 presents the pH measurements obtained after mellowing periods of 1, 7, 28, and 90 days in both recipes, using different concentrations of cementation solutions. The most favorable environmental condition for precipitating $CaCO_3$ is the alkaline environment. For urease microbes, the approximate neutral pH environment is optimal (pH=7) [6].

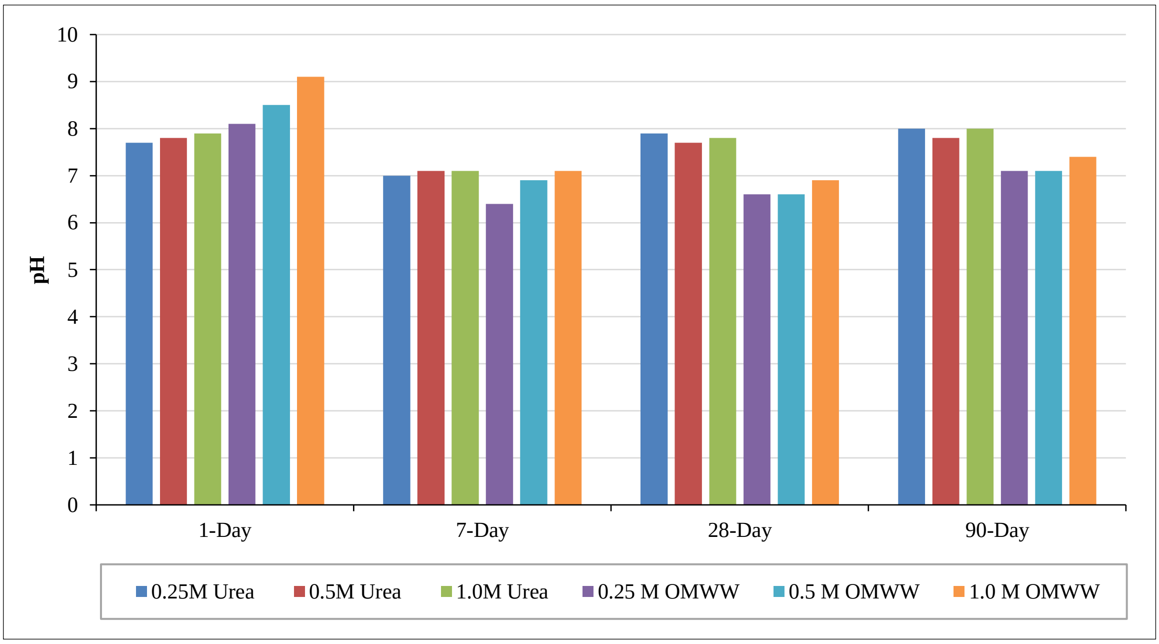


Figure 4. pH Results after (1, 7, 28, and 90 Days Mellowing Period)

Different researchers have reported varying optimal pH ranges for the activity of S. pasteurii, a common bacterium used in bio cementation processes. Some studies indicate that S. pasteurii has an optimal pH range between 8-9.5. These deviations in optimum pH could be due to factors such as strain differences, experimental conditions, and specific research aims. By-products like hydroxide (OH^-) ions and ammonium ($NH4^+$) are made through urea hydrolysis. These by-products increase the pH level, making it more suitable for $CaCO_3$ precipitation to occur [30].

Figure 4 shows the fluctuation in pH values in the bio-treated soil with different mellowing periods. The pH values were not consistent and exhibited changes over time. This behavior can be explained by the microbial activity and various reactions occurring during the MICP process.

In the urea hydrolysis process, carbon dioxide and ammonia are produced initially; the production of these by-products increases with the urea concentration. This results in a rapid increase in the pH values during the initial mellowing periods, as shown in Figure 4. With time, these factors of urea hydrolysis, bacterial activity, and the precipitation of $CaCO_3$ balance out and result in more stable pH values inside the system [31, 32]. The behavior shown here is consistent with Moradi et al.'s (2022) [33] findings, which reported similar fluctuations in pH values at different mellowing periods. These pH fluctuations indicate the complex interactions between the microorganisms, the medium, and the MICP process.

On the other hand, for the proposed recipe, the addition of calcium hydroxide at the initial stages leads to an increase in the pH values due to the exiting of hydroxide and calcium ions, the dissolving of lime with time, and the acidic nature of OMWW resulted in a dynamic system with flocculation's in the pH values until the system stabilize and reach the equilibrium during the progress of calcite precipitation.

3.2. Atterberg's Limits Results

Figure 5 shows the Atterberg's limits results after mellowing periods of 1, 7, 28, and 90 days using different concentrations of cementation solutions for both recipes. The figure shows that a decrease in Liquid Limit (LL) and an increase in Plastic Limit (PL) after the seven-day mellowing period culminated in a decrease in Plasticity Index. Therefore, in the proposed MICP recipe, a concentration of 1.0 M resulted in the maximum reduction in plasticity index, meaning less soil plastic deformation. Additionally, there was a slight reduction in PI due to slight increments in LL and further reductions of PL after the mellowing period of 28 and 90 days. The increase in the LL values is attributed to bacterial populations' increased mellowing over time, thus requiring more moisture for their activities since they use it for their metabolic activities.

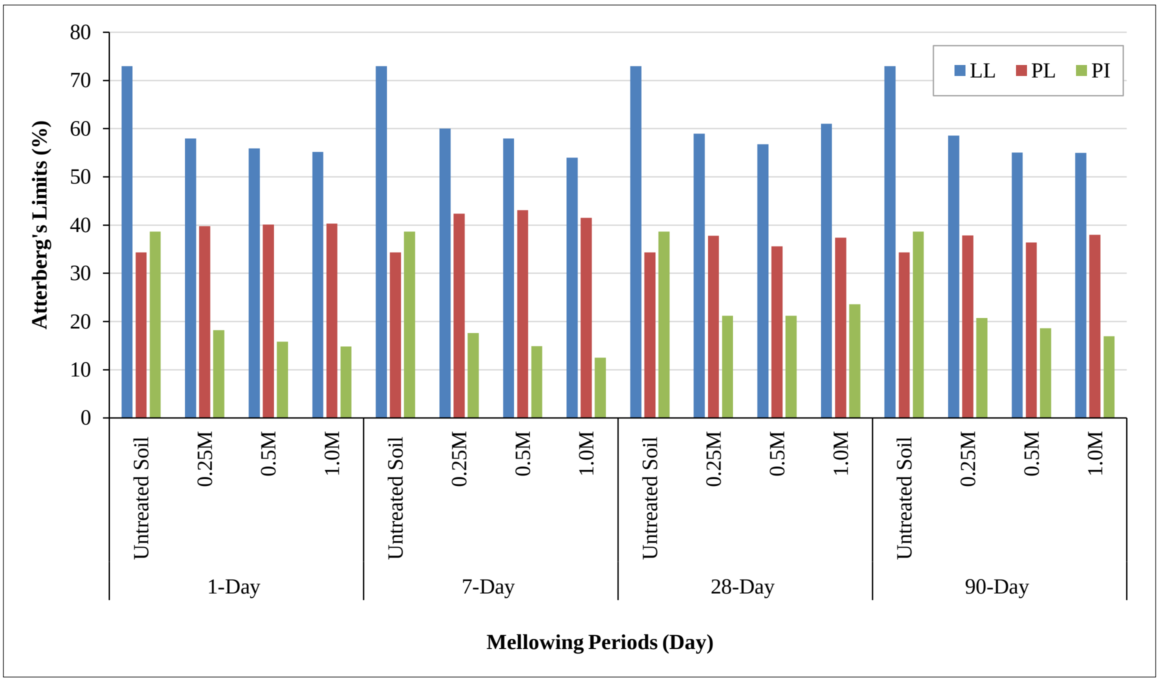


Figure 5. Atterberg's Limits Results (1, 7, 28, and 90 Days Mellowing Period)

The reduction in soil plasticity can be attributed to various factors within the MICP process. One explanation is the behavior of aerobic bacteria during MICP. These bacteria generate slime-filled voids between soil particles, effectively clogging the soil and promoting the precipitation of $CaCO_3$. This process increases coherence between soil particles, thereby reducing the LL of soils [32].

An additional explanation for improved Atterberg's limits in both recipes is the cation exchange capacity between soil and cations such as Ca^{+2} and K^{+} in OMWW in the new cementation solution compared to the $CaCl_2$ traditional cementation solution. An increase in cation exchange capacity leads to a reduction of diffuse double-layer thicknesses and a decline in the ability of soil particles to retain water [33].

The results obtained after using the proposed recipe were consistent with those obtained by other researchers [28, 29, 34]. On the other hand, a slight increase in Atterberg's limits was observed after a long mellowing period due to the complex and dynamic nature of the MICP process. In addition, different factors may affect the biofilm formation, the calcite dissolving due to changes in the environmental conditions, the decomposition of organic matter, which requires more water, and the adjustment of the soil's microstructure. The formation of $CaCO_3$ bonding between soil particles limited the organic matter's impact on swelling-shrinkage behavior [34].

3.3. Free Swell Results

Figure 6 shows the results of free swell tests conducted after mellowing periods of 1, 7, 28, and 90 days, using different concentrations of cementation solutions in the two recipes. The initial free swell percentage of the untreated soil was measured at 15.5%. However, a notable decrease in the free swell percentage was seen following the bio-stabilization process. This reduction indicates an improvement in the soil's ability to resist swelling when exposed to water.

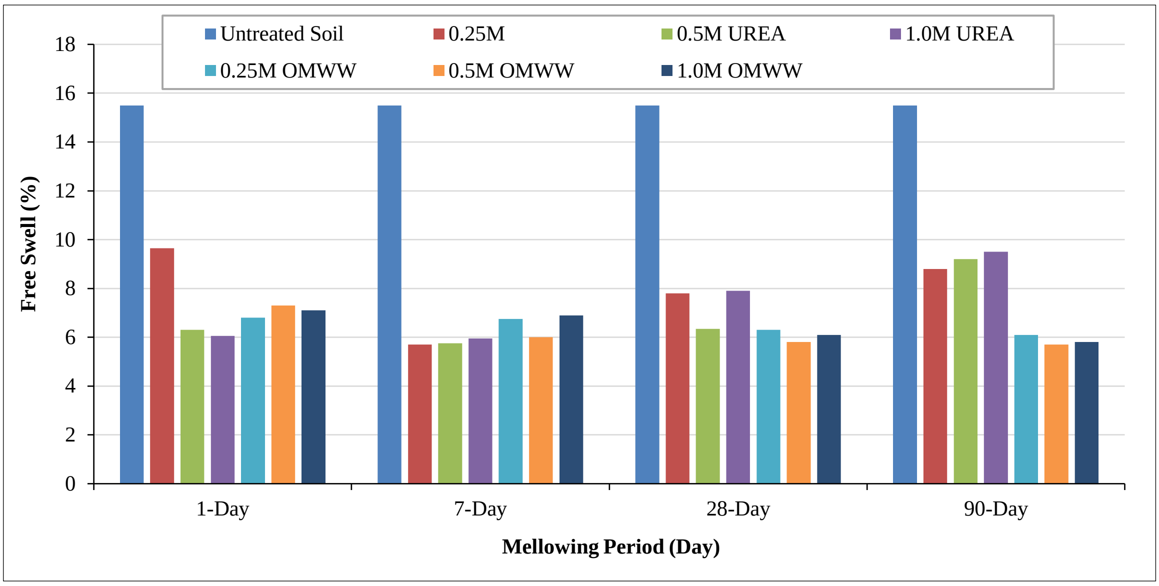


Figure 6. Free Swell Results after (1, 7, 28, and 90 Days Mellowing Period)

During the one- and seven-day mellowing periods, the free swell percentage in the traditional MICP recipe decreased as the concentration of cementation solution increased. However, free swelling was slightly increased after the 7-day mellowing period.

This behavior can be attributed to two reasons. The first reason is the non-uniform distribution of the precipitated $CaCO_3$ through the soil's matrix, which means some areas through the soil matrix are less stabilized and more prone to local swelling. The second reason is due to the presence of urea. Urea hydrolyzes to produce ammonia and carbonate ions, which contribute to the precipitation of $CaCO_3$.

$$(NH_2)_2CO + H_2O \rightarrow 2NH_3 + CO_2 \quad (5)$$

The released ammonia (NH_3) reacts with the existing water in the soil to form ammonium ions (NH_4^+):

$$NH_3 + H_2O \rightarrow NH_4^+ + OH^- \quad (6)$$

Expansive soil, especially the montmorillonite, can react and absorb the ammonium. The existence of ammonium between the interlayer spaces attracts the water, and then the soil expands.

This behavior can be attributed to the presence of urea. Urea hydrolyzes to produce ammonia and carbonate ions, which contribute to the precipitation of $CaCO_3$. This increases voids and gaps within soil structure, resulting in higher free swell percentages. Several researchers have studied the impact of urea on geotechnical properties of soils. According to a study by Narasimha Rao & Chittaranjan (2010) [26], increasing urea content caused an increase in swelling pressure.

A free swell test was carried out using soils with different levels of urea contents to evaluate the effect of urea concentration increase on swelling potential. The results in Figure 7 indicate that higher urea concentrations in the bio-treated soil increase swelling potential.

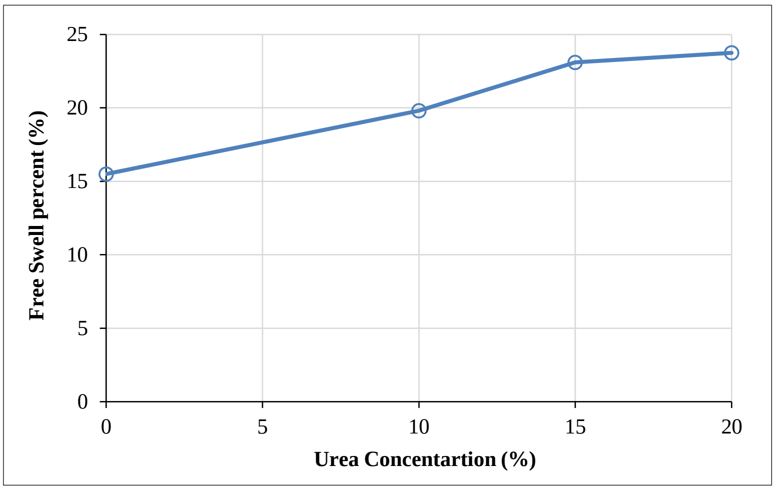


Figure 7. The effect of urea on the free swell percent

In the proposed MICP recipe, a consistent reduction in the free swell percentage was observed as the mellowing period and cementation solution concentration increased. This decrease in free swell can be attributed to the formation of $CaCO_3$, which acts as a bonding agent, enhancing the cohesion between soil particles and improving the overall stability of the soil structure. For instance, the presence of $CaCO_3$ minerals on the external surface of the soil particles alters the interaction between water and the hydrophilic soils. This alteration reduces the inter-spacing between particles and decreases the thickness of the water film surrounding the soil particles, in addition to the absence of ammonium cations, which attract water molecules. As a result, the soil experiences reduced swelling potential. The same behavior was obtained by several researchers [17, 28, 34–36].

3.4. Unconfined Compressive Strength (UCS) Results

The results of UCS tests after mellowing periods of 1, 7, 28, and 90 days are shown in Figure 8, using different concentrations of cementation solutions for both traditional and new MICP recipes. It can be observed that the increase in the concentration of cementation solution and extension of the mellowing period significantly increased the UCS values. For example, in the traditional treatment recipe for MICP, it was about four times higher than untreated soil. Whereas in the proposed MICP recipe, the strength was boosted by approximately eight times that of untreated soil material. The increase in UCS values can be attributed to several causes: firstly, metabolic reactions during MICP lead to water loss, which reduces the overall moisture content within soil particles and enhances their stability. Secondly, Microbial activities contribute to calcium carbonate precipitation, which fills pores, enhances soil cohesion, reduces voids, and densifies the soil matrix [32, 37, 38].

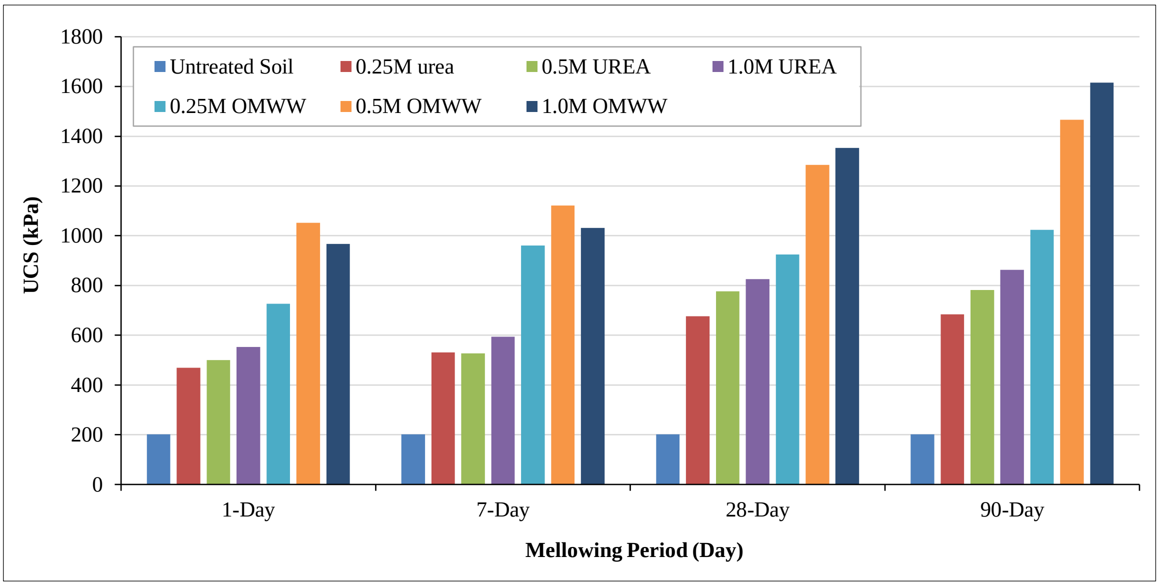


Figure 8. UCS Results after (1, 7, 28, and 90 Days Mellowing Period

As shown, the improvement in the UCS is related to time and concentration. Firstly, the precipitation of $CaCO_3$ in the MICP technique is time-dependent. Therefore, increasing the mellowing period allows more $CaCO_3$ precipitation and further improves UCS [39]. Secondly, increasing the concentration of the cementation solution improves UCS [40]. This improvement is attributed to the increased availability of calcium ions, which are associated with carbonate ions that precipitate $CaCO_3$.

The same trend was observed in several studies for the UCS test results obtained in this study. Xiao et al. (2020) [37] studied the increase in the soil's strength due to the MICP treatment technique. The results showed that the UCS values increased approximately 2.42 times the UCS of the untreated soil. They explained this increase in the UCS through two processes. First, the water content is reduced during the microorganisms' reactions. Second, precipitation of the calcium carbonate minerals inside the pores and between particles solidified and strengthened the soil particles. Tiwari et al. (2021) [28] observed an increase in the UCS values for bio-treated soil specimens with the mellowing period and explained this increase due to the increase in the precipitated calcium carbonate, which strengthens the clayey soil.

3.5. Thermogravimetric Analysis (TGA) Results

The $CaCO_3$ content for natural soil and 1.0 M biotreated soil in both recipes with increasing mellowing period are represented in Figure 9.

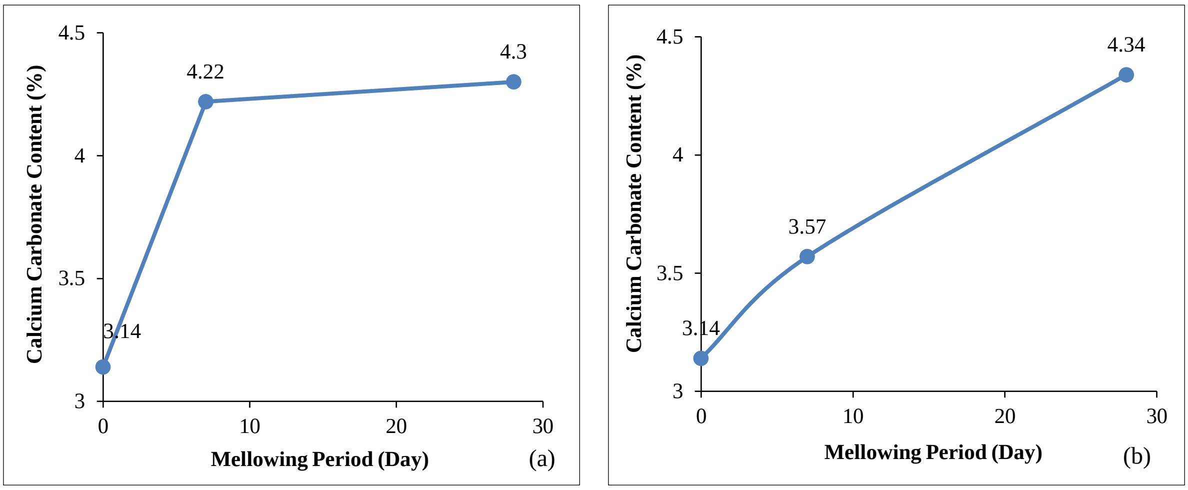


Figure 9. $CaCO_3$ results after (7 and 28 Days Mellowing Period) for a) Urea recipe, b) OMWW recipe

In the traditional MICP recipe, the bio-treated specimens exhibited increased CaCO3 content, a total increase of 1.16%. This increase in CaCO3 content played a significant role in improving the geotechnical properties of the soil. Similarly, in the proposed MICP recipe, the bio-treated specimens showed an increase in CaCO3 content, with a total increase of 1.2%.

In the traditional recipe, the notable increase in $CaCO_3$ content occurred during the initial seven days, primarily due to the availability of nutrients for bacterial growth and activity. After consuming the available nutrients, the rate of $CaCO_3$ precipitation remained relatively steady. On the other hand, there was a slower rate of $CaCO_3$ precipitation during the first seven days in the proposed recipe. This slower rate can be attributed to the adaptation period required by the bacteria in new environments. After this adaptation period, the precipitation of $CaCO_3$ increased continuously over time due to the availability of nutrients and organic compounds in the proposed MICP recipe.

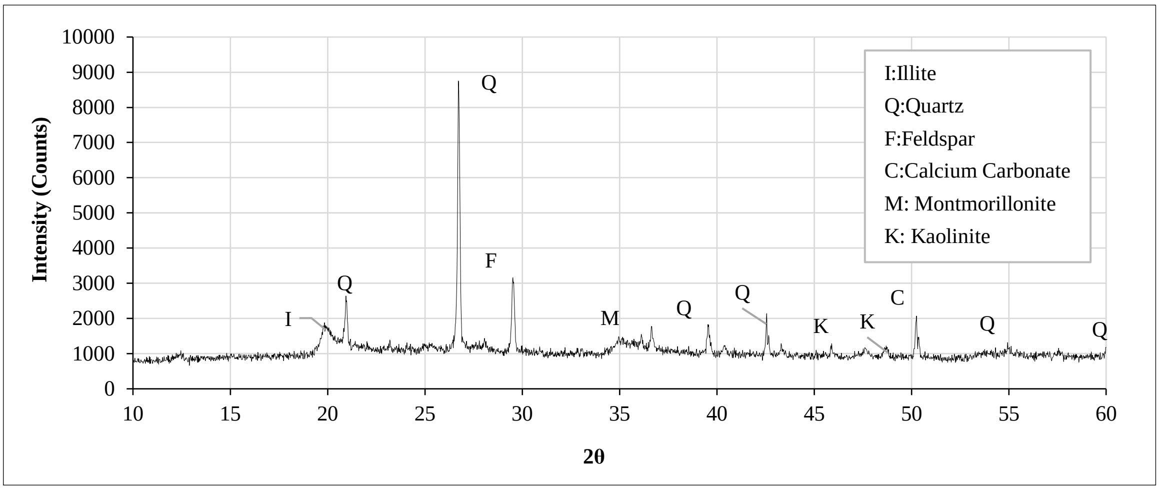
Overall, the increase in $CaCO_3$ content observed in both the traditional and proposed MICP recipes contributes to the improvement in geotechnical properties, and the variation in precipitation rates can be attributed to factors such as nutrient availability and the adaptation period of bacteria. The longer curing time allows the bacteria to produce more $CaCO_3$ precipitation [35].

The findings from previous studies align with the results obtained in this study, providing further support for the observed trends in $CaCO_3$ content and its influence on soil properties. Soon et al. (2013) measured the $CaCO_3$ content for two types of soil: residual soil and sand [41]. The $CaCO_3$ content in the bio-treated sand was in the range of 2.66% to 6.102%, but in the residual soil, it was in the range of 1.75% to 2.559%. The pore space size can explain this high variation in $CaCO_3$ content in these two soils, with residual soil having a pore size of less than 2 µm that inhibited the free movement of bacteria (1.3-4) µm in size. Therefore, the precipitation in $CaCO_3$ was more efficient in sandy soil.

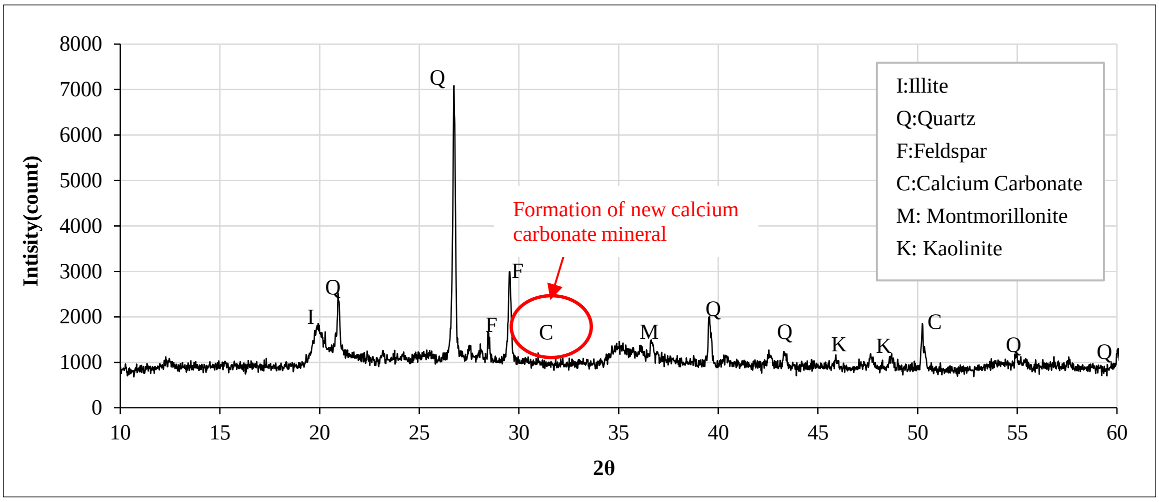
Islam et al. (2020) [34] measured the $CaCO_3$ content for two types of CH natural soil and two types of CL natural soil. The maximum increase in the $CaCO_3$ content in the bio-stimulated specimens was 1.41%. Narasimha Rao & Chittaranjan (2010) [26] Measured the $CaCO_3$ content for two types of soil: residual soil and sand. The $CaCO_3$ content for the untreated soil was 0.67% and 0.27% for residual soil and sand, respectively. After the bio-treated process, the calcium carbonate content in the sand was 2.66% to 6.10%, but in the residual soil, the calcium carbonate content was 1.75% to 2.56%.

3.6. X-Ray Diffraction (XRD) Results

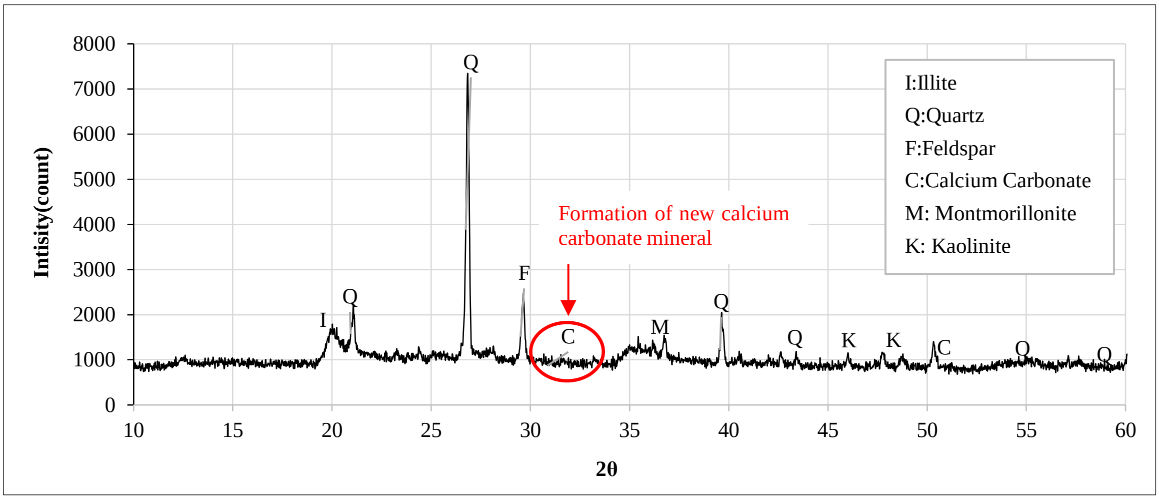
The XRD results in Figure 10-a demonstrate significant changes in soil composition between the natural and bio-treated soil samples after a 7-day mellowing period with a cementation concentration of 1.0M in both recipes.



(a)



(b)



(c)

Figure 10. XRD results for untreated soil, (b) XRD results for biotreated soil in the traditional MICP recipe, and (c) XRD results for biotreated soil in the proposed MICP recipe

The observed shifting of peaks and the presence of new calcite minerals indicate alterations in the mineralogy of the soil. The formation of calcite minerals, a crystalline form of $CaCO_3$, indicates the bio-treated soil undergoing mineralogical changes. This transformation is attributed to the bio-stimulation process and the metabolic activities of microorganisms, particularly the precipitation of $CaCO_3$ through MICP.

Overall, the XRD results confirm that the bio-treatment has induced notable changes in the soil composition, including the formation of Calcite minerals and modifications in the natural soil mineralogy. These findings provide valuable insights into the geotechnical improvements observed in the bio-treated soil samples and support the efficacy of the MICP process in enhancing soil properties.

The consistency between the obtained results after implementing the proposed MICP recipe and the traditional MICP recipe aligns with the findings reported by various researchers. Several studies, including those conducted by previous studies [28, 30, 37, 42–44], have reported that the changes in soil mineralogy following MICP treatments can be attributed to the precipitation of $CaCO_3$ and subsequent interaction with the soil minerals.

3.7. Scanning Electron Microscopy (SEM) Results

Figures 11 and 12 compare images of natural and bio-treated soil that reveal significant improvements in the soil structure. The improvement includes a reduction in volume and the precipitation of $CaCO_3$ on the surface of soil particles. Moreover, the distribution of $CaCO_3$ crystals is not even. This means that soil particles are closer to each other in some areas, and this irregular arrangement improves the overall cohesion and compactness of soil, thus making it more stable.

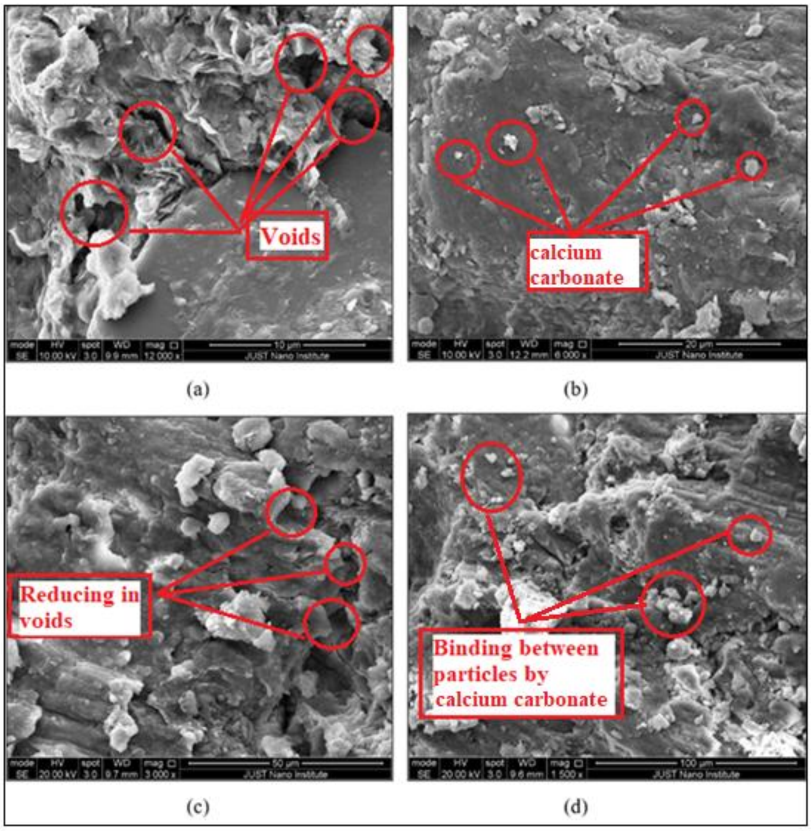


Figure 11. SEM results for (a) untreated soil (b, c, d) 0.5M bio-treated soil at different magnifications in the traditional MICP recipe

The proposed MICP recipe showed notable improvements in the soil structure. These improvements were attributed to several factors. Firstly, the precipitation of $CaCO_3$ within the voids and on the surface of the soil particles effectively filled the voids, reducing their volume. Additionally, the formation of $CaCO_3$ acted as a binding agent, effectively binding the soil particles together. The observations presented in Figure 12 provide visual evidence of these improvements in the soil structure. The altered size, shape, and closer arrangement of the soil particles highlight the positive impact of the proposed MICP recipe on the soil's physical properties.

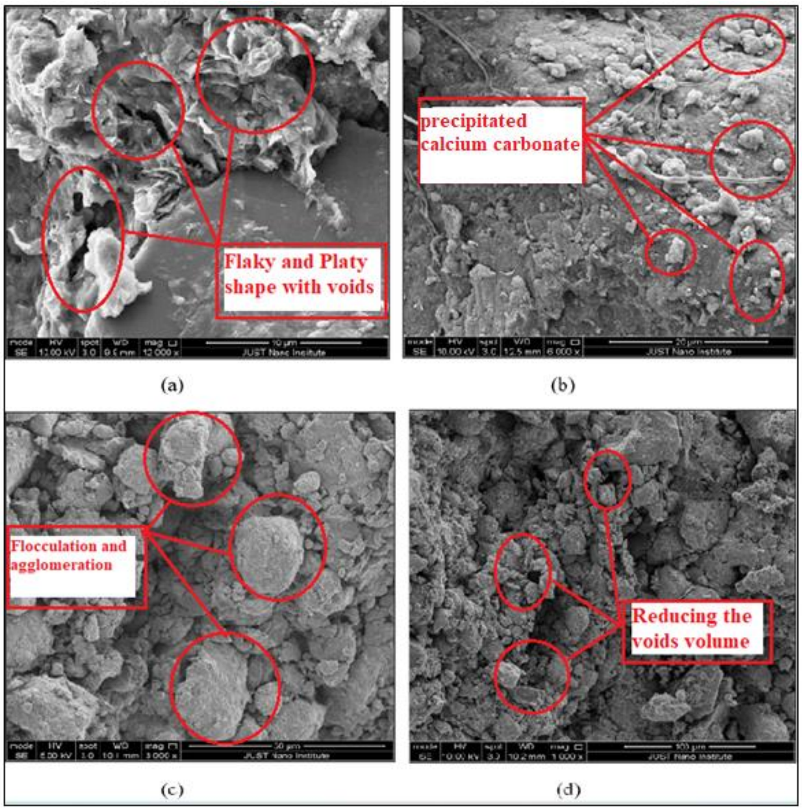


Figure 12. SEM results for (a) untreated soil (b, c, d) 0.5M bio-treated soil at different magnifications in the proposed MICP recipe

3.8. The Optical Microscopic Images

The optical microscope was used to examine the minute features of the bio-treated specimen. Figure 13-a shows the microscopic images of the untreated soil. In these images, the $CaCO_3$ crystals are small and limited to the outer surface of the clay particles. Despite the presence of natural $CaCO_3$, the untreated soil specimen exhibits voids and pores, as shown in the figure. It is important to note that $CaCO_3$ alone does not possess cementing properties that can bind and interlock the soil particles. Consequently, the untreated soil specimen exhibits undesirable geotechnical properties due to the lack of cohesive forces among the particles.

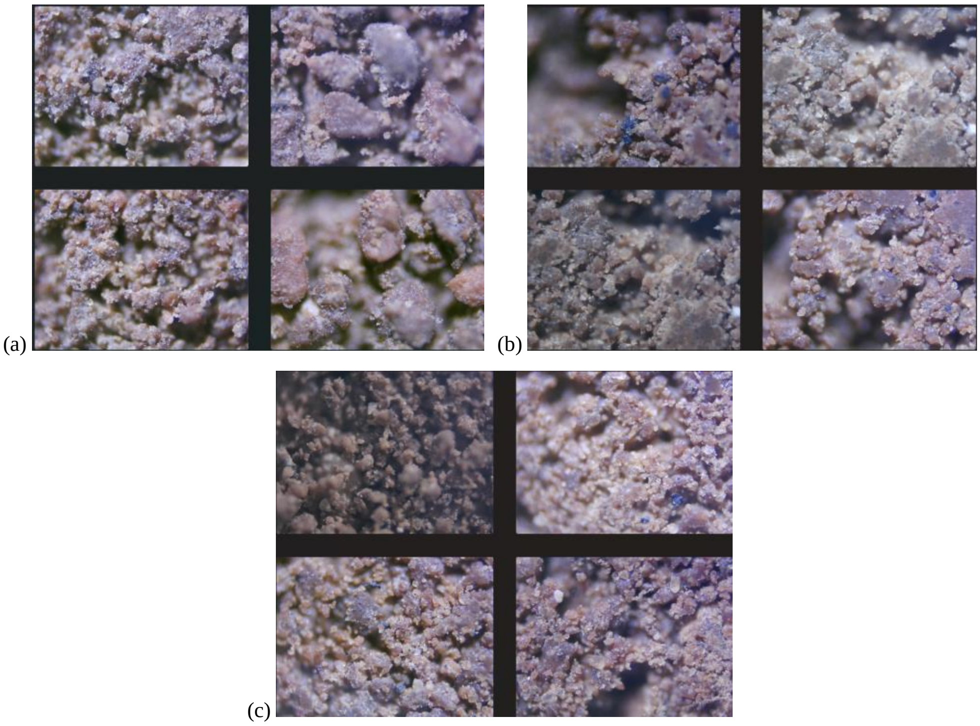


Figure 13. The Microscopic Images for (a) untreated soil, (b) 1.0 M bio-treated soil in the traditional MICP recipe, and (c) 1.0 M bio-treated soil in the proposed MICP recipe

Figure 13-b shows a noticeable increase in the precipitated $CaCO_3$ content compared to untreated soil. Additionally, the size of the $CaCO_3$ crystals increases after the bio-treatment. Moreover, the precipitated $CaCO_3$ effectively covers the external surface of the soil particles and fills the voids between them. The presence of precipitated $CaCO_3$ improves the stabilization of soil structure and geotechnical properties. It serves as a binding material that increases interparticle bond strength, reduces voids, and enhances soil's overall cohesion and compactness.

The bio-treated soil shows a similar pattern when treated with the suggested MICP recipe, as seen in Figure 13-c. The image illustrates that $CaCO_3$ precipitated is evenly spread among soil particles. This even spread benefits the particles by clustering them together and making the $CaCO_3$ crystals stick well onto outside soil particle surfaces. Thus, uniform distribution of these $CaCO_3$ precipitates in the soil acts like a bond-enhancing cementitious material. It strengthens interparticle connections and thus enhances the stability of soil structure. In this way, the geotechnical qualities of soil, such as shear strength, permeability, and load-bearing capacity, are improved.

3.9. Bacterial Growth Rate Results

The suitability of alternative nutrient media for S. pasteurii using OMWW and lime for bacterial growth was assessed by evaluating the growth rate of the bacterium in the proposed MICP recipe and comparing it with the growth rate in the traditional MICP recipe, as shown in Figure 14. The results show that the bacterial count in both recipes went up over the study time. This result confirms the effectiveness of the suggested MICP recipe as a nutrient medium for bacteria to survive and multiply.

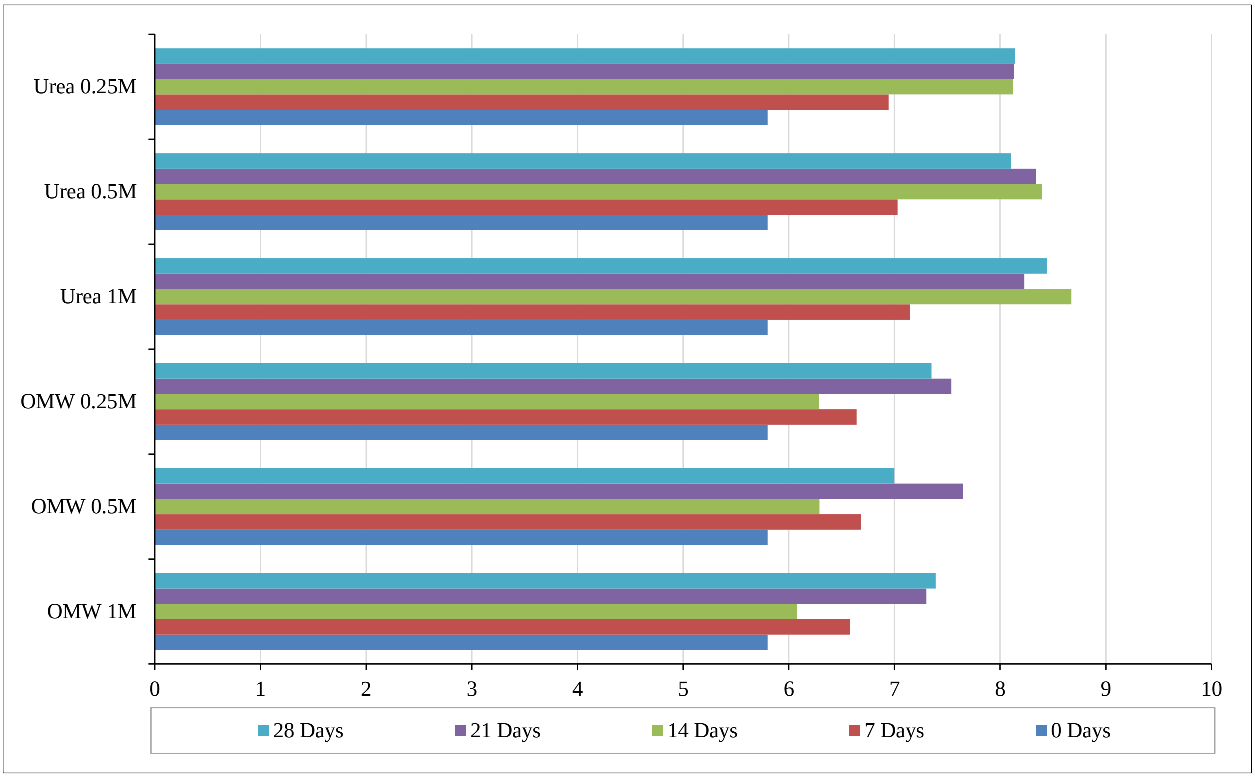


Figure 14. A comparison between the growth rate in the proposed MICP recipe and the traditional MICP recipe

MICP's traditional method results demonstrate significant growth in the bacteria population over time. It is important to highlight that this increase was most noticeable within the initial 14 days because enough nutrition was present in the soil mixture. However, when the population of bacteria increased, they required additional nutrients to survive [45]. In Figure 14, it can be observed that the bacteria's growth was somewhat non-significant. There was a non-significant number alteration between day 21 and day 28, which is believed to be due to nutrient availability. Also, the bacterial populations only experienced a small rise in numbers. This implies that the nutrient supply could have turned insufficient to encourage more growth.

The results of the proposed MICP recipe are consistent with the literature. AL-Eitan et al. (2021) [46] showed an increase in the Bacillus population after treating clayey and sandy soil with OMWW. The results obtained from the growth rate of bacteria were consistent with the experimental results for the geotechnical properties, as observed after 28 days, and the improvement effect was diminished.

4. Conclusions

This study focused on two main objectives. The first is to identify the potential of MICP technology as a viable solution to geotechnical engineering and related areas. In its second intent, this research suggested a new recipe for MICP. This researcher studied the soil improvement by incorporating Sporosarcina pasteurii at various concentrations of cementation solution (0.25M, 0.5M, 1.0M) and different mellowing periods (1, 7, 28, and 90 days). The innovative MICP recipe for stabilizing expansive soil yielded promising results. It can be concluded that the suggested MICP recipe could substitute the traditional MICP recipe based on these outputs. The findings of this investigation can be summed up as follows:

- The introduction of MICP has improved the liquid limit and plasticity index values. As a result, it exhibited reduced plasticity, indicating improved stability and less susceptibility to moisture-induced volume changes.
- The implementation of MICP yielded positive results by effectively reducing the liquid limit of the soil and simultaneously increasing the values of the plastic limit. This led to a decrease in the soil's plasticity, which indicates enhanced stability and decreased vulnerability to volume changes resulting from fluctuations in moisture levels.
- A significant reduction was observed in free swell percentage, particularly following the 7-day mellowing period. However, it is worth noting that after the 28-day and 90-day mellowing periods, the free swell exhibited a slight increase, potentially attributed to the urea effect. Despite this minor increase, the overall trend demonstrated a notable decrease in the free swell percentage.
- The proposed recipe was a suitable medium for increasing the bacteria population. The effectiveness of the proposed MICP recipe can be affirmed based on the laboratory results. The experimental results showed a more significant improvement than the traditional MICP recipe. Specifically, the proposed recipe resulted in higher levels of precipitated $CaCO_3$ compared to the traditional method. Furthermore, changes in microstructure and mineralogy were observed, suggesting soil composition and structure modifications.
- The microscopic image of bio-treated soil showed $CaCO_3$ crystals covering the external surface and filling the voids between particles. On the other hand, the SEM images show flocculation and agglomeration in the soil particles, enhancement in interparticle bonding, and the voids becoming smaller and filled with $CaCO_3$. There was also a notable change in the shape and size of soil particles.
- There are many reasons to improve the MICP recipe. Economically, replacing the traditional chemicals and ingredients in the well-known MICP recipe with OMWW, a wastewater material with enough nutrients and materials to help bacteria grow, reduces costs. Moreover, it helps mitigate the risks and health hazards associated with traditional MICP technology, such as ammonia releases and urea-induced soil swelling. Given the rising water scarcity and the increased demand for water, the safe disposal of large amounts of OMWW and the reduction of freshwater use are environmentally beneficial. A practical MICP recipe for stabilizing expansive soil yielded promising results.

Considering these outcomes, it can be confidently stated that the proposed MICP recipe holds potential for practical applications in MICP processes. The observed enhancements in calcium carbonate precipitation and alterations in soil characteristics provide evidence for the efficacy of the proposed recipe in facilitating soil stabilization and addressing the challenges associated with expansive soils.

5. Declarations

5.1. Author Contributions

Conceptualization, S.R., A.A., B.A., and E.I.; methodology, S.R., A.A., B.A., H.A., and E.I.; software, S.R., B.A., and E.I.; formal analysis, S.R. and E.I.; investigation, E.I. and S.M.; resources S.R., A.A., and H.A.: writing—original draft preparation, S.R. and E.I.; writing—review and editing, S.R., H.A., and E.I.; project administration, S.R., A.A., and E.I. All authors have read and agreed to the published version of the manuscript.

5.2. Data Availability Statement

Data sharing is not applicable to this article.

5.3. Funding

Funding information is not available. The work present in this paper was supported by the Jordan University of Science and Technology (Grant No. 20220347).

5.4. Conflicts of Interest

The authors declare no conflict of interest.

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