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# Bio-cementations: A Review on Enzyme and Microbially Induced Calcite Precipitation Mechanisms and Applications in Geotechnical Engineering

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## ABSTRACT

Modern methods to improve soil must deal with sustainable and green building purposes, and precipitated calcite is the most modern bio-cementation method to improve soil sustainably. Enzyme-induced calcite precipitation and microbially-induced calcite precipitation are precipitated calcite's best branches in bio-cemented soil improvement. EICP and MICP contain calcium chloride, urea, water, urease enzyme and other chemical materials (for MICP As nutrients for bacteria) that are mixed together to precipitate calcium carbonate in the soil. The precipitated calcium carbonate was worked as a binder between soil particles, filling the void and improving the soil's properties. By utilizing this technique in the field, several researchers were able to achieve favorable outcomes in the areas of soil improvement and other engineering applications, including concrete repair and other applications, while also taking into consideration the objectives of sustainability. treatment carried out on soils with one or many cycles of the treatment solution. The treatment revealed a significant improvement in the value of unconfined compression strength, compared to untreated soils. During certain experiments, the unconfined compression strength revealed that soils treated with the enzyme approach exhibited higher values compared to soils treated with a mixture containing a certain proportion of regular Portland cement. The encouraging outcomes that were achieved through the utilization of this test in both the laboratory and the field can be utilized in large-scale operations. Till now, this method has been considered under study. In Iraq there are no any research in term of EICP treatment while there are several research about using MICP in soil treatment.

**Keywords:** EICP, MICP, Calcite precipitations, Calcium carbonate, Bio-cemented.

## 1. INTRODUCTION

The concept of sustainability has a lengthy historical heritage. The United Nations Brundtland Commission, in 1987, defined sustainability as the act of satisfying the current requirements while ensuring that future generations can also fulfill their own needs without

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any hindrance. A consortium of over 140 developing nations is actively implementing methods to meet their individual developmental needs. Given the increasing danger of climate change, it is crucial to take tangible actions to ensure that current advancements do not have negative effects on future generations **(Holdgate, 1987).**

Finding effective solutions to ground improvement challenges is becoming progressively complex due to sustainability attentions **(Ayarkwa et al., 2022).** Conventional materials and methods must often be changed or enhanced by advanced materials and be environmentally friendly to address sustainability **(Fahy and Rau, 2013).** Portland cement is an example of a widely used building material that is almost indispensable, yet it also raises serious problems regarding sustainability. Portland cement is extensively utilized in ground improvement applications. One application of Portland cement is to directly treat soil in order to reinforce it through soil binding. Regrettably, the production of Portland cement is highly energy-intensive, serving as a significant contributor to carbon dioxide ($CO_2$) emissions. Cement manufacture, specifically Portland cement, is the second largest contributor to global greenhouse gas emissions within the industry sector, accounting for 18% of the total **(Water Resources, 2005).** According to estimates, the cement industry is considered one of the two leading manufacturing businesses that contribute to world $CO_2$ emissions, accounting for around 5% of the total global $CO_2$ emissions **(van Oss and Padovani, 2003)**. Cement is an indispensable component for numerous construction projects, and its necessity is expected to persist indefinitely. Nevertheless, significant progress towards achieving sustainability objectives could be made by implementing strategies that involve either directly replacing or supplementing the usage of Portland cement with ecologically conscious technologies and materials **(Song et al., 2018)**. This study investigated the impact of altering the quantities of urea ($H_2NCONH_2$), calcium chloride ($CaCl_2$), and the urease enzyme on the characteristics of the mixture formed from the interaction of these substances in soil remediation.

**(Arab et al., 2021a)** studied bio-bricks production in the construction area using (EICP) and sodium alginate biopolymer. The findings demonstrate that the manufactured bio-bricks are an environmentally friendly replacement compared to traditional bricks and are comparable to cement-treated beams in terms of their mechanical assets. **(Bernardi et al., 2014)** uses MICP in construction materials, specifically in the creation of bio-bricks. (**Lee and Kim, 2020)** conduct a laboratory test to determine the applicability of (EICP) using yellow soybean for soil stabilization as a source of urease enzyme. **(Zhao et al., 2014)** studied experimentally the effect of several parameters on the engineering properties of MICP-treated soil injected by bacteria and ureases; research aimed to select the best precipitation amount of calcium carbonate by mixing different ratios of urea, $CaCl_2$, and urease enzyme conjunction with studying changes in pH, electrical conductivity.

Following the finding of CIP, the process of carbonate production by urea hydrolysis has been utilized in a variety of different fields **(Tiano, 1995; Stocks-Fischer et al., 1999; Castanier et al., 1999; Rodriguez-Navarro et al., 2003),** bioremediation **(Ferris et al., 2004; Fujita et al., 2004),** wastewater treatment (**Hammes et al., 2003)**, and concrete reinforcement (**Ramachandran et al., 2001)** are some examples of studies that have focused on various methods of restoring calcareous stone materials using multiple techniques (**Knorr, 2014),** a recent shift in emphasis in the field of geotechnical engineering toward the utilization of calcite precipitation produced by the hydrolysis of urea**.**

Using techniques in geotechnical engineering has been recommended to improve soil strength and decrease soil permeability. The suggestions have recognized funding from studies conducted (**DeJong et al., 2006; van Paassen, 2009; Yasuhara et al., 2012)**, (**Hamdan et al., 2013)** to strengthen soil. **(Nemati and Voordouw, 2003; Larsen et al., 2008; Beser et al., 2017)** studied the applications for reducing permeability by using MICP treatment applications.

In the field of geoenvironmental engineering, the techniques of EICP and MICP have been presented as potential methods for decreasing soil erosion, managing flying dust, and co-determining the precipitation of contaminants. **(Hamdan and Kavazanjian, 2016; Shanahan and Montoya, 2016; Kumari et al., 2016; Ossai et al., 2020; Lo et al., 2020)** studies that have been conducted on this topic. Cracks in rock and concrete can also be repaired using the methods described above **(Van Tittelboom et al., 2010; Dakhane et al., 2018)**. Additionally, sandstone monuments can be restored using these methods **(Yang et al., 2011).**

Despite the presence of undesirable precipitation material and remains in the precipitation process, extensive research has consistently verified that this method is environmentally sustainable and meets all the principles for green buildings **(Javadi, 2021)**. EICP considers the development of MICP, EICP usages free urease enzyme. In contrast, the MICP method prepares the urease enzyme with a specific bacteria solution to start the precipitation procedure **(Neupane et al., 2013)**.

In Iraq, there are local efforts to employ chemical substances for soil treatment. In their study, **(Al-Abdullah and Al-Dulaimi, 2008)** used a calcium chloride solution to treat gypsum soil. The approach yielded encouraging outcomes in this treatment, whereas **(Ali and Karkush, 2021)** employed the bacterial technique to remediate clay soil. **(Jasim et al., 2021)** conducted a study on the impact of incorporating high-density polyethylene polymer on the engineering characteristics of sandy soil. The findings indicated an 18% reduction in permeability, along with an elevation in both the angle of internal friction and the CBR value. **(Mekkiyah, 2013)** conducted a study on enhancing soil behavior by incorporating polymeric fibers beneath square foundations. The findings demonstrated a significant enhancement in the failure load values of soil treated with a combination of soil and fibers. This phenomenon represents the current resurgence in soil treatments using nontraditional approaches. These studies exhibit good results and demonstrate significant advancement for local research in Iraq.

This research includes advantages and disadvantages of using EICP and MICP, urease enzyme extraction and solution preparation also including mixing and adding materials to the soil, treatment curing time, and the problematic calculation methods in the precipitation process.

## 2. CHEMICAL PROCESS

### 2.1 Urease Extraction Process and Urease Activity

**(Pratama et al., 2021)** conducted a study on soybean powder derived from soybean seeds prior to their utilization and storage in the refrigerator. To create a uniform mixture, combine a precise quantity of soybean powder with a suitable amount of water. Stir the mixture using a magnetic stirrer for 6 minutes. Then, the solution was centrifuged at room temperature using a centrifuge machine set at a speed of 3000 rpm for 20 minutes. Next, the transparent liquid portion of the solution that contained the urease enzyme was gathered and utilized. The activity of urease was assessed by comparing it to commercial pure urease using an electrical conductivity test **(Whiffin et al., 2007)** in **Eq. 1.** The amount of commercial pure urease used is 1-4 g /L, while the amount of centrifuge urease is 10-50 g/L.

$$\text{Urease activity (U)} = \left(\frac{\theta_{ms}}{\theta_{sc}}\right)(V)(N) \qquad (1)$$

where $\theta_{ms}$: Rate of change of electrical conductance, $\theta_{sc}$: Rate of change of the standard curve, V: Sample volume in liters, N: Final ammonia concentration in millimoles per liter.
The study result shown in **Fig.1** the standard curve for commercial and centrifuged urease, while **Fig.2** shows the relationship between urease concentration and urease activity for three types of ureases **(Pratama, 2021).**

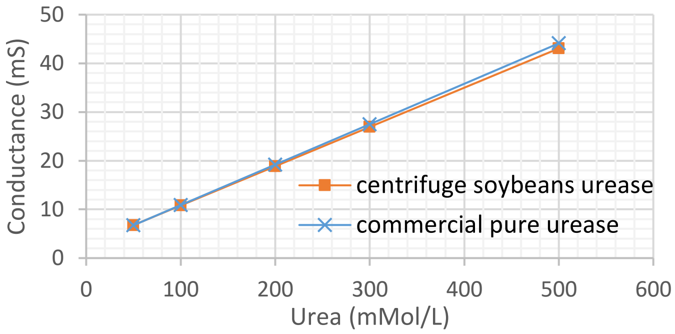



**Figure 1**. Variation between the urea concentration and conductance (**Pratama, 2021).**

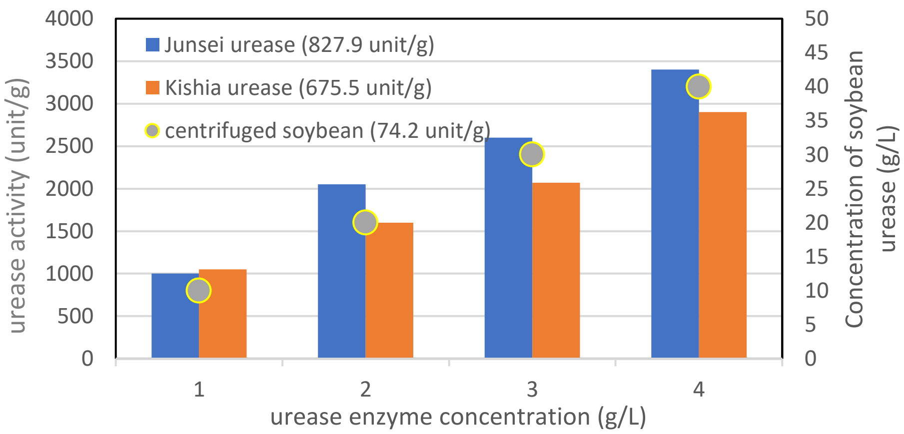



**Figure 2.** Relation between urease concentration and urease activity for different types of urease enzymes (**Pratama, 2021).**

The study's finding is that the soybean urease product can be used in soil improvement instead of commercial urease. **(Shu et al., 2022)** used a urease enzyme extracted from soybean powder by treating the powder with tap water with a pH of 7.5. Diverse concentrations and variable temperatures were applied during the production process. Using a food-grade additive, calcium sulfate dihydrate ($CaSO4 \cdot 2H2O$), with a minimum content of 98%, to precipitate excessive protein in a powdered soybean suspension. The solution was exposed to stirring for five minutes, the resulting solution was centrifuged at 4000 rpm for 15 minutes at a temperature of 4 ◦ C. The supernatant was collected, while the insoluble constituents were removed through filtration. The soybean crude urease was refrigerated at 4◦ C. Five distinct ranges of powder particles were employed to examine the effect of different particle sizes on urease activity, and the test results are depicted in **Fig. 3.** The study revealed that increased particle size beyond 0.25mm decreased urease activity.

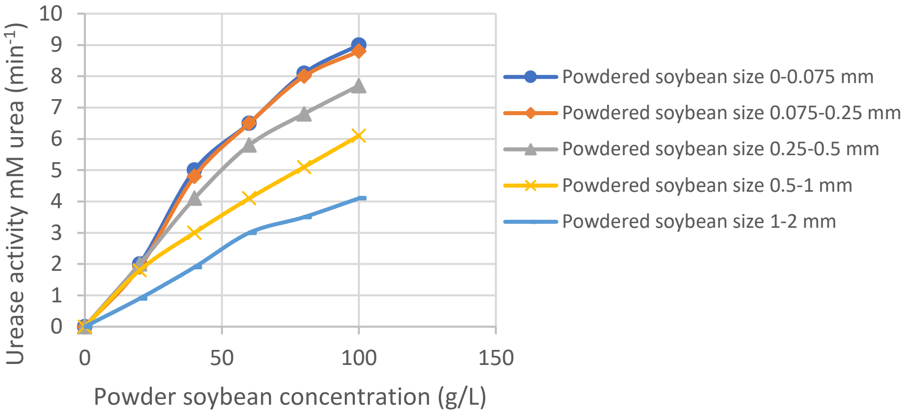



**Figure 3**. Effect of particle size of soybeans powder on the urease activity.

## 2.2 ECIP Mixing Procedure

The EICP technique involves the hydrolysis of urea in the presence of calcium chloride. In the following section, a comprehensive examination will explore various methodologies employed in the scientific mixture of chemicals to acquire calcium carbonate. **(Neupane et al., 2013)** clarified that the mixing procedure involves dissolving the entire quantity of urease in half of the total volume. In the remaining half, urea and calcium chloride are dissolved. These two portions combined to obtain the complete EICP solution, as shown in **Fig. 4**.

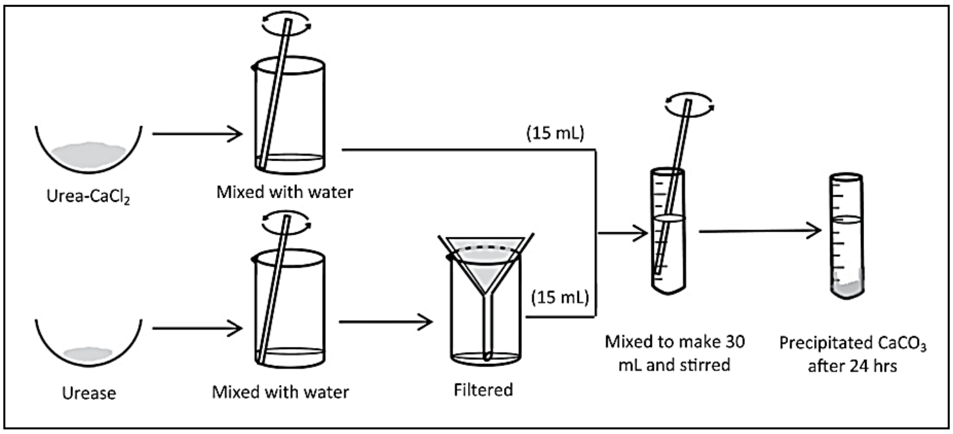



**Figure 4**. Schematic of mixing procedure (**Neupane et al., 2013**).

**(Cuccurullo et al., 2019)** conducted a study wherein an ECIP solution was prepared by combining soybeans, centrifuged urease, and urea. The mixture remained undisturbed for 24 hours, after which the complete quantity of calcium chloride was introduced into the solution.

## 2.3 Calcium Carbonate Precipitation Ratio (PR%)

The precipitation ratio represents the relationship between the observed amount of precipitation and the expected or calculated amount. The calculation of the theoretical value is determined by **Eq. 2**.

$$\text{Theoretical precipitation amount} = M * C * V \tag{2}$$

were M molecular weight of calcium carbonate, (100.087) g/mol, C concentration of urea-$CaCl_2$, in mol/liter. V The total volume in liter.

**(Ahenkorah et al., 2020)** conducted a series of tests to evaluate and improve the influence of urease enzyme and activity and the concentration of chemical (urea-$CaCl_2$) on the applications of (EICP). The laboratory procedures involved the measurement of pH, electrical conductivity (EC), and micro-structure by utilizing an SEM test (scanning electron microscope). The experiment tested two specimens of urea and calcium chloride concentrations at 0.5M and 1M, respectively. Two types of urease enzymes, with two activity levels (40.15 kU/g and 3.5 kU/g), were used in the experiment. 48 tests were examined to evaluate the effects of varying enzyme prescriptions. The test outcome is depicted in **Fig. 5**.

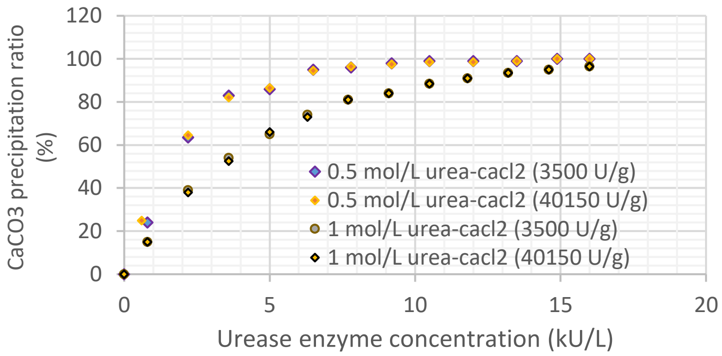



**Figure 5**. Urease enzyme with calcite precipitation ratio (**Ahenkorah et al., 2020).**

The findings suggest that the PR% percentage depends on the urease enzyme quantity. The amount of PR exhibits a progressive increase. Eventually, it reaches an asymptote at around 10 kU/L when the urea-$CaCl_2$ concentration is low (0.5 mol/L) compared to high (1.0 mol/L), keeping the urease enzyme concentration constant. Insufficient levels of urease enzyme in EICP at high concentrations of urea- $CaCl_2$ can lead to this result.

## 3. SMALL SOIL COLUMN

### 3.1 Unconfined Compression Tests

The unconfined compression test conducted on sand is widely regarded as a prevalent examination method among researchers in this field. **(Almajed et al., 2019)** Conducted nine unconfined compression strengths (UCS) on Ottawa (20/30) sand with a relative density 76%. The three treatment solutions were used; the first one was EICP solutions contain urea, calcium chloride, and urease enzyme with concentrations equal to 1 M, 0.67 M, and 3 g/l respectively. The two other solutions are called modified EICP solutions; the first contains a 1 M of urea, 0.67 M of calcium chloride, 3 g/l of urease and enzyme, and 4 g/l of non-fat milk, while the second modified EICP solutions contain 0.37 M urea, 0.25 M calcium chloride, 0.85 g/l urease enzyme and 4 g/l of urease enzyme. Urease enzyme activity was equal to 3500 U/g, and the volume of treatment solution used was equal to one pore volume. The sample was covered to minimize the evaporation process of the treatment solutions, three days of curing time were used, and then the specimen was rinsing with one pore volume of deionized water and then dried until constant mass were achieved. Unconfined compression strength with an axial strain rate of 1.27 mm/minute was applied, as shown in **Fig. 6.**

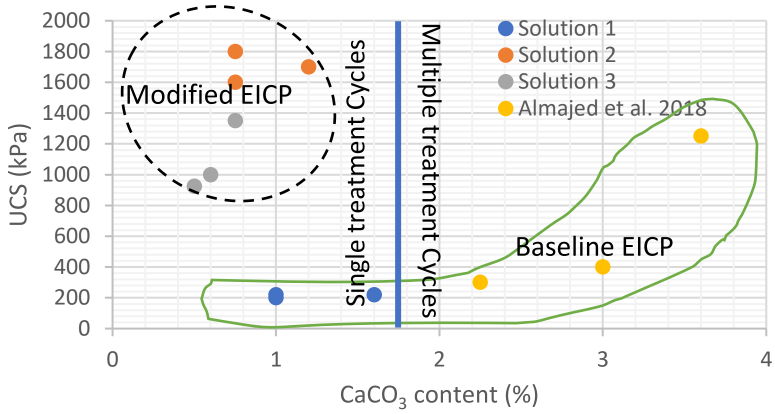
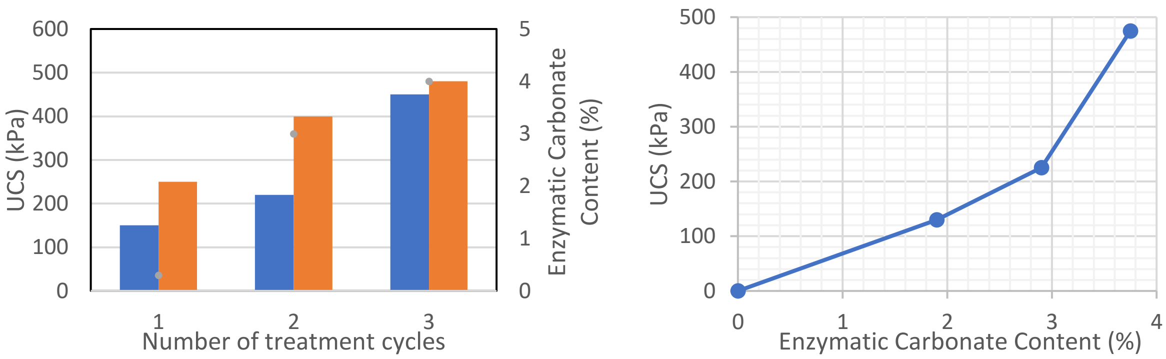



**Figure 6**. Unconfined compressive strength with calcium carbonate ($CaCO_3$) content for specimens treated by EICP **(Almajed et al., 2019).**

The result of study indicates that the enhancement of unconfined compressive strength in soils due to carbonate precipitation is contingent upon the quantity of carbonate precipitated and how precipitation occurs. If the distribution and shape of the precipitates are favorable, it is possible to get a relatively high strength at a relatively low carbonate concentration. **(Miftah et al., 2019)** Nine specimens of sand columns were conducted with dimensions of 5*10 cm. Natural beach sand with 62.5% carbonate mineral was used with one, two, and three cycles were, the sand treated with an EICP solution containing 1M of urea, 0.67 $CaCl_2$, and 10 ml/l of urease enzyme. Unconfined compression strength (UCS) for sand relative density was 60% and mixed with one pore volume of EICP for four days were shown in **Fig.7.**

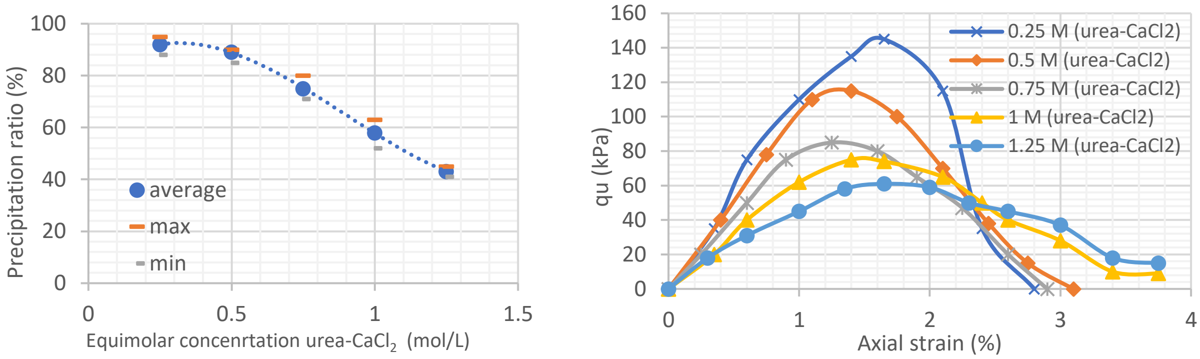



a b

**Figure 7.** (a) UCS and $CoCa_3$ with treatment cycles, (b) UCS with $CoCa_3$ content **(Miftah et al., 2019).**

The findings indicated that the three treatment cycles yielded UCS of approximately 0.5 MPa, accompanied by calcium carbonate precipitation above 3% of the soil mass.

**(Carmona et al., 2016)** conduct five unconfined compression tests on five different concentrations of urea-calcium chloride while keeping the enzyme content constant. Sandy soil with low grading was combined with EICP treatment solution in 8 layers. The treatment solution consisted of five different concentrations of urea-calcium chloride, with the concentrations being 0.25, 0.5, 0.75, 1, and 1.25. The urease enzyme concentration remained constant at urease activity of 34,310 U/g. The specimens cured for 14-day in a chamber with exactly controlled humidity (60±5%) and temperature (20±2ºC). The specimen dimension was equal to 37 mm diameter and a height of 76 mm. **Fig. 8** shows the result of the test.

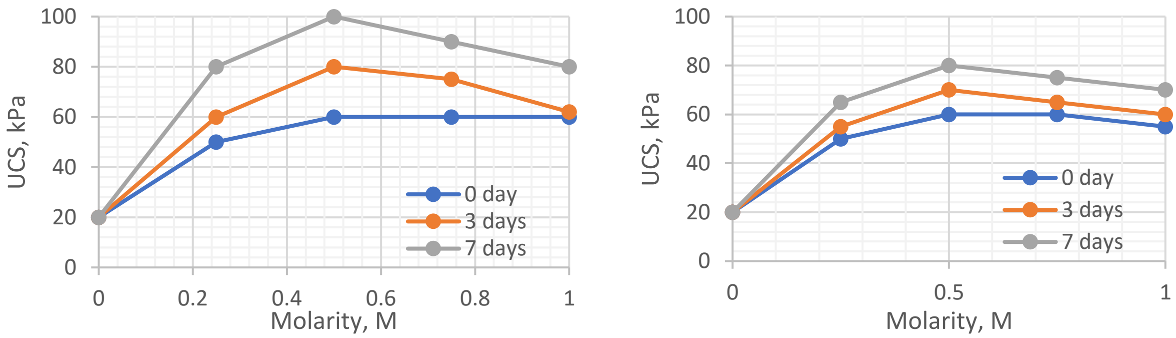



**(a)** **(b)**

**Figure 8**. (a) The concentration of equimolar solutions with a PR% precipitation ratio**,** (b) Stress-strain curves with urea- $CaCl_2$ concentration. **(Carmona et al., 2016)**

The results indicate that higher concentrations of urea- $CaCl_2$ over 0.25 M result in a decline in the precipitation ratio and, subsequently, a decrease in the strength of the stabilized soil. The cause of this phenomenon can be ascribed to insufficient levels of urease and/or the suppression of urease activity at greater concentrations of urea- $CaCl_2$.

**(Ali and Karkush, 2021)** studied the improvement of UCS of Soft soil using MICP. Clay soil CL and two different Bacillus Sporosarcina bacterial concentrations were used in the test to produce calcite with varying concentrations of equimolar solution (urea-$CaCl_2$). Three different curing times were studied 0, 3, 7 days; the result of the test is shown in **Fig. 9.**




**(a)** **( b)**

**Figure 9**. Compares UCS for soil treated with EICP (a) =1*10$^4$ and (b)= 1*10$^7$.

The test results demonstrate a positive correlation between the molarity of the equimolar solution and UCS, within the range of 0.25 to 0.5. However, beyond this range, the rise in molarity has a detrimental influence on endurance. Increased bacterial concentration results in a corresponding rise in UCS at equimolar concentrations. **Fig. 9** illustrates a positive correlation between curing time and strength growth.

### 3.2 Direct Shear Test

**(Putra et al., 2018)** studied a series of direct shear tests on small specimens to examine the changes in cohesion and friction of sandy soil when subjected to Enzyme-Mediated Calcite Precipitation (EMCP). The EMCP solution concentration comprises 1 M of urea, 2 g/l of urease enzyme, and a set total concentration of $CaCl_2$-$MgSO_4$ at 1.00 mol/L. A range of $MgSO_4$

concentrations, ranging from 0.02 to 0.10 mol/L, were employed to determine the optimal combination in the quantity of precipitated minerals. The silica sand exhibited inadequate grading, as indicated by a relative density of 50%. The methodology employed by the researchers in this study is consistent with the approach described in a previous study by (**Neupane et al., 2013),** and the curing period lasted for three days under ambient conditions while the material was introduced into the soil samples through the uppermost portion. The normal stresses of 0.80, 1.60, and 3.20 kN/cm$^2$ were administered to establish the correlation between normal and shear stress for each condition. The test result is shown in **Fig. 10**.

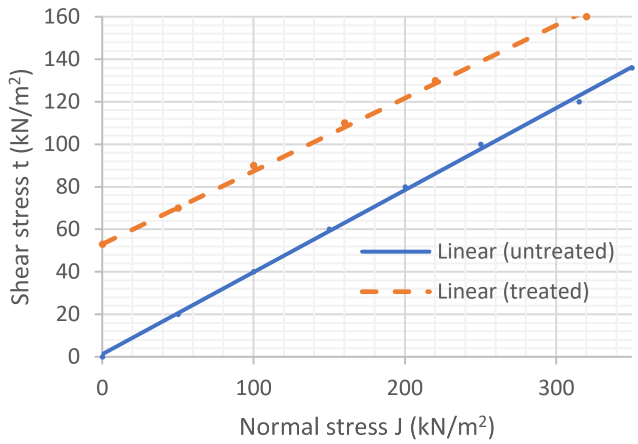
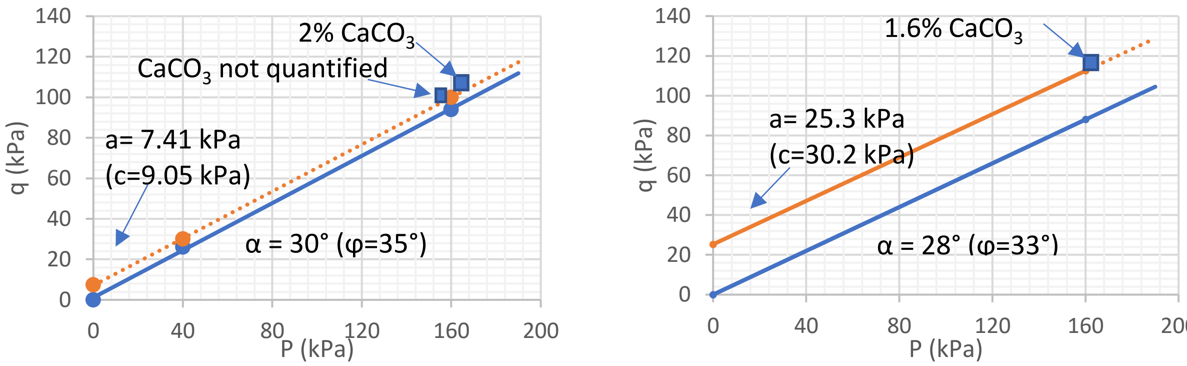



**Figure 10**. Shear Stress-normal Stress Curve and Shear Strength Parameters.

The findings demonstrated that a soil cohesion value of 53 kN/m$^2$ and a precipitation content of 4.1% calcium carbonate inside the soil mass was utilized. Simultaneously, the friction angle exhibited a near-constant value.

### 3.3 Triaxial Test

**(Hamdan, 2015)** conducted a series of drain triaxial compression tests on three sand columns, consisting of two Ottawa 20-30 and one Ottawa f -60. The first sand sample subjected to a dry pluvial process using a funnel with a drop height of approximately 3 inches. Subsequently, treated with a cementation solution consisting of urea and calcium chloride, mixed with an enzyme concentration of 1.4g/L. The total volume of the solution used was approximately 300 ml. The second sand sample was added to the funnel in the same manner as the first sample, and then two applications of the cementation solution were received, with a total volume of about 150 ml. The third specimen in the lower set was filled with approximately 3g of sand and dry enzyme. The remaining tube portion was filled with dry sand without any urease enzyme. Then, the sample was subjected to two applications of the cementation solution, adding approximately 150 ml, without adding any enzyme. The amount of the precipitate of calcium carbonate was recorded as 11.8 g, 2.07 g, and 3.57 g for the first, second, and third specimens, respectively. The triaxial test results are presented in **Fig .11**.

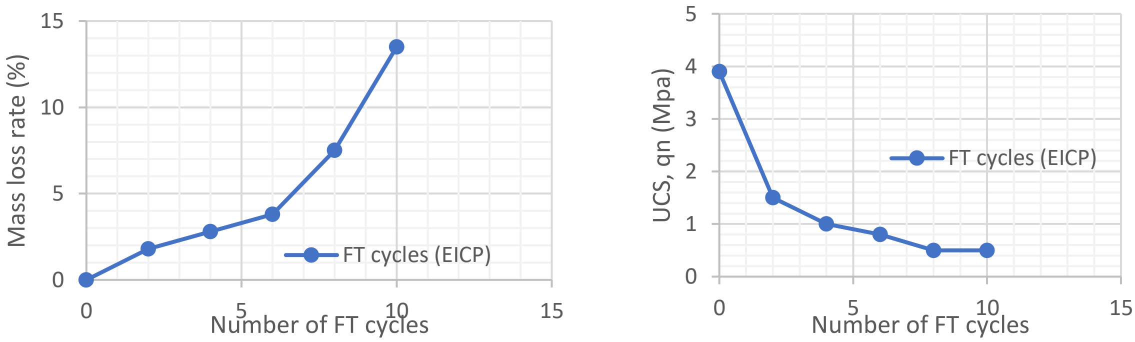



**(a)** **(b)**

**Figure 11.** (a) p-q Plot for 20-30 silica sand: ■ Cemented (Dr = 60%); ○ Uncemented (Dr = 60%), (b) p-q plot for F-60 silica sand: ■ Cemented (Dr= 35%); ○ Uncemented (Dr = 37%)

## 4. DURABILITY TESTS

**(Ahenkorah et al., 2023)** investigated the impact of wetting-drying and freezing-thaw cycles on poorly graded sand characterized by a relative density of 33%. The EICP solution consists of a of 0.5 M for urea- $CaCl_2$ and 0.25 g/l of enzyme with an activity level of 40.15 KU/L and 4 g/l of nonfat powder milk. The percolation approach was utilized for both wetting-drying and freezing-thawing cycles. Multiple treatment cycles were administered, with a cure duration of 48 hours at 30 degrees Celsius.
The wetting and drying cycle followed the (**ASTM D559**, **2015)** standard for soil treatment with portland cement. The sample underwent exposure for several cycles, specifically 2, 4, 6, 8, and 10 wetting-drying cycles. Each experimental cycle involves immersing the specimen completely in distilled water for one day, then the specimen placing in oven at 60°C for 48 hrs. The (**ASTM D560/D560, 2016)** standard was utilized to incorporate the freezing-thawing cycle in the testing of soil treatment with Portland cement. The specimens underwent exposure to a series of Freezing Thaw cycles for 2, 4, 6, 8, and 10 cycles. The saturated specimen was frozen at -18°C for 24 hours, followed by a subsequent thawing period of 24 hours at room temperature (25°C ±1). Mass loss was determined following each treatment cycle by measuring the sample's dry weight. The mass loss was determined after completing the specified number of wetting-drying and freezing- thaw cycles. Subsequently, the unconfined compression strength was calculated to measure the precipitation of calcite in each specimen, the result of the test shown in **Fig. 12**.




a b

**Figure 12. (a)** The relationship between the mass loss rate with many FTs and **(b)** the unconfined compression strength with several FT cycles (**Ahenkorah et al., 2023**).

The test result indicates that after 10 cycles of treatment, the precipitation reaches 17 % of the sample weight. The sample underwent mass loss with an increasing number of cycles also the mass loss rate increased with increasing cycles.

## 5. LIQUEFACTION STUDY

(**Simatupang and Okamura, 2017)** studied the effects of the degree of saturation during the precipitation of calcite on the behavior of sand that has been lightly cemented using EICP and investigated through a series of undrained cyclic triaxial tests. Liquefaction strength curves correlating the cyclic stress ratio with the number of cycles needed to cause 5% double amplitude (DA) axial strain were compared for treated and untreated sand. The way used to mix EICP solution is the same way previously mentioned in **(Neupane et al., 2013).**

## 6. EXPERIMENTAL MODEL

**(Gao et al., 2019)** performed four experimental trials on soil samples comprising 10% clay, 50% silt, and 40% sand. The experimental model used for the examination is shown in **Fig. 13**. The vacuum pressure was provided at a maximum of 100 kPa, and the treatment cycle involved applying vacuum pressure and percolating the EICP fluid treatment at the soil surface. The vacuum pressure enhanced the movement of the treatment liquid through the soil samples. After immersion of the treatment liquid into the soil sample, the valve was closed, ensuring the retention of the liquid within the soil sample for three days, thereby facilitating the completion of the reaction. The previously utilized treatment liquid was evacuated for the succeeding treatment, and a new treatment liquid was introduced, employing the identical procedure described previously. The samples were created using several treatment passes, specifically 0, 5, 10, and 15 passes, triaxial test results for different treatment are shown in **Table 1**.

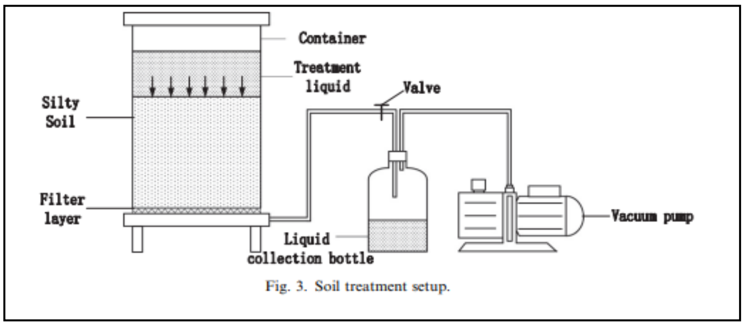



**Figure 13.** Soil treatment model **(Gao et al., 2019).**

**Table 1**. Peak deviatoric stresses in the triaxial tests **(Gao et al., 2019)**

| **Effective confining pressure σc (kPa)** | | **Peak deviatoric stress. Q peak (kPa)** | | |
|---|---|---|---|---|
| | | **100** | **150** | **200** |
| Treatment passes | 0 | 120.8 | 178.9 | 205.7 |
| | 5 | 191.7 | 208.6 | 377.9 |
| | 10 | 206.6 | 269.6 | 280.2 |
| | 15 | 250.0 | 314.6 | 375.5 |

The experimental result showed that applying vacuum pressure resulted in a uniform distribution over the entire soil volume; also, the deviatoric stress exhibits an upward trend when the cycle treatment is intensified.

A cylindrical bio-cemented soil column with a diameter of 0.3 m and a length of 0.9 m was constructed within a rectangular box measuring 0.6 m in width, 0.6 m in depth, and 1.2 m in height. The column was shaped using the EICP technique, in which dry quarry sand was used as the filling material. The EICP solutions contained urea, calcium chloride urease enzyme, and non-fat milk with a concentration of 1.5 M, 1 M, 9900 U/L, and 6 g/L, respectively. These solutions were then subjected to three treatment cycles in the large model. Tap water was utilized in a substantial container as a substitute for distilled water to ascertain that the EICP technique remains unaffected by variations in the water quality. A purpose-built PVC tube-a-manchette (TAM) with a diameter of 24.5 mm was utilized for the experiment **(Martin et al., 2020)**

The tube was equipped with five injection ports, evenly spaced at a distance of 15.2 mm center-to-center between each port. Each injection port consisted of four holes arranged orthogonally. Sand relative density was measured to be 50%, and the injection process was carried out using a peristaltic pump set at an injection rate of 1.7 liters per minute. Each set of injections, consisting of one injection per port, was administered within approximately one hour. There was a one-day interval between each round of injections. The injected fluid volume into each port exceeded the anticipated pore volume of a 0.3 m cylinder by 20% within the 152 mm length of the injection interval. After the completion of a round of injections, the box was either left outside until the initiation of a following cycle of injections or disassembled. The penetration resistance of the bio-cemented sand was measured using a Mecmesin shotcrete needle penetrometer (AFG 1000N), calibrated based on the unconfined compression strength. **Fig. 14** depict the test box and test instrument.

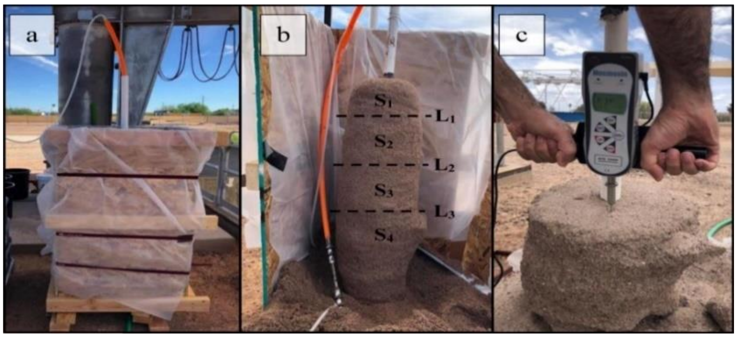



**Figure 14.** (a) Box test set-up during injection process with packer inside TAM; (b) Bio-cemented soil column; (c) Needle penetrometer test on L3 **(Martin et al., 2020).**

The findings indicate that the highest recorded measurement for the uncemented sand was 35 N, whereas the average maximum reading following the treatment cycle with EICP was 348 N. **(Salman et al., 2022)** explored gypseous soil strengthening with bacterial calcium carbonate precipitation. Iraqi gypseous soils from Al-Najaf (42% gypsum) and Al-Samawa (54% gypsum) were tested. Two bacterial solution ratios (4% and 8% of soil dry weight) improve soil cohesion and internal friction angle. Researchers injected the bacterial solution into gypseous soil samples using gravity. Samples were treated for 4 days. Urea medium solution was made from 4 g nutrient broth, 25 g urea, 15 g $NH_4Cl$, 3 g $NaHCO_3$, and 500 ml distilled water. Prior to autoclaving, 5 N HCL adjusted the pH of the urea medium solution to

6.0.Mix 500 mL of distilled water thoroughly dissolved solids. A 20 mL calcium chloride solution with 18.5 g of $CaCl_2$ per 100 mL of filtered water was mixed with 200 mL of air-infused urea. This combination significantly lowered the solution pH. The suspended bacterial solution was added to urea–calcium chloride. The specimens received 500 ml of urea, calcium chloride, and B. pasteurii cells by gravity. Untreated soil samples were tested after two hours of soaking. The stress-strain relationship of treated and untreated soil was examined at 75, 150, and 225 kPa confining pressures. All samples stabilized by bacterial calcium carbonate precipitation showed shear failure, including micro-crack initiation, formation, and aggregation. **Table 1** shows direct shear experiments on **(Salman et al., 2022)** explored gypseous soil strengthening with bacterial calcium carbonate precipitation.

**Table 2.** Variation of shear strength parameters for tested soil sample treated by MICP.

| **Al-Najaf soil** | | | | | | |
|---|---|---|---|---|---|---|
| **Parameter** | **Untreated** | | **Treated** | | | |
| | **Dry** | **Soaked** | **4% dry** | **8% dry** | **4% soaked** | **8% soaked** |
| c (kPa) | 15 | 0 | 40.44 | 60.66 | 23 | 34 |
| φ (°) | 32 | 28.5 | 33.75 | 40 | 30 | 34.5 |
| **Al-Samawa soil** | | | | | | |
| **Parameter** | **Untreated** | | **Treated** | | | |
| | **Dry** | **Soaked** | **4% dry** | **8% dry** | **4% soaked** | **8% soaked** |
| c (kPa) | 12 | 0 | 38 | 55 | 18.55 | 33.88 |
| φ (°) | 33 | 29.45 | 35 | 36.5 | 31.6 | 33 |

**(Almurshedi and Karkush, 2023)** tested two types of gypseous soil from Iraq, as shown in **Table 2**. The first soil sample from Al-Najaf City had 42% gypsum (USCS: SM). The second soil sample from Samawa City had 54% gypsum (USCS: SP-SM). Starting with urea medium, the bacteria treatment solution was made. A solution of urea medium was made by mixing 4 g nutrient broth, 25 g urea, 15 g $NH_4Cl$, 3 g $NaHCO_3$, and 500 ml distilled water. The gradient solids were thoroughly mixed in 500 mL of distilled water until dissolved. The urea medium solution was autoclaved after 5 N HCL was added to make it pH 6.0. Distilled water was added until the volume reached 1 liter. The urea medium pH was seven after autoclaving. Aerating the 1L solution raised its pH from 7 to 8. Thus, the solution became bacterial-friendly. Each soil type received 4% and 8% bacterial solutions. A single collapse test showed that an 8% bacteria solution gradually decreased collapsibility. At these bacteria percentages, collapse potential improved by over 50%. Gypseous soils lose 50%–60% compressibility. Laboratory tests showed that these soils are collapsible, and bacteria treatment increases shear parameters under dry and soaking conditions. A summary of some research on EICP and MICP techniques for soil was shown in **Table 3**.

**Table 3.** Summary of some research on EICP and MICP techniques for soil

| **Authors** | **MICP or EICP** | **Soil type, relative density %** | **Equimolar solution concentration** | **Bacterial, urease concentration** | **Curing time** | **Applied tests** |
|---|---|---|---|---|---|---|
| **(Montoya et al., 2012)** | MICP | Ottawa 50-70, 40% | Urea= =1 M $CaCl_2$= 0.5M 0.13 M (molarity) Tris Buffer (pH=9.0),10 g | Sporosarcina pasteurii | Until the target shear | Shear wave velocity, Dynamic centrifuge |

| | | | | | | |
|---|---|---|---|---|---|---|
| | | | $(NH_4)_2SO_4$, and 20g Yeast Extract | | wave velocity | model tests liquefaction |
| **(Meyer et al., 2011)** | MICP | well-graded sand, density =1245.6 kg/m$^3$ | Nutrient broth (NB) containing 3 g NB, 20 g urea, and 10 g $NH_4Cl$ per L (pH 6.0). $CaCl_2$ is 1.5% (100 Mm), | Sporosarcina pasteurii, 1 × 10$^7$ cells/Ml | 1, 2, 4, 7, and 14 days | EDS, wind erosion tests |
| **(Hoang et al., 2020)** | MICP | Two types of sand coarse-grained sand #20/30 fine-grained sand #50/70 | trypticase soy broth (20g/L), ammonium sulfate(10g/L), and Tris buffer (0.13 mol=L) | Sporosarcina pasteurii | 4, 8, 12, or 16 days | UCS, permeability, SEM, EDS |
| **(Carmona et al., 2016)** | EICP | Sand SP, | Urea- $CaCl_2$ from 0.25 – 1.25 M | urease concentration (4 Ku/L) | 14 days | UCS, XRD |
| **(Miftah et al., 2020)** | EICP | natural beach sand, 60% | Urea = 1 M $CaCl_2$ =0.67 M | Urease =10 Ml/l , (activity =4650 U/l) | 4 days | UCS, XRD, SEM, |
| **(Chandra and Ravi, 2021)** | EICP | 1-silty sand (SM) 2- clayey sand 3-low compressible silt (ML) | Urea=0.5 M $CaCl_2$ =0.5 M | Urease = 0.2 g/l (activity =40.150 kU/g) | 14 days | UCS, SEM, XRD |
| **(Zhao et al., 2014)** | EICP | Sand F-60, loose | Urea=1.5 M $CaCl_2$ =1 M | 0.45 g/l Urease enzyme with activity = 15,000-50,000 units/g | 14 days | SEM, EDX XRD, ammonia concentration |
| **(Krishnan et al., 2018)** | EICP | three batches of Ottawa 20-30 sand,75% | Urea = 1 M $CaCl_2$= 0.67 M | Urease = 3 g/l (activity= 4200 U/g),milk=4 g/l | 36-48 hrs. | UCS, SEM |
| **(Dawoud et al., 2014)** | MICP | Medium Sand, density was 1700 kg/m$^3$ | Urea-calcium chloride From 0.25 M to 1 M 20 g yeast extract 10 g $(NH_4)_2SO_4$ per 1 L of 0.13 M Tris solution in Ph 9.0 | Sporosarcina pasteurii | | Permeability, shear wave velocity, hydraulic conductivity |
| **(Oliveira et al., 2017)** | EICP | five types of soil (SP-SM), silty sands (SM) silty sands (SM) (ML), (OL) | Urea- $CaCl_2$ 0.25-0.5 M | Two of urease concentration (4, 8 KU/g) | 14 days | SEM/EDX, UCS |

## 7. CONCLUSION

1. The extraction of urease enzyme solution from select plant seeds offers a viable method to obtain an enzyme solution with comparable efficacy to commercially available, expensive pure urease. This research holds significant potential for advancing the application of this technology on a broader scale.
2. The most commonly employed methods involve separating the mixing process into two distinct sections: the enzyme and stabilizers section and the urea and calcium chloride section. These methods are preferred because they effectively eliminate insoluble components and impurities from any given mixture before initiating the reaction process.
3. The precipitation of materials is primarily influenced by the enzyme's activity and urea and calcium chloride concentrations. Extensive research has consistently demonstrated that increasing precipitation becomes challenging when the urea or calcium chloride percentage is elevated beyond 1 M, because increasing conductivity.
4. The potential for enhancement using this approach is contingent upon multiple factors, including enzyme activity, enzyme concentration, the concentration and purity of additional substances, mixing techniques and the specific soil type targeted for improvement. Consequently, a discernible disparity exists in the precise values obtained from this mixture.
5. This technique was applied to different soils and all soils except organic soils showed improvement in soil properties.
6. As in soil treated with cement unconfined compression strength could be applied on sandy soil.
7. The bearing capacity of all treated soil increase at least from 1.5 times as shown in the above researches.
8. These method of treatment in experimental work stage and not used in field application till now.
9. The precipitated calcium carbonate amount depends on the reaction activity and the concentration of reactant.

## NOMENCLATURE

| Symbol | Description | Symbol | Description |
|---|---|---|---|
| $\theta_{ms}$ | Rate of change of electrical conductance | UCS | Unconfined compression |
| $\theta_{sc}$ | Rate of change of the standard curve | Dr | Relative density |
| V | Sample volume in liters | Q peak | Peak deviatoric stress (kPa) |
| $\sigma c$ | Effective confining pressure (kPa) | c | Cohesion (kPa) |
| M | molecular weight of calcium carbonate | $\varphi$ | Friction angle ° |
| C | concentration of urea-$CaCl_2$, in mol/liter | U | Urease activity |
| N | Final ammonia concentration in millimoles per liter | | |

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## Credit Authorship Contribution Statement

Mustafa S. Ali: Conceptualization, Investigation, Review & editing Methodology, Validation.

Alaa D. Almurshedi: Review & editing Methodology, Validation.

## Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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# الأسمنت الحيوي: مراجعة لآليات وتطبيقات ترسيب الكالسيت الناجم عن انزيم اليورياز بطريقة مباشرة او عن طريق البكتيريا في الهندسة الجيوتقنية

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**الخلاصة**

يجب أن تتعامل الطرق الحديثة لتحسين التربة مع أغراض البناء المستدامة والابنية الخضراء، ويعتبر ترسيب الكالسيت هو أحدث طرق التدعيم الحيوي لتحسين التربة بشكل يلبي معايير الاستدامة. ترسيب الكالسيت الناتج عن الإنزيمات وترسيب الكالسيت الناتج عن الميكروبات من أفضل طرق ترسيب الكالسيت في تحسين التربة. يحتوي *EICP* و*MICP* على كلوريد الكالسيوم واليوريا والماء وإنزيم اليورياز ومواد كيميائية أخرى (لـــ *MICP* كمواد مغذية للبكتيريا) يتم خلطها لترسيب كربونات الكالسيوم في التربة. تعمل كربونات الكالسيوم المترسبة كرابط بين جزيئات التربة، وكذلك لملى الفراغ بين جزيئات التربة ويحسن خواص التربة. ومن خلال استخدام هذه التقنية ميدانيًا، تمكن عدد من الباحثين من تحقيق نتائج إيجابية في مجالات تحسين التربة والتطبيقات الهندسية الأخرى، بما في ذلك إصلاح الخرسانة والتطبيقات الأخرى، مع الأخذ في الاعتبار أيضًا تحقيق معايير الاستدامة. اظهرت جميع الفحوصات التي اجريت على الترب المعالجة بدورة او عدة دورات من خليط المعالجة ان قيمة فحص الانضغاط اللامحصور قد ارتفعت بنسبة كبيرة حيث جدا مقارنه مع الترب غير المعالجة. في بعض الفحوصات تجاوزت قيمة فحص الانضغاط اللامحصور للترب المعالجة بطريقة الانزيم عن الترب المعالجة بخلط نسبة من الاسمنت البورتلاندي الاعتيادي. يمكن الاستفادة من النتائج المشجعة التي تم تحقيقها من خلال استخدام هذه الطرق في المعالجة في كل من المختبر والميدان في عمليات واسعة النطاق. لغاية الان، تعتبر طرق المعالجة أعلاه قيد الدراسة. في العراق لغاية الان لايوجد أي بحث باستخدام طريق التحسين بواسطة الانزيم. بحوث قليلة في العراق باستخدام الترسيب باستخدام البكتيريا.

**الكلمات المفتاحية:** انزيم اليورياز ، ترسيب الكالسيت، كربونات الكالسيوم.