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**Improvement of Unconfined Compressive Strength of Soft Clay using
Microbial Calcite Precipitates**

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ABSTRACT

The precipitation of calcite induced via microorganisms (MICP) is a technique that has been developed as an innovative sustainable ground improvement method utilizing ureolytic bacteria to soil strengthening and stabilization. Locally isolated *Bacillus Sonorensis* from Iraqi soil samples were found to have high abilities in producing urease. This study aims to use the MICP technique in improving the undrained shear strength of soft clay soil using two native urease producing bacteria that help in the precipitation of calcite to increase the cementation between soil particles. Three concentrations of each of the locally prepared *Bacillus sonorensis* are used in this study for cementation reagent (0.25M, 0.5M, and 1M) during the period of treatment. The results showed that the native isolated bacteria have high activity in bindings the soil particles together. The results of unconfined compressive strength tests showed that using MICP helps increase the undrained shear strength of soil by (3-5 times) for C11 types of native isolates, but the D11 was (1.5-2 times) because two types have different activity. This study's main finding is using the native urease-producing bacteria isolated from Iraqi soil in the MICP technique for the biocementation of soil, which is considered one of the sustainable techniques in the construction industry.

Keywords: Microbially induced calcite precipitation (MICP), biocementation, soft soil, sustainability, *Bacillus sonorensis*.

تحسين مقاومة الانضغاط غير المحصور للطين الرخو باستخدام رواسب الكالسيت الميكروبية

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الخلاصة

ترسيب الكربونات الكالسيوم بالميكروبات (MICP) هو عملية برزت كتقنية حديثة ومستدامة لتحسين التربة باستخدام البكتيريا لتقوية التربة وتثبيتها. تم تشخيص بكتريا نوع *Bacillus sonorensis* المعزولة محلياً من عينات التربة العراقية والتي تمتلك قدرات عالية في إنتاج انزيم اليورييز. الهدف من هذه الدراسة هو استخدام تقنية MICP في تحسين مقاومة القص للتربة الطينية

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الرخوة باستخدام نوعين من البكتريا المحلية المنتجة لليوريبيز (D11 and C11) والتي تساعد في ترسيب الكالسيت لزيادة الترابط بين جزيئات التربة. تم استخدام ثلاثة تراكيز من كل من *Bacillus sonorensis* المحضر محلياً في هذه الدراسة لكاشف التدعيم (0.25 مولاري، 0.5 مولاري، 1 مولاري) خلال فترة العلاج. أظهرت النتائج أن البكتيريا الأصلية المنتجة لليوريبيز ذات النشاط العالي في ربط جزيئات التربة ببعضها البعض. أظهرت نتائج اختبارات مقاومة الانضغاط غير المحصورة أن استخدام MICP يساعد على زيادة مقاومة القص غير المصبوبة للتربة بمقدار (3-4 مرات) لكلا النوعين من العزلات الأصلية، ولكن للعزلتين نشاط مختلف. تتمثل النتيجة الرئيسية لهذه الدراسة في استخدام البكتيريا المحلية المنتجة لليوريبيز المعزولة من التربة العراقية بتقنية MICP للتثبيت بايولوجياً للتربة والتي تعتبر إحدى التقنيات المستدامة في البناء.

الكلمات الرئيسية: ترسيب الكالسايت باستخدام البكتريا، التدعيم بايولوجياً، تربة طينية رخوة، استدامة، *Bacillus sonorensis*.

1. INTRODUCTION

The microbiologically induced calcite precipitation method is one of the newest methods of ground improvement, in which calcium carbonate crystal was formed between soil particles using bacteria in order to improve soil properties. This procedure can stabilize the soil or other small particles (porous material) without disturbing the initial structure. In this method, penetration reduction and the cost of implementation are low. Moreover, it is environmentally compatible, and a wide range of materials and microorganisms can be used in this method without harmful environmental consequences. Many studies have been carried out up to now on the capability of this method in different applications. MICP was used to enhance sandy soils' properties, such as increasing shear strength and minimizing soil permeability (DeJong et al., 2006; Whiffin et al., 2007; Van Paassen, 2009). Scaled-up experiments have also been conducted on sandy soil, and good results have been achieved (Van Paassen, 2009), strength and toughness enhancement for concrete and mortar, building cracks remediation (Achal et al., 2011). Dejong et al. (2006) have shown an improvement in sandy soil's strength in microbially cemented specimens. Their findings suggest that their activity was close to the typical cemented sand behavior of the gypsum-cemented specimen.

Chou et al. (2011) performed a laboratory analysis to specify the impact on sand's geomechanical properties for growing, dead, and restful cells. The (CBR) testing was performed on sand samples, and bacteria were found to enhance sand's geo-mechanical properties effectively. The sand analysis of CBR specimens, which were treated with growing cells, showed that the microbial processes were helping to block the medium of porous. Al Qabany et al. (2012) studied the impact on the efficacy of calcite formation in the sand by *S.pasteurii* of the various treatment parameters (reactive concentration, retention time, and reagent input rates). The efficiency of calcite formation was evaluated on the basis of stoichiometry reactions. Al Qabany et al. (2012) found that the use of a reagent input below $0.042M = h_{21}$, irrespective of its reagent concentrations, can achieve high chemical efficiency of over 90 percent (up to 1.0 M (Mollary)).

Al-Qabany and Soga (2013) performed Unconfined compressive strength tests on sand samples treated with solutions of urea and calcium chloride 0.1, 0.25, 0.5, and 1M. After MICP treatment, depending on the reagent concentration used, the tested samples' strength improved, and the low concentration solution (such as urea and calcium chloride) led to stronger samples. The permeability test results showed that the use of a solution of high concentration of calcium chloride and urea led to a rapid minimize in the permeability in the beginning stage of calcium carbonate precipitation. At the same time, it was observed that the use of a solution of low chemical concentration led to a regular and uniform decrease in permeability.

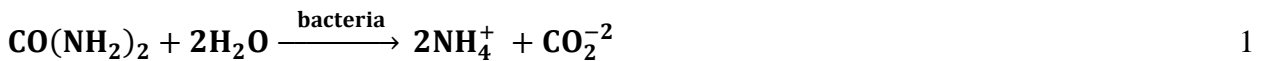
Lee et al. (2012) studied the effect of concentration of cementing media in biocementation enactment. Soil strength, calcite, pH, and ammonium substances were measured under the influence of three levels of cement: 1 M, 0.5 M, and 0.25 M. Soil, 0.5 M, and 0.25 M. The findings



showed that the treatment is deferred if the cementation media concentration reaches 1M. This test's objective was to examine two native urease-producing bacteria previously isolated by **Ali et al. 2020** and conducted a Microbial Induced Calcite Precipitates (MICP) for improving the shear strength parameters of soft clayey soil. The selected ureolytic bacteria used in this experiment are C11 and D11.

2. BIOCEMENTATION MECHANISM

In MICP, hydrolysis of urea through the urease enzyme is the most important and widely studied mechanism that increases pH and carbon dioxide production. Bacterial cells are to a rise in pH and carbon dioxide production. Bacterial cells are the best nucleation positions to evolving crystals in addition to changes in environmental effects due to the presence of several negative loaded cell wall groups resulting in positively loaded metal ions attached to bacterial surfaces (**Douglas and Beveridge 1998; Ehrlich 1998**). Many studies verified that bacterial cell surface causes CaCO₃ precipitation in the porous media. Most research on soil bio-treatment is also focused on the hydrolysis of urea. The microbial urease catalyzes the ammonium and carbonate hydrolysis from urea;



Calcite crystals precipitate as a result of the interaction of carbonate ions resulting from the hydrolyzing of urea with calcium ions on, which form bonding bridges between existing soil grains:



The remainder of the solution of ammonium chloride is removed. When the calcium carbonate has been precipitated, it dissolves very slowly, whether in acidic groundwater or by acidifying pores (e.g., biomass degradation). If enough calcium carbonate is precipitated, the stabilization of the soil would be long-lasting.

3. EXPERIMENTAL WORK

3.1 Soil Sampling

A clay soil sample was brought from the site Al Nahrawan city, which is located 35 km to the east of Baghdad city at depths 9 m. Standard tests have been conducted to specify the chemical and physical characteristics of the soil samples. The physical and mechanical soil tests were conducted on the soil samples at the laboratory of soil mechanics at the Department of Civil Engineering/the University of Baghdad. In contrast, the chemical tests are conducted for both treated and untreated soil samples in the Department of Materials Research/Ministry of Science and Technology. Table 1 shows the physical, mechanical, and chemical tests.

Table 1. The physical and chemical properties of natural soft soil.

Index property	Test standard	Value
Liquid limit (LL), %	ASTM D 4318	49
Plastic limit (PL), %	ASTM D4318	24
Plasticity index (P.I.), %	ASTM D4318	25
Specific gravity (Gs)	ASTM D 854	2.77



Sand, %	ASTM D 422	6
Silt, %	ASTM D 422	26
Clay, %	ASTM D 422	68
Classification (USCS)	ASTM D 2487	CL
Optimum moisture content, %	ASTM D1557	21.54
Maximum dry unit weight, kN/m ³	ASTM D 1557	17.13
Hydraulic conductivity, cm/s	ASTM D 2434	2.45×10 ⁻⁷
SO ₃ Content, %	B.S. 1377:1990	0.52
Total soluble salt (TSS), %	B.S. 1377:1990	2.541
pH Value	ASTM D 4972	8.45
Chloride content (Cl), %	B.S. 1377:1990	0.628
Organic Material (O.M), %	ASTM D 2974	0.732
Gypsum content, %	B.S. 1377:1990	1.17
Unconfined compressive strength (kPa)	ASTM D 2166	20

3.2 Identification of Used Bacteria

The selected bacteria isolated from the local soil were cultivated using urea-nutrient agar for the treatment test. The bacteria obtained from local sources denoted to be C11, D11 were characterized to be *Bacillus sonorensis*. These isolates were routinely kept on a cultivated media called nutrient agar which was supplied with 20g urea substrate, working as a source of energy and nitrogen. The media was stored at 4C° in the fridge before being used for subsequent tests. The bacteria were cultivated in the same way from a bacterial culture not grown for more than one month (Cuzman et al., 2015).

3.3 Bacterial Cementation Solution

The concentration of cement reagent is shown in Table 2. To prepare the cementation solution, the components were mixed well in 450 mL of distilled water until it had dissolved to produce the urea solution. After autoclaving, distilled water with urea was then added to reach the final required volume (500ml) after sterilizing using a bacterium filter (diameter of this filter 0.25 μm). The flasks containing the growth medium were then cultivated for 48 hours with agitation (130 rpm) in an incubation shaker under aerobic batch conditions at 37 C. after incubation, add sterilized calcium chloride (Al Qabanny et al., 2012; Lee Min Lee et al., 2012).

Table 2. Bio-cement treatment components.

Component	Amount
Nutrient broth powder	3 g
NH ₄ Cl	10 g
Na.HCO ₃	3 g
Distilled water	1 L
Urea (NH ₂ (CO)NH ₂)	0.25 M, 0.5 M, 1 M
CaCl ₂ . 2H ₂ O	0.25, 0.5 M, 1 M



3.4 Testing Procedure

The main procedure of the test is:-

- 1) Preparation the soft soil with undrained shear strength (c_u) of 10 kPa.
- 2) Preparation of the urea nutrient agar and bacteria culturing for the treatment solution of bacteria
- 3) Adding the bacteria and mixing with soil, then adding reagent solution (urea+CaCl₂) to the soil.
- 4) Placing the soil with bacteria and reagent solution in CBR mold.
- 5) Leaving the samples to curing for 1, 3, and 7 days.
- 6) Finally, extracting the sample from the mold and conducted the unconfined compression strength (UCS).

4. RESULTS AND DISCUSSION

The value of the unconfined compressive strength of the original soil sample was 20 kPa, for which the MICP found an increase in the treatment with more. **Table 3** shows the results of a soil treatment test with *Bacillus sorensonis* C11. It was observed from the test results that with the increase in the duration of treatment, the UCS values increased. For soil treated with *Bacillus sorensonis* C11, the highest increase of bacterial concentration 12×10^7 cfu/ml and molar concentration of 0.5 M of the cementation reagent was observed. Also, the UCS value increased significantly with increasing treatment duration. **Table 4** presents the soil test results treated with *Bacillus sorensonis* D11, where the results showed that the highest increase was recorded for the molar concentration of the cement reagent 0.5 M and bacterial concentration 4.1×10^4 cfu/ml. However, in the soil treated with *Bacillus sorensonis* C11, a comparatively higher percentage increase was observed in the UCS value of both types of bacteria. This can be due to the high urease activity, so that the formed sediments are closely connected with the soil compound, resulting in an increased bonding between soil particles. This increased bond-forming in the soil increases the soil's cohesion and is one of the criteria of soil shear strength, thus increasing the strength of the soil.

Table 3. Results of UCS tests for soil samples treated by MICP of C11.

Bacillus C11	Cementation reagent	UCS (kPa)	UCS (kPa)	UCS (kPa)	Rate of increase (%)
		0 days curing	3 days curing	7 days curing	
1×10^7 (cfu/ml)	0.25M	60	70	80	200-300
	0.5M	70	88	100	250-400
	0.75M	65	80	90	225-350
	1M	60	75	85	200-325
1×10^4 (cfu/ml)	0.25M	55	65	70	175-250
	0.5M	65	75	82	225-325
	0.75M	62	68	76	200-250
	1M	55	65	70	175-250

A comparison of U.C. tests with varying MICP concentrations in the soil sample is illustrated in **Fig. 1**. Clearly show the Unconfined Compression (U.C.) profile of the treated soft soil was higher than the treated soft clay regarding different concentrations. For the soil treated with *Bacillus*



sonorensis concentration $C11(10^7 \text{ cfu/ml})$. The 3 and 7 day test profile is consistent across the samples, which lies above the control value increase from (70, 80) kPa with 0.25 M to (88, 100) kPa with 0.5 for 3 and 7 day, and the decrease to (80, 90) kPa. The 7 days test shows the greatest improvement, which increased from 80 kPa to 100 kPa for 0.25 M and 0.5 M and decreased to 85 kPa for 1 M. Also, as shown in Fig. 1, in the soil treated with bacterial concentration $1 \times 10^4 \text{ cfu/ml}$ system, the trend was almost similar to the $1 \times 10^7 \text{ cfu/ml}$ but at a lower value.

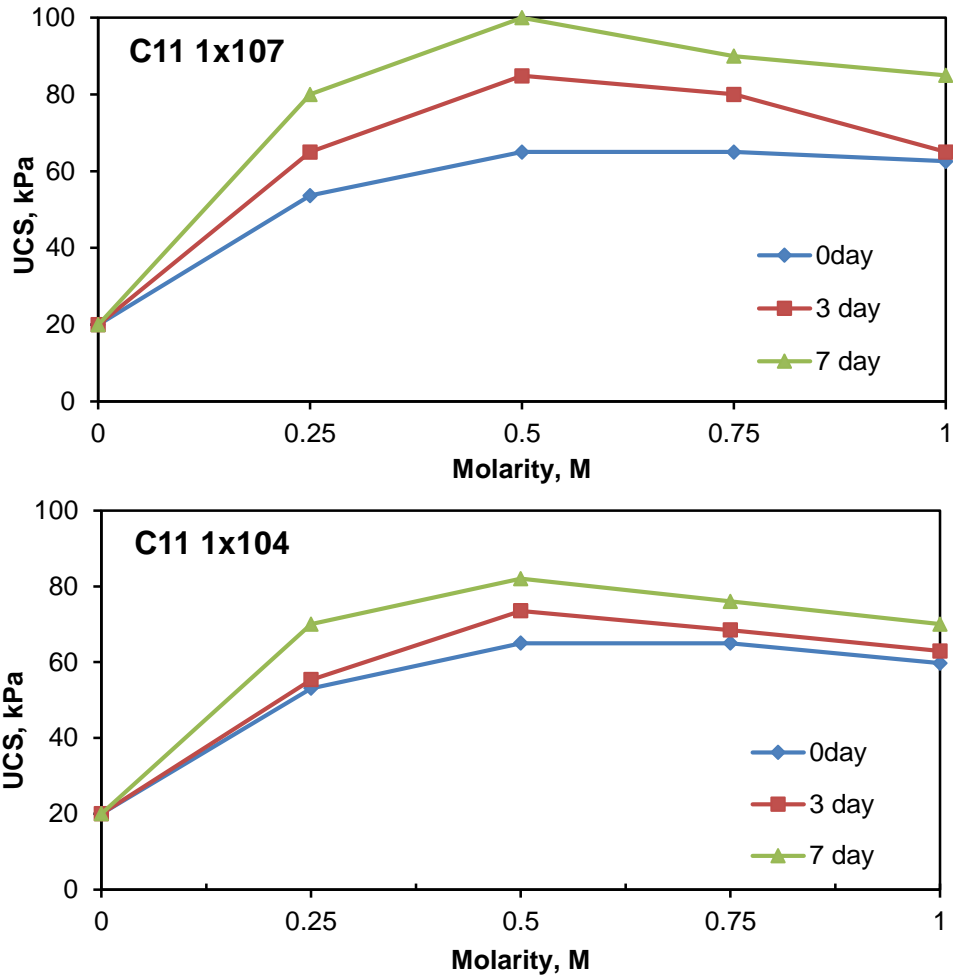


Figure 1. Comparison of UCS of soil sample C11 treated with MICP with $1 \times 10^7 \text{ cfu/ml}$ and $1 \times 10^4 \text{ cfu/ml}$.

Table 4. Summaries the results of UCS test for soil sample D11 treated by MICP.

Bacillus D11	Cementation reagent	UCS (kPa)	UCS (kPa)	UCS (kPa)	Rate of increase (%)
		0 days curing	3 days curing	7 days curing	
1×10^7 (cfu/ml)	0.25 M	45	50	55	125-175
	0.5 M	58	65	76	125-250
	0.75 M	55	60	65	175-225
	1 M	50	56	60	150-200



1×10 ⁴ (cfu/ml)	0.25 M	35	45	50	75-150
	20.5 M	44	55	65	120-225
	0.75 M	41.5	55	60	100-200
	1 M	40	45	55	100-175

As shown in **Fig.2**, for the soil treated with a bacterial concentration of 10⁴ cfu/mL system, the trend was almost similar to 10⁷ cfu/ml but at a lower value. From **Fig.2** that MICP improved the UCS of soil sample treated with D11 noticeably, with an increased ratio of cement reagent. However, Bacillus sonorensis concentration D11 lowers than C11. Calcite precipitation is usually due to produced urease in the presence of positive bacteria, which increases the strength between soil and fills up the remaining voids, which in turn increases the strength of the soil. The strength increases with the duration of treatment that can be observed as well.

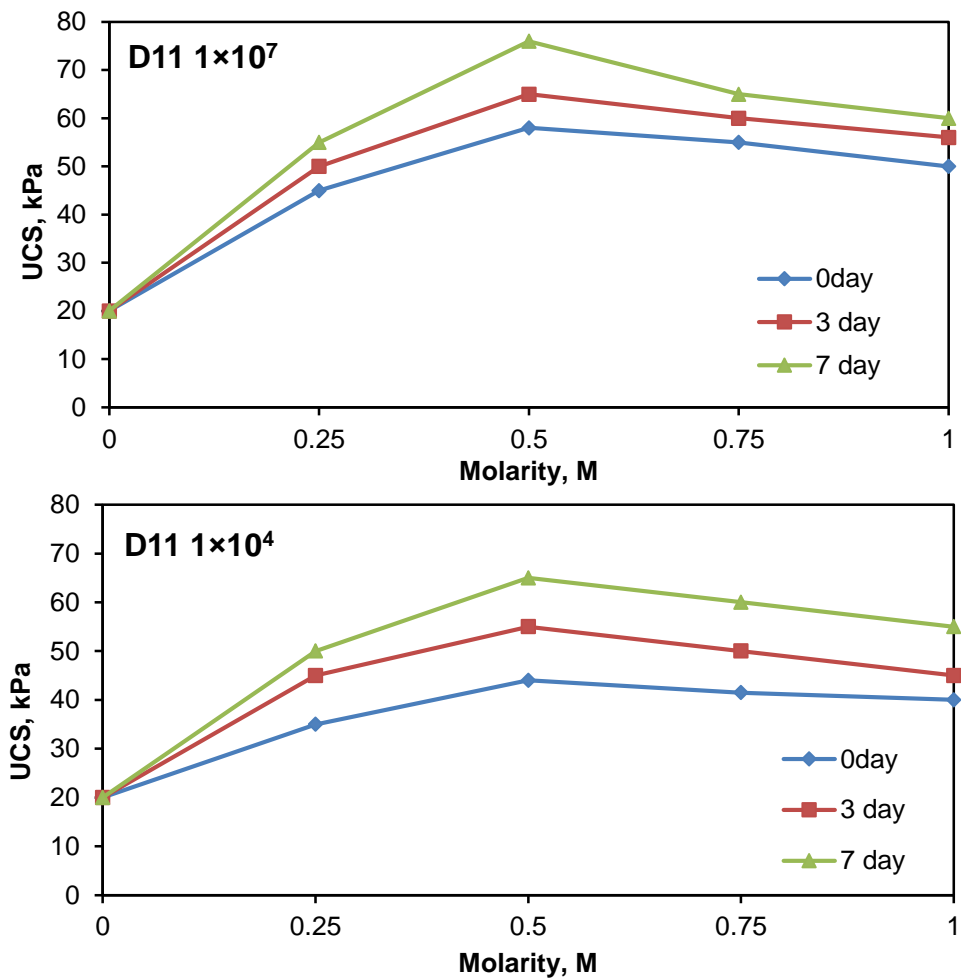


Figure 2. Comparison of UCS of soil sample D11 treated with MICP of 1×10⁷ cfu/ml and 1×10⁴ cfu/ml.



5. CONCLUSIONS

The following conclusions can be drawn from examining two native urease producing bacteria and utilizing the (MICP) technique for enhancing the shear strength of soft clay soil.

1. The value of unconfined compressive strength value increased treatment with the MICP technique, and both the bacteria kinds offered high rates of treatment. Further increase can be obtained by increasing the treatment duration.
2. For the soil with *Bacillus sonorensis* C11, the highest rates of treatment have been noticed for the bacterial concentration of 12×10^7 cfu/ml and 0.5 M of (urea + calcium chloride).
3. The results of UCS treated with C11 (3-5) were slightly more than the soil sample treated with D11 (1.5-2).

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