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Application of Enzyme-Induced Carbonate Precipitation for Dune Sand Stabilization

Dima Fatooh[1*], *Mohamed* Arab[1], *Mohamed* Shahin[2], and *Emran* Alotaibi[3]

[1]Department of Civil and Environmental Engineering, University of Sharjah, Sharjah, United Arab Emirate
[2]Department of Civil Engineering, Curtin Univ., Bentley, WA 6102, Australia
[3]Department of Public Health, Canadian University Dubai, Dubai, United Arab Emirate

Abstract: Enhancing the mechanical properties of dune sand is critical for improving its stability and load bearing capacity in geotechnical applications. This study investigates the treatment of dune sand using the Enzyme-Induced Carbonate Precipitation (EICP) technique, focusing on the percolation method as a sustainable and effective approach. In this method, dune sand samples were first fully saturated with water, followed by the sequential addition of urease enzyme and a cementing solution to facilitate carbonate precipitation within the sand matrix. Comprehensive laboratory experiments were conducted to evaluate the efficacy of the treatment. Unconfined Compressive Strength (UCS) tests were performed to assess the mechanical improvement in treated samples, while ammonia activity measurements provided insight into the enzymatic efficiency of the EICP process. The calcium content percentage was also analyzed to quantify the extent of carbonate precipitation and its influence on the structural integrity of the sand. The results demonstrate significant improvements in the strength and stability of dune sand, correlating the observed mechanical enhancements with the precipitation of calcium carbonate within the pore spaces. This study provides valuable insights into the optimization of the EICP process and its potential for practical applications in ground improvement techniques. This research contributes to advancing the use of bio-inspired soil stabilization techniques, offering a sustainable alternative for improving the engineering properties of problematic soils.

Introduction

Enzyme-Induced Calcite Precipitation (EICP) is an innovative and sustainable soil stabilization technique gaining significant attention in geotechnical engineering. EICP uses urease enzymes to hydrolyze urea, releasing carbonate ions that react with calcium to form calcium carbonate ($CaCO_3$). This calcite binds soil particles, increasing strength, stiffness, and erosion resistance. Unlike Microbially Induced Calcite Precipitation (MICP), EICP does not require cultivating bacteria, making it simpler, more efficient, and eco-friendly. Early studies, such as [1], confirmed EICP's effectiveness in improving unconfined compressive strength (UCS) and erosion resistance, particularly in sandy soils. Laboratory experiments demonstrated that increasing reagent concentrations enhances soil strength, where UCS ranged from 161 kPa at 0.25 M to 552 kPa at 1.00 M. This effectiveness made EICP a preferred option for applications such as erosion control in coastal and riverine areas, as evidenced by [2], where calcite crusts resisted high hydraulic stresses. Beyond sandy soils, EICP has shown promise for stabilizing fine-grained, highly plastic soils, reducing their liquid limit and plasticity index while increasing shear strength [3]. EICP's applications extend beyond stabilization, including bio-brick production, groundwater sealing, and archaeological conservation, such as preserving sites along the Silk Road [4]. It also offers advantages over traditional methods like chemical grouting

*Corresponding author: dfatooh@sharjah.ac.ae

by using biodegradable reagents and natural enzymes, minimizing environmental harm. Recent research focuses on optimizing EICP for maximum calcite precipitation and improved soil properties. Studies have identified ideal urea and calcium chloride concentrations, while advanced techniques like Scanning Electron Microscopy (SEM) have revealed the spatial distribution of calcite in treated soils. Additionally, curing conditions such as temperature and pH significantly impact EICP efficiency, with higher temperatures and alkaline pH yielding improved strength [5]. These advancements highlight EICP's potential for large-scale and environmentally sustainable geotechnical applications.

The environmental benefits along with the economic advantages of EICP have also been contributing to its increasing popularity. In this technique, only natural enzymes are used, and no environmental hazards arise thus, EICP proves to be a green alternative to traditional soil stabilization methods. Moreover, the EICP's lower costs and the fact that it is easy to implement make it a widely available tool for geotechnical engineers in many parts of the world particularly in the developing countries where resources for the traditional stabilization methods are limited. In addition, the use of EICP in innovative applications such as groundwater sealing and plugging cracks in concrete structures showcases its adaptability and brilliance, which may allow it to tackle a wide range of technical issues.

Moreover, EICP has undergone modifications from a new way of bio-cementation into a method that has been broadly used over the years for soil stabilization and ground improvement. Since its roots in early studies, including urease-induced calcite precipitation studies, the technology made its paths in a variety of geotechnical applications. The examples include sandy soil stabilization and archaeological trove conservation. The proper development of EICP processes as well as the diversification of its use in geotechnical areas will make it one of the major contributors to sustainable engineering practices. The chemical reactions involved in EICP are as follows:

Urease Hydrolysis Reaction:

$$(NH_2)_2CO + H_2O_{(l)} \xrightarrow{urease\ enzyme} 2NH_{3(aq)} + CO_{2(aq)} \quad (1)$$

Ammonia Dissolution and Hydrolysis:

$$NH_3 + H_2O \rightarrow NH_4^+ + OH^- \quad (2)$$

Carbonate Formation:

Carbon dioxide reacts with water:

$$CO_2 + H_2O \rightarrow H_2CO_3 \quad (3)$$

Carbon acid dissociates:

$$H_2CO_3 \rightarrow HCO_3^- + H^+ \quad (4)$$

$$HCO_3^- \rightarrow CO_3^{-2} + H^+ \quad (5)$$

Calcium Carbonate Precipitation:

$$CO_3^{-2} + Ca^{+2} \xleftrightarrow{Precipitation} CaCO_3(solid) \quad (6)$$

The reaction sequence in the Enzyme-Induced Calcite Precipitation (EICP) process begins with the urease hydrolysis reaction (Eq.1). In this step, urea is catalytically hydrolysed by the enzyme urease, producing ammonia (NH_3) and carbon dioxide (CO_2) [1]. The ammonia dissolves in water, generating ammonium ions (NH_4^+) and hydroxide ions (OH^-) through ammonia hydrolysis (Eq.2). This reaction is crucial, as it increases the pH of the solution, thereby providing an alkaline environment that favors the generation of carbonate ions. In another reaction, carbon dioxide reacts with water to form carbonic acid (H_2CO_3) (Eq.3); this then further dissociates into bicarbonate (HCO_3^-) and hydrogen ions (H^+) (Eq.4). Bicarbonate further dissociates into carbonate ions (CO_3^{-2}) and extra hydrogen ions (Eq.5). The produced carbonate ions react with calcium ions, Ca^{+2}, usually supplied by dissolving calcium chloride, to form a solid precipitate of calcium carbonate, ($CaCO_3$) (Eq.6). This can cement soil particles together, minimizing porosity and increasing the compressive strength of the soil [6]. The interaction of these reactions leads to a bio-cementation process characterized by both efficiency and environmental sustainability, attributable to its dependence on enzymatic mechanisms as opposed to chemical or microbial systems [7].

Methodology

Table 1 summarizes methodologies and outcomes from various studies on EICP-treated soils, highlighting differences in treatment conditions, unconfined compressive strength (UCS) results, and practical considerations. [8] used a 1.0 M solution in a closed-bottom, saturated setup with wet testing to prevent unintended precipitation, achieving UCS values up to 220 kPa after three cycles. [8] conducted four cycles with a 1.0 M solution, using oven drying at 50°C and effluent circulation, which resulted in a UCS of 1268 kPa. [9] employed a 0.3 M solution over 16 cycles, adding urease before urea and calcium, and achieved a UCS of 1690 kPa with drying at 50°C. [10] utilized a 0.5 M solution with drying at 60°C and noted that saturated conditions might not reflect field conditions, reaching a UCS of 4230 kPa after 10 cycles. [11] adopted an open-bottom, unsaturated approach with 0.5 M solution and enzyme addition every five cycles, achieving a UCS of 10500 kPa after 40 cycles with drying at 50°C. In contrast, this study used a 2.0 M solution in an open-bottom, unsaturated setup with wet testing and no enzyme addition after the first cycle, achieving a UCS of 800 kPa after three cycles. This comparison underscores the impact of testing conditions, treatment efficiency, and drying methods on EICP performance and field applicability.

Table 1: Previous EICP treatment tests conducted using percolation method.

Ref	Molarity	Type	UCS	Drying	Notes
[12]	1.0	Closed bottom, saturated	1 cycle - 20kPa 2 cycles 120kPa 3 cycles – 220kPa	Wet testing to avoid any unexpected precipitation that may occur when samples are intentionally dried out	Enzyme was added with cycles

[8]	1.0	Closed bottom, saturated	1 cycle - 99kPa 2 cycles - 291kPa 3 cycles - 430kPa 4 cycles - 1268kPa	$50C^0$	Enzyme was added with cycles Circulated effluent
[9]	0.3	Closed bottom, saturated	4 cycles - 400kPa 8 cycles - 600kPa 12 cycles - 1250kPa 16 cycles – 1690kPa	$50C^0$	Enzyme was added with cycles Urease was added before urea/calcium Circulated effluent
[10]	0.5	Closed bottom, saturated	4 cycles - 550kPa 6 cycles – 1470kPa 8 cycles - 1890kPa 10 cycles – 4230kPa	$60C^0$	Enzyme was added with cycles Circulated effluent
[11]	0.5	Opened bottom, unsaturated	10 cycle - 1500kPa 20 cycles - 4200kPa 30 cycles - 7500kPa 40 cycles - 10500kPa	$50C^0$	Enzymes were added each 5 cycles
This Study	2	Opened bottom, unsaturated	1 cycle – 400kPa 3 cycles – 800kPa	Wet testing	Enzyme WAS NOT added to each cycle only urea + calcium chloride

This study investigated the application of Enzyme-Induced Calcite Precipitation (EICP) for the treatment of ASTM-graded sand and natural sand imported from Maliha, UAE. Cylindrical specimens were prepared using PVC moulds with dimensions of 50 mm in diameter and 150 mm in height. To prevent seepage of sand after the addition of the EICP and cementation solutions, all moulds were positioned on sieves, as illustrated in (Fig.1). Dry sand was first poured into the moulds, followed by the addition of 70 mL of tap water to enhance water retention within the sand matrix [11]. Subsequently, 70 mL of the EICP solution, comprising enzyme, urea, calcium chloride, and non-fat milk, was added to the moulds.

This step constituted the first treatment cycle (Fig.2). The second and third treatment cycles involved the addition of 70 mL of the cementation solution, consisting solely of urea and calcium chloride, with one and two repetitions, respectively. A 24-hour interval was maintained between each cycle, during which all specimens were rinsed with 500 mL of tap water to ensure consistency and mitigate salt accumulation, as depicted in (Fig.3). The composition of the cementation solution included 2 M urea with a urea-to-calcium chloride molar ratio of 1.5:1. Additionally, 9 g/L of enzyme (supplied by Fisher Inc.) and 12 g/L of non-fat milk were separately added after thorough mixing with tap water.

After completing the curing process (24 hours per cycle), the specimens were washed and air-dried for 4 hours on a clean cloth. To assess the unconfined compressive strength (UCS) of the treated specimens, tests were conducted using an unconfined testing machine available in the Civil Engineering Laboratories at the University of Sharjah. The loading rate was set at 0.5 mm/min.
Unlike prior studies, which predominantly employed oven drying for sample preparation [10, 9], this study adopted wet testing conditions to better simulate field scenarios. This

approach was chosen to avoid any unintended precipitation that might occur during oven drying [12]. Furthermore, the samples were left open at the bottom during testing to replicate field conditions, diverging from the fully saturated conditions often employed in laboratory studies (Table 1). This methodology provides a more practical representation of real-world applications.

Figure 1: (a) PVC mold placed on top of sieves to prevent soil seepage, (b) 15 samples of Maliha sand during treatment (right after solution pouring)

Figure 2: Extracted samples after 1 cycle of treatment and washing with tap water

Results and Discussion

The UCS test results presented in (Fig.4) highlight the stress-strain behaviour of ASTM sand and dune sand (from Maliha) treated with EICP under one-cycle and three-cycle treatments. The graph demonstrates a clear distinction in the response of the two sand types and provides insights into the impact of treatment cycles on soil strength. For both sand types, the stress increased with strain during the initial loading phase, reaching

a peak before gradually decreasing. This peak represents the maximum strength achieved by the treated soil, while the post-peak behavior indicates the material's capacity to retain its structure under further strain.

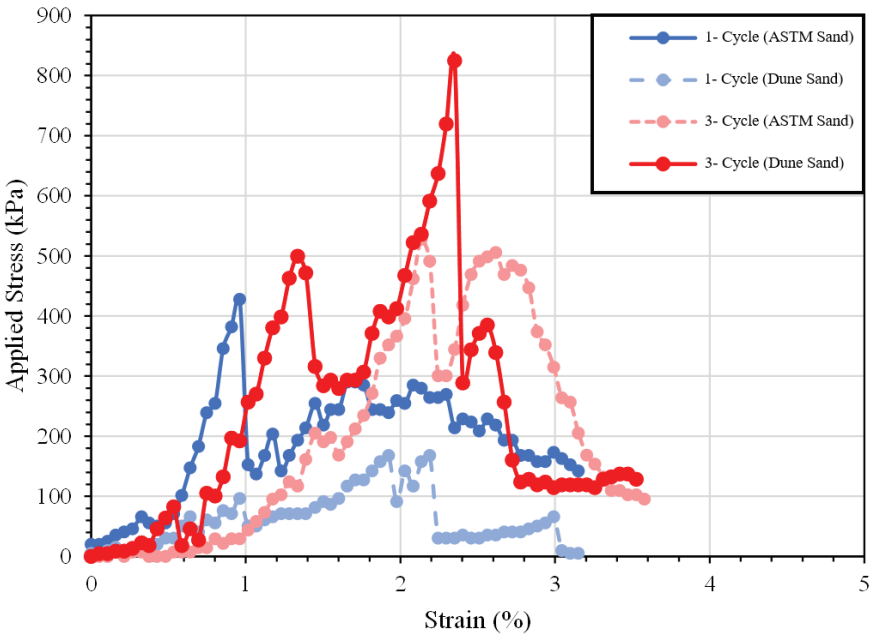

Figure 3: UCS results for tested sand samples treated using EICP at 1 and 3 cycles of treatment

In comparing the sand types, ASTM sand consistently exhibited higher peak stress values than dune sand in both the one-cycle and three-cycle treatments. In the one-cycle treatment, ASTM sand achieved a peak stress of approximately 400 kPa, while dune sand reached around 300 kPa. With three cycles of treatment, the peak stress of ASTM sand increased significantly to approximately 800 kPa, whereas dune sand improved to around 600 kPa. These differences suggest that ASTM sand, with its likely more uniform gradation and coarser texture, responds better to EICP treatment, resulting in stronger particle bonding and higher UCS values. On the other hand, dune sand, with its finer particles and potentially higher surface area, demonstrated a more modest increase in strength, indicating that its characteristics may limit the efficiency of EICP in achieving uniform calcite precipitation. The results also reveal the cumulative benefits of additional treatment cycles. The three-cycle treatment clearly outperformed the one-cycle treatment for both sand types, showing that successive cycles contribute to increased calcite precipitation and improved particle bonding. This observation underscores the importance of optimizing the number of treatment cycles in EICP applications, particularly for soils requiring enhanced strength for engineering purposes.
Post-peak behavior in both sands reflects strain-softening, with a noticeable reduction in stress as strain increases beyond the peak. Dune sand exhibited a sharper decline in stress after the peak compared to ASTM sand, indicating that it may be less effective in retaining its structure under loading. This behavior points to potential challenges in stabilizing fine-grained or marginal sands with EICP, as their properties might hinder uniform calcite distribution or create weaker bonds.
Overall, the UCS test results demonstrate the effectiveness of EICP as a soil stabilization technique, with the number of cycles and soil type playing critical roles in its success. ASTM sand showed superior performance, achieving higher strength and better post-peak

stability, while dune sand, despite its limitations, still benefited significantly from EICP treatment. These findings suggest that EICP can be tailored to suit different soil types, making it a versatile and sustainable option for improving soil properties in various geotechnical applications.

a) b)

Figure 4: Treated ASTM Sand under UCS testing: a) 180 kPa 1-cycle treatment; and b) 800 kPa 3-cycle treatment

Figure 5: Maliha EICP treated sand at 1 cycle

The one-cycle treatment process did not achieve uniform cementation throughout the soil column, as evidenced in (Fig.5a). The failure occurred at the edge of the specimen, indicating uneven distribution of calcite precipitation. In contrast, the three-cycle treatment resulted in a more homogeneous sample, with failure occurring at the centre of the specimen (shear failure). This central failure suggests that the cementation was distributed uniformly across the entire soil column, significantly enhancing the structural integrity of the treated sand.

In the case of Maliha natural sand, the treatment did not yield strength comparable to ASTM sand. The difference is attributed to the unique gradation of Maliha sand, which hindered the uniform distribution of the cementation solution throughout the sand column, as shown in (Fig.6). This issue was particularly evident after one cycle of treatment. However, subsequent cycles are expected to improve the distribution of the cementation solution, potentially enhancing the strength of the treated Maliha sand. These observations

underscore the importance of soil gradation in achieving effective and uniform EICP treatment.

Conclusion

The application of Enzyme-Induced Carbonate Precipitation (EICP) for dune sand stabilization has demonstrated its efficacy in enhancing the unconfined compressive strength (UCS) and overall mechanical properties of treated soils. This study established that the effectiveness of EICP is highly dependent on treatment parameters, including solution molarity, number of cycles, and soil gradation. The experimental results showed that ASTM sand, with its uniform particle size distribution, achieved a UCS of 800 kPa after three cycles, outperforming dune sand, which reached a UCS of 600 kPa under similar conditions. The disparity underscores the critical role of gradation in facilitating uniform calcite precipitation and effective particle bonding.

The methodology employed, including the use of open-bottom molds and wet testing conditions, better replicated field scenarios compared to conventional saturated or oven-dried methods. The exclusion of enzyme addition after the initial cycle provided insight into the efficiency of calcite precipitation with minimal enzymatic input. Despite these innovations, the results indicated non-uniform cementation in single-cycle treatments, emphasizing the necessity of multiple cycles to achieve homogeneity and enhanced structural integrity. This study confirms that EICP can be optimized through precise control of treatment variables to improve its applicability in field conditions, particularly for sandy soils. Further research into the interaction between soil properties and precipitation dynamics could refine EICP protocols, enabling its broader application in geotechnical engineering, including erosion control, subgrade stabilization, and structural reinforcement.

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