State-of-the-art Review of Enzyme Induced Calcite Precipitation in Soils

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ABSTRACT

Soil improvement is considered as an alternative when the natural soil cannot meet the engineering requirements. Previously, applying cement or lime to the soil was one of the most popular ways to improve it. Many modified soil improvement techniques are now available. But some of these methods are not environmentally friendly, while others are hard to carry out. The way for the development of bio-stabilization techniques has been created by the discovery of alternatives to soil stabilisation by mechanical and chemical stabilisation. The process of bio-stabilization, which encourages ureolysis and results in the precipitation of calcite in the soil mass, commonly makes use of enzymes. According to recent research on environmentally friendly ground improvement methods, Enzyme Induced Calcite Precipitation (EICP)has recently gained popularity as a suitable option for improving soil. Bio-cementation via EICP increases the strength and stiffness by clogging the voids and binding the soil particles with calcium carbonate.

Keywords: Enzyme-induced calcite precipitation, Soil improvement, Calcite precipitation, Soil stabilization

1. INTRODUCTION

When it comes to building infrastructure in emerging nations or ancient cities where there are geographical restrictions on site expansion, soil stabilization is essential. Due to the tremendous engineering challenges this poses for meeting human requirements, it is especially important to improvise a sustainable foundation for construction activities [10]. Soil stabilization aims to improve soil bearing capacity while reducing settlement and deformation [6]. Chemical and mechanical approaches are among the traditional techniques for stabilising soil. For better performance, mechanical stabilisation involves eliminating air spaces from the soil mass while varying the water content to a minimum. Chemical stabilisation, on the other hand, involves supplementing the soil with additives to reach the required density, lower permeability, can promote stronger soil [19]. Densification and compaction of the soil are achieved using mechanical techniques such as blasting, vibroflotation, rammering, rollers, and preloading of the soil. The implementation of this approach in the field may result in significant energy release along with high production and installation costs. The chemical improvement technique involves the grouting and deep mixing of chemical admixtures like cement, lime, fly ash, etc [10]. Portland cement, silicates, asphalt, polymers, and lime are all used in chemical stabilisation. These alterations improve the chemical composition of the soil, improving its geotechnical behaviour [5]. The use of chemical stabilisation has grown in acceptance because of how well it improves soil. It can employ conventional calcium-based binders such as fly ash, cement, ash, and new stabilisers like lignosulfonates, acids, salts, and enzymes

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petroleum-based polymers, resins, and emulsions [22]. Another new development in soil stabilisation is biologically mediated soil modification. In order to improve soil performance, soil stabilisation includes domesticating micro-organisms. Mineralization by microbes like bacteria, fungus, and algae occurs naturally and has numerous engineering applications [11]. Bacterial intrusion called Microbial-induced calcite precipitation (MICP) is a bio-mediated ground improvement technique which improves soil geotechnical properties by precipitating calcium carbonate ($CaCO_3$) and binding soil grains together [18]. MICP treatment improves various geotechnical engineering challenges in various fields like improvement of shear strength, soil liquefaction, carbon dioxide sequestration, erosion control and remediation of contaminated soils. The non-homogeneity of the calcium carbonate distribution is one of the limitations of the MICP [10]. Enzyme-induced calcite precipitation (EICP) is an innovative and bio-inspired technique for ground improvement. EICP is a low-carbon-emission, sustainable, and ecologically friendly technique. Enzyme induced calcite precipitation (EICP) uses enzymes to precipitate $CaCO_3$, rather than microorganisms [17]. This method has numerous engineering applications for soil treatment, including stabilizing slopes, preventing wind and water erosion, reducing soil scouring, monitoring seepage beneath levees, improving soil bearing capacity, tunnelling, and controlling seismic settlement [13].

2. CONCEPT OF EICP METHOD

2.1 ENZYME INDUCED CALCITE PRECIPITATION

The EICP approach improves the geotechnical qualities of soil by using an aqueous chemical solution to precipitate calcite in voids. The precipitations help to improves the strength and stiffness of soil by binding and roughening the grains of soil and filling the pores. The MICP method uses bacteria to create urease enzymes, whereas the EICP method applies urease enzymes directly to the soil. Using bacteria as living organisms in soil necessitates a specific environment for their survival because they need laboratory equipment to sterilise the environment in order to create their colonies. One advantage of this research is the elimination of the challenges related to using bacteria to manufacture calcite precipitation. The EICP approach becomes more practical for use in the field, which is crucial for civil engineering research projects, due to the direct application of urease enzymes and the constraints of laboratory apparatus [25]. The EICP approach is different from MICP by substituting free urease instead of bacteria. Enzymes can come from plants, bacteria, and agricultural resources [2]. EICP is an innovative, biologically inspired method of ground improvement. EICP is a sustainable, environmentally friendly, low-carbon method. The cementation solution (urea and calcium chloride) and urease enzyme make up the EICP treatment solution. The precipitation of calcium carbonate improves the soil's shear strength, stiffness, and dilatancy qualities at the end of the treatment by binding the soil particles together [10]. Using EICP could be a method to induce bio-cementation in soils consisting of significant amounts of fines due to enzymes not requiring the larger void spaces microbes require because enzymes are water soluble [14]. Considerable reviews concerning MICP and EICP treatment have primarily focused on soil stabilization and strength improvement [7].

2.2 UREA HYDROLYSIS

EICP uses urease enzymes produced by microbes or derived from plants to catalyse the hydrolysis of urea [1], [2]. Urea can be broken down by the urease enzyme into ammonium and carbonate anions (Eq. (1)). When carbonate anions and external calcium sources are present, the released ammonium helps to create an alkaline environment that is favourable for the precipitation of calcium carbonate ($CaCO_3$) [24]. The $CaCO_3$ precipitation created by urea hydrolysis can act as cementation materials to bind soil particles together, that's how EICP for controlling soil erosion works. Compared to traditional soil stabilisation materials, EICP procedures are more environmentally friendly because the primarily generated $CaCO_3$ crystals have less of an impact on the soil matrix's environmental conditions. EICP reaction is more suitable for high temperature environments such as arid and semi-arid regions. The cost of purified urease enzyme is relatively high for practical EICP applications [2].

$$CO(NH_2)_2 + 2H_2O \longrightarrow 2NH_4^+ + CO_3^{2-} \quad \text{………………………….... (1)}$$

$$Ca^{2+} + CO_3^{2-} \longrightarrow CaCO_3 \quad \text{………………………………..……….(2)}$$

The urea hydrolysis process is accelerated by enzymes by a factor of millions as compared to the rate of an uncatalyzed reaction. The catalytic mechanism and, consequently, the rate of the reaction or precipitation are determined by the concentration and activity of the urease enzyme. Urea ($CO(NH_2)_2$) is first hydrolysed into ammonium (NH_4^+) and carbonate (CO_3^{2-}) ions by the urease enzyme as part of the EICP process. Additionally, this causes the pH of the solution to rise. These products of ureolysis can precipitate carbonate when there are enough divalent cations present, such as calcium ions. Calcium chloride is one source of calcium ions. Ca^{2+} is produced in the solution when calcium salt ($CaCl_2$) is present, and this causes $CaCO_3$ to form.

2.3 PLANT DERIVED UREASE ENZYME

The amount of urease produced by crude jack bean extract, as determined by testing it on crude and refined extracts from watermelon seeds, soybeans, jack beans, and jack bean meal in test tubes, is the highest unit yield among these four plant sources [23]. A comparison between the strength of soil samples treated with crude and commercially available urease extract revealed that the crude extract is more successful because of the substantial impact that the impurities in both extracts play in strengthening the soil. Test tube experiments and soil column investigations have shown that the level of enzyme purity in addition to urease activity can affect the efficiency of bio cementation via EICP, though not always in the way one might anticipate. Higher q_u outcomes were observed in specimens that were bio cemented using crude extracts from jack beans, which were far less pure than enzymes that were sold commercially. This finding implies that the presence of organic contaminants in the bio cementation solution can actually improve the efficiency of EICP in bio cementation.

Urease enzyme is a cheap source for cementation treatments and can be isolated from a variety of plant sources. Baiq investigated the precipitation efficiency of crude urease enzyme extracts by substituting extracts from cabbage and soy pulp for commercial urease enzyme. The precipitation findings using commercial products and crude extracts were compared under the same reagent solution concentration conditions, i.e., 1M of urae-$CaCl_2$ solution and 15 g/l urease enzyme over many curing days. The test-tube investigations' findings showed that 40–60% of the materials may be precipitated when using soy pulp and cabbage as bio-catalysts without first being purified. The precipitated material's mineralogical analysis using FTIR showed that it was calcium carbonate, which was identified by a range of wave numbers. This was the outcome of applying the unrefined extracts. XRD data verified the promoted polymorph of calcite as well. According to SEM investigations, using soy pulp and cabbage leads to precipitated materials with varying sizes and shapes; some agglomerations have a spherical shape, while others have a rhombohedral shape [4].

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3. EFFECT OF EICP TREATMENT IN SOIL

3.1 EFFECT OF EICP TREATMENT ON COARSE GRAINED SOIL

The primary parameters influencing the effectiveness of EICP are the type of soil, the composition of treatment solutions, the source of urease enzyme, and the treatment technique. In a baseline study, sand columns were treated with 0.67M calcium chloride, 1M urea, and 3 g/L enzyme done by Almajed in order to evaluate the influence of the content of the enzyme-induced carbonate precipitation (EICP) treatment solution on the efficiency of carbonate precipitation. Test tube investigations on the precipitation efficiency of various urease enzyme concentrations were used to determine the concentration. The treatment sample's effluent solution contained urea and unconsumed calcium chloride, but since no free urease was found in the effluent, it cannot be employed as a source of urease enzyme. The findings of the undrained shear strength test indicated a critical carbonate content above which the strength of the EICP-treated soil increases significantly. Loss of strength from organic matter and ammonium chloride salt precipitates being flushed out of the treated soil was similarly affected by rinsing the EICP samples with deionised water. The desired dimension and the study's predetermined target qu value of 500 kPa were achieved by the columns in the shortest amount of treatment time, according to the results of a mid-scale dry sand biocemented soil utilising EICP. When the specimens were removed from the columns, their q_u values decreased below the desired value. The sample disturbance is responsible for this. Moreover, the low enzyme activity in the samples treated with low enzyme concentration prevented them from reaching the desired q_u value of 500 kPa. As a result, the sample disturbance has significantly impacted on the strength improvement of soil samples [2].

Dilrukshi improved soil by the usage of plant-derived urease-induced calcium carbonate precipitation. Urea, calcium chloride, and a crude extract of crushed watermelon seeds were utilised as urease sources for the precipitation of calcium carbonate. According to the estimated undrained shear strength of Mikawa sand, which is sold commercially, the strength increased as the content of urea-$CaCl_2$ increased. Samples cured after 14 days at a concentration of 3.912 U/mL urease and 0.7 M $CaCl_2$-urea had the highest q_u values [8]. Javadi experimented with EICP treatment of sand columns by using urease enzyme obtained from watermelon seeds. The sample was rinsed with deionised water and acid digestion. After extraction, the treated samples broke down, indicating inadequate carbonate precipitation to bind the particles together. The findings of the SEM investigation showed that precipitated $CaCO_3$ was present, but no proof of interparticle binding was discovered. The findings led to the conclusion that watermelon seed urease can be employed as a low-cost method of causing calcium carbonate precipitation. Larger urease enzyme concentrations and more treatment cycles, however, can generate enough calcium carbonate to increase the soil's strength [12]. Park investigated the unconfined compressive strength of poorly graded sand (SP), urea, calcium chloride, and a jack bean solution. The results showed that the EICP treated sand specimen's undrained shear strength (200–300 kPa) was almost as high as that of sand mixed with Portland cement, which hardens rapidly at 4% by weight [16].

Shu studied poorly-graded Ottawa sand. Crude urease extracted from soybeans was used for all the EICP treatment methods in this study [21]. The precipitation of $CaCO_3$ in sand samples was catalysed by four distinct types of EICP treatment procedures, and the effectiveness of each approach was compared. Method 1: 36 ml soybean crude urease solution (SCUS) prepared with 60 g/L powdered soybean was premixed with the same volume of 1 M cementation solution (CS), and then the mixture was introduced from the top of the sand samples. Method 2: 72 ml SCUS prepared with 30 g/L powdered soybean was firstly percolated onto the sand, after 6 h retention time, 72 ml of 0.5 M CS (1 MCS diluted with an equal volume of water) was injected. Method 3: dry sand corresponding to a relative density of 45 % was quickly mixed with 36 ml SCUS (prepared with 120 g/L powdered soybean) and 36 ml CS (1 M) and then the mixture was placed in the PVC column as suggested by Almajed [2]. Method 4: the proposed method consisted of four cycles of bio-cementation. In the first cycle bio-cementation, a premixed percolation was conducted (similar to that performed in Method 1), and in the following three cycles, only 72 ml CS (0.5 M) was percolated each time. After completing the last treatment, 500 ml of tap water was introduced to the top of the column to remove the soluble agent in the sand. It was evident that, for the same number of treatments, the chemical conversion efficiency and $CaCO_3$ concentration of the EICP treated sand produced by Method 2 were much lower than those produced by Methods 1, 3, and 4. Moreover, statistical analysis showed that for Methods 1, 3, and 4, the $CaCO_3$ content and chemical conversion efficiency were comparable ($p > 0.05$). One possible explanation for this occurrence is that the second CS injection washed away a significant amount of previously injected urease. The proposed multiple-phase method produced an equal dispersion of $CaCO_3$ in the EICP technique. After multiple treatments, bio-stabilized sand samples maintained good permeability while also obtaining high strength. In addition, the multiple-phase method could significantly improve the efficiency of urease usage. Based on the mechanical and physical responses of biotreated samples, the premix-and-compact method was suitable for backfill reinforcement.

Most of the researchers focused on using EICP for improving shear strength of soil. Crude urease derived from various plant sources was found to be more economical than commercially available urease. UCS strength of sand treated using EICP was higher than the sands stabilized using cement and lime combinations.

3.2 EFFECT OF EICP TREATMENT ON FINE GRAINED SOIL

Anie studied into the effect of adding montmorillonite nano clay in weak clayey soil and the change in the soil properties. The results showed that the ideal moisture content had decreased and the total dry density had increased. This is because there is now less space between the particles as a result of the addition of nano clay [3]. Yuan conducted an experimental study of EICP combined with organic materials for silt improvement in the area which is an improved EICP method that involves adding an appropriate mass concentration of organic materials into urease solution was proposed and applied to reinforce silt in the Yellow River flood area of China. The strength obtained using the enhanced EICP technique with organic material is approximately four times greater than the original soil's strength and 25–33% greater than that of the conventional EICP approach. Through modified-EICP treatment, the calcium carbonate content changes by about 1%, the strength increases by about 33%, and the strength improvement efficiency increases by 32%. This indicates that the optimisation of the calcium carbonate crystal structure is primarily responsible for the improved modified EICP [26].

Gao employed EICP to increase the compacted clay liners' shear strength. On soil treated with four separate cementing concentrations at various water contents, compaction was carried out. The qu value of the treated soil samples was higher than 200 kPa, which was the minimal level advised for compacted clay liner; the qu value of the untreated soil samples was lower than 200 kPa. It was found that the shear strengths increased together with the molarity of the urea-$CaCl_2$ solution. When the sample was created at 2% water content in proportion to OMC, the highest strength of 643.5 kPa was reached at 1.0 M cementation solution [9].

Yuan studied the effects of Na-montmorillonite (Na-Mt) content and curing age on enzyme-induced carbonate precipitation (EICP)–treated soil.First, tube tests were used to examine how the addition of Na-Mt affected the pH, Ca^{2+} precipitation rate, and urease activity of the solution. Next, in China's Yellow

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River flooding region, silty sand was reinforced with Na-Mt-modified EICP. The unconfined compressive strength (UCS) test, calcium carbonate content (CCC) measurement, X-ray diffraction, and scanning electron microscope analyses were used to investigate the solidification effect and action mechanism of Na-Mt. Soil treated with conventional EICP and soil treated with Na-Mt alone were considered the control group. By decreasing pH and increasing $CaCO_3$ production through chelation, Na-Mt enhanced urease activity and Ca^{2+} precipitation rate, controlled $CaCO_3$ crystal shape, and promoted the development of densely aggregated calcite. During the first seven days of curing, there was a fast increase in the CCC and mechanical characteristics, which subsequently decreased. When 8% Na-Mt was added, the UCS and Ca^{2+} utilisation ratio at 7 days of curing age increased by 1.4 and 2.72 times, respectively, in comparison to typical EICP; 8% Na-Mt was found to be the ideal concentration. The Na-Mt-modified EICP technique is an effective way to solidify fine-grained soil, as demonstrated by the fact that, at Na-Mt contents of less than 8%, the mathematically expressed improvement effect of the Na-Mt modified EICP on the soil strength was greater than the arithmetic sum of that when these two approaches were applied separately [26]. Oliveira uses sand, silty soil, and organic soil using a mix-and-compact technique with EICP. In the preliminary round of testing, they employed an EICP solution containing 0.25 M urea, 0.25 M calcium chloride, and 4 KU/L urease enzyme (equal to around 0.12 g/L high activity enzyme). They then performed additional tests with 0.5 M urea, calcium chloride, and 8 KU/L urease enzyme (equal to 0.23 g/L high activity enzyme). They discovered that whereas EICP treatment has a negative effect on organic soil, it strengthens sand and silty soil [15].

For the EICP technique, Roksana used crude soybean extract for the purpose of urea hydrolysis and conducted the test on soil sample collected from a local construction site in New Jersey. For the investigation, a number of fluid samples were evaluated, including a control sample, a cementation solution comprising 1 M urea, 0.675 M CaCl2, and 4 g/L milk, as well as different doses of enzyme solutions (3–80 g/L). The approach comprised continuous observation and photographing with a high-resolution camera with the help of image processing software in order to assess the surface cracking patterns. The results demonstrated that when the EICP approach was applied, fine-grain soils improved due to increased calcite precipitation and decreased desiccation cracking intensity. Low quantities of cementation and enzyme solution (3 g/L and 10 g/L) produced comparable results on crack repair, indicating a moderate impact. In this instance, the crack network did not change in comparison to the sample that was treated with water. As the concentration of the enzyme increased (30 g/L, 50 g/L, and 80 g/L), CaCO3 precipitation within the void area maintained the fracture network in situ even if the void thickness reduced. In the EICP-treated sample, it was discovered that wetting and drying cycles reduced the crack ratio, crack width, and crack length, especially at higher urease enzyme concentrations. While they efficiently prevent the production of new cracks, lower enzyme doses of 3 g/L and 10 g/L have no effect on crack repair [20].

In fine-grained soil, most of the researchers focused on improving shear strength with the help of EICP treatment. But the durability and role of EICP in controlling soil erosion in fine grained soil have not been explored widely.

4. COMPARISON OF TESTS IN EICP

4.1 UNCONFINED COMPRESSION TESTS

The maximum axial compressive stress that a specimen may withstand with zero confining force is known as Unconfirmed Compressive Strength (UCS). The unconfined compressive strength of the soil is the compressive load per unit area needed to cause the specimen to fail. The treated soil is levelled on top and bottom to give the specimen a smooth sitting position. The specimen is then placed under a testing machine, where its strength is measured and recorded, in accordance with the work procedure, and rinsed and dried. The UCS is typically measured at a strain rate of 1.27 mm/min during EICP treatment. Values are noted, and strength and ability are evaluated [15].

Table 1 shows the comparison of the optimum value of UCS tests with different additives. And the results displayed that the addition with the EICP solution has produced a great strength in soil and thus stabilizing it.

Table 1. Optimum values of UCS test conducted in different experiments in Kpa

Components	Method	q(KPa)	Author
1M urea, 0.67 M $CaCl_2$, and 3 g/L enzyme	Mix compact and percolation (EICP). When comparing mix and compact, percolation revealed strength.	1268	(Almajed et al., 2018)
0.5 M $CaCl_2$, 0.875 M urea, 0.85 g/L urease enzyme	Addition of 0.2%,0.3%,0.4%,0.75% and 0.85% of sisal fibre and mixes with and mix and compact method	296	(Almajed et al., 2018)
0.67 M $CaCl_2$-dihydrate, 4 g/L nonfatmilk powder, and around 13,000 U/L of free urease enzyme.	Crude extract in water	1100	(Tirkolaei et al., 2020)

4.2 SEM ANALYSIS

High-resolution imaging is provided by scanning electron microscopy, which is helpful for assessing different materials for surface fractures, defects, impurities, or corrosion. These tests are used in studies to better understand how soil particles interlink and how crystalline structures (polymorphs of calcite, aragonite, vaterite, monohydrocalcite, and ikaite) form in soil. The existence of aggregated rhombohedral calcite crystals was shown by the SEM investigation. They are able to detect the existence of biological materials that could weaken the force. The findings of the experiments indicated that washing the soil before to testing will facilitate comprehension of the crystalline structure. When excess chemicals are present in the test material it will make the study difficult and prevent from getting exact results [2]. After being applied to soil, the crude urease extract from plant seeds is examined under a scanning electron microscope. In contrast to the test tube tests, it demonstrated the precipitation of rhombohedral crystals and revealed that none of the biocemented soil specimens included vaterite or spherical calcite. It is possible

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that the lack of vaterite or spherical calcite in the bio-cemented sand will either encourage calcite to precipitate or prevent it from forming in the EICP solution [23].

5. CONCLUSIONS

The observation in different aspects of EICP are the addition of substrates will increase in strength of the soil. But it depends on the concentrations of urea, and urease enzyme. Optimum concentrations should be taken for research. An unconfined compression test is a way to compare the strength of treated soil. It was observed that comparing with other studies the crude extract resulted in less strength but there was a noticeable increase compared to untreated soil. In some cases, the soil might not give the best results with the UCS test, in that case different tests such as triaxial drained test can be included. Organic soil or soil containing any inhibiting material could reduce soil strength. The majority of silica-containing, well-graded soil has excellent strength test results. As the cost of industrial urease production is higher, urease extraction from various plant sources could be an acceptable alternative to this problem. It is also an ecofriendly solution.

Conflict of Interests

The authors declare that they have no conflict of interest.

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