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# Improvement in Plasticity and Strength Characteristics of Kuttanad Soil using Enzyme Induced Calcite Precipitation

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**Abstract:** *This work presents an inquiry into Enzyme Induced Calcite Precipitation (EICP) as a way for enhancing the engineering properties of highly plastic soil, in keeping with previous studies on eco-friendly ground improvement methods. The highly plastic soil samples were collected from Kuttanad, Alapuzha. In order to precipitate calcium carbonate (CaCO<sub>3</sub>) through urea hydrolysis, an equimolar mixture of cementation solution, consisting of urea and calcium chloride along with varying amounts of urease enzyme as catalyst, was added to the collected soil samples. The plasticity, strength, and microstructure of soils will be examined using a variety of tests, including the Atterberg limits test, Unconfined Compressive strength (UCC) testing, and Scanning Electron Microscopy (SEM) analysis. After EICP treatment and 7-day curing, the highly plastic soils showed a significant improvement in their plasticity and strength properties, as evidenced by a decrease in liquid limit and plasticity index, an increase in plastic limit, and a notable improvement in undrained shear strength. The results of the study proves that EICP can be used as an excellent treatment method to improve the plasticity and strength characteristics of highly plastic soils.*

**Keywords:** *Enzyme Induced Calcite Precipitation, Urease enzyme, Calcium carbonate, Urea hydrolysis*

## I. INTRODUCTION

Ground improvement is required to support a growing population and rising worldwide demand for civil infrastructure. While being widely utilized, traditional ground improvement techniques including vibration, preloading, compaction, and chemical grouting are generally expensive and frequently have high energy and CO<sub>2</sub> footprints (Ahenkorah et al., 2021). In order to improve the mechanical qualities of soil, chemical agents or Portland cement have been frequently utilised for soil stabilisation. However, due to environmental problems including groundwater contamination, the use of chemical agents has been limited. Several factors have led to a rise in environmental concerns over the use of Portland cement as a soil stabilising substance, such as production of carcinogens and carbon emission, despite the fact that it has traditionally been recommended. These factors have led to a steady rise in the need for affordable and environmentally friendly soil stabilising products in the geotechnical engineering sector (Lee et al., 2020). However, the soil properties have a great impact in stability of foundations and structures (Aparna et al. 2018). In order to meet the demands of social infrastructure, there is a need for contemporary, long-lasting, and environmentally responsible ground restoration technology.

A relatively new field and potentially sustainable alternative strategy for ground improvement is the utilization of biological processes, particularly bio-cementation. Lekshmi et al. (2019) studied the use of biopolymers as an alternative to conventional soil stabilizers. In the study, Xanthan gum and Guar gum was added to Kuttanad soil at different concentrations. An increase in the dry unit weight and reduction in optimum water content was found with the increasing concentrations for both Guar gum and Xanthan gum. Compressive strength was increased by as much as 60% when 2% of Xanthan gum and 1.5% of guar gum were added to the soil. Anie et al. (2020) investigated the effect of montmorillonite nano clay on geotechnical properties of weak clayey soil. As a result of the addition of nano clay, clay absorbs more water and has fewer spaces between its particles, increasing its overall dry density but lowering its optimum moisture content. The outcomes demonstrated that the dry density of the materials being handled is positively impacted by nano clay.

The biological soil improvement methods can be divided into two categories: bio-mediated and bio-inspired methods. Living organisms are introduced directly into the soil as part of bio-mediated soil improvement approaches, and the byproducts of their biological activity can be exploited to change the engineering qualities of the soil. While using bio-inspired procedures, living creatures are not directly applied to the soil in order to stabilize it.

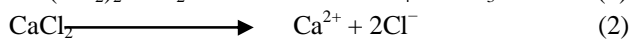
To transfer identical reactions and products into the soil, different materials are used. Microbial induced calcium carbonate precipitation (MICP) and Enzyme induced calcium carbonate precipitation (EICP) are two emerging biological soil improvement techniques mainly used for increasing strength and stability of soils and for decreasing the permeability and liquefaction effects. A study conducted by Punnoi et al. (2021) used *Bacillus pasteurii* bacterium for MICP treatment where the bacteria was added in the form of bacterial spores and vegetative cells. The result showed an improvement in Undrained shear strength ( $q_u$ ) of treated soil compared to the strength of untreated soil. After 3 days and 7 days of curing, the  $q_u$  of treated samples values were higher than the  $q_u$  values of the untreated samples. The  $q_u$  values of the treated clay were enhanced by 2.6 and 2.0 times, respectively, by MICP by bacterial spores and vegetative cells. Also, it was demonstrated that vegetative cells increase clay strength prior to bacterial spores, due to delay in time for the spores to reactivate to active cells. Similarly, Xiao et al. (2020) found an increase in undrained shear strength by 2.42 times on the treatment of soft clay specimens. Kannan et al. (2020) experimented the behavior of marine clays treated using MICP method. Both bioaugmentation and bio stimulation methods were experimented and it was found that bioaugmentation was effective for soil stabilization in marine clays. A reduction of 29% and 47% were observed for liquid limit and plasticity index values respectively after 25 days bioaugmentation treatment. An improvement of 148% and reduction of 32% were found for undrained shear strength and compressibility for marine clays.

A comparative study on the efficiency of carbonate precipitation of MICP using ureolytic bacteria and EICP using soybean solution for urea hydrolysis was done by Lee et al. (2020). From the results, it was found that over time, the rate of EICP decreases whereas that of MICP increases. This was due to degradation of urease and increase in microbial growth. By changing the proportion of yellow soybean to distilled water, the problem can be fixed. The rate of EICP can be altered to precipitate the highest amount of calcium carbonate possible in 24 hours by increasing the yellow soybean concentration. The microbial population can also control the rate of MICP, although more difficult than with EICP due to the complexity of microbial cultivation. The 28-day-cured sample treated with 3g/L of soybean solution and 140g/L of urea- $\text{CaCl}_2$  solution showed the greatest strength in the case of EICP. Gao et al. (2020) used EICP method to improve the strength of compacted lay liners. Cementing solutions of four different concentrations were used to conduct compaction at different water contents. While the  $q_u$  values of the untreated soil samples were less than 200 kPa, the  $q_u$  values of the treated soil samples were larger than 200 kPa, which was thought to be the minimal level required for compacted clay liners. It was seen that the shear strengths rose together with the molarity of the urea- $\text{CaCl}_2$  solution. When the sample was created at -2% molding water content in reference to OMC, the strongest strength of 643.5 kPa was reached at 1.00 M cementation solution. The soil matrix of the treated soil contained the mineral calcite, according to an XRD analysis. Similar results were obtained for study conducted by Dilrukshi et al. (2018). Along with urea and calcium chloride, a crude extract of crushed watermelon seeds served as the urease source. Commercially available Mikawa sand's estimated Undrained shear strength showed that it did rise in response to an increase in urea- $\text{CaCl}_2$  concentration. The samples that were cured for 14 days with 0.7 M  $\text{CaCl}_2$ -urea and 3.912 U/mL urease concentration had the greatest  $q_u$  values. Crude urease from crushed watermelon seeds could take the place of commercially available urease for carbonate precipitation and use as a low-impact method of soil improvement.

Another source of urease enzyme, along with watermelon seeds and soybeans seeds was jack bean seeds, which was found by Tirkolaei et al. (2020) in their study. The refined and crude extracts of jack beans, soybeans, watermelon seeds and jack bean meal were tested and the results revealed that in terms of urease production per initial mass of source material, crude jack bean extract produces the highest unit yield than the other three plant sources. Comparing the strength of soil samples treated with crude and commercial urease extracts revealed that the contaminants in both extracts significantly contribute to soil strengthening, making the crude extract more effective. The test tube experiments and soil column investigations show that the level of enzyme purity, in addition to urease activity, can affect the effectiveness of bio cementation by EICP, although not always in the way that might be anticipated. The specimens with the higher  $q_u$  results were bio-cemented utilizing jack bean crude extracts, which were significantly less pure than commercially available enzymes. According to this finding, organic contaminants in the bio cementation solution may actually increase how well EICP works for bio cementation.

## II. ENZYME INDUCED CALCIUM CARBONATE PRECIPITATION (EICP)

Enzyme induced calcium carbonate precipitation (EICP) is a novel bio geotechnical ground improvement method that improves the bio geotechnical properties of soil by precipitating calcium carbonate from an aqueous solution into the soil pores. The strength, stiffness, and dilatancy of the soil are improved by calcium carbonate precipitation through pore filling, particle roughening, and interparticle binding (Almajed et al. 2018). The EICP process has the potential to be used as bioremediation and bio-cementation solutions in many geotechnical, building, and environmental and civil engineering problems, such as reducing soil liquefaction potential, enhancing soil strength, controlling surface erosion, remediating heavy metal contaminants, reducing permeability and so on. (Lee et al. 2020).



Due to the smaller size of the urease enzyme crystals, which are typically 12 nm or 120 in size, EICP has the advantage of being effective for a wider variety of soils, including fine-grained soils. Despite the fact that some studies have used cheap sources of enzyme, such as crude urease extract, some extraction techniques may require additional steps or chemicals and occasionally only provide small amounts of urease enzyme. A sustainable adaptation of EICP as a bio-cementation process focuses on the optimization of chemical ingredients and curing time (reaction/precipitation time) in order to save building costs and time. The CaCO<sub>3</sub> that has precipitated inside the soil matrix creates bridges and linkages between the grains of the soil particles in bio cemented soils, limiting their movement and boosting the strength and stiffness of the soil. EICP can be used for granular soil strengthening, concrete crack repair, and liquefaction mitigation in soil stabilization and strengthening.

The current paper discusses about the potential application of bio-inspired ground improvement technique to improve the plasticity and strength characteristics of Kuttanad soils. The experimental investigation that was conducted, the discussion of the findings, and the key findings of the current study are presented in the following parts.

### III. EXPERIMENTAL WORK AND TESTING

Soil sample was collected from Kuttanad regions of Alapuzha district, Kerala at a depth of 2m below ground level. Basic soil tests such as particle size distribution, and Atterberg limits were done as per IS codes. The strength characteristics of the soil was conducted by Unconfined compressive strength (UCC) test as per IS standards. The collected samples were preserved at their natural water contents as drying is reported to alter basic properties of these clays (Vinod and Bindu 2010). Table 1 presents the properties of samples used for the study.

TABLE 1:  
Properties Of Collected Soil Samples

Property	Value
Initial moisture content (%)	114.28
Specific gravity	2.18
Liquid limit (%)	98.9
Plastic limit (%)	43.6
Plasticity index (%)	55.3
Percentage of clay	8
Percentage of silt	76.2
Percentage of sand	13.8
Soil classification	MH
Undrained shear strength(kPa)	12.9
Activity	5.6

For conducting the EICP treatment, the Urea- CaCl<sub>2</sub> solution, hereby referred to as cementation solution, and urease enzyme solution was prepared. CaCl<sub>2</sub> solution is used as the source of Ca<sup>2+</sup> in the study. An equimolar concentration of cementation solution was prepared and the concentration was fixed as 1M. For preparing urea and CaCl<sub>2</sub> solutions of 1M concentration, the molecular weights of the components are dissolved in 1 liter of deionized water (110.9 g of CaCl<sub>2</sub> and 60 g of urea respectively). ‘Jack bean meal Urease extra pure’ enzyme was used for the study since urease enzyme derived from jack bean seeds are found to be most effective among plant derived urease enzymes (Tirlokai et al. 2020). The urease enzyme solution was added to the soil samples at two different concentrations, i.e., 2.5 g/l and 5g/l.

For EICP treatment, 20% by volume of the cementation solution and urease enzyme solution were added to soil and was mixed thoroughly. The soil samples, along with the solutions, were filled into a thick plastic container. The container was fashioned with a tight lid to reduce moisture loss through the spaces in between. The container was partially immersed in water to ensure the maintenance of constant temperature and was subjected to curing for 7 days.

Experiments were done to see the change in plasticity and strength characteristics of the soil samples as a result of treatment. Liquid limit ( $w_L$ ) and plastic limit ( $w_P$ ) were determined by Casagrande’s method and rolling thread method, respectively as per IS 2720: Part 5 (1985). The liquid limit test was performed by air drying the soil samples to adjust the water content and subsequently plot the flow curve. The plastic limit was established by rolling the soil to a diameter of approximately 3 mm, kneading, and rerolling it until the thread breaks under pressure after being reduced to a consistency that could be rolled without clinging to hands with the aid of an electric fan. The Undrained shear strength of the soil was determined by Unconfined compressive strength (UCC) test as per IS 2720: Part 10 (1991). The tests were conducted for the soil samples treated at both concentrations of the urease enzyme. The plasticity and strength characteristics of the treated soils were analysed.

#### IV. EFFECT OF EICP TREATMENT ON THE PLASTICITY CHARACTERISTICS

The liquid limit of the collected soil sample was 99% which was very close to its natural water content. After EICP treatment with 1M cementation solution and 2.5 g/l urease enzyme concentration, the liquid limit was reduced to 79%, whereas the treatment with 1M cementation solution and 5 g/l urease enzyme concentration reduced the liquid limit of the sample to 68%. However, the plasticity indices of the samples reduced to 33.5% and 18.1% for treatment with 2.5g/l and 5g/l urease enzyme concentrations respectively. There was an increment in the values of plastic limits to 45.03% and 50.8% for treatment with 2.5g/l and 5g/l urease enzyme concentrations respectively. Figure 1 represents the variation in consistency limits of the soil samples after EICP treatment with both 2.5g/l and 5g/l urease enzyme concentrations respectively. With a considerable difference in the consistency limits, the designation of the soil sample changes from the higher regions of MH classification to the lower regions of MH classification (IS 2720: Part 5 (1985)), indicating a significant difference in soil characteristics.

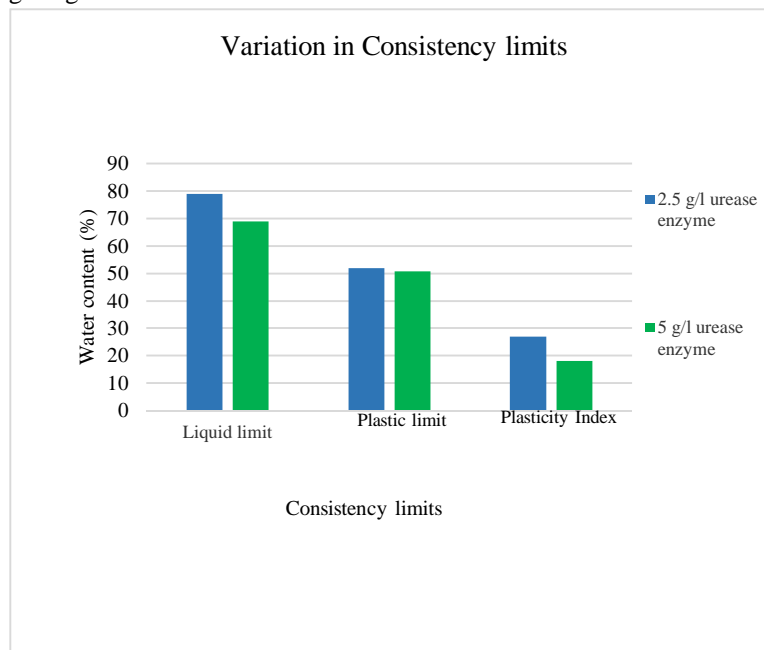


Fig. 1: Variation in consistency limits of EICP treated soil samples after 7 days of curing

#### V. EFFECT OF EICP TREATMENT ON THE PLASTICITY CHARACTERISTICS

The Undrained shear strength ( $q_u$ ) value of the virgin soil sample is 12.9 kPa. After EICP treatment for 7 days, the strength was increased to 38.5 kPa and 49.3 kPa for treatment with 2.5g/l and 5g/l urease enzyme concentration respectively. Thus, a considerable improvement in strength characteristics were observed for the soil samples after treatment. Figure 2 shows the stress-strain curves EICP treated soil samples after 7 days of curing. Table 2 represents the percentage variations in plasticity and strength properties of the EICP treated samples after 7 days of curing.

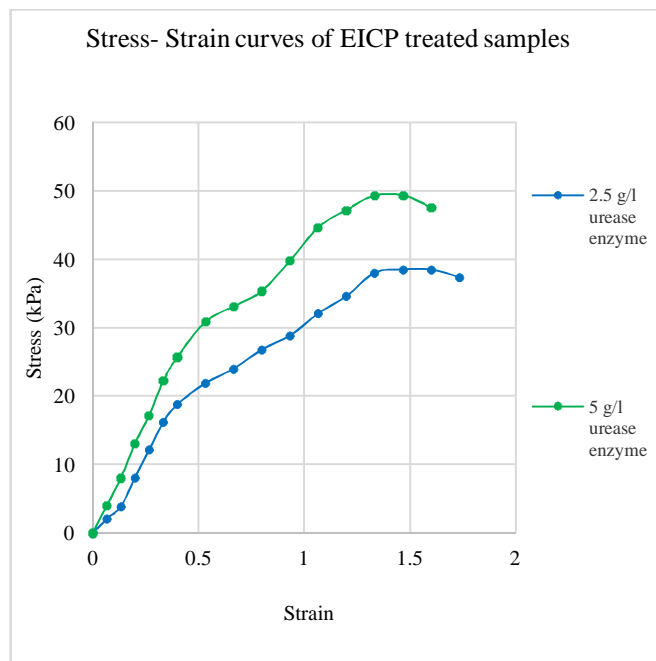


Fig. 2: Variation in consistency limits of EICP treated soil samples after 7 days of curing

TABLE 2:  
PERCENTAGE VARIATIONS IN PLASTICITY AND STRENGTH PROPERTIES  
OF THE EICP TREATED SAMPLES AFTER 7 DAYS OF CURING

Urease enzyme concentration	% reduction in liquid limit	% increase in plastic limit	% reduction in plasticity index	% increase in UCS
2.5 g/l	27.2	32.4	63.8	198.4
5 g/l	36.3	46.6	82.3	282.1

## VI. RESULTS AND DISCUSSION

The liquid limit of the sample reduced by 27-37% and plastic limit of the sample increased by 32-47% after EICP treatment with various concentrations of urease enzyme. There was a prominent reduction in the plasticity index of the sample by 63-83% after the treatment. The UCS values of the treated sample exhibited a considerable improvement of 2.96-fold and 3.38-fold after EICP treatment with 2.5g/l and 5g/l urease enzyme concentration respectively. The above results clearly suggests that EICP is an effective treatment method for improving the plasticity and strength characteristics of fine-grained soils. The above observed variations in the properties can be attributed to the precipitation of CaCO<sub>3</sub> in the soil (Kannan et al. 2017). As the soil sample contains a higher fraction of fine-grained particles, it provides a larger surface area and a large amount of contact points, or nucleation sites, thus providing higher particle-particle interaction compared to coarse grained soils. Hence, the better plasticity and strength characteristics of the treated soil can be justified. Previous study on the effect of Microbial Induced Calcite Precipitation (MICP) on strength of fine-grained soils showed an increase of UCS values by 1.50-2.91-fold. Hence, it can be concluded that EICP can be used as a better alternative to MICP for strength improvement.

## VII. CONCLUSION

Biological ground improvement techniques have been gaining a huge appraisal among the geotechnical engineers worldwide. Two new biological soil enhancement methods, microbial-induced calcium carbonate precipitation (MICP) and enzyme-induced calcium carbonate precipitation (EICP), are primarily utilized to improve soil strength and stability while reducing permeability and liquefaction impacts.

From the literature reviews, it was found that EICP can be an excellent alternative to MICP due to its precipitation efficiency and the ease of controlling the precipitation rate. For the present study, an equimolar concentration of cementation solution, i.e., Urea-CaCl<sub>2</sub> solution of 1M was prepared for the study. Urease enzyme solution was added to the soil in two different concentrations, 2.5g/l and 5g/l. The solutions were added to the soil and was subjected to curing for 7 days. From the results, it was found that the liquid limits and plasticity indices of the sample reduced considerably whereas plastic limits of the sample increased. Also, comparing the two concentrations of urease enzyme, it was found that the combination of 1M cementation solution and 5g/L urease enzyme concentration showed better improvement in properties.

All the results obtained were for the laboratory scale treatment conditions. A variation in results can be expected for field scale testing and implementation due to varying site conditions, soil conditions and other factors. The present study was conducted at fine grained soil, which mainly had silt particles. The study can be conducted on clay specimens of different regions and conditions for the better understanding of the efficiency of EICP in fine grained soils. Also, the concentration and compositions of the cementation solution and urease enzyme solution can be varied and a correlation of the concentrations can be discovered according to the soil type and conditions.

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