Enzyme Induced Calcium Carbonate Precipitation and Addition of Strengthening Materials: A Review

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ABSTRACT Increasing concerns regarding sustainable soil improvement point towards biocementation. Different biocementation methods are in the field today. Without disturbing the soil structure and its natural habitat calcium carbonate precipitation provides soil stabilization. The bonding of particles in combination with urea, water and calcium chloride produce calcium carbonate precipitation which is applied to the soil with urease enzyme can result in the binding of soil particles. The process Enzyme Induced Carbonate Precipitation is a process using chemicals to improve soil engineering properties like strength, shear strength, permeability. The improved versions of Enzyme Induced Carbonate Precipitation with the addition of magnesium chloride, sisal fibres and biopolymer can provide high strength to soils in vulnerable conditions. Unconfined Compressive Strength test and Scanning Electron Microscopy are the tests to measure the strength and particle binding respectively in soils.

KEYWORDS: Soil improvement; Biocementation; Carbonate Precipitation; Urease Enzyme; EICP; Unconfined Compression Test; SEM

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INTRODUCTION

Soil improvement is the alteration of any property of the soil to improve its engineering performance. Conventional ground improvement techniques are soil reinforcement, mechanical stabilization, chemical stabilization etc. The suitability of a selected ground improvement technique depends on various factors such as type of soil, degree of improvement required, cost and construction time, availability of materials and equipment, etc. Biocementation with the Enzyme Induced Carbonate Precipitation (EICP) treatment is one of the emerging trends in soil stabilization. This ground improvement technique contains calcium chloride, urea, and urease enzyme in different amounts to improve current situations of soils properties. In calcium carbonate precipitation, properties like stiffness, strength, pore filling and intermolecular binding are better. MICP (Microbiologically Induced Calcite Precipitation) method has been studied for analysing the calcium carbonate precipitation comparing EICP. In EICP, the hydrolysis of urea in the presence of free urease in an aqueous solution produces carbonate ions (Equations (1)-(5)). The free urease is produced from plants fungal and microbial sources (Blakeley & Zerner, 1984; Hamdan *et al.*, 2013). In MICP, the treatment solution with appropriate nutrients is needed for the bacterial growth or the ureolytic bacteria is directly applied to the soil to encourage chemical reactions. In both EICP and MICP, the calcium salt is used in the form of calcium chloride ($CaCl_2$). Bacterial cells in MICP acts as nucleation sites where adsorption of calcium ions to their negatively charged surface occur by creating localized supersaturation (Al-Thawadi, 2008). Relying on the bacterial method has its pros and cons. The risk in this method includes non-homogeneous distribution, difficulties in controlling bacterial growth and their enzymatic activity interaction (Burbank *et al.*, 2012; Phillips *et al.*, 2013).

BACKGROUND THEORY

During the EICP process, free urease enzyme from plant sources (including beans, leaves, melon seeds and squash) is used to catalyze the reaction of urea hydrolysis to form ammonium (NH_4^+) and carbonate ions (CO_3^{2-}) (Krajewska, 2018). The reaction involved in $CaCO_3$ precipitation via urea hydrolysis by urease enzyme during the EICP process are shown in the following reaction equations.

$$CO(NH_2)_{2\,(s)} + H_2O_{\,(l)} \xrightarrow{\text{urease enzyme}} NH^+_{4(aq)} + CO^{2-}_{3\,(aq)} \tag{1}$$

$$CaCl_{2\,(s)} \xrightarrow{H_2O} Ca_{2+(aq)} + 2Cl^-_{(aq)} \tag{2}$$

$$Ca_{2+((aq)} + CO^{2-}_{3\,(aq)} \overset{\text{Precipitation}}{\leftrightarrow} CaCO_{3\,(s)} \tag{3}$$

The precipitated $CaCO_3$ within the soil matrix usually binds the soil particles thereby enhancing its engineering properties. Precipitation ratio (PR) has been commonly used to quantify $CaCO_3$ precipitation efficiency (Ahenkorah *et al.*, 2020).

$$\text{Precipitation Ratio} = \frac{\text{Actual Mass of precipitated } CaCO_3}{\text{Theoretical mass of } CaCO_3} \times 100 \tag{4}$$

The theoretical mass of $CaCO_3$ is evaluated as C×V×M, where C and V represent the concentration of the urea-$CaCl_2$ solution in moles per litre and the total volume of EICP solution in litres, respectively, and M is the molar mass of $CaCO_3$ (100.087 g/mol) (Ahenkorah *et al.*, 2020). Different terms are used to describe the concentration of urease enzyme in EICP solution. Urease activity is defined as the micromoles of ammonia liberated per minute by 1 mL (if liquid) or 1 g (if powder) of the urease enzyme (i.e., U/mL or U/g).

METHODS AND PROCEDURES IN EICP

Enzyme Induced Calcium Carbonate Precipitation

In EICP, the measurement of enzyme properties is carried out by using both continuous and discontinues methodologies. The discontinuous method involves mixing the substrates (urea and $CaCl_2$) and enzyme and measuring the product ($CaCO_3$) formed after a set period (Ahenkorah *et al.*, 2020). Bio stabilization aims to improve the engineering properties like strength, stiffness, and dilatancy of the soil. MICP has been greatly discussed by researchers because of its benefits in improving the structure of soils (Montoya *et al.*, 2015; Chu *et al.*, 2012; Burbank *et al.*, 2011). Microbial Induced Calcium Carbonate Precipitation uses exogenous ureolytic bacteria either into the soil directly or introduces it into the treating solution (Mujah *et al.*, 2019). There is a risk for microorganisms if some other microorganisms are present in soil which inhibits its growth. There are some other difficulties such as controlling the growth of bacteria and their enzymatic activity, cell attachment to soil particles (Gomez *et al.*,2014; Liu *et al.*, 2019). So, the addition of urease directly into the soil was a prominent option. Using urease into soil decreases the challenges faced by the addition of microorganisms such as providing nutrients to, oxygen availability for growth cell binding to soil particles (Khodadadi *et al.*, 2017). Studies regarding the EICP treatment had been investigated by several researchers. The baseline study about EICP was started by test-tube experiments. A series of 37 precipitation tests were conducted with 20mL EICP solution (urea, $CaCl_2$

and urease enzyme) by varying the urea concentration from 0.25-1.5M, $CaCl_2$-urea concentration from 1:1 to 1:1.75 and urease enzyme was varied from 1 to 6 g/L. The urease enzyme extracted from jack been had an activity of 3500U/g. They are incubated at 200rpm for 72h in a shaking incubator. Out of these 37 tests, the combination with the highest precipitation rate (1 M urea, 0.67 M calcium chloride, and 3 g/L urease enzyme) was used to conduct a soil column test. Ottawa 20-30 sand for the test was treated by both percolation and mix and compact method with 3 specimens of each by treating with 1-4 cycles of enzyme solution. The top of the soil columns was covered tightly and kept for 7 days for curing to conduct tests UCS, XRD and SEM (Almajed *et al.*, 2018b). Soil prepared by the percolation method with a relative density of 45% shows higher interparticle binding than the mix and compact method. SEM analysis concludes that the rinsing of soil is important to avoid strength loss due to the flushing of organic matter and ammonium salts. A comparison of microbe and enzyme precipitation was examined on two types of soil Ottawa 20-30 and Ottawa 50-7.0 A chemical solution of urea, NH_4CL, $CaCl_2$ at a ratio of 333 mM: 374 mM: 100 mM with jack bean urease enzyme powder from Fischer Scientific Co. with the activity of 200 U/g and 0.30 g of stabilizer (non-fat milk powder) (Hamdan *et al.*, 2015) and the solution is applied to the triaxial soil column by injection. Shear wave velocity was measured by a pair of piezoelectric bender elements that were used to propagate and receive a 10-V, 10-kHz sine wave using a digital signal generator. The travelling time was recorded using an oscilloscope. After the tests, the result proved that the shear wave velocity occurred at a faster rate and the distribution of calcium carbonate was uniform in EICP (Nafisi *et al.*, 2019). The large-scale experiment of the soil was done using the Drum-cans. The usual combination of chemicals (urea-$CaCl_2$) with urease enzyme of activity 2970 units/g and silica sand with specific gravity 2.645 were used for preparing soil specimen. A steel drum-can with a diameter and height of 57 cm 85 cm respectively attached to a flexible tube for the passage of CO_2 and water for saturation. Soil is filled with 6 layers which have an injection tube for the injection of chemicals. The gap between the injection tube and outer tube is filled with gypsum to avoid shortcutting of chemicals. The whole arrangement is sealed with a mortar layer of 3cm thickness (Neupanea *et al.*, 2015; Montoya *et al.*, 2019). Table 1 represented below is the most used and effective concentrations and combination for soil stabilizing.

Table 1. Different concentrations of EICP components used different studies

Urease	$CaCl_2$	Urea	Enzyme Activity	References
1.5 g/L + 4 g/L of non-fat dry milk	0.335 M $CaCl_2 \cdot 2H_2O$	0.5 M	1500 U/g (Sigma-Aldrich reagent)	(Almajed *et al.*, 2020)
3 g/L + 4 g/L of non-fat dry milk	0.67 M $CaCl_2 \cdot 2H_2O$	1 M		
6 g/L + 4 g/L of non-fat dry milk	1.34 M $CaCl_2 \cdot 2H_2O$	2 M		
3 g/L	0.67 M	1 M		Cui *et al.*, 2020)
15g/L	1.0 M	1.0 M		
0	1. 0.5 M 2. 1.0 M	1.0.5 M 2.1.0 M		
4 and 8 kU/L	urea- $CaCl_2$ of 0.25 and 0.5 mol/L		34,310 U/g (Sigma-Aldrich reagent)	(Oliveira *et al.*, 2017)
3g/L	0.67 M	1.0M	3,500 U/g.	(Almajed *et al.*, 2018b)
50 ml of enzyme solution with 550 ml of chemical solution	374 mM NH_4Cl, and 100 $CaCl_2$	333 mM		(Nafisi *et al.*, 2019)
400 U/L to 2000 U/L	0.25, 0.5, or 0.75 mol/L.	0.25 to 2.0 mol/L	1030 U/g	(Sun *et al.*, 2020)

Enzyme Induced Calcium Carbonate Precipitation From Plant Derived Urease Enzyme

Researches were done to understand the effect of urease enzyme derived from plants such as soybeans, watermelon seeds, sword seeds etc., in the EICP treatment (Park *et al.*, 2014) (Dilrukshi *et al.*, 2016). Jack bean meal was directly used in pervious oil wells and they reported activity of 1700 U/g and it was understood that the other proteins stabilize the urease protein in it and thus increases the precipitation (Larsen *et al.*, 2008). The extract from watermelon seeds was used to treat sandy soil (Javadi *et al.*, 2018). The seeds were finely grounded using a mortar and pestle and the seeds were immersed in deionized (DI) water and the resulting supernatant were centrifuged at a rate of 500 rpm and 1000 rpm for 1 hour and 3 minutes respectively. The supernatant containing urease enzyme showed an activity of 80 U/g and they were used to prepare a soil column (Dilrukshi *et al.*, 2018). A combined extract of jack bean meal, watermelon seeds and soybeans were obtained using three-step chemical extraction. The initial step of the process was the dehusking of the seeds. Then those were soaked overnight in the extraction solution. The solution was having a pH of 7.5. 2 mm of Ethylenediaminetetraacetic Acid (EDTA) and 20 mm of phosphate buffer constituted the extraction solution. A kitchen blender was used to homogenize the seeds and beans. Then a fabric of cheesecloth was used to filter out the larger solid particles. The next step was the use of centrifugation to remove the finer particles. After that, it was passed through glass wool as the final step of filtration. In this step, the excess fat was removed. The crude extract generated was subjected to multiple fractionation steps. In the first fractionation, also called the acetone fractionation the urease is precipitated out of the solution by adding prechilled acetone (−20°C) to the crude extract. This is done in an ice chamber and the solution is continuously stirred. Post this step, the mixture is again subjected to centrifugation. The pellets are collected and the supernatant is discarded. The collected pellets are then suspended overnight and again centrifuged. The resulting yield is the product of the first fractionation. The second fractionation is carried out on this yield for further purification. The only difference in this step is that the concentration of the chemicals used will be a bit different from the previous. For comparison commercially available urease enzyme Sigma-Aldrich enzyme (U1500, Type III, powder), 42,700 U/g activity, Sigma-Aldrich enzyme (U1875, Type III, supplied in glycerol solution), 800 U/mL activity and the Fisher Scientific enzyme was studied. A 0.3ml of urease solution was added to 9.7 mL of urea together with phosphate buffer solution in three serum bottles as well as covering the bottle using an aluminium seal and shake it. The reaction is stopped by adding 5mL of trichloroacetic acid. After the reaction ends the bottles were opened and diluted with DI along with Nessler's reagent into a cuvette. The optical density of the solution was measured using a spectrophotometer at a wavelength of 412 nm. The ammonium concentration vs decay function was plotted using the equation:

$$Y = a\,(1 - be^{-cx}) \tag{5}$$

where Y - ammonium concentration, x - time, and a, b, and c are the asymptote, scale, and decay rate of the reaction respectively. The enzyme activity was calculated by dividing the slope of the initial linear part of the ammonium-time curve by enzyme concentration. Test tube experiments were conducted to obtain the optimum result and concentration of 1.0 M urea, 0.67 M $CaCl_2$-dihydrate, 4 g/L non-fat milk powder, and around 13,000 U/L of free urease enzyme (Almajed *et al.*, 2018b; Almajed *et al.*,2019) The crude extract and the commercial enzymes were tested on soil Ottawa 20-30 silica sand and the results were reported using UCS, SEM and XRD. A comparison of different UCS results is provided in Table 2. The presence of impurities with non-fat milk powder significantly increases the strength comparing to studies without the non-fat milk powder. The impurities containing different proteins results in coagulation with milk proteins which increases the crystal bindings in the oil. The formation of vaterite and calcite were not visible in the soil experiment as in test-tube experiments. This may be due to the adhesion of the combined proteins. (Tirkolaei *et al.*, 2020).

Biopolymer and Hydrogel Assisted EICP

EICP is best for surface stabilization. A three-dimensional network of hydrophilic polymers composed of water and can change large volumes. In the food industry and medicine, the introduction of synthetic and non-synthetic hydrogels was a very big asset and hydrogel contains both synthetic polymers and biopolymers (Anseth *et al.,* 2002; Chou *et al.,*2011). Biopolymers addition were introduced as biofilm into geotechnical applications. (Chou et al., 2011).

Studies are conducted to observe the effects of EICP assisted with hydrogel. An improved water retention and reaction time will increase the utilization of the substrate and accelerate the precipitation of $CaCO_3$. Studies show that hydrogels can produce a favourable environment for mineral precipitation. (Decho *et al.,*2010; Chen *et al*.,2013) The hydrogels xanthan and guar gum in mine tailings showed an increase in liquid limit and shear strength. The treated soil was analysed for surface stabilization in fine-grained soil. The hydrogel selected for the study with EICP is xanthan, guar gum and inert polyol-cellulose hydrogel. Five different specimens with $CaCl_2$ of 2M and high and low enzyme activity. Sigma Aldrich Type III Jack Bean, 26,100 units/g of higher activity and Fisher Chemical (Waltham, Massachusetts) low-activity Jack Bean 200 units/g of low activity were selected respectively for high and low concentrated solutions. The test with xanthan and guar gum was started by adding 15 mL of the urea-$CaCl_2$ solution to a glass beaker and slowly adding the hydrogel powder and 3mL of urease enzyme while stirring. The enzyme is added after the gel gets fully dissolved in the urea-$CaCl_2$ solution. The polyol-cellulose hydrogels were started by adding 15 mL of urea-$CaCl_2$ into a glass beaker and then 3 mL of dilute liquid polyol-cellulose gel and 3mL of urease enzyme were added while stirring. The paper cup experiments with soil were conducted with a mixture of the hydrogel-urea- $CaCl_2$-enzyme solutions (Five cups of xanthan, guar and polyol-cellulose with high and low activity and no enzyme). The solution of hydrogel-urea-$CaCl_2$ was stirred for the 30s and mixed with soil, later a 3mL of urease enzyme was added to the hydrogel-EICP- soil specimens. The cups were covered and kept for 7 days (Hamdan *et al.,*2016). The addition of the hydrogel didn't improve the hydrolysis although it improves the precipitation due to the water retention ability. Research conducted on glycerol and xanthan gum portrayed that xanthan gum was more effective in water retention (Pasillas *et al.,* 2018). Another biopolymer sodium alginate (SA) biopolymer was used in investigating the water retention and compressive strength of the soil. Soil samples were prepared by mixing SA with soil and then the enzyme solution was mixed with soil with a target density. The top and bottom were covered and cured for 6 days and on the 7th day, it was oven-dried (Refaei *et al.,* 2020). Three sets of the specimen with a non-enhanced soil sample and the other two with an aqueous solution of glycerol and xanthan gum were investigated. The soil selected was Ottawa 20/30 with silica content and columns were used in the experiment instead of paper cups. They were kept for 3 days curing and covered with a plastic cap to reduce evaporation of EICP solution. The analysis is done by UCS, SEM and Water vapour pressure test (Pasillas *et al.,*2018).

Sisal Fibre Enhancement in EICP Treated Soil

In EICP multiple cycles may disturb the treated soil. The strength of soil with the addition of enzyme solution in a single cycle with the enhanced sisal fibres can improve soil strength (Yasuhara *et al.,* 2012; Neupanea *et al.,* 2015). Properties like length, modulus of fibres and amount can determine the degree of improvement (Santos *et al.,*2010; Li *et al.,* 2016). The enzyme solution was prepared using 0.5M calcium chloride, 0.875 M urea, and 0.85 g/l urease enzyme (≈ 3500 U/g activity). The selected solution is mixed together with sisal fibres and they were placed in a column. The soil column was prepared by mix and compact method and kept three days for curing. After curing the specimen were rinsed with deionized water before testing. Five fibre contents of 0.2%, 0.3%, 0.4 %. 0.75% and 0.85% (w/w, mass of fibre per dry mass of soil) of 20mm and 10mm were the natural fibres obtained from the sisal plant. The effects were measured using tests UCS and SEM (Almajed *et al.,* 2018a). The results from UCS are compared to other UCS tests conducted in different scenarios in Table 2.

Magnesium Substrate Addition

The precipitation rate of the carbonate can be increased to 90% by the addition of magnesium in a small amount (Neupane *et al.*,2013). Some of the studies showed that the addition of magnesium to the carbonation process delayed the carbonate precipitation rate (Yasuhara *et al.*,2011). The magnesium shows a similar cementing character as calcium carbonate and denser microstructure with a lower porosity and higher compressive strength (Rong *et al.,* 2013).

For the study related to magnesium, the chemicals used were urea, $CaCl_2$, $MgCl_2$ and urease with the activity of 2950 U/g and poorly graded silica sand. First, the test tube experiments were done to analyse the activity with $MgCl_2$ and the effect of magnesium chloride in the hydrolysis of urea. They were prepared by mixing the urea, $CaCl_2$ and $MgCl_2$ and later the urease was added by filtered urease powder mixing with distilled water. The grout solution was the one that showed the optimum result in test-tube experiments. Sand specimen were prepared in PVC cylinders by pouring soil and the grout solution. They were kept for curing as per the test tube experiments. They were examined by the UCS, acid leaching method (Putra *et al.,* 2016).

Effect of Soil Types in EICP Treatment

Often the EICP tested are conducted in Ottawa 20-30, Ottawa 50-70 type of soil which have silica content. The study regarding the effects of treatment in the local soil is very important. The analysis was conducted by researchers and they selected soils that are poorly graded slit, silty soil, sand with silt and organic sand. The EICP solution was prepared and mixed to form a homogenous mixture and these soils were filled in a PVC mould by Standard Proctor Test and they were covered for 14 days for curing. The results showed that there was an increase in compressive strength for all soils except the sand containing organic matter. It was concluded that the increase in urease does not increase the strength of soil instead may show a decreasing effect. An optimum amount of urea-$CaCl_2$ and urease enzyme should be used to get a better strength in soils. In soil type poorly graded, silty soils, sand with silt show a considerable increase in strength while the solution containing organic matter had a very noticeable decrease in strength outcome. Tests indicate that soils that are well graded have better $CaCO_3$ precipitations (Oliveira *et al.*,2017).

COMPARISON OF TESTS IN EICP

Unconfined Compression Tests

Unconfirmed Compressive Strength (UCS) stands for the maximum axial compressive stress that a specimen can bear under zero confining stress. The compressive load per unit area required to fail the specimen is called the unconfined compressive strength of the soil (Oliveira *et al.*,2017). The treated soil is prepared by level the top and bottom to provide a smooth sitting position for the specimen and according to the procedure of the work the rinsing, as well as drying of the specimen, should be completed and the specimen is placed under testing machine the strength is measured and recorded. In EICP treatment usually, the UCS is tested at a strain rate of 1.27 mm/min. The values are recorded and strength ability is compared (Oliveira *et al.*,2017).

Table 2 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. The inclusion of the SA polymer is the key factor in increasing strength.

Table 2. Optimum values of UCS test conducted in different experiments in KPa

Components	Method	q(KPa)	Experiment/Author
1 M urea, 0.67 M $CaCl_2$, and 3 g/L enzyme	Mix compact and Percolation (EICP). Percolation showed strength comparing mix and compact	1,268	(Almajed *et al.*, 2018b)
1:0.67 Urea: $CaCl_2$,Enzyme 9(g/l) Organic Stabilizer (g/l) 12,SA content 1%	First mixes with SA and mixes the soil with EICP solution	1613	(Refaei et al.,2020)
0.67 M $CaCl_2$-dihydrate, 4 g/L nonfat milk powder, and around 13,000 U/L of free urease enzyme	Crude Extract in water	1100	(Tirkolaei et al.,2020)
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 soil and mix and compact method and optimum at 0.3%	296	(Almajed *et al.*,2018b)

SEM Analysis

Scanning Electron Microscopy, or SEM analysis, provides high-resolution imaging useful for evaluating various materials for surface fractures, flaws, contaminants or corrosion. Scanning Electron Microscopy, or SEM analysis, provides high-resolution imaging useful for evaluating various materials for surface fractures, flaws, contaminants or corrosion.

In the studies, these tests help to understand the interlinking of soil particles and to learn about the formation of crystalline structures in soil (polymorphs of calcite, aragonite, vaterite, monohydrocalcite, and ikaite). The SEM analysis showed the presence of agglomerated rhombohedral calcite crystals. They can identify the presence of organic matter that can reduce the strength. The test results showed that rinsing of the soil before tests will help to understand the crystalline structure. When excess chemicals are present in the test material it will make the study difficult and prevent from getting exact results (Almajed *et al.*,2018b). In one phase enzyme precipitation study, it showed the EICP have the presence of rhombic calcium carbonate crystals and the crystallisation rate of calcium carbonate by EICP treatment is much faster comparing MICP thus the sizes of crystals will be smaller (Gomez *et al.*, 2014). The evidence of the $CaCO_3$ precipitation is proved with SEM analysis and it showed that the use of xanthan gum helps in soil formation which is due to the interparticle binding (Hamdan *et al.*, 2016). The soil treated with sodium alginate forms larger calcite crystals and the soil is uniformly distributed. It increases the soil bond and improves surface texture (Refaei et al.,2020). The crude extract of urease from the plant seeds after application in soil are scrutinized under SEM. It showed that precipitation of rhombohedral crystals and contrary to the test tube experiments, neither vaterite nor spherical calcite were observed in any of the biocemented soil specimens. The absence of vaterite or spherical calcite in the bio cemented sand might somehow either promote the precipitation of calcite or inhibit the formation of vaterite or spherical calcite from the EICP solution (Tirkolaei *et al.*, 2020).

CONCLUSION

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, $CaCl_2$ and urease enzyme. Optimum concentrations should be taken for research. On the other hand, the $CaCO_3$ precipitation appears to have a nullifying effect.
- An unconfined compression test is the best 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, water vapour pressure test, etc., can be included.
- Organic soil or soil containing any inhibiting material could reduce soil strength. Mostly well-graded soil with silica content shows very positive results in the strength test.
- As the cost of industrial urease production is higher, urease extraction from various food wastes could be an acceptable alternative to this problem. It is also an eco-friendly solution.

REFERENCES

[1] Ahenkorah, I., Rahman, M., Karim, R. & Teasdale, P. R. 2020. Optimization of Enzyme Induced Carbonate Precipitation (EICP) as a Ground Improvement Technique. *Geo-Congress 2020: Foundations, Soil Improvement, and Erosion*. 25–28 February, 2020. Minneapolis, Minnesota. pp 52–56.

[2] Almajed, A., Abbas, H., Arab, M., Alsabhan,A., Hamid,W., & Al-Salloum, Y. 2020. Enzyme-Induced Carbonate Precipitation (EICP)-Based methods for eco-friendly stabilization of different types of natural sands. *Journal of Cleaner Production*, 274(1), 1-47.

[3] Almajed, A., Khodadadi, H & Kavazanjian, E. Jr. 2018a. Sisal Fiber Reinforcement of EICP-Treated Soil. *IFCEE 2018: Recent Developments in Geotechnical Engineering Practice*. 5–10 March, 2018. Orlando, Florida. pp 29-36.

[4] Almajed, A., Tirkolaei, H. K & Kavazanjian, E. Jr. 2018b. Baseline Investigation on Enzyme-Induced Calcium Carbonate Precipitation. *Journal of Geotechnical and Geoenvironmental Engineering*, 144 (11), 1-11.

[5] Almajed, A., Tirkolaei, H. K., Kavazanjian, E. Jr. & Hamdan, N. 2019. Enzyme Induced Biocementated Sand with High Strength at Low Carbonate Content. *Scientific Reports*, 9(1) 1135, 1-7.

[6] Al-Thawadi, S. 2008. *High strength in-situ biocementation of soil by calcite precipitating locally isolated ureolytic bacteria*. PhD Thesis, School of Biological Sciences and Biotechnology, Murdoch University.

[7] Anseth, K. S., Metters, A. T., Bryant, S. J., Martens, P. J., Elisseeff, J. H. & Bowman, C. N. 2002. In situ forming degradable networks and their application in tissue engineering and drug delivery.*Journal of Controlled Release*, 78(1-3), 199-209.

[8] Blakeley, R. L., & Zerner, B. 1984. Jack bean urease: the first nickel enzyme. *Journal of Molecular Catalysis*, 23(2-3), 263-292.

[9] Burbank, M. B., Weaver, T. J., Green, T. L., Williams, B. C. & Crawford, R. L. 2011. Precipitation of Calcite by Indigenous Microorganisms to Strengthen Liquefiable Soils. *Geomicrobiology Journal*, 28(4), 301-312.

[10] Burbank, M. B., Weaver, T. J., Williams, B. C. & Crawford, R. L. 2012. Urease Activity of Ureolytic Bacteria Isolated from Six Soils in which Calcite was Precipitated by Indigenous Bacteria.*Geomicrobiology Journal*, 29(4), 389-395.

[11] Chen, R., Zhang, L. & Budhu, M. 2013. Biopolymer Stabilization of Mine Tailings. *Journal of Geotechnical and Geoenvironmental Engineering*, 139(10), 1802-1807.

[12] Chou, C.W., Aydilek, A. H., Lai, M., Seagren, E.A. 2011. Biocalcification of Sand through Ureolysis. *Journal of Geotechnical and Geoenvironmental Engineering,* 137(12), 1179–1189.

[13] Chu, J., Stabnikov, V. & Ivanov, V. 2012. Microbially induced calcium carbonate precipitation on the surface or in the bulk of soil. *Geomicrobiology Journal*, 29(6), 544-549.

[14] Cui, M. J., Lai, H. J., Hoang, T. & Chu, J. 2020. One-phase-low-pH enzyme induced carbonate precipitation (EICP). *Acta Geotechnica*, 16, 481–489.

[15] Decho, A. W., 2010. Overview of biopolymer-induced mineralization: What goes on in biofilms? *Ecological Engineering*, 36(2), 137-144.

[16] Dilrukshi, R. A. N. & Kawasaki, S. 2016. Soil improvement using plant-derived urease-induced calcium carbonate precipitation. *Journal of Civil & Environmental Engineering,* 6(1),1-13.

[17] Dilrukshi, R.A.N., Nakashimab, K. & Kawasakib, S. 2018. Soil improvement using plant-derived urease-induced calcium carbonate precipitation. *Soils and Foundations,* 58(4), 894-910.

[18] Gomez, M. G., Anderson, C. M., DeJong, J. T, & Nelson, D. C. 2014. Stimulating In Situ Soil Bacteria for Bio-Cementation of Sand. *Geo-Congress 2014 Technical Papers: Geo-Characterization and Modeling for Sustainability*. 23-26 February, 2014. Atlanta, Georgia. pp 1674-1682.

[19] Hamdan, N. M. 2015. Applications of enzyme induced carbonate precipitation (EICP) for soil improvement. *In:* Gali, M. L. & Rao, P. R. (Eds). *Problematic Soils and Geoenvironmental Concerns*. Singapore: Springer.

[20] Hamdan, N. M., Kavazanjian, E. Jr., & O'Donnell, S. 2013. Carbonate cementation via plant derived urease. In the *18th International Conference on Soil Mechanics and Geotechnical Engineering (ICSMGE 2013).* 2-6 September, 2013. France, Paris. pp 2489-2492.

[21] Hamdan, N., Zhao, Z., Mujica, M., & Kavazanjian, E.Jr. 2016. Hydrogel-Assisted Enzyme-Induced Carbonate Mineral Precipitation. *Journal of Materials in Civil Engineering,* 28(10), 1-9.

[22] Javadi, N., Khodadadi, H., Hamdan, H. & Kavazanjian, E. Jr. 2018. EICP Treatment of Soil by Using Urease Enzyme Extracted from Watermelon Seeds. *IFCEE 2018: Innovations in Ground Improvement for Soils, Pavements, and Subgrades*. 5–10 March, 2018. Orlando, Florida. pp 1-10.

[23] Khodadadi, H. T., Kavazanjian, E., Paassen, L. E. & DeJong, J. 2017. Bio-Grout Materials: A Review. *Grouting 2017: Grouting, Drilling, and Verification*, 9–12 July 2017, Honolulu, Hawaii. pp 1-12.

[24] Kimberly, K. M., Tirkolaei, H. K., Chester, M. and Kavazanjian, E. Jr. 2020. Hotspot Life Cycle Assessment for Environmental Impacts of EICP for Ground Improvement. *Geo-Congress 2020: Biogeotechnics*. 25–28 February, 2020. Minneapolis, Minnesota. pp 321-329.

[25] Krajewska, B. 2018. Urease-aided calcium carbonate mineralization for engineering applications: A review. *Journal of Advanced Research*,13(1) 59-67.

[26] Larsen, J., Poulsen, M., Lundgaard, T. & Agerbaek, M. 2008. Plugging of fractures in chalk reservoirs by enzyme-induced calcium carbonate precipitation. *SPE Production & Operations,* 23(4), 478–483.

[27] Li, M., Li, L., Ogbonnaya, U. & Wen, K. 2016. Influence of Fiber Addition on Mechanical Properties of MICP-Treated Sand. *Journal of Materials in Civil Engineering*, 28(4), 1-10.

[28] Liu, S., Wen, K., Armwood, C. & Bu, C. 2019. Enhancement of MICP-Treated Sandy Soils against. *Journal of Materials in Civil Engineering,* 31(12), 1-13.

[29] Montoya, B. M. & DeJong, J. T. 2015. Stress-Strain Behavior of Sands Cemented by Microbially Induced Calcite Precipitation. *Journal of Geotechnical and Geoenvironmental Engineering,* 141(6), 1-9.

[30] Mujah, D., Cheng, L. & Shahin, M. A. 2019. Microstructural and Geomechanical Study on Biocemented Sand for Optimization of MICP Process. *Journal of Materials in Civil Engineering,* 31(4), 1-10.

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[31] Nafisi, A., Safavizadeh, S. & Montoya, B.M. 2019. Influence of Microbe and Enzyme-Induced Treatments on Cemented Sand Shear Response. *Journal of Geotechnical and Geoenvironmental Engineering*, 145(9), 1-8.

[32] Neupanea, D., Yasuhara, H., Kinoshita, N. & Unno, T. 2013. Applicability of Enzymatic Calcium Carbonate Precipitation as a Soil-Strengthening Technique. *Journal of Geotechnical and Geoenvironmental Engineering*, 139(12), 2201-2211.

[33] Neupanea, D., Yasuharaa, H., Kinoshitaa, N. & Andob, Y. 2015. Distribution of mineralized carbonate and its quantification method in enzyme-mediated calcite precipitation technique.*Soils and Foundations*, 55(2), 447-457.

[34] Oliveira, P. J. V., Freitas, L. D. & Carmona, J. P. S. F. 2017. Effect of Soil Type on the Enzymatic Calcium Carbonate Precipitation Process Used for Soil Improvement. *Journal of Materials in Civil Engineering*, 29 (4), 1-9.

[35] Park, S. S., Cho, S. G. & Nam, I. H. 2014. Effect of Plant-Induced Calcite Precipitation on the Strength of Sand. *Journal of Materials in Civil Engineering*, 26 (8), 1-5.

[36] Pasillas, J. N., Khodadadi, H., Kimberly, K. M. & Bandini, P. 2018. Viscosity-Enhanced EICP Treatment of Soil. *IFCEE 2018: Innovations in Ground Improvement for Soils, Pavements, and Subgrades*. 5–10 March, 2018. Orlando, Florida. pp 145-154.

[37] Phillips, A.J., Gerlach, R., Lauchnor,E., Mitchell, A. C., Cunningham, A. B., & Spangler, L. 2013. Engineered applications of ureolytic biomineralization: a review. *Biofouling*, 29(6), 715-733.

[38] Putra, H., Yasuhara, H., Kinoshita, N., Neupane, D. & Lu, C. V. 2016. Effect of Magnesium as Substitute Material in Enzyme-Mediated Calcite Precipitation for Soil-Improvement Technique. *Soil-Improvement Technique*, 4(37), 1-8.

[39] Refaei, M., Arab, M. G. & Omar, M. 2020. Sandy Soil Improvement through Biopolymer Assisted EICP. *Geo-Congress 2020: Foundations, Soil Improvement, and Erosion*. 25–28 February, 2020. Minneapolis, Minnesota. pp 612-619.

[40] Rong, H., Qian, C. X. & Li, L. Z. 2013. Influence of Magnesium Additive on Mechanical Properties of Microbe Cementitious Materials. *Materials Science Forum*, 743–744, 275–279.

[41] Santos, D., Silva, A. P., Consoli, N. C., & Baudet, A. 2010. The mechanics of fibre-reinforced sand.*Géotechnique*, 60(10), 791-799.

[42] Sun, X., Miao, L. & Wu, L. 2020. Applicability and Theoretical Calculation of Enzymatic Calcium Carbonate Precipitation for Sand Improvement. *Geomicrobiology Journal*, 37(4), 389-399.

[43] Tirkolaei, H. K., Javadi, N., Krishnan, V., Hamdan, N. & Kavazanjian E. Jr. 2020. Crude Urease Extract for Biocementation. *Journal of Materials in Civil Engineering*, 32 (12), 1-12.

[44] Yasuhara, H., Hayashi, K., Okamura, M. 2011. Evolution in Mechanical and Hydraulic Properties of Calcite-Cemented Sand Mediated by Biocatalyst. *Geo-Frontiers 2011: Advances in Geotechnical Engineering*, 13-16 March, 2011. Dallas, Texas, United States. pp 3984-3992.

[45] Yasuhara, H., Neupanea, H., Hayashib, K. & Okamuraa, M. 2012. Experiments and predictions of physical properties of sand cemented by enzymatically-induced carbonate precipitation. *Soils and Foundations*, 52 (36), 539-549.