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# STRENGTHENING OF SAND CEMENTED WITH CALCIUM PHOSPHATE COMPOUNDS USING PLANT-DERIVED UREASE

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**ABSTRACT:** The effect of plant-derived urease enzyme to induce the precipitation of calcium phosphate compounds (CPCs) and hence, to improve the unconfined compressive strength (UCS) of sand was examined as a novel, eco-friendly ground improvement method. Initially, Toyoura sand test pieces were cemented only from CPC solution. Furthermore, another sand test pieces were cemented by different concentrations of urea, concentration fixed plant seeds extract (Watermelon) to obtain optimal cementation, and different concentrations of CPC solutions made from calcium and phosphate stock solutions. All test pieces were cured up to 28 days in an airtight container at high humidity at 25$^{o}$C. The UCS tests and scanning electron microscope (SEM) observations of sand test pieces were carried out. The UCS of test pieces cemented with CPC and plant extract were significantly higher than that of test pieces cemented without plant extract and increases with time. The best ratio of Ca: P in CPC solution was 0.75 M: 1.5 M, reaching a maximum UCS of 125.6 kPa after 28 days of curing. In addition, pH concentration was measured after UCS test and it has increased with time. Results indicate that the best pH for optimal cementation is 8.0. A specific crystal structure could not be identified from SEM observations in the segments of the test pieces cemented with CPC in all cases in this study. These results suggest that the addition of plant extract to CPC significantly enhances the mechanical properties of sand.

***Keywords:*** *Plant-derived Urease, Calcium Phosphate Compound, Unconfined Compressive Strength, Ground Improvement*

## 1. INTRODUCTION

In recent years, development of infrastructures with scarcity of useful land has become a major issue and the engineers are compelled to resolve the problem. Possible alternative solutions may be one of among as avoid the particular land, removal/ replace unsuitable soils, designing the planned structures (flexible/ rigid), and modifying existing ground using ground improvement techniques. However, currently, the engineers are focusing on modifying existing ground using ground improvement techniques by ensuring sustainability in land use. Ground improvement refers to a technique that improves the engineering properties of the soil mass treated and hence, to enhance bearing capacity of soil for successful foundation design and use as a countermeasure against natural disasters, including ground liquefaction of saturated sand.

Currently, ground improvement techniques such as grouting via cement, chemical, compaction, fracture and jet, micro piles, jacked piers, driven piers, ground anchors, shoring, soil nailing, vibro compaction, concrete columns, piers, etc. are practicing widely [1]. However, these techniques are related with major environmental issues such as large $CO_2$ emissions during cement production, and high energy cost for cement production and re-excavation of cement improved ground. Therefore, in recent years, biogrout- based ground improvement practices have been introducing.

The process of using biological means to obtain ground improvement is known as biogrouting [2]. Different mineral formation mechanisms are involved in the formation of biogrouts. Carbonate precipitation using urea and ureolytic bacteria [3] or urea and purified/crude extracts of plant species having urease activity [4]-[6] or using glucose and yeast [7], iron/manganese compound precipitation through iron-oxidizing bacteria [8], siloxane bond formation using glucose and yeast [9], calcium phosphate compound (CPC) based chemical grouts (CPC-Chem) formation by its self-setting mechanism [10], CPC biogrout (CPC-Bio) formation in relation to the addition of ureolytic microorganisms and an ammonia source to CPC-Chem [11]. The solubility of CPC-Bio which is generated as a result of the biological action is dependent on its pH (Fig.1 and Table 1) [12]. CPC-Chem is easy to obtain, safe to handle, non-toxic, and can be recycled in the form of a fertilizer. These advantages make it suitable for geotechnical applications [10]. When CPC-Chem was converted to CPC-Bio by the addition of urease producing bacteria and an ammonia source, the unconfined compressive strength (UCS) increased from 42.9 kPa to 57.6 kPa [11].

The purpose of this study was to discover a plant species that contains urease activity unrelated to ureolytic microorganisms. This activity can then be used to increase the pH that is favorable for

CPC precipitation by catalyzing the hydrolysis of urea, which can then use to precipitate CPC and increase the UCS of small scale, cylindrical Toyoura sand test pieces more than 100 kPa, which is the required strength for mitigating ground liquefaction [14].

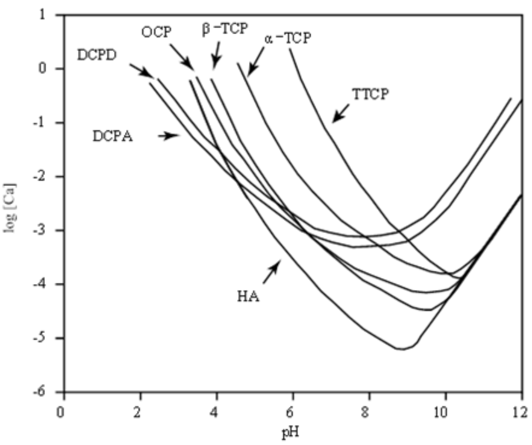


Fig. 1 Solubility phase diagrams for the ternary system, $Ca(OH)_2$-$H_3PO_4$-$H_2O$, at $25^0$C, showing the solubility isotherms of $CaHPO_4$ (DCPA), $CaHPO_4.2H_2O$ (DCPD), $Ca_8H_2(PO_4)_6$. $5H_2O$ (OCP), α-$Ca_3(PO_4)_2$ (α-TCP),β-$Ca_3(PO_4)_2$ (β-TCP), $Ca_4(PO_4)_2O$ (TTCP), and $Ca_{10}(PO_4)_6.(OH)_2$ (HA) [12]

## 2. SELECTION OF A PLANT-DERIVED UREASE SOURCE FOR CPC PRECIPITATION

The importance of urease enzyme is to catalyze the reaction of urea hydrolysis to form ammonium ($NH_4^+$) ions and carbonate ($CO_3^{2-}$) ions (Eq. (1)). The $NH_4^+$ ions and $CO_3^{2-}$ ions produced from this reaction represent the final products of the reaction. However, $NH_4^+$ ions actually start out as $NH_3$. When $NH_3$ reacts with water, it creates $OH^-$ ions, which raise the pH of the system. A number of common plant families are very rich in urease; for example, melons and squash, the pine family, bean varities [15] such as Jack beans (*Canavalia ensiformis*), soybean (*Glycine max*) leaf and seed, pigweed (*Chenopodium album*) and mulberry leaf (*Morus alba*) [16].

$$CO(NH_2)_2 + 2H_2O \rightarrow 2NH_4^+ + CO_3^{2-} \qquad (1)$$

In this study, we investigated urease active plant species to increase the pH that is favorable for CPC precipitation. Hence, initially, we studied three types of plant seeds in cucurbit family such as watermelon, melon and pumpkin. Out of them, a solution made by dissolving 0.08 g of urea in 3.44 mL of watermelon seed extract (prepared by soaking 1g of crushed seeds about 30 min. in 10 mL of distilled water) showed pH value ranging from 8.5 to a constant value of 9.5 within 1 hour. This range of pH is favorable for CPC precipitation (See Fig. 1), and therefore, watermelon seeds were selected for further investigations of this study.

Table 1 Biologically relevant calcium orthophosphates [13]

| Ca/P ratio | Compound | Abbreviation |
|---|---|---|
| 0.5 | Monocalcium phosphate monohydrate ($Ca(H_2PO_4)_2.H_2O$ ) | MCPM |
| 0.5 | Monocalcium phosphate anhydrate ($Ca(H_2PO_4)_2$) | MCPA |
| 1 | Dicalcium phosphate dihydrate ($CaHPO_4.2H_2O$) | DCPD |
| 1 | Dicalcium phosphate anhydrate ($CaHPO_4$) | DCPA |
| 1.33 | Octacalcium phosphate ($Ca_8(HPO_4)_2(PO_4)_4.5H_2O$ ) | OCP |
| 1.5 | A-tricalcium phosphate (α- $Ca_3(PO_4)_2$) | α-TCP |
| 1.5 | B-tricalcium phosphate β- $Ca_3(PO_4)_2$ | β-TCP |
| 1.2-2.2 | Amorphous calcium phosphate ($Ca_x(PO_4)_y.nH_2O$) | ACP |
| 1.5-1.67 | Calcium-deficient hydroxyapatite ($Ca_{10-x}(HPO_4)_X(PO_4)_{6-x}(OH)_{2-x}$) ($0 < x < 1$) | CDHA |
| 1.67 | Hydroxyapatite ($Ca_{10}(PO_4)_6(OH)_2$) | HA |
| 2 | Tetracalcium phosphate ($Ca_4(PO_4)_2O$) | TTCP |

## 3. MATERIALS AND METHODS

Chemical reagents such as calcium acetate-$Ca(CH_3COO)_2$ (CA) and dipotassium phosphate-$K_2HPO_4$ (DPP) were used as CPC chemicals. Urea was selected as the ammonia source and watermelon seeds (*Citrullus vulgaris*) that leftover as food waste were used for catalyzing the hydrolysis of urea. Seeds extract was prepared by soaking crushed seeds in water or another liquid as required, about 30 min. and collected the filtrate. Toyoura sand with particle density $\rho_s = 2.64$ g/cm$^3$, minimum density $\rho_{min}$ = 1.335 g/cm$^3$, maximum density $\rho_{max}$ = 1.645 g/cm$^3$, mean diameter $D_{50}$ = 170 μm was used for preparing UCS test specimens. The final concentrations of CA: DPP = 0.75 M: 1.5 M and CA: DPP = 0.75 M: 0.75 M, which have yielded largest UCS at the initial investigation were used for further investigations of this study.

Initially, the pH variability in relation to different concentrations of plant seeds extract (different solid-liquid ratios) and urea was investigated to ascertain optimal concentrations for maximum CPC precipitation. Solid-liquid ratio (dry weight of crushed seeds, (g) /volume of liquid, (mL)) ranging from 0.0005 to 0.005 were selected and prepared in a CA solution with 0.75 M. Amount of urea ranging from 0.08 g to 0.8 g was selected and dissolved in a DPP solution (0.75 M and 1.5 M). Small scale sample mixtures consisting of 15 g of Toyoura sand, 1.72 mL of plant seed extract prepared using CA solution and 1.72 mL urea solution made by dissolving urea in 1.72 mL DPP solution were used for the pH determination. Hence, the final volume of the solution was 3.44 mL.

$Ca^{2+}$ ion concentration in a CPC solution gives an indication about CPC precipitation. A low $Ca^{2+}$ ion concentration in the solution indicates high CPC precipitation rather than high $Ca^{2+}$ ion concentration. Therefore, $Ca^{2+}$ ion concentration in a mixture consisted with CPC- Chem, seed extract and urea was measured with time using a $Ca^{2+}$ ion meter to get an indication about CPC precipitation with time. Best solution mixture that gives favorable pH from first investigation explained in above paragraph was considered for assessing $Ca^{2+}$ ions. According to that, 5 mL of plant seeds extract having best solid-liquid ratio, was prepared using CA solution with 0.75 M and 5 mL urea solution was made by dissolving urea in 5 mL of DPP solution (0.75 M and 1.5 M). Hence, the volume of the final solution was 10 mL. $Ca^{2+}$ ion concentration of prepared solution was measured just after mixing, after 1 day, 3 days, 7 days, 14 days, and 28 days. Controls were prepared using only CPC solution for the comparison purpose and measured $Ca^{2+}$ ion concentration with time.

Urease activity test was conducted according to a method based on conductivity [17]. Conductivity was measured using a conductivity meter to investigate the change in urease activity using different solid-liquid ratios of seeds extract and urea. Urea dissolved in 10 mL of distilled water was mixed with 10 mL of seeds extract and the final mixture with the volume of 20 mL was used for measuring conductivity and hence to calculate urease activity of watermelon seed extract contacted with urea.

After completing basic investigations, such as pH variability test, $Ca^{2+}$ ion measurement and urease activity test, best solid-liquid ratio and amount of urea were selected to prepare of test specimens for UCS test. As mentioned above, CA with 0.75 M and DPP with 0.75 M and 1.5 M concentrations were selected to prepare of test specimens for UCS test. Crushed seeds were soaked in the concentration known CA solution by maintaining required solid-liquid ratio. The required weight of urea, which had been weighted in advance, was then dissolved in the concentration known DPP solution. A volume of 36.65 mL from both the seeds extract in the CA solution and the urea in the DPP solution were added to 320.09 g of Toyoura sand. The mixture was uniformly mixed in a stainless steel ball for 2 min and then divided into quarters. Each quarter was then placed in to a plastic mold (inner diameter $\varphi$ = 5 cm, height h = 10 cm). The inner wall of each mold was covered with an overhead projector sheet (0.1 mm thick) to avoid any disturbances of the test pieces during their removal from the mold. The mixture was tamped down 30 times by a hand rammer when each quarter was filled into the mold. Finally, the upper edges of the test pieces were slightly trimmed so that they were flat, and covered with Parafilm M (Structure Probe, Inc., West Chester, PA) to avoid desiccation. The molded test pieces were subsequently cured in an airtight container at high humidity. After curing, test pieces were removed carefully from the mold, and the UCS of the test pieces was measured with a UCS apparatus T266-31100 (Seiken-sha Co. Ltd., Japan) at an axial strain rate of 1% /min. All test pieces were prepared, cured and tested at 25 $^{o}$C. Two test pieces were made for each test case under same condition, in order to investigate the repeatability of measurements of UCS test. The pH of the test pieces was calculated as an average of three measurements (at the top, bottom, and middle of each test piece) using a pH Spear (Eutech Instruments Pte., Ltd., Singapore).

## 4. RESULTS AND DISCUSSION

According to the results of pH variability with different amount of urea and solid-liquid ratios, pH was increased and become constant with time and it has shown favorable pH for CPC precipitation, in the mixture of 0.08 g of urea and 0.005 of solid-liquid ratio.

$Ca^{2+}$ ion concentration of the mixture of CPC solution consisted with 0.233 g urea (0.08 g*5 mL/1.72 mL) instead of 0.08 g of urea used in pH variability test, and 0.005 of solid-liquid ratio was decreased gradually with the time and that indicated the increment of the precipitation of CPC with time. The behavior of control solutions were similar and decrement rate with the time was low compared with the solution consisted with urea and seeds extract. Furthermore, this investigation showed that CPC precipitation was closely related with pH. As an example, $Ca^{2+}$ ion concentration with time in the test case only with CPC (CA = DPP = 0.75 M) and the test case with CPC (CA = DPP = 0.75 M), urea and seeds extract are shown in Fig. 2. According to the Fig. 2, low $Ca^{2+}$ ion concentration (high CPC precipitation) could be expected with urea and seed extract rather than using CPC only. The reason to get high precipitation is the availability of favorable pH for CPC precipitation. (See Fig. 1). Moreover, the precipitation of CPC could be observed in the solution only with CPC. However, the amount and the rate of precipitation were low compared with CPC having urea and seeds extract due to low pH (pH around 6). There is a tendency to solubilize CPC at pH around 6 rather than the pH in the range of 8-9 (See Fig.1).

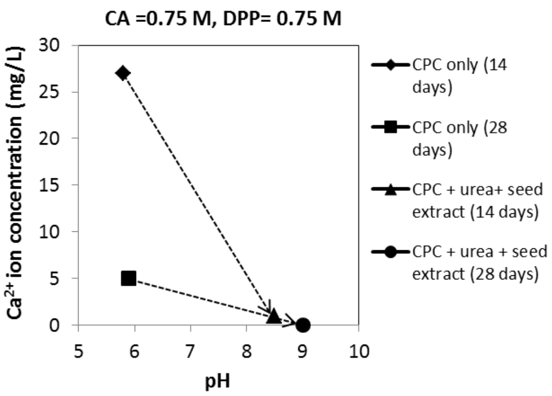


Fig.2 Temporal variation of $Ca^{2+}$ ion concentration

Urease activity values of the mixture of watermelon seeds extract and urea are presented in Table 2. As shown in Table 2, when the urea content increased, then the urease activity decreased and when the solid-liquid ratio of the seeds extract was increased, the urease activity increased. Furthermore, best combination of urea and seeds extract (0.08 g of urea/1.72 mL and 0.005 of solid/liquid ratio) selected from pH variability test showed a high urease activity from urease activity test (Case No. 4 in Table 2).In Case No. 4, weight of urea was 0.465 g instead of 0.08 g to match the concentration of urea (0.08 g*10 mL/1.72 mL). It is clear that the property of urease activity supported to increase the pH by catalyzing urea in to $NH_4^+$ and $CO_3^{2-}$ ions.

Table 2 Quantitative values of urease activity

| Case No. | Weight of urea (g) | Solid/liquid ratio | Conductivity (mS/cm) | Urease activity (u/L) |
|---|---|---|---|---|
| 1 | 4.65 | 0.005 | 0.0438 | 520.54 |
| 2 | 2.33 | 0.005 | 0.0617 | 733.28 |
| 3 | 0.93 | 0.005 | 0.0608 | 722.58 |
| 4 | 0.465 | 0.005 | 0.0722 | 858.06 |
| 5 | 0.0465 | 0.005 | 0.0798 | 948.39 |
| 6 | 4.65 | 0.0025 | 0.0149 | 177.08 |
| 7 | 4.65 | 0.0005 | 0.0013 | 15.45 |

Finally, the UCS test was conducted for the test specimens made using best combination of urea and seeds extract. According to that, 1.71 g (0.08g * 36.65 mL /1.72 mL) was used to prepare the first UCS test pieces, and later, more test pieces were made by changing urea in the range of 1.71 g-17.07 g to observe the behavior of UCS. As shown in Fig.3 (A), while using CA = DPP = 0.75 M, the increment of urea caused to increase the UCS up to certain level and afterward increment of urea caused to decrease the UCS. When the maximum UCS achieved was decreased by adding more urea, the pH of the sample has changed from around 8 to more than 9. It was clear that an increase of urea caused the release of greater number of $NH_4^+$ ions in relation to urea hydrolysis, and that caused an increase in the pH of the specimen that was unfavorable for CPC precipitation. It was thus ascertained that the UCS value has been decreased

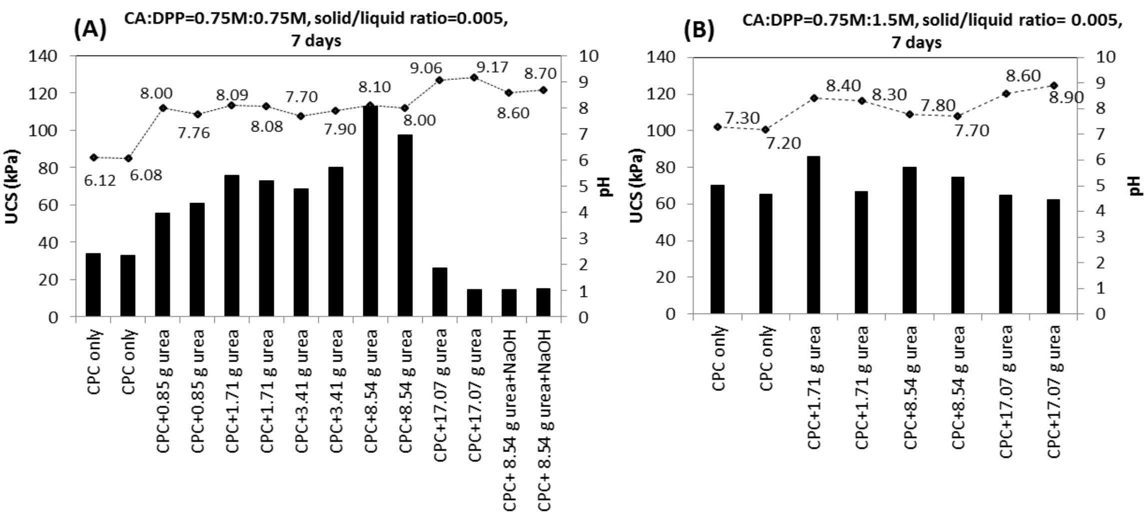


Fig.3 Effect of pH on UCS

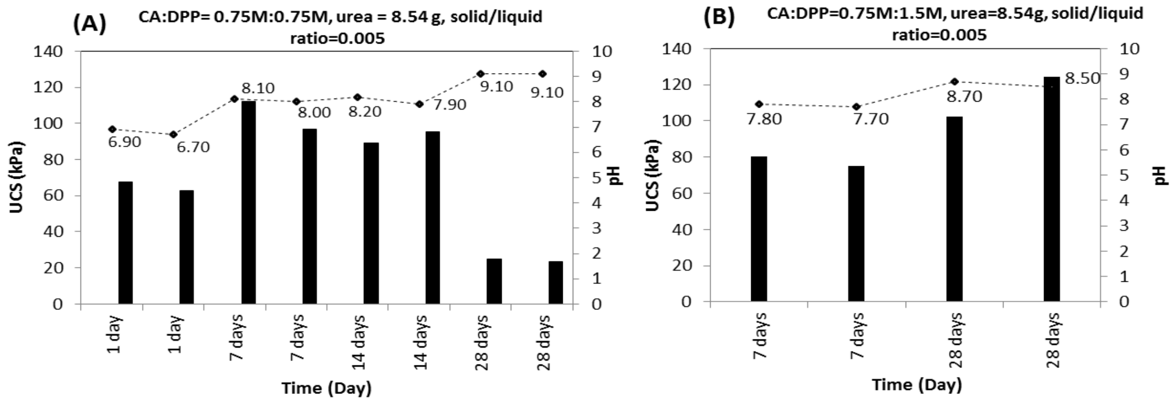


Fig.4 Temporal variation of UCS

after a certain level of urea was used. The maximum value of UCS was obtained after 7 days with 8.54 g of urea. At the same way, while using CA: DPP = 0.75 M: 1.5 M, the behavior of UCS was observed by changing the amount of urea. The maximum average value of UCS was obtained after 7 days with 8.54 g of urea (Fig.3 (B)) as in the case with CA = DPP = 0.75 M. We therefore, further considered temporal variations in values of the UCS using 8.54g of urea for both cases (CA = DPP = 0.75 M and CA = 0.75 M, DPP = 1.5 M) (Figs. 4 (A) and 4 (B)). In the case of CA = DPP = 0.75 M, the UCS began to decrease gradually after 14 days and dramatically decreased after 28 days. The pH of the test pieces after 28 days was more than 9. That was unfavorable for precipitating CPC relevant to CA = DPP = 0.75 M that means Ca/P = 1. The possible precipitates are DCPA and/or DCPD (See Table 1). Unfavorable pH caused to decrease the UCS value dramatically after 28 days. Furthermore, the decrease of the UCS after 14 days was not related to the value of pH. As we mentioned earlier, it is possible to precipitate two CPCs in the case of Ca/P ratio of 1. The solubility of DCPD is higher than that of DCPA for the same value of pH and this could thus cause a decrease in UCS after 14 days rather than 7 days, even if both cases indicated nearly the same pH values (See Fig. 4 (A)). In case of CA = 0.75 M and DPP = 1.5 M, the pH value of the test pieces increased and pH of around 8 was observed after 28 days. This favorable pH caused to precipitate maximum CPC. In this respect, the UCS value increased with time and had a value of more than 100 kPa after 28 days by achieving our goal (See Fig. 4 (B)).

According to the compressive stress (σ) – strain (ε) curves for the test pieces made using 8.54 g of urea, (Fig. 5) to study the temporal variations of the UCS, the two stress- strain curves obtained for two identical test pieces made at each test case showed almost the same behavior in terms of shape. Furthermore, the compressive strain (ε) was

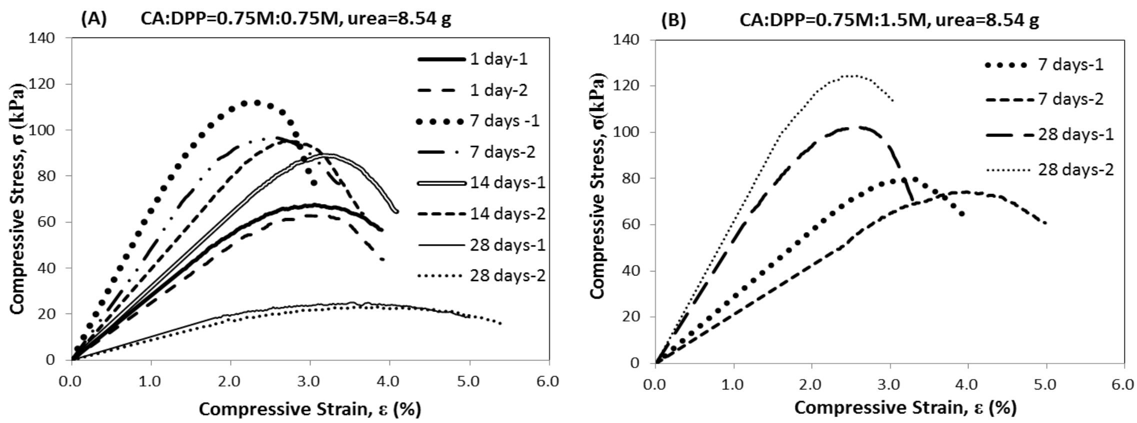


Fig.5 Compressive stress (σ) - compressive strain (ε) curves of test pieces with different curing time (Day) – (Solid/liquid ratio =0.005)

decreased and moved leftward, and Young's modulus increased with the increments of UCS. The comparison of all the stress (σ) - strain (ε) curves in relation to the maximum UCS values, shows a distinctive peak at approximately 2% of the failure strain. The above discussion confirms that the aim of this study was achieved after 28 day curing period with CA: DPP = 0.75 M: 1.5 M in relation to the use of seed extract at solid-liquid ratio of 0.005 and 8.54 g of urea for 320.09 g of Toyoura sand.

## 5. CONCLUSIONS

The efficacy of plant-derived urease enzyme to induce the precipitation of CPC-Bio and hence, to improve the UCS of sand was examined in small scale, cylindrical, laboratory samples. The results show that,

- the UCS of more than 100 kPa was achieved after curing of 28 days, due to generated CPC-Bio with the help of CA: DPP = 0.75 M: 1.5 M in relation to the use of watermelon seeds extract at a solid-liquid ratio of 0.005 and 8.54 g of urea for 320.09 g of Toyoura sand.
- the pH was the governing factor to precipitate CPC as CPC-Bio and watermelon seeds extract having urease activity helps to increase the pH by catalyzing the process of urea hydrolysis and best pH observed for optimal cementation was around 8.0.

According to the results of this study, plant-derived urease induced CPC precipitation technique has the potential to be utilized as an environmental friendly grouting material to respond to soil liquefaction by improving the ground. In addition to the geotechnical engineering issues, knowledge gathered from this technique may also provide satisfactory solutions for solving problems in the fields of geoenvironmental and rock engineering.

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