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Implementation of eggshell extracted calcium acetate in biocementation via soybean urease

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Abstract. Biocementaion, a promising soil improvement technique, mostly utilizes calcium chloride ($CaCl_2$) as its calcium source. However, using $CaCl_2$ poses a critical environmental problem of producing ammonium chloride as a by-product. Alternatively, when calcium acetate ($Ca(CH_3COO)_2$) is used as the calcium source, the production of ammonium chloride during biocementation can be reduced. $Ca(CH_3COO)_2$ can be easily derived from waste eggshells. Nevertheless, such research has been conducted only with Microbial Induced Carbonate Precipitation (MICP) to date. This paper aims to study the implementation of eggshell $Ca(CH_3COO)_2$ in the more effective biocementation approach: Enzyme Induced Carbonate Precipitation (EICP) via soybean urease. For this study, EICP solutions of varying molar ratios and concentrations were studied for their precipitation efficiency and reinforcement effect on poorly graded sandy soil. The findings showed that the EICP solution composition of 1:1 molar ratio between $Ca(CH_3COO)_2$ and urea, added with 50 g/L soybean solution reached the maximum precipitation efficiency. It also produced the highest 7-day UCS of 371 kPa at a calcium carbonate content of 0.40%. Furthermore, the eggshell-soybean EICP produced 8.5% higher UCS than $CaCl_2$-soybean EICP despite having similar calcium carbonate content.

1. Introduction

Biocementation is an emerging technique of soil improvement. In this method, the pore spaces of the soil are filled with calcium carbonate ($CaCO_3$), thereby improving the mechanical properties of the soil. The $CaCO_3$ is produced as precipitation by a series of biochemical reactions; hydrolyzation of urea by urease in the presence of calcium, as depicted by Equation 1.

$$CO(NH_2)_2 + H_2O + Ca^{2+} \rightarrow CaCO_3 + 2NH^{4+} \quad (1)$$

The precipitation of $CaCO_3$ can cement two soil particles already in contact [1], bridge between two soil particles [2], coat the soil grains [3] or just fill the pore spaces between the soil particles [4]; all of these improve bonding between the soil particles. This improvement leads to the enhancement of soil's unconfined compressive strength (UCS) [5-8], shear strength [9-12] and also reduction of permeability [7, 13]. The urease enzyme for $CaCO_3$ precipitation can be acquired by either using ureolytic bacteria to produce urease, which is referred to as Microbial Induced Carbonate Precipitation (MICP), or using urease enzyme directly, which is known as Enzyme Induced Carbonate Precipitation (EICP). MICP has been widely adopted and proved to enhance soil's strength in both laboratory investigations [1, 14-16] as well as large-scale investigations [17-19]. However, it has a number of disadvantages, including the high cost of bacterial cultivation [20], deceleration of MICP due to the presence of indigenous microorganisms [21], disruption in the ecological balance of the soil due to the injection of bacteria [22] and its inapplicability to fine-grained soils [23, 24]. Unlike MICP, EICP does not disturb the ecosystem of soil and it can be effectively applied to fine-grained soils [25-27]. Though EICP can significantly strengthen any type of soil

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[5, 28-31], it can also be expensive as the price of commercially available urease is very high. In this case, extracting urease from some plant species can be more convenient. Urease derived from plants such as soybean seeds [32-36], watermelon seeds [37, 38], jack bean [39], cabbage and soy pulp [40] has been applied in biocementation. Due to reasons like uniform distribution and difference in microstructure of precipitated $CaCO_3$, soybean based EICP has more strengthening capability than MICP [41].

However, both EICP and MICP have a critical environmental drawback; the most used calcium source for biocementation is calcium chloride ($CaCl_2$), which causes the production of ammonium chloride (NH_4Cl) as a by-product. NH_4Cl has adverse environmental effects such as groundwater contamination and greenhouse gas emission [42]. However, using calcium acetate ($Ca(CH_3COO)_2$) as the calcium source in MICP can reduce ammonia emission by 54.2% [43]. The strengthening effect of MICP using $Ca(CH_3COO)_2$ is also higher than $CaCl_2$ and calcium nitrate [44]. $Ca(CH_3COO)_2$ can be produced from eggshells and when it is used in MICP, a better reinforcing effect on soil than $CaCl_2$ is observed [45, 46]. Also, when $Ca(CH_3COO)_2$ is prepared from limestones and utilized in MICP, 10.61% higher UCS than using $CaCl_2$ is demonstrated [47]. Utilization of $Ca(CH_3COO)_2$ in EICP has also been investigated and like MICP, $Ca(CH_3COO)_2$ in EICP also produces higher UCS than using $CaCl_2$ [48]. But, no study has been yet conducted on soybean based EICP utilizing $Ca(CH_3COO)_2$. Also, EICP studies with $Ca(CH_3COO)_2$ to this date have been conducted with commercially available $Ca(CH_3COO)_2$ and utilization of $Ca(CH_3COO)_2$ from other sources is yet to be investigated. This study aims to utilize the positive aspects of using soybean EICP and using $Ca(CH_3COO)_2$ in biocementation together.

This paper is a short study of biocementation by EICP using eggshell $Ca(CH_3COO)_2$ and soybean urease. The scope of this paper is to find out how eggshell $Ca(CH_3COO)_2$ and soybean urease perform together in EICP and its effect on soil reinforcement. Also, the scope includes an evaluation of the efficiency of eggshell-soybean EICP by comparing it with $CaCl_2$-soybean EICP as well as other previous biocementation related studies.

2. Materials and Methods

2.1 Soil

The grain size distribution of the soil used in this study is shown in Figure 1. The soil was collected from

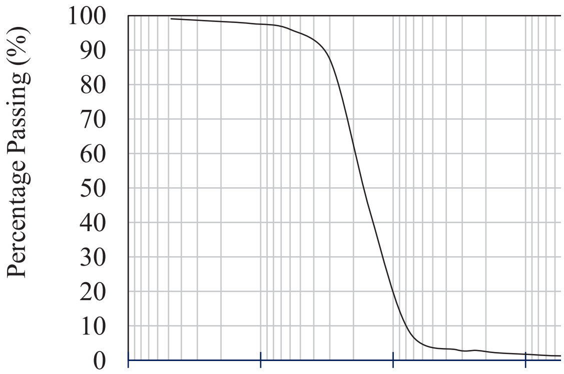


Figure 1. Particle size distribution of the soil used in this study

the campus area of Khulna University of Engineering and Technology, Bangladesh. 8% of the total mass of the collected soil had passed through sieve no. 200. The other properties of the soil found were: $G_S = 2.68$, $D_{60} = 0.21$, $D_{30} = 0.12$, $C_u = 2.62$, $C_c = 0.90$. According to the unified soil classification system, the soil was classified as poorly graded sand (SP) [49].

2.2 Eggshell Solution

Waste eggshells were collected from the kitchen of a local restaurant. The eggshells were washed, oven-dried at 105°C for 24 hours, crushed and passed through sieve no. 100. Then, it was mixed with vinegar at 1:8 ratio by weight. Upon mixing, $Ca(CH_3COO)_2$ was formed and carbon dioxide bubbles were observed. The reaction is shown in Equation 2.

$$CaCO_3 + 2CH_3COOH \rightarrow Ca(CH_3COO)_2 + H_2O + CO_2 \quad (2)$$

The solution was left for 7 days. After 7 days, the solution was filtered to remove any undissolved eggshell powder. This filtered solution is referred to as the eggshell solution throughout this paper. Several eggshell solutions were prepared for this study, and the molarity of $Ca(CH_3COO)_2$ in each solution varied. The highest molarity among the eggshell solutions was 0.39 mol/L and the corresponding eggshell solution used in this study.

2.3 Soybean Solution

Soybean seeds used in this study were bought from the local market. To extract the urease enzyme, the soybean seeds were first crushed and passed through sieve no 100 to make soybean seed powder. Then, the soybean seed powder was mixed with distilled water at three different concentrations: 25 g/L, 50 g/L and 75 g/L. Such three different concentrations were prepared because the strength of the urease enzyme extracted from soybean seeds depends on the concentration of the solution [32, 33]. Finally, these solutions went through centrifugation at 3000 rpm for 30 mins. After centrifugation, the supernant was collected. It contained the extracted urease enzyme and is referred to as soybean solution for the rest of this study.

2.4 Precipitation Efficiency Test

The quantity of precipitated $CaCO_3$ depends on the molar ratio of Ca^{2+} to urea and also on the strength of urease solution. In this study, 9 different compositions of eggshell-soybean EICP solution were tested to find the composition that produces the highest $CaCO_3$ precipitation. The compositions were based on the molar ratio of $Ca(CH_3COO)_2$ to urea and the concentration of soybean solutions, as shown in Table 1.

Table 1. Compositions of eggshell-soybean EICP solutions for precipitation efficiency test

Composition	$Ca(CH_3COO)_2$:Urea (mol/L)	Soybean Solution Concentration (g/L)	Duration (days)
1:1/25	1:1	25	7
1:1/50	1:1	50	7
1:1/75	1:1	75	7
1:1.3/25	1:1.3	25	7
1:1.3/50	1:1.3	50	7
1:1.3/75	1:1.3	75	7
1:1.7/25	1:1.7	25	7
1:1.7/50	1:1.7	50	7
1:1.7/75	1:1.7	75	7

For each composition, an 8mL eggshell-soybean EICP solution was prepared in a 15mL test tube by mixing 4mL urea-eggshell solution with 4mL soybean solution. The test tubes were left for 7 days. After 7 days, the mass of the precipitated $CaCO_3$ was measured. The maximum theoretical precipitation was 0.14g. The Precipitation efficiency was calculated by Equation 3.

$$\text{Precipitation Efficiency (\%)} = \frac{\text{Actual Precipitation (g)}}{\text{Theoritical Precipitation (g)}} \times 100\% \quad (3)$$

The result of the precipitation efficiency test is shown in Table 2. It is observed that the precipitation efficiency varied in different eggshell-soybean EICP compositions, which will be discussed in later section.

Table 2. Results of precipitation efficiency test

Composition	Precipitated $CaCO_3$ (g)	Precipitation efficiency (%)
1:1/25	0.08	57.14
1:1/50	0.14	100
1:1/75	0.12	85.71
1:1.3/25	0.12	85.71
1:1.3/50	0.10	71.43
1:1.3/75	0.12	85.71
1:1.7/25	0.08	57.14
1:1.7/50	0.13	92.86
1:1.7/75	0.05	35.71

2.5 Unconfined Compressive Strength Test

UCS test was conducted to study the reinforcement effect of eggshell-soybean EICP. From the precipitation efficiency test results, four compositions were selected for the UCS test: four high efficient compositions of 1:1/50, 1:1.7/50 and 1:1.3/75 and one relatively lower efficient composition of 1:1.7/25. The one low efficient composition was selected to observe how low efficient composition treatment effects the strengthening of biocemented soil compared to the high efficient composition treatment.

For UCS tests, total five specimens were prepared; one was non-treated specimen which was prepared by mixing 15% distilled water with the sandy soil by weight. The other four specimens were mixed with 15% eggshell-soybean EICP solution (7.5% urea-eggshell solution and 7.5% soybean solution) by weight. The specimens were cylindrical; 2 inches diameter and 4 inches height. Unit weight of ~1.35 g/cm^3 was maintained for all five specimens. Soil treatment details for the UCS tests are shown in Table 3. After preparing the specimens, they were left for curing for 7 days. After 7 days, UCS tests were conducted on the specimens [50].

Table 3. Soil treatment details for UCS test

Treatment type	Composition	$Ca(CH_3COO)_2$:Urea (mol/L)	Soybean Solution Concentration (g/L)	Curing Duration (days)
Non-treated	-	-	-	7
Eggshell-soybean EICP treated	1:1/50	1:1	50	7
	1:1.3/75	1:1.3	75	7
	1:1.7/25	1:1.7	25	7
	1:1.7/50	1:1.7	50	7

2.6 Calcium carbonate content (CCC) measurement test

CCC of each specimen was determined by EDTA titration method. To determine the CCC, first, the failed biocemented specimens from UCS tests were oven dried at 105°C for 24h. Then, chunks of soil (around 5g) were taken from the oven-dried specimens and immersed into 2M 20mL hydrochloric acid solution. In the solution, $CaCl_2$ was formed through chemical reaction as shown by Equation 4.

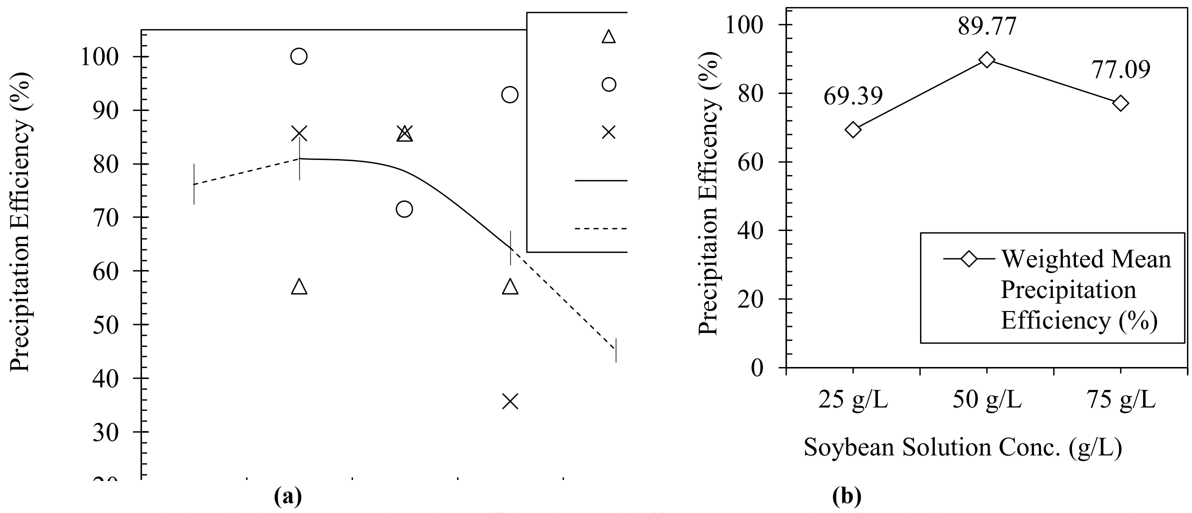
$$CaCO_3 + 2HCl \rightarrow CaCl_2 + CO_2 + H_2O \quad (4)$$

The $CaCl_2$ was dissolved in the solution. After the solution stopped seething, 5mL of solution was taken out and added to 30mL of distilled water. Then the quantity of $CaCl_2$ of the 30mL solution was measured through EDTA titration. Then, the percentage of CCC in the 5g soil chunk was determined through backward calculation.

3. Results and Discussion

3.1 Precipitation efficiency of eggshell-soybean EICP

As previously stated, the precipitation efficiency varied among the different tested compositions, with only one out of nine achieving 100% precipitation efficiency. Figure 2(a) shows the effect of the $Ca(CH_3COO)_2$ to urea ratio on precipitation efficiency.



(a) **(b)**

Figure 2. (a) Relationship between precipitation efficiencies and different molar ratios of eggshell $Ca(CH_3COO)_2$ and urea, **(b)** Average precipitation efficiencies of different soybean solution concentrations

It can be seen from the trendline in Figure 2(a) that the increment of urea portion in the ratio from 1:1 to 1:1.7 decreased the precipitation efficiency. The composition 1:1/50, which had the highest precipitation efficiency, also had the lowest ratio of 1:1. The reason for this decrement of precipitation efficiency is that higher concentration of urea increases the pH of the solution and therefore slows down the biochemical reaction [8]. It is possible that the compositions with higher urea concentration can reach higher precipitation efficiency even after 7 days if the urease stays strong enough [51]. However, it wasn't studied in this paper. The prediction line in Figure 2(a) suggests a predicted trend line; it shows that the precipitation efficiency may drop not only by increasing the urea portion beyond 1:1.7, but also by decreasing urea portion lower than 1:1. It is probably because of lower quantity of urea will lead to lower hydrolyzation of urea, which will lower the quantity of CO_3^{2-} production and consequently lower $CaCO_3$ carbonate precipitation.
Figure 2(b) shows the weighted mean precipitation efficiency for three concentrations of soybean solution. It suggests that the optimal precipitation-producing ratio was 50g/L. Deviations from this concentration, either higher or lower, reduced the precipitation efficiency. This supports what was reported by Shu et al., that although increasing the concentration of the soybean solution generally increases the urease activity, increasing beyond 60 g/L does not further increase precipitation efficiency [33].

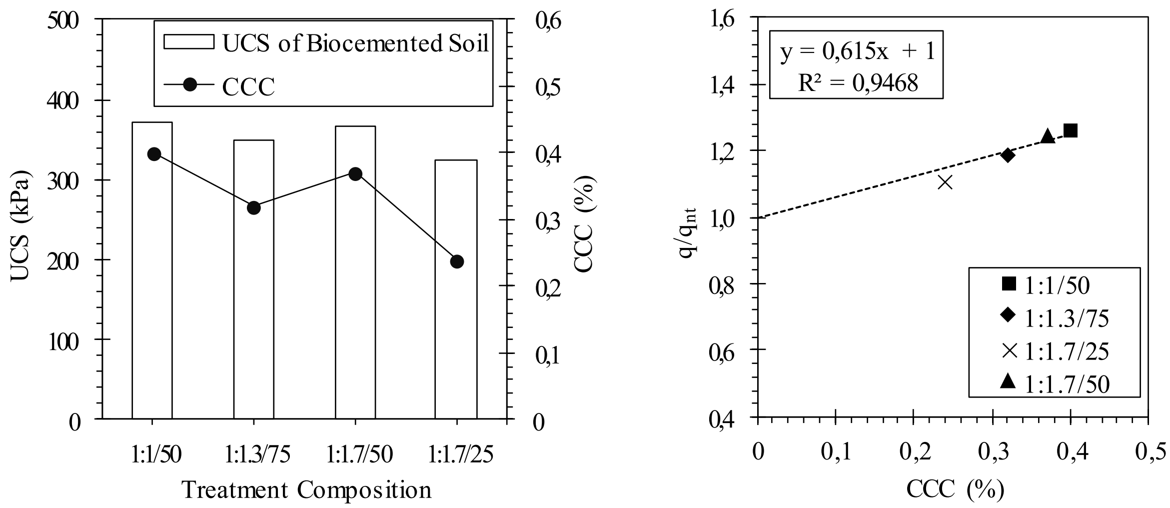
3.2 Unconfined Compressive Strength

The 7-day UCS test results, along with the respective moisture contents and CCC are presented in Table 4. It is observed from the 7-day UCS results that the application of eggshell-soybean EICP treatment led to significant improvements of the soil's UCS. The non-treated soil specimen achieved a UCS of 294 kPa. Among the biocemented soil specimens: the 1:1/50 treatment produced the highest 7-day UCS of 371 kPa, which is approximately 26% improvement over the non-treated soil. The 1:1.7/25 treatment showed the lowest improvement of 324 kPa, which is approximately 10% improvement. All the soil specimens had very low and almost similar moisture content, in the range of 0.62%-0.78%, so the possibility of the effect of different moisture content on UCS can be discarded.

Table 4. 7-day UCS Test Results

Treatment type	Composition	UCS (kPa)	CCC (%)	Moisture Content (%)
Non-treated	-	294	-	0.62
Eggshell-soybean EICP treated	1:1/50	371	0.40	0.69
	1:1.3/75	349	0.32	0.63
	1:1.7/25	324	0.24	0.77
	1:1.7/50	367	0.37	0.78

Generally, the strength of biocemented soils depends on their CCC quantity [52]. The relation between UCS and CCC of this study is shown in Figure 3(a), where it is observed that the higher CCC-containing specimens also have higher UCS. Accumulated $CaCO_3$ between the pore spaces of soil binds the nearby soil particles together, which increases the soil's strength. Figure 3(b) is plotted based on the instructions by Lee et al. [32]. The term q/q_{nt} denotes a UCS increment ratio biocemented soil over non-treated soil, where q is the UCS of biocemented soils and q_{nt} is the UCS of non-treated soil.



(a) **(b)**

Figure 3. Effect of CCC on **(a)** the UCS of biocemented soil specimens for different eggshell-soybean EICP treatments, and **(b)** the UCS increment ratio q/q_{nt}.

Figure 3(b) shows that the q/q_{nt} increases linearly as the CCC increases and a linear relation can be formed from it. It is important to note that the eggshell solution used in this study is quite low in concentration. Choi et al. mixed eggshell powder in vinegar and left it mixer for 3 days but achieved a $Ca(CH_3COO)_2$ molar concentration of only 0.45 mole/L [45]. With such a low concentration of calcium, it is not possible to achieve a higher CCC in biocemented soil; unless percolation method with multiple treatment cycle is adopted.

If a mathematical relation between q/q_{nt} and CCC is known, a prediction about the UCS of a particular soil at a certain CCC can be made. In this paper, based on the linear relationship shown in Figure 3(b), four predictions of q/q_{nt} and UCS for four CCC of 0.5-2% have been calculated, which is shown in Figure 4. It can be seen that for 1% CCC, the UCS is about 500 kPa and for 2%, the UCS can reach close to 700 kPa.

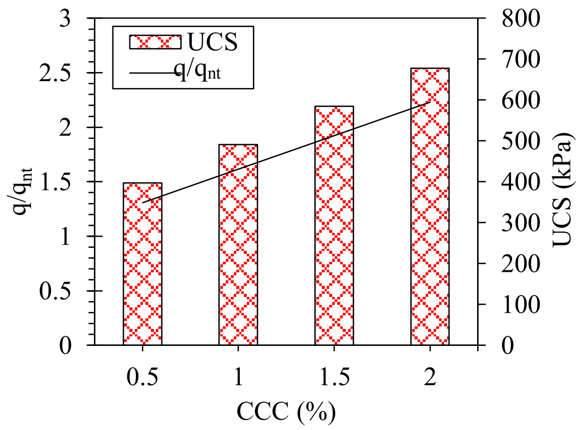


Figure 4. Predicted q/q_{nt} and UCS for different CCC

3.2.1 Comparison with $CaCl_2$-soybean EICP

A new soil specimen for UCS test was prepared to find out whether using eggshell $Ca(CH_3COO)_2$ instead of $CaCl_2$ in soybean EICP has any better strengthening effect. It was mixed with 15% $CaCl_2$-soybean EICP solution (7.5% $CaCl_2$-urea solution and 7.5% soybean solution). The molar ratio in the urea-$CaCl_2$ solution was kept to 1:1 where both $CaCl_2$ and urea had a molarity of 0.39 mol/L. After preparation, the specimen was left for 7 days curing. Then, after 7 days, UCS test was conducted. After that, CCC was determined by EDTA titration method. The results of UCS and CCC determination tests are shown in Figure 5, along with the UCS and CCC of 1:1/50 treated specimen.

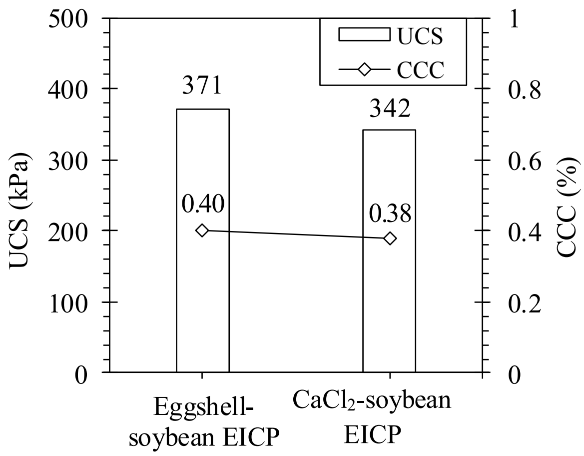


Figure 5. Comparison between UCS and CCC produced by eggshell-soybean EICP and $CaCl_2$-soybean EICP

It can be observed from Figure 5 that, the CCC of both eggshell-soybean EICP and $CaCl_2$-soybean EICP had produced very close CCC of 0.40% and 0.38% respectively. But, the UCS produced by eggshell-soybean EICP had approximately 8.5% higher UCS than $CaCl_2$-soybean EICP. The reason behind the higher UCS of eggshell-soybean EICP was not studied in this paper. However, previous studies have reported that using $Ca(CH_3COO)_2$ increases the biomineralization rate because CH_3COO^- has higher relative molar mass, which inhibits the Ca^{2+} to cluster and consequently improves the biomineralization rate [43]. No previous literature has carried out such comparative microstructural investigation to find the morphology of eggshell $Ca(CH_3COO)_2$ based biocementation. Future study is suggested to investigate the microstructure of $CaCO_3$ produced by eggshell-soybean EICP.

3.3 Comparison with previous studies

Figure 6 shows a comparison between the relationship of UCS and CCC of this study and previously published literatures on biocmentation. Other than CCC, the particle size of soil plays an important role in the strength characteristics of biocemented soil [53, 54]. So, in this comparison, only those previous literatures are selected which has similar soil characteristics to the soil used in this study.

Figure 6 shows that almost all the other studies have reported higher UCS value than this study. However, most of them had significantly higher CCC than in this study. In fact, in the UCS range of 300-400 kPa, the CCC of this study was the lowest; no other compared previous study has observed UCS of 300-400 kPa range with this low CCC. The predicted UCS values from Figure 4 have also been plotted and they are closest to the study by Lee et al., where soybean EICP with $CaCl_2$ was used [32]. However, Lee et al. had a slightly higher CCC than this study [32]. It aligns with the discussion in Section 3.2.1 that, using eggshell $Ca(CH_3COO)_2$ in soybean EICP can strengthen soil better than $CaCl_2$ at same CCC. Kulantheival et al. used eggshell calcium with MICP, and the reported UCS was similar to this study, but had a significantly higher CCC of 11.2-17.9% [46].

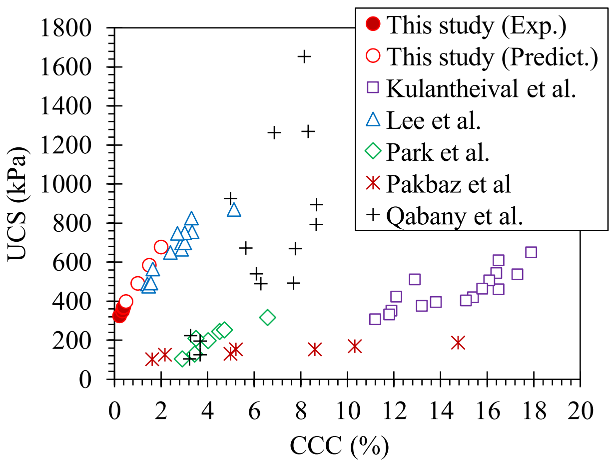


Figure 6. Comparison of UCS at different CCC between this study and previously published studies: Kulantheival et al. [46], Lee et al. [32], Park et al. [6], Pakbaz et al. [55], Qabany et al. [52] (data taken from Shu et al. [33])

4. Conclusions

This paper studied the applicability of using eggshell $Ca(CH_3COO)_2$ for biocementation via soybean urease. Using $Ca(CH_3COO)_2$ prepared from eggshell-vinegar solution and urease extracted from soybean seeds, nine compositions of EICP-treatment solutions were prepared and tested for precipitation efficiency. To explore the reinforcing effect of eggshell-soybean EICP, poorly graded sandy soil was treated with the compositions that showed higher precipitation efficiency and after 7 days of curing, UCS tests were conducted. To compare with $CaCl_2$-soybean EICP, one specimen treated with $CaCl_2$-soybean EICP was prepared and it went through UCS test after 7 days of curing. UCS results of the eggshell-soybean EICP treated specimens were compared with previously published studies to observe where eggshell-soybean EICP stands among the other approaches of biocementation. Following conclusions could be made,

1. With eggshell-soybean EICP, 100% precipitation efficiency can be reached when treatment composition 1:1/50 ($Ca(CH_3COO)_2$:Urea is 1:1 and treated with soybean solution of 50g/L) is used. Deviating from this composition reduces the precipitation efficiency.
2. The application of eggshell-soybean EICP treatment on poorly graded sandy soil led to significant improvement of the UCS of the soil. The treatment of 1:1/50 composition allowed the soil to reach a 7-day UCS of 371 kPa at 0.40% CCC, which is 26% improvement over the non-treated soil.
3. Eggshell-soybean EICP treatment showed 8.5% higher UCS than $CaCl_2$-soybean EICP. Also, similar UCS at such low CCC is unprecedented in any biocementation-related previous study with a similar soil type.
4. Eggshell $Ca(CH_3COO)_2$ should be used in EICP by percolation method with multiple treatment cycles. It is because mixing method limits the overall precipitated CCC in the soil and consequents in lower UCS.

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