Research Article

Sand Improvement by Surface Percolation Method Using Urease-Containing Substance Extracted from Soybean Seed

Amirreza Pirghazani,[1] **Hooshang Katebi** (ID),[2] **Hossein Samadi Kafil** (ID),[3] **and Ahmad Fahmi** (ID)[4]

[1]*University of Tabriz, Tabriz, Iran*
[2]*Faculty of Civil Engineering, University of Tabriz, Tabriz, Iran*
[3]*Drug Applied Research Center, Tabriz University of Medical Sciences, Tabriz, Iran*
[4]*Faculty of Engineering, University of Bonab, Bonab, Iran*

Correspondence should be addressed to Hooshang Katebi; katebi@tabrizu.ac.ir, Hossein Samadi Kafil; kafilhs@tbzmed.ac.ir, and Ahmad Fahmi; fahmi.ahmad@ubonab.ac.ir

Background. In recent decades, the microbial and plant-derived urease have been used for sand stabilization by the calcium carbonate precipitation method, especially with *Sporosarcina pasteurii*. However, calcium carbonate precipitation using plant-derived urease has received less attention. Purchasing the extracted and purified commercially available plant-derived urease for the sand improvement is costly. The plant-derived urease-containing crude extract (enzyme substance) is cheaper than purified (commercial) urease. In the present study, the feasibility of sand improvement by enzyme-induced calcium carbonate precipitation method was investigated with urease-containing crude extract (extracted from soybean). *Methods.* The distilled water, instead of phosphate buffer, was used to provide the main enzyme extraction medium. Also, the effects of temperature, time, and dilution on the activity of the urease-containing crude extract by the electrical conductivity meter were investigated. *Results.* The results showed that the extraction temperature had a significant effect on the behavior of the enzymes, and according to the results, the temperature range between 19 and 25°C is suitable for the enzyme extraction. The four-layer surface percolation method was used to improve the sand, and 0.75 equimolar (eqM) concentration of urea-CaCl$_2$ cementation solution is suitable for sand improvement using the UCE extracted from soybean seed. *Conclusion.* Silica sand was successfully improved by the EICP method using the four-layer surface percolation method, and significant unconfined compressive strength and elasticity modulus were obtained. XRD and XRF analysis also confirmed the successful precipitation of calcium carbonate between the sand particles.

1. Introduction

Drying up of lakes, rivers, and wetlands, soil erosion, desertification, and dust storms are among the environmental problems. Geotechnical engineering always attempts to solve environmental problems, such as the drifting process of moving sands (through stabilization) in desert areas and in the margins of dry lakes [1], and to prevent the occurrence of dust storms. One of the important issues in geotechnical engineering is to deal with the liquefaction of saturated loose sand. To stabilize the moving sands and to deal with the liquefaction, the use of microbial and enzymatic improvement methods has been suggested by researchers [2–10].

Advanced soil improvement techniques such as bio-grouting via microbial-induced calcium carbonate precipitation (MICP) and enzyme-induced urease carbonate precipitation (EICP) are now available for the sand stabilization and improvement. Bio-cementation (by MICP or EICP method) has many advantages over chemical methods. The advantages of bio-cement include eco-friendliness [11], less viscosity of bio-slurry and easier penetration into soil pores [12], and in-situ cementation without changing or disturbing the main soil structure [13].

MICP has grown up to be a hot topic in the field of improvement and bio-geo-technics [3, 4, 14–19]. There have been many studies on carbonate precipitation (mainly

calcite) caused by the activity of *Sporosarcina pasteurii* [3, 4, 20–23] because it has been more effective in improving than other microorganisms. Also, an eco-friendly and cost-effective method for soil improvement using bone meal and acid urease was studied by researchers [24]. In addition to microorganisms, urease is also found in some plants. Several families of common urease-rich plants include some types of beans (soybean), jack-bean melon and watermelon, pumpkin, and pine family [25]. Urease accelerates the hydrolysis of urea by 1014 times compared to the spontaneous reaction [26]. This enzyme (urease) breaks down the urea into ammonia and carbonate ions (equation (1))

$$CO(NH_2)_2 + 2H_2O \xrightarrow{\text{urease}} 2NH_4^+ + CO_3^{2-} \tag{1}$$
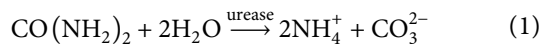
Eventually, adding calcium ions to the system (as a common method) will result in the precipitation of calcium carbonate (equation (2)).

$$Ca^{2+} + CO_3^{2-} \longrightarrow CaCO_3 \tag{2}$$
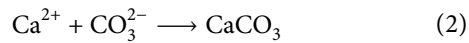
Improvement by the EICP method has more advantages than the MICP method. The soluble plant urease (12 nm) is smaller than the size of the urease-producing bacteria (300–5000 nm) which can penetrate into very small voids and be used for much finer soils. Also, the smaller size of plant urease (than ureolytic bacteria) allows the urease to be used in the soil depths. It is a protein and is not a living organism; therefore, it has no limitations related to the microorganism like *Sporosarcina pasteurii*. The plant urease has a limited lifespan, and its activity decreases over time [27, 28]. Compared with the microbial method, the direct use of the plant urease does not cause microbial contamination of the environment and does not need government authorization and monitoring [29].

So far, numerous works have been conducted on the purified plant-derived urease [30–34]. However, purchasing the extracted and purified commercially available urease is also costly despite the high yield. However, the use of plant urease as a crude extract is cheap and a good alternative for commercially purified urease [35–37], while the costs of production, isolation, storage, and transfer of urease-producing bacteria are very high [11, 38, 39]. Therefore, the cost of sand improvement by urea hydrolysis mechanism using the crude extract of urease is lower than that in the MICP method. Until now, the Jack bean crude extract and crushed watermelon crude extract have been used to improve the sand with the production of calcium phosphate [40] and calcium carbonate [41–43] precipitation among the sand particles. Östvold also used a highly cost-effective method by means of the distilled water (instead of buffer phosphate) for extracting the plant-derived enzyme from Jack bean and its lyophilization [44].

Production of ammonia by-products is a major problem of the EICP/MICP technology. Some innovative methods, including the use of natural zeolite to remove the ammonium ions from the aqueous solution and struvite precipitation technology, were used to solve this problem [45, 46].

Given the aforementioned advantages of using the plant method over the microbial one and the potential benefits of using the urease-containing crude extract (UCE), the challenge of this study is to evaluate the feasibility and efficiency of sand improvement using the crude extract of urease from the UCE. To reduce the costs, unlike the other studies, the distilled water instead of phosphate buffer was used to provide the main enzyme extraction medium. In this study, the effects of temperature, time, and dilution on the amount of urease activity extracted by the electrical conductivity meter were investigated (which was common only in MICP research). To confirm the results, the mechanical parameters of soil and the advanced X-ray diffraction (XRD) and X-ray fluorescence (XRF) analyses were considered.

2. Materials and Methods

In this study, the UCE and the cementation solution (urea-CaCl$_2$) were used to improve the silica sand [47–49]. First, in the material section, the soybean plant seeds traits used to extract the UCE, sand characteristics, and substances required for preparing the cementation solution are presented. The second section deals with the experiments on the extraction, measurement, and evaluation of the UCE of the plant seed. In this section, the method of extraction, isolation, and storage of UCE from soybean seed and the method of measuring the urease activity of UCE are presented. The experimental program was also performed to investigate the effect of extraction temperature, elapsed time, and dilution with distilled water on the urease activity of the extracted UCE. The third section deals with the sand improvement using the UCE and cementation solution (EICP method). This section describes how to prepare the sand samples and the experimental program of sand improvement to investigate the effect of the cementation solution concentration and urease activity of the UCE on the mechanical strength of the samples. This section also describes how to measure the mechanical strength and the analyses performed on the improved samples.

It should be noted that all the experiments were repeated three times and the average of three experiments was reported. More experiments are performed to reduce the effect of probable errors whenever some outliers are detected.

2.1. Plant Seed Used for Extracting UCE (Urease). To improve the sand using the EICP method, the soybean seeds purchased from the Ardabil market were used for the urease extraction (Figure 1). The distilled water was used to prepare the main enzyme extraction medium [50–52].

2.2. Sand Used for Improvement. To improve the sand using the EICP method, the Firuzkuh silica sand (Firuzkuh standard fine-grained sand #161) provided by the casting sand supplier company was used for the sand improvement. The grading curve of Firuzkuh sand #161 is shown in Figure 2. 92% of the particles were 130–500 μm in diameter. This sand has 97.5% mineral silica, 1.76 uniformity coefficient (C_U), and 0.86 curve coefficient (C_C); the name of sand in the unified classification system is SP (poor-graded sand).
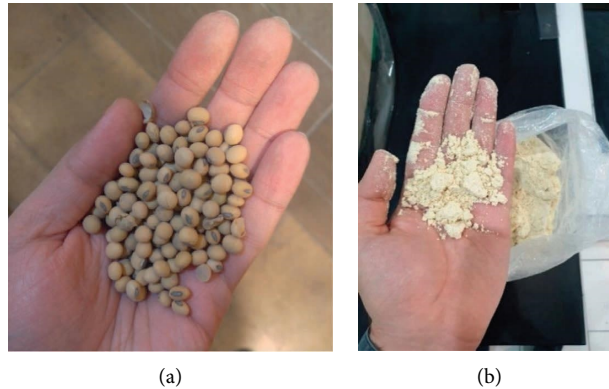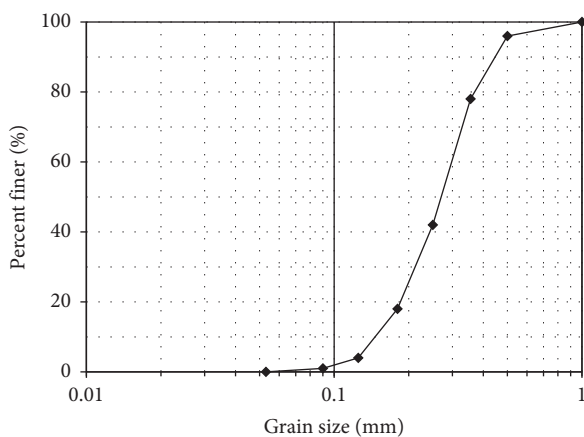
FIGURE 1: Ground soybean (b). Soybean seed (a).

FIGURE 2: Firuzkuh sand properties and grading curve.

2.3. Cementation Solution Used for Sand Improvement.

To improve the sand using the EICP method, the cementation solution of urea and calcium chloride was used. For the cost-effectiveness of making cementation solution, instead of using experimental grade, industrial grades of the substances (agricultural urea and industrial calcium chloride dehydrate with 60.06 g/mole and 147.02 g/mole molecular mass, respectively) were used. The price of the industrial type of the substances was lower than the laboratory price. The 0.3 and 0.75 eqM urea and calcium chloride solutions were used to prepare the cementation solution according to Table 1.

2.4. Method of Extraction, Separation, and Storage of UCE.

Initially, the soybean seeds were powdered and finely ground using an industrial mill. Then, 200 g of soybean was brought to volume in 1 liter of water, and the similar samples were stirred in a refrigerated shaking incubator at 15–30°C for 20 h in a stirrer (magnetic stirrer with hot plate) (Figure 3(a)). After 20 h of extraction, a milky urease-containing suspension (MUS) was obtained (Figure 3(b)). A part of it was used to measure the urease activity, and the rest was filtered through a fabric filter, and a new filtered urease-containing suspension (FUS) with less solids was obtained (Figure 3(c)). A part of the MUS was used to measure the urease activity, and the rest was poured into the Falcon 50 ml

and centrifuged at 25°C for 15 min at 10,000 rpm (Figure 3(d)). Immediately after the centrifugation, the supernatant (liquid phase), UCE, was removed and the solid phase was discarded (Figures 3(e) and 3(f)). A part of the UCE was used to measure the urease activity, and the rest was stored at 4°C in the refrigerator before use for the sand improvement and necessary measurements. In this way, three types of enzymatic substances including the MUS, filtered MUS, and the UCE (filtered and centrifuged MUS) were used to measure the urease activity.

2.5. Method for Measuring Suspension Urease Activity and UCE.

PrismaTech Benchtop Conductivitymeter (model: BPTC-500) at the Microbiology Laboratory of Tabriz University of Medical Sciences was used to measure the urease activity. In this respect, 5 ml of unfiltered milky suspension, filtered suspension, and UCE (separately in different measurements) was added to 45 ml of 1.11 M urea solution, and the rate of electrical conductivity changes was measured in 5 min at 22°C (Figure 4) [53]. The slope of the electrical conductivity graph over time yielded the urease activity of the urease suspension (in mS/cm/min). The results were multiplied by 10 because it was diluted 10 times.

2.6. Experimental Program to Investigate Effect of Extraction Temperature on Urease Activity.

According to Table 2, MUS, filtered MUS, and UCE (filtered and centrifuged MUS) of soybean were extracted at various temperatures in 6 tests. Then, their urease activity was measured and calculated according to the method described in the previous section.

2.7. Experimental Program to Investigate Effect of Elapsed Time on MUS Urease Activity.

Similar to Test 2 (Table 2), the UCE of soybean was extracted at 15°C according to the method described in the previous sections. During the extraction process of UCE (for 20 was described before), the urease activity was measured 2, 4, 6, 8, 10, and 20 h from the start. After 20 h, the prepared UCE was collected and stored in the refrigerator at 4°C for further measurements. To investigate the effect of elapsed time on the change in the

TABLE 1: Quantities of calcium chloride and urea used for preparing cementation solution at different concentrations.

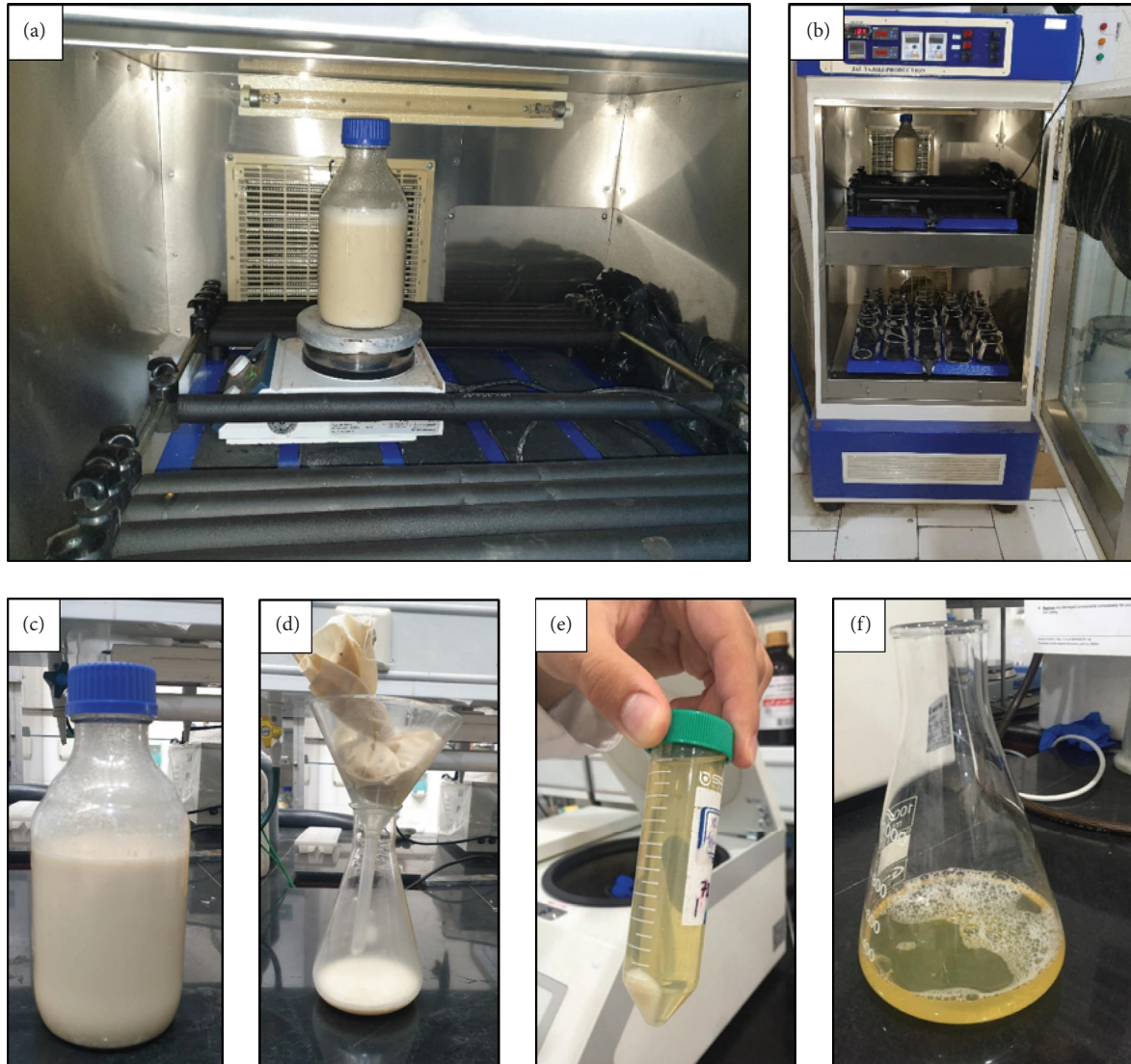| No.	Cementation solution	CO $(NH_2)_2$ (g/l)	CaCl$_2$.2H$_2$O (g/l)
1	0.3 eqM CaCl$_2$.2H$_2$O & urea	18.018	44.1
2	0.75 eqM CaCl$_2$.2H$_2$O & urea	45.045	110.26

FIGURE 3: Extraction of urease from soybean powder in an incubator on stirrer (a, b), milky suspension after 20 h of extraction and before filtering with fabric filter (c), use of fabric filter for filtering the solids (d), centrifugation of filtered suspension and separating residual solids (e), and UCE (f).

urease activity of UCE, its urease activity was measured after 24, 48, 72, 96, 120, 144, 168, and 192 h (accounting for the time of the curing process). At each specific time, the urease suspension was taken out of the refrigerator and its urease activity was measured when its temperature reached the ambient temperature. For example, the first measurement of the urease activity of the UCE kept in the refrigerator was carried out 24 h after starting the curing process (after 3.5 h of refrigeration and half an hour waiting for the suspension temperature to reach the ambient temperature).

2.8. Experimental Program to Evaluate the Effect of UCE Dilution on Urease Activity. Similar to Test 1 (Table 2), the UCE of soybean was extracted at 15°C according to the method described in the previous sections. Then, to evaluate the effect of UCE concentration including 100, 75, 50, 25, 10, and 5% on the urease activity, the prepared UCE was diluted with the distilled water and the urease activity was measured. For example, for preparing UCE 25%, 75 weight percentage of distilled water was mixed with 25 weight percentage of pure UCE.

FIGURE 4: Measuring urease activity for UCE using an electrical conductivity meter.

TABLE 2: Name of tests to investigate the effect of change in extraction temperature on their urease activity.

Test	Test1	Test2	Test3	Test4	Test5	Test6
Temperature (°C)	15	17	20	25	28	30

2.9. Preparing Sand Samples for Treatment (Improvement). A wooden injection table with 10 sections for the placement of samples next to each other was used to improve the sand (Figure 5). On the injection table, a drainage duct was placed in the base of the samples, which allowed drainage of the samples through these ducts. The sample diameter of 23 mm and sample height of 130 mm were selected, and a disposable transparent plastic mica sheet was used to make the cylindrical molds of the samples. Also, the 25 mm scourer was used as the drainage on the base of samples, 85 gr of sand was slowly poured into the molds using a funnel, no compaction was applied to the molds, and only slight vibration was applied to fully place the sand in the molds during the sand pouring. Due to the uniform grading of sand, the physical properties of sand, such as bulk density (1.51 g/cm^3), were identical at different depths of the cylindrical sample.

2.10. Experimental Program of Sand Improvement, Evaluation of Cementation Solution Concentration, and UCE Urease Activity Affecting Mechanical Strength of Samples. The sand samples were improved according to the experimental program presented in Table 3 using the UCEs with different urease activities and cementation solutions with different concentrations by the four-layer surface percolation method. According to Table 3, for each test design, three replications (for measuring the average unconfined compressive strength of three samples for each design) were prepared.
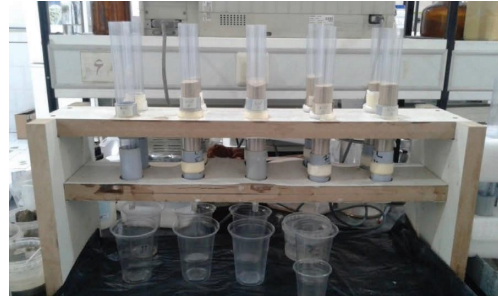

FIGURE 5: Putting cylindrical molds on the injection table.

In this method, the UCE and cementation solutions were added to the sand sample in four steps. The volume of void space of each cylindrical sand sample (containing 85 g of Firuzkuh sand #121) was measured by the 50 mm drainage method. Therefore, 12.5 ml of UCE was passed through the sand by the surface percolation method. Immediately after the complete passage of UCE through the sand, 12.5 ml of cementation solution was passed through the sand. After the complete passage of the cementation solution through the sand, 12.5 ml of UCE and, then, 12.5 ml of other cementation solution were again passed through the sand. The passage of 50 ml of UCE and cementation solutions was considered in four steps of an improvement cycle. The subsequent improvement cycles were performed similarly in 24-h intervals. The samples were improved in 6 cycles. After 6 cycles of the improvement, the molds were opened and immersed in water for 7 days to eliminate any binding effects of the presence of a high concentration of organics (proteins and fats) between the sand particles on the compressive strength of the treated samples. After the immersion process, the samples dried in an oven at 105°C, and the unconfined compressive strength of the improved cylindrical samples was measured.

2.11. Measuring Mechanical Strength of Improved Samples. In this study, the uniaxial loading device was used to measure the unconfined compressive strength of the improved cylindrical samples by strain control method according to ASTM-D2166 standard. Each improved sample was divided into two upper and lower samples. The height-to-diameter ratio of the samples was set to 2–2.5. The loading rate of the samples was selected as 1 mm/min (about 1.5% strain/min).

2.12. XRD and XRF Analyses. XRD analysis of the dried powder of the treated sample (UCE6) in the angular region (2θ) of 10–80° was recorded by X'Pert Pro diffractometer (Panalytical, the Netherlands) with Cu anode at the room temperature, step size of 0.026°, rate of 1°/min, and wavelength of 0.154056 nm, 40 kV, and 40 mA. The dried powder of natural (nontreated) and treated samples was pressed and formed into tablets with boric acid as an additive to determine the elemental composition with S4 EXPLORER XRF (X-ray fluorescence spectrometer). For the treated sample, XRF and XRD analyses were examined after the immersing-

TABLE 3: Experimental design for sand improvement using UCE and cementation solution (triple samples per design).

No.	S1	S2	S3	S4	S5	S6	S7	S8
Concentration of cementation solution (eqM)	0.3	0.75	0.3	0.75	0.3	0.75	0.3	0.75
Urease activity of UCE (mS/cm/min)	0.20	0.20	0.33	0.33	0.50	0.50	1.00	1.00

drying process. In this process, the treated sample was immersed in water for 7 days to eliminate organics (proteins and fats) and unreacted $CaCl_2$ and urea between the sand particles. After the immersion process, the samples were dried in an oven at $105°C$.

3. Results and Discussion

First, the effects of various parameters such as extraction temperature, elapsed time, and dilution with distilled water on the urease activity are presented and discussed. In the following, the influence of the urease activity of UCE and concentration of cementation solution on the mechanical strength of different sand samples was investigated, and the results of XRD and XRF analyses were also considered.

3.1. Results of the Effect of Extraction Temperature on Urease Activity. In this study, as described before, three types of enzymatic substances, including the MUS (initial milky urease-containing suspension before any filtration and centrifuge), filtered MUS (which is still in suspension state due to uncompleted solids removal), and UCE (filtered and centrifuged MUS) were extracted and prepared from soybean seed at different temperatures.

According to Table 4 and Figure 6, the extraction temperature had a significant effect on the behavior of the extracted enzymatic substances:

(1) For MUS, in the extraction temperature range from 15 to 17°C, urease activity before filtration increased from 1.18 to 1.62 mS/cm/min. By increasing the extraction temperature from 17 to 25°C, the urease activity of MUS decreased from 1.62 to 1.1 mS/cm/min. The highest urease activity for both of them was obtained at the extraction temperature of 17°C.

(2) In the extraction temperature range from 15 to 17°C, because the prepared MUS was very viscous (single-phase) and it was technically not possible to completely separate the liquid and solid phases to produce a clear UCE, the filtration process was not possible, and after centrifugation, only a very small amount of the solid precipitates and, as a result, the values of urease activity for the prepared nonclear UCE were close to MUS.

(3) In the temperature range from 20 to 25°C, the prepared MUS was not viscose and was two-phase (including solid and liquid particles). Therefore, clear UCE was produced possible by filtration and

centrifugation. It is concluded that the urease was related to the liquid phase and that the separation of solids during the filtration and centrifugation had no significant effect on the reduction of the urease activity. In this temperature range, the urease activity of UCE and MUS was slightly different.

(4) In the extraction temperature range from 28 to 30°C, the urease activity of UCE and MUS was significantly different in this range. After filtration and centrifugation of MUS to prepare UCE in this temperature range, the urease activity dropped significantly (68 and 82.7% according to Figure 6) because the extracted MUS consisted of three phases, and in addition to the liquid and solid phases, it contained the gas phase, which was removed by opening the container lid (containing MUS).

According to the results, the production of clear UCE was possible only at 20–25°C extraction temperature. From the sand improvement perspective, the use of clear UCE which is liquid and could pass easily through the sand particles was appropriated for the uniform sand improvement in depth. However, the use of MUS would cause sand blockage and inhomogeneous urease distribution and cementation solution at the depth of the sand sample. It should be noted that if the MUS (prepared at 15–30°C) or nonclear UCE (prepared at 15–17°C) is diluted with distilled water and the concentration is reduced, it can be used for the sand improvement.

3.2. Results of the Effect of Elapsed Time on Urease Activity. As shown in Figure 7, the urease activity of the enzyme increased rapidly in the 2–12 h curing time period. In the time period from 12 to 96 h, the urease activity of the enzyme decreased. The urease activity of the enzyme reached the maximum of 1.76 mS/cm/min after curing for 10 h. The urease activity decreased by 18% from 1.34 to 1.1 mS/cm/min via maintaining the urease suspension at 4°C in the refrigerator for 20–96 h. Over time, the urease activity decreased.

3.3. Results of the Effect of Diluting Urease Suspension with Distilled Water on Urease Activity. According to Table 5, after dilution of nonclear UCE provided at 15°C with distilled water, the urease activity of diluted UCE decreased as it was diluted and the concentration was reduced. By 95% dilution of UCE (5% UCE concentration), the urease activity decreased to 0.14 mS/cm/min. An important result was that dilution of the UCE with

TABLE 4: Results of experiments to compare enzyme activity at different extraction temperatures.

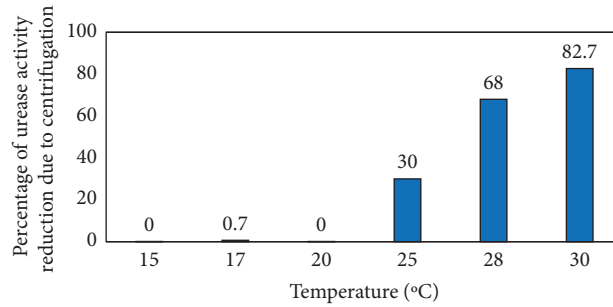| Test		Test1	Test2	Test3	Test4	Test5	Test6
Extraction temperatures (°C)		15	17	20	25	28	30
Urease activity (mS/cm/min)	MUS	1.18	1.62	1.55	1.4	1.2	1.1
	Filtered MUS	—	—	1.5	1.2	0.46	0.34
	UCE (filtered and centrifuged MUS)	1.2	1.613	1.55	1.1	0.52	0.273

FIGURE 6: Percentage of drop in urease activity of UCE relative to MUS at different extraction temperatures.
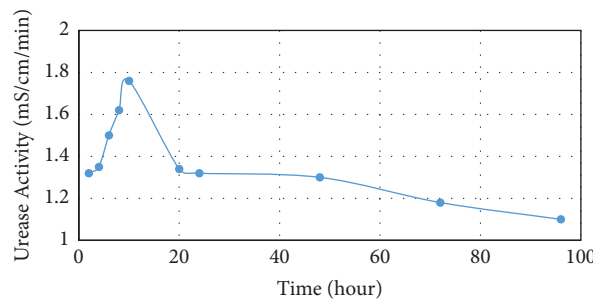

FIGURE 7: Diagram of changes in urease activity of UCE over time.

TABLE 5: Effect of UCE concentration on urease activity.

UCE concentration (%)	100	75	50	25	10	5
Urease activity (mS/cm/min)	1.34	0.90	0.68	0.42	0.20	0.14

distilled water provided the urease activity required for the sand improvement. Dilution of UCE made it easier to inject into the sand.

3.4. Results of Sand Improvement Experiments via EICP Method.
Effect of UCE concentration and cementation on the unconfined compressive strength were as below:

(1) According to Figure 8, the axial stress-strain diagram of an improved sample (one of triple samples with experimental design S6 according to Table 3) is presented. This sample was treated using UCE with 0.5 mS/cm/min urease activity and 0.75 eqM urea-CaCl$_2$ cementation solution (in the upper part of the sample). The remarkable unconfined strength of 4.62 MPa and the modulus of elasticity of 2.72 MPa were obtained by using the 6-cycle EICP improvement method. Also, the average of unconfined

compressive strength for triple similar samples was obtained at 4.35 MPa. However, in the field of sand improvement, such a large amount is usually not required for the unconfined compressive strength, and reducing the number of improvement cycles can reduce its value. One of the results of reducing improvement cycles will be reducing the time and costs of improvement.

(2) According to Figure 9, for treatment using the 0.75 eqM urea-CalCl$_2$ cementation solution, with increasing the urease activity from 0.2 to 0.333 mS/cm/min, the unconfined compressive strength and the elasticity modulus of the upper part of treated sand samples increased. By increasing the urease activity from 0.33 to 1 mS/cm/min, 68% reduction in unconfined compressive strength, and 53% reduction in elasticity modulus were achieved because in the high urease activities, due to the high rate of

urea hydrolysis and enzyme accumulation, the blockage occurred in the upper part of the sample and the distribution of calcium carbonate precipitation in the sample depth was uneven and resulted in the reduced mechanical strength of the sample. For the lower part of the sample treated with UCE with urease activities of 0.2 and 0.33 mS/cm/min, unconfined compressive strength of 1.42 and 1.53 MPa and elasticity moduli of 1.02 and 1.69 MPa were obtained. Also, for the urease activities of 0.5 and 1 mS/cm/min, compressive strength and modulus of approximately were not increased.

(3) According to Figure 10, for treatment using the 0.30 eqM urea-CalCl$_2$ cementation solution, the compressive strength of the upper part of treated sand samples increased with increasing urease activity from 0.33 to 0.5 mS/cm/min. Compressive strength decreased with a further increase of urease activity from 0.5 to 1 mS/cm/min. The highest unconfined compressive strength was obtained for 0.5 mS/cm/min urease activity equaling to 1.49 MPa. The lower part of these samples did not offer much resistance.

(4) By comparing the diagrams of Figures 9 and 10, the maximum unconfined compressive strength for the 0.75 eqM concentration was about 2 times higher than the maximum unconfined compressive strength for the 0.3 eqM concentration, indicating that for deep improvement, it was better to use the concentration of 0.75 eqM, which is consistent with other results [41].

(5) By comparing the diagrams of Figures 9 and 10, it can be seen that the maximum unconfined compressive strength and the elasticity modulus were obtained for the concentration of 0.75 eqM of urea and calcium chloride cementation solution and urease activity of 0.33 mS/cm/min (which is considered as low urease activity). Therefore, the use of UCE extracted from soybean for improving the sand, the dilution process, and its effect on the urease activity had a significant effect on the mechanical strength of the improved sand.

(6) By diluting the UCE (with distilled water), the urease activity was reduced to 0.333 mS/cm/min; in addition to increasing the mechanical strength of the sample, it helped reduce the costs of improvement. It is also shown in Figure 9 that for the urease activity of 0.333 mS/cm/min, the maximum unconfined compressive strength was obtained in the upper part of the sample and the unconfined compressive

strength of the lower part was less than the upper part due to the occurrence of partial blockage in the higher part and the less reaching of the enzyme and cementation solution to these parts.

3.5. XRD and XRF Analyses. As shown in Figure 11, the bonding between the improved sand particles (created sandstone) treated with UCE6 and the 0.75 eqM cementation solution of urea and calcium chloride.

As shown in Figure 11, the XRD analysis shows that all major peaks were related to the mineral quartz (Firuzkuh silica sand composition). Some of the peaks were related to calcite and vaterite, related to the enzymatic calcium carbonate precipitation (EICP) between the sand particles.

According to Table 6, the XRF analysis results on the untreated silica sand samples contained 94% silica (SiO$_2$), and the sand sample improved by the EICP method containing 59% silica (SiO$_2$), 22.7% carbon dioxide (CO$_2$), and 13.8% calcium oxide (CaO). Reduction of SiO$_2$ and increment of CO$_2$ and CaO indicated the precipitation of calcium carbonate among the silica sand particles.

3.6. Innovations of This Study. The enzyme-induced calcium carbonate precipitation (EICP) method presents a novel approach to sand improvement, offering several advantages over other methods like microbially induced calcium carbonate precipitation (MICP). With plant-derived urease's smaller size compared to ureolytic bacteria, the EICP method enables easier penetration into small voids without microbial contamination, making it ideal for field-scale applications. Moreover, using crude extract (UCE) of plant-derived urease from soybean seeds proves more cost-effective than commercially extracted urease, as purification is unnecessary. Distilled water replaces phosphate buffer for urease extraction, further reducing costs. Innovative use of an electrical conductivity meter successfully measures urease activity in crude extracts, while research identifies optimal conditions for urease activity. Silica sand improvements using the EICP method yield significant increases in strength and modulus. Dilution of the urease-containing substance enhances sand improvement quality, with an optimal concentration identified. This study's focus on evaluating the feasibility and efficiency of sand improvement using crude urease extract from UCE, alongside investigations into temperature, time, and dilution effects on urease activity, highlights its innovative approach. Advanced analyses, including XRD and XRF, confirm the findings, emphasizing the EICP method's potential for cost-effective and efficient sand improvement.
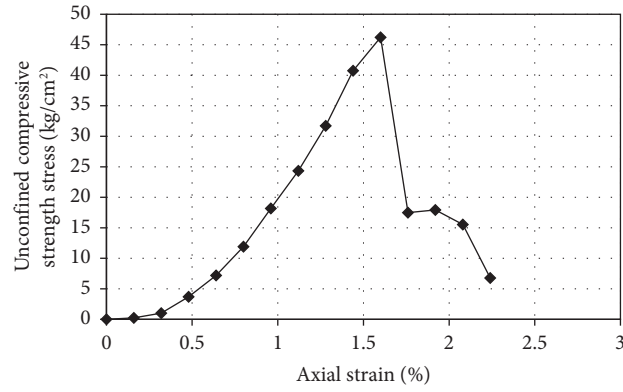
Figure 8: Stress-strain diagram of the improved sand sample (treated using enzyme UCE6 and 0.75 eqM urea cementation and calcium chloride solution).
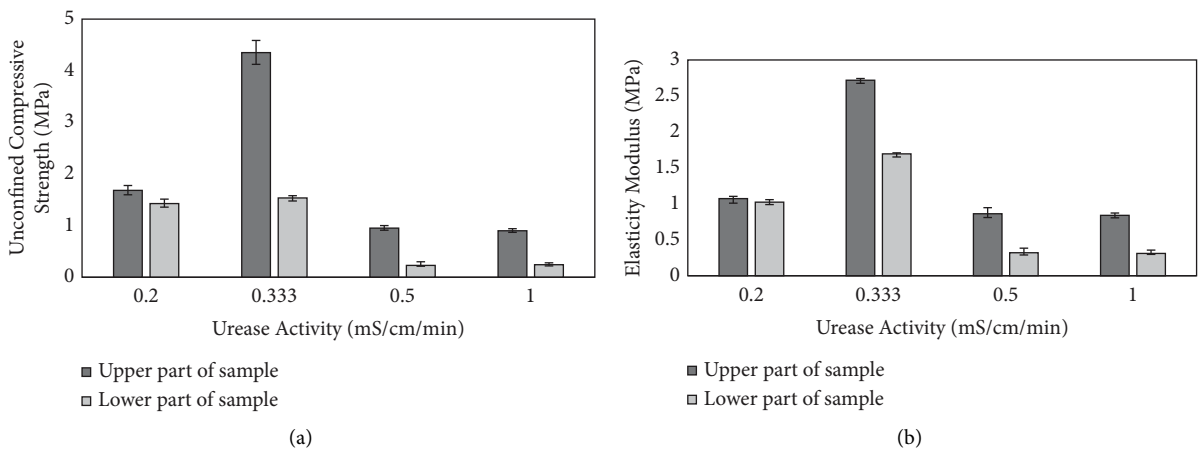

(a)

(b)

Figure 9: Unconfined compressive strength values (a) and elasticity modulus (b) of improved samples using UCE with different urease activity and 0.75 eqM cementation solution.
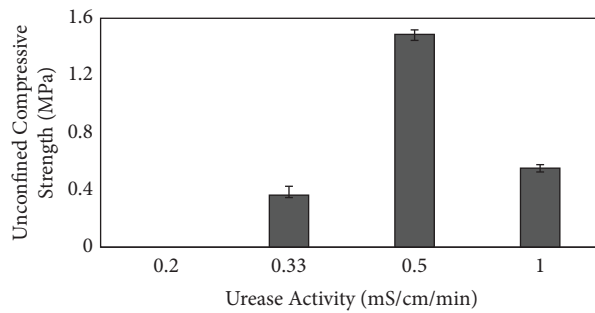

Figure 10: Unconfined compressive strength values of improved samples using UCE with different urease activity and 0.30 eqM cementation solution.
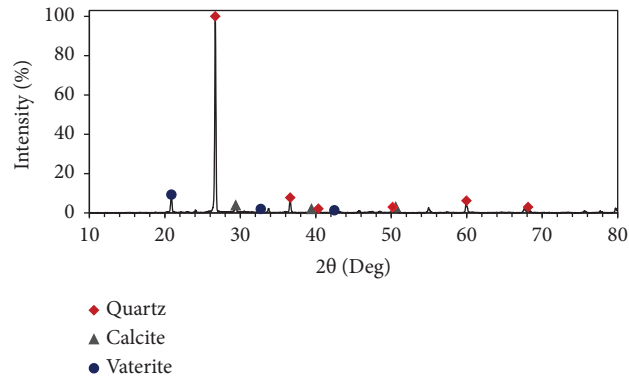
Figure 11: XRD analysis result of treated sand.

Table 6: Chemical composition of nontreated and treated samples (XRF examination).

Composition		SiO$_2$	CO$_2$	CaO	Cl	Al$_2$O$_3$	P$_2$O$_5$	Fe$_2$O$_3$	Other
% by weight	Untreated	94	2.42	1.48	0.017	0.84	—	0.76	0.483
	Treated	59.0	22.7	13.8	2.03	0.783	0.712	0.450	0.525

4. Conclusion

Due to various advantages of the EICP method over the MICP method such as the smaller size of plant-derived urease than the size of ureolytic bacteria, the possibility of easier penetration into very small voids without environmental microbial contamination using the EICP method for the sand improvement was recommended on the field scale. The use of plant-derived urease as a crude extract (UCE) was much cheaper than the commercially extracted plant urease. On the other hand, to improve the sand, it is not necessary to use the purified urease. Therefore, using the results of this study, it is possible to extract the urease-containing substance from the soybean seed at a lower cost (than purified urease) to improve the sand by the EICP method. The important results of this research are summarized as follows:

(1) The possibility of successfully extracting the MUS and UCE from soybean seed using the distilled water, instead of phosphate buffer, was proposed to further reduce the extraction costs

(2) Innovative and successful use of the electrical conductivity meter to measure the urease activity of urease-containing crude extract (from soybean seed) was evaluated

(3) The significant effect of extraction temperature on the behavior of the extracted enzymes (urease) was identified, and the suitable range of temperature (20–25°C) and time (20 h after the enzyme curing) for the UCE with the most optimal urease activity (0.333 mS/cm/min) is suitable

(4) Silica sand was successfully improved by the EICP method using the four-layer surface percolation method, and significant unconfined compressive strength and elasticity modulus were obtained

(5) The dilution of urease-containing plant-derived substance (UCE) leads to an increase in the quality of sand improvement

(6) 0.75 eqM concentration of urea-CaCl$_2$ cementation solution is suitable for sand improvement using the UCE extracted from soybean seed

Data Availability

All data and analysis results related to this study are available from the corresponding author upon request.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

Acknowledgments

References

[1] H. Katebi, A. Fahmi, H. Samadi Kafil, and M. Hajialilue Bonab, "Stabilization of calcareous sand dunes using phosphoric acid mulching liquid," *Journal of Arid Environments*, vol. 148, pp. 34–44, 2018.

[2] P. Xiao, H. Liu, Y. Xiao, A. W. Stuedlein, and T. M. Evans, "Liquefaction resistance of bio-cemented calcareous sand," *Soil Dynamics and Earthquake Engineering*, vol. 107, pp. 9–19, 2018.

[3] V. S. Whiffin, *Microbial CaCO3 Precipitation for the Production of Biocement*, Murdoch University, Murdoch, Australia, 2004.

[4] J. T. DeJong, M. B. Fritzges, and K. Nüsslein, "Microbially induced cementation to control sand response to undrained

shear," *Journal of Geotechnical and Geoenvironmental Engineering*, vol. 132, no. 11, pp. 1381–1392, 2006.

[5] S. Gowthaman, T. H. K. Nawarathna, P. G. N. Nayanthara, K. Nakashima, and S. Kawasaki, "The amendments in typical microbial induced soil stabilization by low-grade chemicals, biopolymers and other additives: a review," *Building materials for sustainable and ecological environment*, pp. 49–72, 2021.

[6] M. Sharma, N. Satyam, and K. R. Reddy, "Rock-like behavior of biocemented sand treated under non-sterile environment and various treatment conditions," *Journal of Rock Mechanics and Geotechnical Engineering*, vol. 13, pp. 705–716, 2021.

[7] M. Sharma, N. Satyam, and K. R. Reddy, "Large-scale spatial characterization and liquefaction resistance of sand by hybrid bacteria induced biocementation," *Engineering Geology*, vol. 302, Article ID 106635, 2022.

[8] M. Sharma and N. Satyam, "Strength and durability of biocemented sands: wetting-drying cycles, ageing effects, and liquefaction resistance," *Geoderma*, vol. 402, Article ID 115359, 2021.

[9] M. Sharma, N. Satyam, and K. R. Reddy, "Liquefaction resistance of biotreated sand before and after exposing to weathering conditions," *Indian Geotechnical Journal*, vol. 52, no. 2, pp. 328–340, 2022.

[10] M. Simatupang, M. Okamura, K. Hayashi, and H. Yasuhara, "Small-strain shear modulus and liquefaction resistance of sand with carbonate precipitation," *Soil Dynamics and Earthquake Engineering*, vol. 115, pp. 710–718, 2018.

[11] V. Ivanov and J. Chu, "Applications of microorganisms to geotechnical engineering for bioclogging and biocementation of soil in situ," *Reviews in Environmental Science and Biotechnology*, vol. 7, no. 2, pp. 139–153, 2008.

[12] V. Ivanov, J. Chu, V. Stabnikov, J. He, and M. Naeimi, "Iron-based bio-grout for soil improvement and land reclamation," in *Proceedings of the 2nd International Conference on Sustainable Construction Materials and Technologies*, pp. p415–p420, Italy, June 2010.

[13] R. Dilrukshi and S. Kawasaki, "Effective use of plant-derived urease in the field of geoenvironmental," *Journal of Geotechnical and Geoenvironmental Engineering*, vol. 6, p. 2, 2016.

[14] M. Sharma, N. Satyam, and K. R. Reddy, "Effect of freeze-thaw cycles on engineering properties of biocemented sand under different treatment conditions," *Engineering Geology*, vol. 284, Article ID 106022, 2021.

[15] M. Dagliya, N. Satyam, M. Sharma, and A. Garg, "Experimental study on mitigating wind erosion of calcareous desert sand using spray method for microbially induced calcium carbonate precipitation," *Journal of Rock Mechanics and Geotechnical Engineering*, vol. 14, no. 5, pp. 1556–1567, 2022.

[16] M. Sharma, N. Satyam, and K. R. Reddy, "Strength enhancement and lead immobilization of sand using consortia of bacteria and blue-green algae," *Journal of Hazardous, Toxic, and Radioactive Waste*, vol. 24, no. 4, Article ID 04020049, 2020.

[17] M. Sharma, N. Satyam, K. R. Reddy, and M. Chrysochoou, "Multiple heavy metal immobilization and strength improvement of contaminated soil using bio-mediated calcite precipitation technique," *Environmental Science and Pollution Research*, vol. 29, no. 34, pp. 51827–51846, 2022.

[18] W. Mwandira, K. Nakashima, and S. Kawasaki, "Bioremediation of lead-contaminated mine waste by Pararhodobacter sp. based on the microbially induced calcium carbonate precipitation technique and its effects on strength of coarse and fine grained sand," *Ecological Engineering*, vol. 109, pp. 57–64, 2017.

[19] M. Sharma, N. Satyam, and K. R. Reddy, "Comparison of improved shear strength of biotreated sand using different ureolytic strains and sterile conditions," *Soil Use and Management*, vol. 38, no. 1, pp. 771–789, 2022.

[20] L. A. van Paassen, R. Ghose, T. J. van der Linden, W. R. van der Star, and M. C. van Loosdrecht, "Quantifying biomediated ground improvement by ureolysis: large-scale biogrout experiment," *Journal of Geotechnical and Geoenvironmental Engineering*, vol. 136, no. 12, pp. 1721–1728, 2010.

[21] L. Cheng and R. Cord-Ruwisch, "In situ soil cementation with ureolytic bacteria by surface percolation," *Ecological Engineering*, vol. 42, pp. 64–72, 2012.

[22] A. Fahmi, H. Katebi, M. Hajialilue Bonab, and H. Samadi Kafil, "Microbial sand stabilization using corn steep liquor culture media and industrial calcium reagents in cementation solutions," *Industrial Biotechnology*, vol. 14, pp. 270–275, 2018.

[23] M. Asgharzadeh, H. Samadi Kafil, A. Fahmi, M. Yousefi, M. Aghazadeh, and M. Pourostadi, "Optimizing the use of Sporosarcina pasteurii bacteria for the stiffening of sand," *Asian Journal of Microbiology, Biotechnology and Environmental Sciences*, vol. 18, pp. 391–394, 2016.

[24] S. Gowthaman, M. Yamamoto, K. Nakashima, V. Ivanov, and S. Kawasaki, "Calcium phosphate biocement using bone meal and acid urease: an eco-friendly approach for soil improvement," *Journal of Cleaner Production*, vol. 319, Article ID 128782, 2021.

[25] N. Das, A. M. Kayastha, and P. K. Srivastava, "Purification and characterization of urease from dehusked pigeonpea (Cajanus cajan L.) seeds," *Phytochemistry*, vol. 61, no. 5, pp. 513–521, 2002.

[26] R. P. Hausinger, *Biochemistry of Nickel*, Springer Science & Business Media, Berlin, Germany, 2013.

[27] C. Marzadori, S. Miletti, C. Gessa, and S. Ciurli, "Immobilization of jack bean urease on hydroxyapatite: urease immobilization in alkaline soils," *Soil Biology and Biochemistry*, vol. 30, no. 12, pp. 1485–1490, 1998.

[28] N. Pettit, A. Smith, R. Freedman, and R. G. Burns, "Soil urease: activity, stability and kinetic properties," *Soil Biology and Biochemistry*, vol. 8, no. 6, pp. 479–484, 1976.

[29] M. Akiyama and S. Kawasaki, "Fundamental study on new grouting material using calcium phosphate compounds—crystal precipitation test and unconfined compression test of sand test pieces," *Jiban Kogaku Janaru*, vol. 6, no. 2, pp. 341–350, 2011.

[30] M. R. Bull, *Bio-inspired Cementation of Soil Using Plant Enzyme*, Arizona State University, Tempe, AZ, USA, 2014.

[31] N. Hamdan, E. Kavazanjian Jr., and S. O'Donnell, "Carbonate cementation via plant derived urease," in *Proceedings of the 18th International Conference on Soil Mechanics and Geotechnical Engineering*, pp. 2–6, Paris, France, September 2013.

[32] B. Knorr, *Enzyme-induced Carbonate Precipitation for the Mitigation of Fugitive Dust*, Arizona State University, Tempe, AZ, USA, 2014.

[33] M. Nemati and G. Voordouw, "Modification of porous media permeability, using calcium carbonate produced enzymatically in situ," *Enzyme and Microbial Technology*, vol. 33, no. 5, pp. 635–642, 2003.

[34] H.-J. Lai, M.-J. Cui, S.-F. Wu, Y. Yang, and J. Chu, "Extraction of crude soybean urease using ethanol and its effect on soil cementation," *Soils and Foundations*, vol. 63, no. 3, Article ID 101300, 2023.

[35] I.-H. Nam, C.-M. Chon, K.-Y. Jung, S.-G. Choi, H. Choi, and S.-S. Park, "Calcite precipitation by ureolytic plant (Canavalia ensiformis) extracts as effective biomaterials," *KSCE Journal of Civil Engineering*, vol. 19, no. 6, pp. 1620–1625, 2015.

[36] L. Liu, Y. Gao, W. Geng, J. Song, Y. Zhou, and C. Li, "Comparison of jack bean and soybean crude ureases on surface stabilization of desert sand via enzyme-induced carbonate precipitation," *Geoderma*, vol. 435, Article ID 116504, 2023.

[37] M. Cui, X. Fu, J.-J. Zheng, H.-H. Xiong, C. Zeng, and S.-Y. Han, "Multivariate experimental study on soybean urease induced calcium carbonate precipitation," *Rock and Soil Mechanics*, vol. 43, p. 2, 2023.

[38] M. Van, G. Van den Ham, M. Blauw, M. Latil, N. Benahmed, and P. Philippe, "Preventing internal erosion phenomena with the BioGrout process," in *Proceedings of the 15th European conference on soil mechanics and geotechnical engineering*, pp. 1079–1084, IOS Press, Athens, Greece, August 2011.

[39] S. M. Al-Thawadi, "Consolidation of sand particles by aggregates of calcite nanoparticles synthesized by ureolytic bacteria under non-sterile conditions," *Journal of Chemical Science and Technology*, vol. 2, pp. 141–146, 2013.

[40] R. A. N. Dilrukshi, J. Watanabe, and S. Kawasaki, "Sand cementation test using plant-derived urease and calcium phosphate compound," *Materials Transactions*, vol. 56, no. 9, pp. 1565–1572, 2015.

[41] R. Dilrukshi, K. Nakashima, and S. Kawasaki, "Soil improvement using plant-derived urease-induced calcium carbonate precipitation," *Soils and Foundations*, vol. 58, no. 4, pp. 894–910, 2018.

[42] N. Javadi, H. Khodadadi, N. Hamdan, and E. Kavazanjian, "EICP treatment of soil by using urease enzyme extracted from watermelon seeds," *IFCEE 2018*, ASCE, Reston, VA, USA, pp. 115–124, 2018.

[43] H. Khodadadi Tirkolaei, N. Javadi, V. Krishnan, N. Hamdan, and E. Kavazanjian, "Crude urease extract for biocementation," *Journal of Materials in Civil Engineering*, vol. 32, no. 12, Article ID 04020374, 2020.

[44] T. Östvold, "Method for water tightening of water bearing zones and stabilization of sand in underground constructions," Google Patents, Washington, DC, USA, EP2516581A1, 2015.

[45] H. A. Keykha, H. Mohamadzadeh, A. Asadi, and S. Kawasaki, *Ammonium-free Carbonate-Producing Bacteria as an Eco-friendly Soil Biostabilizer*, ASTM International, West Conshohocken, PA, USA, 2018.

[46] A. Mohsenzadeh, E. Aflaki, S. Gowthaman, K. Nakashima, S. Kawasaki, and T. Ebadi, "A two-stage treatment process for the management of produced ammonium by-products in ureolytic bio-cementation process," *International journal of Environmental Science and Technology*, vol. 19, pp. 449–462, 2021.

[47] P. Oktafiani and H. Putra, "Scale-up soybean crude urease calcite precipitation (SCU-CP) method for sandy soil improvement," in *IOP Conference Series: Earth and Environmental Science*, IOP Publishing, New York, NY, USA, September 2023.

[48] K. Roksana, S. A. Hewage, M. Montalbo-Lomboy, C.-S. Tang, C. Zhu, and W. Xue, "Bioremediation of desiccation cracking in clayey soils using enzyme induced calcite precipitation," *Geo-Congress 2023*, ASCE, pp. 603–613, Reston, VA, USA, 2023.

[49] S. Shu, B. Yan, B. Ge, S. Li, and H. Meng, "Factors affecting soybean crude urease extraction and biocementation via enzyme-induced carbonate precipitation (EICP) for soil improvement," *Energies*, vol. 15, p. 5566, 2022.

[50] N. E. Dixon, C. Gazzola, R. L. Blakeley, and B. Zerner, "Jack bean urease (EC 3.5. 1.5). Metalloenzyme. Simple biological role for nickel," *Journal of the American Chemical Society*, vol. 97, no. 14, pp. 4131–4133, 1975.

[51] B. Krajewska and W. Zaborska, "The effect of phosphate buffer in the range of pH 5.80–8.07 on jack bean urease activity," *Journal of Molecular Catalysis B: Enzymatic*, vol. 6, no. 1-2, pp. 75–81, 1999.

[52] B. Krajewska and W. Zaborska, "Jack bean urease: the effect of active-site binding inhibitors on the reactivity of enzyme thiol groups," *Bioorganic Chemistry*, vol. 35, no. 5, pp. 355–365, 2007.

[53] V. S. Whiffin, L. A. van Paassen, and M. P. Harkes, "Microbial carbonate precipitation as a soil improvement technique," *Geomicrobiology Journal*, vol. 24, no. 5, pp. 417–423, 2007.