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Multivariate experimental study on soybean urease induced calcium carbonate precipitation

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Abstract: The plant-derived enzyme induced calcium carbonate precipitation (EICP) can significantly improve the engineering mechanical properties of sand. However, there is no specification for choosing the parameter value in the specific operation, and the solidification effect needs to be improved. Based on soybean urease, the effects of temperature, urease concentration, urea concentration, calcium concentration, pH value, calcium source type and other variables on urease activity and calcium carbonate precipitation were studied, and the SEM and XRD tests on precipitated calcium carbonate crystals were carried out. On this basis, the unconfined compressive strength and solidification effect of soybean urease-solidified sand were tested. The results showed that the urease activity increased linearly with the increase of urease concentration, but there was a temperature threshold. When the temperature exceeded the threshold, urease activity was completely inactivated, and the threshold decreased with the increase of urease concentration. Urea concentration and pH value affected urease activity together, and there was an optimum combination, i.e. the optimum pH value is 7 when urea concentration is 0.1–1 mol/L, and 8 when urea concentration is 1.0–1.5 mol/L. Urease is the catalyst of a precipitation reaction. The higher the urease concentration was, the more complete the reaction was, and the higher the precipitation rate of calcium carbonate was. For urea and calcium solutions, the dosage mainly affected the precipitation of calcium carbonate, and the dosage ratio should be 1:1. The concentration and pH value of urea and calcium solutions can affect the precipitation of calcium carbonate by affecting urease activity. Different calcium sources had little influence on the amount of precipitated calcium carbonate. The composition and density of precipitated calcium carbonate crystals from different calcium sources were basically the same, but the crystal structure was different. The calcium carbonate crystals with calcium chloride as calcium source are mainly massive, with spherical and spheroidal crystals on the surface and large cementation surface, which can be used as an ideal calcium source in EICP technology. The unconfined compressive strength of the sand solidified with urease from soybean and calcium chloride as calcium source was about 6 times that of the sand mixed with coal fly ash. SEM images show that the precipitated calcium carbonate crystals wrap and bond the sand into a whole, and the solidification effect is ideal.

Keywords: soybean urease; urease activity; calcium carbonate precipitation; solidified sand; experimental study

1 Introduction

Soil is a naturally loose medium, and its improvement has always been a popular direction in the field of geotechnical engineering. As conventional improvement methods (e.g. adding lime, cement, and chemical reagents) can bring about a certain degree of pollution to the soil, the groundwater and the surrounding environment, many scholars have paid more attention to novel environmentally friendly and economic solidification materials and methods. In recent years, microbially induced calcium carbonate precipitation (MICP) has been rapidly developed. The principle of this method is that urea is hydrolyzed into carbonate and ammonium ions by urease catalyzed by the metabolic activity of microorganisms, and then calcium carbonate crystals are produced under alkaline conditions with the participation of external calcium ions to achieve the solidification effect. Fruitful achievements have been accumulated in laboratory tests and engineering applications for this technology[1–3], and the solidification effect is relatively apparent. However, the microorganisms as the source of urease are expensive, the inoculation and cultivation process is complicated, and its environmental safety is still unclear[4].

In addition to the metabolism of microorganisms, urease can also be purchased directly from commercial urease or extracted from plants, but commercial urease is more costly. Therefore, Pratama et al.[5] extracted urease from soybean flour to reduce the cost and found that soybean urease could be used as a substitute for commercial urease for soil improvement by testing urease activity, amount of precipitated calcium carbonate, and strength. Nam et al.[6] also extracted urease from jack bean and found that jack bean urease could be used as a substitute for commercial urease for crack repair and strength improvement at a lower cost by comparing it with commercial urease. Similar research showed that the engineered properties of plant-derived urease can replace commercial urease at a much lower

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cost. This has led to the development of plant-derived enzyme induced calcium carbonate precipitation (EICP).

At present, many studies concerning the feasibility of EICP in different scenarios have been conducted, and a series of research results has been obtained. Gao et al.[7] and Almajed et al.[8] found that EICP can significantly improve the surface strength and wind erosion resistance of sand through field tests and has good application prospects in the control and management of desertification. Li et al.[9] compared and analyzed the effects of EICP and MICP on the improvement of dispersive soils and found that EICP was more effective and could be used for the improvement of hydraulic dam construction materials. By comparing the solidification effect of EICP with MICP on sand columns, Hamdan et al.[10] found that the small size of plant-derived urease resulted in less bio-clogging during solidification and better application in fine-grained soils. Moghal et al.[11] found that EICP can improve soil properties and pollutant remediation capacity and has a good applicability in heavy metal soil improvement by studying the effect of EICP treatment on swelling and permeability of two Indian clayey soils, as well as the adsorption response and desorption effect on heavy metals. Kavazanjian et al.[12] found that the small size and high stability of plant-derived urease allow the EICP to be used in practical projects such as pile foundations, embankment support, and soil liquefaction control. Liu et al.[13] investigated the erosion resistance of EICP-solidified sands during coastal erosion through model tests, and the results showed that EICP treatment significantly reduced the erosion of sand by seawater.

Meanwhile, the solidification processes and parameters of EICP have also been investigated, and some results have been achieved. Cui et al.[14] proposed a low-pH one-phase injection method. The experimental results showed that this method could significantly improve the conversion efficiency of calcium ions and a more uniform distribution of calcium carbonate compared with the conventional injection method is obtained. Wu et al.[15] tested the activity of soybean urease at different temperatures and pH values and the solidification effect of soybean urease on sand samples of different grain sizes. The results showed that the optimum pH value of soybean urease was 8, the urease activity increased with temperature from 15 ℃ to 75 ℃, and the solidification effect was the best for sand samples of 0.25–0.50 mm grain size. Neupane et al.[16] investigated the effects of urea and calcium salt concentrations on the solidification effect by fixing the urease concentration, and the optimum combination of EICP treatment solution was determined.

On the whole, previous studies have focused more on the feasibility of EICP technology, which has been verified in the laboratory and in many engineering scenarios. However, less research has been conducted on the influence patterns of various factors in EICP technology and the intrinsic mechanisms of strength growth, and some consistent conclusions have not been obtained yet in the literature. Based on this, a systematic and comprehensive multivariate experimental study on the soybean urease was carried out in this paper, and many variables such as urease concentration, temperature, pH value, urea concentration, calcium concentration, pH value of the cementing solution, and type of calcium source were considered. This study investigated the influences of experimental variables on urease activity, amount of precipitated calcium carbonate, calcium carbonate crystal structure and unconfined compressive strength (UCS) to provide foundation for further standardization and promotion of the EICP technique.

2 Experimental methods

2.1 Experimental principle

Urease was separated from the soybean flour solution by centrifugal force and was then used to catalyze the production of carbon dioxide (CO_2) and ammonia (NH_3) from urea, which was hydrolyzed to ammonium ions (NH_4^+) and hydroxide ions (OH^-). Meanwhile, carbon dioxide is dissolved in water to produce bicarbonate ions (CO_3^{2-}) and hydrogen ions (H^+). Calcium carbonate precipitation can be formed under conditions suitable for the reaction of divalent cations (e.g. calcium ions) to achieve soil solidification. The reaction equations are written as follows:

$$CO(NH_2)_2 + H_2O \rightarrow 2NH_3 + CO_2 \tag{1}$$

$$2NH_3 + 2H_2O \rightarrow 2NH_4^+ + 2OH^- \tag{2}$$

$$CO_2 + H_2O \rightarrow HCO_3^- + H^+ \tag{3}$$

$$HCO_3^- + H^+ + 2OH^- \leftrightarrow CO_3^{2-} + 2H_2O \tag{4}$$

$$Ca^{2+} + CO_3^{2-} \rightarrow CaCO_3 \downarrow \tag{5}$$

2.2 Experimental materials

2.2.1 Urease preparation

The soybeans used in the experiment were commercially available from Ganzhou, Jiangxi Province, China, which were non-genetically modified agricultural products with round and full grains, as shown in Fig. 1(a). The soybeans were dried and milled into powder, as shown in Fig. 1(b), and passed through a sieve with 0.150 mm opening. The soybean powder was mixed with deionized water and stirred on an electromagnetic stirrer for 0.5 h, and then centrifuged in a centrifuge at 3000 r/min for 0.5 h. After centrifugation, the supernatant was the soybean urease solution, which must be stored at a low temperature.

(a) Soybean grains (b) Soybean powder

Fig. 1 Soybean

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2.2.2 Cementing solution

The cementing solution was a mixture of urea and calcium source solution. In the calcium carbonate precipitation test, there were three types of calcium sources, i.e. calcium chloride, calcium acetate and calcium nitrate, while calcium chloride was used as the source of the cementing solution in other variable tests. In addition, these three calcium sources were also used in the crystal structure tests for precipitated calcium carbonate.

2.2.3 Sand

The sand used in this test was the well graded China ISO standard sand (GB/T 17671–1999)[17] with a relative density d_s of 2.652. The effective particle size d_{10}, median particle size d_{30}, average particle size d_{50} and restricted particle size d_{60} of the sand were 0.14, 0.50, 0.76 and 0.90 mm, and the uniformity coefficient C_u and curvature coefficient C_c of the sand were 6.43 and 1.98.

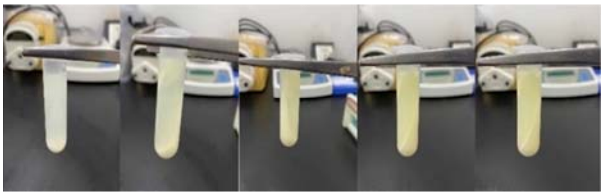
2.3 Experimental program

2.3.1 Urease activity test

Urea solutions are electrically conductive when catalyzed by urease, and urease activity can be obtained by testing the electrical conductivity of the urea–urease mixture. In combination with the key conditions in the test, two combined test variables of urease concentration and temperature, and urea concentration and pH value were selected.

(1) Urease concentration and temperature

To explore the synergistic effect of urease concentration and temperature on urease activity, five mass concentrations of urease solutions including 20, 40, 60, 80 and 100 g/L were prepared, as shown in Fig. 2.



(a) 20 g/L (b) 40 g/L (c) 60 g/L (d) 80 g/L (e) 100 g/L

Fig. 2 Urease solution with different concentrations

In this test, 3 mL urease solution was taken from each concentration of urease solution and then mixed with 27 mL 1.0 mol/L urea solution. The five mixtures were placed in a constant temperature incubator, and the temperature was adjusted to 5, 10, 20, 30, 40, 45, 50, 55 and 60 ℃ in turn. The electrical conductivity of each mixture was monitored by a conductivity meter after each temperature level was maintained for 8 min to deduce the urease activity.

(2) Urea concentration and pH value

To explore the synergistic effect of urea concentration and pH value, eight concentrations of urea solutions including 0.1, 0.3, 0.5, 0.7, 0.9, 1.0, 1.3 and 1.5 mol/L were prepared. For each concentration of urea solution, eight pH values of 4.0, 5.0, 6.0, 7.0, 8.0, 9.0, 10.0 and 11.0 were prepared, making a total of 64 different combinations of urea solutions with different concentrations and pH values. In this test, 27 mL urea solutions were mixed with 3 mL of 100 g/L urease solution, and the electrical conductivity was tested separately to deduce the urease activity.

2.3.2 Calcium carbonate precipitation test

In combination with the key factors affecting calcium carbonate precipitation in the experiment, five test variables including urease concentration, urea concentration, calcium concentration, pH value of the cementing solution, and type of calcium source were selected.

(1) Urease concentration

The urease solutions were prepared at mass concentrations of 20, 40, 60, 80 and 100 g/L, and a cementing solution was also made by mixing 1 mol/L calcium chloride solution with 1 mol/L urea solution in equal volumes. During the test, 10 mL urease solution was taken from each concentration of urease solution to mix with 10 mL cementing solution, and then the amount of precipitated calcium carbonate was measured after 1 d. The resting time in Section 2.3.2 was chosen to be 1 d, as the pre-tests have shown that the urease reacted with the cementing solution in a test tube, and the reaction rate was rapid, with no significant change in the amount of precipitated calcium carbonate after 1 d.

(2) Urea concentration

Twenty concentrations of urea solutions were prepared, i.e. 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9, 1.0, 1.1, 1.2, 1.3, 1.4, 1.5, 1.6, 1.7, 1.8, 1.9 and 2.0 mol/L. Each concentration of urea solution was mixed with 1 mol/L calcium chloride solution in equal volumes to produce 20 cementing solutions and a 100 g/L urease solution was prepared. Then, 10 mL of the cementing solution was mixed with equal volume of urease solution, and the amount of precipitated calcium carbonate in each mixture was measured after 1 d.

(3) Calcium concentration

Eighteen concentrations of calcium chloride solutions were prepared, i.e. 0.10, 0.15, 0.20, 0.25, 0.30, 0.35, 0.40, 0.50, 0.60, 0.70, 0.80, 0.90, 1.00, 1.10, 1.20, 1.30, 1.40 and 1.50 mol/L. Each concentration of calcium chloride solution was mixed with 1 mol/L urea solution in equal volumes to produce 18 cementing solutions, and a 100 g/L urease solution was prepared. Then, 10 mL cementing solution was mixed with equal volume of urease solution, and the amount of precipitated calcium carbonate in each mixture was measured after 1 d.

(4) pH value of cementing solution

Since the pH value of the cementing solution and the urea concentration synergistically affect the urease activity, three urea concentrations of 0.5, 1.0 and 1.5 mol/L, a 1.0 mol/L calcium chloride solution and a 100 g/L urease solution were prepared. The three concentrations of urea solution were mixed with calcium chloride solution in equal volumes, and each mixture was adjusted to four pH values of 6.0, 7.0, 8.0 and 9.0 to produce a total of 12 cementing solutions. Then, 10 mL cementing solution was mixed with equal volume of urease solution. The amount of precipitated calcium carbonate in each mixture was measured after 1 d.

(5) Type of calcium source

The calcium sources used in the above tests were calcium chloride solutions, but here three calcium sources including calcium chloride, calcium acetate and calcium nitrate were selected to investigate the effect of the different calcium sources on the amount of precipitated calcium carbonate. Each calcium source solution was prepared at a concentration of 1 mol/L and mixed with 1 mol/L urea solution in equal volumes to form a cementing solution with three calcium sources, and a 100 g/L urease solution was also prepared. Then, 20 mL cementing solution was mixed with equal volume of urease solution, and the amount of precipitated calcium carbonate in each mixture was measured after 1 d. Three parallel sets of samples were carried out for each of the three calcium sources, and the results were averaged.

2.3.3 Crystal structure test of precipitated calcium carbonate from different calcium sources

To investigate the effect of different calcium sources on the crystal structure of precipitated calcium carbonate, the precipitated calcium carbonate described in the calcium source variable test in Section 2.3.2 was used to test the morphology, distribution, density and mineral composition of the calcium carbonate crystals by SEM and XRD tests. The SEM and XRD tests were carried out on the precipitated calcium carbonate in accordance with the specimen requirements.

2.3.4 Sand solidification test with EICP

In this test, the solidification parameters will be taken as the optimum combination based on the results of Sections 2.3.1 and 2.3.2. A 100 g/L urease solution, 1 mol/L calcium chloride solution (the calcium source), and 1 mol/L urea solution were prepared. The urea solution and calcium chloride solution were mixed in equal volumes to produce a cementing solution with a pH value of 7. Then, 197 g of standard sand was dried and molded into a cylindrical specimen of 8 cm in height and 3.91 cm in diameter. The pore volume of the specimen was calculated to be 59.35 cm^3, and the cementing solution and urease solution were taken in equal volumes at 1.1 times the pore volume, mixed with the testing sand in succession using the mixing method, and compacted in layers in a mold. After compaction, the prepared specimens were maintained at a constant temperature of 30 ℃ for 7 d. The UCS test was then carried out at a loading rate of 1 mm/min. Two specimens were made for each group and the average value was taken. Meanwhile, the sand and fly ash were mixed to prepare a specimen for UCS test under the same loading conditions as a comparison test. Finally, SEM tests were carried out on the failed specimens to obtain the microstructure of the solidified sand specimen.

3 Experimental results

3.1 Urease activity test

3.1.1 Urease concentration and temperature

The variations of urease activity with temperature for five concentrations of urease solutions are shown in Fig. 3.

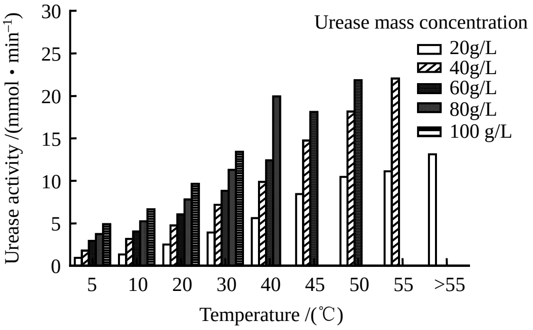


Fig. 3 Urease activity at different temperatures

It can be seen from Fig. 3 that: (1) There was a temperature threshold for soybean urease activity. When the actual temperature exceeded this threshold, the urease will lose its activity. (2) The temperature thresholds for 100, 80, 60 and 40 g/L urease solutions were 40, 45, 50 and 55 ℃, respectively. One can see that the temperature threshold was related to the urease concentration; the greater the mass concentration of urease solution, the lower the temperature threshold. (3) Before the inactivation of urease, or when the temperature is below the threshold, the urease activity in all five concentrations of urease solutions increased with the increasing of temperature. (4) When the temperature was within 30 ℃, none of the five concentrations of urease solutions were inactivated. The urease activity increased linearly with the urease concentration at the same temperature, as shown in Fig. 4.

The expressions and coefficients of determination for relationship between urease activity and urease mass concentration at 5, 10, 20 and 30 ℃ are given in Fig. 4, where the slope of the fitting line at 30 ℃ was the largest, i.e. 2.313 7. The relationship between urease activity and urease mass concentration is written as follows:

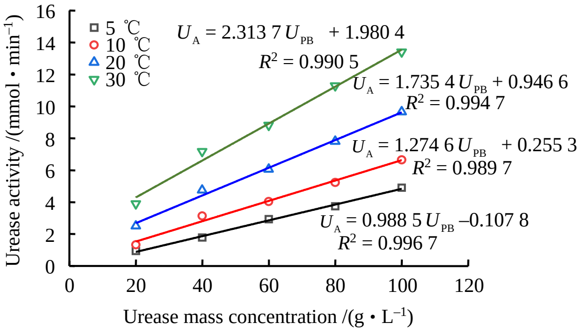


Fig. 4 Relationships between urease activity and mass concentration

$$U_{\mathrm{A}} = 2.313\,7 U_{\mathrm{PB}} + 1.980\,4 \tag{6}$$

where U_{A} is the urease activity; and U_{PB} is the urease mass concentration.

The urease activity was significantly influenced by the temperature. This is mainly because urease is the protein and increasing the temperature can increase the rate of enzymatic reaction, but too high temperature also causes protein denaturation and lead to enzyme inactivation. Therefore, combining the above experimental results and the practical application conditions, it is recommended that the urease concentration in the EICP technique should be controlled at around 100 g/L and the temperature at around 30 ℃.

3.1.2 Urea concentration and pH value

The variations of urease activity with urea pH value for eight concentrations of urea solutions are shown in Fig. 5.

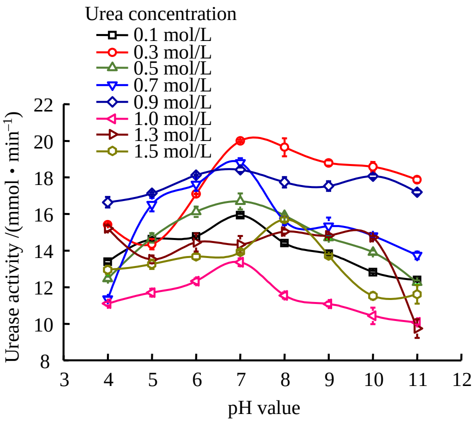


Fig. 5 Urease activity in different pH environments

One can see from Fig. 5 that: (1) The urease activity of urea solutions with different urea concentrations showed a general pattern of increasing and then decreasing with pH value, thus there was a peak urease activity and a corresponding optimum pH value. (2) The optimum pH value was related to the urea concentration, which was 7 at 0.1–1.0 mol/L urea concentration and 8 at 1.0–1.5 mol/L urea concentration. Accordingly, the optimum combination of urea concentration and pH value can be set.

3.2 Calcium carbonate precipitation test

3.2.1 Urease concentration

At constant parameters of the cementing solution, urease affects the amount of precipitated calcium carbonate within a range of concentrations. Herein, the calcium carbonate precipitation rate was used to represent this process, and the expression is as follows:

$$C_{\mathrm{CE}} = \frac{m'}{m} \tag{7}$$

where C_{CE} is the calcium carbonate precipitation rate; m is the theoretical amount of precipitated calcium carbonate; and m' is the actual amount of precipitated calcium carbonate. The theoretical amount of precipitated calcium carbonate (m) can be calculated by

$$m = C \times V \times M \tag{8}$$

where C is the concentration of the cementing solution, i.e. the concentration of calcium chloride in the cementing solution; V is the volume of the cementing solution; and M is the molar mass of calcium carbonate, equal to 100.09 g/mol.

Figure 6 shows the variation curve of calcium carbonate precipitation rate with urease concentration. It is apparent that the calcium carbonate precipitation rate was roughly linear with urease concentration when the urease concentration was in the range of 20–100 g/L. The expression is as follows:

$$C_{\mathrm{CE}} = 0.535\,5 U_{\mathrm{PB}} + 21.89 \tag{9}$$

where C_{CE} is the calcium carbonate precipitation rate.

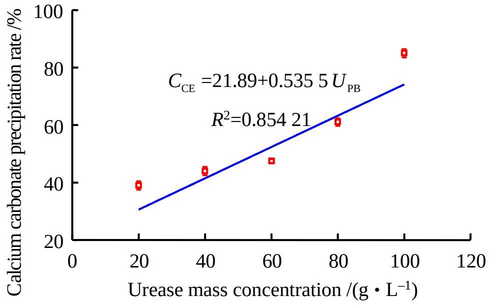


Fig. 6 Calcium carbonate precipitation rates at different urease concentrations

3.2.2 Urea concentration

The amount of precipitated calcium carbonate varies with urea concentration, as shown in Fig. 7. It is evident that the amount of precipitated calcium carbonate increased approximately linearly with increasing urea concentration in the range of 0–1.0 mol/L. When the urea concentration was in the range of 1.0–1.5 mol/L, the amount of precipitated calcium carbonate fluctuated slightly, but remained at a high level overall. Therefore, to obtain more calcium carbonate precipitated, the urea concentration must be controlled in the range of 1.0–1.5 mol/L.

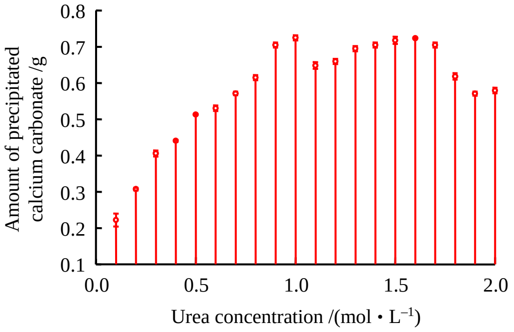


Fig. 7 Amount of precipitated calcium carbonate at different urea concentrations

3.2.3 Calcium concentration

The variation of the amount of precipitated calcium carbonate with calcium concentration is shown in Fig. 8. One can observe that the amount of precipitated calcium carbonate increased with increasing calcium concentration. The first peak occurred at a calcium concentration of 0.35 mol/L and the second peak occurred at a calcium concentration of 1.0 mol/L. When the calcium concentration was greater than 1.0 mol/L, the amount of precipitated calcium carbonate remained at approximately the same level, at which point the amount of precipitated calcium carbonate was mainly limited by the amount of CO_3^{2-}, i.e. the amount of urea in the cementing solution. Therefore, from an economic point of view, an upper limit should be set for the calcium concentration, the size of which should be combined with the amount of urea in the binder.

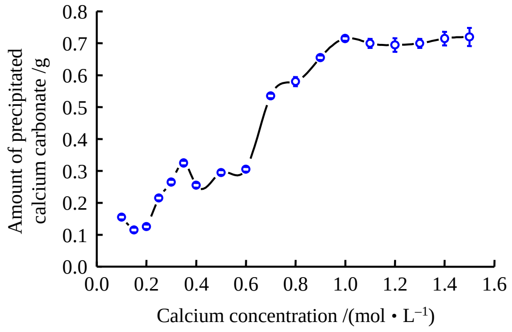


Fig. 8 Amount of precipitated calcium carbonate at different calcium concentrations

3.2.4 pH value of cementing solution

The amount of precipitated calcium carbonate at different pH values for the cementing solutions prepared from three urea concentrations is shown in Fig. 9. The peak amount of precipitated calcium carbonate was presented in the cementing solutions at different urea concentrations. This value increased with increasing urea concentration, but the pH value corresponding to the peak amount of precipitated calcium carbonate (optimum pH value) was different, with the optimum pH value of 7.0 for 0.5 mol/L and 1.0 mol/L cementing solution and 8.0 for 1.5 mol/L cementing solution, which also verified the results in Section 3.1.2. Therefore, the pH value of the cementing solutions should be set according to the urea concentration, which is 7.0 when the urea concentration is less than or equal to 1.0 mol/L and 8.0 when the urea concentration is greater than 1.0 mol/L.

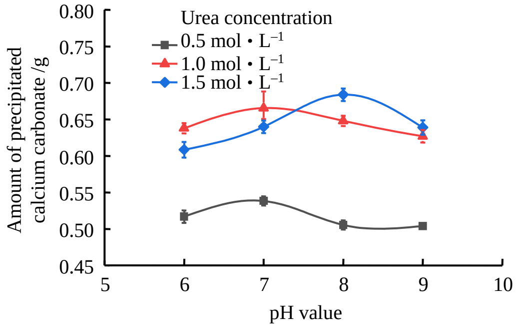


Fig. 9 Amount of precipitated calcium carbonate at different pH values

3.2.5 Type of calcium source

Under the same conditions for all other parameters, the amount of precipitated calcium carbonate from three calcium sources is shown in Fig. 10. It can be observed that the type of calcium source affected the amount of precipitated calcium carbonate, with calcium chloride being the largest, calcium acetate the smallest and calcium nitrate in the middle. This effect was less obvious in terms of the magnitude of the values, which fluctuate within 5%. Therefore, for reasons of efficiency and economy, calcium chloride is the recommended source.

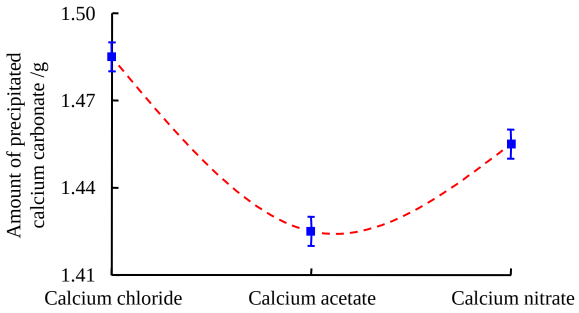


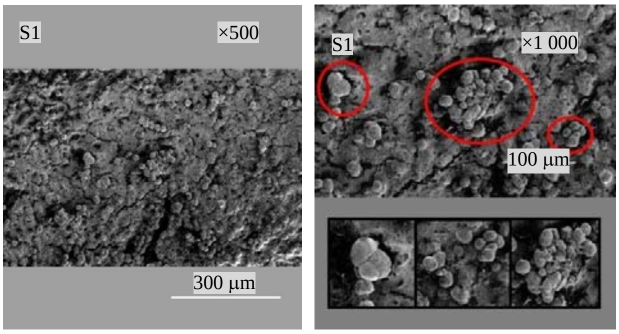
Fig. 10 Amount of precipitated calcium carbonate from different calcium sources

3.3 Crystal structure test of precipitated calcium carbonate from different calcium sources

3.3.1 SEM

(1) Calcium chloride

Calcium carbonate crystals cemented with calcium chloride as a calcium source were represented by S1. SEM images at different magnifications are shown in Fig. 11, with the lower image in Fig. 11(b) being an enlargement of the circled area above. As can be observed, the crystals of calcium carbonate were interconnected to form a predominantly large aggregate structure with small spherical, sphere-like and massive crystals of uneven size distributed on the surface of the large masses.

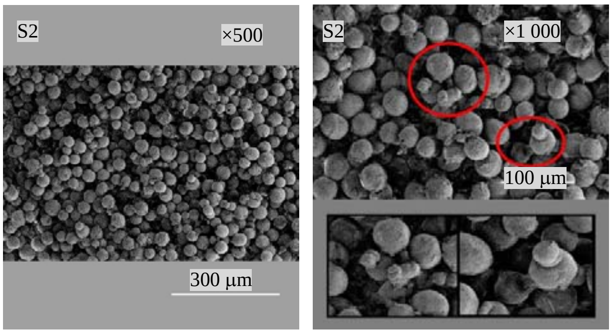


(a) 500× (b) 1 000×

Fig. 11 Crystal structure of calcium carbonate with calcium chloride as a calcium source

(2) Calcium acetate

Calcium carbonate crystals cemented with calcium acetate as a calcium source were represented by S2, and SEM images at different magnifications are shown in Fig. 12. As can be seen, the precipitated calcium carbonate crystals were mainly small, relatively independent spherical and sphere-like crystals, which were closely distributed and relatively uniform in size, with contact between the crystals mainly through point-to-point and face-to-face forms.



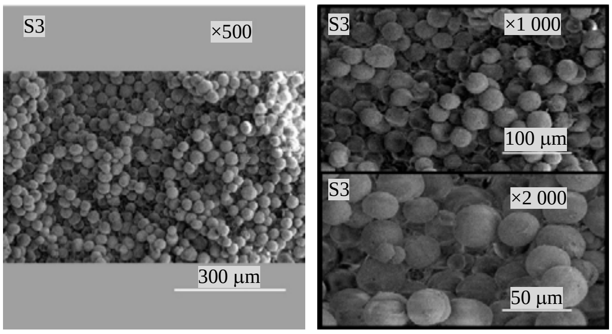
(a) 500× (b) 1 000×

Fig. 12 Crystal structure of calcium carbonate with calcium acetate as a calcium source

(3) Calcium nitrate

The calcium carbonate crystals cemented with calcium nitrate as the calcium source were represented by S3, and SEM images at different magnifications are shown in Fig. 13. It is shown that similar to calcium acetate, the precipitated calcium carbonate crystals were predominantly small, relatively independent and closely distributed crystals with a predominantly spherical crystal structure, a uniform size, and the same point-to-point and face-to-face contact between the crystals.

A comparative analysis of SEM images of precipitated calcium carbonate crystals from three calcium sources revealed that the calcium source affected the crystal structure of precipitated calcium carbonate, with calcium nitrate producing the most uniform structure of calcium carbonate crystals and calcium chloride producing the largest colloidal area of calcium carbonate crystals.



(a) 500× (b) 1 000×

Fig. 13 Crystal structure of calcium carbonate with calcium nitrate as a calcium source

3.3.2 XRD

(1) Composition analysis

The precipitated calcium carbonate crystals were ground into powder, and 1–3 mg of the sample was placed in an X-ray diffractometer for physical phase analysis with the following parameters: emission current of 40 mA, voltage of 40 kV, scanning range of $2\theta = 10°–90°$, scanning speed of 5° per minute and step size of 0.02°. The diffraction data were imported into the Jade6 software for analysis, and the mineral composition corresponding to each diffraction peak was obtained and identified, as shown in Fig. 14.

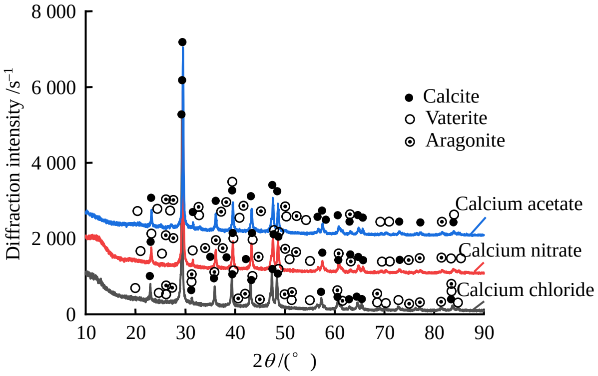


Fig. 14 XRD diffraction curves of precipitated calcium carbonate crystals from different calcium sources

The diffraction angles of the precipitated calcium carbonate crystals from three calcium sources were slightly different but generally consistent, with the diffraction angles distributed between 20° and 85°, e.g. strong diffraction peaks occurred at 2θ = 22.969°, 29.418°, 31.260°, 35.983°, 39.339°, 43.166°, 47.064°, 47.489° and 48.401°. The mineral compositions of the precipitated calcium carbonate crystals from three calcium sources were generally consistent and mainly consisted of calcite, sphalerite and aragonite, dominating by calcite, suggesting that the precipitated calcium carbonate crystals were a mixture of calcite-based crystals.

(2) Density analysis

The density of precipitated calcium carbonate crystals can be calculated by

$$\rho = \frac{N_{\mathrm{c}} \times M_{\mathrm{c}}}{N_{\mathrm{A}} \times V_{\mathrm{cell}}} \tag{10}$$

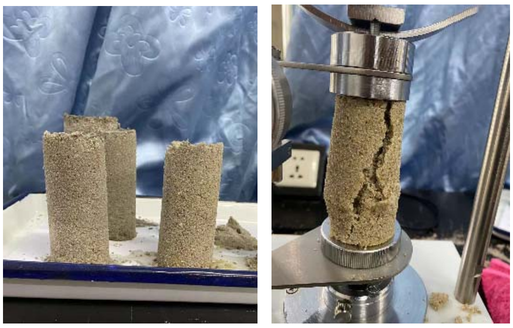
where N_{A} is the Avogadro constant, $N_{\mathrm{A}} = 6.02 \times 10^{23}$; N_{c} is the structural unit in which a cell exists; V_{cell} is the cell volume; and M_{c} is the relative molecular mass of the crystal.

The density of precipitated calcium carbonate crystals from different calcium sources can be calculated using Eq. (10). The densities of calcium carbonate crystals generated from calcium chloride, calcium acetate and calcium nitrate as calcium sources were 2.703 0, 2.701 3 and 2.703 2 g/cm^3, respectively, with little difference.

The XRD tests showed that the composition and density of the precipitated calcium carbonate crystals from different calcium sources were approximately the same, with no particularly significant differences, which again verified that the precipitates were calcium carbonate crystals.

3.3.3 EICP solidified sand

EICP solidified sand specimens and blended fly ash–sand specimens were prepared according to the test program, as shown in Fig. 15(a). The UCS tests were conducted on both specimens, and the failure pattern of the solidified sand specimen is shown in Fig. 15(b).



(a) Specimen preparation (b) Failure of specimen

Fig. 15 Solidified sand column

The stress–strain relation curves for the EICP solidified sand specimens and the blended fly ash–sand specimens are shown in Fig. 16. The UCS of the blended fly ash–sand sample was 3.2 kPa, while the strength of the sand solidified by the EICP technique was up to 18.5 kPa, with an increase of nearly six times. Meanwhile, the strain values at the peak stress of the two sand samples were relatively close, in the range of 1.8% to 2.3%, and both showed more obvious brittle damage.

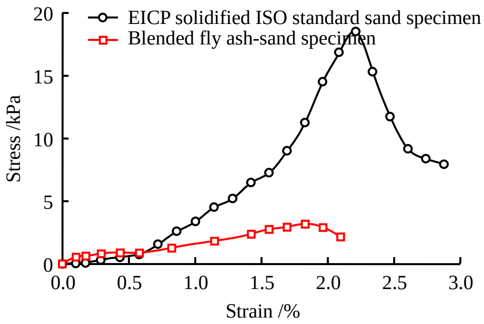
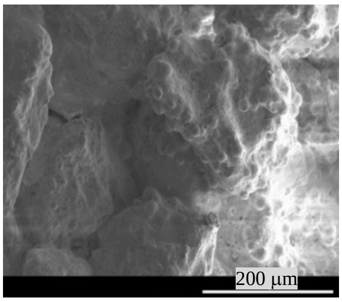
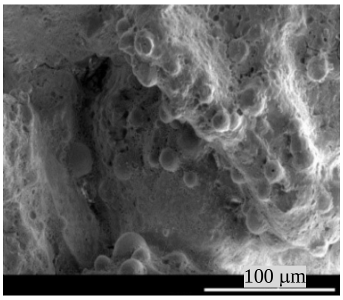


Fig. 16 UCS test

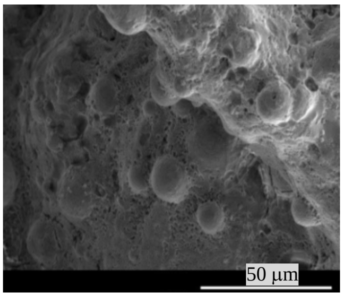
In addition, the microstructure of the EICP-solidified sand was tested by SEM to better visualize the solidification effect of the EICP technique, and the structural images at different magnifications are shown in Fig. 17. As can be seen, the calcium carbonate crystals formed by cementation were more evenly distributed on the surface of the sand and between the sand grains, bonding the separated grains into a single unit, and the strength of the solidified sand sample depended on the bonding force between the crystals and the sand grains and the strength of the crystals themselves. The cemented calcium carbonate crystal was mainly massive, with a certain number of spherical and sphere-like crystals on the surface.



(a) 800×



(b) 1 600×



(c) 3 200×

Fig. 17 Microstructures of solidified sand

4 Conclusions

In this paper, an experimental study was carried out to investigate the variables influencing urease activity and calcium carbonate precipitation in EICP technology, and the microstructure, composition, density of precipitated calcium carbonate crystals as well as the UCS of EICP solidified sand were tested. The following conclusions are drawn:

(1) There was a temperature threshold for urease activity. When the temperature exceeded this threshold, urease will be completely inactivated. This threshold was related to the urease concentration; the greater the urease concentration, the lower the threshold. Urease activity increased linearly with increasing urease concentration at the same temperature when urease was not inactivated. Urease activity was influenced by the combination of urea concentration and pH value, with an optimum pH value of 7 when the urea

concentration was 0.1–1.0 mol/L and 8 when the urea concentration was 1.0–1.5 mol/L.

(2) Urease was the catalyst for the precipitation reaction of the cementing solution, and the higher the urease concentration, the greater the calcium carbonate precipitation rate, with a roughly linear relationship between them. The urea and calcium solutions in the cementing solution affected the amount of precipitated calcium carbonate mainly through the amount of admixture, the admixture ratio should be 1:1, and the urea concentration and pH value of the cementing solution can change the amount of precipitated calcium carbonate by affecting the urease activity. The type of calcium source will affect the amount of precipitated calcium carbonate, with the highest amount of precipitated calcium carbonate occurring when calcium chloride was used as the calcium source.

(3) The composition of calcium carbonate crystals precipitated by different calcium sources was basically the same, all of them were mainly calcite and contained a mixture of spherulite and aragonite, and the crystal densities were basically the same. However, the crystal structures of calcium carbonate precipitated by different calcium sources differed greatly. When the calcium acetate and calcium nitrate were used, the precipitated calcium carbonate crystals were mainly spherical and spherulite-like, uniform in size and with small cementation surfaces. When the calcium chloride was used, the precipitated calcium carbonate crystals were mainly massive, with a small number of spherical and spherulite-like crystals distributed on the surface of the masses and large cementation surfaces. The precipitated calcium carbonate crystals with calcium chloride as a calcium source were predominantly massive, with a small number of spherical and sphere-like crystals on the surface of the masses, which were not uniform in size and had a large cementation surface. When comparing the amount of precipitated calcium carbonate, crystal structure and composition of the three calcium sources, calcium chloride was found to be the preferred calcium source for the EICP technique.

(4) The EICP-solidified sand based on soybean urease and calcium chloride source could achieve a UCS of 18.5 kPa, which was about 6 times the strength of the blended fly ash–sand sample. SEM images of the solidified sand showed that the precipitated calcium carbonate crystals wrapped and adhered to the sand particles to form a unit, which was a more satisfactory solidification effect.

References

[1] YANG Si-meng, PENG Jie, WEN Zhi-zhi, et al. Application of concentrated seawater as calcium source in microbial-induced calcium carbonate reinforcement of sandy soil[J]. Rock and Soil Mechanics, 2021, 42(3): 746–754.

[2] DONG Bo-wen, LIU Shi-yu, YU Jin, et al. Effect evaluation of natural seawater reinforcement of calcareous sand based on microbe-induced calcium carbonate precipitation[J]. Rock and Soil Mechanics, 2021, 42(4): 1104–1114.

[3] LIU Lu, SHEN Yang, LIU Han-long, et al. Application of bio-cement in erosion control of levees[J]. Rock and Soil Mechanics, 2016, 37(12): 3410–3416.

[4] LIU Yang, GAO Yu-feng, HE Jia, et al. Experimental study on the wind break and sand fixation effect using soybean urease induced calcium carbonate precipitation[J]. Henan Science, 2019, 37(11): 1784–1789.

[5] PRATAMA G B S, YASUHARA H, KINOSHITA N, et al. Application of soybean powder as urease enzyme replacement on EICP method for soil improvement technique[C]//IOP Conference Series: Earth and Environmental Science. [S. l.]: [s. n.], 2021.

[6] NAM I H, CHON C M, JUNG K Y, et al. Calcite precipitation by ureolytic plant (Canavalia ensiformis) extracts as effective biomaterials[J]. KSCE Journal of Civil Engineering, 2015, 19(6): 1620–1625.

[7] GAO Y F, MENG H, HE J, et al. Field trial on use of soybean crude extract for carbonate precipitation and wind erosion control of sandy soil[J]. Journal of Central South University, 2020, 27(11): 3320–3333.

[8] ALMAJED A, LEMBOYE K, ARAB M G, et al. Mitigating wind erosion of sand using biopolymer-assisted EICP technique[J]. Soils and Foundations, 2020, 60(2): 356–371.

[9] LI Chi, SHI Guan-yu, WU Hui-min, et al. Experimental study on bio-mineralization for dispersed soil improvement based on enzyme induced calcite precipitate technology[J]. Rock and Soil Mechanics, 2021, 42(2): 333–342.

[10] HAMDAN N, KAVAZANJIAN Jr E, O'DONNELL S. Carbonate cementation via plant derived urease[C]// Proceedings of the 18th International Conference on Soil Mechanics and Geotechnical Engineering. Paris: [s. n.], 2013.

[11] MOGHAL A, LATEEF M A, MOHAMMED S, et al. Heavy metal immobilization studies and enhancement in geotechnical properties of cohesive soils by EICP technique[J]. Applied Sciences, 2020, 10(7568): 1–21.

[12] KAVAZANJIAN E, HAMDAN N. Enzyme induced carbonate precipitation (EICP) columns for ground improvement[C]//IFCEE 2015. San Antonio: [s. n.], 2015.

[13] LIU K W, JIANG N J, QIN J D, et al. An experimental study of mitigating coastal sand dune erosion by microbial-and enzymatic-induced carbonate precipitation[J]. Acta Geotechnica, 2021, 16(2): 467–480.

[14] CUI M J, LAI H J, HOANG T, et al. One-phase-low-pH enzyme induced carbonate precipitation (EICP) method for soil improvement[J]. Acta Geotechnica, 2021, 16(2): 481–489.

[15] WU Lin-yu, MIAO Lin-chang, SUN Xiao-hao, et al. Experimental study on sand solidification using plant-derived urease-induced calcium carbonate precipitation[J]. Chinese Journal of Geotechnical Engineering, 2020, 42(4): 714–720.

[16] NEUPANE D, YASUHARA H, KINOSHITA N, et al. Applicability of enzymatic calcium carbonate precipitation as a soil-strengthening technique[J]. Journal of Geotechnical and Geoenvironmental Engineering, 2013, 139(12): 2201–2211.

[17] China Building Materials Academy. GB/T 17671－1999 Test method for strength of cement mortar (ISO method)[S]. Beijing: Standards Press of China, 1999.