Application of soybean powder as urease enzyme replacement on EICP method for soil improvement technique

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Abstract. Many researchers have confirmed the application of calcite precipitation method by urease enzyme induction as a potential soil improvement technique. However, this method needs an expensive investment due to the urease enzyme issue. The enzyme itself brings 90% of the total materials cost. It means that finding new inexpensive material for replacing the enzyme is considered essential for the development of this method. In this study, a potential material, which is soybean powder, was evaluated through several experimental tests. A comparison of urease activity value between EICP using soybean powder and commercial urease as a catalyst for hydrolysis of urea compound was made through a series of conductivity tests. It was found that EICP using soybean urease can be a potential alternative to a commercial product in terms of urease activity. The precipitation materials were analysed using a microscale test to analyses the minerals type. Besides, the reinforcing effect of EICP solution on soil specimens was evaluated by conducting unconfined compressive strength (UCS) test and acid leaching test. The results of the UCS test were indicated that the soybean powder is a potential material to be used in soil improvement technique.

1. Introduction

Recently, enzyme-induced carbonate precipitation (EICP) has become one of the innovative and potential techniques for soil improvement. The application of carbonate precipitation method by utilizing the purified urease enzyme induction as a sandy soil improvement technique has been confirmed by some researchers [1-4]. It can significantly improve the strength of the soil [2-4] and reduce the permeability and porosity of soil [1]. The grouting solutions is injected into the sand, then form the CaCO₃ formation. The formation of CaCO₃ compounds in the soil produce ties between the sand grains, limiting their motion, and then increasing the soil strength. The deposited of CaCO₃ fills the spaces between soil grains, then contribute for reducing the porosity and permeability[1,5].

In this technique, the grouting solution is composed of urease, calcium carbonate, and urea. The urease, which is the catalyst for dissociation of urea into the carbonate compound (Equation 1) derived from the purified commercial enzyme. The carbonate compound merges with the calcium ion to form a calcium carbonate precipitation (Equation 2). The improvement mechanism in soil strength using the carbonate precipitation method is describe in Figure 1.

\[ \text{CO(NH}_2\text{)}_2 + 2\text{H}_2\text{O} \xrightarrow{\text{urease}} 2\text{NH}_3^+ + \text{CO}_3^{2-} \quad (1) \]

\[ \text{Ca}^{2+} + \text{CO}_3^{2-} \rightarrow \text{CaCO}_3 \quad (2) \]
Since enzyme-induced carbonate precipitation EICP technique utilizing a purified urease enzyme, it was uneconomical method due to its high expense. The enzyme itself brings almost of the total materials cost. Therefore, finding new inexpensive material for replacing the urease enzyme is considered essential for the development of this method. In the present study, a potential inexpensive material, which is soybean powder, was evaluated through several experimental tests. Soybean powder was derived from soybean seeds. It was including in the bean’s family, which is one of the urease-rich family such as jack beans[7]. Moreover, the urease activities in the soybean seed seem like high enough to be used for carbonate precipitation technique[8]. A comparison of urease activity value between EICP using soybean powder and commercial urease was made through a conductivity test. The test tube experiment was performed to produce precipitated materials for the microscale test to evaluate the minerals form and type. Besides, the reinforcing effect of grouting solution on soil specimens was evaluated by conducting unconfined compressive strength (UCS) test and acid leaching test. Finally, the applicability of soybean powder for replacing the current purified urease enzyme in the EICP method was explicitly evaluated.

2. Materials and Method

2.1. Materials
Urea (CO(NH$_2$)$_2$) and calcium chloride (CaCl$_2$) were obtained from Kanto Chemicals Co. Inc, Japan. The new material, which is soybean powder (Gasol Soybean Flour 200 gr) was obtained from PT. Gasol Organik, South Jakarta, Indonesia. In addition, two purified commercial ureases (020-83242, Kishida Chemical, Osaka, Japan and 16040-1210, Junsei Chemical, Tokyo, Japan) were used for the comparison purpose. The silica sand number 4 with specific dry density, water absorption rate, and unit volume mass of 2.63 g/cm$^3$, 0.27%, and 1.67 kg/L, respectively, was used to evaluate the reinforcing effect of the grouting solution on the soil specimens.

2.2. Crude urease extraction
In this present study, soybean powder purchased from Indonesia was chosen for EICP method as it is an inexpensive materials and potential urease source as a species of beans. The soybean powder was kept in refrigerator before its used. The crude urease was made by mixing the soybean powder with distilled water at a specific concentration. The solution was mixed using a magnetic stirrer for 6 minutes to obtain homogenous suspension solution. The mixed solution was then centrifuged by centrifuge
machine at a rate of 3,000 rpm for 20 minutes at room temperature. Then, the clean supernatant solution containing the urease enzyme was collected and used in the next experiments.

2.3. Urease activity test
A comparison of the urease activity between soybean crude urease and the commercial product was investigated. The purpose of this comparison is to evaluate the potential replacement of new material in the case of urease activity. The urease activity was estimated using the electrical conductivity (EC) test developed by Whiffin et al. [9]. Each testing sample was prepared by mixing a 50 mL solution containing urease enzyme and a 50 mL of solution containing 1 mol/L of urea. In the solutions, urea was hydrolysed by the urease to ammonium and carbonate compounds, then bring the increase in conductivity. The change in conductivity at mS/cm/min was recorded for 10 minutes using Hanna HI5522 apparatus. A standard curve for both soybean urease and commercial urease was made by fixing the final conductivity of the complete hydrolysis process of several urea concentration. The urease activity of the material was determined by using Equation (3).

\[
\text{Urease activity (U)} = \frac{\theta_{ms}}{\theta_{sc}} \times V \times N
\]

\(\theta_{ms}\) is the gradient of electrical conductance changes, \(\theta_{sc}\) is the gradient of the standard curve, \(V\) is the volume of sample (L), and \(N\) is the final concentration of ammonia (mMol/L). In this experiment, crude urease solution derived from soybean powder at the concentration of 10-50 g/L and commercial urease solution containing 1-4 g/L concentration were prepared for the activity test.

2.4. XRD and SEM test
After the comparison with commercial urease product, a certain concentration of soybean crude urease was chosen for the following experiment. The criteria of concentration selection are the equalization activity with commercial urease and the economic side. For the microscale test, a precipitated material was provided through a test tube experiment. 20 mL of soybean crude urease was mixed with 20 mL of a testing solution containing 1 M of urea and 1 M of calcium chloride, then cured for 5 days at room temperature (± 20°C). After the curing process, the precipitated material was dried at 80°C oven for 1 day before it was used. X-ray powder diffraction (XRD) with Co-Ku radiation and scanning electron microscopy (SEM) were used to evaluate the mineral crystal phase and morphology of the mineral form, respectively.

2.5. Unconfined Compressive Strength (UCS) test
In order to evaluate the reinforcing effects of EICP solution using soybean crude urease on soil specimens, EICP-treated soil samples under various injection numbers listed in Table 1 were prepared for the UCS test. The experimental methods developed by Putra et al. [10] were adopted in this experiment. The PVC cylinder was utilized in diameter of 5 cm and height of 10 cm. Firstly, 324 g of the Keisha sand was poured into the PVC cylinders by using funnel to obtain a 50 % relative density of soil sample. Then, the 80 mL of grouting solutions were injected to the cylinder’s samples from the top by using a syringe tube. The EICP-treated sample was took after seven days curing time and then washed using distilled water. The surface of the sample was flattened using a spatula before the UCS test was conducted. Two tests for each condition were performed to check the reproducibility.

| Table 1. Experimental condition for EICP-treated specimens |
|------------------|--------|--------|----------------|--------|
| Case   | CaCl₂ (mol/L) | Urea (mol/L) | Soybean crude urease (g/L) | Cycle number |
| S5.1   | 0.5       | 0.5     | 20              | 1       |
| S5.2   | 0.5       | 0.5     | 20              | 2       |
| S5.3   | 0.5       | 0.5     | 20              | 3       |
3. Results and Discussion

3.1. Urease activity

The activity of soybean crude urease solutions made by various concentration of soybean powder were evaluated. Firstly, the standard curve for both commercial urease and soybean crude urease were provided. The standard curve is depicted in Figure 2.

![Figure 2. Standard curve of (a) commercial urease and (b) soybean crude urease](image)

The gradient of conductance changes in Figure 2 seems straight enough with R2 close to 1, for both commercial urease and soybean urease. Both of them also close each other with the gradient slope of 0.081 for commercial urease and 0.083 for soybean crude urease. The result indicates that using soybean powder as a urease enzyme could hydrolyse the urea solution into the ammonium compound as well as using commercial urease enzyme. By using Equation (3), urease activity can be estimated. For the comparison purpose, the result for both commercial urease and soybean crude urease is depicted in Figure 3.

![Figure 3. The urease activity result](image)
As apparent, the activity is linearly related to the concentration of soybean urease, and commercial urease added. The specific activity for soybean crude urease, Junsei urease, and Kishida urease was estimated at 827.9 U/g, 675.5 U/g, and 74.2 U/g, respectively (1 U activity corresponded to 1µmol/L urea hydrolysed per minute). The results indicate that the activity of soybean crude urease with high concentration (more than 10g/L) is high enough to be used for replacing the urease enzyme on the EICP technique.

3.2. Microscale analysis
In the following experiment, a certain concentration of soybean crude urease was chosen. Based on the previous EICP study, the amount of 2 g/L commercial urease enzyme was needed to hydrolyze 1.0 M reagent in the test tube experiment [20]. Since the activity of 2 g/L Kishida and Junsei urease was estimated at 1615.7 U and 2057 U, the concentration of 20 g/L soybean crude urease (corresponded to 1668.7 U activity) was chosen due to close enough with those activity values. The result of the microscale analysis test is depicted in Figure 4. As in appearance, the XRD test result shows that in the precipitation materials was found CaCO$_3$ compounds including calcite and vaterite. It also confirmed in the SEM result that the appearance of calcite was seen and pointed to by the red arrow. Otherwise, the appearance of vaterite is almost seen on the figure in the form of bubble shapes.

![Figure 4. XRD and SEM test result for precipitation material](image)

3.3. Reinforcing effect of EICP solution
The same concentration of soybean crude urease that used in microscale analysis was used for the soil specimen treatment. The UCS tests were performed on the treated samples with various injection number. A relation between the injection number of grouting solution and UCS strength is depicted in Figure 5.

The UCS strength of the treated sand improved between 64.71-623.18 kPa when 1-3 injection of EICP solution was applied in the sand samples. The increasing rate of the UCS strength from one injection to two injections of grouting solution is more than 200%. In another hand, the improvement rate decreases to 180% when one more injection was applied. It may be caused by the formation of calcite precipitations, which have already filled some gaps of sand particles and cause the difficulties of the new grouting solution to infiltrate into the soil specimens. However, although the improvement rate was decreased, it still indicates that applying multiple injection number of grouting solutions can significantly improve the soil strength.
4. Conclusion
The application of soybean powder in the EICP method was evaluated for soil improvement technique. Several experimental series were conducted to confirm the potentiality of soybean powder as new material. The urease activity of crude urease is linearly related to the concentration of soybean powder that was added. The results indicate that the soybean crude urease with high concentration (more than 10g/L) is much enough to be used for soil improvement technique. The microscale analysis confirmed that using soybean urease could produce the carbonate compound, including calcite and vaterite in the test tube experiment. The UCS test series also proved that the strength of treated soil specimens gradually increased when several injection numbers were applied. The UCS strength of treated samples was increased more than 100% when one more injection was applied. The result of this study shows that the soybean powder is a great potential material for replacing commercial urease enzyme in the EICP method for soil improvement technique.

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