Effect of increasing urease enzim concentration on shear strength properties sand clay biocementation

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Abstract. Environmentally friendly soil improvement materials have been developed over the past decade. Utilization of biotechnology in biocementation for engineering geotechnical materials has been proven to increase the shear strength of soils. This biocementation method uses the urease enzyme mixed with a solution of urea and calcium chloride. The purpose of this study was to determine the effect of increasing the concentration of the urease enzyme in sand soil stabilized with biocementation and clay addition. This research will be compared with previous studies with a ratio of 1:4. Mixing is done manually and tested using direct shear and permeability testing. Curing time for the biocementation process is carried out for two and four weeks. The result of this test is the increase in shear strength of soil due to the addition of clay and the biocementation process of the urease enzyme.

1. Introduction

Rapid economic growth in Indonesia has triggered to optimize every available land, including land in the coastal areas. The coast of Indonesia has characteristics of coarse-grained sand and uniformly grained granules. Sand that is uniformly graded tends to have a low shear angle value. Coastal sand also has no cohesion due to little or no clay content, loose grains, and no cementation. The low value of shear strength causes loose sand soil can not be used as a pile material due to the large value of the total deformation produced. Stabilization needs to be done before it is used as a construction material.

Stabilization is often used in uniformly graded loose sand using chemical grouting methods. Grouting material commonly used is Portland cement which is injected and fills pores in sandy soil. Although it can increase the shear strength and compressive strength of the soil, the use of chemical materials such as grouting has a significant environmental impact and relatively expensive [1].

Biotechnology methods for engineering geotechnical materials have been developed in the last decade. The use of biotechnology is considered to be more environmentally friendly, cheaper than chemical repairs [2], and low energy requirements. Microorganisms used in biotechnology are non-pathogenic [3]. The biotechnology method commonly used is the MICP biocementation method (microbially induced calcite precipitation). This method utilizes microorganisms to hydrolyze urea.
Hydrolyzed urea is mixed with a solution of Calcium Chloride to produce calcite (calcium carbonate) as filler and cementation. Microorganisms used in the biocementation method are gram-positive bacteria with high urease activity such as Bacillus and Sporosarcina. These bacteria produce the urease enzyme, which can hydrolyze urea (CO(NH$_2$)$_2$) [6].

Several tests on MICP in sandy soil have been carried out on a laboratory scale [5-9]. Biocementation methods used in sandy soil can increase soil cohesion. The cementation of calcite produced also acts as a filler that fills the sand pores [3].

This study observes the effect of increasing the concentration of the urease enzyme in stabilized sand soils by methods and the addition of clay soils. The addition of clay soil can increase the cohesion and fill the pores of sand soil [4]. Increasing the concentration of the urease enzyme is expected to improve the increase calcite production and cementation. This study compares the changes in the shear strength of the soil by increasing the concentration of the urease enzyme. The shear strength of soil before and after exposed to water was compared to determine the durability of the cemented soil.

2. Material characteristics

2.1. Soil used in this study

The soil used in this study, sand and clay, is the same as soil used by Putri A.R. et al. [5]. The sand soil used was taken from Sirandah Island, West Sumatra. This loose sand soil has a uniform grain gradation (Cu = 2.4 and D50 = 0.35) and its specific gravity value is 2.8. This sand includes poorly graded sand (SP) with <5% fine grains according to the USCS classification system (Unified Soil Classification System).

![Figure 1. Graph of grain size distribution on the soil used in this study.](image)

The clay soil used was taken from Depok City with a specific gravity value of 2.69. This clay is included in the classification of high plasticity silt (MH) based on the USCS classification with LL = 90.93% and PI = 46.45%. The clay used has an activity value of 1.18, so it does not include expansive clay. Grading graphs of clay and sand soil used are shown in Figure 1.

2.2. Urease enzyme

Urease enzyme is an enzyme that can be a catalyst in the hydrolysis of urea to ammonium bicarbonate [(NH$_4$)$_2$CO$_3$]. Urease enzymes are formed from a number of higher plants and bacteria with a carbonate deposition process that is environmentally friendly. Some microbes that can synthesize urease come from the genera Micrococcus, Bacillus, Sarcina, Achromobacter, Pseudomonas, Clostridium, Klebsiella, and Corinebacterium. The reactions that occur in hydrolyzed urea are as follows:
Urea + H₂O $\xrightarrow{\text{Urease}}$ CO₂ + NH₃

In this study, the urease enzyme was derived from the synthesis of Oceanobacillus sp bacteria which is grown on B4 medium. There are several factors that influence the activity of the urease enzyme [10]. The performance of urease enzyme is affecting by nutrients, type of bacteria, geometric compatibility of bacteria, bacteria cell concentration, fixation and distribution of bacteria in soil, temperature, reactant concentration, pH, and injection method [10].

2.3. Cementation solution

The cementation solution is used to produce calcite and the cementation effect biologically. The cementation solution commonly used is a solution of urea and calcium chloride (CaCl₂). The urea solution hydrolyzed using urease is then added with calcium chloride to produce calcite.

In the reaction of ammonia formation, the pH of the mixture has expanded. The increase in pH improves the rate of calcite precipitation. The reaction between calcium ions and ammonia is shown as follows:

$$\text{Ca}^{2+} + 2\text{HCO}_3^- \rightarrow \text{CaCO}_3 + \text{CO}_2 + \text{H}_2\text{O}$$

3. Methods

3.1. Soil preparation

The soil used in this study was a mixture of sand soil added 15% clay soil based on its dry weight. Both types of soil must pass sieve No. 4. Moisture content of each type of sample is calculated before mixing process.

3.2. Cementation Solution Preparation

The cementation solution used is urea and calcium chloride with a percentage of 1: 1 each. The final concentration of cementation solution is 1.1 M. The required amount of urease enzyme and cementation solution is calculated based on the total pore volume of the sample. The sample become saturated after mixing it with the cementation solution and the urease enzyme.

This study continues the previous research by Putri, A.R. et al. [5], which uses a 1: 4 concentration mixture between the urease enzyme and cementation solution. This study uses a mixed concentration of urease enzyme and cementation solution of 1: 3. The results of this study will be compared with the previous testing with different concentrations.

3.3. Sample Preparation

The Sample is prepared with a proportion of sand and 15% clay soils based on their dry weight. The soil is then manually mixed. After evenly mixed, the cementation solution is added to the soil mixture until it reaches saturation. The addition of cementation solution is only given once at the beginning of mixing the solution with the urease enzyme.

This sample is compacted with the same compaction energy in a cylindrical sample container with a diameter of 5.5 cm and a height of 11 cm in accordance with standards [13]. After compacting, samples are stored for two and four weeks.

3.4. Direct shear test

The direct shear test is conducted to determine the effect of cementation time on the value of the shear strength. In addition, the effect of water on cementation is also determined through the shear strength parameters of the direct shear test. The test equipment used was an electric direct shear test instrument that was operated by following standard procedures [11-12].

Samples used in direct shear test are prepared under two conditions, non-watering and watering sample. After the sample is cured, non-watering samples were removed from the container. Then, the
The sample is cut into three parts and formed into a direct shear sample size. The watering sample is watered according to permeability testing [13]. The sample is watered until it reaches a constant flow. After the flow is constant, then the samples are re-stored for one week for curing.

![Figure 2](image)

Figure 2. Direct shear test sample (A) before cutting (B) after cutting.

After the sample is cured, the sample is removed from the sample container and then cut into three parts. The watering sample formed into the size of a direct shear sample. This sample is used to determine the effect of water on cementation.

4. Result and discussion

4.1. Comparison with control soil

The non-watering sample was compared to control sand and sand-clay sample. Shear strength properties of control sand and sand-clay sample were obtained from previous tests by Putri, A.R. et al [5]. The comparison of the shear strength properties of the non-watering samples with the control soil is shown in Table 1.

Table 1. Comparison shear strength properties of non-watering sample with control soil.

| Parameter | Control sand | Sand + Clay | Sand + Clay + Urease (4 weeks of cementation) |
|-----------|--------------|-------------|-----------------------------------------------|
| c (kPa)   | 0            | 0           | 34.83                                         |
| ϕ (°)     | 18.96        | 15.99       | 34.15                                         |

The cohesion of the four weeks cured sample increased by 34.83 kPa. The enhancement also occurred in the friction angle. The value of the improved soil friction angle increased to 34.15° for four weeks curing time. This result show that biocementation is more affected than stabilization with clay.

Watering sample that curing for two and four weeks compared with control soil. The sample are also compared based on curing time and resistance of biocementation to water. The result of cementation in watering sample is shown in the following table 2.

Table 2. Comparison shear strength properties on watering sample with control sand.

| Parameter | Control Sand | Sand + Clay | Sand + Clay + Urease Cementation (weeks) |
|-----------|--------------|-------------|------------------------------------------|
| c (kPa)   | 0            | 0           | 37.25                                    |
| ϕ (°)     | 18.96        | 15.99       | 27.74                                    |

Increased shear strength parameters also occur in watering samples, both samples that are cured for two weeks or four weeks. The cohesion of watering samples increased after being re-cured for a week.
The cohesion of watering sample cured for two and four weeks increased by 37.25 kPa and 49.7 kPa, respectively, larger than that of the non-watering sample. The friction angle of the watering sample has increased even though it is not as large as the non-watering sample. In watering sample, the friction angle enhanced by 8.78˚ and 9.3˚ in two and four weeks curing time.

The soil shear strength properties of the watering sample is also compared to the comparison of cementation period. The test results show that sample with longer curing period (four weeks) produce better cementation. This was proven by higher shear strength in sample.

The increase in cohesion from week 2 to week 4 is not significant compared to the increase in the first 2 weeks. The cohesion of the 4th week sample increased by 12.45 kPa compared to the 2nd week sample which each sample had been watered.

Figure 3 clearly shows the comparison of the shear strength values of the test samples clearly.

![Figure 3](image)

**Figure 3.** Comparison of sample shear strength properties with control soil.

Shear strength properties of sand were improved by biocementation. The optimum calcite production occurred in the first two week. The most significant increase is the cohesion value due to the presence of clay and calcite of the resulting cementation. The calcite and clay also acts as a filler that fills the pores of the soil. So that gradations of soil grains become better and increase the friction angle of the soil that is improved.

4.2. **Comparison with previous research**

The shear strength properties of the test results are compared with the results of previous studies by Putri, A.R et al [5] with different concentration. The difference in shear strength properties as a result of differences in the concentration of the urease enzyme is shown in Table 3 and Figure 4.
Table 3. Comparison of shear strength properties at different concentrations.

| Parameter | Concentration | 1:4 | 1:3 |
|-----------|---------------|-----|-----|
|           | Curing Time (weeks) | 2 | 4 | 2 | 4 |
|           | non-watering | 2 | 4 | non-watering | watering | watering | non-watering |
| c (kPa)   | 73.04 | 55.93 | 37.25 | 49.7 | 34.83 |
| $\phi$ (°) | 11.25 | 0 | 27.74 | 28.26 | 34.15 |

Figure 4. Comparison of sample shear strength properties with previous research.

Figure 4 shows that the increase in the concentration of the urease enzyme tends to increase the value of the friction angle of the improved soil. The friction angle of the four weeks curing sample increased to 34.15°. The friction angle of the watering sample has decreased, but it is still higher than the friction angle of the non-watering sample with a concentration of 1:4.

But, the increase in the concentration of the urease enzyme does not increase its cohesion. The cohesion value of samples with concentrations of 1:3 is smaller than samples with concentrations of 1:4. The decrease in the cohesion of non-watering samples that were cured for four weeks was 21.1 kPa.

The cohesion value of this study is not as good as previous studies. This is because the quality of the enzymes used decreases due to being stored for a long time. The urease enzyme used in previous studies was more effective because it was used immediately after the extraction. The quality of the urease enzyme has a big role in improving cohesion than the quantity of the enzyme itself.

5. Conclusion
The improvement in the value of shear strength properties occurred in samples that have cured both for two and four weeks. Samples with longer cementation period show better cohesion and friction angle. The optimum cementation occurred in the first two week. Watering and re-curing process the sample...
did not eliminate the calcite that has formed. Increasing the concentration of urease enzyme can produce more calcite and increase the friction angle of the sample. However, cohesion of the sample tends to be influenced by the quality of the urease enzyme.

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