Effect of urease enzyme and clay mixture in shear strength properties of sand

Aulina Reza Putri[1], *Wiwik* Rahayu[2,*], and *Puspita* Lisdiyanti[3]

[1] Undergraduate student, Universitas Indonesia, Department of Civil Engineering, 16424 Depok, Indonesia
[2] Lecturer, Universitas Indonesia, Department of Civil Engineering, 16424 Depok, Indonesia
[3] Researcher, Indonesian Institute of Sciences (LIPI), Research Center of Biotechnology, 16911 Cibinong, Indonesia

Abstract. The utilization of environmentally friendly materials in soil stabilization has grown in the last decade. Recent studies have shown that adding urease enzyme in calcium chloride and urea solution will improve the shear strength properties of sand. The purpose of this paper is to introduce the usage of clay to improve the performance of calcite produced in the mixture of urease enzyme and calcium chloride-urea solution. This mixture is then mixed manually with the clay-sand mixture and its effect is further tested using a direct shear test conducted at every two weeks of curing. The test is carried out to determine the development of the shear strength properties of the stabilized sand. The results from the test show that there is a rise in the cohesion value of the sand due to the addition of the clay mixture.

1 Introduction

The high demand for land is increasing the requirement to exploit coastal areas as land for economic activity. Coastal areas are dominated by loose sand. The main characteristic of sand is the lack of cohesion between granules and it has a high permeability value. Therefore, sand, if used directly as a construction material, can result in large total deformation and a low bearing capacity. This means that sand in coastal areas must be stabilized prior to construction on it.

Stabilization in sand is often done by chemical grouting, which involves using chemicals to fill the pores in the sand so that it results in a stronger sand mass that is better to bear loads. The use of chemicals such as Portland cement and epoxy has been proved to improve the mechanical properties of sand such as its permeability, shear strength, and compressive strength. However, the use of chemicals for soil stabilization is considered relatively expensive and is not environmentally friendly [1].

In the last decade, there has been an innovation in stabilizing sand by utilizing biotechnology, which is more environmentally friendly. The popular biotechnology method

* Corresponding author: wiwikftui@gmail.com

used is Microbially Induced Calcite Precipitation (MICP), which is the formation of calcium carbonate from microbial activity in a saturated solution [2].

Studies of MICP of sand in laboratory scale have been done several times [2-9]. MICP was performed by injecting *Bacillus pasteuri* bacteria on sand samples that had been mixed with urea and dissolved calcium sources [3]. Besides using urease-producing bacteria, MICP is also done by injecting urease produced by *Oceanobacillus sp.* and cementation solution in sand samples [4, 5]. From the studies that have been conducted, MICP can increase the shear strength capacity [3], decrease the permeability [4], and increase the value of sand's cohesion [5]. The latest innovation on MICP in sand is the addition of clay to fill the pores between sand grains. This method can reduce the sand's permeability due to pore clogging by clay and calcium carbonate [6].

In this research, MICP was performed by adding urease from *Oceanobacillus sp* and clay to coastal sand samples. The addition of clay can reduce the porosity of the sand so that the calcium carbonate formed does not have to cover large sand pores. The purpose of this study was to observe changes in the shear strength properties of sand before and after stabilization using urease enzyme and the addition of clay. The mechanical properties are tested performed using direct shear test.

2 Characteristics of the materials

2.1 Urease

Urease (urea amidohydrolase) is a catalytic enzyme in the hydrolysis process of urea to ammonium bicarbonate [$(NH_4)_2CO_3$] [10]. Urease can be produced by microbes such as marine carbonate sequestering bacteria [11] and several high-level plants. The potential use of urease has been studied in several laboratory studies, ie in biotechnology and engineering applications [12]. The direct use of urease compared to the use of urease-producing bacteria has the advantage of not having to consider the growth and storage of the bacteria [12].

The urease used in this study is the result of extraction from the bacteria *Oceanobacillus sp.* with the isolate P3BG41. *Oceanobacillus sp*. were grown in B4 medium whose nutritional content comes from 0.3% nutrient broth, 2% urea, 0.212% $NaHCO_3$, 1% NH4Cl, and 0.441 % CaCl2 [5].

2.2 Cementation solutions

The cementation solution consists of urea ($CO(NH_2)_2$) and calcium chloride ($CaCl_2$) solution [3, 7] with a final concentration of 1.1 M [8]. Using a cementation solution, the urease enzyme catalyzes the process of urea hydrolysis then produces ammonia (NH_4OH) and carbon dioxide (CO_2). The presence of ammonia will raise the pH and initiate the precipitation of calcium carbonate or calcite ($CaCO_3$) [5].

2.3 Soils used

In this research, the soils used were sand and clay soil. The loose sand from the coast of West Sumatra was used with uniform gradations of grain size (Cu = 2.4 and D50 = 0.35 mm). The specific gravity value of the sand is 2.80. According to the Unified Soil Classification System, this sand is classified as poorly-graded sand (SP) with <5% fine grains. The cohesion values and shear angle of the sand used are 0 kPa and 18.96 °.

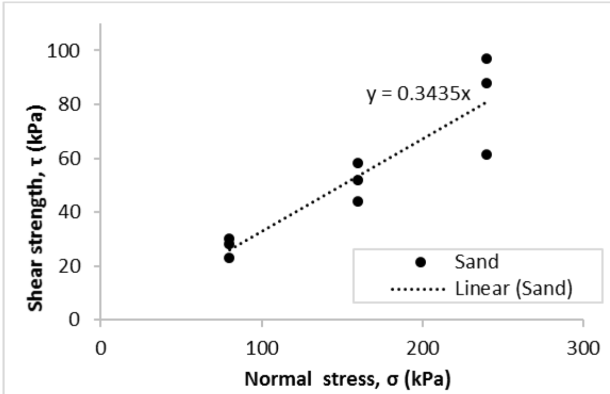

Fig. 1. Direct shear test for sand used.

Meanwhile, the clay used is Depok's clay with a specific gravity of 2.69. The distribution of grain size through hydrometer and sieve analysis test (ASTM D 421) is shown in Fig. 1. According to the Unified Soil Classification System, this clay soil is classified as high plasticity silt, MH (LL = 90.93% and PI = 46.45%). The activity value of the clay soil is 1.18 and it is classified as non-expansive clay, so it can be used as a stabilization material.

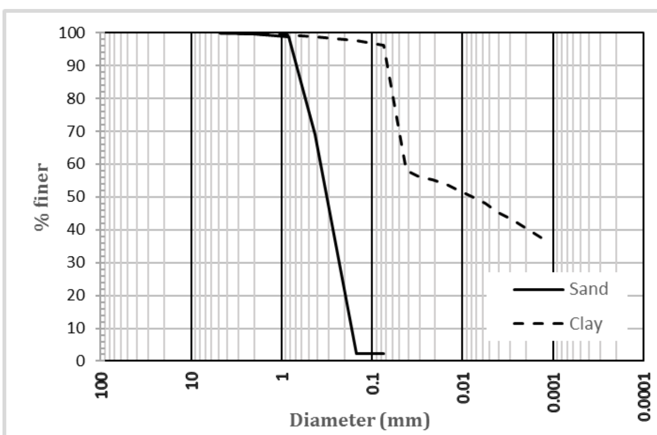

Fig. 2. Grain size distribution curves of the soils used.

3 Methods

3.1 Soil preparation

Two types of samples were prepared, sand samples and sand–clay samples. To make a clay-sand sample, the percentage of clay used is 15% of the sand's dry weight. To simplify the mixing process, the sample is made until oven dry so it has moisture content of 0%.

The porosity value of the sample at 0% moisture conditions can be used to calculate the urease and cementation requirements [5]. Based on the porosity value, the pore volume of the sand sample is 19.47 cm^3 and the pore volume of the sand–clay sample is 16.89 cm^3.

This pore volume will be filled by urease and cementation solution based on a 1: 4 ratio [13].

3.2 Urease and cementation solution preparations

Oceanobacillus sp. was cultured in B4 medium and its urease activity was tested until it reached the optimum urease production. The urease was extracted from the bacteria and then stored at -20 °C. The amount of urease prepared was approximately 1 L. Meanwhile, the cementation solution was prepared by mixing urea solution and calcium chloride solution at a 1: 1 ratio. The final concentration of the cementation solution was 1.1 M.

3.3 Sample preparation and curing

Before the mixing process, oven-dried sand samples and sand–clay samples were prepared in two separate containers. Both samples were mixed first with the cementation solution and stirred manually. When the cementation solution was well-mixed, the urease was poured onto the sample and stirred again. The direct mixing method was selected so that the cementation occurred evenly on the sample. By mixing the cementation solution and the urease, the pores in the sample were filled and the sample became saturated [5].

Samples that had been mixed with the cementation solution and urease were then put into acrylic cylinders until it reaches γ = 1.9 g/cm^3 by compacting it using a temper. The prepared acrylic cylinder has a diameter of 5.5 cm and height of 2 cm, where the size of this cylinder closely resembled the size of the direct shear sample according to SNI standard. The samples were left open in cylinders for up to 4 weeks of curing.

After two weeks of curing, cementation and urease solutions needed to be added to the sample so that urea hydrolysis and calcium carbonate precipitation continued. Because the sample was porous enough, this process was done by directly pouring the cementation solution and urease in sequence. The amount of urease volume and cementation solution added was 50% of the initial mixing volume.

3.4 Direct shear test

The direct shear test was performed at every 2 weeks of curing using an electrical direct shear testing device in accordance with the procedures of ASTM D 3080 and SNI 2813: 2008. The test was performed for both samples, so it could be compared to the sand shear strength properties before and after being stabilized by urease and the addition of clay soil. Prior to testing, both samples were first removed from the acrylic cylinder.

4 Results and discussion

4.1 Soil cementations

The cementation results in the sample were observed visually, as shown in Figs. 3 and 4. Visual observations were performed by observing the sample when removed from the acrylic cylinder. Cementation had occurred if the sample had become harder or denser so that when removed from the acrylic cylinder it was still in the form of solid pieces.

After 4 weeks of curing, the cementation of sand–clay sample was better than the sand sample. This was evident because when the samples were removed from the acrylic cylinders, the sand sample was broken while the sand–clay sample remained firm. In addition, cementation happened in all parts of the sand-clay sample, whereas cementation of the sand sample occurred only at the bottom of the sample.

According to the curing time, the cementation of the sand clay samples after 2 weeks of curing was not different from the cementation after 4 weeks of curing. However, the cementation of the sand samples after 4 weeks of curing was better than after 2 weeks of curing. This indicates that the addition of clay helps to improve the cementation of sand samples by MICP.

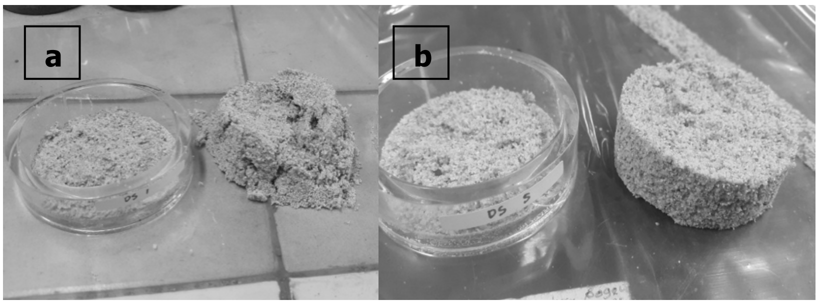

Fig. 3. Sand sample: (a) after 2 weeks of curing; (b) after 4 weeks of curing.

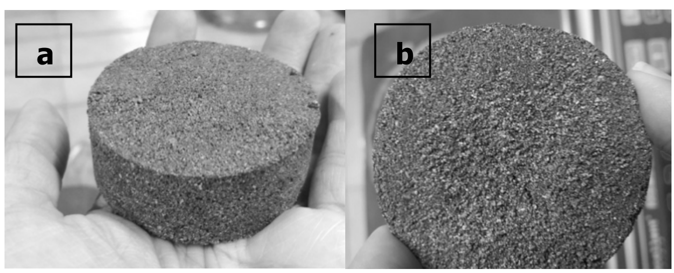

Fig. 4. Sand-clay sample: (a) after 2 weeks of curing; (b) after 4 weeks of curing.

4.2 Direct shear test results

The change in shear strength parameters is shown in Table 1. Compared to the sand before the stabilization, the cohesion in the clay–sand samples increased significantly from 0 kPa to 73.04 kPa after 2 weeks of curing. The increase in the cohesion value was not significant after 4 weeks of curing, when it became 55.93 kPa. Meanwhile the shear angle increased from 18.96° to 11.25° after 2 weeks of curing. By contrast, the shear angle dropped to 0° after 4 weeks of curing.

For the sand samples, stabilization with urease until 4 weeks of curing did not increase cohesion, but increased the shear angle. The shear angle increased from 18.96° to 22.06° after 2 weeks of curing and to 21.21 ° after 4 weeks of curing.

Table 1. Direct shear test after 2 weeks of curing.

Curing time	2 weeks			
Sample	Sand (control)	Sand + urease	Sand-clay (control)	Sand-clay+ urease
Cohesion (kPa)	0	0	0	73.04
Friction angle (°)	18.96	22.06	15.99	11.25

Table 2. Direct shear test after 4 weeks curing.

Curing time	4 weeks			
Sample	Sand (control)	Sand + Urease	Sand-clay (control)	Sand-clay+ Urease
Cohesion (kPa)	0	0	0	55.93
Friction angle (°)	18.96	21.21	15.99	0

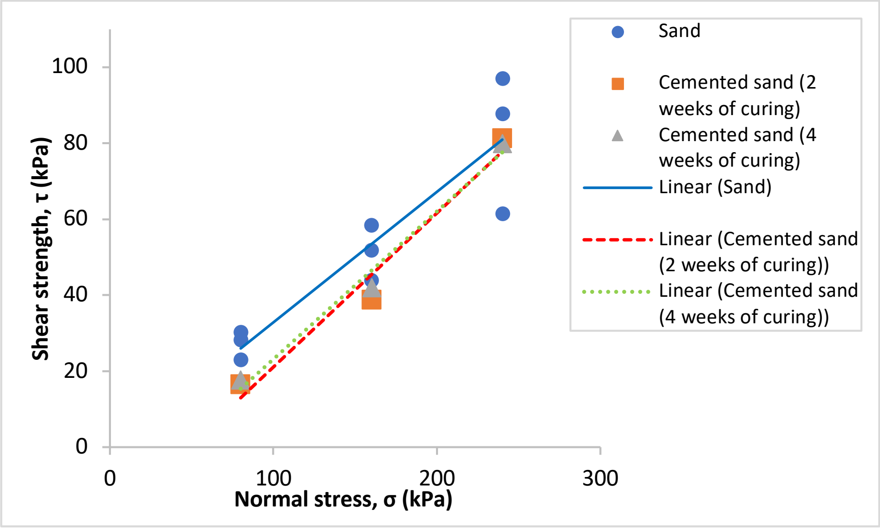

Fig. 5. Development of shear strength parameter of cemented sand sample after 4 weeks of curing.

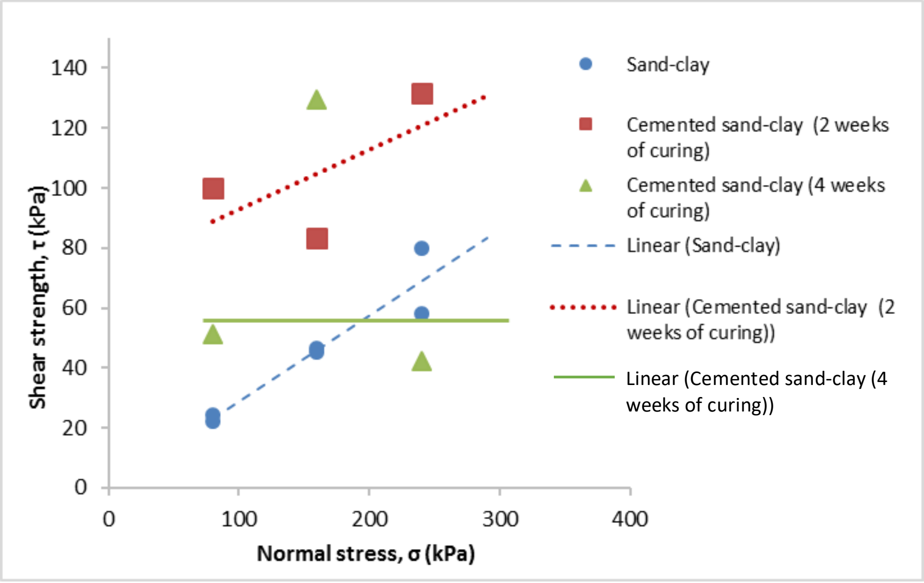

Fig. 6. Development of shear strength parameter of cemented sand-clay sample after 4 weeks of curing.

4 Conclusions

Sand stabilization using urease and the addition of clay affected the shear strength parameters, specifically the value of cohesion and shear angle. From the result of the research, there is an increase of cohesion in the sand that has been stabilized with urease and the addition of clay soil. By contrast, the shear angle value decreases. The cementation in the clay–sand samples is better than the sand samples. This indicates that the addition of clay helps to improve the cementation of sand samples by MICP.

Acknowledgment

The experimental laboratory study was conducted by a collaboration between Universitas Indonesia and Indonesian Institute of Sciences (LIPI) through the Soil Mechanics Laboratory of Universitas Indonesia and Biotechnology Laboratory of LIPI. This study was funded by the Donation of International Indexed Publication for Universitas Indonesia's Student Final Project (PITTA) No. 5000/UN2.R3.1/HKP.05.00/2018

References

1. R. H. Karol, *Chemical grouting and soil stabilization, revised and expanded*, 3rd ed, (CRC Press, 2003)
2. T. Bosak, *Encyclopedia of Geobiology*, 223-227 (2011)
3. J. T. DeJong, M. B. Fritzges, K. Nüsslein, *J. Geotech Geoenviron*, **132**, 1381-1392, (2006)
4. S. Ainiyah, P. Lisdiyanti, M. Pertiwi, E. Nugroho Prasetyo, *Jurnal Sains Dan Seni ITS*, **2**, 1, 2337-3520 (2014)
5. Y. Heri Suprapto, H. Mroueh, J. M. Lacroix, E. Madec, B. Susilo Soepandji, W. Rahayu, *International Conference on Civil, Biological and Environmental Engineering* (CBEE), 21-26 (2014)
6. R. Cardoso, I. Pires, S. O .D. Duarte, G. A. Monteiro, *Appl Clay Sci*. **156**, 96-103 (2018)
7. L. A. van Paassen, R. Ghose, T. J. M. van der Linden, W. R. L. van der Star, M. C. M van Loosdrecht, *J. Geotech. Geoenviron*. **136**, 1721-1728 (2010)
8. V. S. Whiffin, L. A. van Paassen, M. P. Harkes. *Geomicrobiol. J.* **24**, 417-423 (2007)
9. V. Stabnikov, V. Ivanov, J. Chu, *World J. Microbiol. Biotechnol*. **31**, 1303-14 (2015)
10. N. Rani Wandansari (2006)
11. Y. Fujita, F. G. Ferris, R.D. Lawson, F. S. Colwell, R. W. Smith, *Geomicrobiol. J.* **17**, 305-318 (2000)
12. B. Krajewska, *J. Adv. Res*. **13**, 59-67 (2017)
13. Y. Syahrifan Mihar (2014)