Experimental Study of Utilization of Bacillus subtilis to improve the Shear Strength of Sand

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***Abstract*—**Soil improvement method using enzymatic activity from bacteria *Bacillus subtilis* that able to catalyze urea and calcium chloride become cementation solution is regarding as an another approach to increase the shear strength of sand. The production of urease enzyme of *B. subtilis* will be increased using specific medium. Determination of the volume of bacteria needed is based on the soil porosity. The preliminary result of *B. subtilis* that use specific medium could increase the urease enzyme activity and already verified using urease measurement kit. The cementation had already started in three days treatment compare to the sand without specific medium that need approximately one month. The result visually observed that the sand particles had increase their cohesion and started to hardened. The calcite that was produced would fill the soil pore and increase its unit weight. Direct shear test is conducted to measure the soil properties after treatment.

Keywords*—B.subtilis*, cementation, direct shear test, precipitation, urease

I. Introduction

USE of microbiological aspect in civil engineering domain has been developed in the last few years. The conception of soil cementation using bacteria is to increase the soil particle bonding through biochemical processes of catalyze urease [19]. The implementation of this method for example bioconsolidation and bioclogging have done in laboratory scale to discover the method of soil improvement. [1], [16], [18], [20], [22].

In this laboratory research will be described the development of the microbial aspect. The activity of the urease enzyme produced was optimized so that the precipitation of calcite could still occur, but the population of bacteria will be reduced significantly. Reduction of the population of bacteria will be able to minimize the contamination of the environment due to the improvement of the soil. Growth nutrition that used generally in the cultivation of bacteria is LB medium [22] – [24].

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The composition of cementation solution consists of urea and calcium chloride with the final concentration 1,1 M. By using the cementation solution, bacteria will produce the urease enzyme that used to catalyze the urea and calcium chloride into calcite particles.

In previous researches, the type of bacteria that used in the soil cementation is *Sporosarcina pasteurii*. In this research, *B. subtilis* is used in the process of soil cementation due to the bacteria has the ability to produce urease enzyme [1] – [4]. In general, LB medium is used as growth nutrition for *B. subtilis*, but when it combined with cementation solutions, the calcite particle did not much performed as expected before. Therefore, the modification of nutrition is needed to increase the amount of urease enzyme significantly.

The research is the preliminary study for the type of bacteria *B. subtilis* used in the sand soil cementation. The result of the research is qualitatively to observe the influence of utilization of *B. subtilis* to improve the properties of the sand.

II. Soil Characteristics

A. Physical Properties

Hostun sand RF is the soil that used in this research has unit weight around 13 kN/m^3 and 16 kN/m^3 in dry condition, but in the saturated condition it reaches 25 kN/m^3. The void ratio (e) of this soil is in range of 0,575 to 1,041. From this void ratio, the porosity of the sand could be determined. The porosity is approximately 36,5% to 48,5% [5],[6].

TABLE I
PHYSICAL PROPERTIES OF HOSTUN RF SAND

Researcher	D_{50} mm	U	e_{min}	e_{max}
Alvarado (2000)	0,35	1,57	0,656	1,00
Al Mahmoud (1997)	0,471		0,575	0,943
Fargeix (1986)			0,648	1,041
Colliat (1986)			0,624	0,961

The chemical composition of Hostun sand RF is consists of silicon (SiO_2) with the presentation more than 98%. The friction angle of the sand is 35° and the cohesion is 0 kPa (cohesion-less) [5]. Hostun sand that used in the laboratory

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has the water content 0% which is possible to calculate the porosity of the soil using the void ratio (e).

TABLE II
UNIT WEIGHT OF HOSTUN RF SAND

Researcher	ρ_s (g/cm^3)	$\rho_{d\ min}$ (g/cm^3)	$\rho_{d\ max}$ (g/cm^3)
Alvarado (2000)	2,65	1,325	1,6
Al Mahmoud (1997)	2,59	1,336	1,648
Fargeix (1986)	2,65	1,298	1,608
Colliat (1986)	2,65	1,351	1,632

B. Shear Strength Parameter

The direct shear test in the laboratory is the common and simple test to observe the shear characteristic of the sand. Shear strength, friction angle, and cohesion could be obtained from this test.

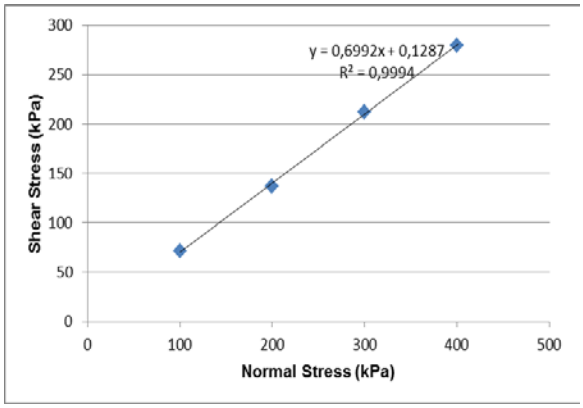


Fig. 1 Graphic of friction angle of Hostun Sand RF

The graphic above is the initial condition of Hostun sand by direct shear test with its unit weight 15,14 kN/m3. The friction angle that obtained is 34,9° and the cohesion is 0,12 kPa. This laboratory result is approaching the value in the literature where the friction angle is 35° and the cohesion is 0 kPa [5].

III. BIOCHEMICAL ASPECTS

A. Bacteria

Type of bacteria that used in the experimental study is *B. subtilis* that has the ability to produce urease enzyme [1]. In general, *B. subtilis* is one type of bacteria that is non-pathogen (did not cause disease), it has the simple cell structure (rod), non-motile or not actively moving, and the most important is it has the ability to produce the urease enzyme [1] – [4].

Bacteria were grown at 37°C in *Luria-Bertani* broth (LB) or in minimal medium M63 supplemented with a carbon source at a concentration of 2 g/L [29]. In this research, *B. subtilis* was grown under medium M63 and medium M63 supported with glutamic acid to observe the production of urease enzyme. The addition of glutamic acid in the medium is expected to increase the urease activity of the bacteria.

The growth result is observed using spectrophotometer. Measurement of the growth is conducted in overnight stay culture. The value of optical unit weight (OD) will describe the number of bacteria cells. The growth rate of *B. subtilis* for one-night stay is OD 0,97 for *B. subtilis* grown under medium M63 and 1,30 for *B. subtilis* grown under medium M63 supported with glutamic acid. This OD value will be the basis reference of injection of the bacteria into the soil. Injection of the bacteria in this phase will give the possibility to bacteria to precipitate more the cementation solution.

B. Urease Test

The measurement of urease activity was conducted using urease assay kit. This test was conducted to measure the urease production of *B. subtilis*. The urease activity is measured based on the optical density value of the enzyme. The calculation of urease activity is describes in the equation below:

$$\text{Urease activity} = \frac{OD_{sample} - OD_{blank}}{Slope\ x\ t}\ (\text{U/L}) \qquad (1)$$

The OD value is read in several degree of density started from OD_{630}, OD_{650}, OD_{670}, and OD_{700}. For the calculation, the reading of OD_{670} will be used.

The urease test was started by destroying the bacteria's cells using sonication equipment. Then, the protein concentration of the bacteria that rest is measured. The protein then put into 96 plates corning-well then measured the OD value.

C. Cementation Solution

Cementation solution that applied in soil cementation is consists of urea ($CO(NH_2)_2$) and calcium chloride ($CaCl_2$). These compositions have been used in the cementation researches [1] – [4], [12], [25] – [27], [28]. The concentration of the cementation solution based on the previous researches is 1,1 M. This experimental study, determination of the concentration is based on the previous research and the volume between the bacteria needed is 1:3 compare to the cementation solution [28].

D. Calcite Precipitation

Calcite precipitation occurs when the urease enzyme from *B. subtilis* is catalyzes the urea and the calcium chloride according to the reaction below [13], [28]:

$$CO(NH_2)_2 + 3H_2O \rightarrow HCO_3^- + 2NH_4^+ + OH^- \qquad (2)$$

$$Ca^{2+} + HCO_3^- + OH^- \rightarrow CaCO^3 + 2H_2O \qquad (3)$$

From the chemical reaction above, the role of urease enzyme is to catalyze the urea and turn it into carbonate ion. This ion will react with calcium ion from calcium chloride solution and produce the calcite grains that fulfill the pore of the sand.

IV. Experimental Modeling

A. Soil Preparation

The acrylic boxes with dimension of 6 x 6 x 2 cm^3 are prepared and filled by Hostun sand RF with the density approximately 1,5 gr/cm^3. These boxes have the same size with the sample of direct shear test according to France standard (*Norme Française* NF 94-071-1). The relative density of the sand (Dr) is 59,5%. It is calculated using the equation which related into the void ratio (e) in the equation below:

$$Dr = \frac{e_{max} - e}{e_{max} - e_{min}} \quad (4)$$

The maximum void ratio is 0,943 and the minimum void ratio is 0,575 [5]. The void ratio for the initial condition is 0,724. The porosity of the sand is approximately 42%. From these parameters, the sand is defined as medium sand [10].

The water content of the sand is 0% which means that the soil was in the non-saturated condition. This condition could be used to calculate the needs of the biochemical solution that includes the bacteria and the cementation solution. Based on the porosity value, the void volume of the sand is approximately 30 cm^3. This void volume will be filled by 7,5 mL of bacteria and 22,5 mL of cementation solution which based on 1:3 comparison.

The boxes sand will be divided into three parts of treatment. The first part is the control sand which filled by medium M63 only. The second part is the sand boxes are filled by *B. subtilis* grown under M63 medium and cementation solution. The third part, the boxes will be filled by *B. subtilis* grown under M63 supported with glutamic acid medium and cementation solution. The treatment will take for 3 days and 7 days under 37°C temperature to obtain the optimum condition for the bacteria.

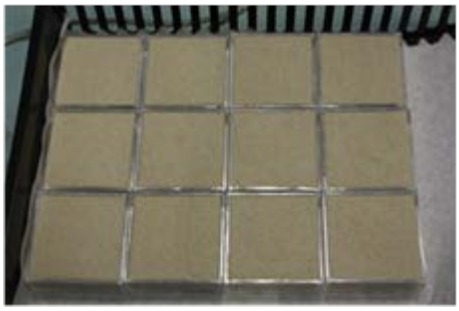


Fig. 2 Sand preparation

There are 20 boxes that divided into 4 control boxes, 4 boxes for 3 days treatment under medium M63, 4 boxes for 3 days treatment under medium M63 supported with glutamic acid. 4 boxes for 7 days treatment under medium M63, and 4 boxes for 7days treatment under medium M63 supported with glutamic acid. The complete formation of the treatment is describes on the table below.

B. Bacteria and Cementation Solution Preparation

B. subtilis is grown under medium M63 and medium M63 supported with glutamic acid. These mediums are added by carbon source 2 g/L. The culture will be used for the urease activity test and for injection into the sand. The volume of bacteria was determined by the correlation with the soil porosity. *B. subtilis* was stored in the 37°C temperature for one night incubation.

TABLE III
SOIL TEST AFTER TREATMENT 3 DAYS

Box	Vol. (cm^3)	W_{sand} (gr)	ɣ (gr/cm^3)	Observation
1	72	108.51	1.5071	No Bacteria (Control)
2	72	108.28	1.5039	
3	72	108.42	1.5058	
4	72	108.03	1.5004	
5	72	108.27	1.5038	Day 3
6	72	108.46	1.5064	
7	72	108.44	1.5061	
8	72	108.25	1.5035	
9	72	108.15	1.5021	Day 3
10	72	108.07	1.5010	
11	72	108.14	1.5019	
12	72	108.20	1.5028	
13	72	108.04	1.5006	Day 7
14	72	108.14	1.5019	
15	72	108.27	1.5038	
16	72	108.27	1.5038	
17	72	108.16	1.5022	Day 7
18	72	108.09	1.5013	
19	72	108.16	1.5022	
20	72	108.31	1.5043	

The culture then separated into two parts. The first part is used for urease measurement using urease kit. The second part is used for injection into the soil. This method is conducted to observe the relation between the urease activity and the result of cementation. The two activities are conducted in the same day to ensure that the result will be comparable.

Cementation solution is consist of urea and calcium chloride with the final concentration of 1,1 M. These solutions are autoclaved under 120°C for 25 minutes.

C. Injection Method

Injection of the bacteria and cementation solution is conducted using syringe. Bacteria's solution and cementation solution are mixed before injected into the sand. This method will give the bacteria to adapt with the environment and start the precipitation directly.

Fig. 3 Injections on the surface of the sand

The points of injection are spread to obtain the homogenization of the cementation result. There are 15 points of injections to simplify the volume of every point of injection. By injecting the amount of bacteria and cementation solution, the sand condition is become saturated. The pore is filled by bacteria and cementation solution.

V. Result

A. Urease Test

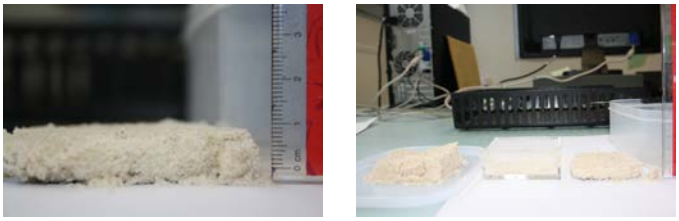
The urease test was conducted parallel with the sand injection. The culture of bacteria for the urease test is the same culture for the sand cementation. From the urease assay kit, the activity of urease for medium M63 and M63 with glutamic acid addition shows that the medium M63 supported with glutamic acid has the urease activity 2 times higher than medium M63 without glutamic acid. Urease activity is measured using the equation (5) below:

$$\text{Urease activity} = \frac{OD_{sample} - OD_{blank}}{Slope \; x \; t}(U/L) \qquad (5)$$

The urease activity for *B. subtilis* grown under medium M63 basic is 0,011574 µmol NH_3 produced/µg protein/minute. The bacteria that grown under medium M63 with glutamic acid as an inducer is 0,020699 µmol NH_3 produced/µg protein/minute. The urease activity is two times higher than *B. subtilis* that grown under M63 medium without glutamic acid support.

B. Soil Cementation

The cementation was already started in three days. Deep of the cementation is approximately 1 to 1,5 cm from the total height of the sample 2 cm. Below the cemented sand, the condition of the sand was humid. The cementation did not form homogeny inside the box of the sand. These non-uniform results would be due to the lack of the oxygen in the bottom of the boxes.



(a) (b)

Fig. 4 (a) and (b) Cementation result of Hostun sand

The treated sand then extruded from the boxes. Direct shear test was conducted to verify the result of the cementation. The weight of the sand was increased that increase the soil density. The augmentation of the weight is approximately 4% from the initial weight. This result is described in the table below.

TABLE IV
WEIGHT ADDITON AFTER TREATMENT

Box	W_{sand} (gr)	ɣ (gr/cm^3)	W_{sand} (gr)	ɣ (gr/cm^3)
Control	108.51	1.5071	108,61	1,5085
	108.28	1.5039	108,89	1,5124
	108.42	1.5058	108,76	1,5106
	108.03	1.5004	108,74	1,5103
3 days with glutamic acid	108.27	1.5038	113,99	1,5832
	108.46	1.5064	114,53	1,5907
	108.44	1.5061	114,02	1,5836
	108.25	1.5035	113,97	1,5829
3 days without glutamic acid	108.15	1.5021	113,66	1,5786
	108.07	1.5010	114,12	1,5850
	108.14	1.5019	113,86	1,5814
	108.20	1.5028	113,92	1,5822
7 days with glutamic acid	108.04	1.5006	113,45	1,5757
	108.14	1.5019	113,69	1,5790
	108.27	1.5038	113,61	1,5779
	108.27	1.5038	113,66	1,5786
7 days without glutamic acid	108.16	1.5022	113,46	1,5758
	108.09	1.5013	113,33	1,5740
	108.16	1.5022	113,61	1,5779
	108.31	1.5043	113,56	1,5772

C. Direct Shear Test

Direct shear test then conducted to obtain the soil properties after treatment. The results for three days and seven days treatment will be explained below.

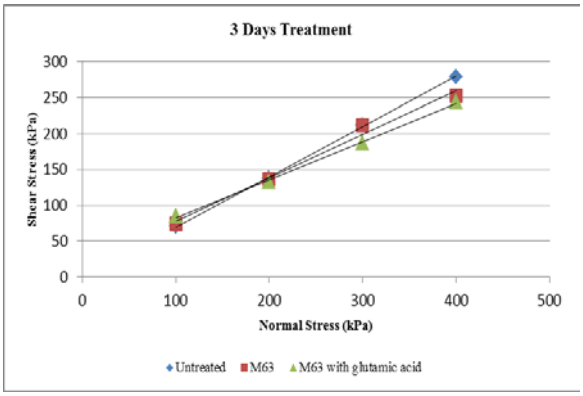


Fig. 5 Three days treatment

From the curves above, the friction angle and the cohesion of treated Hostun sand RF are:

TABLE V
SOIL TEST AFTER TREATMENT 3 DAYS

Parameters	Medium M63 basic	Medium M63 with glutamic acid
Friction Angle (°)	31,29	27,95
Cohesion	17,01	29,44

The cohesion for three days treatment has increased significantly from initial condition 0,12 kPa to 17,01 and 29,44 kPa. On the other hand, the friction angle was reduced from 34,9° for initial condition to 31,29° and 27,95°.

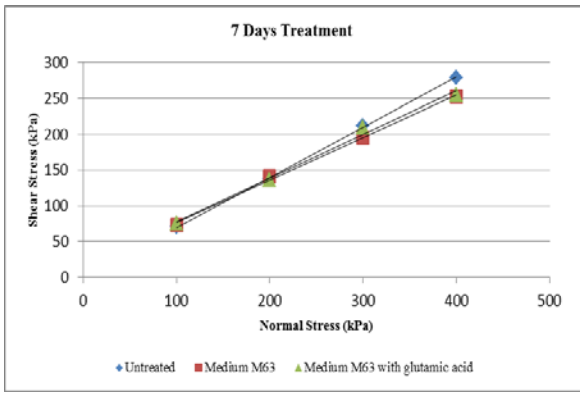


Fig. 6 Seven days treatment

From the curves above, the friction angle and the cohesion of treated Hostun sand RF are:

TABLE VI
SOIL TEST AFTER TREATMENT 7 DAYS

Parameters	Medium M63 basic	Medium M63 with glutamic acid
Friction Angle (°)	30,65	31,41
Cohesion	17,712	16,508

Compare to the untreated sand, the cohesion of the soil was increased significantly from 0,12 kPa to 16 kPa and 17 kPa kPa for 7 days treatment. The friction angle was reduced from 34,9° for initial condition to 30,65° and 31,41°.

VI. CONCLUSION

Utilization of medium M63 and medium M63 supported by glutamic acid and carbon source for growth nutrition of *B. subtilis* is an effective way to increase the production of urease enzyme. The urease activity of bacteria grown under M63 supported with glutamic acid was increased the urease activity two times higher than medium M63 which did not support by glutamic acid.

From the cementation result, *B. subtilis* was able to increase the cohesion between the sand particles. The cementation of the sand was started in three days treatment under 37°C temperature. The cementation was well performed on the surface of the sand with 1 to 1,5 cm deep.

The direct shear test shows that biological treatment is able to change the behavior of the sand to be more cohesive. The cohesion is increase significantly for three days and seven days treatment. The calcite that formed due to precipitation is fulfills the pore of the soil and increase the particle binding. Otherwise, the friction angle of the sand has slightly decreased than untreated soil.

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