Improvement of Soil Cohesion Using Microbial Acitvity

Yustian Heri Suprapto[1], Budi Susilo Soepandji[1], Wiwik Rahayu[1], and Puspita Lisdiyanti[2]
[1]*Department of Civil Engineering, Faculty of Engineering Universitas Indonesia, Indonesia*
[2]*Central Research of Biotechnology of Indonesian Institute of Sciences, Indonesia*
E-mail: yustian.heri11@ui.ac.id

ABSTRACT: Bio-cementation process or commonly known as MICP (Microbial Induced Calcite Precipitation) on soil is strongly influenced by *urease* enzyme activity. High of *urease* activity the precipitation of urea and calcium chloride into calcite particles will also increase. The *urease* enzyme is obtained from *B.subtilis* bacteria with isolate number P3BG41 and P3BG43. The bacteria was grown under B4 urine medium at 37°C and pH +7 for five days observation. The *B.subtilis* was then daily measured its optical density and *urease* activity. The bacteria and combination of urea ($CO(NH_2)_2$) and calcium chloride ($CaCl_2$) were daily injected into the sand to obtain the optimum results from the calcite precipitation. The highest value of *urease* enzyme activity occurs on the second day incubation. While the optical density was reduced on the second day, the soil cohesion reaches the highest value at that day. However, the friction angle value on the second day has the lowest point compared to the other day.

KEYWORDS: B.subtilis, Calcium chloride, Cohesion, Friction angle, Urea.

1. INTRODUCTION

The application of bacteria for soil improvement was developed in last 20 years. Starting from the concept of soil remediation to clean up the contamination inside the soil by forming clogging process inside the soil's pore (Baveye et al. 1998). The application then expands to the concept of bio-sealing for in situ scale (Van Beek, 2008). In 2010, this bio-sealing was applied at Danube River, Austria to eliminate the seepage along the river (Lambert, et al. 2005). Both concepts bio-clogging and bio-sealing are based on the ability of the bacterial to produce *urease* enzyme that used for calcite precipitation from urea and calcium chloride. This concept is also known as Microbial Induced Calcite Precipitation (MICP) or bio-cementation where the calcite particles are fulfilling the soil's pores. The species of bacteria that was used in these applications is *Sporosarcina pasteurii* (De Jong et al. 2006,2010; Ivanov and Chu, 2008). This bacteria has the ability to produce *urease* enzyme that was used to precipitate the urea and calcium chloride. Another species of bacteria that could be used in this application is *B.subtilis* that has the same ability to produce *urease* enzyme (Whiffin, 2007; Achal and Pan, 2010). This bacteria lives in the soil or alkaline source like lime soil (Achal and Pan, 2010).

2. LITERATURE REVIEW

2.1 Biochemical Aspect

B.subtilis is a non-pathogen bacteria that has ability to produce *urease* enzyme. This bacteria has a simple cell structure, rod shape, and non-motile behaviour. The *B.subtilis* that is used for application of bio-cementation must be verified its ability to grow under specific medium and its ability to produce *urease* enzyme. The bacteria with isolate number P3BG41 and P3BG43 is well known as *Oceanobacillus sp.* which has characteristic rod, motile with lateral flagella and give the positive result of urease test. This bacteria culture was isolated from Pari Island, Indonesia and stored at the collection of Indonesia Institute of Sciences. These bacteria were grown under B4 urine medium at 37°C and pH 7 (Ainiyah, et al. 2014). According to Achal and Pan (2010), the comparison of *urease* enzyme between Bacillus megaterium, Bacillus simplex, and Bacillus subtilis were concluded that the all the bacteria have reached their optimum *urease* production at 120 hours of incubation.

From the Figure 1 below, the *B.megaterium* (AP6) has the highest production of *urease* enzyme. The *B.subtilis* (AP4) has the second grade of the *urease* production rate and the *B.simplex* (AP9) has the lowest activity of *urease* enzyme.

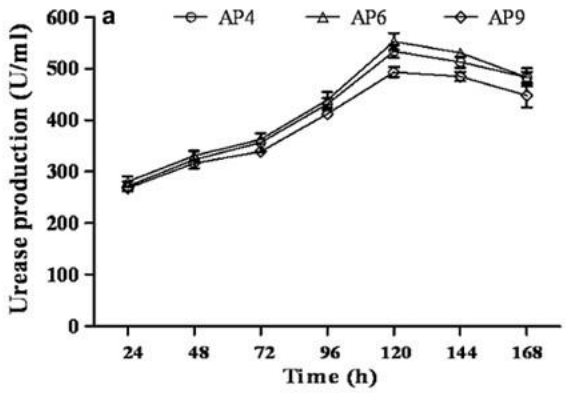

Figure 1 Result of *urease* enzyme activity from three isolate bacteria (Achal and Pan, 2010)

The fifth day of measurement (120 hours) shows that the production of calcite is reach its highest value. Therefore, to obtain high production of calcite, the bacteria must produce high activity of *urease* enzyme.

Other comparison is the calcite production between the three isolate bacteria as describe the Figure 2 below.

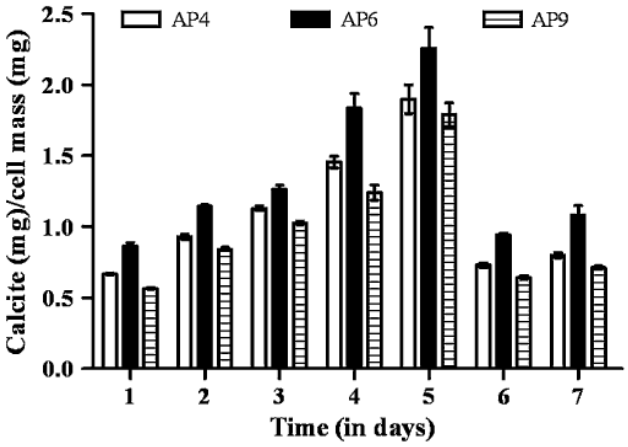

Figure 2 Production of calcite from three isolate bacteria (Achal and Pan, 2010)

The process of calcite form is the reaction between the enzyme with 2,2 M urea ($CO(NH_2)_2$) and 2,2 M calcium chloride ($CaCl_2$). The Urea and Calcium Chloride then mixed to obtain the final concentration of 1,1 M. (De Jong et al. 2006; Whiffin, 2007; van Paassen, 2009; and Achal et al. 2010).

2.2 Soil Characteristic

According to De Jong (2009), the sand particle size was 0,42 mm (D50) and the density relative (DR) is 35%. This sand is classified as loose sand (Budhu, 2010). On the other hand, an experiment by Shirikawa et al. 2011, the criteria of the sand that is used must free from organic compound and has particle size 150 – 300 µm and its porosity is 40%.

Both of the researches were using the existing soil directly without any treatment. However, Chu et al. 2011 observe a specific treatment to the sand with HCl and NaOH to obtain the pH 7 (neutral). The sand is then put into autoclave to obtain sterile condition. The treated sand then tested by using the direct shear apparatus to obtain the cohesion and the friction angle.

In this experiment, the sand was taken from Pari Island, Indonesia and mesh with maximum diameter of 0.84 mm or passing no.18 ASTM sieve and without any sterilization. This process of filtering is to separate the sand from other materials like shells and small coral, also to obtain medium size particle of sand.

3. METHODOLOGY

3.1 Biochemical Preparation

The *B.subtilis's* culture with isolate number P3BG41 and P3BG43 were grown under B4 medium and incubated at 37°C. The medium B4 is composed from minerals and urea $(NH_4)_2CO_3$. The urea contains the ammonium which may cause the calcite precipitation. The optical density as the measurement of the growth of the bacteria were daily measured using spectrophotometer. The *urease* enzyme activity is also measured daily parallel with the optical density of the bacteria. For the cementation solution is urea and calcium chloride were prepared with final concentration of 1.1 M.

3.2 Soil Preparation

The soil was prepared in the PVC pipe mould with diameter of 60 mm. This mould suits the shear box in the direct shear apparatus. The sand prepared with the unit weigh of 1.4 gr/cm^3. Before the treatment, the sand is filtered using Number 18 ASTM sieve to obtain the particle size is less than 1 mm.

The initial condition of Pari Island sand is described as figure below.

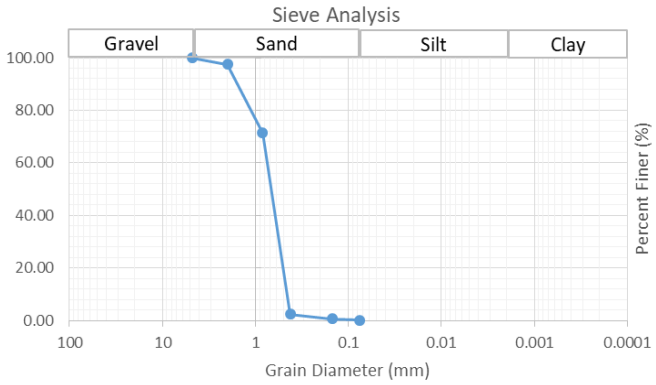

Figure 3 Sieve analysis test

From the sieve analysis above, the concentration of sand is 99% and the silt/clay is only 1%. The coefficient curvature (Cc) value is 0.89 where this sand is classified as poorly graded sand. Furthermore, the coefficient uniformity (Cu) is 1.67 shows that the size of sand particle is almost uniform.

The injection of the biochemical liquid was done after the measurement of optical density of the culture. The injection is using the pipet of 1000µL. The biochemical liquid is injected at from the surface to approximately 0,5 cm depth for 15 points of injection. After the injections were done, the sand samples then stored under ambient temperature for one month. The process of treatment is continued by using the direct shear test to obtain the mechanical parameters which is the cohesion and the friction angle.

3.3 Direct Shear Test

Direct shear test is a simple test to observe the mechanical properties of granular soil. The cohesion and the friction angle of the sand could be obtained from this test. Direct shear was done by putting specific load above the soil samples and then half of the sample height was pushed slowly to let the soil defend the shear force. Data acquisition was conducted every deformation until the deformation is constant for three times read. The load is varied from 0.8 kg, 1.2 kg, 1.6 kg, and 2 kg.

4. RESULT AND DISCUSSION

4.1 Biochemical Result

The isolate P3BG41 and P3BG43 are measured their optical density day by day during the treatment process for five days.

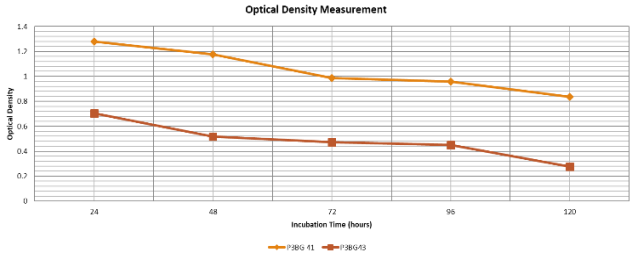

Figure 4 The optical density measurement

From the curve above, the growth of *B.subtilis* at the first day is the highest. The optical density has reached 1,279 for isolate P3BG41 and 0,703 for isolate P3BG43. The *urease* measurement result is described below.

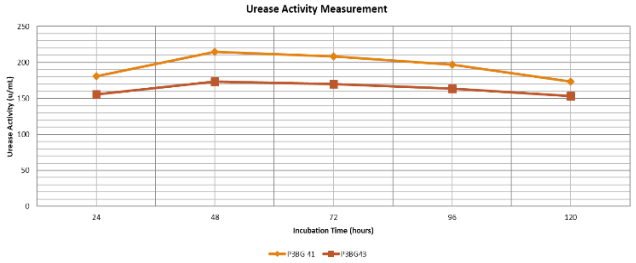

Figure 5 The *urease* activity measurement

From the curve above, the *urease* activity has occurred better at the 48 hours of incubation than the 24 hours incubation as the bacterial growth. For isolate P3BG41, the highest *urease* activity is 214.6 U/mL. The *urease* activity for isolate P3BG43 produce 173.4 U/mL of *urease* enzyme. Compare to the result of Achal and Pan, 2010 where the highest of *urease* enzyme activity has occurred at the 120 hours incubation. Hence, different strain of bacteria leads to different growth behaviour of the bacteria itself.

4.2 Sand Treatment Result

Several test to observe the initial condition of sand was conducted like sieve analysis and direct shear test. The direct shear test for initial condition is conduct to obtain the cohesion and friction angle before the treatment. From the test, the cohesion is 0 kPa and the friction angle is 31°.

The figure shows that the initial condition of sand did not have the cohesion due to the characteristic of the loose sand. The sand then treated by injecting the bacteria and the cementation liquid with composition 1:3 (bacteria:cementation liquid). From the treatment, the direct shear test was conducted after a month storage under ambient temperature.

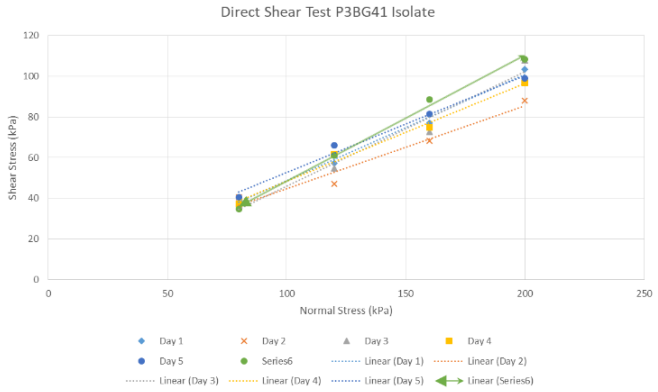

Figure 6 Direct shear test result for P3BG41 isolate

From the direct shear test, the cohesion at the second day is 7.9 kPa where this is the highest value during the treatment. On the contrary, the friction angle at the second day is the lowest value. The friction angle is only 19 º. The change of cohesion from 0 kPa to 7.9 kPa shows that the behaviour of the sand is more cohesive than the initial condition.

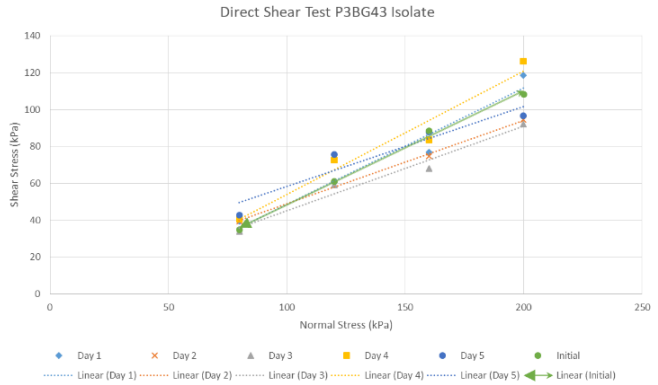

Figure 7 Direct shear test result for P3BG43 Isolate

The cohesion at the second day incubation is 5.14 kPa. This value is lower than the isolate P3BG41. But, the friction angle is the lowest among the isolate P3BG43. The sand behaviour is less cohesive than the treatment using isolate P3BG41.

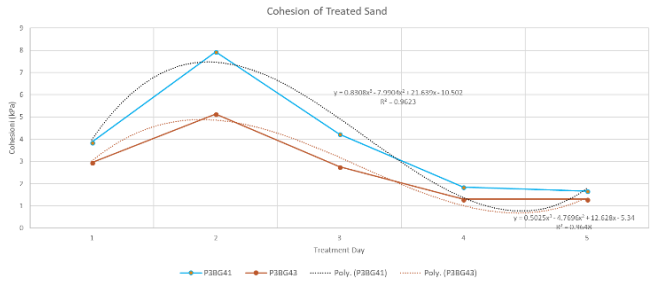

Figure 8 Cohesion result of the treatment

The cohesion at the second day incubation is 5.14 kPa. This value is lower than the isolate P3BG41 7.93 kPa. But, the pattern shows that the cohesion at the second day treatment is highest and the third to the fifth day treatment is reduced until 1.3 kPa.

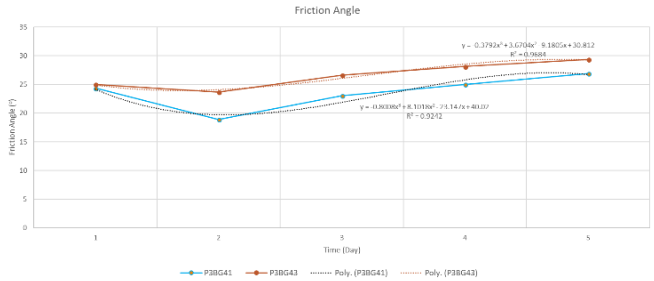

Figure 9 Friction angle result of the treated sand

The friction angle for P3BG41 isolate is varying from 18º to 26º. on the other hand, for P3BG43 isolate, the friction angle is varying from 23º to 29º. Hence, from Figures 8 and 9 it can be concluded that the value of cohesion and the friction angle is antithetical.

5. CONCLUSION

From the result of *urease* enzyme activity, the isolate P3BG41 has the higher activity than isolate P3BG43. But, the day of incubation to obtain the highest activity is on the second day of incubation. The cohesion results at the 48 hours of incubation is the highest from other duration of incubation. On the other hand, the friction angle is the lowest value at the second day incubation compare to another duration of incubation. Hence, the cohesion value of treated sand is the opposite of its friction angle value. High *urease* activity lead to the cohesion of the sand, but also may reduce the friction angle.

6. ACKNOWLEDGEMENTS

The experimental laboratory was conducted in collaboration between Universitas Indonesia and Indonesia Institute of Sciences. This article's publication is partially supported by the United States Agency for International Development (USAID) through the Sustainable Higher Education Research Alliance (SHERA) Program for Universitas Indonesia's Scientific Modeling, Application, Research and Training for City-centered Innovation and Technology (SMART CITY) Project, Grant #AID-497-A-16-00004, Sub Grant #IIE-00000078-UI-1.

7. REFERENCES

Achal, V., and Pan, X. (2010) “Characterization of *Urease* and Carbonic Anhydrase Producing Bacteria and Their Role in Calcite Precipitation”, Springer Science + Business Media.

Ainiyah, S., Lisdiyanti, P., Pertiwi, M., and Prasetyo, E. N. (2016) Biogrouting: Produksi *Urease* dari Bakteri Laut (Oceanobacillus sp.) Pengendap Karbonat. Biota Vol. 21(1). ISSN 0853-8670.

Baveye, P., Vandevivere, P., Hoyle, B. L., DeLeo P. C., and de Lozada, D. S. (1998) “Environmental impact and mechanisms of the biological clogging of saturated soils and aquifer materials”, Crit Rev Environ Sci Technol 28, pp123–191 .

Budhu, M. (2010) Soil Mechanics and Foundation 3rd Edition

Chu et al. (2011) "Biocalcification of Sand Through Ureolysis”

Craig R. F. (2004) Craig’s Soil Mechanics.

DeJong Jason, T., Fritzges Michael, B., Nusslein Klaus (2006) “Microbially Induced Cementation to Control Sand Response to Undrain Shear”.

Ivanov, V., Chu, J. (2008) “Applications of microorganisms to geotechnical engineering for bioclogging and biocementation of soil in situ”, Springer Science+Business Media B.V.

Laboratorium Teknik Penyehatan dan Lingkungan, Universitas Indonesia. 2009. Modul Praktikum Mikrobiologi Lingkungan.

Lambert, J., Veenbergen, V., Ross, N. (2005) “Biosealing to reduce risks in tunnelling”, Tunn Tunn Int, 37: pp34–35, 37.

Mahanty, B., Kim, S., Kim, C.G. (2013) “Biocalcification Mediated Reduction of PAHs Bioavailability in Artificially Contaminated Soil”. Springer Sciences + Business Media Dordrecht.

Martinez, B. C., DeJong, J. T. (2009) "Bio-Mediated Soil Improvement Load Transfer Mechanisms at the Micro- and Macro- scales".

Mitchell, J. K., Santamaria, J. C. (2005) "Biological Considerations in Geotechnical Engineering".

Shirakawa, M. A., Kaminishikawahara, K. K., et.al. (2011) "Sand Bioconsolidation Through the Precipitation of Calcium Carbonate by Two Ureolytic Bacteria", Mater Lett. Elsevier.

Stocks-Fischer, S., Galinat, J. K., Bang, S.S. (1999) "Microbial Precipitation of CaCO3".

van Beek, V. M., Lambert, J. W. M., Blauw, M., et al. (2008) "Application of bi-osealing for saltwater seepage reduction", 10th International Confer-ence on Soil-Water System, Milano.

van Paassen, L. A., et al. (2009) "Potential Soil Reinforcement by Biological Denitrification".

Whiffin, V, (2007) "Microbial CaCO3 Precipitation".