

# Applicability of Soybean Crude Urease-Calcite Precipitation Method in Various Liquefiable Sandy Soils

Akbar Renaldi Loebis<sup>1\*</sup>, Heriansyah Putra<sup>2</sup>

<sup>1</sup>Special Course on Disaster Mitigation Study for Asian Students, Graduate School of Science and Engineering, Ehime University, Matsuyama, Japan, 790-8577; akbar.renaldi.loebis.22@cee.ehime-u.ac.jp.
<sup>2</sup>Department of Civil and Environmental Engineering, Faculty of Agriculture Engineering and Technology, IPB University, Bogor, Indonesia, 16680; heriansyahptr@apps.ipb.ac.id
\*Correspondence: akbar.renaldi.loebis.22@cee.ehime-u.ac.jp

SUBMITTED 1 March 2023 REVISED 26 April 2023 ACCEPTED 18 May 2023

**ABSTRACT** Liquefaction is a process in which pore-water pressure generated in the soil, usually by earthquake, is equal or almost equal to the total stress in the soil. This process reduces the effective stress to zero or near-zero, leading to reduction in shear strength and stiffness of soil. Hence, when liquefaction occurs, structures above and within the liquified soil, buildings, bridges, tunnels, docks, underground pipelines, and many other structures can get damaged. Soils which are susceptible to liquefaction are granular soil such as sand and silt. One of the methods that can be adopted to prevent liquefaction is soybean crude urease-calcite precipitation. This method uses urease enzyme in soybean crude as a biocatalyst to hydrolyze urea to produce carbonate ions and ammonium ions. In the presence of calcium ions, the carbonate ions react to produce calcium carbonate (calcite) precipitate. This research investigates the applicability of soybean crude ureasecalcite precipitation method to improve various liquefiable sandy soil (fine, medium, coarse sand) at different relative density (40-60%). The soil strength after improvement was evaluated by using unconfined compressive strength (UCS) test, while calcite content was measured using acid leaching method. The results of the UCS test and calcite content analysis varies depending on the type of sand and relative density. The UCS value obtained from medium sand is higher than 50 kPa, potentially sufficient to prevent liquefaction. However, treatment on coarse sand failed to develop UCS, whereas treatment on fine sand produces limited UCS (< 10 kPa).

KEYWORDS Liquefaction; Calcite; Soil gradation; Relative density

# **1 INTRODUCTION**

Liquefaction generally happens when fine granular soil, such as sandy soil and silt, is subjected to strong enough earthquake. Liquefaction can cause damage to buildings, bridges, tunnels, docks, underground pipelines, and many other structures. Some examples of past liquefaction disasters were Niigata 1964, Alaska 1964, Tangshan 1976, Wenchuan 2008, Darfield 2010, Tohoku 2011, Padang 2009, and Palu 2018 (Hakam and Darjanto, 2013; Xu *et al.*, 2020). These disasters could have been prevented with soil improvement techniques.

One of the soil improvement techniques that can be adopted to treat liquifiable soil is calcite precipitation method. This method uses urease enzyme as a biocatalyst to hydrolyze urea to produce carbonate ions and ammonium ions. In the presence of calcium ions, carbonate ions react to produce calcium carbonate or calcite precipitate (Yasuhara *et al.*, 2012). The urease enzyme can be injected into liquefiable soil, and the produced calcite precipitate can act as a bridge between the soil grains, improving the strength of the soil. Previous work conducted by the co-author shows unconfined compressive strength (UCS) of treated soils ranging from 0.2 to 1.6 MPa (Putra et al., 2020).

However, laboratory-manufactured urease enzyme is expensive and can contribute up to 90% of the soil improvement expenses. To reduce the cost, cheaper means to obtain urease enzymes need to be sought. Previous research has reported the potential of using urease enzymes contained in soybean

crude (soybean oil/solution) (Dilrukshi, et al., 2018; Gao et al., 2019; Baiq et al., 2020; Lee and Kim, 2020; Pratama et al., 2021). This method is called the soybean crude urease-calcite precipitation method, and this method has been shown to improve the strength of treated fine-grained soil by 65 to 870 kPa (Lee and Kim, 2020; Pratama et al., 2021).

In previous studies, soybean mixture (soybean powder or crushed soybean mixed with water) was purified using centrifugation technique to separate the soybean crude from undissolved soybean (Dilrukshi et al., 2018; Gao et al., 2019; Baiq et al., 2020; Lee and Kim, 2020; Pratama et al., 2021). However, this technique takes a long time to separate sufficient amount for soil treatment, thus not feasible. Another technique that can be used to extract the soybean crude was the filter technique (Lofianda et al., 2021; Loebis and Putra, 2022). Soybean crude extracted by the filter technique had been successfully implemented to treat soil by Loebis and Putra (2022). They reported UCS of soybean crude-treated soils as well as the percentage of calcite content produced. The UCS varied from 59-172 kPa with calcite content ranging from 1.3 to 6.1%. Lofianda et al. (2021) conducted tests to compare the effectiveness of soybean crude extracted by centrifugation vs filtration technique. Soybean powder was initially mixed with water to produce soybean mixture at varying concentration (10-50 g/L). After extracting the soybean crude, it was mixed with urea and calcium chloride dihydrate to produce calcium carbonate precipitate. It was found that the soybean crude extracted by filtration technique produces the most precipitate when using soybean mixture of 20 g/L concentration, while for centrifugation technique, soybean mixture of 40 g/L concentration was optimum. The soybean crude obtained from 20 g/L mixture separated by each respective technique was used to treat sand. After 28 days of curing, the UCS of treated sand by filtered soybean crude was 53.6 kPa, while the one treated by centrifugation soybean crude was 65.6 kPa. Despite the lower UCS obtained by filtration technique, the results show a potential for utilizing soybean crude obtained by filtration technique to treat sandy soil.

In the previous work by Lofianda et al. (2021), the focus of the tests was to find the ideal separation technique as well as concentration of soybean mixture. Only a single sand gradation was tested. To expand field applicability of soybean crude urease-calcite precipitation method to treat liquefiable soil, further research on sand with varying gradations and relative densities is needed.

Previous studies that have investigated the effects of particle size on the mechanical property of biotreated sand foundations used microbially induced calcite precipitation (MICP) technique. The results show that calcite distribution was more uniform for sand with larger particle size. This was due to the solution being able to flow easier when the pore volume is larger (Yang et al., 2020). The bacteria used as bio-catalyst in the MICP method has a size of 500-5000 nm. Thus, due to the large size of bacteria, MICP method is more effective when used in coarse-grained soils, and less effective in fine-grained soils (Hamdan et al., 2013). Unlike the MICP method, urease enzyme molecule is much smaller, with a size of 12 nm. Therefore, urease enzyme has the potential to be applicable even in fine-grained compacted soil (Blakely and Zerner, 1984; Saif et al., 2022).

This research focuses on soybean crude-urease calcite precipitation method on treating various liquefiable sandy soil. The sand gradations and relative densities were varied from fine, medium course and 40-60% respectively. The soil strength of treated soil was evaluated using UCS test and its calcite content was tested using acid leaching method.

# 2 MATERIALS AND METHODS

# 2.1 Preparation and Properties of Sand Specimen

Potentially liquefiable sand with various gradations was used in this research. Soil samples of different gradations were prepared by separating the soil using sieve number 30 for fine sand (0.075-0.600 mm), sieve number 16 for medium sand (0.600-1.180 mm), and sieve number 8 for coarse sand (1.180-2.360 mm mm). Figure 1 shows the photograph of the prepared sand specimen. Based on Unified Soil Classification System (USCS) these sands were classified as poorly graded sands (SP) (ASTM, 2006). The soil gradation curve is shown in Figure 2. Based on the boundaries of

liquefiable soil gradation chart by Tsuchida (1970), fine sand has the greatest potential for liquefaction, followed by medium sand, and lastly coarse sand has the lowest potential for liquefaction. The properties of sand specimen are presented in Table 1.



Figure 1. Sand used in this research, (a) fine sand, (b) medium sand, and (c) coarse sand.



Figure 2. Soil gradation curve of fine, medium, and coarse sand. Note: potential and very potential line refers to the liquefaction potential by Tsuchida (1970).

Parameters	Fine sand	Medium sand	Coarse sand	
D <sub>10</sub> (mm)	0.40	0.60	0.75	
D <sub>50</sub> (mm)	0.60	0.89	0.95	
e <sub>max</sub>	0.82	0.83	0.85	
e <sub>min</sub>	0.57	0.59	0.60	
Cu	1.75	1.53	1.33	
Gs	2.63	2.64	2.65	
$\rho_{\rm dmax}$ (g/cm <sup>3</sup> )	1.67	1.66	1.65	
$\rho_{\rm dmin}$ (g/cm <sup>3</sup> )	1.45	1.44	1.43	

Table 1. The properties of sands

Each gradation of sand was prepared in a cylindrical mold with a height and diameter of 10 cm and 5 cm respectively. The sand was poured using a funnel with different falling height to obtain relative densities ( $D_R$ ) of 40, 50, and 60%. The difference in gradation and relative density affects the void volume of sand in the mold sample. The void volume of sand in each mold sample is shown in Table 2.

Tuble 2. The volu volume of suite in mole sumples					
$D_{R}(\%)$	Fine sand (cm <sup>3</sup> )	Middle sand (cm <sup>3</sup> )	Coarse sand (cm <sup>3</sup> )		
40	82.10	83.30	84.20		
50	80.45	81.69	82.60		
60	78.75	80.03	80.95		

Table 2. The voi	d volume of sand	in mold	samples
------------------	------------------	---------	---------

#### 2.2 Soil Treatment

The procedure to prepare soybean crude urease-calcite precipitation solution was adopted from Putra *et al.*, (2021). The materials used to make soybean crude urease-calcite precipitation solution were calcium chloride (CaCl<sub>2</sub>), urea (CO(NH<sub>2</sub>)<sub>2</sub>), and dry soybean. First, dry soybean purchased from a local market was grilled, then grinded to powder form (0.1-0.5 mm). The soybean powder was then mixed with distilled water at 20 g/L concentration, the optimum concentration for filter technique based on Lofianda et al., 2021). After 5 minutes of stirring with magnetic stirrer, the soybean was filtered using sieve number 400 (38  $\mu$ m). The filtered solution is the soybean crude. For the reagent solution, calcium chloride (CaCl<sub>2</sub>) and urea (CO(NH<sub>2</sub>)<sub>2</sub>) with purity levels > 95% from Kanto Chemical Co. Inc were mixed and stirred for 5 minutes with distilled water. The reagent solution was prepared at a concentration of 1 mol/L. Subsequently, 50 ml of the soybean crude and 50 ml of reagent solutions were combined, totaling to 100 ml. The solution was added into the sample by soil percolation, followed by 7 days of curing.

Thereafter, the unconfined soil strength was then evaluated by UCS test and calcite content by acid leaching method. After curing time, the sample was retrieved by pulling out the sample from the mold and the UCS test was conducted directly on the sample. After the UCS test, the sample was placed in a beaker and distilled water was added to the sample to dissolve the ammonium chloride (NH<sub>4</sub>Cl) produced. The distilled water was then drained out. After that, the samples were dried in an oven at 100°C for 1 day. Then, 25 grams of each dried sample are placed on a filter paper to be washed repeatedly using 0.1 mol/L HCl solution until there were no more bubbles. After the HCl washing process, the sample was then dried again in an oven at 100°C for 1 day. The final dry weight of the sand in the beaker and filter paper was measured and the reduction in dry weight was assumed to be the weight of calcite formed in the soil. The procedure of the sample preparation, reagents preparation, as well as sample testing are summarized in Figure 3.



Figure 3. The procedure of soybean crude urease-calcite precipitation soil treatment method in this research (Putra *et al.*, 2016, 2021).

### **3 RESULTS AND DISCUSSION**

The calcite content value is defined as the percentage ratio of the mass of calcite formed in the soil to the mass of the soil (Putra *et al.*, 2017). The UCS test and calcite content results are shown in Figure 4.



Figure 4. The UCS test and calcite content results

The test results for different sand gradations and relative densities show different values of soil strength and calcite content. The soil strength values of fine sand with relative densities 40, 50, and 60% are 6, 9, and 6 kPa, respectively. For medium sand with relative densities 40, 50, and 60%, the UCS are 53, 53, and 47 kPa, respectively. The UCS test cannot be conducted on coarse sand. Based on soil gradation, it shows that medium sand has the highest soil strength value and coarse sand has the lowest soil strength value compared to other sands. Based on relative density, it shows that higher relative density is associated with a decrease in soil strength values.

The calcite content values of fine sand with relative densities 40, 50, and 60% are 4.33, 6.14, and 6.43% respectively, medium sand with relative densities 40, 50, and 60% are 1.84, 1.91, and 1.97% respectively, and coarse sand with relative density 40, 50, and 60% are 1.21, 1.31, and 1.58% respectively. It can be seen that fine sand has the highest calcite content value, while coarse sand has the lowest calcite content value. It can also be seen that higher relative density leads to an increase in the calcite content value. This result is very different compared to the MICP method by Xiao et al. (2020). They reported that coarser sand produced higher calcite content , while finer sand produced lower calcite content value. Tsukamoto et al. (2013) also reported that the calcite content increases as the relative density of soil decreases. This is due to the size of bacteria, which is 230 times bigger than the urease enzyme molecule, restricting the flow of the bacteria (bio-grouting) solution from entering smaller pore size of soil (Saif et al., 2022).

It is shown that soil strength and calcite content evaluation vary with sand gradation and relative density. This is because the void volume of the different sand samples (Table 2) affects the pattern of calcite formation, which in turn affects the soil strength and calcite formed. Neupane et al., (2015) reported that the pore size of sand influences the uniformity of calcite distribution in the sand. Yang et al., (2020) also stated that higher pore size in soil enables bio-grouting solution to pass through easier, resulting in uniform calcite distribution in the soil.



Figure 5. Fine sand in different relative densities after 7 days of curing



Figure 6. Medium sand in different relative densities after 7 days of curing

The effect of the void volume value of the sand variation in the mold sample can be visually seen from Figure 5 and 6. Figures 5 and 6 show the appearance of the sample after being extracted from the mold. Differences in gradation and relative density affect the permeability of the sand samples, affecting the infiltration of soybean crude urease-calcite precipitation solution. These differences in turn affects the position of wet and dry sides in each sample. The wet side is relatively more brittle than the dry side, thus the larger area of the wet side of fine sand samples resulted in smaller soil strength value. Fine sand has a larger wet side area compared to medium sand, as a result fine sand has a smaller soil strength value compared to medium sand.

The calcite content evaluation results showed that void volume caused by sand variation (gradation and relative density) affects calcite formation in the sand. Fine sand has a smaller void volume and grain size diameter compared to medium sand and coarse sand Therefore, fine sand has the highest calcite content than medium sand and coarse sand and coarse sand has the smallest calcite content than fine sand and medium sand. Xiao *et al.*, (2020) stated that smaller void volume creates small space between the soil grains, thus trapping more bio-grout solution in the soil. Hence, higher calcite content obtained compared to sample with larger void volume. Pan *et al.*, (2020) also reported that larger void volume means larger gap between soil grains, thus the calcite formed cannot bind the soil grains easily. Thus, less calcite content obtained compared to sample with least calcite content.

The UCS of soil treated by calcite precipitation method is one of the key indicator to prevent liquefaction. Kawasaki and Akiyama, (2013) reported a value of 50-100 kPa (equivalent to medium sand) in UCS can prevent liquefaction disaster during an earthquake. The UCS values of treated fine sand and coarse sand are lower than 50 kPa. However, there is still calcite formed in fine sand and

coarse sand (fine sand: 4.33-6.43%; coarse sand: 1.21-1.58%). This means that the soybean crude urease-calcite precipitation method can be used to prevent medium sand from liquefaction. Further research is needed to find out the better method to increase the UCS of fine sand and coarse sand.

# 4 CONCLUSIONS

Applicability of the soybean crude urease-calcite precipitation method to improve various liquefiable sandy soil has been conducted. The soil samples tested were fine sand separated by aggregate sieve number 30 (0.075-0.300 mm), medium sand by sieve number 16 (0.075-1.180 mm), and coarse sand by sieve number 8 (0.600-1.180 mm).

The UCS of treated medium sand shows the largest UCS of > 50 kPa, with higher relative density giving higher UCS. Treated fine sand exhibits low UCS of less than 10 kPa, whilst treated course sand fails to develop any UCS. Calcite content test results show that fine sand produced the most calcite, followed by medium sand and course sand. Despite the highest calcite produced for fine sand, the UCS of find sand is lower than medium sand. Likewise, course sand does not exhibit any UCS despite the calcite which had formed. UCS of > 50 kPa has been shown to be sufficient to prevent earthquake-induced liquefaction. Meaning, treated medium sand has sufficient strength to resist liquefaction. Whereas more research is needed to investigate why the calcite formed in treated fine sand and course sand exhibits little to no UCS.

# DISCLAIMER

The authors declare no conflict of interest.

# AVAILABILITY OF DATA AND MATERIALS

All data are available from the author.

## ACKNOWLEDGMENTS

The research described in this paper was financially supported by the Minister of Education, Culture, Research, and Technology of the Republic of Indonesia, grant number 1584/IT3.D10/PT.01.02/P/T/2023. Their support is gratefully acknowledged.

# REFERENCES

ASTM, 2006. Standard practice for classification of soils for engineering purpose (unified soil classification system). ASTM D:2487-06, Pennsylvania (US).

Baiq, H.S., Yasuhara, H., Kinoshita, N., Putra, H. and Johan, E., 2020. Examination of calcite precipitation using plant-derived urease enzyme for soil improvement *GEOMATE* Journal, 19(72), pp. 231–237.

Blakely, R.L. and Zerner, B., 1984. Jack bean urease: the first nickel enzyme, *Journal of Molecular Catalysis*, 23(2–3), pp. 263–292.

Dilrukshi, R., Nakashima, K. and Kawasaki, S., 2018. Soil improvement using plant-derived ureaseinduced calcium carbonate precipitation. *Soils and Foundation*, 58(4), pp. 894–910.

Gao, Y., He, J., Tang, X. and Chu, J., 2019. Calcium carbonate precipitation catalyzed by soybean urease as an improvement method for fine-grained soil. *Soils and Foundation*, 59(5), pp. 1631–1637.

Hakam, A. and Darjanto, H., 2013. Penelusuran potensi likuifaksi Pantai Padang berdasarkan gradasi butiran dan tahanan penetrasi standar. *Jurnal Teknik Sipil ITB*, 20(1), pp. 33–38.

Hamdan, N., Kavazanjian, E. and O'Donnell, S., 2013. Carbonate cementation via plant derived urease. *Proceedings of the 18th International Conference on Soil Mechanics and Geotechnical Engineering*. Paris, France, pp. 2489–2492.

Kawasaki, S. and Akiyama, M., 2013. Effect of addition of phosphate powder on unconfined compressive strength of sand cemented with calcium phosphate compound. *Materials Transactions*, 54(11), pp. 1–6.

Lee, S. and Kim, J., 2020. An experimental study on enzymatic-induced carbonate precipitation using yellow soybeans for soil stabilization. *KSCE Journal of Civil Engineering*, 24(7), pp. 1–12.

Loebis, A.R. and Putra, H., 2022. Efektivitas metode calcite precipitation dengan biocatalyst bubuk kedelai sebagai metode biogrouting untuk mencegah likuifaksi tanah pasir. *TERAS JURNAL: Jurnal Teknik Sipil*, 12(1), pp. 1–14.

Lofianda, L., Putra, H., Erizal, S. and Yasuhara, H., 2021. Potentially of soybean as bio-catalyst in calcite precipitation methods for improving the strength of sandy soil. *Civil Engineering and Architecture*, 9(7), pp. 2317–2325.

Neupane, D., Yasuhara, H., Kinoshita, N. and Putra, H., 2015. Distribution of grout material within 1-m sand column in insitu calcite precipitation technique. *Soils and Foundation*, 55(6), pp. 1512–1518.

Pan, X., Chu, J., Yang, Y. and Cheng, L., 2020. A new biogrouting method for fine to coarse sand. *Acta Geotech*, 15(1), pp. 1–16.

Pratama, G.B.S., Yasuhara, H., Kinoshita, N. and Putra, H., 2021. Application of soybean powder as urease enzyme replacement on EICP method for soil improvement technique. In *IOP Conference Series: Earth and Environmental Science*, pp. 1–7.

Putra, H., Yasuhara, H., Kinoshita, N., Neupane, D. and Lu, C.W., 2016. Effect of magnesium as substitute material in enzyme-mediated calcite precipitation for soil-improvement technique. *Frontiers in Bioengineering and Biotechnology*, 4, pp. 1–9. https://doi.org/10.3389/fbioe.2016. 00037.

Putra, H., Yasuhara, H., Kinoshita, N. and Hirata, A., 2017. Application of magnesium to improve uniform distribution of precipitated minerals in 1-m column specimens. *Geomechanics and Engineering*, 12(5), pp. 803–813.

Putra, H., Yasuhara, H., Erizal, Sutoyo and Fauzan, M., 2020. Review of enzyme-induced calcite precipitation as a ground-improvement technique. *Infrastructures*, 5(5), pp. 1–14.

Putra, H., Erizal, Sutoyo, Simatupang, M. and Yanto, D.H.Y., 2021. Improvement of organic soil shear strength through calcite precipitation method using soybeans as bio-catalyst. *Crystals*, 11(9), pp. 1–14.

Saif, A., Cuccurullo, A., Gallipoli, D., Perlot, C. and Bruno, A.W., 2022. Advances in enzyme induced carbonate precipitation and application to soil improvement: a review. *Materials*, 15(950), pp. 1–30.

Tsuchida, H., 1970. Evaluation of liquefaction potential of sandy deposit and measures against liquefaction induced damage. *Proceedings of the Annual Seminar of the Port and Harbour Research Institute*, pp. 1–3.

Tsukamoto, M. and Oda, K., 2013. Influence of relative density on microbial carbonate precipitation and mechanical properties of sand. in *Proceedings of the 18th International Conference on Soil Mechanics and Geotechnical Engineering*, Paris, pp. 2613–2616.

Xiao, Y., Zhao, C., Sun, Y., Wang, S., Wu, H., Chen, H. and Liu, H., 2021. Compression behavior of MICP-treated sand with various gradations. *Acta Geotech*, pp. 1–10.

Xu, C., Feng, C., Du, X. and Zhang, X., 2020. Study on liquefaction mechanism of saturated sand considering stress redistribution. *Engineering Geology*, 264, pp. 1–11.

Yang, D., Xu, G. and Duan, Y., 2020. Effect of particle size on mechanical property of bio-treated sand foundation. *Applied Science*, 10(22), pp. 1–16.

Yasuhara, H., Neupane, D., Hayashi, K. and Okamura, M., 2012. Experiments and predictions of physical properties of sand cemented by enzymatically-induced carbonate precipitation. *Soils and Foundations*, 52(3), pp. 539–549.

- This page is intentionally left blank -