The use of eggshell powder as calcium source in stabilizing expansive soil using *Bacillus subtilis*

M Sugata¹, J Widjajakusuma²*, A Augestasia², A Zacharia² and T J Tan¹

¹ Department of Biology, Universitas Pelita Harapan, Tangerang, Indonesia
² Department of Civil Engineering, Universitas Pelita Harapan, Tangerang, Indonesia

*Corresponding author: jack.widjajakusuma@uph.edu

Abstract. Expansive soils are commonly characterized by low strength and high swelling potential. Thus, to use this type of soil as foundation, stabilization process is needed. This research aimed to improve the stability of expansive soils by using *Bacillus subtilis*. The bacteria have been reported for its ability to form calcium carbonate precipitation which could bind soils particles, hence, increase the soil strength. In this study, the bacteria were grown in medium containing eggshell as calcium source. Then, the bacterial liquid culture was injected into expansive soil samples, followed by curing period of 30, 60 and 90 days. The swelling potential of the stabilized soil samples were evaluated with free swell index (FSI) test. Meanwhile, the soil strength was analysed with unconfined compressive strength (UCS) as well as direct shear (DS) tests. The results showed that *Bacillus subtilis* could use eggshell as calcium source to improve the stability of expansive soils. The use of higher bacterial cell concentration and longer curing period showed higher improvement in soil strength. After 90 days of curing period, there was a 30% decrease in the FSI, 74.32% increase in the UCS, and 77.27% increase in the cohesion of soil samples.

1. Introduction

Since construction has been growing rapidly in Indonesia, the use of problematic soils as foundation is inevitable. Expansive soil, which is one of the problematic soils, is mainly characterized by its high swelling potential [1]. In the presence of water, expansive soils could swell and cause serious damage to structures. Therefore, stabilization of expansive soils must be done before any construction is started. Conventionally, soil improvement is done by the application of chemical grouting, such as cement, lime and resins. Although many of those additives have proven successful and economically benefit, chemical grouting is not environmentally friendly. The additives could alter the pH of soils and contaminate the groundwater [2]. With increasing awareness of environmental issues, a soil improvement method based on biological process has emerged.

In nature, biomineralization is a common phenomenon when mineral precipitations are formed by microbial activities. Among various mechanisms involved in the production of biominerals, microbiobally induced calcite precipitation (MICP) has gained interest from engineers and microbiologist. The presence of microbial cells and their biochemical activities results in supersaturated solution which leads to the formation of calcium carbonate. During MICP, microbes secrete metabolic products such as carbonate (CO$_3^{2-}$) that could react with calcium ions (Ca$^{2+}$) in the environment and form mineral precipitations [3, 4]. Those precipitates are considered as biogrout that could bind soil particles and strengthen the soils.

The amount of calcite precipitation produced by microbes depends on several factors, including bacteria type, cell concentration, pH, temperatures and calcium concentration [5, 6]. In the past years,
several studies have employed various factors that affecting calcite precipitation in soil stabilization. Lee et al. [7] employed Bacillus megaterium on silty residual soil and found that higher concentration of calcium chloride resulted in higher soil shear strength. Widjajakusuma et al. [8] found that the longer curing period and the more Bacillus subtilis cells injected to the soil, the higher soil strength was obtained.

Most previous studies usually used calcium salts such as calcium chloride and calcium acetate. However, the use of those calcium salts in large amount is very expensive. To lower the cost, many waste materials containing calcium could be used. Choi et al. [9] reported that the application of Bacillus sp. on sand soils using calcium source from eggshell could increase compressive strength and decrease permeability of the soil. Studies related to soil stabilization using Bacillus sp. and various calcium sources on different type of soils are still very limited. Therefore, the present experimental work evaluated the stabilization of expansive soil using Bacillus subtilis as biogrouting agent and eggshell as calcium source.

2. Materials and Method

2.1. Soil samples characteristics
Soil samples were obtained from Cikarang Selatan, Indonesia. According to Unified Soil Classification System (USCS), the soil samples were CH type soil or inorganic clay soil with high plasticity. This type of soil could experience considerable volumetric deformation due to the alteration in moisture content [1]. To check the characteristics of the soil sample before and after curing period, analysis using X-Ray Diffraction (XRD) and Scanning Electron Microscope (SEM) were carried out.

2.2. Pre-treatment of eggshell
Eggshell was obtained as a waste from a bakery at Pasar Modern BSD City, Tangerang Selatan. Eggshells contain calcium carbonate which could not dissolve in water with neutral pH. Thus, acid solution must be used to dissolve calcium ion from eggshells [9]. After washed with tap water, the eggshells were incubated at 105°C for 24 h. The dried eggshells were then crushed into smaller flakes, followed by sieving using sieve number 20 and number 200. The flakes that passed sieve number 20 and held by sieve number 200 were soaked in 5% vinegar solution and incubated with 100 rpm agitation at 37°C. After 3 days, the mixture was filtered using filter paper to separate the flakes and liquid containing calcium ion. The liquid was dried at 118°C for 4 h until calcium rich flour was obtained (Figure 1). According to the analysis result from PT Saraswati Indo Genetech, the concentration of calcium ion in the obtained flour was 1361.77 mEq/l.

![Figure 1. Pre-treatment of eggshell. (A) Crushed eggshell, (B) Crushed eggshell soaked in 5% vinegar solution, (C) Eggshell flour.](image_url)

2.3. Bacterial strain and cultivation
Bacillus subtilis SM10.1 was obtained from Basic Biology Laboratorium, Biology Department, Universitas Pelita Harapan. The bacterial strain is basil Gram positive and endospore forming. It was maintained constantly on 85% glycerol solution and were preserved in deep freezer (-80°C). Prior experiment, the bacteria was grown in medium containing (per liter) 4 g of yeast extract, 5 g of dextrose, 2.5 g of eggshell. The culture was incubated at 37°C with 100 rpm agitation for 24 h.

2.4. Measurement of calcium concentration
A modified method of Wei et al. [10] was applied in this experiment. The liquid bacterial cultures were centrifuged for 3 min at 8,000 xg. The pellets containing calcium carbonate precipitates and bacterial cells were then re-suspended using 200 µl TE buffer (10 mM Tris, 1 mM EDTA pH 8.5). Lysozyme (1 mg/ml) was added to the suspended samples, followed by incubation at 37°C for 1 h. The mixtures were then centrifuged to separate the cell debris from the calcium carbonate precipitates. The supernatant were discarded and dH$_2$O (pH8.5) was added to wash the pellets, which were then air-dried at 37°C for 24 h. The dried pellets were then weighted. To ensure the presence of calcium carbonate qualitatively, NaOH and HCl was added separately to the dried pellet. Calcium carbonate precipitates are not dissolved in alkali solution. To measure calcium carbonate concentration, titration method was used. The dried pellet was dissolved in two drops of 5 N HCl and then heated using hot plate until the solution boiled. pH of the solution was then adjusted to 6.0 - 8.0 and two drops of maurexide (pH indicator) was added. Titration was done using 0.01 M Na-EDTA until the colour of the solution changed from red to purple. The volume of Na-EDTA needed to change the colour was used in the calculation of calcium concentration.

2.5. Biogrouting stabilization on expansive soils

The medium containing overnight grown bacterial cultures was measured for its optical density using spectrophotometer at 600 nm. The bacterial liquid culture with different cell concentration was then injected to expansive soil specimens, followed by curing period of 30, 60, 90 days at room temperature. After curing period, the treated soil samples were tested for its swelling potential with free swell index (FSI) and its strength with test unconfined compressive strength (UCS) as well as direct shear (DS) tests.

3. Results and Discussion

*B. subtilis* exhibited the ability to form calcium carbonate precipitates using calcium from eggshell. Qualitative test showed that the pellet obtained from bacterial liquid culture could dissolve in acidic solution, but not in alkali solution (Figure 2). The carbonate will tend to dissolve rather than precipitate at low pH level [11]. Quantitative analysis using titration showed that the concentration of calcium ion in the liquid solution was 35 ppm.

After ensure that *B. subtilis* could use eggshell to form precipitates, then medium containing eggshell and overnight grown bacterial cultures was injected into the soil samples. Two different bacterial cell concentrations were used in this study. After 30, 60, and 90 days curing period, the swelling potential and the strength of soil samples were tested.

![Calcium precipitates](image)

**Figure 2.** Calcium precipitates after the addition of (A) acidic and (B) alkali solution.

3.1. Swelling potential of expansive soil

Swelling potential was analysed with Free Swelling Index (FSI) test [12]. Degree of expansion from the soils samples was decreased after the treatment, either by medium or bacterial liquid culture. However, higher decrease in FSI value, which was 30%, was found in treated soil with bacteria (Figure 3).
Based on X-Ray Diffraction (XRD) test, untreated soil specimen contained various minerals, such as quartz, albite, montmorillonite, illite and halloysite. After 60 days of curing period with B. subtilis, FSI value of the soil samples reduced from 100% to 70% (Figure 3). Although there was a decrease in FSI value, the soils samples still had medium degree of expansion. XRD test on treated soil samples showed the presence of montmorillonite, which is considered as the main cause of swelling properties in expansive soil. The montmorillonite interlayer could adsorb water molecules and swell [13, 14].

Calcium could fill the montmorillonite interlayer and reduce the ability of soil to adsorb water molecules. This might explain the decrease in FSI value of the treated soil samples with only medium. In the presence of bacteria, calcium carbonate could be formed so more montmorillonite interlayer was filled. After 90 days, there was no significant decrease in FSI value from treated samples with different bacterial cell concentration. Calcium source in the samples might be used up so that no more precipitates could be form to strengthen the soil. However, after 60 days of curing period, the decrease in FSI value of treated samples with more bacterial cell concentration (4x) was slightly higher than that of treated samples with less bacterial cell concentration (1x). In this case, the cell concentration might affect the rate of precipitate formation, but calcium sources acted as limiting factor.

3.2. Soil strength analysis
The stability of expansive soil samples was evaluated using Unconfined Compressive Strength (UCS) test. Figure 4 shows that the strength of soil samples increased 19.47% for treated soil with medium, 62.05% for treated soil with bacteria (1x) and 74.32% for treated soil with bacteria (4x) after 90 days of curing period. The results indicated that the more bacterial cells injected into the soil and the longer curing period, the higher increase in soil strength. Calcium in the medium was used by B. subtilis or indigenous bacteria in the soil samples to form calcium carbonate precipitates. The precipitates acted as binding agents for soil particles, causing the stability of soil samples improved after curing period [3]. However, there was only a slight increase in the strength of treated soils with medium, indicating that only a few indigenous bacteria in the soil that can form precipitates.

3.3. Cohesion value analysis
Besides UCS test, the stability of soil could be evaluated by cohesion value obtained from Direct Shear (DS) test [15]. After 90 days of curing period, cohesion value of the soil samples increased 13.64% for treated soil with medium, 59.09% for treated soil with bacteria (1x) and 77.27% for treated soil with bacteria (4x). At different curing period, the highest cohesion value of soil samples was always obtained in treated samples with higher bacterial cell concentration. However, after 60 days of curing period, the cohesion value remained stable in 0.39 kg/cm² (Figure 5). The results indicated that
calcium in the medium had been used up for calcium carbonate precipitation at 60 days of curing period.

![Figure 4](image.png)

**Figure 4.** The strength of soil samples after 30, 60, and 90 days of curing period.

![Figure 5](image.png)

**Figure 5.** Cohesion value of the soil samples after 30, 60, and 90 days of curing period.

3.4. Soil morphology

Figure 6 shows the image of untreated and treated soil sample from Scanning Electron Microscope (SEM). Treated soil samples were the soil that had been cured for 60 days with *B. subtilis* using eggshell as calcium source. The granules of treated soil sample are more lumpy compare to untreated soil. The lump might be the precipitate of calcium carbonate.

![Figure 6](image.png)

**Figure 6.** SEM image of (A) untreated and (B) treated soil sample.
4. Conclusion

_Bacillus subtilis_ exhibited the ability to use calcium from eggshell and form calcium carbonate precipitates. The application of _B. subtilis_ via injection improved the stability of expansive soils by decreasing Free Swell Index (FSI) and increase the unconfined compression strength as well as cohesion value. After 90 days of curing period, the improvement of soil stability was shown by a 30% decrease in the FSI, 74.32% increase in the UCS, and 77.27% increase in the cohesion value of soil samples. Furthermore, in this study, the expansive soil stabilization was affected by several factors such as bacterial cell concentration and curing period. The more bacterial cells injected into the soil and the longer curing period, the higher increase in soil strength.

5. Acknowledgments

This study was conducted at Basic (202) and Advanced (407) Biology Laboratorium, Soil Mechanics Laboratorium and Chemistry Laboratorium, Universitas Pelita Harapan. The authors would like to thank Universitas Pelita Harapan (UPH), Faculty of Science and Technology (FaST), Civil Engineering Department and Biology Department for their support. Special thanks to Directorate General of Strengthening Research and Development, Ministry of Research, Technology and Higher Education Republic of Indonesia for funding this study and publication through Hibah Penelitian Unggulan No. 188/LPPM-UPH/V/2019 entitled “Perbaikan Tanah dengan Bakteri Indigenous” (Soil Stabilization using Indigenous Bacteria). The authors also appreciate the support from Head of Biology Department Universitas Pelita Harapan, Dr. Reinhard Pinontoan, who allowed the use of bacteria from laboratory collection.

References

[1] Soltani A, Taheri A, Khatibi M and Estabragh A R 2017 Geotech. Geol. Eng. 35 1717
[2] Soon N W, Lee L M, Khun T C and Ling H S 2013 KSCE J. Civ. Eng. 17 718
[3] Phillips A J, Gerlach R, Lauchnor E, Mitchell A C, Cunningham A B and Spangler L 2013 Biofouling 29 715
[4] Whiffin V S, Van Paassen L A and Harkes M P 2007 Geomicrobiol. J. 24 417
[5] Ng W-S, Lee M-L and Hii S-L 2012 World Acad. Sci. Eng. Technol. 62 723
[6] Kim D, Park K and Kim D 2013 Materials (Basel) 7 143
[7] Lee L M, Ng W S, Tan C K and Hii S L 2012 Appl. Mech. Mater. 204 326
[8] Widjajakusuma J, Sugata M, Changgrawinata A, Jap L, Zacharia A and Tan T J 2019 IOP Conf. Ser. Mater. Sci. Eng 620 1
[9] Choi S-G, Wu S and Chu J 2016 J. Geotech. Geoenviron. 142 1
[10] Wei S, Cui H, Jiang Z, Liu H, He H and Fang N 2015 Braz. J. Microbiol. 46 455
[11] Seifan M, Samani A K and Berenjian A 2017 Biocatal. Agr. Biotechnol. 12 299
[12] Mohan D and Goel R K 1959 J. Ins Eng. India 40 58
[13] She J, Lu Z, Yao H, Fang R and Xian S 2019 Appl. Sci. 9 1233
[14] Norrish K and Quirk J P 1954 Nature 173 255
[15] Das B M and Sobhan K 2012 Principles of Geotechnical Engineering. 8th ed. (Stamford: Cengage Learning)