Study on tropical organic soil stabilization based on biogrouting

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Abstract. Biogrouting is a new environmental friendly stabilization method to stabilize soft soils by applying microorganisms. The microorganisms produce CaCO₃, which fill voids of soil particles and bond the particles. This work studied biogrouting of high plasticity tropical organic soil applying Bacillus subtilis bacteria. In order to study the effectiveness of biogrouting using Bacillus subtilis, unconsolidated undrained triaxial and direct shear tests were conducted on the untreated and stabilized soils. The curing time for the stabilized soil specimens were 7, 14 and 28 days before the tests were conducted. In order to study the influence of the amount of Bacillus subtilis, 6 ml and 12 ml of Bacillus subtilis liquid culture was injected into the soil specimens. The soil became stronger as the amount of Bacillus subtilis used increased. The results indicated that the longer curing time and the higher amount of Bacillus subtilis reflected better soil improvement in term of cohesion, friction angle and shear stress. After 28 days of curing time, injection of 6 ml and 12 ml Bacillus subtilis liquid culture increased the effective stress cohesion values by 180% and 270%, respectively.

1. Introduction
Civil engineering structures on soft soils are usually associated with substantial difficulties. In order to support these civil engineering structures, the soft-soils have to be stabilized. The traditional soil stabilization techniques including compaction, installing sheet piles, using nails, stimulating mussel beds, mixing the soil with cement, deep mixing, chemical grouting or ground freezing [1] have some disadvantages such as limited treating area, high cost, requiring heavy equipments, disturbing urban area, or polluting environment [2, 3]. Due to these disadvantages, some sustainable and environmental friendly techniques such as replacing part of cement with rice husk ash, planting trees or grasses, or biogrouting are introduced.

Biogrouting is soil stabilization technique involving microorganism induced calcium carbonate (CaCO₃) precipitation [4]. Calcium carbonate precipitation acts as an intercellular binder crystal that stimulates the cementation process between the soil grains [5]. In applying biogrouting technology, it is necessary to consider the type of soil to be stabilized and the type of microorganisms used as biogrouting
Several studies related to biogrouting have been extensively assayed during the past years. DeJong et al. [5] employed Bacillus pasteurii to stabilize loose, collapsible sand. Nur and Sofyan [8] showed that biogrouting via Bacillus subtilis could reduce the permeability of sandy clay. Bacillus subtilis was also described to stabilize marine sandy clay soil by strengthening and decreasing the permeability of the soil [9].

Bacillus are Gram-positive, rod-shaped bacteria with optimum temperature for growth between 25-35°C [10]. Although Bacillus was thought to be a strict aerobe, it was discovered later that they could subsist anaerobically in a defined condition. Bacillus are naturally found in soil, they colonize on root systems and compete with other microorganisms such as fungus [11]. Bacillus subtilis are known to be safely applied on food products as probiotics and part of food ingredient [12]. Under harsh condition, Bacillus could form stress-resistant endospores as a defense mechanism. The spores are resilient against the exposure of heat, radiation, chemicals and withstand desiccation.

The mechanism of soil stabilization by Bacillus subtilis is commonly achieved through the expression of urease enzyme. Urease decomposes urea (CO(NH$_2$)$_2$) through hydrolysis and induces the production of ammonium (NH$_4^+$) that results in the increase of soil local pH.

\[
\text{CO(NH}_2\text{)}_2 + 2\text{H}_2\text{O} \rightarrow 2\text{NH}_4^+ + \text{CO}_3^{2-}
\]  

(1)

When pH is elevated, the ideal condition for calcium carbonate (CaCO$_3$) crystals to form is created.

\[
\text{Ca}^{2+} + \text{CO}_3^{2-} \rightarrow \text{CaCO}_3
\]  

(2)

Calcium carbonate crystals are required for biocementation process to take place which strengthen soil specimens. However, ureolytic activity produces ammonium ions (NH$_4^+$) and releases nitrogen oxide into the atmosphere [13]. The presence of excessive ammonium in the concrete matrix aggravates the risk of salt damage by the liberation of nitric acid. Therefore, metabolic conversion of organic compound (organic acid salt) to calcium carbonate has been proposed [14].

In this approach, aerobic oxidation of organic acids produces carbon dioxide which results in the generation of carbonate in alkaline environment. The existence of a calcium source as cation leads to the production of calcium carbonate [4, 5, 15]. Chemical reactions to form calcium carbonate in the presence of calcium acetate as a source of low molecular weight acid and calcium ion are listed below [16].

\[
\text{CH}_3\text{COO}^- + 2\text{O}_2 \rightarrow 2\text{CO}_2 + \text{H}_2\text{O} + \text{OH}^-
\]  

(3)

\[
2\text{CO}_2 + \text{OH}^- \rightarrow \text{CO}_3^{2-} + \text{HCO}_3^-
\]  

(4)

\[
2\text{HCO}_3^- + \text{Ca}^{2+} \rightarrow \text{CaCO}_3 + \text{CO}_2 + \text{H}_2\text{O}
\]  

(5)

The effects of Bacillus subtilis on engineering properties of organic silt are still not fully discovered. The organic silt and the sandy clay demonstrate different characteristics. In general, organic soil is a problematic soil associated with low unit weight, unsatisfactory strength characteristics and high compressibility. These undesirable organic soil properties may result to serious foundation problems. Therefore, organic soil need to be stabilized before civil infrastructures are built on them.

In this paper, the stabilization of the tropical organic silt through the employment of Bacillus subtilis was studied. The soil specimen was collected from the residential area in Parung Panjang region, which was a former plantation area. The obtained soil had a light brown color, mixed with a soft textured
gray color and slightly black component with a coarser texture. Bacillus subtilis was chosen because it is known to be soil resident, non-pathogenic and reported to be ubiquitous in Indonesia [10].

2. Experimental Studies

2.1. The tropical organic silt
To study the effect of biogrouting, various laboratory tests were carried out. The geotechnical properties of the soil were determined according to American Society for Testing and Materials (ASTM) standards [2, 17].

The specific gravity test (ASTM D854-14) indicated that the soil was an organic soil (Gs = 2.43). The standard grain size analysis (ASTM D6913-04) showed that 96.59% of the soil was silt and 3.41% of the soil was clay, meaning that the soil was the fine-grained soil. The complete results of the grain size analysis were shown in Fig. 1 as the particle size distribution curve. The soil consistency test (ASTM D4318-05) of the fine-grained soil displayed liquid limit (LL) = 71% and plastic limit (PL) = 38.31%. By combining the obtained data properties of soil specimen, it is concluded that the soil was an organic silt with high plasticity according to the Unified Soil Classification System. The classification symbol for this soil is denoted by OH.

![Figure 1. Particle size distribution curve.](image)

The next step was to measure the shear strength of the organic soil by direct shear and triaxial test. The soil shear strength can be represented by the following line equation

\[ s' = c' + \sigma' \tan \phi' \] (6)

Where \( s' \) is the shear stress and \( \sigma' \) is the normal effective stress. The effective stress cohesion \( c' \) is also called the cohesion intercept because \( c' \) is the intercept on the vertical axis. The internal friction angle \( \phi' \) is the slope of the line, i.e. the angle which the line (1) makes with the horizontal line (cf. Fig. 2). The direct shear test was performed in accordance with ASTM D6528. Since the direct shear test gives only one point (one value) on the shear strength line (1), hence, it is necessary to conduct at least two direct shear tests to gain \( c' \) and \( \phi' \). In this study, three consecutive direct shear stress tests were run using load of 3 kg, 6 kg and 9 kg, respectively. The effective stress cohesion \( c' = 0.2143 \) kg/cm² and the internal friction angle \( \phi' = 23' \) (Fig. 2).

Another apparatus to determine the shear strength parameters and the stress-strain behaviour of soils is the triaxial apparatus. In the triaxial test, a cylindrical specimen of soil is performed to either
controlled increases in axial stresses or axial displacements and radial stresses. The specimen is laterally confined by a membrane and radial stresses are applied by pressuring water in a chamber.

In this study, two pure soil specimens were tested employing triaxial test according to ASTM D5311. The failure envelope can be determined using Mohr’s circle. The cohesion intercept c’ for two specimens were 0.1688 kg/cm² and 0.2598 kg/cm², meanwhile, the internal friction angle were 26˚ and 20˚, respectively.

![Figure 2. Direct shear test strength results for organic silt.](image)

2.2. Preparation of Culture Medium for Bacillus subtilis

B4 liquid medium of the following composition (w/v) was used: 0.4% yeast extract, 0.5% dextrose, 0.25% calcium acetate, and 0.0025% phenol red [18]. To obtain solid medium, 2% agar was added. Before medium was inoculated with bacteria, it was sterilized using autoclave at 121°C for 15 minutes. The buffering capacity of the B4 medium played a critical role in supporting or preventing formation of the crystals. Increase in pH facilitates transformation of carbon dioxide to carbonate [19], which promoted calcium carbonate precipitation.

Due its high concentration of proteins, yeast extract was the only component able to affect the buffering capability [20]. Titration experiments revealed that buffering zone of yeast extract was between pH 8.1 and 10, which was characteristic for amino groups [21]. Calcium acetate was used as a source of calcium for precipitation of calcium carbonate. Phenol red was used as pH indicator.

2.3 Bacterial Inoculation on Soil Specimens

In this study, Bacillus subtilis was used to produce CaCO₃ crystals on B4 medium. The bacterial culture was prepared by inoculating a bacteria colony into sterilized B4 liquid medium, followed by incubation at 37°C with 150 rpm agitation. After 24 hours, optical density of the culture was adjusted to 0.7 – 0.9 using spectrophotometer at 600 nm. Ten ml of bacterial liquid culture obtain then injected to soil specimens.

3. Results and Discussion

3.1. Effect of Curing Time

![Table 1. Triaxial tests for untreated and treated soils.](table)
Table 1 shows the influence of curing time on shear strength of the specimens for 7, 14 and 28-days curing. All samples showed that the shear strength reduced after 7-days of injection liquid of bacterial. The strength reduction was caused by the increasing of water content. Water content significantly altered soil cohesion, because increasing water content caused greater separation of silt particles, hence, led to soften the soils. After the water dried up and Bacillus subtilis produced enough calcium carbonate crystals to bind the soil particles, the soil shear strength significantly increased (after 14- and 28-days). Fig. 3 showed that the soil shear strength could further increase due to the further production of calcium carbonate by Bacillus subtilis.

The friction angle did not change with the increasing curing time. The friction angles of the untreated soil specimens were 20˚ and 26˚ depending on the location of specimens, meanwhile, the friction angles of the treated soils were between 19˚ to 31˚. These results meant that calcium carbonate binded the soil particles but did not change the roughness of soil surfaces.

3.2. Effect of Amount of Bacterial Liquid Culture
In order to study the effect of amount of bacterial culture, 6 ml and 12 ml amount of bacterial liquid culture were injected to the tropical soil specimens. After 28 days of curing time, the direct shear tests were performed on the soil specimens.

These results indicated that the higher number of bacteria was injected in the specimen, the more calcium carbonate would be produced. Hence, the more soil particles would be bound together. Furthermore, the higher the amount of injected bacteria, the higher the effective stress cohesion could be obtained (cf. Fig. 4). Fig. 4 shows that the shear stress would continue to increase by injecting the soil with the higher amount of bacterial cultures.
Table 2 shows that the effective stress cohesion of untreated tropical organic silt was 0.2317 kg/cm², meanwhile those of the injected soil with 6 ml and with 12 ml were 0.4132 kg/cm² and 0.6155 kg/cm², respectively. The results indicate that the effective stress cohesion values increased by 180% and 270%, respectively. The friction angle increased from 25° (untreated soil) to 26° (injected soil with 6 ml bacteria culture) and to 27° (injected with 12 ml bacteria culture).

| Specimen           | Cohesion, $c'$ [kg/cm²] | Friction Angle, $\phi'$ |
|--------------------|------------------------|------------------------|
| Untreated          | 0.2317                 | 25                     |
| Treated with 6 ml  | 0.4132                 | 26                     |
| Treated with 12 ml | 0.6155                 | 27                     |

4. Concluding Remarks

As a conclusion, the application of Bacillus subtilis via injection improved the engineering properties of the tropical organic soil. The longer the soil is cured and the higher amount of Bacillus subtilis is injected to the soil, the higher soil strength is obtained. After 28 days of curing time, injection of 6 ml and 12 ml Bacillus subtilis liquid culture increased the effective stress cohesion values by 180% and 270%, respectively. However, the optimal values for curing time and the amount of Bacillus subtilis are still not obtained. It is recommended to study further about those optimal values.

For future work, it is recommended to monitor pH in order to create optimal environment for the Bacillus species. The Scanning Electron Microscope test (SEM) and X-Ray Diffraction test (XRD) are needed to study the microstructures of untreated and treated soils.

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