International Journal of GEOMATE, Dec., 2019 Vol.17, Issue 64, pp. 224- 229
ISSN: 2186-2982 (P), 2186-2990 (O), Japan, DOI: https://doi.org/10.21660/2019.64.63608
Geotechnique, Construction Materials and Environment

EFFECTIVENESS OF DECREASING PERMEABILITY AND INCREASING SHEAR STRENGTH OF SANDY SOIL USING EXOPOLYSACCHARIDE BIOPOLYMER

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*Corresponding Author, Received: 25 July 2019, Revised: 06 Aug. 2019, Accepted: 26 Aug. 2019

ABSTRACT: Sand is a type of soil with high porosity and a tendency toward a high permeability rate and low shear strength, which often causes problems in terms of piping and low slope stability. Permeability and shear strength have a very close relationship with soil stability. In this paper, we analyzed the addition of the bacterial producer exopolysaccharide on sandy soil for increased instability. The addition of this bacterial producer is expected to improve soil stability by reducing its permeability and increasing its shear strength. To find an alternative approach to overcome the problems of low permeability and shear strength, experiments were conducted using five types of non-pathogenic inoculated bacterial producer, namely, *Lactobacillus sakei*, *Agrobacterium tumifaciens*, *Bacillus subtilis*, *Pseudomonas sp*, and *Nitrobacter sp*, added to five samples of river and beach sand. After 30 days of bacterial inoculation, the permeability of the sand samples was tested using a constant head test, and the shear strength was tested using a direct shear test, which was confirmed through scanning electron microscopy (SEM). Based on the permeability test, the permeability of the sand samples inoculated with *Bacillus subtilis* was reduced the most at 74.43%. Based on the direct shear test, the highest increase in shear strength, i.e., about 58.91%, was in the samples inoculated with *Lactobacillus sakei*. SEM photographs with 10,000 x magnification showed the presence of bacteria and the formation of extracellular polysaccharides in the walls of the sand, which affected the permeability reduction rate and increased the shear strength of the sand samples. Based on the permeability and shear strength results, it can be seen that the correlation between the two is weak, and thus when the shear strength of the sample increases, the permeability does not necessarily decrease; the correlation depends on the type and characteristics of the bacterial producer.

Keywords: Permeability; Shear strength; Inoculation; Biopolymer exopolysaccharide

1. INTRODUCTION

In construction work, the soil structure is a major factor in the ability of soil to support any buildings standing on top of it. A good soil structure has an aggregate constancy improving its support stability. Until now, no dam has been built without a geotechnical engineering assessment on the soil structure used as the toe of the dam. A geotechnical engineering assessment is also focused on the permeability (seepage) and shear strength coefficients of the soil [1].

Over the last few years, a significant amount of research on improving the stability of soil has been conducted. One of the most exciting research topics is improving the stability of the soil by adding bacteria producing exopolysaccharide into the ground. Exopolysaccharides have been defined [2] as organic polymers of microbial origin, which in biofilm systems, are frequently responsible for binding cells and other particulate materials to each other (cohesion) and to the substrate (adhesion). The production of bacterial exopolysaccharides in the soil can be used to modify the soil properties [3]. These microorganisms are of great importance in the cement production of stabilizing substances.

On geotechnical engineering applications commonly microbial exopolysaccharides use for biocloging and biocementation. The purpose of biocloging is to reduce soil permeability and soil porous due the microbial activity or their products, whereas biocementation used to enhance the strength and stiffness properties of soil and rocks [4].

In Indonesia, chemicals are generally used for reducing the permeability and increasing the shear strength; in this study, however, this research was to analyze the addition of bacteria producing exopolysaccharide into the soil as an alternative to chemical materials. This research aims to prove that adding bacteria can improve the stability of the toe dam by reducing the soil permeability and increasing the soil shear strength.

2. MATERIAL AND METHODS

2.1. Bacterial growth culture medium

The culture medium used for EPS production contained 1 L of pure mineral water, 5 g of peptone, 0.25 g of $CaCl_2$, 0.25 g of $MgSO_4$, 2.50 g of K_2HPO_4, and 25.00 g of sucrose at room temperature. This media is specially designed for the nutrient requirements of bacterial. The growth kinetics of bacteria incubated under a room temperature of 30°C for 72 hours.

2.2. Inoculation Bacteria

Sandy soil, collected from Malang City, East Java, Indonesia, was passed through a 2 mm mesh sieve. This soil was sterilized using an autoclave (SV-302 II; Advantec Toyo, Ltd, Japan) at 138 kPa for 30 min. The sterilized soil was allowed to cool to room temperature before use.

The soil samples were then divided into six sections, each weighing 2,500 g, and placed in a plastic poly bag. Five soil samples were inoculated with five different kinds of bacteria, with one sample used as a control. Each part was mixed with a bacterial growth culture medium until blended and aged for approximately 1 h. Soil samples that were mixed with the growth medium were then inoculated with 25 mL of colony bacteria at a concentration of 10^{-9} CFU and left at room temperature for 30 days. Soil without culture medium and bacterial content as control were also maintained. The soil samples were kept moist by wetting them with pure mineral water.

2.3. Analysis of constant head permeability test

The permeability of the soil samples inoculated with the tested bacteria was tested using a constant head permeameter. The permeability of the soil samples inoculated with bacteria was analyzed to determine the effectiveness based on test results of samples with natural permeability as a control. The hydraulic conductivity was determined using a constant head method with an apparatus design based on an ASTM 2434 (Standard Test Method for Permeability of Granular Soil)[5].

2.4. Analysis of shear strength using direct shear test

In addition to testing for permeability, the shear strength of soil inoculated with bacteria was also tested using direct shear testing. The shear strength test results of soil samples that had been inoculated with bacteria were then analyzed to determine the effectiveness based on test results from the shear strength of natural samples as a control.

2.5. Scanning Electron Microscopy Test

To determine the potential use of bacteria in soil pore filling, the soil samples with inoculation bacteria that were shown to have low permeability and high shear strength values were analyzed using a scanning electron microscopy test (SEM-EDX, Inspect S50, FEI).

3. RESULT AND DISCUSSION

During the 30 days of inoculation, bacteria produced an exopolysaccharide synthesis of different chemical compositions and physical properties. Exopolysaccharide production is influenced by the growth and development of bacteria, nutrients in the medium available during development, and the pH and temperature. The synthesis process is one in which polymers and bacteria cell wall peptidoglycan form. The unity of the polymers and peptidoglycans are then referred to as exopolysaccharide [6]. Exopolysaccharide-exopolysaccharide is secreted by the communal bacteria in the sample and forms a glue that can clog the pore structure of the sample through the sandy soil grains. During the bacteria inoculation in sandy soil, three processes occur [3]: A process in which bacteria bioclogging produces a mass of material, and the bacteria cells are capable of filling the cavities within the sandy soil, which can reduce the porosity and permeability of the soil; A process in which bacteria biocementation produces an extracellular polysaccharide slime material formation that can improve the soil shear strength and stiffness of the sand; A process producing a biogas in which the bubbles (air) can reduce the risk of soil liquefaction potential of sand through bacteria activity. The accumulation of cell mass that occurs in bacteria, and the slime and gas produced by bacteria in the sandy soil, make the soil more impermeable to water sand [3]. This occurs because the extracellular polysaccharide produced by the bacteria changes the composition of the sandy soil structure. The production of extracellular polysaccharides will bind the soil particles of the sand and fill in the cavities between the soil grains.

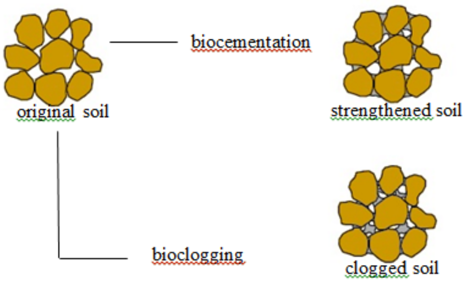

Figure 1 Effect of bacterial inoculation on soil structures [3].

Based on Figure 1, it can be seen that during the process of biocementation, the material produced by the bacteria will bind the soil particles so that the soil becomes denser, and the ability to withstand a shear force increases. In addition, during the process of clogging or blockage, bioclogging occurs from the material produced by the bacteria, or by the bacteria clogging or filling in the cavities between the soil particles of sand, thereby impeding the flow of water as it passes through the sandy soil particles and decreasing the permeability of the sandy soil [7].

The factors affecting the production of extracellular polysaccharide produced by bacteria are generally the medium growth, environmental conditions, and the formation of side reactions. The medium used to produce the polysaccharide compounds typically contains high amounts of carbon, limiting the comparison of nutrition with nitrogen. Changes in 60–80% of the carbon source applied complex into simple polymer compounds (hydrolysis), obtaining high yields from the fermentation of the polysaccharide compound. An important step in the growth of microorganisms and the formation of products is a good medium formulation. Medium growth and good production should contain elements of carbon, nitrogen, phosphorus, sulfur, potassium, and magnesium salts [8]. In addition, according to De Jong in [9], microorganisms are often in the cementation of sediment containing calcium, magnesium, iron, manganese, and aluminum in the form of crystalline carbonate, silica, phosphate, sulfide, hydroxides, and especially iron hydroxide.

In this study, the medium used is in the form of sugar (sucrose) and mineral salts, as well as in the form of $CaCl_2$, $MgSO_4$, K_2HPO_4, and sucrose. Carbon elements are required in the production process of extracellular polysaccharide obtained from sucrose ($C_{11}H_{22}O_{11}$). In a previous experiment, EPS polymers produced in a culture media containing sucrose were stickier and could bind to the soil particles more strongly. Therefore, increasing the amount of sucrose as a carbon source has a marked effect on exopolysaccharide production [9]. Salts required are obtained from K_2HPO_4 potassium, magnesium is obtained from $MgSO_4$, and calcium is obtained from $CaCl_2$. Calcium (Ca) serves as an ingredient in the formation of calcite precipitation, which will affect the process through bioclogging or impairment of the permeability. Magnesium (Mg) participates in anaerobic fermentation in biocementation.

3.1. Effects of bacterial inoculation on soil permeability reduction

Referring to Figure 2, it can be seen based on the differences between the six samples that the permeability decreases in samples that had been inoculated as compared against some types of natural bacterial samples used as a control. The percentage of reduction was obtained by calculating the difference between the size and magnitude of the permeability in natural samples after bacterial inoculation. The percentage of reduction obtained will determine the effectiveness of the bacteria used. The recapitulation percentage of the decrease in permeability of the samples inoculated with several types of bacteria was compared with the control, as shown in the following table. Based on the results, we can see that the lowest permeability in sandy beach soil was for a sample with *Bacillus subtilis* inoculation, where the percentage of reduction of the permeability rate was 74.43%. This condition was possible because *Bacillus subtilis* is one of the various bacteria that are able to produce a calcium carbonate precipitate, called biomineralization [11]. According to Muthukkumaran in [12] as the voids get reduced, the calcite precipitated connects soil grains and the strength of treated soil increase significantly. This condition allows *Bacillus subtilis* to produce more exoplysaccharide than other bacteria. This biopolymer has an important role in reducing permeability.

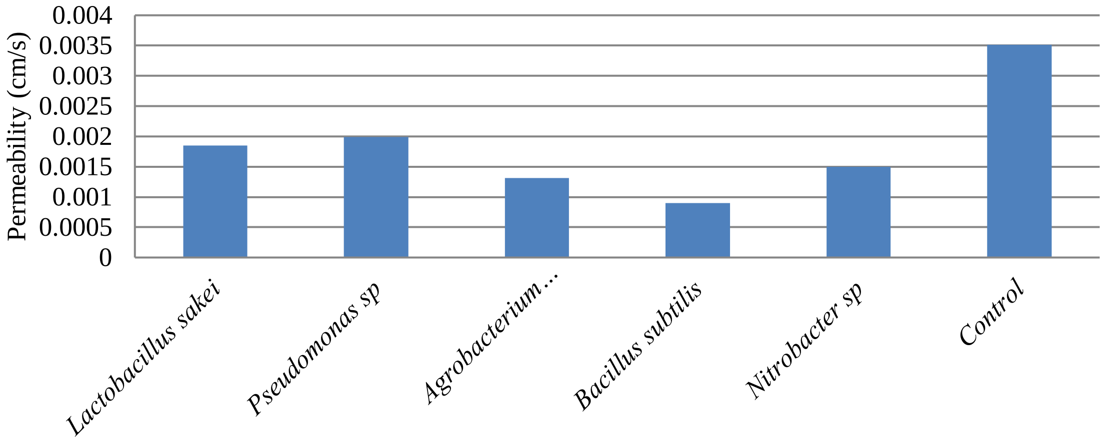

Figure 2 Permeability value of sandy soil after treatment as compared with the control

Table 1 Reduction of permeability after bacteria inoculation in sandy beach soil

Bacteria	Reduction of permeability (%)
Lactobacillus sakei	47.293
Pseudomonas sp	43.305
Agrobacterium tumifaciens	62.678
Bacillus subtillis	74.359
Nitrobacter sp	57.265

3.2. Effects of bacterial inoculation on shear strength

Referring to Table 1, the difference in shear strength between the six samples inoculated with a type of bacteria increased compared with the natural sample used as a control. The percentage of increase is obtained by calculating the difference between the magnitude of the amount of post-inoculation bacteria permeability and permeability of natural samples. The percentage of improvement obtained determines the effectiveness of the bacteria used. Recapitulation of the percentage of increase in the shear strength of the samples that had been inoculated with some type of bacteria as compared with the natural control sample can be seen in table 3. Based on result of calculation, we know that the highest shear strength in sandy soil is sample of *Lactobacillus sakei* inoculation with the percentage reduction of the permeability rate was 58.91%.

Tabel 2. Shear strength of sandy soil after treatment as compared with control

Bacteria	Shear strength (kg/m^2)
Lactobacillus sakei	0.731 x 10^4
Pseudomonas sp	0.508 x 10^4
Agrobacterium tumifaciens	0.578 x 10^4
Bacillus subtilis	0.472 x 10^4
Nitrobacter sp	0.590 x 10^4
Control	0.460 x 10^4

Table 3 Increase in shear strength after bacteria inoculation in sandy beach soil

Bacteria	Increase in shear strength (%)
Lactobacillus sakei	58.913
Pseudomonas sp	10.522
Agrobacterium tumifaciens	20.360
Bacillus subtillis	2.609
Nitrobacter sp	28.174

In this research, we also examined the relationship between the permeability and shear strength in both natural samples and control samples that had been inoculated with bacteria. From the correlation analysis, the following results were obtained: In general, each sample inoculated with one of five types bacteria had lower permeability and higher shear strength; in addition, a comparative analysis of the permeability and shear strength was also conducted. From these correlations, it can be concluded that the correlation coefficient (r) between the shear strength and permeability has a weak link. That is, in the case of bacteria inoculation, if the shear strength of the sample was high, the permeability did not necessarily decrease.

3.3. Analysis of Scanning Electron Microscopy Test

A SEM test is a microscopic examination with magnification of up to more than 2,000 x, and in this study was applied to determine the presence of extracellular polysaccharides formed on the samples that were inoculated with a particular type of bacteria for 30 days. Testing was conducted on soil samples inoculated with *Bacillus subtilis*, *Agrobacterium tumefaciens*, and *Lactobacillus sakei*. Soil samples used in the SEM test were selected based on having the lowest permeability and high shear strength after the inoculation process was completed. At constant high test results obtained soil inoculation of *Bacillus subtilis* and *Agrobacterium tumefaciens* has the lowest permeability values and the direct shear test results obtained inoculating *Lactobacillus sakei* has the highest shear strength results. An SEM test result shows an image of the sample tested.

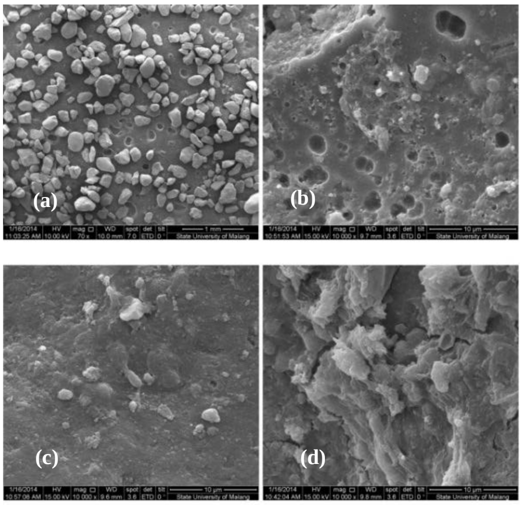

Figure 3. SEM test results for inoculation of *Bacillus subtilis*

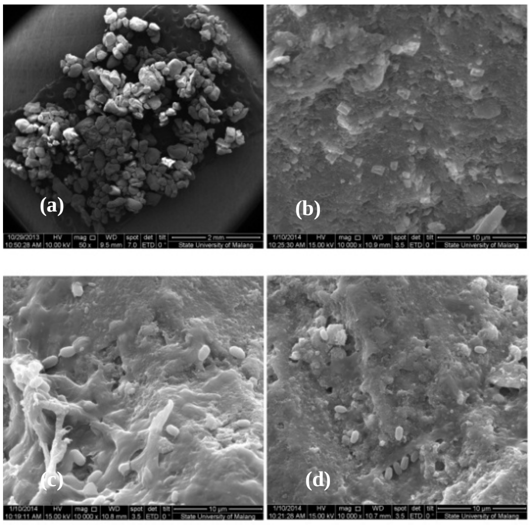

Figure 4. SEM rest results for inoculation of *Agrobacterium tumefaciens*

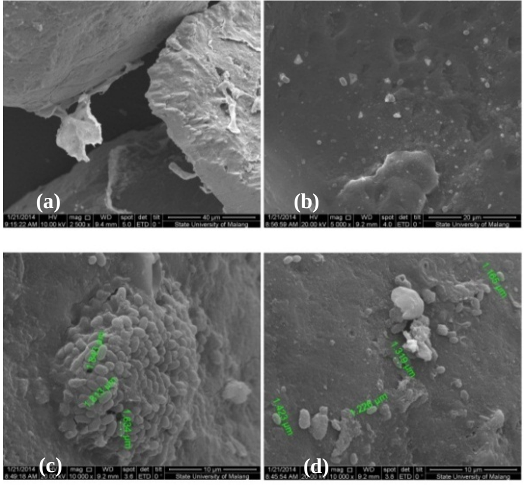

Figure 5. SEM test results for inoculation of *Lactobacillus sakei*

Figures 3(a), 4(a), and 5(a) show SEM tests of sandy soil with a magnification of 50–70x for samples inoculated with *Bacillus subtilis*, *Agrobacterium tumifaciens*, and *Lactobacillus sakei*, respectively. In Figure 5(a), it can be clearly seen that *Lactobacillus sakei* produces slime as an adhesive between the soil particles, which increases the density of the particles, and thereby increases the shear strength. In Figures 3(b), 4(b), and 5(b) mineral salt crystals can be seen on the sandy beach soil. The samples used for SEM testing were taken randomly. The samples were placed on a metal layer with a size of approximately 1 cm x 1.5 cm, and magnification of the desired object was applied up to the point shown in the images. From Figures 5(c), it can be seen the amount of *Lactobacillus sakei* colony more than *Bacillus subtilis* colony and *Agrobacterium tumifaciens* colony. This proves that *Lactobacillus sakei* bacteria that inoculated in samples of beach sand can grow and develop better and faster than two kind another bacteria, and produces exopolysaccharides more higher. Proceeding with the SEM test results in Figures 3(d), 4(d), and 5(d), it can be seen that exopolysaccharide begins to form on the wall of the sand soil samples. *Bacillus subtilis* is able to produce the enzyme urease, which then can produce the exopolysaccharide compound of calcium carbonate as an adhesive in the pores of the sample. In contrast to *Bacillus subtilis*, *Agrobacterium tumefaciens* ammonification reactions and the precipitation of carbonate form particles of exopolysaccharide plugging the pores of the sample. Exopolysaccharide-formed soil particles stick to the wall of the sand, filling in and sealing the pores between the soil particles [13]. Biofilm accumulation in porous media is the overall result of microbial cell adsorption, desorption, growth on surfaces detachment and filtration [14]. The SEM test results prove that these findings are consistent with the expected results, namely, that bacteria produce exopolysaccharide.

4. CONCLUSION

Bacteria produce a synthesis of polymers and peptidoglycan in their cell wall. Unity and peptidoglycan polymers are thus referred to as exopolysaccharide. Exopolysaccharide bacteria, a communal secreted by bacteria in a sample, acts as a glue, and can clog the pore structure of the sample through the sandy soil grains. Because blockage and adhesion occur, the ability of a fluid to seep through the samples can be reduced. Conversely, the ability of the sample to withstand a force or load can be increased.

Based on test results using an SEM magnification of 10,000 x, it can be seen that bacteria attached to the walls of the sand solitarily and reproduced. This proves that bacteria bred or inoculated into river sand samples can reproduce and develop. In addition, looking at the wall of the soil samples, the sand began to form exopolysaccharide. Exopolysaccharide sticks to the walls of sandy soil particles, filling in and sealing the pores between soil particles. The SEM test results prove that the present research is consistent with the expectation that bacteria produce exopolysaccharide.

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