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A STUDY ON CLAY SOIL IMPROVEMENT WITH BACILLUS SUBTILIS BACTERIA AS THE ROAD SUBBASE LAYER

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ABSTRACT: This study aims to investigate the bearing capacity of the high-plasticity clay soil with bacteria stabilization (*Bacillus subtilis*). The growth of bacteria with the phase of 6 days was used as a stabilizing agent. The assessment of bearing capacity was based on unconfined compressive strength value, California Bearing Ratio (CBR), and modulus of soil reaction as a subbase layer. The test results show that the compressive strength value tends to increase with the addition of 2% to 6% bacteria and decrease with 8% bacteria. This phenomenon corresponds to the increase of CaO value due to Microbial-Induced Calcite Precipitation (MICP) microstructurally. The compressive strength value curve pattern increases proportionally with curing time of 3, 7, 14, and 28 days. According to the California Bearing Ratio (CBR) value range criteria (20% ≤CBR<40%) for subbase layer, the use of 3.5 to 6% bacteria can technically be functionally justified as a road material with the modulus of stabilized clay soil reaction value of 68 to 110 kN/m^2/mm. The results of this study have technical implications for the significance of the use of *Bacillus subtilis* bacteria as the biotechnology stabilization material to increase the bearing capacity and modulus of soil reaction with high plasticity clay.

Keywords: Clay soil, Bacillus subtilis, stabilization, reaction modulus

1. INTRODUCTION

Moving forward as the time progresses, the stage of development in the country, particularly in the field of construction, is also growing rapidly, that is the increasing demand for qualified soil specification. However, it is often found that the soil condition is not quite well such as soft clay soil. This type of soil is found in places where the soil condition contains a high water content such as in the swamp or riverbank areas. The construction problem of road foundation layer with soft clay soil is that it has a low bearing capacity and a high potential to the pavement depression. This condition will cause the road to be unable to support the construction above. Therefore, a method of soil improvement to enhance the soil structure is urgently necessary.

The soil that has met the specification is very influential at the beginning of construction planning, in which a good planning will give its own satisfaction on the achieved results. The improvement of soil physical properties from poor to prosper in the field of civil engineering is known as soil stabilization. Soil stabilization can be done by adding a certain additive to the poor soil. Some of the most widely used mixtures in the additive include limestone, Portland cement, asphalt, fly ash and sand. Furthermore, this study aims to engineer the stages of soil stabilization in general by using microorganism (bacteria) as the stabilization material. The bio-stabilization method of clay soil with Bacillus subtilis bacteria is believed to improve soil characteristics by increasing the bearing capacity of the subbase layer and also reducing the compressibility and permeability. The benefit of Bacillus subtilis bacteria is that it can reduce the contamination of soil and environment risk. Looking at the strategic issues of clay soil problems, this research emphasizes the study on the use of Bacillus subtilis bacteria as the stabilization material in clay soil.

Microorganisms are the living organisms that have a very small size [6]. Every single cell of microorganisms has the ability to carry out life activities such as being able to experience growth, produce energy, and reproduce by itself. Microorganisms have high metabolic flexibility because they must have great adaptability. Thus, if there is a high interaction with the environment, it will cause a high conversion of the substance as well. However, because of its small size, there is no place to store the enzymes that have been produced. Therefore, the unnecessary enzymes will not be stored in the inventory form. Certain enzymes that are necessary can be used for clay soil improvement.

Microbial-Induced Calcite Precipitates (MICP) is a new and sustainable technology used to improve the properties of building materials. This technique works by introducing a bacterial solution (e.g. Sporosarcina pasteurii, Bacillus megaterium, Sporolactobacillus, Clostridium, and Desulfotomaculum) into the soil matrix, and then injecting a chemical solution consisting of urea and one of the calcium salts (e.g. calcium chloride and calcium acetate) into the soil matrix several times [3]. A number of factors should be considered to allow the use and control of Microbial-Induced Calcite Precipitates process in field application, including the concentration of the bacterial solution, the concentration of the chemical solution, and also the method for introducing bacteria and chemical solution, Besides to provide an overview of the various factors affecting Microbial-Induced Calcite Precipitates in the soil, this research also aims to identify the effect of bacteria, soil particle size, nutrients, chemical solutions, pH, temperature, and injection [5].

2. LITERATURE REVIEW

Clay soil is one type of problematic soil and must be handled properly as the bearing capacity of the soil and also the soil deformation can cause a failure or damage to the structure above it. Therefore, it is necessary to employ a method of soil improvement that can be applied to overcome these problems occurred in the clay soil. Several methods of handling clay have been conducted, i.e. by replacing materials with other stabilizers [4].

Stabilization method by using added materials such as cement, limestone, and fly ash has the main objective of improving the technical properties of subgrade and subbase layer to be better according to the needed specifications and requirements. Therefore, it is necessary to invent alternative environmentally friendly stabilization methods. One of them is by utilizing microorganisms derived from bacteria since it can produce calcium carbonate calcite/crystal which is able to alter the granular behavior. Various studies on the stabilization method in improving the soil have been conducted, one of which is Bio-Mediated Soil Improvement: Cementation of Unsaturated Sample [5]. This research uses Bio-Mediated Calcite Precipitation as the soil improvement material. Furthermore, a study on Experimental Biogrouting Stabilization on Marine Sandy Clay Soil [12] has also been conducted. This study presents an application used to increase the bearing capacity of the soil by employing the stabilization [9], and the result indicates that a bacteria species, i.e. Bacillus subtilis can be used as the stabilization material. This bacteria derived from Papua region can breed in the temperature in Indonesia and able to produce most of the calcium carbonate calcite/crystal [16]. In addition, the result also shows that the urease which catalyzes the conversion of urea to ammonium and carbonate produce a precipitate with calcium as calcium carbonate crystals. These crystals form a bond between the sand grains can increase the strength and stiffness of the sand. Meanwhile, the remaining ammonium chloride is extracted and removed.

Furthermore, bacteria are microscopic organisms of 1.25 micrometer (μm) in diameter on the average. Bacteria can grow at a maximum temperature of 25-75°C. It can be found in air, water, soil, animal fur, and carcasses, in which the optimum pH of its growth is of 5.5-8.5 [2]. These microorganisms are bred by inoculating the microorganisms into a new medium. The culture medium used consisted of nutrient jelly and nutrient broth (NB) [10]. The role of bacteria in stabilizing the soil is at the time of precipitation of calcium carbonate

The subbase course on highway pavement planning lies between the subgrade layer and the upper base layer which functions to receive the traffic load on it [1]. Therefore, it must have an optimum bearing capacity and be able to receive the pressure due to traffic load without any damage occurring. The bearing capacity is expressed by the California Bearing Ratio (CBR) value. California Bearing Ratio was first introduced by California Division of Highway in 1928. How to improve the CBR and minimize the swelling of subgrade soil is explained in this research.

3. MATERIALS AND METHODS

The materials used in this research are clay soil and *Bacillus subtilis* bacteria. The collection of soil samples was done conventionally by using a crowbar and shovel, then soil samples were placed in the sample sacks and wrapped with plastic to keep the original water content condition. The laboratory test was conducted to identify the physical and mechanical properties of the clay soil based on the *American Standard Testing and Material* (ASTM) standard as summarized in Table 1. Meanwhile, the compaction test was conducted by standard Proctor compaction, and the soil bearing capacity test was done by CBR and unconfined compressive strength. The procedure of the tests is as in the following.

1. Growth culture test was done by making a liquid media with the help of shaker and incubator. The culture medium used consisted of nutrient jelly and nutrient broth (NB), i.e. 1 liter of water was mixed with 20g of urea, 3g of nutrient broth, 2.12g of $NaHCO_3$, 4.1g of $CaCl_2.2H_2O$, 10g of NH_4CI, and H_2O. Furthermore, it was put in the Autoclave tool

with a temperature of 121ºC in 15 minutes with the pressure of 1 ATM.

2. The California Bearing Ratio (CBR) test in this study was conducted by mixing the clay soil with water based on the optimum moisture content of the Proctor compaction result and mixing it with Bacillus subtilis bacteria in the concentrations of 2%, 4%, 6%, and 8% for 7 and 28 days tests.
3. Unconfined compressive strength test used the cylindrical test specimens with the dimension of H ≥ 2D, i.e. the clay soil was mixed with water based on the optimum moisture content of the Proctor compaction result and added the Bacillus subtilis bacteria in the concentrations of 2%, 4%, 6%, and 8%. The clay mixture with bacteria addition was inserted layer by layer into a 4 cm diameter polyvinyl chloride (PVC) pipe mold and then it was pounded 25 times per layer for 3, 7, 14 and 28 days tests.
4. The model was made in the form of a cylinder with a diameter of 80 cm with 120 cm in height. Moreover, the soil layers consisted of the bottom layer which was a layer of soil deposits with the thickness of 80 cm, and above it, a layer of clay soil with Bacillus subtilis bacteria as the stabilizer with the thickness of 30 cm, a steel plate with the thickness of 20 mm. furthermore, the model was given a static loading with varying loads as shown in Figure 1.

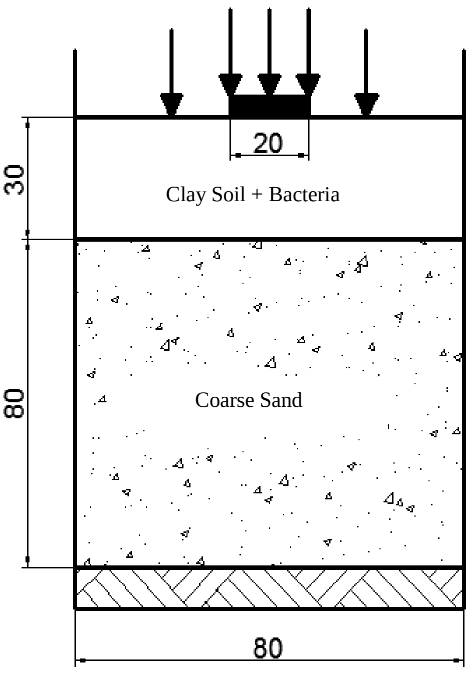

Fig.1 Physical model sketch for the road subbase layer test

Table 1 The standard method of physical properties soil testing

Type of Testing	ASTM Standard
Water content	ASTM D 2216-71
Specific gravity	D-162
Liquid limit	D-423-66
Plastic limit	D-424-74
Grain size analysis	C-136-06
Proctor compaction	D-698
Unconfined compression strength	D-633-1994
Dry density (γ_{dry})	D-854-72
Saturated density (γ_{sat})	D-2216-98
Bearing capacity Ratio (CBR)	D-1833
XRD test	D3906-03 (2013)
SEM test	E986-04 (2010)

4. RESULTS AND DISCUSSIONS

4.1. Clay Soil Characteristics

Visually observed, soft clay has black ash color, the hard texture so it is not easy to squeeze in dry conditions, whereas if exposed to water, the soil becomes slippery and sticky in hand, easy to mold and plastic impressed. From the results of research in the laboratory as seen in Table 2.

Table 2 Test results of physical and mechanical properties of soft soil

Soil Characteristics	Unit	Test Results
Water content (w)	%	78.12
Specific gravity (Gs)	-	2.68
Grain size analysis		
- Sand	%	4.25
- Silt	%	35.21
- Clay	%	60.55
Liquid limit (LL)	%	67.89
Plastic limit (PL)	%	30.8
Plasticity index (PI)	%	37.08
Maximum dry density (γd)	kN/m^3	1.48
Optimum moisture content	%	26.5
California Bearing Ratio (CBR)		
- 10x blows	%	1.84
- 25x blows	%	2.34
- 56x blows	%	8.96
Unconfined compressive strength (qu)	kN/m^2	26
Cohesion (C)	kN/m^2	25
Internal friction angle (θ)	(º)	23
AASHTO classification		A-7-5
USCS classification		CH

4.2. The capacity of Clay Soil Stabilized by Bacillus Subtilis Bacteria

4.2.1. CBR Test Results

The California Bearing Ratio test result of clay soil with Bacillus subtilis bacteria stabilization in the concentration of 2% - 8% is shown in Fig. 2 as follows.

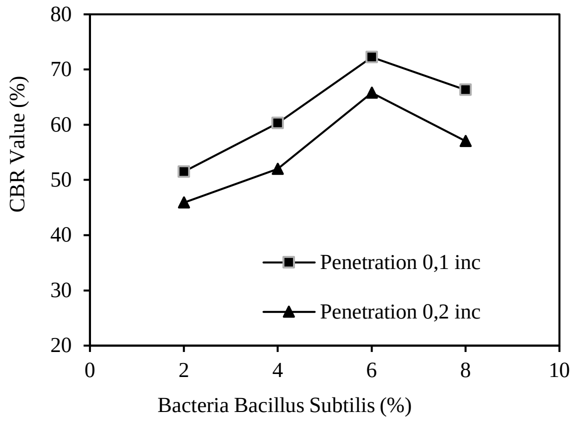

Fig.2 The relationship between bacteria addition and penetration of CBR value

As shown in Figure 2, the California Bearing Ratio value increases optimally in the use of 6% Bacillus subtilis bacteria and decreases after 6%, both for penetration of 0.1 inc and 0.2 inc. The phenomenon of the decrease in CBR value corresponds to the decrease of calcite deposits in the clay so that the pore space becomes larger and causes the soil strength to decrease.

As can be seen in the result of the California Bearing Ratio (CBR) test shown in Fig**.** 3, the CBR value curve pattern increases proportionally with curing time of 7 and 28 days.

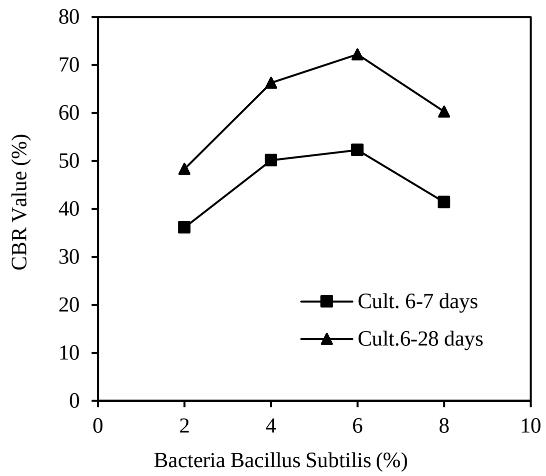

Fig.3 The relationship between CBR value and addition of bacteria to 6-day culture cured

This phenomenon indicates that the CBR value increases from the 2%-6% Bacillus subtilis bacteria in 6 days culture and after the concentration of 6%, the CBR value decreases. Clay soil with the addition of 6% bacteria which has 6 days of culture and 28 days of curing time is of 72.23%. Furthermore, the increase in the number of bacteria percentage and the time of humidity causes increased soil support. The pozzolanic reaction that causes pozzolanic strength gain increases the soil strength and also increases the bearing capacity of the soil [9].

4.2.2. Unconfined Compressive Strength Test Result

From the test results, it can be interpreted that the clay soil which is not mixed with the bacteria has a very small value of bearing capacity and is classified as very soft soil.

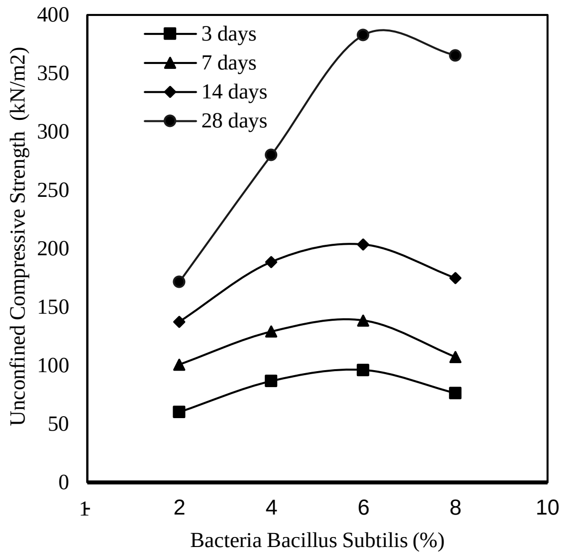

Fig.4 Relationship of q_u value and bacteria addition to the curing time

The test result of the unconfined compressive strength of clay soil with the stabilization of Bacillus subtilis bacteria in the concentration of 2%-8% is shown in Figure 4. The value of compressive strength increases significantly along with the addition of bacteria and the increase of the curing time [17]. This phenomenon is due to several causes, i.e. Bacillus subtilis bacteria hydrolyze the urea which is catalyzed by the urease enzyme produced from by bacteria. With dissolved Ca_2^+ in the surrounding environment, it produces solid calcite/calcium carbonate ($CaCO_3$) crystals that will bond closely and cause the soil to become hard [14]. The compressive strength value increases optimally during the use of bacteria of 6% that is 382 kN/m^2, due to the increasing particle density caused by Microbial-Induced

Calcite Precipitation process. Meanwhile, the precipitated calcite is used to fill the cavity at soil mass and bond between weak soil grains. As when the cavity decreases, precipitate calcite binds the solid soil. Therefore, when the concentration of bacteria used is 8%, the value of unconfined compressive strength decreases. Furthermore, the soil acidity level (pH) has an effect on the exchange capacity of the positive ions (cations) and makes the particle size become smaller as the compressive strength decreases.

4.2.3. Chemical Test Results of Clay Soil with Bacillus Subtilis bacteria stabilization

The composition of the chemical element and the description of the microstructured surface of clay soil with stabilization of Bacillus subtilis bacteria were observed by using SEM-EDS (scanning electron microscopy with energy dispersive spectroscopy). Furthermore, the test results are shown in Table 3.

Table 3 Chemical contents of clay soil with Bacillus Subtilis Bacteria Stabilization

Chemical Contents	Bacteria Addition (%)		
	2	4	6
Na_2O	3,59	3,59	3,59
MgO	4,81	3,81	3,77
Al_2O_3	19,72	23,5	25,15
SiO_2	33	40,98	42,12
SO_3	6,98	4,98	2,26
K_2O	2,03	2,07	2,48
CaO	0,42	4,56	6,13
TiO_2	1,06	1,9	1,79
FeO	10,86	17,09	18,4

Furthermore, the microstructure surface image of the SEM test result is shown in Figure 5. SEM displays an image by firing a high-energy electron beam of 1-20 keV.

As can be seen from Figure 5, the SEM/EDS test shows that the chemical content of clay soil with the addition of 6% Bacillus subtilis bacteria was dominated by the elements of 42.12% SiO_2, 25.15% Al_2O_3, 18.40% FeO, and 6.13% CaO. Moreover, it is seen that the microstructure of the soil becomes denser and more compact. Silica (SiO_2) is the main constituent element in the formation of pozzolanic cement that has the behavior of binding other minerals in clay so that it becomes harder within a certain period of time. Meanwhile, calcium can neutralize the excess organic matter content on the soil.

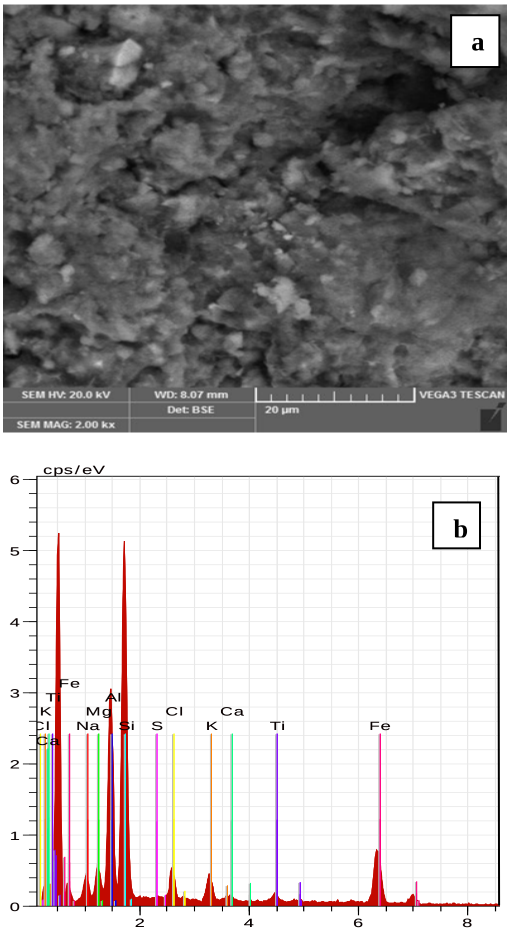

Fig.5 Photos of SEM and EDS (energy dispersive spectroscopy) of soft soil with 6% Bacillus subtilis bacteria stabilization

4.3. Soil Reaction Modulus of Clay with Bacillus Subtilis Bacterial Stabilization

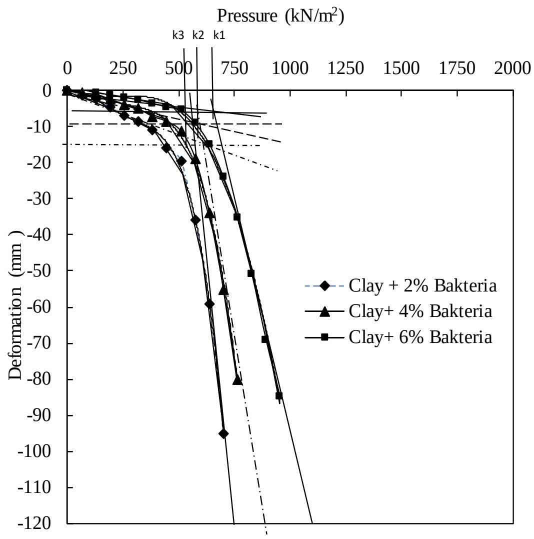

Fig. 6 The relationship of pressure (q) to the vertical deformation of clay soil with Bacillus subtilis stabilization

Figure 6 shows the value of soil reaction modulus (k) from the correlation of pressure (q) with the vertical deformation of clay soil with of Bacillus subtilis bacteria stabilization. The value of k = 40 kPa/mm is obtained with the pressure (q) of 550 kPa and vertical deformation of 14 mm. Meanwhile, for the pressure value (q) of 610 kPa and vertical deformation of 9.0 mm, the value of k is 68 kPa/mm, and for the pressure value (q) of 660 kPa and vertical deformation of 6 mm, the value of k is 110 kPa/mm. It can be seen that the value of the voltage increases along with the increased value of k and the opposite, the decreased value of vertical deformation [13]. This is due to in the MICP process, the micro pores become smaller and the soil fabric becomes solid.

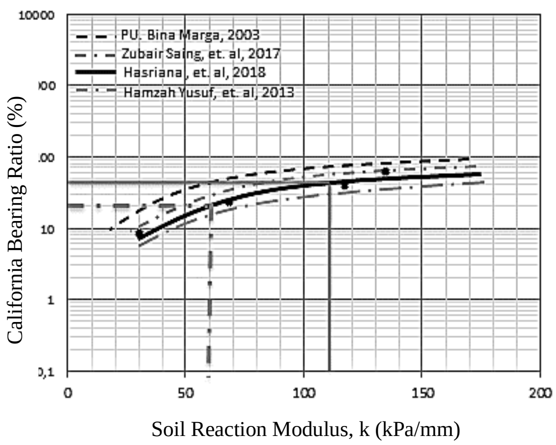

Fig.7a The relationship of CBR and clay soil reaction modulus (k) values, result of a study in 2018 [15, 16]

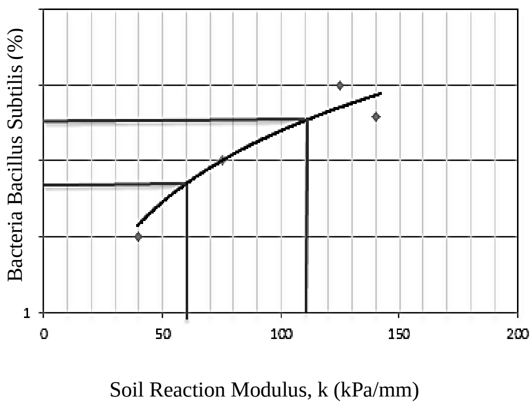

Fig.7b The correlation of k value and bacteria percentage of stabilized soil

In Fig. 7a, the correlation of the modulus of soil reaction value (k) and the California Bearing Ratio value for 4 (four) types of soil, i.e. a general soil according to Bina Marga Office of Highways, a Bili-bili DAM sediment soil with cement stabilization, a laterite ferrous soil with limestone stabilization, and a clay soil with Bacillus subtilis bacteria stabilization. The graph shows that the clay soil with Bacillus subtilis bacteria stabilization is between the Anzub charts with HMZ analysis. From the criteria of Bina Marga Office of Highways, the materials used for the lower foundation (subbase), the CBR value is ≥ 20%, and for the base layer, the CBR value is ≥ 40%. Meanwhile, in Fig. 7b, the graph depicts the composition of 6% Bacillus subtilis bacteria for the material base. Subgrade modulus of soil reaction of 110 kN/m^2/mm was observed for 6% of bacteria in conformity with the CBR value of 40%. This result proves that the clay soil with 6% Bacillus subtilis bacteria stabilization could be recommended as the bottom layer (subbase) of the road that with the Microbial-Induced Calcite Precipitation influence, it can make the modulus of soil reaction (k) increases.

5. CONCLUSION

The material characteristics of clay soil used in this study were classified as a high plasticity clay with unconfined compressive strength value of 26 kN/m^2 and bearing capacity or California Bearing Ratio (CBR) of 8.96%, indicating that the clay soil had a low bearing capacity and did not meet the requirements as the subbase layer of the road. Furthermore, the California Bearing Ratio (CBR) value and the modulus of subbase layer reactions of the clay soil with 2, 4, and 6% bacteria stabilization increase significantly and start to decrease at 8% bacteria with the curing age of 3, 7, 14 and 28 days. The compression strength value also decreases after 6% bacteria stabilization. The support capacity (unconfined compressive strength = 382.84 kN/m^2 and California Bearing Ratio (CBR) value = 72.23%) decreases at the use of bacteria exceeded 6% in line with the increase of CaO value with calcite due to the microbial process of Microbial-Induced Calcite Precipitation (MICP). Subgrade modulus of soil reaction of 110 kN/m^2/mm was observed for 6% bacteria stabilization in conformity with California Bearing Ratio (CBR) value of 40%. In brief, it shows that technically it can be functioned as the subbase layer on a road structure. Furthermore, the result also shows that the use of *Bacillus subtilis* bacteria has a technical implication significance in the improvement and increase of the bearing capacity and the modulus of high plasticity clay soil reaction.

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