

SOIL MECHANICAL CHARACTERISTICS IMPROVEMENT WITH BACTERIAL BIOCEMENTATION TECHNOLOGY

*Alkadri¹, Abdul Rachman Djamaluddin¹, Tri Harianto¹ and Ardy Arsyad¹

¹ Faculty of Civil Engineering, Hasanuddin University, South Sulawesi, Indonesia

*Corresponding Author, Received: 30 Sep. 2023, Revised: 11 Jan. 2024, Accepted: 12 Jan. 2024

ABSTRACT: The aim of this study is to establish a subgrade layer by field-scale testing using a 6% *Bacillus subtilis* bacterial solution in Microbially Induced Calcite Precipitation (MICP), altering the bacterial culture's age by four days and curing it for 150 days. Field-scale testing was carried out, starting with soil property tests, followed by tank tests, bacterial growth, and soil mechanical tests using bacterial solutions. The study found that on the field model scale, the unconfined compressive strength increased with the lengthening of the curing period. However, because of the influence of rainfall, high rainfall lowers the soil's carrying capacity. In the 14-day to 45-day test, there is a difference in carrying capacity between layer 1 (0–15 cm) and layer 2 (15–30 cm). Comparing Layers 1 and 2, Layer 1 has a higher (q_u) value, but Layer 2 has a higher (q_u) value during the 60-150 days test. The optimal value of CBR with MICP stabilization is 20% after 150 days of curing. This increased by four times as compared to soil without bacterial stabilization, while the optimum value of compressive strength increased by three times. The SEM analysis reveals that MICP caused calcite to appear on the soil's surface. The results indicated that bacterial bio cementation stabilization is an alternative method of soil improvement that can enhance the mechanical characteristics of the soil, beneficial for the construction of infrastructure, and is environmentally benign.

Keywords: *Subgrade, Microbially Induced Calcite Precipitation (MICP), Field test.*

1. INTRODUCTION

The discovery of unfavorable soil conditions can lead to geotechnical issues like instability, long-term settlement, low shear strength, and high compressibility, as well as the characteristic of swelling and shrinking during the rainy and dry seasons. The effects of inadequate bearing capacity can result in losses, beginning with losses from rising building costs, risks to construction safety where the structure being built cannot stand steadily, and a decrease in the age of construction. In the case of road construction needs where material replacement and the use of chemicals for the subgrade layer are not efficient, it is necessary to use other methods to improve the quality of the existing soil for road construction purposes without replacing the existing soil and damaging the environment. One of the potential soil stabilizing methods that have lately attracted the attention of many researchers is biocementation, which is based on microbially induced calcite precipitation (MICP) [1–3]. MICP is a recent, sustainable, and cost-effective soil improvement technique with low CO₂ emissions [4].

The use of microorganisms is considered an alternative amidst the prevalence of chemical and synthetic materials in civil construction [5,6]. The effective formation of CaCO₃ will increase the strength and stiffness of subgrade layers and embankments while maintaining their permeability characteristics. The formation of CaCO₃ results in an

increase in the dry density of the sample, which increases particle bonding and contributes greatly to the improvement of soil mechanical properties and liquefaction resistance [7]. Microbial calcite precipitation can be caused by organisms involved in the nitrogen cycle through the hydrolysis of urea by the enzyme urease, which results in the production of carbonate ions in the presence of ammonium. Calcium carbonate is readily precipitated under these conditions. In the presence of calcium [8,9], it was found that tropical organic soil, along with the addition of sand using the pre-mixing treatment method with a 3-day curing period, increased the value of Unconfined Compressive Strength (UCS).

Biocementation is a soil improvement method that utilizes the ability of bacteria living in the soil to produce the enzyme urease, which catalyzes the hydrolysis of urea into carbon dioxide and ammonia. MICP is quite flexible and can be tailored for different applications using different injection methods. Its use in geotechnical applications has been studied by several researchers over the past few years to examine its potential for strength enhancement and permeability reduction [10]. Through bacterial biocementation conducted on a lab scale, it was discovered that bacteria (*Bacillus subtilis*) stabilized the bearing capacity of highly flexible clay soil. Bacterial growth in phases 3 and 6 is used as a stabilizing agent. The bearing capacity assessment is

based on the unconfined compressive strength, California Bearing Ratio (CBR), and soil reaction modulus as a subbase layer. The test results indicate that adding 2% to 6% bacteria tends to raise the compressive strength value while adding 8% bacteria causes it to drop. This phenomenon is consistent with the increase in CaO values due to the MCP [11] and Unconfined Compression Test (UCT). Variations in *Bacillus subtilis* concentration were applied to as much as 3%, 4.5%, and 6% of the coal-contaminated sand in order to observe the impact of CaCO_3 calcite precipitation on the unconfined compressive strength characteristics of the coal-contaminated sand. The bacteria used were 3-day cultures still in the stationary phase and 6-day cultures in the dead phase. After 28 days of treatment, there was a significant increase in the unconfined compression test value of the soil stabilized with MICP compared to the untreated soil [12]. MICP was a commonly utilized method of soil stabilization that decreased sand's hydraulic conductivity and increased shear strength characteristics [13]. It is imperative to carry out developments related to field testing on the biocementation ability of bacteria against seasonal changes (rainy season and dry season) because several previous laboratory testing studies have found that the biocementation potential of bacteria can increase the carrying capacity. Using a solution of *Bacillus subtilis* bacteria for bacterial biocementation stabilization (MICP), which is cured for 150 days and tested for soil-bearing ability, the goal of this research is to establish a subgrade layer using field-scale testing.

2. RESEARCH SIGNIFICANCE

This research covers the application of subgrade bacterial biocementation for highways. In the case of road construction needs where the placement of materials and the use of chemicals for the subgrade layer are inefficient, it is necessary to use other methods so that the quality of the existing soil becomes better without replacing the existing soil and damaging the environment. Bacterial biocementation using *Bacillus subtilis* bacteria is an alternative and is expected to increase the carrying capacity of the soil. The research results can be used as a reference in developing an alternative soil improvement method using bacterial biocementation stabilization.

3. MATERIALS AND METHODS

This study uses soil from Gowa Regency South Sulawesi Indonesia, while the type of bacteria used is *Bacillus subtilis*. *Bacillus subtilis* bacteria are one type of gram-positive bacteria and are bacillus (sticks) that can form oval-shaped endospores in the central part. *Bacillus subtilis* was cultured in a B4 medium containing urea (20 g/L), nutrient broth (3 g/L), NaHCO_3 (2.12 g/L), $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$ (4.14 g/L), and NH_4Cl (10 g/L) dissolved in distilled water. Cultured

bacteria were collected at the stationary phase of culture growth after 3 days and at the death phase of culture growth after 6 days. Endospores are a phase carried out by bacteria such as *Bacillus* and *Clostridium* that produce a form of survival defense in unfavorable conditions. These endospores are resistant to extreme environmental conditions such as high temperatures, drought, and toxic chemical compounds. *Bacillus subtilis* can live in the presence or absence of oxygen and is therefore referred to as an anaerobic microorganism.

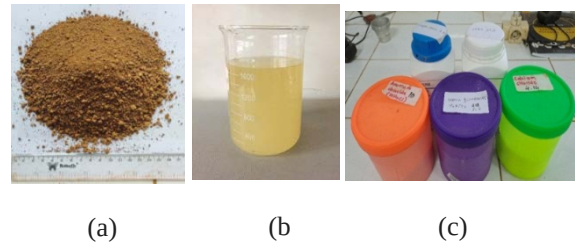


Fig.1 (a) Soil sample. (b) *Bacillus subtilis* bacteria (c) B4 media

Figure 1 shows (a) the original soil (b) the bacterial solution of *Bacillus subtilis* (c) growing media (B4) consisting of 20 gr urea, 3 gr nutrient broth, 2.12 g NaHCO_3 , 4.14 g CaCl_2 , and 10 g NH_4Cl . Calcium carbonate induced by microbes is influenced by 4 factors, namely, calcium concentration, dissolved inorganic carbon concentration, pH, and nucleation availability [14].

3.1 Excavation of Field Test

The prepared soil is then mixed with a 6% bacterial solution (dry density of soil with a 4 day bacterial culture) then the soil and bacterial solution are mixed until evenly distributed then compacted in the test tank that has been prepared. The excavation of the field test, as shown in Fig.2



Fig.2 (a) Soil filling for field test (b) Compacted soil

Figure 2 shows a cross-section of the excavation measuring 200 cm in length, 100 cm in width, and 30 cm in depth to simulate conditions of substandard CBR value (below 6%), which is frequently observed in soft soil [15]. Using a CBR value of 5.3%, the subgrade layer was prepared. Before excavating the test basin, soil property tests were first carried out to determine the physical properties of the soil. The test plan of the model is shown in Fig. 3, shows the field test placed

outdoors so that it is exposed to heat and rain. This testing tank is to serve to see the condition of the soil that has been mixed with bacteria. Field testing was conducted for 14 to 150 days. The field test has been marked with a test location that will be carried out during 150 days. The mechanical tests that will be carried out are UCT and CBR.

3.2 Sampling Process

UCT sampling in the excavation field is done by coring at the location of the tub that has been placed. For each test sample taken, as many as 2 samples are taken at a layer 1 depth of 0–15 cm and a layer 2 depth of 15–30 cm, as shown in Fig. 4.

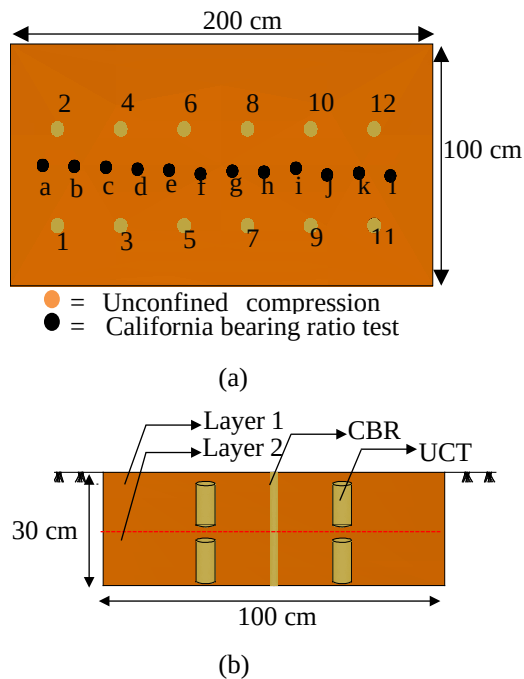


Fig.3 (a) Longitudinal section (b) Cross section

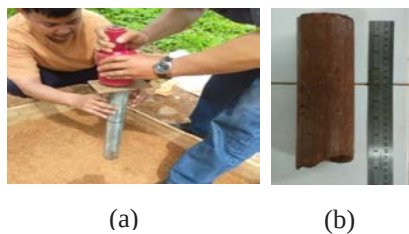


Fig.4 (a) UCS field sampling, (b) UCS samples

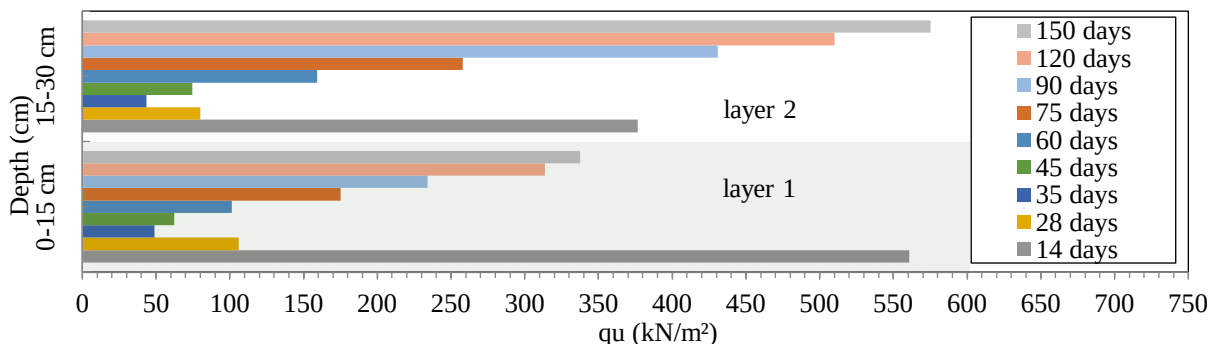


Fig.5 Unconfined compression test results on field test samples for 150 days

4. RESULTS AND DISCUSSION

To determine the best characteristics to apply to a particular situation, it is very important that we start by analyzing the relationship between the mechanical properties of the soil [16]. Before digging the test tub, according to standard ASTM Procedures D422-63, D854-58, D4318-84, and D427-61, the fundamental properties of soil, such as grain size analysis, specific gravity of soil solids, and Atterberg limits (liquid limit, plastic limit, and shrinkage limit), were determined [17]. The results of soil testing were shown in Table 1.

Table 1. Summary of soil characteristics investigation

Test	Test results	
	Unit	Value
Specific gravity (Gs)	-	2.69
Water content (w)	%	29.64
Wet density (γ_{wet})	kN/m ³	17.08
Dry density (γ_{dry})	kN/m ³	13.17
Atterberg limit		
Liquid Limit (LL)	%	28.9
Plastic Limit (PL)	%	12.35
Plasticity Index (PI)	%	16.55
Shrinkage Limit (SL)	%	10.85
Sieve analysis and hydrometer :		
Gravel	%	0
Sand	%	19.2
Silt	%	27.04
Clay	%	53.76
Classification USCS: CL		
Engineering Properties of Soft Soil		
Unconfined Compressive Strength (qu)	kN/m ²	26.9
California Bearing Ratio (CBR)	%	5.38

4.1 Unconfined Compression Test Results Of Bacteria-Stabilized Soil Layer

The most often used test to characterize the strength of biocemented soils is the unconfined compressive strength (UCS) [18]. Sample testing was carried out at the ages of 14, 28, 35, 45, 60, 75, 90, 120, and 150 days; soil depth of 30 cm (0–15 cm and 15–30 cm) in layers 1 and 2, and daily rainfall information during a 150-day period as shown in Fig. 5.

Figure 5 illustrates, where q_u values vary. Figure 5 illustrates the greatest value of 560.71 kN/m^2 for the q_u value (depth 0–15 cm) throughout the 14-day testing period. For 150 days, the q_u value in layer 2 (depth 15–30 cm) is 575 kN/m^2 , and for layer 1 (0–15 cm), it is 338 kN/m^2 . After 35 days, the q_u value decreased; in the first surface layer, the q_u value was 49 kN/m^2 , while in the second layer, the q_u value was 43 kN/m^2 . When comparing the q_u values in the first and second layers at 90 days of testing, there is a difference between the first layer's 234 kN/m^2 and the second layer's 15–30 cm q_u value of 431 kN/m^2 , which is an increase of 10 times from the test at 35 days. This difference is observed at 45 days of testing. This

discrepancy results from low rainfall intensity as well as the depth of the second layer of bonding that forms between soil particles and CaCO_3 calcite biocementation. Bacteria's endospores have the ability to endure both dry and wet circumstances during the rainy and dry seasons. Extending the curing period can improve the soil's compressive strength in comparison to unstabilized soil. Fig. 6 illustrates the link between stress and strain. The relationship between stress and strain is plotted on the graph in Figures 6 and 7. The amount of rainfall increases between 35 and 75 days into the test, then declines between 120 and 150 days. The interactions between soil and additional materials were mostly responsible for the rise in UCS [19].

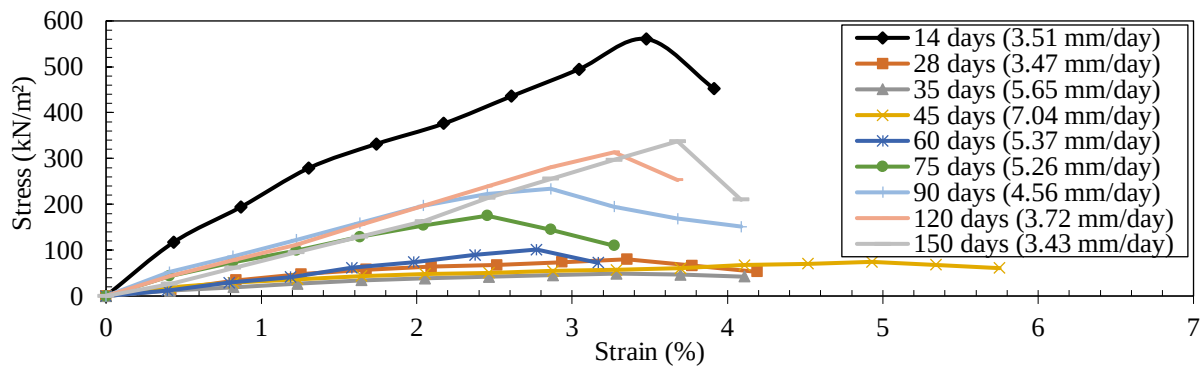


Fig.6 Stress-strain relationship depth (0-15 cm)

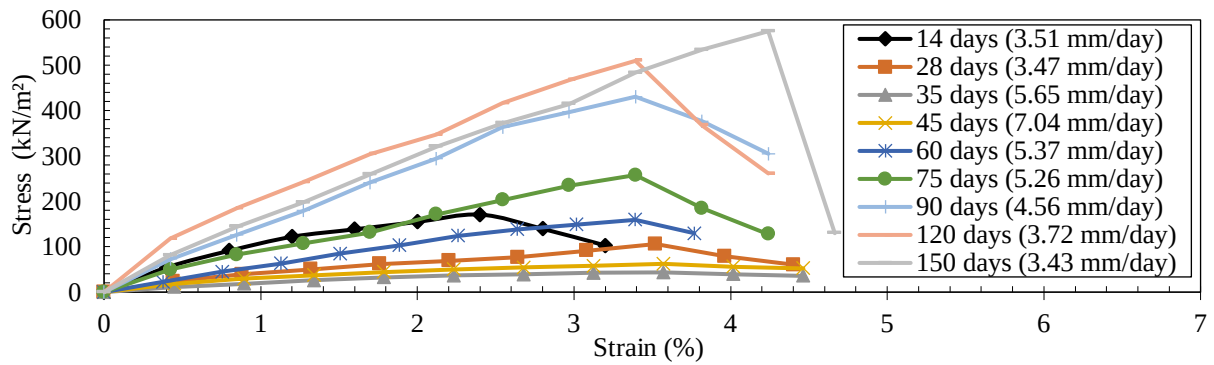


Fig.7 Stress-strain relationship depth (15-30 cm)

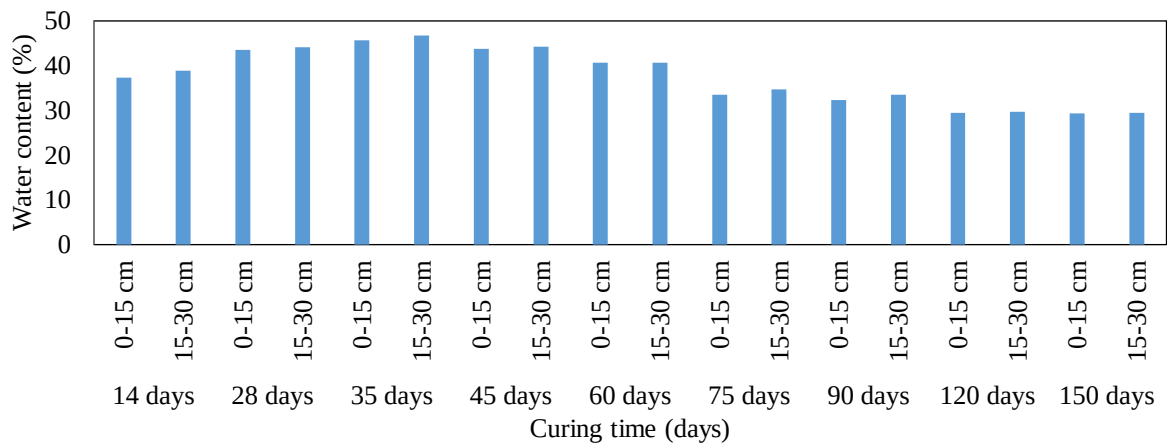


Fig.8 Relationship curing time and water content

Figure 8 demonstrates the relationship curing time and water content, in comparison to layer 2 (15–30 cm), which has a water content of 37–46%, layer 1 (0–15 cm) has a higher q_u value at 14–35 days. Rainfall has a big impact on this, a lot of it changes the soil's compressive strength.

4.2 Results of Bacterial Biocementation Field CBR Tests

CBR testing is used to evaluate the strength of foundation, subgrade, and subbase materials, including recycled materials for pavements on roads and airports. The basic soil condition is improved with a higher CBR value. CBR testing was carried out over a 150 day period. The results of the CBR test carried out using the DCP (Dynamic Cone Penetrometer) tool

are shown in Fig. 9, displays the CBR results in an excavation tub that has been 150 days cemented with bacteria. The results vary according on the season (rainy or dry). As observed in tests 28, 35, 45, and 60 days, Figure 10 illustrates how high rainfall affects the CBR value. The maximum rainfall of 51 mm and the CBR value of 3% result in weakened soil particle bonds, decreased soil bearing capacity, and a maximum CBR value of 20% or increased four times. For the duration of the 90–150 day test, the CBR value remains fixed at 20%. According to the Indonesian general specification, subgrade and subbase should have minimum CBR values of 6 and 20%, biocementation increases the carrying capacity of the soil. The relationship between depth and changes in bearing capacity can be seen in Fig. 10.

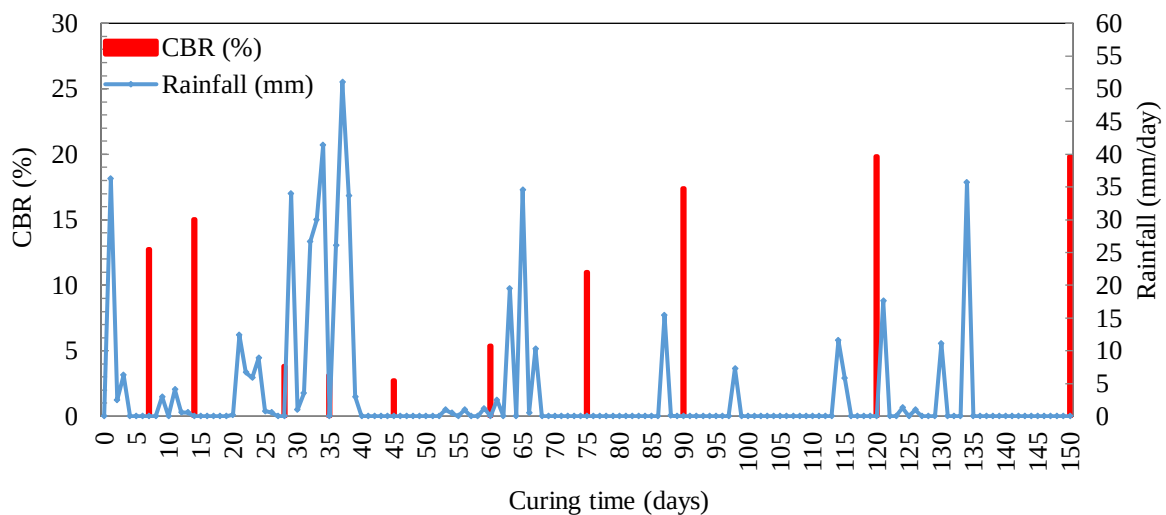


Fig.9 Relationship between CBR and daily rainfall during a 150-day testing period

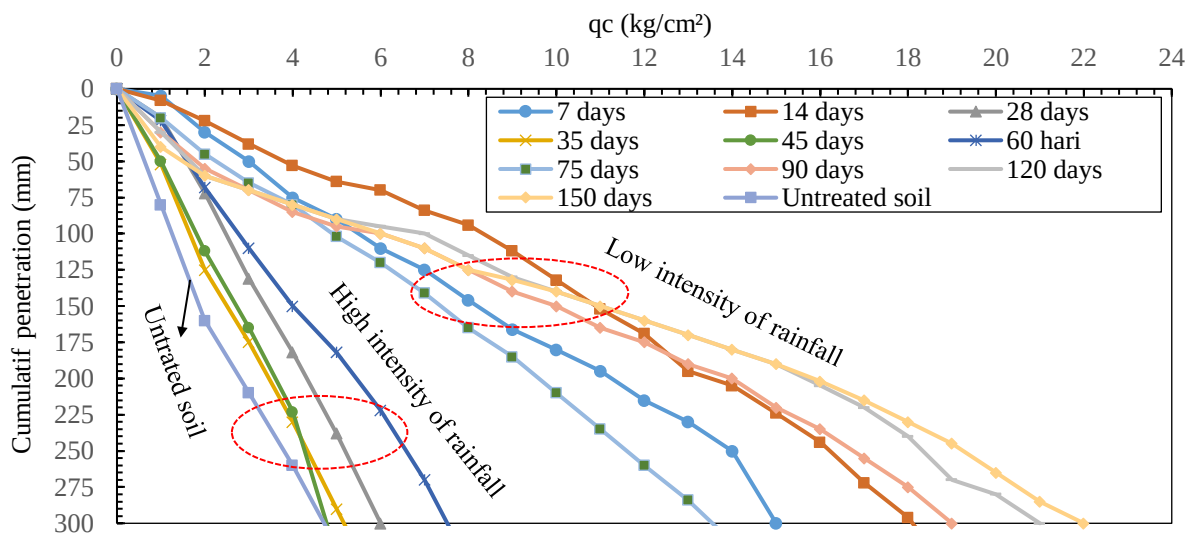


Fig.10 Relationship between depth and change in soil bearing capacity

Figure 10 shows the results of the 30 cm depth. There are differences in the value of each test, such as testing at 14, 90, 120, and 150 days. At 14 days, the strength of the first layer (0–15 cm) is stronger than the second layer, but at 90, 120, and 150 days, the second layer is stronger than the first layer. This shows that the CaCO_3 cementation under the surface layer is increasing. This is caused by the condition of the soil, which is not exposed to direct sunlight, so that soil moisture is able to optimize the bonds between soil particles.

4.3 Microstructure Test Results Scanning Electron Microscope (SEM)

The examination of microstructure used SEM, which is a method for examining the surface and cross-sectional morphology of materials under magnification. As seen in Fig. 11, SEM analysis was performed on both bacterially stabilized and unstabilized soil samples. SEM give up-close view of the CaCO_3 connections formed at the inter-particle soil grains and sheds light on the mechanisms that improve biocemented soils. Figure 11 shows the results of SEM testing with 10 μm magnification on soil that is not cemented by bacteria: (a) there is no calcite (whitish color) and there is no bond calcite that binds soil particles; the color of the material tends to be blackish; and (b) the results of SEM testing on the original soil with bacterial cementation show that the soil cemented by bacteria has whitish calcite. The shape of the precipitated crystals (CaCO_3) acts as a binding agent between soil particles, serving as the principal soil stabilizing factor [20]. Figure 11 shows the growth and development of CaCO_3 crystals. Bond calcite forms links between soil particles to cement the soil, and bond calcite on soil particles to produce sturdiness in the material. This is consistent with the findings of [21]. The result of the bio-cementation process are carbonate ion deposits known as bio-cements or calcium carbonate crystals [22], who used SEM testing of soil samples to discover that bacterial biocementation leads to the creation of soil particle bonds.

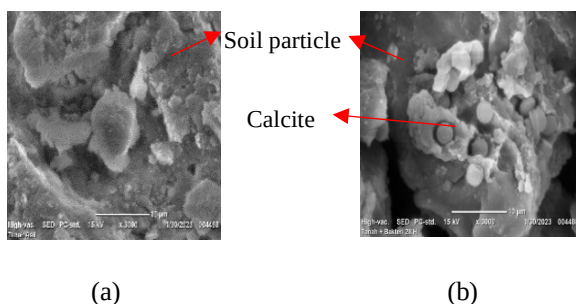


Fig.11 Microstructural analysis Scanning Electron Microscope (SEM) (a) Untreated soil (b) Treated soil

5. CONCLUSION

The results of the field test model indicated that the subgrade layer of soil produced compressive strength values that increased with the curing period. However, the soil's bearing capacity was impacted by rainfall, with high rainfall causing a decrease in the soil's bearing capacity. At 14 to 45 days following testing, layer 1 had a higher q_u than layer 2. When testing had been performed 60–90 days on, layer 2's q_u value was higher. The increase in CBR values occurred due to bacterial cementation, but high rainfall affected the CBR values, as in the 28, 35, and 45-day tests, the CBR was 3%. During 150 days of curing, the optimum bearing capacity of the MICP-stabilized soil increased 4 times that of the soil without bacterial stabilization, while the optimum compressive strength increased 3 times that of the soil without bacterial stabilization. SEM test showed calcite on the soil surface due to MICP. The research shows that the stabilization of *Bacillus subtilis* bacteria in soil increase the soil's carrying capacity as a subgrade and as an environmentally benign.

6. REFERENCES

- [1] Moqsd M. A., Slope Soil Stabilization Through Biocementation By Native Bacteria in Chugoku Region, Japan. *International Journal of GEOMATE*, Vol. 21, No. 83, 2021, pp. 36–42.
- [2] Gowthaman S., Mitsuyama S., Nakashima K., Komatsu M., and Kawasaki S., Biogeotechnical Approach for Slope Soil Stabilization Using Locally Isolated Bacteria and Inexpensive Low-Grade Chemicals: A Feasibility Study on Hokkaido Expressway Soil, Japan. *Soils and Foundations*, Vol. 59, No. 2, 2019, pp. 484–99.
- [3] DeJong J. T., Mortensen B. M., Martinez B. C., and Nelson D. C., Bio-Mediated Soil Improvement. *Ecological Engineering*, Vol. 36, No. 2, 2010, pp. 197–210.
- [4] Røyne A., Phua Y. J., Le S. B., Eikjeland I. G., Josefsen K. D., Markussen S., Myhr A., Throne-Holst H., Sikorski P., and Wentzel A., Towards a Low CO₂ Emission Building Material Employing Bacterial Metabolism (1/2): The Bacterial System and Prototype Production. *Plos One*, Vol. 14, No. 4, 2019, pp. 1–24.
- [5] Oliveira P. J. V., Freitas L. D., and Carmona J. P. S. F., Effect of Soil Type on the Enzymatic Calcium Carbonate Precipitation Process Used for Soil Improvement. *Journal of Materials in Civil Engineering*, Vol. 29, No. 4, 2017, pp. 1–7.
- [6] Carmona J. P. S. F., Oliveira P. J. V., Lemos L. J. L., and Pedro A. M. G., Improvement of a Sandy Soil by Enzymatic Calcium Carbonate Precipitation. *Proceedings of the Institution of Civil Engineers. Geotechnical Engineering*, Vol.

- 171, No. 1, 2018, pp. 1–13.
- [7] Yu T., Souli H., Pechaud Y., and Fleureau J. M., Review on Engineering Properties of MICP-Treated Soils. *Geomechanics and Engineering*, Vol. 27, No. 1, 2021, pp. 13–30.
- [8] Achal V. and Pan X., Characterization of Urease and Carbonic Anhydrase Producing Bacteria and Their Role in Calcite Precipitation. *Current Microbiology*, Vol. 62, No. 3, 2021, pp. 894–902.
- [9] Phang I. R. K., Wong K. S., Chan Y. S., and Lau S. Y., Effect of Microbial-Induced Calcite Precipitation towards Tropical Organic Soil. *AIP Conference Proceedings*, Vol. 2020, 2018, pp. 1–6.
- [10] Qabany A. A. and Soga K., Effect of Chemical Treatment Used in MICP on Engineering Properties of Cemented Soils. *Geotechnique*, Vol. 63, No. 4, 2013, pp. 107–115.
- [11] Hasriana, Samang L., Djide M. N., and Harianto T., A Study on Clay Soil Improvement with *Bacillus Subtilis* Bacteria as the Road Subbase Layer. *International Journal of GEOMATE*, Vol. 15, No. 52, 2018, pp. 114–120.
- [12] Indriani A. M., Harianto T., Djamaluddin A. R., and Arsyad A., Bioremediation of Coal Contaminated Soil As the Road Foundations Layer. *International Journal of GEOMATE*, Vol. 21, No. 84, 2021, pp. 76–84.
- [13] Hasan H. A., Lafta S. H., Majeed M. W., Khabbaz H., and Aghayarzadeh M., Numerical Simulation of Pervious Concrete Pile in Loose and Silty Sand After Treating With Microbially Induced Calcite Precipitation. *International Journal of GEOMATE*, Vol. 22, No. 90, 2022, pp. 32–39.
- [14] Hammes F. and Verstraete W., Key Roles of PH and Calcium Metabolism in Microbial Carbonate Precipitation. *Reviews in Environmental Science and Biotechnology*, Vol. 1, No. 1, 2002, pp. 3–7.
- [15] Harianto T., Performance of Subbase Layer with Geogrid Reinforcement and Zeolite-Waterglass Stabilization, *Civil Engineering Journal*, Vol. 8, No. 2, 2022, pp. 251–262.
- [16] Harianto T., Hayashi S., Du Y. J., and Suetsugu D., Experimental Investigation on Strength and Mechanical Behavior of Compacted Soil-Fiber Mixtures, *Geosynthetics in Civil and Environmental Engineering-Geosynthetics Asia 2008: Proceedings of the 4th Asian Regional Conference on Geosynthetics*, 2008, pp. 392–397.
- [17] Harianto T., Hayashi S., Du Y. J., and Suetsugu D., Effects of Fiber Additives on the Desiccation Crack Behavior of the Compacted Akaboku Soil as a Material for Landfill Cover Barrier. *Water, Air, and Soil Pollution*, Vol. 194, No. 1–4, 2008, pp. 141–149.
- [18] Mujah D., Shahin M. A., and Cheng L., State-of-the-Art Review of Biocementation by Microbially Induced Calcite Precipitation (MICP) for Soil Stabilization. *Geomicrobiology J.*, 2016, pp 1–15.
- [19] Harianto T. and Utami W. D., Effect of Mineral Additives on the Strength Characteristics of a Laterite Soil. *Lecture Notes in Civil Engineering*, Vol. 144, 2021, pp. 423–431.
- [20] Imran A., Nakashima K., Evelpidou N., and Kawasaki S., Improvement of Using Crude Extract Urease From Watermelon Seeds for Biocementation Technology. *Int. Journal of GEOMATE*, 20(78), 2021, pp. 142–147.
- [21] Soon N. W., Lee L. M., Khun T. C., and Ling H. S., Factors Affecting Improvement in Engineering Properties of Residual Soil through Microbial-Induced Calcite Precipitation. *Journal of Geotechnical and Geoenvironmental Engineering*, Vol. 140, No. 5, 2014, pp. 1–11.
- [22] Mortazavi H., Kariminia T., Shahbodagh B., Rowshanzamir M. A., and Khoshghalb A., Application of Bio-Cementation to Enhance Shear Strength Parameters of Soil-Steel Interface. *Construction and Building Materials*, Vol. 294, 2021, pp. 1–11.

Copyright © Int. J. of GEOMATE All rights reserved, including making copies, unless permission is obtained from the copyright proprietors.
