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SLOPE SOIL STABILIZATION THROUGH BIOCEMENTATION BY NATIVE BACTERIA IN CHUGOKU REGION, JAPAN

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ABSTRACT: In this research, a study has been carried out to investigate the potential use of native bacteria from the Chugoku region of Japan for slope soil stabilization. In the biocementation process, soil improvement occurs due to the crystallization of calcium carbonate, which binds soil particles by the enzyme urease of ureolytic bacteria. The precipitated carbonate cements the particle contacts and fills the pores with or without bridging the adjacent soil particles, thereby eventually stiffening the soil matrix. A 60 x 20 mm mold has been used with the natural slope soil collected from Ube City, Yamaguchi, Japan. The treatment was carried out for 21days at the constant room temperature. A direct shear test has been carried out to examine the strength of the bio-cemented soil under the normal stress of 50, 100 and 150 kPa. It was observed that the angle of friction has increased after the biocementation of soil (28 to 34 degrees). Test results indicated the effectiveness of the native bacteria for increasing the strength of the slope soil. The generated calcium carbonate revealed that the native bacteria of Chugoku region could use biocementation for potential slope stabilization.

Keywords: Biocementation, Direct shear test, Native bacteria, Slope stabilization

1. INTRODUCTION

Biocementation, based on microbial induced calcite precipitation (MICP), is one of the promising soil stabilization techniques that has recently gained the interests of many researchers [1-3]. In biocementation process, soil improvement occurs due to the crystallization of calcium-carbonate-cement soil particles by the enzyme urease of ureolytic bacteria. Initially, the enzymatic hydrolysis of urea releases ammonium and carbonate ions in the medium. The produced carbonate ions precipitate in the presence of calcium ions as calcium carbonate crystals on the surface of the soil particles. The bacteria play another vital role: because the cell surfaces of bacteria are negatively charged, the calcium ions are attracted, and nucleation for calcium carbonate precipitation begins on the surfaces [4].

$$CO(NH_2)_2 + 3H_2O \rightarrow 2NH_4^+ + 2OH^- + CO_2 \quad (1)$$

$$CO_2 + H_2O \rightarrow HCO_3^- + H^+ \quad (2)$$

$$HCO_3^- + Ca_2^+ + OH^- \rightarrow CaCO_3 + H_2O \quad (3)$$

In MICP, the bio-mediated cementation process can bind soil particles at contact locations, coat soil particle surfaces, and reduce void space in porous media [4, 5]. The generated calcite makes bonds between the soil particles [6-9]. This biocementation has a wide range of applications that are mainly considering slope protection [10-13].

Recently, rainfall-induced geo-disasters are increasing in the Chugoku region (Yamaguchi, Hiroshima areas) of Japan [14]. The slope protection methods which are currently being used are mainly not suitable for the environment. They mostly use cement which is not ideal for the environment. The natural biocementation method can be a sustainable solution. Up to now, many studies have focused on MICP based soil stabilization to mitigate the potential of erodibility [15-20]. Most of them have been performed based on a bioaugmentation strategy by introducing non-native ureolytic bacteria to the soil. Among them, *S. pasteurii* is the most researched bacteria; it enables a highly active urease enzyme associated with urea hydrolysis [5, 21]. The solidification of sand using this bacterium can control surficial sediment erosion and reduce hydraulic conductivity while increasing the confined compressive strength [22-25].

However, the bio-augmentation of exogenous bacteria which have not been adapted to the native environment is associated with uncertainties in bacterial survival and performance. The weather factors have also affected the performances of the biocementation if the non-native bacteria are used. Sometimes, it is not allowed to use foreign bacteria to improve the soil due to biosafety. So, it is crucial to use the native bacteria to bio-cement the local slope. This study aims to assess the feasibility of the use of native bacteria to improve the soil strength in natural slope in the Chugoku region of Japan.

2. RESEARCH SIGNIFICANCE

This research aims to evaluate the utilization of the native bacteria to improve soil bio-stabilization in Western Japan. This method may reduce the problem of biocementation due to the imported bacteria from other countries or other parts of the country. It will also decrease the total cost of the biocementation process. So, the application of this method undoubtedly impacts the future as it is an environmentally friendly approach of soil erosion protection in the slope and reduces the geo-disasters.

3. MATERIALS AND METHODS

3.1 Slope Soil Sample Collection

The natural soil was collected from a natural slope of Ube city, Yamaguchi Prefecture, Japan (33.956 ^{0} N, 131.272 ^{0} E) in the southern part of Honshu island. The grain size analysis of the collected soil has been shown in Fig. 1. The other three samples were collected from Shimonoseki, Iwakuni, and Hiroshima. The Ube slope soil is similar to the other natural slope soils in the Chugoku region in Japan, stated in Table 1 and Fig.1 in terms of basic soil properties and grain size analysis. The samples were collected in sterile tubes and transported to the laboratory. The basic physicochemical properties of collected samples have been measured, which are shown in Table 1. The collected soil has been cleaned to remove any dry roots or other waste materials that might hamper the sample preparation. To ensure the optimal results, soils with natural slope soil free from organic contents were used in this experiment.

Table 1 Basic soil properties of the slope soil

Slope Soil sample	WC (%)	pH	LOI (%)	Density (g/cm^3)	Uc
Ube	12.1	7.11	6	2.575	77.8
Shimonoseki	10.3	6.99	5	2.622	12.4
Iwakuni	16.1	7.14	7	2.633	48
Hiroshima	12.8	6.98	10	2.610	33

3.2 Culture Solution

The culture solution was prepared using pure chemicals, which are generally used in standard laboratory experimentations. The compositions of the culture solutions are clearly described and presented in Table 2. The amounts were expressed for 1 liter of distilled water. All the chemicals were added to the distilled water and shook adequately to mix. The prepared mix was filtered before applying it to the soil.

3.3 Cementation Solutions

The cementation solution was prepared by using pure chemicals. The detail of the composition of cementation solutions is summarized in Table 3. The composition is shown for 1 liter of distilled water. The chemicals were mixed with the distilled water and filtered.

Table 2 Composition of the culture solution

Substance	Amount (g)
Ammonium sulfate	10
Yeast Extract	20
Tris-buffer	15.70
Distilled water	1 L

Table 3: Composition of cementation solution

Substance	Amount (g)
Calcium chloride	55.5
Urea	30
Nutrient broth	3
Distilled water	1 L
Sodium Acetate	7

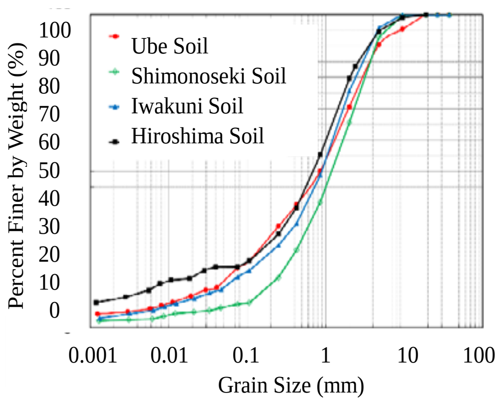


Fig. 1 Grain size analysis of slope soils in Chugoku region, Japan.

3.4 Laboratory Experiment

Figure 2 shows the experimental laboratory setup for this test. The biogrouting potential of the slope of Chugoku areas has been demonstrated by recognizing native ureolytic bacteria. The details of the investigations regarding bio stabilization efficiency are focused only on Ube slope soils. Similar criteria may apply to other slope soils in the different parts of the Chugoku areas and can be focused on the future. A 60 mm× 20 mm cylindrical size mold was adapted for the bio stabilization test (Fig. 2). The relative density was set at 80% while preparing the biocementation and direct shear test sample. A two-stage injection was performed to confine the bacteria for subsequent cementation. During the first stage, bacteria culture was added to fill the soil column, and a cementation solution was added during the second stage. All the solutions were simply applied to the surface of the soil columns and allowed to infiltrate by gravity and capillary forces [11-14]. The cementation solution of 8 ml was poured every day for 21days. The culture solutions were poured 2 times (8 ml) during the test, beginning, and on the 10th day of the test. After 21days of treatment, the sample was collected and dried before conducting the direct shear test. The direct shear test was conducted as it is a simple method and less time needed to measure the strength than the triaxial test and the simple shear test conducted previously. The deviation of the strength was not differed much [3].

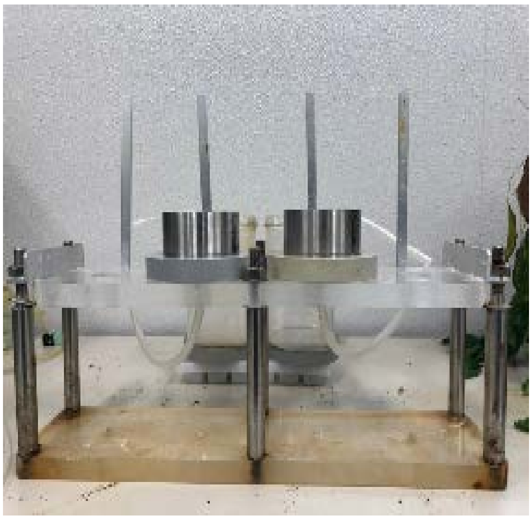


Fig. 2 Laboratory experiment of the bio-cementation of slope soil.

3.5 Direct Shear Test

Direct shear tests were conducted as it is a simple method for measuring the strength according to ASTM D 3080. In general, the test is performed by deforming a specimen at a controlled strain rate on or near a single shear plane determined by the configuration of the apparatus. Three or more samples were tested, each under different normal stresses, to determine the effects upon shear resistance and displacement and strength properties. In this research, the direct shear test was conducted under the condition of shear displacement of 7 mm, speed of 0.2 mm/min, and the three normal loads were 50,100 and 150 kN, respectively. The loss on ignition test was conducted to check the generated calcium carbonate amount.

3.6 Scanning Electron Microscope Images

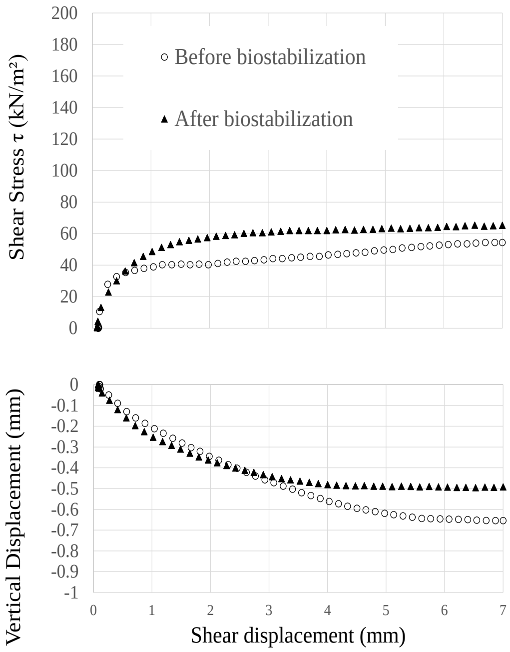
Scanning electron microscope images were completed with a Hitachi S-4100 field emission SEM at an acceleration voltage of 2 kV and magnification of 100x. Before imaging, cemented samples were oven-dried for at least 1-day. Soil samples were collected from the bio-cemented and un-cemented soil samples.

3.7 Identification and Isolation of Ureolytic Bacterium

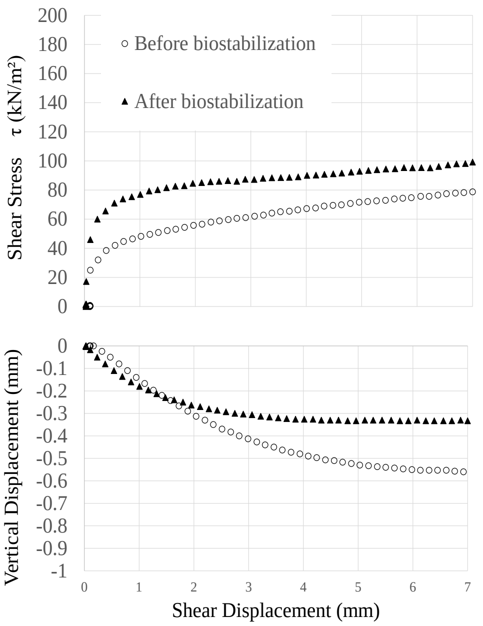
The native bacteria that are dominantly found in the Chugoku region's natural slope are mainly *Bacillus sp.*, *Psychrobacillus sp.*, and *Lysinibacillus sp.* which were similar in the other parts of Japan [1]. The process of isolation of ureolytic bacteria is followed by Gowthaman et al. 2019 [1]. In short, at first, the slope soil was collected in sterile test tubes, transported to the laboratory. Then, to identify the microorganisms, 5.0 g of the natural soil sample was taken and mixed with 45 mL of distilled water, then diluted 10^1-10^4 times using distilled water in separate autoclaved sterile test tubes. Subsequently, 10-micro liters of each dilution were applied to an NH_4–YE agar medium prepared by combining tris-buffer, ammonium sulfate, yeast extract, agar, and distilled water. The cultured plate mediums were placed in an incubator for 3 days at 30 ^{0} C. About 28 colonies were identified in the plate mediums at the end of the incubation period. A cresol-red test solution was prepared by combining 20 mL of cresol red solution, 0.4 g of cresol red with distilled water, and 25 g of $CO(NH_2)_2$ with distilled water (for preparation of 1 L solution). Afterward, each isolated colony was transferred to a 20 mL solution, well shaken, and incubated for 2 h at 45ºC. The basis of this urease activity test is that the cresol red changes from yellow to purple when the pH changes from 7.2 to 8.8, thereby confirming the urease activity of the bacteria. Thus, the ureolytic bacteria were isolated from the natural slope soil.

4. RESULTS AND DISCUSSION

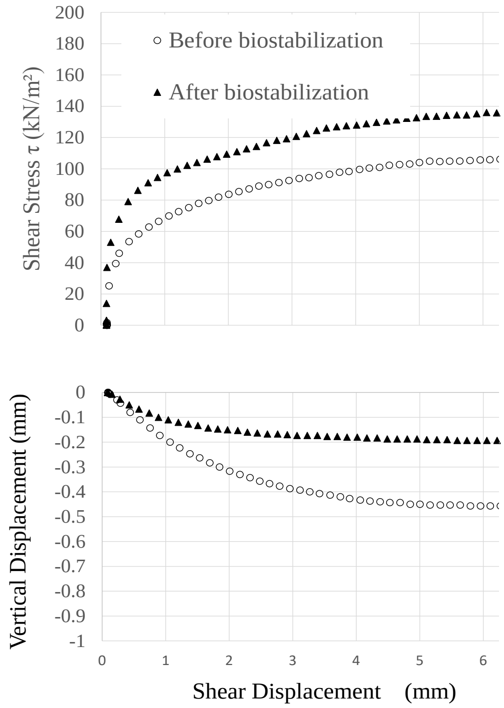
Figure 3 illustrates the result of the direct shear test of the samples which are bio cemented by the native bacteria of the Chugoku region of Japan. It is observed that the shear stress increases gradually with the shear displacement (in Fig 3) for all the 50,100 and 150 kN normal loads. By comparing the two samples, (non-solidified and bio-solidified), it was observed that the bio-cemented soil has increased strength.



a) 50 kN/m² normal stress



b) 100 kN/m² normal stress



c) 150 kN/m² normal stress

Fig. 3 Direct shear test result at 50,100,150 kN/m² normal stress (a),(b), (c), respectively

The shear stress is increased with the increased of normal stress at 21 days of treatment. The native ureolytic bacteria are responsible for the strength increment. The natural ureolytic bacteria in the natural slope of the Chugoku region in Japan can bio-solidify the slope soil. The trend of the vertical displacement for all the 3-stress (50, 100, 150kPa) indicated the densification of the bio-cemented soil after the treatment. The peak shear stress reached at 50,100,150 KN/m^2, respectively. However, none of the trends showed a distinctive peak or softening behavior. This type of trend was also found in the direct shear test experiment previously [3]. The soil properties might influence this type of trend. The vertical displacement always showed the denser trend, and the feeling of bio-cemented soil is hard when touched by hand. Even though the softening behavior was not observed, bio-stabilization has occurred, which has been revealed in the shear displacement and shear stress curve. In biocementation, soil improvement occurs due to the crystallization of calcium carbonate by the enzyme urease of ureolytic bacteria and eventually increases the strength.

The shear stress increased with the increase of the normal stress, which is illustrated in Fig. 4. Figure 4 shows the variation of shear stress with the normal stress after and before bio-stabilization of the slope soil. The rate of this increasing trend was similar to the non-treated and treated soil after 21 days. The internal friction angles increased, and consequently, the shear strength was also increased. The peak friction angle has calculated as 28-degree, 34-degree before and after the biocementation, respectively. The friction angle has been calculated by drawing the slope from shear stress and normal stress. It was depicting a relatively linear increase. The tendency of peak friction angle and carbonate content is in good agreement with the results reported by many researchers [18-22].

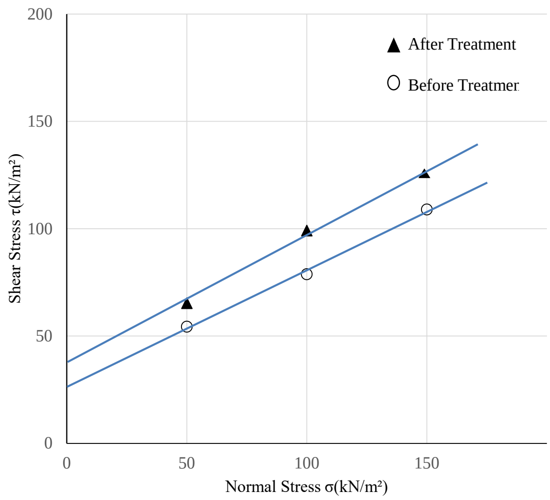


Fig. 4 Relationship of shear stress and the normal stress

It was also observed that after 21days of treatment, the friction angle had increased about 6 degrees. This phenomenon has proved that by bio-cementation, the strength can be increased gradually. The native bacteria of the slope soil in the Chugoku region in Japan can improve the soil's strength to protect it from slope erosion or other geo-disasters if fed with the nutrients and the cementation media. The generated calcium carbonate was measured, and it was found that 4.1% of calcium carbonate had been developed after 21 days of treatment. The native ureolytic bacteria can produce calcium carbonate and increase the slope soil's strength in the northern part of Japan, which was reported before [1].

However, in this current study, the native bacteria of the southern part of Japan's main island can be also actively participated in the biocementation process. To use the non-native bacteria in MICP is not effective due to the weather factors [9]. Many countries in the world do not permit foreign bacteria to use in their native land. In Japan, foreign ureolytic bacteria are not allowed to use in the soil for biocementation process. In the previous studies [3], *Sporosarcina pasteuri* (American Type Culture Collection ATCC11859 was used) due to the high ability to synthesize urea. However, due to the non-native bacteria, it is not suitable in the field application and the environmental protection point of view. The performance of native bacteria is satisfied as they can generate more than 4% Calcium Carbonate during treatment by using the native slope soil. So, the foreign bacteria can be replaced by the native bacteria easily.

To further understand the improvement in shear strength of cemented specimens, effective friction angle and cohesion were calculated from the shear test results based on the Mohr-Coulomb failure criteria. The internal friction angles obtained herein are likely to be higher than the typical friction angle values reported to sandy soils [13,14]. For instance, uniform sand with a similar mean particle diameter has been reported to have a friction angle of around 24 deg. [15]. The soil's friction angle is governed by several factors such as grain size, grain shape, surface roughness, mineralogy, fines, organic content, and moisture level [16,17]. In this current study, the soils were used at their natural state, and no pretreatments were done to remove the impurities from the soil. An observable quantity of organic materials was easily witnessed in the soil, and the presence of organic fines on shearing planes also could affect the result on friction angle. Another reason for this type of result is that the total time for the biocementation process is maintained 21 days in this research, which was longer than the previous studies with the sand.

It was found that the most optimal range of grain size for the biocementation process is between 50 and 400μm because bacterial activity cannot take place in very fine soils, and larger amounts of nutrients are needed to increase the stiffness and strength in coarser soils [31].

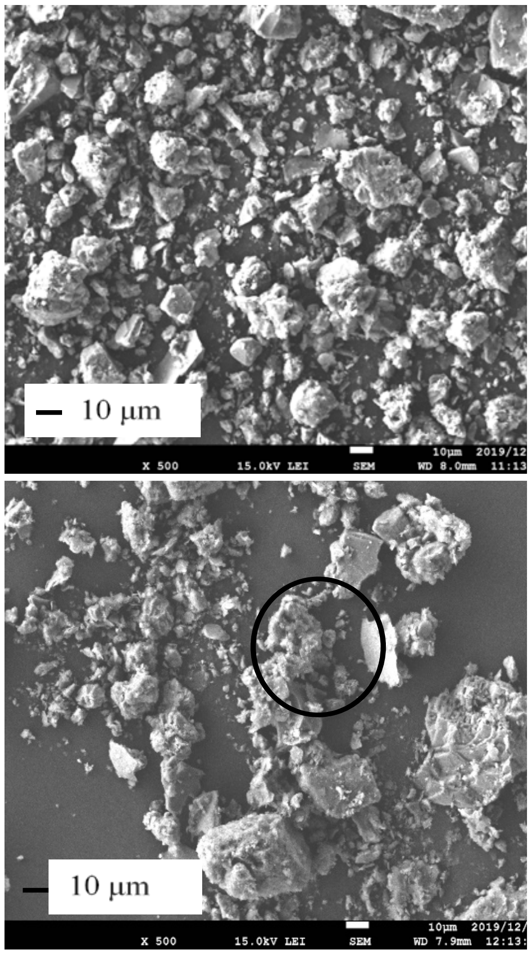


Fig. 5 Scanning electron microscopic view of biocementation of soil; before treatment (top) and after treatment (bottom)

4.1 SEM Analysis

Figure 5 shows that the scanning electron microscope (SEM) view of the biocementation slope soil. The cementation reaction of the specimens was confirmed by the microscopical observations using the SEM [18-21]. Samples were oven-dried at 105ºC for 24h initially, and observations were made at the solidified zone ranges up to 1 cm depth of treated samples of different cases to highlight the effects of treatment [22-24]. The SEM photos of the sample treated under conditions 25ºC show the precipitation of irregular rhombohedral crystals ranges 14-21 micrometer, which is the typical form of crystalline calcites. The test results were validated by similar observations made by another research [25-28].

The crystals are spherical with an average size of around 15-20 micrometers and are probably vaterite crystals. They are surrounded by abundantly accumulating nanocrystals commonly referred to as extracellular polymeric substances with an extensive range in general [29-31]. Further evidence of similar accumulation is shown in Fig 5. Vaterite is the least stable than calcite and can be transformed into calcite very rapidly with time.

The field application of this method needs a model test which is currently conducting by the author. To make the model slope saturated with the nutrient is a great challenge in the future applicability of this method in the natural slope.

5. CONCLUSIONS

In this research, the feasibility of using the emerging microbial induced calcium precipitation technology for soil stabilization of natural slope has been studied to mitigate the erosion of slope soil in the Chugoku region of Japan.

The biostability of the local slope soil has been carried out by using the native bacteria. Direct shear tests have been carried out to check the strength of the bio-cemented soil. It was observed that the soil strength increased significantly by treating the soil for 21days. The native bacteria of the Chugoku region slope soil can increase the strength of the soil if they are fed with nutrients for at least 21days. The generated calcium carbonate was around 4.1% which is the main reason for the bio stabilization and can be compared with the foreign (non-native) bacterial activity [30,31].

The angle of friction was increased with the increase of the treatment for 21 days. There was a relationship between the strength of the treated samples and the global average calcium carbonate. In this research, it was clear that the strength can be increased with the application of microbes in the slope soil. This biocementation method using the native bacteria has clear potential to improve the residual strength by solidifying soil, which can be used to prevent slope soil erosion and other geo-disasters.

Future works must be done to see the seasonal variation of the biocementation in the natural slope with the native bacteria. The effect of different weather conditions and the effect of rainfall must be carried out for biocementation by native bacteria in the Chugoku region of Japan.

6. ACKNOWLEDGEMENTS

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7. REFERENCES

[1] Gowthaman, S., Mitsuyama, S., Nakashima, K., Komatsu, M., 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, 2019, pp. 484-499.

[2] DeJong, J.T., Mortensen, B.M., Martinez, B.C., Nelson, D.C., Biomediated soil improvement. Ecol. Eng. Vol. 36, 2010, pp.197–210.

[3] Moqsud, M.A., Soga, K., Hyodo, M., Nakata, Y.. Evaluation of bio-cemented sand for landslide disaster prevention. International symposium on bio geotechnology. September 12-13, Atlanta, USA. 2018.

[4] Bao, R., Li, J., Chen, L.. Effect of microbial induced calcite precipitation on surface erosion and scour of granular soils proof of concept. Journal of transportation research board, Vol. 2657, 2017,pp.10-18.

[5] Stocks-Fisher, S., Galinat, J. K. , Bang, S. S. Microbiological precipitation of CaCO3. Soil Biology and Biochemistry Vol. 31(11),199, pp. 1563–1571 .

[6] DeJong, J., Fritzges, M. & Nüsslein, K. Microbial induced cementation to control sand response to undrained shear. Journal of Geotechnical and Geoenvironmental Engineering Vol.132(11), 2006, pp.1381–1392 .

[7] Gomez, M.G., Anderson, C.M., Graddy, C.M.R., DeJong, J.T., Nelson, D.C., Ginn, T.R. Large scale comparison of bioaugmentation and biostimulation approaches for biocementation of sands. Journal of geotechnical and geoenvironmental engineering, Vol.143, 2017, 4016124.

[8] Jiang, N.J., Soga, K. The applicability of microbially induced calcite precipitation for internal erosion control in gravel-sand mixtures. Geotechnique, Vol. 67, 2017, pp. 42-55.

[9] Kawasaki, S.. Coral sand solidification test through microbial calcium carbonate precipitation using *Pararhodobacter sp*. Intl. Journal of Geomate. Vol.11 (4): 2016, pp. 2667-2670

[10] Imran, M.A., Kimura, S., Nakashima, K., Evelpidou, N., Kawasaki, S. Feasibility study of native ureolytic bacteria for biocementation towards coastal erosion protection by MICP method. Applied science, Vol. 9, 2019, pp. 4462-4475.

[11] Jonkers, H.M. Toward bio-based geo and civil engineering for a sustainable society. Procedia Engineering. Vol.171, 2017, pp. 168-175.

[12] Gomez, M.G., Graddy, C., DeJong, J. and Nelson, D. Biogeochemical changes during bio-cementation mediated by stimulated and augmented Ureolytic Microorganisms. Scientific Reports. Vol. 9, 2019,11517.

[13] Cui, M.J., Zheng, J.J., Zhang, R.J., Lai, H.J., Zhang, J. Influence of cementation level on the strength behavior of bio-cemented sand. Acta Geotech. Vol. 12, 2017, pp. 971–986.

[14] Montoya, B.M., De Jong, J.T. Stress-strain behavior of sands cemented by microbially induced calcite precipitation. J. Geotech. Geoenvironmental Eng. Vol. 141. 2015

[15] Cheng, L., Cord-Ruwisch, R., Shahin, M.A. Cementation of sand soil by microbially induced calcite precipitation at various degrees of saturation. Can. Geotech. J. Vol. 50,2013, pp. 81–90.

[16] Bareither, C.A., Edil, T.B., Benson, C.H., Mickelson, D.M. Geological and physical factors affecting the friction angle of compacted sands. J. Geotech. Geoenvironmental Eng. Vol. 134, 2008, pp. 1476– 1489.

[17] Canakci, H., Hamed, M., Celik, F., Sidik, W., Eviz, F. Friction characteristics of organic soil with construction materials. Soils Found. Vol. 56,2016, pp. 965–972.

[18] DeJong, J.T., Fritzges, M.B., Nu¨sslein, K. Microbially induced cementation to control sand response to undrained shear. J. Geotech. Geoenvironmental Eng. Vol. 132, 2006, pp. 1381–1392.

[19] DeJong, J.T., Mortensen, B.M., Martinez, B.C., Nelson, D.C. Biomediated soil improvement. Ecol. Eng. 36, 2010, pp. 197–210.

[20] Dhami, N.K., Reddy, M.S., Mukherjee, A. Significant indicators for biomineralization in sand of varying grain sizes. Constr. Build. Mater. 104, 2016, pp. 198–207.

[21] Farukh, M.A., Yamada, T.J. Synoptic climatology of winter daily temperature extremes in Sapporo, northern Japan. Int. J. Climatol. 38, 2018, pp. 2230–2238.

[22] Feng, K., Montoya, B.M.,. Quantifying level of microbial-induced cementation for cyclically loaded sand. J. Geotech. Geoenvironmental Eng. Vol. 143, 2017, 06017005.

[23] Feng, K., Montoya, B.M. Influence of confinement and cementation level on the behavior of microbial-induced calcite precipitated sands under monotonic drained loading. J. Geotech. Geoenvironmental Eng. Vol. 142, 2016, 04015057.

[24] Soon, N.W., Lee, L.M., Khun, T.C., Ling, H.S., Factors affecting improvement in engineering properties of residual soil through microbial-induced calcite precipitation. J. Geotech. Geoenvironmental Eng. Vol. 140, 2014, 04014006.

[25] Van Paassen, L.A., Ghose, R., van der Linden, T.J.M., van der Star, W.R. L., van Loosdrecht, M. C. M. Quantifying biomediated ground improvement by ureolysis: large-scale biogrout experiment. J. Geotech. Geoenvironmental Eng. 136, 2010, 1721–1728.

[26] Viklander, P. Permeability and volume changes in till due to cyclic freeze/thaw. Can. Geotech. J. 35, 2011, 471–477.

[27] Vutukuri, V.S., Lama, R.D., Saluja, S.S.,. Handbook on Mechanical Properties of Rocks: Testing Techniques and Results, Vol. 1. 1974. ed. Trans Tech Publications, Clausthal, Germany.

[28] Wang, Y., Soga, K., Dejong, J.T., Kabla, A.J., Microscale visualization of microbial-induced calcium carbonate precipitation processes. J. Geotech. Geoenviron. Eng. Vol. 145, 2019,pp. 1–13.

[29] Feng K., Montoya B.M., Influence of confinement and cementation level on the behavior of microbial-induced calcite precipitated Sands under monotonic drained loading. J. Geotech. Geoenvironmental Eng, Vol. 2, 2015, 4015057.

[30] Al Qabani, A., Soga, K.. Factors affecting efficiency of microbially induced calcite precipitation. Journal of geotechnical and geoenvironmental engineering, American society of civil engineers (ASCE), Vol. 138 (8), 2012, pp. 992-1001.

[31] Gowthaman, S., Mitsuyama, S., Nakashima, K., Komatsu, M., Kawasaki, S. Microbial induced slope surface stabilization using industrial grade chemicals: A preliminary laboratory study. International Journal of GEOMATE. Vol.17 (60) , 2019, pp. 110-116.