

MICROBIAL INDUCED SLOPE SURFACE STABILIZATION USING INDUSTRIAL-GRADE CHEMICALS: A PRELIMINARY LABORATORY STUDY

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*Corresponding Author, Received: 25 Dec. 2018, Revised: 04 Feb. 2019, Accepted: 18 Feb. 2019

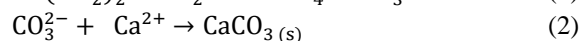
ABSTRACT: One of the promising soil improvement techniques that have recently gained increased attention in Geotechnical and Civil Engineering is microbial induced carbonate precipitation (MICP). The MICP is mediated by ureolytic bacteria through a chain of biochemical reactions which lead to the formation of calcium carbonate cement in soil matrix and persuades the substantial bonds between the soil particles. The study presented herein focuses on surficial stabilization of slope soil (Hokkaido, Japan) by mediating industrial-grade chemicals through two different scales of preliminary laboratory investigations: small-scale columns and bench-scale slopes. Locally isolated *Psychrobacillus* sp. was cultivated in both industrial-grade media (beer yeast) and laboratory-grade media (NH₄-YE: tris-buffer, ammonium sulfate and yeast extract), and the urease activities were compared. The results showed that the cultivation of bacteria in beer yeast resulted in higher urease activity (0.9 U/mL) compared to that in conventional laboratory media (0.4 U/mL). Also, UCS of the specimen treated using industrial-grade chemicals (urea fertilizer, beer yeast and snow melting reagent) was about two times higher than the specimen treated using conventional laboratory-grade chemicals (urea, CaCl₂ and nutrient broth). The benchtop-scale test revealed that the highest surface strength (UCS of 1.02 MPa) was achieved while treating the soil by 0.5 M cementation solution at 30°C. Sets of colorimeter measurements were undertaken on treated slope models to compare precipitation profile at different locations. These findings suggest that industrial-grade chemicals can contribute as potential candidates in MICP applications from the perspective of cost reduction.

Keywords: Microbial induced carbonate precipitation (MICP), Industrial-grade chemicals, Laboratory-grade chemicals, Locally isolated ureolytic bacteria, Cost reduction

1. INTRODUCTION

Microbial induced carbonate precipitation (MICP) is one of the most promising soil stabilization techniques being investigated nowadays. The researchers have used the principle of bio-metabolism to generate the precipitation of calcium carbonate to enable the bridging between soil particles [1-4]. Cementing the particle contacts with a subsequent change of mechanical properties of soil matrix is found to be an innovative approach in sustainable geotechnics with significant scope for future developments [1,2]. The mechanism primarily requires the existence of ureolytic bacteria and calcium source to drive the biochemical reactions. The urease enzyme produced by the bacteria catalyzes the hydrolysis of urea into ammonium and carbonate ions in the aquatic medium (Eq. 1). At the supply of dissolved calcium ions or in the presence of calcium ions, the calcium carbonate mineralization would occur as given in Eq. 2. Eventually, the desired mechanical properties can be achieved, when the calcium carbonate crystals are precipitated

appropriately in the soil matrix.



MICP is nondestructive and less hazardous over conventional soil improvement methods. The unique advantages of the technique provide a wide range of applicability for the scenarios including liquefaction prevention, settlement reductions, piping prevention of dams and levees, slope stabilizations, erosion control, beach rock formations, land stabilizing prior to tunneling, immobilization of hazardous contaminants, facilitating impermeable barriers and carbon sequestrations [3,4]. Up to now, the feasibility of MICP for the above applications has been demonstrated mostly in the laboratory stage using small-scale experiments [5-7]. The studies have widely investigated and addressed the injection procedures, the concentration of reagents and bacterial controls in achieving the desired behavior of the soil matrix. The next step of the MICP technique is to scale up the process using treatment conditions

to prove the feasibility of this technique for real applications. Only a very few studies have attempted the scaling up of MICP as a ground improvement method [1,8,9].

The final goal of this research work is to introduce the MICP as a potential candidate for slope surface stabilization against surface erosion in a cost-effective way. The study attempted to upscale the MICP investigation from elementary-scale (columns) to benchtop-scale (slope models). The benchtop-scale experiments presented herein can be considered as an intermediate step between elementary-scale experiments and large-scale/ field in-situ. However, the elementary-scale experiments are very essential to experience the feasibility as well as to enable the optimization of treatment before upscaling as reported by DeJong et al. [4].

At the same time, the viability of MICP strategy does not hinge only on technical aspects regarding treatment conditions, but also highly associates with economical contests. The previous studies have widely demonstrated the feasibility of MICP by using expensive laboratory-grade reagents such as urea, calcium chloride and conventional nutrient media [2,3,5,7,8]. But, the cost of the required materials is the biggest challenge in assessing the complete feasibility of MICP [8], particularly at field scale implementations. Thus, this paper also aims to investigate the feasibility of using industrial-grade chemicals in place of laboratory-grade chemicals in MICP to overcome the economic challenges. This research work would be an important benchmark to endorse future MICP applications in a cost-effective way.

2. MATERIALS AND METHODS

2.1 Ureolytic bacteria and MICP reagents

The bacteria used in this study is a gram-positive strain, locally isolated from slope soil of Asari Expressway (Hokkaido, Japan). The detection and isolation methods of the strain are the same as Danjo and Kawasaki [5]. The isolated ureolytic strain was characterized as *Psychrobacillus* sp. by sequencing its 16S rDNA and comparing the results to sequences available in the Apollon DB-BA 9.0 database, GenBank, DDBJ (DNA Data of Japan) and EMBL (European Molecular Biology Laboratory).

The soil collected from the expressway slope of Asari was used in the solidification tests presented herein. Asari slope soil is a well-graded soil with D_{10} , D_{30} and D_{50} of 0.36, 0.75 and 1.4 mm respectively. Particle density of the soil is 2.29 g/cm³. Also, natural moisture content and pH of the soil are respectively 21.8 ± 1.30 and 7.029.

Two types of culture mediums (CM) and cementation solutions (CS) were used in current experiments and are described below. $CM_{laboratory-grade}$

and $CS_{laboratory-grade}$ were prepared using laboratory-grade reagents, whereas industrial-grade chemicals were used to prepare $CM_{industrial-grade}$ and $CS_{industrial-grade}$ solutions.

Culture mediums CM

$CM_{laboratory-grade}$: 15.7 g/L tris-buffer, 10 g/L ammonium sulfate, 20 g/L yeast extract, distilled water

$CM_{industrial-grade}$: 30 g/L beer yeast, distilled water

Cementation solutions CS (0.5 mol/L)

$CS_{laboratory-grade}$: 30 g/L urea, 55.5 g/L CaCl₂, 3 g/L nutrient broth, distilled water

$CS_{industrial-grade}$: 30 g/L urea fertilizer, 55.5 g/L snow melting agent, 2 g/L beer yeast, distilled water

Purity is the major difference between laboratory-grade chemicals and industrial-grade chemicals. The urea fertilizer, comprised of 46.0% purity in nitrogen, is widely used in the agriculture industry. The snow melting agent (calcium chloride of 74% purity) is applied for melting the ice. Beer yeast is primarily used in the food industry to break down sugars.

2.2 Urease activity test

The activity of the bacterial urease enzyme was measured using the Indophenol method. 1 mL of bacterial culture was added to the 0.1 mol/L urea prepared in phosphate buffer solution (100 mL). The ammonium ions produced from hydrolyses of urea react with phenol and hypochlorite, which produce the blue color indophenol dye in the weak alkaline-alkaline medium. The intensity of indophenol dye was measured at different uniform intervals of catalyzation. Using the calibration curve developed between the concentration of ammonium ions and intensity, the rates of urea hydrolysis were assessed.

2.3 Column solidification test

The syringes (30 mL in capacity and 25 mm in diameter) were positioned vertically and packed with 45 g by filling with three compacted layers of oven dried (105°C for 48 hours) soil as shown in Fig. 1. All the solutions were injected to the top of the soil columns and allowed to percolate by gravity and capillary forces. The movement of the front reaction fluid was permitted under constant flow conditions. Two test conditions were investigated and are presented in Table 1. In both tests, the bacteria culture medium of 10 mL was injected at the beginning of the experiment. The cementation solution of 6 mL was applied every 24 hours to the column specimens throughout the 10 days of treatment.



Fig.1 Syringe Columns

Table 1 Conditions of column solidification tests

Test	Bacteria Culture	Cementation solution	Temp. (°C)
1	<i>CM_{laboratory-grade}</i>	<i>CS_{laboratory-grade}</i>	30
2	<i>CM_{industrial-grade}</i>	<i>CS_{industrial-grade}</i>	30

2.4 Slope solidification test

The size of the slope model used in the experimentation study is 10 cm × 12 cm in the bottom and 10 cm in height as shown in Fig.2. In fact, the slope model test is the scaling-up of previous elementary-scale column tests. At the same time, the gradient of 1: 1.2 was incorporated in the scaled-up slope model in order to represent the standard cut slope of the expressways, which is generally critical in stability compared to that of standard filling/embankment slope (1: 1.8) [10,11]. The slope-mold was filled in properly tamped five layers of soil (without oven-dry/sterilization).

Four test cases were investigated to optimize the slope model solidification, and the test conditions are clearly summarized in Table 2. Based on the observations made at column solidification tests, industrial-grade chemicals (*CM_{industrial-grade}* and *CS_{industrial-grade}*) were chosen for the slope model solidification tests. All the solutions were continuously and uniformly applied to the slope surface at a slow rate without formation of ponding on the surface.

Considering the large volume of soil needed to be treated, repeated bacteria culture injection was performed (once in every 5 days during the 10 days treatment period). At the same time, the cementation solution was injected every 24 hours similar to the column tests.



Fig.2 Slope model

Table 2 Conditions of slope solidification tests

Test	Bacteria Culture (mL)	Cementation solution (mL)	Temperature (°C)
1	225	135	30
2	100	67	30
3	50	33	30
4	100	67	30

2.5 UCS measurement

Similar to the previous studies [5,7,12,13], needle penetrometer (SH-70, Maruto Testing Machine Company, Tokyo, Japan) was used to estimate the UCS of the treated specimens. Regression relationship given in Eq. 3, which has been developed by analyzing 114 natural rock samples and 50 improved soils with cement, was used to estimate the UCS of each treated sample by using the ratio between applied force and settlement of needle.

$$\log (y) = 0.978 \log (x) + 2.621 \quad (3)$$

where x is the logarithm of “penetration gradient” when the logarithm of y is unconfined compressive strength. Penetration gradient (N/mm) can be determined using penetration and penetration resistance of the needle.

2.6 Colorimeter measurement

Color measurements were undertaken using spectro-colorimeter (CM-2600 d, manufactured by Konica Minolta), as to compare the carbonate precipitation on the surface of solidified slope specimen. Color space is defined by the three components: L^* , a^* and b^* (“ a^* ” from green ($-a$) to red ($+a$), “ b^* ” from blue ($-b$) to yellow ($+b$) and “ L^* ” from

black (-L) to (+L)). The precipitation of calcium carbonate significantly affects the lightness measure (L^*) among the three components of the colorimetric system. Thus, the lightness changes (L^*) were measured from six different locations (five readings per each location) of the solidified slope surface.

3. RESULTS AND DISCUSSION

3.1 Effect of growth media in urease activity

Two types of culture media ($CM_{\text{laboratory-grade}}$ and $CM_{\text{industrial-grade}}$) were inoculated with the pre-cultured *Psychrobacillus* sp. at 30°C in a shaking incubator at 160 rpm. Bacterial population (in terms of optical density (OD_{600})) and the urease activity of both media were measured every 24 hours for 5 days. The highest bacteria growths observed in $CM_{\text{laboratory-grade}}$ and $CM_{\text{industrial-grade}}$ were 4.5 and 1.5 respectively. In both medias, the growth of the bacteria peaked at around 2nd day and remained relatively stable until the 5th day. The measured urease activities are presented in Fig. 3. Results show that the cultivation of bacteria in industrial-grade media constituted the highest urease activity of 0.9 U/mL. At the same time, the highest urease activity assessed in laboratory-grade media was 0.4 U/mL. Although the biomass concentration is lower in industrial-grade growth media, the urease activity of industrial growth media is more than two times higher than that in laboratory-grade media.

The industrial-grade growth media used herein was beer yeast media. Basically, beer yeast consists of various chemical substances including proteins, fats, carbohydrate, sugar and fibers. Also, beer yeast largely consists of ions including potassium, phosphorus, sulfur, calcium, iron and copper. Unlike the laboratory-grade media, the impurity of the industrial-grade beer yeast media supplied the various nutritious to the bacteria and contributed to produce the urease enzyme more effectively.

3.2 Column solidification test

Two number of columns tests were conducted to investigate the feasibility of industrial-grade chemicals in MICP. In order to monitor the internal biochemical condition of the soil sample during treatment, Ca^{2+} ion concentration and pH were measured every day from the percolated solution. The concentrations and pH are presented in Fig. 4. Observations suggest that weak alkali pH (7.0-8.5) conditions were maintained during the treatment process in both test cases. The calcium ion concentration decreased continuously after around 2-3 days of the process. The calcium ion reduction coupled with pH increment indicated that the chemical reaction of urea hydrolysis and calcium carbonate precipitation started by the injected

ureolytic bacteria.

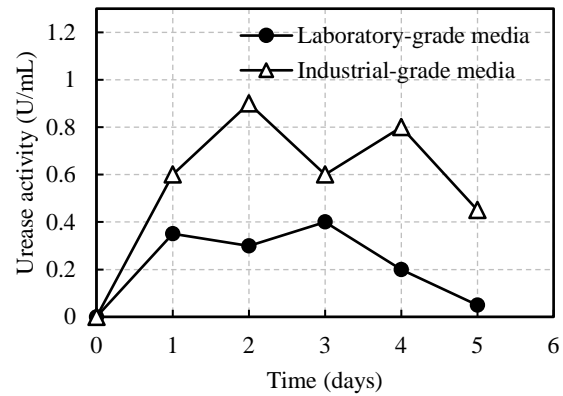


Fig.3 Urease activity of culture media with time

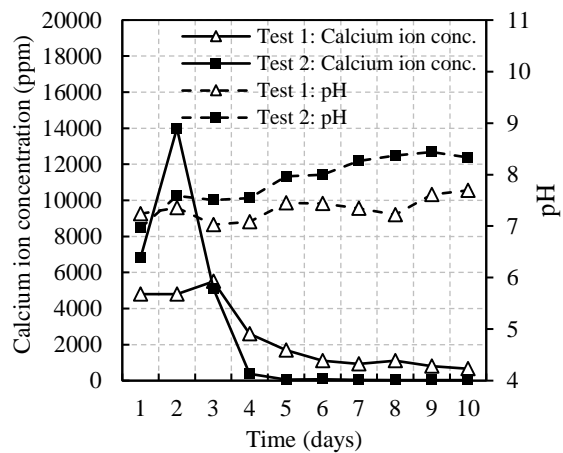


Fig.4 Calcium ion concentration and pH at the treatment

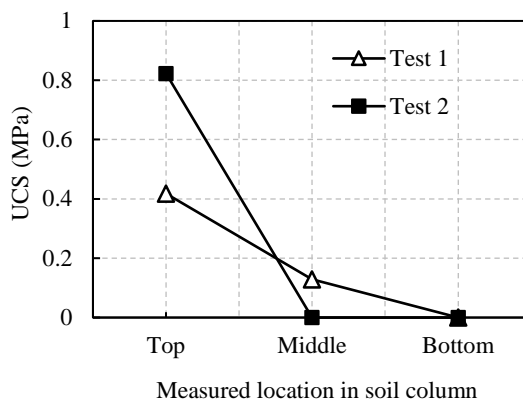


Fig.5 Strength measures of solidified columns

The strength measurements were undertaken on column specimens after ten days of treatment. The values of UCS, obtained using needle penetrometer for the samples treated using laboratory-grade reagents (Test 1) and industrial-grade chemicals (Test 2), are compared in Fig. 5. The results show that the

specimen strength decreases with the column depth in both cases. At the slow flow rates, the top part of the column was exposed to higher concentration of reactants compared to that of the bottom of the column [2], which tends to precipitate relatively high amount of calcium carbonate at top of the column. Also, the injected bacteria cells were filtered through the soil with a reduction of bacteria concentration along the path. As the result, a larger concentration of bacteria cells was retained at the top compared to the bottom. Thus, the highest strength was obtained at the top and decreased over the length. The similar observation is reported in previous studies also [1,14]. At the same time, the industrial-grade chemicals have exhibited a significant enhancement in solidification. The surface strength of the sample treated under industrial-grade chemicals is around two times higher than that of the sample treated using laboratory chemicals.

3.3 Slope solidification test

Four cases were undertaken in the slope model solidification test. Based on the positive observations made at column tests, all the slope solidification tests were incorporated with industrial-grade chemicals. The first three tests (Test 1 - Test 3) were performed to assess the effect of the injection volume of chemicals in cementation. A large quantity of injection was made in Test 1 by assuming that the implementation of a large number of bacteria would significantly enhance the solidification. In Test 2 and Test 3, the injection volume was respectively set to 1/2 and 1/4 of the volume considered in Test 1. The lightness (L^*) of the slope surface was measured every 24 hours using spectro-colorimeter to experience the formation of calcium carbonate, and the results are given in Fig. 6. The trend of average L^* values of Test 1 slope shows an initial increment and remains relatively stable thereafter. But, the average L^* values of the Test 2 slope increase gradually with the time. At the same time, there were no considerable changes regarding lightness (L^*) obtained in the treated Test 3 slope, which indicates that there was no adequate precipitation of calcium carbonate obtained on the slope surface due to insufficient supply of reactants.

The surface strength (UCS) distribution of the treated slope specimens of Test 1 and Test 2 are illustrated in Fig. 7. It is well understood that there is a close relationship between color measurement (L^*) and UCS, similar to that suggested by Amarakoon and Kawasaki [7]. As the solidification occurred only at the certain locations of the slope surface of Test 1 evidenced in Fig. 7 (a), the L^* values failed to exhibit the increasing trend (Fig. 5). In the case of Test 2, the unsolidified surface area reduced (Fig. 7 (b)), thereby resulted in the considerable increment in L^* value with the duration. It is very clear that injection

volume plays a vital role in the solidification process. Injection of the reactants in large quantity might lead to washing out of cells from the soil matrix during the percolation i.e. prior to bacterial immobilization. At the same time, injecting an inadequate volume of reactant, would not be able to contribute significant and uniform cementation.

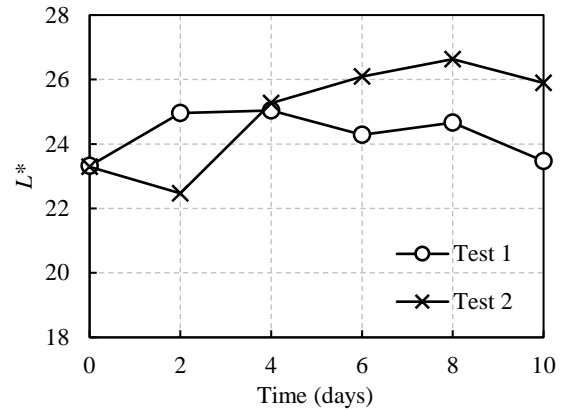


Fig.6 Lightness (L^*) measure on slope surface using spectro-colorimeter

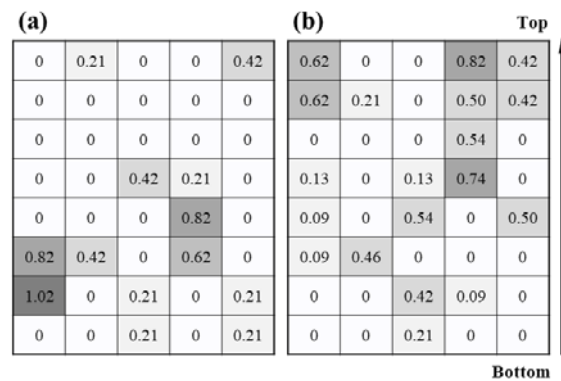


Fig.7 Obtained UCS values with respect to their locations on the slope surface of the specimen (a) Test 1 and (b) Test 2.

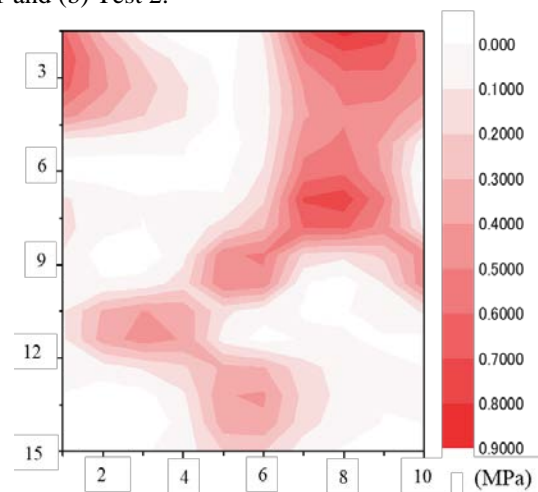


Fig.8 Contour of UCS values measured on the slope surface of the specimen (Test 2).

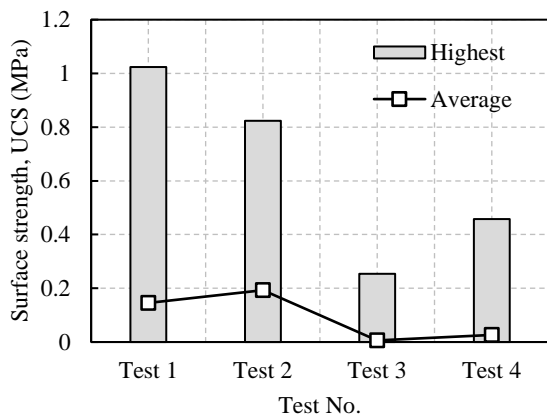


Fig.9 Surface strength of slope solidified at different test conditions

Furthermore, the contour plot of UCS measured on the slope surface for the specimen from Test 2 is presented in Fig. 8. Among the four test cases, the slope treated under Test 2 reveals relatively a homogeneous surface, although the highest strength is less than that obtained in Test 1 (Fig. 9). The highest and average strength of the slopes solidified under all four test conditions are compared in Fig. 9. The average surface strength value comparatively lower in all the cases.

The most important factor for achieving uniform calcium carbonate precipitation on the surface is the distribution of microbes [14]. Therefore, the selection of injection volume and injection rate is highly important to avoid the ponding, surficial flow and transport of bacterial cells due to the excess injection of solutions. Basically, MICP treated well-graded soils exhibited higher strength compared to that of uniformly-graded soils, which is due to the effective packing and a high number of particle contacts in the soil matrix [15-17]. Although the soil reported herein is a well-graded soil, the presence of a significant amount of gravel material (about 20%) affects the ideal behavior of the well-graded matrix. Gravel material requires a higher amount of calcium carbonate content compared to the sands to make a strong contact cementing [18]. Therefore, a limited level of uniformity in surficial stabilization was achieved in natural slope soil reported in this research work.

Test 4 was undertaken at the similar injection conditions of Test 2, additionally placing a non-woven fabric on the slope surface. In fact, this was done for two reasons: (i) to keep a higher water content at the surface zone, as Cheng et al [19] have proven that the calcium carbonate precipitation is proportional to the saturation amount of soil, (ii) to prevent the disturbance of slope soil material at the supply of reactant solutions. However, no considerable improvement in solidification was observed at the implementation of non-woven fabric material.

On the whole, continued research must still address several challenges associated with implementing industrial-grade chemicals and upscaling the process for the *in-situ* applications and for the performance of the induced cementation.

4. CONCLUSIONS

In elementary and benchtop-scale experiments, it has been explored that the industrial-grade chemicals can be potentially used instead of laboratory chemicals for the soil stabilization purposes. The elementary-scale column tests reveal that the surface strength obtained from industrial-grade chemicals is around two times higher compared to that of laboratory chemicals. However, further research should demonstrate what mechanisms are responsible for the observed enhancement of strength. Also, the feasibility of MICP for the slope soil stabilization has been demonstrated by up-scaling the treatment process from column tests to bench-scale slope models. The solidified slopes were analyzed using both non-destructive and destructive methods: spectro-colorimeter measurement and UCS measurements respectively. The slope model tests reveal that the volume of the injection reactants plays an important role in microbial stabilization. Injecting either large quantity of reactants or inadequate volume of reactants, would not be able to contribute significant cementation within soil matrix at the MICP process. Although the solidified slope exhibits higher strength, a wide range of heterogeneity in the deposition of calcium carbonate is observed. Further exploration of field implementation strategy and a deeper understanding of industrial-grade chemicals reaction mechanism in MICP are needed to promote this benchtop-scale investigation to the *in-situ* investigation levels.

5. REFERENCES

- [1] Cheng L., and Cord-Ruwisch R., Upscaling Effects of Soil Improvement by Microbially Induced Calcite Precipitation by Surface Percolation. *Geomicrobiology Journal*, Vol. 31, 2014, pp. 396-406.
- [2] Wiffin V.S., van Paassen L.A. and Harkes M.P., Microbial Carbonate Precipitation as a Soil Improvement Technique. *Geomicrobiology Journal*, Vol. 24, 2014, pp. 417-423.
- [3] DeJong J.T., Fritzges M.B., and Nusslein K., Microbial Induced Cementation to Control Sand Response to Undrained Shear. *Journal of Geotechnical and Geoenvironmental Engineering*, Vol. 132, Issue 11, 2006, pp. 1381-1392.
- [4] DeJong J.T., Martinez B.C., Mortensen B.M., Nelson D.C., Waller J.T., Weil M.H., Ginn T.R.,

- Weathers T., Barkouki T., Fujita Y., Redden G., Hunt C., Major D., and Tanyu B., Upscaling of Bio-Mediated Soil Improvement. Proceedings of 17th International Conference on Soil Mechanics and Geotechnical Engineering, 2009.
- [5] Danjo T., and Kawasaki S., Microbially Induced Sand Cementation Method Using *Pararhodobacter* sp. Strain SO1, Inspired by the Beachrock Formation Mechanism. *Material Transactions*, Vol. 57, 2016, pp. 428-437.
- [6] Mahawish A., Bouazza A., and Gates W.P., Biogrouting Coarse Materials using Soft-lift treatment strategy. *Canadian Geotechnical Journal*, Vol. 53, 2016, pp. 2080-2085.
- [7] Amarakoon G.G.N.N., and Kawasaki S., Factors Affecting Sand Solidification Using MICP with *Pararhodobacter* sp. *Material Transactions*, Vol. 59, 2018, pp. 72-81.
- [8] van Paassen L.A., Ghose R., van der Linden T.J.M., van der Star W.R.L. and van Loosdrecht M.C.M., Quantifying Biomediated Ground Improvement by Ureolysis: Large-Scale Biogrout Experiment, *Journal of Geotechnical and Geoenvironmental Engineering*, Vol. 136, 2010, pp. 1721-1728.
- [9] Martinez B.C., Barkouki T.H., DeJong J.D., and Ginn T.R., Upscaling of microbial-induced calcite precipitation in 0.5 m columns: Experimental and Modeling results. *Geo-Frontiers*, 2011, pp. 4049-4059.
- [10] Road Earthworks - Cutting, Earthquake, Slope Stabilization Guidelines, Japan Road Association, 2009.
- [11] Road Earthworks - Sheng Earth Direction Guidelines, Japan Road Association, 2010.
- [12] Fukue M., Ono S., and Sato Y., Cementation of Sands due to Microbiologically-induced Carbonate Precipitation. *Soils and Foundations*, Vol. 51, Issue 1, 2011, pp. 83-93.
- [13] Mitsuyama S., Nakashima K., and Kawasaki S., Evaluation of Porosity in Biogrouted Sand Using Microfocus X-Ray CT. *Journal of GEOMATE*, Vol. 12, 2017, pp. 71-76.
- [14] Mortensen B.M., Haber M.J., Dejong J.T., Caslake L.F., Nelson D.C., Effects of environmental factors on microbial induced calcium carbonate precipitation. *J. Appl. Microbiol*, 2011, Vol. 111, pp. 338-349.
- [15] 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*, 2014, Vol. 140, 4014006.
- [16] Oliveira P.J.V., Freitas L.D., Carmona J.P.S.F., 2016. Effect of soil type on the enzymatic calcium carbonate precipitation process used for soil improvement. *J. Mater. Civ. Eng*, 2016, Vol. 29, pp. 1-7.
- [17] 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.
- [18] Ng W., Lee M., Hii S., An overview of the factors affecting microbial-induced calcite 20 precipitation and its potential application in soil improvement. *World Acad. Sci. Eng. Technol*, Vol. 62, 2012, pp. 723-729.
- [19] Cheng L., Cord-Ruwisch R. and Shahin M.A., Cementation of sand soil by microbially induced calcite precipitation at various degrees of saturation, *Canadian Geotechnical Journal*, 2013, Vol 50, pp. 81-90.

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