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BIO-INSPIRED STABILIZATION OF EMBANKMENT SOIL MEDIATING *PSYCHROBACILLUS* SP. AND LOW-GRADE CHEMICALS: PRELIMINARY LABORATORY INVESTIGATION

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ABSTRACT

Microbial induced carbonate precipitation (MICP) is one of the foremost bio-inspired soil stabilization technique mediated by ureolytic microorganisms through a chain of chemical reactions, which leads to the formation of calcium carbonate bio-cement in soil matrix, and persuades the substantial bonds between the soil particles. This research aims to examine the achievability of embankment soil stabilization by mediating low-grade chemicals via different scales of preliminary laboratory investigations. Psychrobacillus sp., indigenous ureolytic bacteria, isolated from the embankment slope of Asari (Hokkaido, Japan), was employed in elementary-scale column and benchtop-scale slope model solidification tests performed at different physio-chemical conditions to optimize the bio-cementing performance. According to the column test results, a better stabilization (UCS of 0.82 MPa) was obtained for the specimen treated mediating low-grade chemicals (urea fertilizer, beer yeast and snow melting reagent), whereas specimen treated mediating pure chemicals resulted only 0.42 MPa. The benchtop-scale test reveals that the highest surface strength of 1.02 MPa was achieved with 0.5 M cementation solution at 30ºC. Sets of colorimeter measurements were undertaken on treated slope models to compare precipitation profile at different locations. The results have evidenced the acceptable level of solidifying possibility of surface layer while using low-grade chemicals, which would be adequate to be occupied as crust-layer for the natural embankments.

Keywords: Bio-inspired soil stabilization, Bio-cement, Embankment soil, Ureolytic bacteria, Low-grade chemicals

INTRODUCTION

Many recent investigations have evidenced that the bio-inspired cementation technique can be potentially applied for ground stabilization/ reinforcement purposes. The researchers and biotechnologists have used the principle of bio-metabolism to generate sufficient calcium carbonate crystals, thereby enabling bridges between soil particles. Sealing the pore voids 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]. In detail, the bacteria which produce urease enzyme are used to catalyze the hydrolysis of urea into ammonium and carbonate ions in aquatic medium as presented in Eq. 1.

$$\mathrm{CO(NH_2)_2} + \mathrm{2H_2O} \rightarrow \mathrm{2NH_4^+} + \mathrm{CO_3^{2-}} \qquad (1)$$

$$\mathrm{CO_3^{2-}} + \mathrm{Ca^{2+}} \rightarrow \mathrm{CaCO_{3\,(s)}} \qquad (2)$$

At the supply of dissolved calcium ions or at the presence of calcium ions in the medium, produced carbonate ions precipitate and form calcium carbonate crystals as given in Eq. 2. Eventually, desired mechanical strength can be achieved, when the calcium carbonate crystals are precipitated appropriately in pore spaces of soil.

Bio-inspired stabilization method is nondestructive, inexpensive as well as less hazarded 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 stabling prior to tunneling, immobilization of hazardous contaminants, facilitating impermeable barriers and carbon sequestrations [3], [4]. Up to this moment, feasibility of Microbial induced carbonate precipitation (MICP) for the above applications has been demonstrated mostly in laboratory using elementary-scale column experiments [5] - [7]. The studies have widely investigated and addressed the injection procedures, concentration of reagents and bacterial controls in achieving the desirable behavior of 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].

In this paper, we aim to upscale the MICP investigation from elementary-scale columns to benchtop-scale slope models. In fact, the benchtop-scale experiments 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 stated by DeJong et al. [4]. On the other hand, cost of required substrates (urea, calcium chloride and nutrients for bacterial growth) would be a challenge, when it comes as large-scale investigations/ applications [8]. Thus, paper also focus to investigate the feasibility of using low-grade chemicals in place of laboratory chemicals in MICP as to overcome the economical challenge.

MATERIALS AND METHODS

Ureolytic Bacteria and MICP reagents

The bacteria used in this study is *Psychrobacillus soli*, gram positive strain isolated from Embankment soil of Asari Expressway (Hokkaido, Japan). The detection and isolation methods of the strain are the same as Danjo and Kawasaki [5]. The urease activity of the strain culture was around 0.4 μmol/min at 30°C. Two types of culture mediums and cementation solutions were used in current experiments, and are described below. CM_{pure} and CS_{pure} were prepared using laboratory pure reagents, whereas low-grade chemicals were used to prepare $CM_{low\text{-}grade}$ and $CS_{low\text{-}grade}$ solutions.

Culture mediums CM

CM_{pure} :	15.7 g/L tris-buffer, 10 g/L ammonium sulfate, 20 g/L yeast extract, distilled water
$CM_{low\text{-}grade}$:	30 g/L beer yeast, distilled water

Cementation solutions CS (0.5 mol/L)

CS_{pure} :	30 g/L urea, 55.5 g/L $CaCl_2$, 3 g/L nutrient broth, distilled water
$CS_{low\text{-}grade}$:	30 g/L urea fertilizer, 55.5 g/L snow melting agent, 2 g/L beer yeast, distilled water

The natural soil collected from expressway embankment of Asari was used for solidification tests. Natural moisture content and pH are respectively 21.8 ± 1.30 and 7.029.

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

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. Excess solutions were allowed to drain through the bottom of the columns. 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, bacteria culture medium of 10 mL was injected only 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.

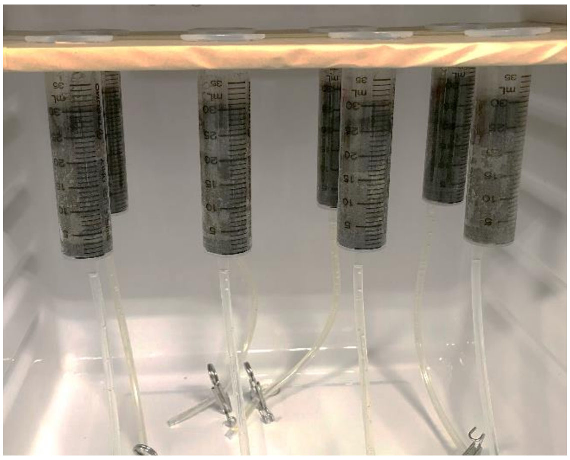


Fig. 1 Syringe Columns

Table 1 Conditions of column solidification tests

Test	Bacteria Culture	Cementation solution	Temp. (°C)
1	CM_{pure}	CS_{pure}	30
2	$CM_{low\text{-}grade}$	$CS_{low\text{-}grade}$	30

Slope model solidification test

The size of the slope model used in the experimentational study is 10 cm × 12 cm in 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, 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-mould was filled in properly tamped five layers of soil (without oven-dry/ sterilization).

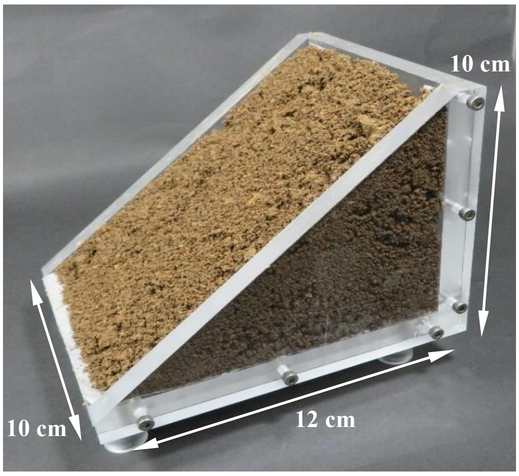


Fig. 2 Slope model

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, low-grade chemicals ($CM_{low\text{-}grade}$ and $CS_{low\text{-}grade}$) 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.

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

Considering 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.

UCS measurements

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, UCS of each treated sample was estimated using the ratio between applied force and settlement of needle.

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

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.

Colorimeter measurements

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 solidified slope surface.

RESULTS AND DISCUSSION

Column solidification

Two number of columns tests were conducted to investigate the feasibility of low grade chemicals in bio-inspired soil stabilization. In order to monitor the internal biogrouting process during treatment, Ca^{2+} ion concentration and pH were measured every day from the percolated solution through syringe column. The concentrations and the pH are presented in Fig. 3. 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 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.

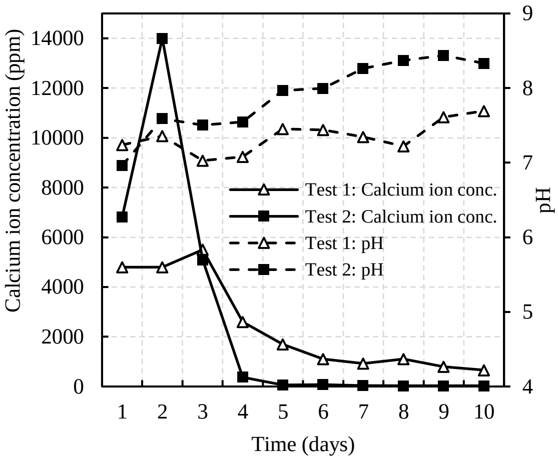


Fig. 3 Calcium ion concentration and pH at treatment

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 pure reagents (Test 1) and low-grade chemicals (Test 2), are compared in Fig. 4. 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 bottom of the column [2], which tends to precipitate relatively high amount of calcium carbonate at top of the column. Thus, the highest strength was obtained at the top, and decreased over the length. At the same time, the low-grade chemicals have exhibited a significant enhancement in solidification. The surface strength of the sample treated under low-grade chemicals is around two times higher than that of sample treated using laboratory chemicals.

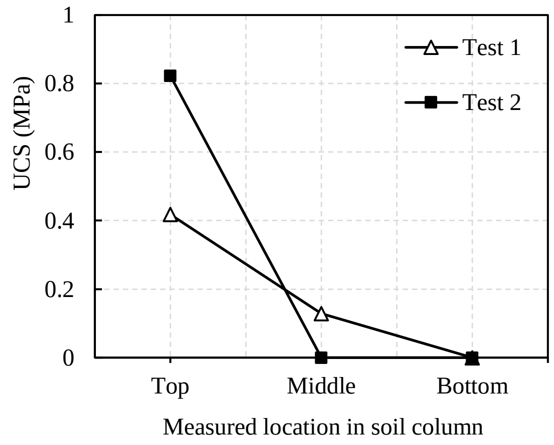


Fig. 4 Strength measures of solidified columns

Slope model solidification

Four cases were undertaken in slope model solidification test. Based on the positive observations made at column tests, all the slope solidification tests were incorporated with low-grade chemicals. The first three tests (Test 1 - Test 3) were performed to assess the effect of the injection volume of chemicals in cementation. Large quantity of injection was made in Test 1 by assuming that implementation of large number of bacteria would significantly enhances 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. 5. The trend of average L* values of Test 1 slope shows an initial increment, and remains relatively stable thereafter. But, average L* values of the Test 2 slope increase gradually with the time. At the same time, there was 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.

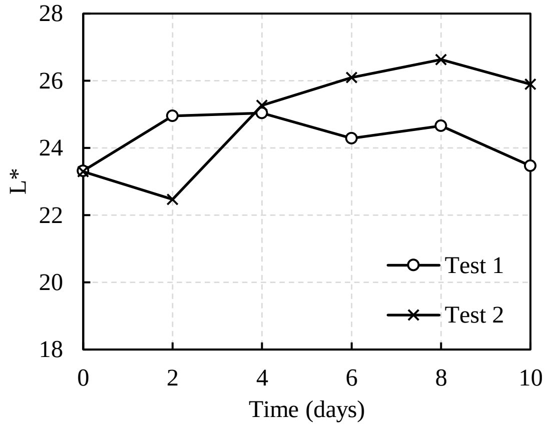
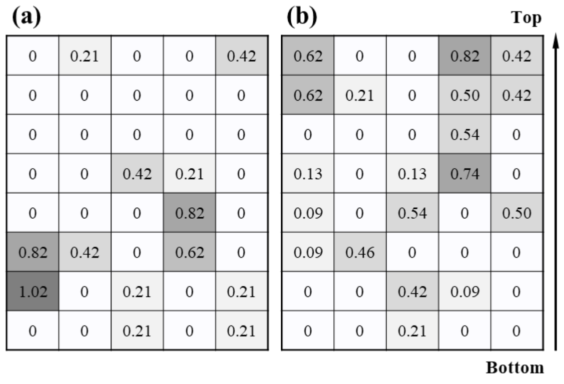


Fig. 5 Lightness (L*) measure on slope surface using spectro-colorimeter

The surface strength (UCS) distribution of the treated slope specimens of Test 1 and Test 2 are illustrated in Fig. 6. It is well understood that there is a close relationship between color measurement (L*) and UCS as stated by Amarakoon and Kawasaki [7]. As the solidification occurred only at the certain locations of the slope surface of Test 1 evidenced in Fig. 6 (a), the L* values failed to exhibit the increasing trend (Fig. 5). In the case of Test 2, the unsolidified surface area reduced (Fig. 6 (b)), thereby resulted the considerable increment in L* value with the duration. It is well understood that injection volume plays a vital role in solidification process. Injection of the reactants in large quantity might lead to wash out of cells from the soil matrix prior to bacterial immobilization. At the same time, injecting inadequate volume of reactant, would not be able to contribute significant and uniform cementation.



(a)

0	0.21	0	0	0.42
0	0	0	0	0
0	0	0	0	0
0	0	0.42	0.21	0
0	0	0	0.82	0
0.82	0.42	0	0.62	0
1.02	0	0.21	0	0.21
0	0	0.21	0	0.21

(b)

0.62	0	0	0.82	0.42
0.62	0.21	0	0.50	0.42
0	0	0	0.54	0
0.13	0	0.13	0.74	0
0.09	0	0.54	0	0.50
0.09	0.46	0	0	0
0	0	0.42	0.09	0
0	0	0.21	0	0

Fig. 6 Obtained UCS values with respect to their locations on the slope surface of the specimen (a) Test 1 and (b) Test 2.

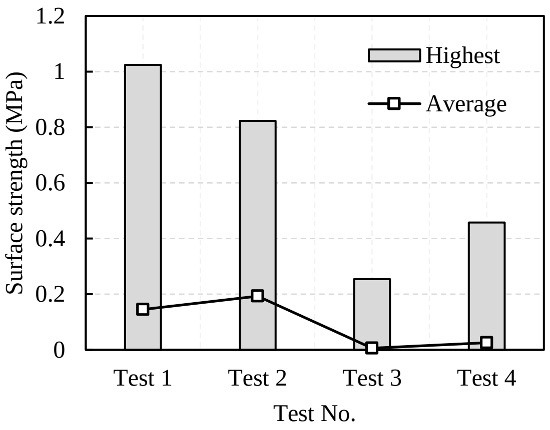


Fig. 7 Surface strength of slope solidified at different test conditions

The highest and average strength of the slopes solidified under all four test conditions are compared in Fig. 7. The average surface strength value comparatively lower in all the cases, whereas the highest value sounds significantly. 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. 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, because Cheng et al [14] 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 upscaling the process for *in-situ* treatment and the performance of the induced cementation. Currently, bench-scale laboratory experiments are ongoing to obtain the homogeneous bio-cementation along the slope profile.

CONCLUSION

In elementary and benchtop-scale experiments, it has been explored that the low-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 low-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 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 with in soil matrix at the MICP process. Although the solidified slope exhibits higher strength, wide range of heterogeneity in the deposition of calcium carbonate is observed. Further exploration of field implementation strategy and deeper understanding about low-grade chemicals reaction mechanism in MICP are needed to promote this benchtop-scale investigation to the *in-situ* investigation levels.

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