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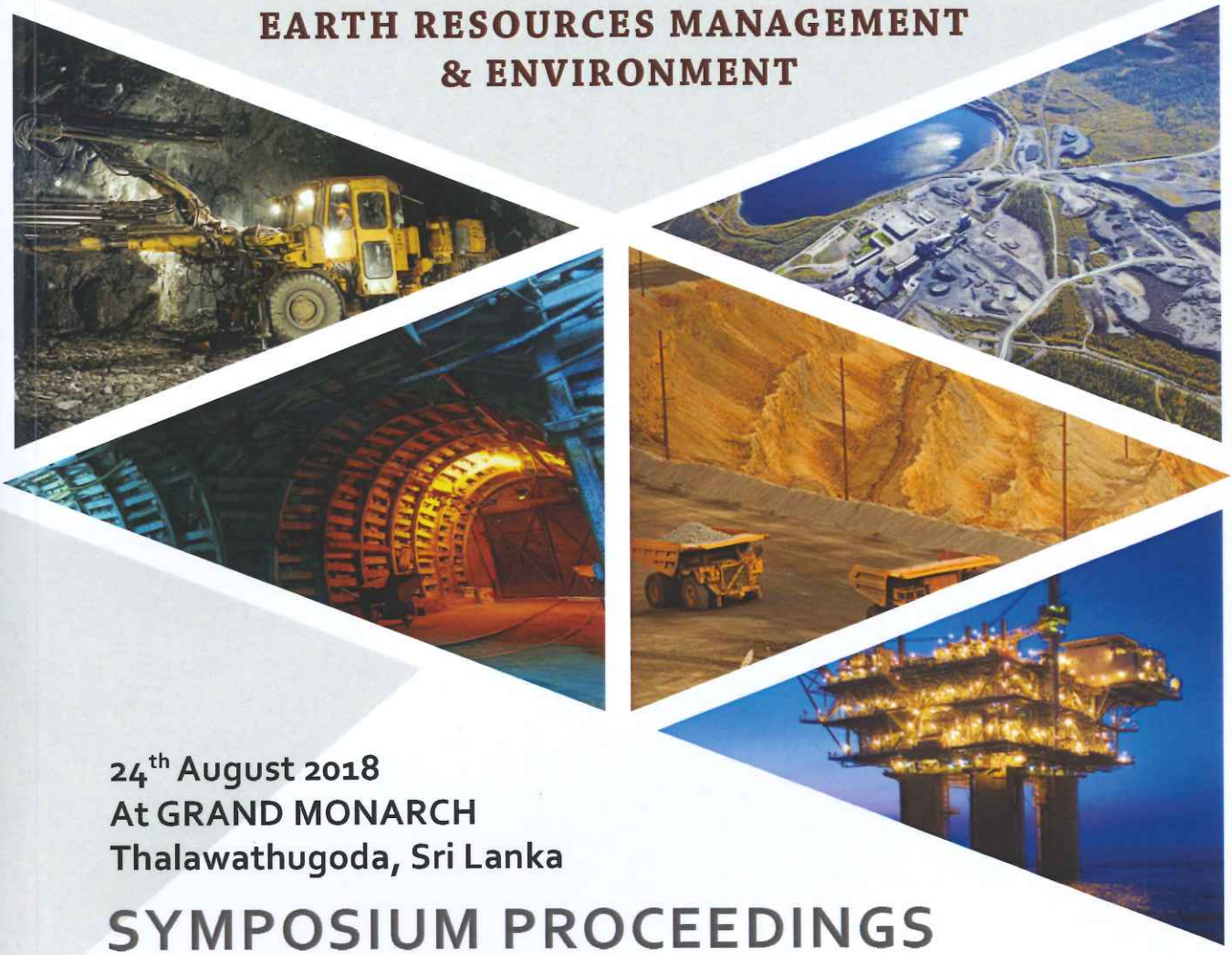

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Experimental Optimization of Biocement Formation: Alternative Countermeasure for Surface Erosion of Cut Slope

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Abstract
The research work aims to assess the feasibility of introducing the microbial induced carbonate precipitation (MICP) as an alternative technique for surface stabilization of the cut slopes by augmenting potential indigenous ureolytic bacteria. A set of column solidification tests was conducted on embankment soil (Hokkaido expressway, Japan) to optimize the performance of bacteria regarding bacterial population of culture solution (optical density (OD_{600}) from 1 to 6), and concentration of Ca^{2+} and urea in cementation solution (0.5 mol/L and 1 mol/L) at the temperature of 20°C. The UCS of treated samples was estimated using needle penetrometer, and the microstructure of the treated specimens was observed using scanning electron microscope (SEM). The results reveal that the UCS of the specimen increases with increasing OD_{600} without any clogging within the samples. Treating the soil using 1 mol/L concentrated (Ca^{2+} and urea) cementation solution and bacterial culture with OD_{600} of 6 results the highest UCS of 7.5 MPa while achieving relatively a homogeneous solidification along the column profile. The micrographs of the treated specimen confirms that the rombohedral calcium carbonate crystals formed within the pores of soil matrix, which has effectively bonded the adjacent soil particles, and contributed to enhance the strength significantly at the optimized treatment condition.

Keywords: Calcium carbonate, Cementation, Microbial induced carbonate precipitation (MICP), Surface stabilization, Ureolytic bacteria

1. Introduction

Slopes are the prime supporting structure of the transportation system, thereby slope stabilization is often an essential part in Geotechnical Engineering. Particularly, surface instability of slopes due to soil erosion has been often documented as a widespread issue all over the world. The erosion due to rainfall and runoff, generally begins with the detachment of soil aggregates at the surface followed by transportation of loose particles to the downslope [1]. It has been reported that the sediment transport and mass-loss, pose severe risks regarding shoulder erosions and collapses of transportation structures [2]. In addition, the aggregates which are transported by runoff, would be

deposited in drainage systems and pavement surfaces, that can obstruct many subsequent exertions and increase the cost of maintenance. To date, there are many mechanical and chemical treatments have been proposed for slope surface stabilization. Majority of these utilize mechanical energy and man-made materials, both of which required high energy for material production and installation [3, 4]. At the same time, enhancing the soil structure of slope surface using chemical additives such as micro-fine cement, epoxy, fly-ash, pheno-plasts, silicates and polyurethane, has been reported to ban in many countries due to their hazardous and toxic effects to the environment [3, 4, 5]. Therefore, demand for new, sustainable methods to improve the slope soil continues to increase worldwide.

Recently, microbial induced carbonate precipitation (MICP) has experienced an increased level of interest due to its sustainability and minimal disturbance to the environment [6, 7, 8]. Basically, MICP is a naturally occurring process wherein calcium carbonate biocement is formed as a result of microbial metabolic activities. The ureolytic bacteria catalyzes the hydrolysis of urea as given in Equation 1 [7, 8], and increase the pH of the reaction medium. Afterwards, calcium carbonate cement is precipitated at the presense of calcium ions in nucleation sites provided by the bacterial cells (Equation 2) [2, 7, 8].

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

$$CO_3^{2-} + Ca^{2+} \rightarrow \text{Cell-}CaCO_3 \quad (2)$$

Formation of stable ground/crust by bonding soil particles using biocement is relatively a novel technique in Geotechnical Engineering, and its potential has been greatly harnessed with many applications.

The final goal of this study as the title stated is to introduce the biocementation technique to cut slopes as an alternative countermeasure for surface erosion. However, preliminary study on optimization regarding microbial performance and cement proliferation is essential in biocementation processes. Therefore, a preliminary laboratory investigation has been undertaken to optimize the biocement formation of the indigenous ureolytic bacteria isolated from considered slope soil. Obviously, biocementation using indigenous bacteria would be more promising and reliable compared to that of exogenous bacteria, particularly for the cold regions like Hokkaido, Japan.

2. Experimental Methods

2.1 Material Tested

Natural soil collected from Expressway slope, Onuma (Hokkaido, Japan) (Figure 1) was used in this investigation. The soil can be classified as fine-grained sand with the average particle size of 0.25 mm, and the grain size distribution is presented in Figure 2.

Figure 1: Location focused in this study

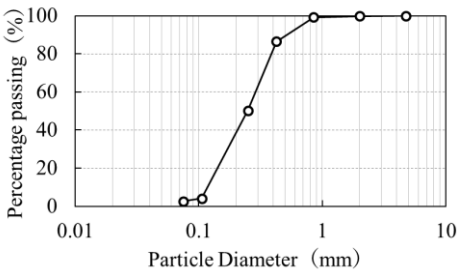


Figure 2 – Partical-size distribution curve of the slope soil

2.2 Bacteria Culture

Lysinibacillus xylanilyticus, ureolytic bacteria, isolated from expressway slope, Onuma (Hokkaido, Japan) (Figure 1), was used in this study. The strain was cultivated under sterile aerobic condition in NH_4-YE medium (10 g/L ammonium sulfate, 20 g/L yeast extract, 15.7 g/L tris-buffer), and kept under dynamic condition (160 rpm) at 20°C. The optical density (OD_{600}), and urease activity of culture medium were measured continuously with the time to perceive the behaviour of the strain.

2.3 Syringe Solidification Test

The syringes of 35 mL capacity (diameter is 25.3 mm) were used to make soil columns. The oven dried (105°C for 48 hours) soil was placed in syringe columns by three layers (15 g per each) and compacted well. The arrangement of syringe solidification test is conceptually presented in Figure 3.

A two-phase injection was performed to the samples. In first phase, bacteria culture was injected (10 mL) to fill the sample. Subsequently, the cementation solution (10 mL) was injected to the soil at the second phase. 0.5 mol/L cementation solution contained a mixture of 55.5 g/L calcium chloride, 30 g/L urea and 3 g/L nutrient broth.

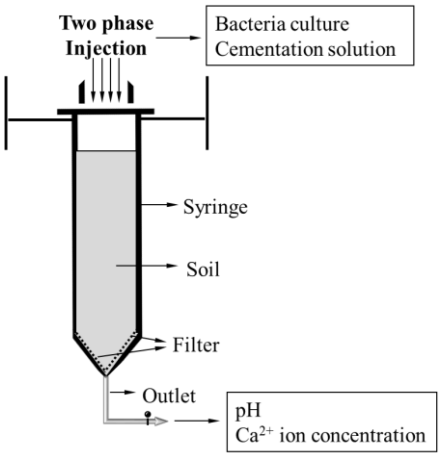


Figure 3 – Conceptual diagram of syringe solidification test

All the solutions were simply applied to the soil surface (at a constant flow rate of 2 ml/ min), and allowed to percolate under gravitational and capillary forces. The solidification tests were undertaken at 20°C for 14 days under fully drained condition by keeping the outlet valve open throughout the experiment. Cementation solution of 10 mL was injected everyday, so that to supply the required substrates to the biochemical reactions. In order to experience the reaction state, Ca^{2+} concentration and pH of the drainage were measured with the time. Number of solidification tests were conducted to optimize the bacterial performance in bio-cementation regarding various aspects. Control sample was treated using distilled water instead of culture/ cementation solutions.

2.4 Needle penetration test

Unconfined compressive strength (UCS) of the solidified samples were estimated using soft-rock needle penetrometer (SH-70, Maruto Testing Machine Company, Tokyo, Japan)

using the same methodology explicated by Danjo and Kawasaki [7].

2.5 Microscopy investigation

In order to make a better understanding on crystal formation and bonding mechanism, microstructures of bio-cemented specimen were observed using scanning electron microscope (SEM) (SuperScan SS-550 Shimadzu Corporation, Kyoto, Japan).

3. Results

3.1 Bacteria Concentration

Figure 4 shows the comparison of UCS estimated at the suface of the samples treated under different bacteria concentration (OD_{600}). 0.5 M cementation solution was used for this initial optimization. It can be seen that the UCS of the soil increases with increase in bacteria population. This observation is in line with the results observed by Amarakoon and Kawasaki [8] while using *Pararhodobacter* sp.

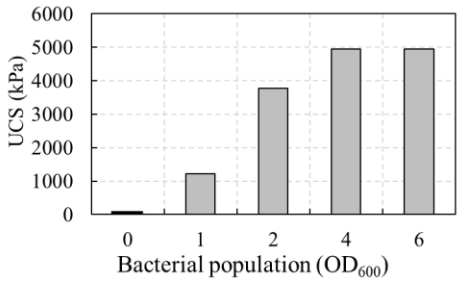


Figure 4 – UCS of the samples treated under different bacteria concentrations

It is clear that the rate of urea hydrolysis has a direct relationship with the bacterial cell concentration. Thereby, the higher bacterial concentration injected to the soil sample increased the amount of carbonate precipitated from MICP, hence increased the UCS.

3.2 Concentration of Cementation Solution

Figure 5 shows the results of UCS obtained (at the surface) versus concentration of cementation solution. It can be seen that the UCS of the specimen treated using high concentrated (1 M) cementation solution is about 35 % higher than that of sample treated using low concentrated (0.5 M) cementation solution. This is in consistence with the previous observation reported by Danjo and Kawasaki [7] and Amarakoon and Kawasaki [8].

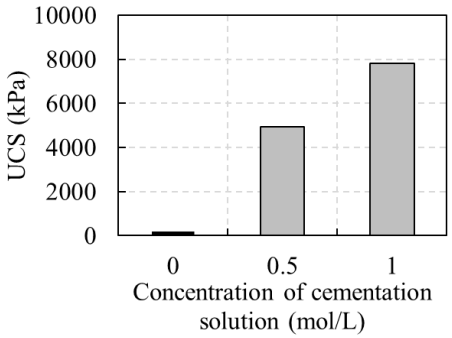
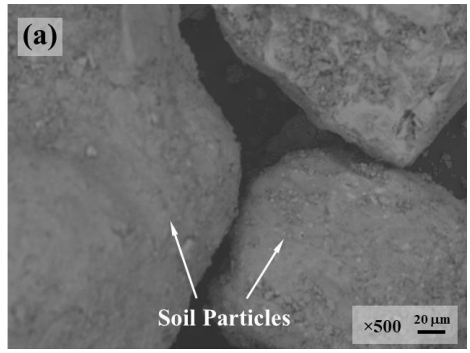
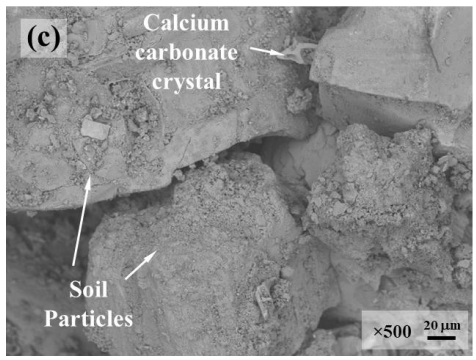


Figure 5 – UCS of samples treated under different concentration of cementation solution

In order to further investigate the impact of concentration of cementation solution on crystallization, SEM analysis was carried out. The unteated soil matrix is presented in Figure 6-a. Both samples treated under 0.5 M (Figure 6-b) and 1 M (Figure 6-c) show the calcium carbonate crystals formed at the particle contact of the soil particles. However, it can be recognized that the distribution of the calcite crystals is denser and more effective in sample treated under 1 M (Figure 6-c) than that of 0.5 M (Figure 6-b). It is understood that higher concentration of urea and calcium chloride extends the amount of

deposition and tends to form the larger crystals in particle contact (similar to Figure 6-c) as reported by Cheng et al. [5] and Ng et al. [5].





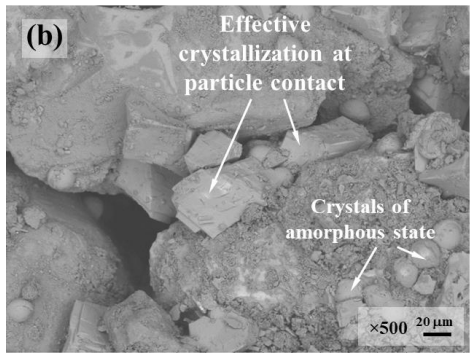


Figure 6 – Micrographs of (a) untreated soil matrix, (b) soil matrix treated under 0.5 M and (c) soil matrix treated under 1 M concentration of cementation solution

3.3 Effect of Reinjection of Culture medium

In all the cases discussed in the above sections, the culture medium was injected to soil only at the beginning (without reinjection). Although the high bacteria and cementation concentration contributed a significant improvement, syringe sample could not achieve a homogenious solidification along the profile. Therefore, a reinjection (again after 7 days) case was incorporated in order to check the feasibility of achieveing homogeneous solidification by distributing more bacteria to the soil, and the results are compared in Figure 7.

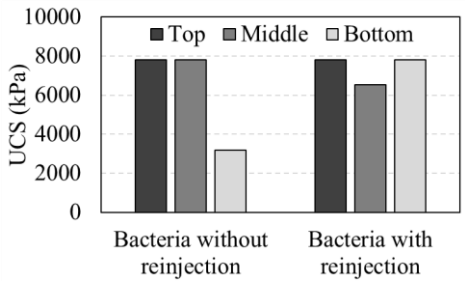


Figure 7 – UCS with the effect of reinjecting bacteria culture medium

The UCS measurements ensure that relatively a homogeneous solidification along the column depth has been achieved at the reinjection of bacteria.

4. Discussions

In most of the cases, the obtained UCS values declined with increasing sample depth, which indicate that relatively high amount of calcium carbonate precipitated at the injection-top zone of the specimen, and declined with the depth. As reported by Wiffin et al. [10], top zone of the sample which is closer to injection point significantly in exposure to solutions

compared to the bottom. The injected solutions diminish their performance continuously with the depth, hence reveals decreasing solidification effect with depth. Threfore, concentration and quantity of cementation solution should be adequate enough to supply the required substances all over the treatment depth. Moreover, distribution of bacteria plays a very important role in achieving homogeneous solidification as reported by Martinez et al. [11]. Small pore throat size would limit their free passage, depends on the size of microbes and soil composition. As an example stated by Ng et al. [9], bacteria with size ranging from 0.3 to 2 µm can move freely within sandy soil with particle size of 0.05 to 2.0 mm. It is thus essential to take into considerations the type of soil, its pore throat size, and size of bacteria when selecting the appropriate type of bacteria for homogeneous treatment. However, the homogeneous solidification for a large depth is not targeted in this study, as the scope of the research focus a surface stabilization of cut slope by establishing a bio-cemented crust with high strength and low permeability for a required depth level. Still, a wide range of laboratory experiments from small to large scales are required for the optimization and quantification of cementation regarding various aspects prior to its real-field execution.

5. Conclusions

The results presented in the current study demonstrated that bio-cementation using indigenous bacteria, *Lysinibacillus xylanilyticus* has a high potential to stabilize the cut slope surface (Onuma, Hokkaido, Japan) at the feasible construction temperature of 20°C with enhanced compressive strength. However, the efficiency of biocementation in improving soil strength vary depends on several treatement factors. Injecting the culture medium with higher bacteria cell concentration (OD_{600} above 4) was found to achieve a greater strength compared to the lower cell concentration. In addition, incorporating higher concentration of cementation solution (1 M) can remarkably enhance the strength by forming larger and denser crystals effectively at particle contacts. Study also indicated that the homogeneous solidification depends on the bacteria distribution in soil, which can be achieved by reinjection (two times in fourteen days treatement) of bacteria culture. By using the optimized conditions, studies are currently carried out at bench-top scale (an intermediate step between small and large scale) to make the technique one step closer to real field application.

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