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Biogeotechnical approach for slope soil stabilization using locally isolated bacteria and inexpensive low-grade chemicals: A feasibility study on Hokkaido expressway soil, Japan

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Abstract

Microbial Induced Calcite Precipitation (MICP) is one of the most popular biotechnological soil stabilization techniques since it results in significant improvements in the geotechnical properties of soil. The current study presents a laboratory-scale MICP investigation performed to demonstrate the feasibility of slope soil stabilization of the Hokkaido expressway through surficial treatment. The objectives of this preliminary study are to investigate the feasibility of (i) augmenting indigenous bacteria, and (ii) implementing commercially available inexpensive low-grade chemicals in microbial induced solidifications. Syringe solidification tests were carried out using indigenous ureolytic bacteria under various temperature condition with the use of different injection sources. A high strength crust layer was achieved on the soil surface with 420 kPa unconfined compressive strength (UCS) as measured by needle penetration test after 10 days of treatment using pure chemicals (30 °C; 0.5 M cementation solution, every 24 h; bacterial culture solution, only at the beginning). However, by substituting pure chemicals with low-grade chemicals, a significant improvement in the UCS of soil (820 kPa at 30 °C) was obtained together with a 96% reduction in the treatment cost. The morphologies and crystalline structures of the precipitated carbonate were characterized by Scanning Electron Microscopical (SEM) observations. This alternative approach of introducing low-grade chemicals in MICP has the potential to provide significant economic benefits in field-scale applications.

Keywords: Microbial induced calcite precipitation; Indigenous bacteria; Pure chemicals; Low-grade chemicals; Slope soil stabilization

1. Introduction

Bio-cementation, based on Microbial Induced Calcite Precipitation (MICP), is a promising soil stabilization technique that has recently gained the interests of many researchers (Chiet et al., 2016; DeJong et al., 2010; Ng et al., 2012; Oliveira et al., 2016; van Paassen et al., 2010). Soil improvement occurs due to the crystallization of calcium carbonate ($CaCO_3$) cement soil particles by the enzyme urease of ureolytic bacteria. Initially, the enzymatic hydrolysis of urea releases ammonium and carbonate ions in the medium (Eq. (1)) while increasing the pH. The produced carbonate ions precipitate in the presence of calcium ions as calcium carbonate crystals on the surface of soil (Eq. (2)). The precipitated carbonate cements the

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particle contacts, clads the particle surface, fills the pores with or without bridging the adjacent soil particles and eventually stiffens the soil matrix. The bacteria play another important role: because the cell surfaces of bacteria are typically negatively charged, the Ca^{2+} ions are attracted, and nucleation for calcium carbonate precipitation begins on these surfaces (Bao et al., 2017).

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

$$CO_3^{2-} + Ca^{2+} \xrightarrow{Bacterial\ \ cell} Cell - CaCO_3 \downarrow \quad (2)$$

Among the wide range of applications of MICP, slope soil stabilization is getting increased attention as slopes are frequently associated with transportation systems (Bao et al., 2017; Jiang and Soga, 2017; Salifu et al., 2016). Surface erosion, one of the key challenges in the stability of slope soil, occurs due to complex interactions of sub processes between the detachment and transport of surface materials (Dai et al., 2018; Zhang et al., 2018). It has been reported that the sediment yield and runoff production of a slope, are significantly impacted by soil texture, slope topography, rainfall intensity and cover condition (Fox et al., 1997; Qing-quan et al., 2001; Rieke-Zapp and Nearing, 2005; Zhang et al., 2018). Since the slopes are typically in an unsaturated state, part of the flow infiltrates the slope, which increases the pore water pressure and seepage forces within the slope. When the water table is close to the surface of the slope, the rise in pore water pressure diminishes the effective stress of the slope soil, reducing the soil strength and may trigger the slope failures (Harden and Scruggs, 2003; Muntohar and Liao, 2010). Also, the infiltration decreases the contribution of matric suction by lessening the negative pore-water pressure and effective stress, thus results the loss in strength of the slope soil, and the effect is crucial in slopes of fine grained soil. Eventually, the failure of embankment slopes can cause direct damage to transportation systems, such as pavement drop-off, the washout of expressway shoulders, or the failure of embankments. This results in an increase in the cost of maintenance, and significant risks to the travelling public (Bao et al., 2017). It is well understood that surface treatment plays a vital role in promoting the cover condition of the slope by achieving aggregate stability and infiltration control. Conventional materials like geotextiles, wire meshes, cable nets, membranes, sheets or nails, which were physically installed to promote slope enforcement, are often expensive and their installment requires a high energy cost (Salifu et al., 2016), whereas chemical grouting methods are reported as environmental-unfriendly and unsuitable for large-scale applications (Gomez et al., 2013). Thus, an alternative remedial action for slope soil stabilization, the implementation of a bio-cement zone of MICP along slope surface, has been considered in this paper.

Up to now, many studies have focused on MICP based soil stabilization in order to mitigate the potential of erodability. Most of them have been performed based on a bio-augmentation strategy by introducing non-native ureolytic bacteria to the soil. Among them, *Sporosarcina pasteurii* is the most researched bacterium: it enables a highly active urease enzyme associated with urea hydrolysis (Gomez et al., 2013). The solidification of sand using *S. pasteurii* allows for significant control of surficial sediment erosion (Bao et al., 2017; Salifu et al., 2016) and reduces hydraulic conductivity while increasing the confined compressive strength (Jiang and Soga, 2017; Whiffin et al., 2007). Also, *S. pasteurii* was shown to form an impermeable stiff crust with a thickness of 2.5 cm which increases resistance to erosion (Gomez et al., 2013). Cheng et al. (2014) reported that *Bacillus sphaericus* can enhance the strength of silica sand with relatively retained permeability when 10 mM urea concentrated artificial sea water was used as cementation solution. However, the bio-augmentation of exogenous bacteria which have not been adapted to native environment is associated with uncertainties in bacterial survival and performance (Sensoy et al., 2017). On the other hand, a very few studies have been attempted to investigate the feasibility of bio-augmentation using indigenous bacteria (Danjo and Kawasaki, 2016; Khan et al., 2016; Stabnikov et al., 2011). Actually, bio-augmentation (the process includes the isolation of bacteria, culture enrichment, and supply back into the ground) conceptionally differs from bio-stimulation strategy in which the indigenous ureolytic bacteria are stimulated *in situ*. Danjo and Kawasaki (2016) and Khan et al. (2016) investigated the feasibility of artificial beach rock formation as the mitigation measure for coastal erosion by augmenting native *Pararhodobactor* sp. isolated from the native sand of Okinawa, Japan. Similarly, using native *Bacillus* sp. VS1, relatively impermeable biocemented crust (maximum flexural strength of 35.9 MPa; permeability of 1.6×10^{-7} m/s) has been achieved on the native sand surface for aquaculture pond construction (Stabnikov et al., 2011). The use of natively adapted bacteria is likely to be both more effective and more appropriate in regions with fluctuating cold climatic conditions, as the urease enzymes of most of the bacteria are temperature-sensitive and can readily be denatured by changes in the environmental temperature. However, due to the uneven presence of ureolytic bacteria in natural soil, stimulating the indigenous bacteria has been reported to result in diffuse zones of improvement with high variability even within the same region (Gomez et al., 2017). At the same time, augmentation has been shown to result in more uniform improvement on a more localized scale due to the reduction in soluble calcium concentrations transported to greater distances (Danjo and Kawasaki, 2016; Gomez et al., 2017). As this research aims for the uniform surface stabilization of slopes, the bio-augmentation of indigenous bacteria is likely to be the better choice among the strategies discussed above.

In fact, the feasibility of MICP does not depend on technical aspects regarding conditions of treatment alone, but accompanies by economical and legislative issues as well. It has been reported that the discharging of vigorous

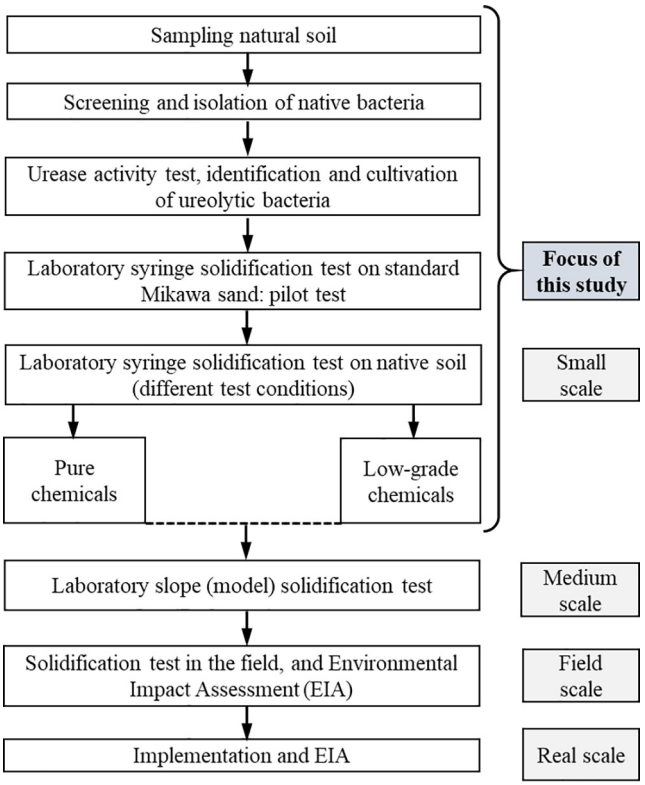

Fig. 1. Schematic outline to understand the scope of the research study.

chemicals and ammonium by-products to the soil-ecosystem in the MICP causes many harmful effects, and the subsequent removal of such harmful products must be taken into account (Soon et al., 2014). The cost of the required substances remains another contest in assessing the complete feasibility of the process (van Paassen et al., 2010). In this research, the feasibility of MICP phenomenon in slope soil stabilization has been the focus at a preliminary stage using small-scale laboratory experiments. The prime objectives of this study are to investigate the efficiency of MICP process (i) by using locally isolated bacteria, and (ii) by introducing commercially available low-grade chemicals instead of pure chemicals. The flowchart of the complete research design up to the real scale application is presented in Fig. 1, and the focus of this article is clearly outlined. The laboratory experiments of different scales listed in Fig. 1 are conceptually represented in Fig. 2, with each laboratory experiment simulating the surface zone of the slope to be treated at different scales.

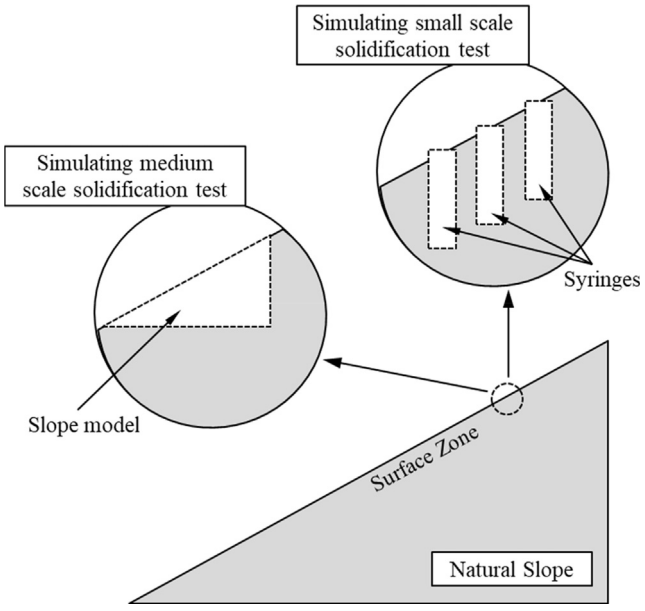

Fig. 2. Conceptional illustration of simulating real field by laboratory scales.

2. Materials and method

2.1. Identification and isolation of ureolytic bacterium

Three potential locations of expressway slopes (Asari, Onuma, Asahikawa) of Hokkaido, Japan were considered in this research. The samples from considered locations were collected in sterile test tubes, transported to laboratory and refrigerated at 4 °C. The intrinsic properties and compositions of soils are summarized in Table 1, and the grain size distributions of the soils are compared in Fig. 3 with standard Mikawa sand. For microbial identification, 5.0 g of the refrigerated natural soil sample was taken and mixed with 45 mL of autoclaved distilled water, then diluted 10^1–10^4 times using autoclaved distilled water in separate autoclaved sterile test tubes. Subsequently, 10 μL of each dilution was 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 °C, and about 30 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). Afterwards, each isolated colony was transferred to a 20 mL solution, well shaken, and incubated for 2 h at 45 °C. 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. Accordingly, the ureolytic bacteria were isolated, and characterized by sequencing their 16S rDNA and comparing the results to sequences available in the Apollon DB-BA 9.0 database, GenBank, DDBJ (DNA Data Bank of Japan) and EMBL (European Molecular Biology Laboratory).

2.2. Laboratory scale solidification test

The biogrouting viability of all three considered slope locations has been demonstrated by recognizing native ureolytic microbes via this research. However, details of the investigations regarding MICP efficiency using solidifica-

Table 1
Intrinsic properties and compositions of soils.

Soils	Basic properties		Composition based on X-ray fluorescence (XRF) analysis											
	Natural Moisture Content, %	pH	MgO	Al_2O_3	SiO_2	P_2O_5	SO_3	K_2O	CaO	TiO_2	V_2O_5	MnO	Fe_2O_3	Na_2O
Mikawa Sand (No. 4)	0	7.010	0.11	0.89	97.65	–	–	0.18	0.03	0.04	–	–	0.43	0.04
Asari	21.8 ± 1.30	7.029	1.29	23.4	60.3	0.203	0.489	1.23	2.14	0.613	0.0338	0.137	9.81	–
Onuma	10.5 ± 0.70	6.997	1.99	25.6	57.5	0.302	0.672	1.05	3.68	0.583	0.0278	0.137	8.1	–
Asahikawa	27.6 ± 1.30	7.306	1.26	27.3	57	0.26	0.755	0.973	4.32	0.7	0.0424	0.135	6.95	–

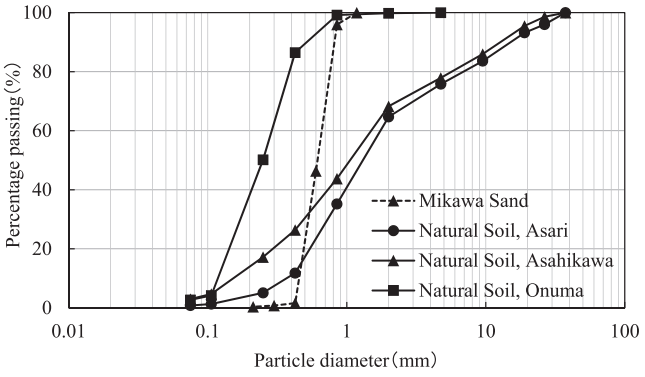

Fig. 3. Grain size distributions of soils.

tion tests are focused only on Asari slope soil in this paper, and similar criterion may be applicable to other slope soils left for the future work.

Standard syringes (1-4908-07) with a diameter of 25.3 mm and a height of 139.6 mm were adopted to perform the syringe solidification tests. At each syringe test, 45 g of soil was packed well into the test syringe, and the experimental set up was as shown in Fig. 4. A two-stage injection was performed in order to confine the bacteria for the subsequent cementation. During the first stage, bacteria culture was injected to fill the soil column, and a cementation solution was injected during the second stage.

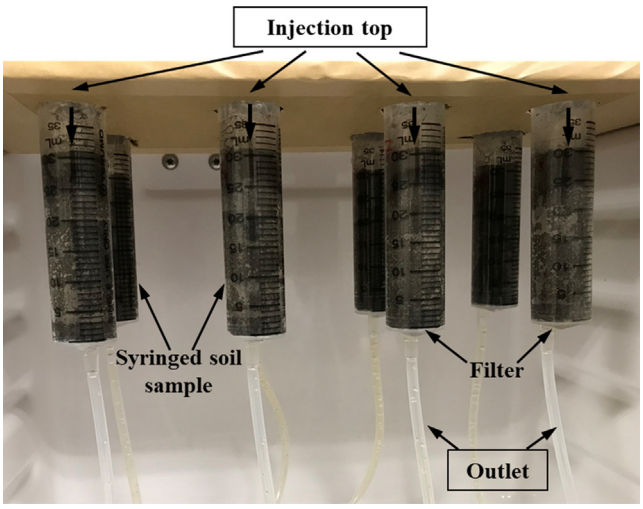

Fig. 4. Syringe solidification test arrangement.

All the solutions were simply applied to the top of the soil columns and allowed to percolate by gravity and capillary forces. The constituents of both culture and cementation solutions are discussed in detail below.

2.2.1. Culture solution

Two types of culture solutions were used: Type 1 and Type 2. The Type 1 solution was prepared using pure chemicals which are used in standard laboratory experimentations, whereas the Type 2 solution was prepared using low-grade chemicals. The compositions are clearly presented in Table 2.

2.2.2. Cementation solution

Likewise, two types of cementation solutions were used. The Type 1 cementation solution was prepared using pure chemicals whereas the Type 2 cementation solution was prepared using low-grade chemicals which are commercially available. Details of the cementation solutions are given in Table 3.

At the end of the treatment, distilled water (five times of sample volume) was applied to each sample in place of bacteria-cementation solutions to rinse out the chemicals from the soil matrix. Syringes were cut carefully, and the samples were taken out from the ports followed by the draining out of solutions. Subsequently, samples were

Table 2
Composition of the culture solutions (per 1 L).

Type 1 (NH_4-YE)		Type 2	
Substance	Amount (g)	Substance	Amount (g)
Tris-buffer	15.70	Beer Yeast	30.00
Ammonium sulfate	10.00		
Yeast extract	20.00		
Distilled Water		Distilled Water	

Table 3
Composition of the cementation solutions (per 1 L, 0.5 M).

Type 1		Type 2	
Substance	Amount (g)	Substance	Amount (g)
$CO(NH_2)_2$ (Urea)	30.00	Urea Fertilizer	30.00
$CaCl_2$	55.50	Snow melting agent	55.50
Nutrient Broth	3.00	Beer yeast	2.00
Distilled Water		Distilled Water	

allowed to cure under the same atmospheric conditions as the treatment conditions. The cementation strength of the specimens was examined using needle penetration device/-soft rock penetrometer (SH-70, Maruto Testing Machine Company, Tokyo, Japan). The needle penetration apparatus is a portable testing device developed in Japan for predicting the UCS of soft, weak to very weak rocks and cemented soil specimens. The specimen was horizontally positioned, and the needle of the device was penetrated into the cylindrical surface of the specimen at three locations (at the distance of 1 cm (top), 3 cm (middle) and 5 cm (bottom) measured from the column top). The penetration resistance (N) and penetration depth (mm) were measured simultaneously. The UCS of the specimen was estimated using the regression relationship given in Eq. (3) which has been developed by analyzing 114 natural soft rock samples and 50 improved soils with cement (Amarakoon and Kawasaki, 2018; Danjo and Kawasaki, 2016; Fukue et al., 2011; Mitsuyama et al., 2017).

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

where y is the UCS; x is the "penetration gradient (N/mm)" which can be determined using penetration resistance and penetration depth.

2.3. Test conditions

In order to investigate the effect of different factors in solidification of soil, eight cases were conducted. Each of them is clearly summarized in Table 4. Case 1 to 2 were carried out on Mikawa sand as an initial step of the study to demonstrate the biocement potential and the effect of saturation in treatment efficiency. In both cases, bacteria culture and cementation solution were injected to the samples every 24 h for ten days (totally 10 pore injections of cementation solution per specimen). In Case 1, the solution level of 2 mm above the top surface of syringed sample was sustained to maintain the saturated condition, and the specimen was drained and refilled every 24 h by cementation solution. In Case 2, the outlet was remained open to keep the fully drained, i.e. in the unsaturated condition.

Cases from 3 to 8 were performed on natural soil (Asari, Hokkaido) in the drained condition with the real field application considered. Cases 3 to 6 were designed to examine the effect of temperature, concentration of cementation solution (Type 1) and injection of culture solution (Type 1). In Cases 3 and 5, the cementation solution was injected every 24 h (with a total of 10 pore injections of cementation solution per specimen), whereas the bacteria culture was injected only once at the beginning of the treatment. In Cases 4 and 6, the specimens were treated in the same way as the specimen in Case 2.

Cases 7 and 8 were designed to study the effect of low-grade injection solutions (Type 2) in MICP treatment efficiency at two different temperatures. The samples were treated by a single injection of bacterial culture and ten pore volumes of cementation solution (in total), the same as Cases 3 and 5. Distilled water was injected instead of culture and cementation solutions to the control samples. Meanwhile, the Ca^{2+} concentration and pH of the drainage were measured for all the cases to determine temporal variations of the parameters in the samples.

3. Results and discussions

3.1. Microbial behavior

The total populations of bacteria in the slope soils of Asari, Asahikawa and Onuma were found to be 10^7 cfu/g, 9×10^5 cfu/g, 16×10^5 cfu/g, respectively. However, ureolytic potential was witnessed only in a few groups of the bacterial strains at each location. The strains are fractionally illustrated in Fig. 5. Table 5 shows the identified ureolytic microbes with respect to their native locations. The culture was maintained in a shaker at 30 °C and 160 rpm, and the growth curves were obtained by monitoring the solutions' optical density at the wave length of 600 nm (OD_{600}) with time (Fig. 6). In consideration of Asari slope (Hokkaido), *Psychrobacillus* sp. strain, at its highest performance among the identified native microbes, was incorporated for detailed experimentations in this research study. The strain has been characterized by motile rods and grew over a wide range of temperatures (−2 to 40 °C) (Pham et al., 2015). Basically, the rate of urea hydrolysis has a direct relationship with the bacterial cell concentration, and high concentration of bacteria produces more urease per unit volume for the commencement of urea hydrolysis (Ng et al., 2012). However, the opposite

Table 4
Test conditions for syringe solidification test.

Case No.	Soil material	Culture solution			Cementation solution			Temp. (°C)	Test duration (Days)
		(mL)	Type	Injection	(mL)	Type	Injection		
1	Mikawa sand	3	1	Every 24 h	3 (1 M)	1	Every 24 h	30	10
2	Mikawa sand	3	1	Every 24 h	3 (1 M)	1	Every 24 h	30	10
3	Asari Soil	10	1	Only at beginning	6 (0.5 M)	1	Every 24 h	30	10
4	Asari Soil	3	1	Every 24 h	3 (1 M)	1	Every 24 h	30	10
5	Asari Soil	10	1	Only at beginning	6 (0.5 M)	1	Every 24 h	20	10
6	Asari Soil	3	1	Every 24 h	3 (1 M)	1	Every 24 h	20	10
7	Asari Soil	10	2	Only at beginning	6 (0.5 M)	2	Every 24 h	30	10
8	Asari Soil	10	2	Only at beginning	6 (0.5 M)	2	Every 24 h	20	10

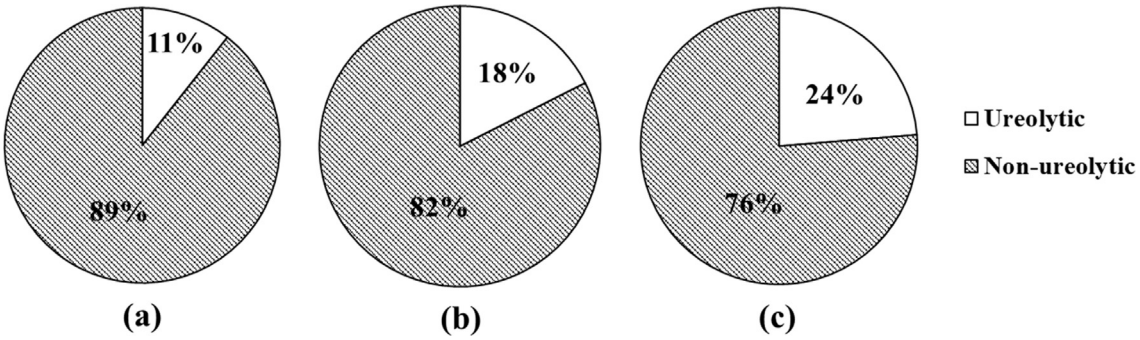

Fig. 5. Proportion of ureolytic bacteria identified from (a) Asari, (b) Asahikawa and (c) Onuma slopes.

Table 5
Identified ureolytic microbes with respect to their native slopes.

Location	Group of Belonging	Sample ID
Asari	*Bacillus* sp.	Asr-1
	Bacillus sp.	Asr-2
	Psychrobacillus sp.	Asr-3
Onuma	*Lysinibacillus xylanilyticus*	Onm-1
	Viridibacillus arvi	Onm-2
	Sporosarcina sp.	Onm-3
Asahikawa	*Sporosarcina* sp.	Asw-1
	Lysinibacillus sp.	Asw-2
	Lysinibacillus sp.	Asw-3

behavior was observed between cell concentration and the urease activity of *Psychrobacillus* sp., which are given in Figs. 7 and 8, respectively. The cell concentration at 20 °C is significantly higher than that at 30 °C, and the urease activities of strain at 20 °C and 30 °C are 0.10 U/ml and 0.41 U/ml, respectively. Based on observations, it can be stated that at higher temperatures (30 °C), bacterial growth is inhibited, whereas bacterial growth is favored at lower temperatures (20 °C). However, 30 °C provides more favorable conditions for the bacteria to produce the protein

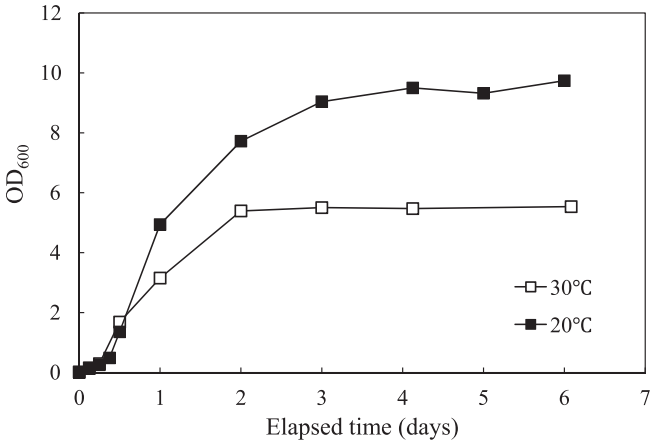

Fig. 7. Cell concentration of *Psychrobacillus* sp. (Asr-3) at two different temperatures.

subunits corresponding to urease activity (urease enzyme) compared to those at 20 °C. Similarly, the rate of urea hydrolysis of *Bacillus Megaterium* and *Sporosarcina pasteurii* is marginally higher in 30 °C compared to 20 °C, and the optimum activity is found to be at around 30 °C

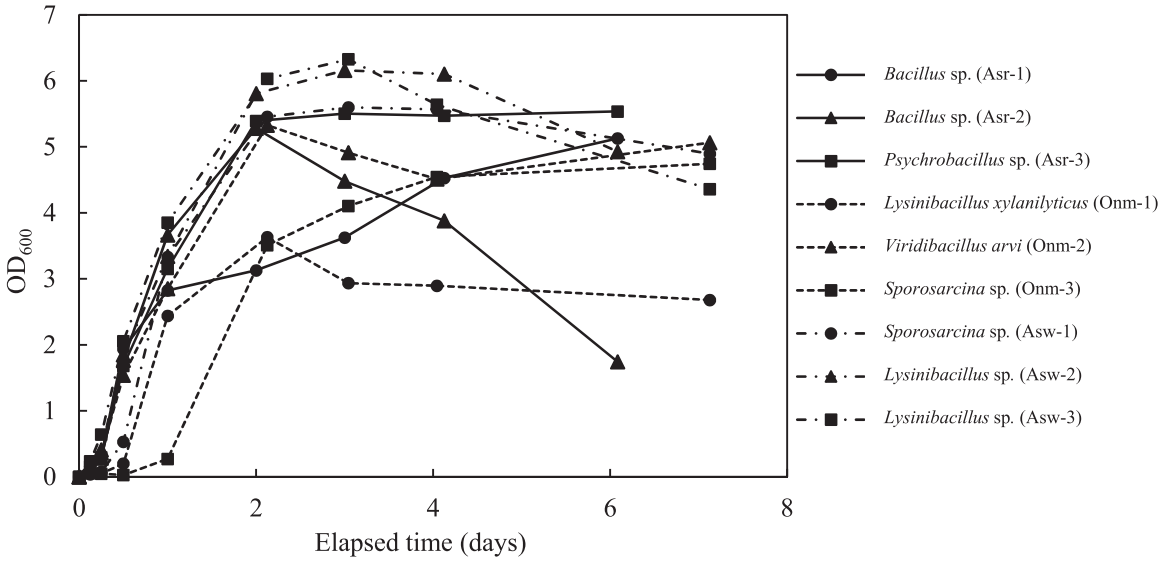

Fig. 6. Variation of cell concentration of isolated microbes with the time.

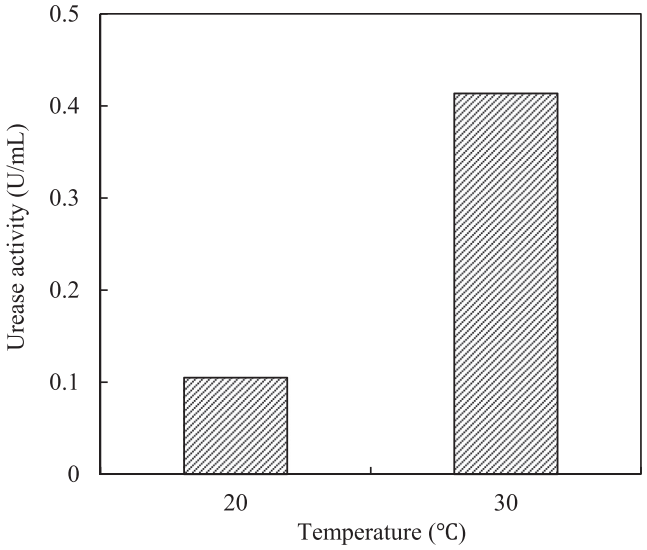

Fig. 8. Urease activity of *Psychrobacillus* sp. (Asr-3) at two different temperatures.

(Ng et al., 2012; Omoregie et al., 2017). At the same time, few strains including *Pararhodobacter* sp., *Deleya venusta* and *Strep-tococcus salivarius* exhibit the optimum urease activity at around 60 °C (Fujita et al., 2017; Ng et al., 2012). However, it is impractical to utilize the optimum activity of bacteria in MICP treatment, as the in-situ soil temperature range cannot be controlled. Rather, optimizing the activity of bacteria in the feasible temperature range is recommended for MICP according to the focus zone. For this reason, solidification tests were considered in detail at two practicable temperatures of Hokkaido.

3.2. Laboratory scale solidification tests

In order to monitor the chemical condition within the samples being treated, continuous outlet measurements were taken during the solidification tests. The Ca^{2+} concentration and pH of Cases 1 and 2 (Mikawa sand) were measured soon after 3, 6, 9 and 10 days, and are presented in Fig. 9a and b, respectively. The measurements of Cases 3 to 8 (natural slope soil) were taken every 24 h, and the results are compared in Fig. 10a and b. The pH of the drainage remained higher in Case 1 than in Case 2, whereas the Ca^{2+} concentration of drainage for Case 1 was lower than Case 2 (Fig. 9). Based on Fig. 10, relatively weak alkali pH conditions were maintained during the test period, with increases over time. At the same time, the Ca^{2+} concentration of drainage in Cases 3 to 8 reduced with time, after an initial raise in the first few days.

3.3. Effect of saturation

Fig. 11 illustrates the effect of saturation in MICP efficiency. The solidified sample under the saturated condition (Case 1) exhibits higher UCS values than those of the unsaturated case (Case 2) at all the depths (1 cm, 3 cm and 5 cm)

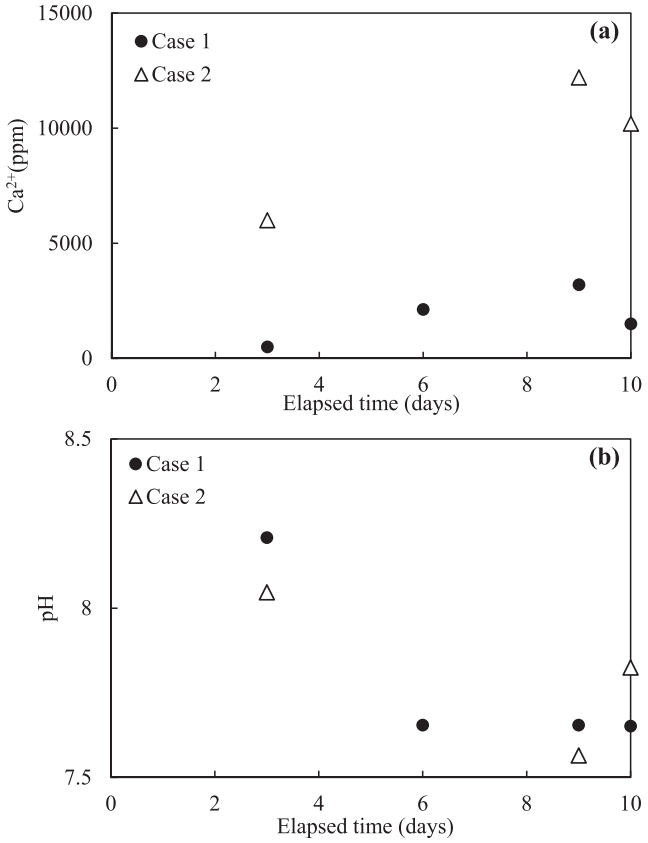

Fig. 9. Measurements of (a) Ca^{2+} concertation and (b) pH at the outlet with the time (Cases 1–2).

measured from the top surface of the sample. Measurements of outlet ensures that the saturated case drains with a higher pH and lower Ca^{2+} concentration than that of the unsaturated case most of the time (Fig. 9). Sustaining the solutions entirely within the soil sample tends to utilize more hydrolyzation of urea (fallouts higher pH), leading to the higher consumption of Ca^{2+} ions for calcite precipitation (fallouts lower Ca^{2+} concentration). It has been reported that the highest $CaCO_3$ precipitation and highest strength occurs with a degree of saturation (%) in sandy soils (Whiffin et al., 2007), which is in line with the results obtained in this study. However, Cheng et al. (2013) concluded that a higher degree of saturation results in the ineffective formation of $CaCO_3$ crystals in pore voids, and the consequent lowering of strength. It is worth noting that since treating the slope soil under the saturated condition is practically impossible in the field, natural soil was treated totally under the unsaturated treatment condition.

3.4. Effect of temperature

In MICP, temperature is one of the important factors as it highly affects the growth, urease activity of microorganisms and nucleation rate of $CaCO_3$ crystals. In consideration of the conditions in the field, MICP efficiency was investigated at two appropriate temperatures (20 °C and

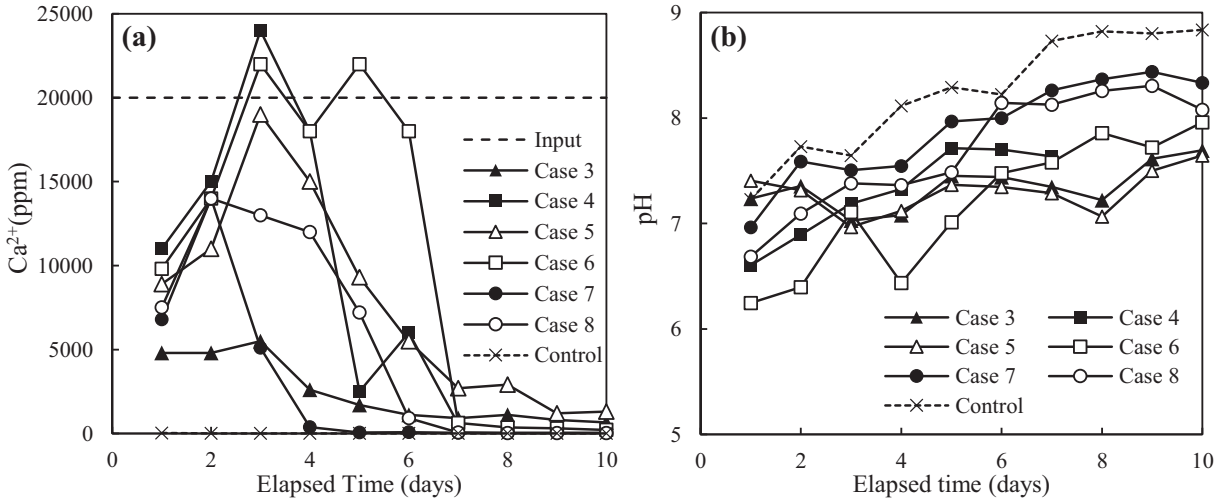

Fig. 10. Measurements of (a) Ca^{2+} concertation and (b) pH at the outlet with the time (Cases 3–8).

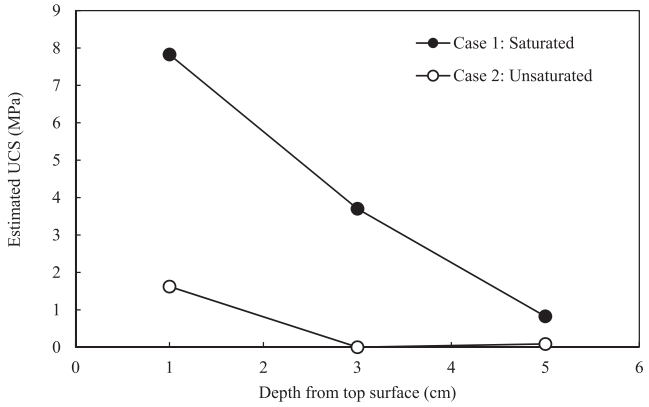

Fig. 11. Effect of saturation in MICP treatment (Cases 1–2).

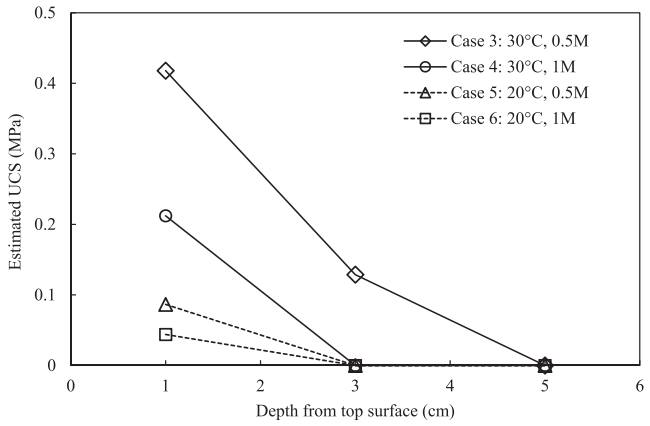

Fig. 12. Effect of temperature and cementation solution in MICP treatment (Cases 4–6).

30 °C) in this study. The effect of temperature obtained at two concentrations of cementation solution (0.5 M and 1 M) are given in Fig. 12. It can be seen that the treated samples exhibit highest UCS at 30 °C in both cases. This is due to the higher urease performance of *Psychrobacillus* sp. at 30 °C, and the performance of bacteria improves by a factor of approximately four when the temperature rises from 20 °C to 30 °C. This is consistent with previous reports, where it was shown that the strength increment increased by folds at optimal temperature of bacteria compared to that in adjacent temperature range (Amarakoon and Kawasaki, 2018; Danjo and Kawasaki, 2016). As well as the favorable temperature of microbial performance contributing to the highest strength, the size and shape of the formed crystals which affects the strength also depends on the temperature range (Mujah et al., 2017).

3.5. Effect of injection solutions

The concentration of cementation solution is one of the controlling factors in effective $CaCO_3$ crystal formation. In this study, two concentrations (0.5 M and 1 M) were considered, and it is observed that the UCS of the soil treated using 0.5 M cementation solution was higher than that treated using 1 M solution at both temperature conditions (Fig. 12). This is consistent with the observations reported by Ng et al. (2012) that the soil treated with 0.5 M cementation reagent more effectively enhanced the UCS than that treated with 1 M. It is clear that lower concentration results in more homogeneous crystal formations at contact spots, which promotes strength, whereas high concentration hinders the establishment of effective bonding by rapid and random formation of crystals in soil voids (Mujah et al., 2017).

Further, the cementation solution was injected by syringe on an every-day basis similar to that reported in previous studies (Amarakoon and Kawasaki, 2018; Danjo and Kawasaki, 2016). Moreover, culture solutions were injected only at the beginning in Cases 3 and 5, whereas it was injected on an every-day basis in Cases 4 and 6. The results indicate that better treatment efficiency is obtained with the single injection of bacteria (Cases 3 and 5). A similar observation was reported by Amarakoon and Kawasaki (2018), with the UCS of a single injection of bacteria approximately twice that of the reinjection case.

3.6. Low-grade chemicals in MICP

For Cases 7 and 8, the solidification effect of natural soil was investigated by incorporating commercially available low-grade chemicals. Basically, purity is the prime difference between standard pure chemicals and low-grade chemicals. Results of an elementary chemical analysis obtained from X-Ray Fluorescence (XRF) Spectrometer (JSX-3100R II JOEL, Japan) for the beer yeast, snow melting salt and urea fertilizer are provided in Fig. 13a, b and c, respectively. The urea fertilizer, a widely used substance in the agriculture industry, is comprised of copper, iron and potassium (45.66%, 39.86% and 14.48% respectively). Based on the manufacturers' specifications, the purity of nitrogen in urea fertilizer is in the vicinity of 46.0%. The snow melting agent/de-icing salt consists of calcium (34.87%), chloride (61.89%), sodium (1.70%) and potassium (1.53%). The purity of calcium chloride is 74% in the de-icing salt commonly applied as bulk to melt the ice deposited on roads and pavements in winter seasons all over the world. Beer yeast is a substance used in the food industry mainly to break down sugars, and added with alcohol to beer as a byproduct of the process. 100 g of beer yeast consists of the following nutrients: 48.6 g of protein, 4.2 g of fats, 39.4 g of carbohydrate, 6.1 g of sugar, 33.3 g of dietary fiber and 6.2 g of ash. Also, an XRF analysis reveals that beer yeast consists of potassium (38.59%), phosphorus (37.15%), sulfur (16.77%), calcium (6.82%), iron (0.45% and copper (0.23%). The MICP feasibility of these low-grade chemicals was investigated since they are inexpensive and less harmful to the geo- environment than pure chemicals.

The UCS values obtained from the treated samples of Case 7 (30 °C) and Case 8 (20 °C) are compared in Fig. 14. The natural soil treated by applying low-grade chemicals at 30 °C shows a remarkable UCS value of 0.82 MPa, whereas solidification is not achieved for the sample treated at 20 °C. The solidification of soil at 20 °C failed while using low-grade chemicals, even though the drainage measures (Ca^{2+} concentration and pH) show a positive precipitation response (Fig. 10).

Fig. 15 shows the comparison of obtained UCS versus $CaCO_3$ content for the samples treated using pure chemicals (Case 3) and low-grade chemicals (Case 7 and 8). It is clearly perceived that the UCS value obtained by implementing low-grade chemicals at 30 °C is approximately twice that of the UCS (0.417 MPa) obtained by implementing pure chemicals. As expected, the marginally higher precipitated $CaCO_3$ content in the pure chemical case (9.5%) than that of the low-grade chemical case (9.3%) is due to the difference in purity among those chemical substances. It should be noted, however, that the precipitated carbonate contents (9.3% and 9.5%) in this study are relatively higher than the results reported in literatures (Feng and Montoya, 2015; Lin et al., 2016a). Feng and Montoya (2015) investigated the effect of cementation level (from 1 to 3.5%) in MICP treated sand under monotonic drained

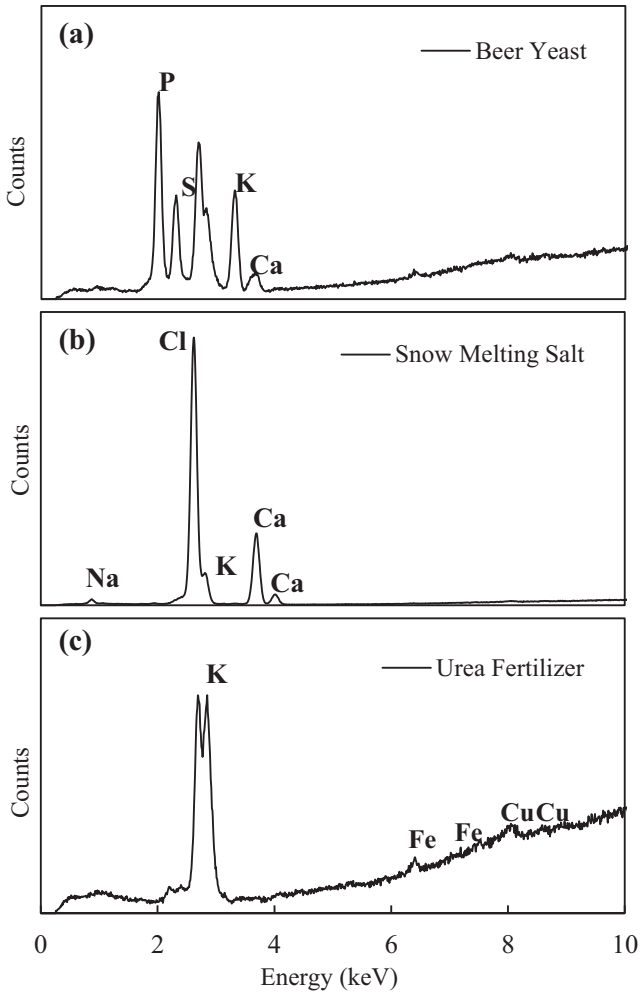

Fig. 13. XRF energy spectrum of the (a) beer yeast, (b) snow melting salt, and (c) urea fertilizer.

conditions and reported that the heavy cementation level (above 3.5%) exhibited significant strain softening, although higher cementation level resulted higher stiffness, strength and dilative tendencies. Also, Lin et al. (2016a, 2016b) have focused on drained response of MICP treated sand and improving the axial capacity of permeable piles respectively with the carbonate content in the same range from 1 to 3%. The discrepancy between the $CaCO_3$ content addressed in this paper and literatures can be explained by many factors, such as the different grain size distribution, the density of the soil, particle shape, and the morphology of the precipitates. Grain size distribution plays a crucial role in the spatial distribution of carbonate cement in pore spaces and the effective formation of bonds at particle contacts (Cheng et al., 2014; Lin et al., 2016a; Oliveira et al., 2016). Actually, MICP is not very effective with gravelly type of soil since the thin layer of $CaCO_3$ precipitated at the contact zone of larger particles is not sufficient to link them (Kim et al., 2014; Rebata-Landa, 2007). The soil from the Asari slope (Hokkaido, Japan) is a well graded soil, with a significant amount of coarse material, including a gravel content of 20–25%, ranging from fine to medium

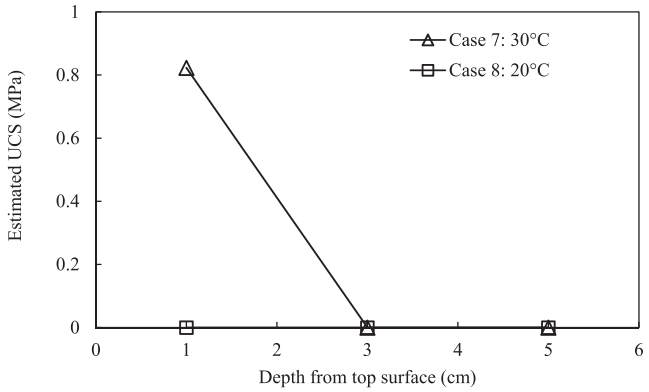

Fig. 14. Effect of temperature in MICP treatment using low-grade reagents (Case 7–8).

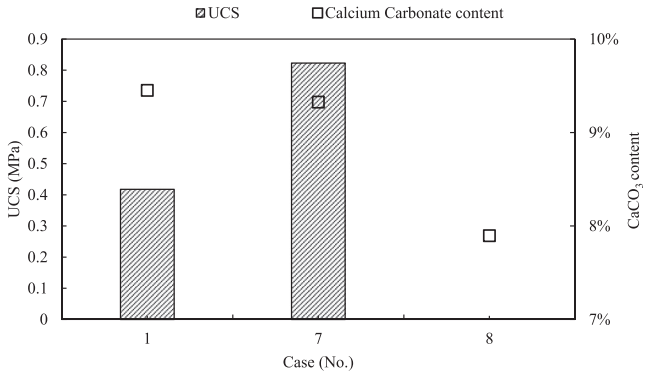

Fig. 15. UCS and $CaCO_3$ content of samples treated using pure chemicals and low-grade reagents.

(Fig. 3). Thus, a relatively higher carbonate content was required to bond the large soil particles, and therefore obtain the required strength in natural slope soil.

The soil reported herein was prepared for a relative density (D_R) of 65%,which is higher than the D_R of the Ottawa 50/70 sand (around 40%) investigated by Feng and Montoya (2015) and Lin et al. (2016a). The dense packing of natural soil could further reduce the void spaces, reduce the percolation rate of injected solutions and eventually facilitate more carbonate precipitation. Mortensen et al. (2011) have also reported that rate of carbonate precipitation is higher in well graded sands than in poorly graded sands. Unlike laboratory sands, the natural soil consisted of particles over a wide range of shapes with significant surface irregularities (observed by SEM), which could provide ideal surfaces for the additional precipitation of carbonate. However, the effect of particle shape in MICP is unclear, and needs to be studied in detail. Furthermore, while the morphologies of the precipitates reported by Lin et al. (2016a, 2016b) are calcite and vaterite, only calcite crystal morphology was observed in treated natural slope soil, and mineral morphology is discussed in detail in the subsequent section.

Moreover, for the same amount of $CaCO_3$ precipitation, the UCS of soil treated using low-grade chemicals was significantly higher than that of pure chemicals. This result is contrary to previous understanding that a higher $CaCO_3$ content can contribute higher strength for a soil treated under the same conditions (van Paassen et al., 2010; Whiffin et al., 2007). During the MICP process, precipitated carbonate crystals deposit at particle contacts, coat the exposed surface of soil particles, fill the voids and provide a matrix to support to the soils, as was clearly explained by Lin et al. (2016a). The formation of crystals at contact points and their crystallographic pattern play the significant role in contributing strength to the cemented soil. Using shear wave velocity measurements, it has been proven that the distribution of $CaCO_3$ at the particle contacts of MICP treated specimens can vary at the equivalent precipitated $CaCO_3$ content (Feng and Montoya, 2017; Qabany et al., 2011; Weil et al., 2012). Thus, the strength response is governed by not the average $CaCO_3$ precipitated, but the effective $CaCO_3$ precipitated at the particle contacts (Feng and Montoya, 2017), and the amount of effective $CaCO_3$ is influenced by the number of bacteria attached at particle contact during treatment (DeJong et al., 2010). Moreover, Cheng et al. (2014) reported that crystals formed under the unfavorable bacterial condition were inadequate and incapable of efficiently forming effective bridges at particle contacts. In this study, although the precipitated crystal content is low in the specimen treated using low-grade chemicals, crystals were driven to support the soil matrix effectively and contributed higher UCS compared to the soil treated using pure chemicals. However, the matrix supporting mechanism is not clear. Therefore, the samples were observed by Scanning Electron Microscopy (SEM) to clearly determine by which the soils treated under low-grade chemicals exhibited higher strength.

To show the cost differences, a detailed cost comparison was performed based on the market price of reagents (in Japan), and the results are presented in Table 6. The total reagent cost of the 10 days of MICP treatment for the 1 m^3 soil using pure chemicals and low-grade chemicals is estimated as 11,972 USD for the former and 468 USD for the latter, which is a 25 fold difference. As well as resulting in a 96% cost reduction, the approach improves the strength significantly. This would certainly be a a considerable advantage for future industrial level MICP advancements.

3.7. Scanning Electron Microscopy analysis

Microscopical observations using SEM were performed to determine the microstructural effects of treatment and to observe cementation matrix using SuperScan SS-550 (Shimadzu Corporation, Kyoto, Japan). Samples were washed and oven dried at 105 °C for 24 h 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.

Photomicrographs of the sample treated under conditions 30 °C, 0.5 M (Case 1) shows the precipitation of irreg-

Table 6
Cost comparison between pure chemicals and low-grade reagents in the MICP treatment of 1 m^3 natural soil.

Reagents	Pure Chemicals				Low-grade reagents			
	Substance	Required amount (kg)	Unit price (JPY/kg)	Price (JPY)	Substance	Required amount (kg)	Unit price (JPY/kg)	Price (JPY)
Cementation Solution	Urea	64.30	1580	101,572	Urea Fertilizer	64.30	150	9643
	$CaCl_2$	118.90	5600	666,000	Snow melting agent ($CaCl_2$)	118.90	124	14,747
	Nutrient Broth	6.40	36,600	235,286	Beer yeast	6.40	2,000	12,857
Culture Solution	$(NH_4)_2SO_4$	3.57	1975	7054	Beer Yeast	7.15	2000	14,286
	Yeast Extract	7.14	34,580	247,000				
	Tris-Buffer	5.62	10,833	60,938				
Estimation	Total material cost			1,317,850 (11,972 USD)	Total material cost			51,533 (468 USD)

ular rhombohedral crystals ranges 12–20 μm (Fig. 16a), which is the typical form of crystalline calcites. This is corroborated by the similar observations made by Kim et al. (2014) and Soon et al. (2014). In contrast, the crystallization obtained for the samples treated under 20 °C (0.5 M and 1 M) (Fig. 16b and c) have different morphologies. The crystals are spherical with an average size of around 15–20 μm and are suspected to be vaterite crystals. It has been clearly reported that vaterite crystals (either solid or hollow) are surrounded by abundantly accumulating nano-crystals commonly referred to as extracellular polymeric substances (EPS) with a large range in general (Chen et al., 2017; Sensoy et al., 2017; Tolba et al., 2016). Further evidence of similar accumulation is shown in Fig. 16b–d. Vaterite is the least stable form of crystalline $CaCO_3$, and is much less stable than calcite, and can be transformed into calcite very rapidly with time (Braissant and Verrecchia, 2002; Tolba et al., 2016). Although vaterite crystallization is promoted by kinetic effects (high supersaturation; role of organic compounds) and thermodynamic effects (energy minimizations; ionotropic effects) (Rodriguez-Navarro et al., 2007), it has been shown that vaterite precipitation is strain-specific (Braissant and Verrecchia, 2002; Sensoy et al., 2017). Based on the observations in this study, it can be stated that calcite crystals were precipitated under the conditions of 30 °C, 0.5 M urea and 0.5 M $CaCl_2$ and tended to involve the contact segments, inducing a strong bond between soil particles, therefore a higher strength in the treated sample (Case 3). At the same time, vaterite was obtained under conditions including 20 °C, 1 M urea, and 1 M $CaCl_2$ (from Cases 4 to 6), forming weaker bonds than the calcite bonds. In previous observations, *Sporosarcina pasteurii* was reported to produce vaterite and amorphous EPS at the conditions of 30 °C, 0.33 M urea, 1 M $CaCl_2$ at the pH range of 6.5–8.17, whereas *Bacillus aerius* has resulted vaterite, calcite and EPS together at the conditions of 20 °C, 0.3 M urea, 1 M $CaCl_2$ at the pH range of 5.5–9.28 (Sensoy et al., 2017). Moreover, Braissant and Verrecchia (2002) concluded by considering *Xanthobacter autotrophicus* and *Alcaligenes eutrophus*, that the precipitation of vaterite instead of calcite occurred due to the absence of the exopolysaccharides produced by bacteria between pH 7 and 9.5. It can be understood that there is a great influence of the physio-chemical conditions in microbial-induced different crystallizations, but the exact formation mechanism of vaterite crystals is still a matter to be explored clearly in future studies.

The observed morphologies of sample treated using low-grade chemicals (Case 7) are presented in Fig. 16e and f. In case the crystallization was not easily distinguished in SEM images, an XRD analysis was performed to build an understanding of crystallization, and the images are shown in Fig. 17. The XRD results confirmed that the precipitated crystals were predominantly calcite. However, the typical crystalline form of calcite was not identified anywhere in the treated soil matrix. Instead, a well packed matrix com-

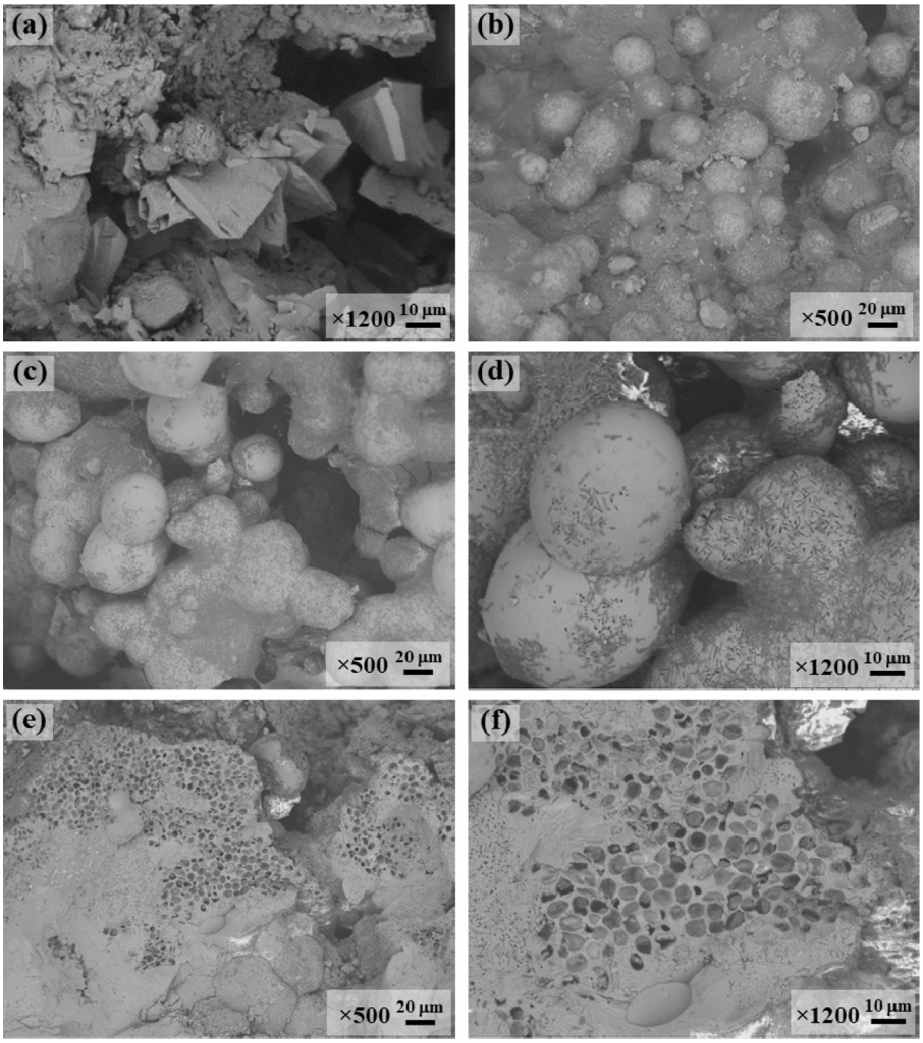

Fig. 16. Scanning electron micrographs of calcium carbonate crystallization by *Psychrobacillus* sp. (a) at 30 °C, 0.5 M from Case 3, (b) at 20 °C, 0.5 M from Case 5, (c) at 20 °C, 1 M from Case 6, (d) enlarged scale of Case 6, (e) using low-grade chemicals from Case 7, and (f) enlarged scale from Case 7.

bined of soil particles and precipitates was widely observed in the treated specimen (Fig. 16e and f). This unusual formation of compacted matrix may be due to the presence of polymeric substances (PS) in low-grade chemicals, and the formed calcite precipitates are thought to have encapsulated the PS, filling the void spaces and bonding the soil particles. This resulted in better compaction and therefore a significant improvement in strength. The above statement is supported by the observation of the voids in the treated matrix. For the same treatment conditions and precipitation content (9.3% and 9.5%), soil treated using low-grade chemicals exhibited a significant reduction in voids compared to that of laboratory chemicals (Fig. 16a and e, respectively). This indicates that the PS from low-grade chemicals was encapsulated with the precipitated carbonate cement and filled the void spaces, effectively providing significant matrix support. However, a detailed future investigation is required to further clarify the discrepancies in the calcite crystal morphology obtained for the implementation of low-grade chemicals.

3.8. Calcium carbonate cement and strength of soil

Since the solidification of soil is achieved through biochemical injections, it is necessary to evaluate the mass of the calcium carbonate cement formed at each condition. The global average calcium carbonate content of the cemented sample was measured based on mass balance using the Ca^{2+} content of the fluid injected and discharged from the specimen. Hence, the UCS results were correlated with global average calcium carbonate content of each solidified sample treated using laboratory grade chemicals. It can be seen that the UCS increases exponentially with the increase of calcium carbonate content (Fig. 18), which is in line with previous studies reported by Amarakoon and Kawasaki (2018), Cheng et al. (2013), Danjo and Kawasaki (2016) and van Paassen et al. (2010). The derived relationship between calcium carbonate precipitation (x) and UCS (y) of natural soil is described in Eq. (4).

$$y = 149.35x^2 - 7.53x \tag{4}$$

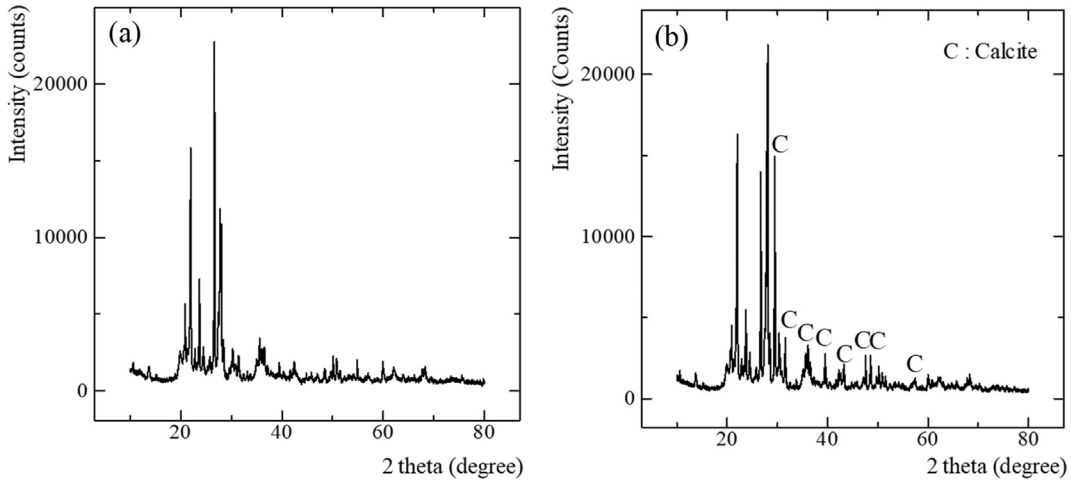

Fig. 17. XRD pattern of natural soil sample (a) before treatment and (b) after treatment using lowgrade reagents (Case 7).

The minimum effective average $CaCO_3$ content, which is not adequate to provide any measurable strength in natural slope soil is 5%. This is higher than that reported in the literature. Whiffin et al. (2007) reported that a $CaCO_3$ content below 3.5% had no significant effect on the strength and stiffness properties of sand relative to untreated sand. However, many researchers have recently shown that the threshold calcium carbonate content required to increase of the strength of sand is in the vicinity of 1% by weight (Feng and Montoya, 2015; Lin et al., 2016a), and the residual strength of MICP treated sand with less than 1% cementation level was close to that of the untreated sand (Soon et al., 2014). Since the slope soil consists of a significant amount of coarse material, including a gravel content of 20–25%, a threshold carbonate content of around 5% was required to obtain any measurable strength, as explained in the previous section. It should also be noted that the relationship in Eq. (4) is applicable only to the soil treated in this study and the method used to treat the soil.

The higher strength of MICP treated well-graded soils than uniformly-graded soils is considered to be primarily due to the effect of increased number of particle contacts (Cheng et al., 2014; Oliveira et al., 2016). Compared to uniformly graded soil, the effective distribution of particles of a wide range of sizes results in fewer void spaces, increases the number of contact points, and improves the strength of the soil. Although the slope soil considered in this study has a gravel content of 20–25%, the presence of a large range of particles from fine to coarse compensates for the MICP behavior. In fact, MICP can be significantly governed by fine content in a soil matrix. Zamani and Montoya (2018) reported that the effect of silt content is

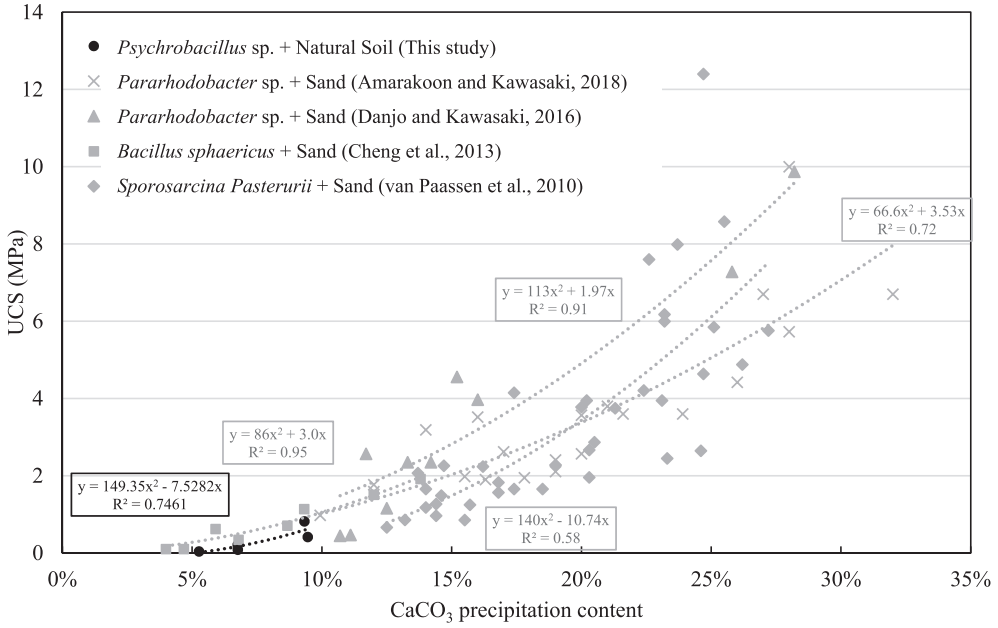

Fig. 18. Comparison of relationships between UCS and global average $CaCO_3$ precipitation content with previous studies.

negligible if the silt content is less than 8%, and only the sand skeleton governs the MICP behavior. Furthermore, the effect of silt content is significant between 20% and 35%, resulting in a large number of contact points which supports the force chain in cemented soil matrix. In Asari slope soil, the fine content was 1–2%, which was inadequate either to fill the voids of the sand matrix effectively or to increase the number of particle contacts. It is well understood that despite having the same amount of $CaCO_3$ content, the mechanical response of MICP treated soil can vary depending on the number of particle contacts involved in cementation.

From the previous studies, it was found that the shear strength improvement of fine sand was linearly proportional with a calcite content between 1 and 2.5%, and improvement became less above 2.5% because almost all of the available particle contact points were bonded by $CaCO_3$ (Soon et al., 2014). In fact, the shear strength of biocemented soil was significantly affected by the increase in soil cohesion resulting from the increase in the cement content, while the friction angle was not greatly affected by the cementation process (Mujah et al., 2017). Once the maximum shear resistance is reached, localized shearing and the breakage of bonds at particle-particle contacts may initiate, and continue to break, resulting in the loss of effective cementation (DeJong et al., 2010).

4. Limitations and future developments

In most of the cases considered in this study, solidification was detected over the length of samples, suggesting that the bacteria and reagents were utilized along the samples. However, because the obtained UCS values declined with increasing sample depth in all cases, it can be concluded that a relatively high amount of calcium carbonate precipitated at the injection-top zone of the sample and that this effect declined with depth. Generally, the solutions were utilized more effectively in the top zone of the sample in closer proximity to the injection point (Whiffin et al., 2007), and their diminished performance with depth reveals a lower solidification effect with depth, as shown by the results (Figs. 11, 12 and 14). Also, Martinez et al. (2013) have suggested that the distribution of bacteria is the most important factor in achieving uniform solidification. The aerobic bacterium *Psychrobacillus* sp. (Pham et al., 2015) was shown to perform poorly in the bottommost zone of samples due to the absence of oxygen (Braissant and Verrecchia, 2002). In this study, a stiff zone of about 3–5 cm in thickness was formed at favorable conditions with an average global calcite content of about 9%. However, it has been reported that a relatively shallow treatment depth of 10–20 cm is required for the stabilization of embankment/expressway slopes to protect them from erosion (Salifu et al., 2016). It has been reported that a solidified sand crust 2.5 cm in thickness (calcite content of 2.1%) provided better resistance in a non-standard field erosion test (water spray for 1 min at 22.7 L per minute from 1 m above ground surface) (Gomez et al., 2013). MICP efficiency with depth of treated soil is one of the widely observed limitations, and more investigations have to be performed to optimize the injection systems/methods in order to enhance the homogeneity of treatment. An economically and environmentally beneficial approach was undertaken in this study by introducing low-grade chemicals rather than pure chemicals. However, since only a limited understanding of the effect of low-grade chemicals in the crystallization mechanism was obtained, further studies are required to provide a deeper understanding of the reliability and morphological effects of such treatment. Scaling-up is required for the next steps (Figs. 1 and 2) to evaluate and overcome the limitations posed by boundary effects before this approach can be promoted for use in industrial-scale applications. The spraying method may be a more suitable option for the large-scale applications than the injection method incorporated in this study. However, this small-scale investigation was essential to determine the feasibility of the method, and to optimize the treatment regimen before scaling-up.

5. Conclusions

In this research study, the feasibility of using the emerging MICP technique for the stabilization of natural soil in order to mitigate the erosion potential of slope soil was investigated. Soil samples treated under different conditions were subjected to a needle penetration test and were characterized using SEM and XRD analysis. Based on the findings of this study, the following conclusions can be drawn:

1. *Psychrobacillus* sp., a native ureolytic bacteria found in slope soil (Asari, Hokkaido), has MICP potential. The urease activity was better at 30 °C than at 20 °C. The optimal treatment conditions for the strengthening of slope soil were 30 °C, 0.5 M concentration of cementation solution (daily injection), and an injection of culture solution only at the beginning.
2. Introducing low-grade chemicals rather than laboratory-grade pure chemicals resulted in a remarkable enhancement in strength. The UCS value was double the value obtained while using pure chemicals. This innovative alternation amounts to a 96% reduction in the material cost, and allows the leaching of harmful chemicals to be avoided.
3. There was a relationship between the surface strength of the treated samples and the global average calcium carbonate content precipitated in samples of slope soil. However, it was not the carbonate precipitation content but the special distribution and the effective formation of cement at the particle contacts which governed the strength of the samples. For the same amount of average $CaCO_3$ content, the mechanical response of MICP treated soil can vary. Moreover, the particle size distribution of slope soil (particularly, presence of fine to medium

gravel material) governed the number of particle contacts and carbonate content for the required strength. The apparent minimum calcium carbonate content required for a measurable increase in strength of natural soil was 5%.

4. On the whole, the MICP process has clear potential ito improve the residual strength by solidification of soil. It can be used as a strategy for the prevention of slope soil erosion, and promises to be an economically feasible alternative after the appropriate scale-up experimentations and environmental impact analysis are complete.

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