



## Field experimentation of bio-cementation using low-cost cementation media for preservation of slope surface

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### ABSTRACT

Microbial induced carbonate precipitation (MICP) is a promising bio-cementation method that involves ureolytic bacteria to improve the geotechnical properties of soil. The laboratory-scale studies carried out in the recent past showed that the MICP can be a potential alternative for slope surface preservation. However, the use of analytical-grade chemicals makes this method too expensive, limiting the applicability of the method especially when implicated at field-scale. The purpose of this research work was to assess the effectiveness of using inexpensive low-grade chemicals for the in-situ stabilization of slope surface. Two test plots were established at the project site (Hokkaido expressway slope, Japan) and subjected to MICP treatment via surficial spraying. One was treated using the cementation media formulated by low-grade chemicals (inclusive of fertilizer urea, snow-melting agent and beer-yeast), while the typical analytical-grade media was used to treat the other plot. After 20 days of treatment, the treated slope surfaces were evaluated by surface strength, CaCO<sub>3</sub> content, scanning electron microscopy, energy dispersive spectroscopy (EDS) and X-ray diffraction (XRD) analyses. The outcomes indicated that the surface was significantly improved by low-grade chemicals; a stiff surface layer was desirably formed to the depth of 5 – 10 cm with the surface strength and CaCO<sub>3</sub> content in the ranges of 0.14 – 1.02 MPa and 0.56 – 3.7%, respectively. The results are compared and discussed, and the challenges in the use of low-grade chemicals are pointed out for the way forward. Cost analysis disclosed that the material cost of the cementation media was reduced by around thirty-seven-fold (by 97%) compared to the analytical-grade media. While demonstrating the potential use of low-grade chemicals, the field-scale experiment could contribute to narrow down the gap between the present-state and real-scale deployment of MICP technology.

### 1. Introduction

Bio-grouting is emerging to be a new soil improvement technology. Involving urease producing bacteria to produce calcium carbonate within soil matrix has become a popular bio-grouting method these days, which is often designated as ‘microbial induced

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carbonate precipitation (MICP) [1–3]. The primary reactions involved in the MICP technology are presented in Eq. 1 and Eq. 2. During the treatment, the enriched bacteria culture and cementation media (mainly consisting of urea and calcium salt) are introduced through the soil surface and allowed to percolate onto the stratum, either one after another or simultaneously [4,5]. The bacteria cells containing urease enzyme facilitate the hydrolysis of urea, resulting in the production of carbonate ions ( $\text{CO}_3^{2-}$ ) and ammonium ions ( $\text{NH}_4^+$ ). Thereupon, the supplied calcium ions ( $\text{Ca}^{2+}$ ) react with carbonates, and mineralize as calcium carbonate ( $\text{CaCO}_3$ ) within the surrounded soil matrix, i.e., on soil surfaces and at particle contacts [6]. Many previous studies suggested that the resulting  $\text{CaCO}_3$  cementation markedly improves the geotechnical properties of soil such as density, gradation, compressive strength, initial shear moduli, peak shear strength, cohesion, liquefaction resistance and S-wave velocity [7–11]. Besides, few researchers used such biologically derived calcium carbonate for remediating the heavy metals in contaminated soils [12,13]. It was shown that the process promotes the transformation of heavy metals [e.g., arsenic (As), cadmium (Cd), copper (Cu) and lead (Pb)] from their mobile-form to stable carbonate minerals, been immobilized into soil matrix.



Preservation of slope surface has been one of the key proposals of MICP in the recent past. One major convenience of bio-grout over conventional grouts (e.g., Portland cement, silicate-based grouts, etc.) is the low viscosity of treatment solutions that allow them to effortlessly percolate through the microchannels of the surface slope. Several laboratory-scale investigations revealed that the MICP treatment enables the formation of crust layer along the outer surface of the slope [8,14–16], and the treated surface was proven to be durable, withstanding for a long-term against various simulated environmental conditions such as tidal waves [15,16], rainfalls [14, 17], heavy winds [18], cyclic freezing and thawing [8], repeated wet-dry actions [19,20] and acid-rain weathering [1,21].

Despite the increased interests in MICP-based surface preservation of slopes, no field-scale trials were reported in the literature so far; the investigations were largely confined to the laboratory. It must be noted that the future of MICP, as a practical surface preservation method, is reliant on the field trials which provide the essential information about the process at metre scale. One major reason that often limits the field-scale testing is the unaffordable cost of the required chemical reagents [22,23]. Once it was indicated that the cost of the reagents alone covers up to 60% of the total operation cost of MICP grouting [24], which is indeed expensive especially when implicated at field-scales. The field-scale treatment requires nutritional sources, calcium source and urea in large quantities during the upstream process. Nutrient source consists of the substances that are essential for the growth of the ureolytic bacteria, such as organic carbon, nitrogen, phosphorous and metal ions [25]. Majority of the previous MICP works relied on the use of laboratory-grade nutrient media (e.g., yeast-extract, nutrient broth, soy broth) that are costly. Few recent studies have reported the use of alternative inexpensive nutrient sources such as corn steep liquor [26], chicken manure effluent [27], lactose mother liquor [22], beer-yeast [28] and food-grade yeast [23] to cultivate various ureolytic bacterial species. Their outcomes indicated that the growth and performance of the species cultivated in the alternative media are pretty much comparable with those in conventional growth medias.

Few previous studies attempted to evaluate the suitability of alternative calcium and urea sources instead of analytical-grade chemicals. Egg shells and calcareous sand were proposed in the past as the replacement for synthetic calcium chloride [29–31]. The researchers showed that the calcium-rich materials can potentially be dissolved in acid and the required soluble calcium can be derived for MICP purpose. Recently, Chen et al. [32] found that the pig urine which is rich in urea can be utilized in MICP treatment instead of analytical-grade urea. In fact, the use of waste materials offers two notable benefits: (i) it desirably reduces the material cost of the treatment and (ii) it contributes to the waste mitigation. Unfortunately, most of the contemporary MICP works still employ expensive analytical-grade chemicals for cementation media, which continue to restrict the technology in proceeding forward. It is worth noting that more alternative sources which are (i) either low-cost or no cost, (ii) available and (iii) accessible must be introduced against synthetic calcium and/or urea sources.

A field-scale experimentation of MICP was performed at an expressway slope site located in the Hokkaido prefecture of Japan. The prime objective of this investigation was to assess the effectiveness of using inexpensive low-grade chemicals for the in-situ stabilization of slope surface. Two test plots were established at the project site and subjected to the surficial treatment. One test plot was treated using low-grade chemicals, wherein fertilizer urea, snow-melting agent and beer-yeast were used to formulate the cementation media. At the same time, analytical-grade chemicals (urea, calcium chloride and nutrient broth) were used to treat the other test plot. By the end of the treatment, both the bio-cemented profiles were evaluated mechanically and chemically. The evaluation program consisted of unconfined compressive strength (UCS) tests, measurements of precipitated calcium carbonate content, scanning electron microscopy (SEM), energy dispersive spectroscopy (EDS) and X-ray diffraction (XRD) analyses. All the results are comprehensively discussed; the discussion also includes the technical and economic soundness of the proposed alternative.

### Significance

While the focus of prevailing MICP research works is confined to the laboratory-scale, this is the first ever milestone project undertaken at field-scale for feasibly promoting MICP technology for the preservation of expressway slope surface. Worth noting that the investigation tends to eliminate one of the long-existed problems of MICP - unaffordable cost of chemical reagents. An alternative direction to make the use of low-grade chemicals (fertilizer urea, snow-melting agent, and beer-yeast) instead of analytical-grade chemicals is successfully shown. A substantial reduction in the cost of required chemicals is evidenced, which aids to establish the

MICP technology more feasible to large-scale applications.

## 2. Materials and methods

### 2.1. Testing site and preparation

The field test reported herein was completed at a representative site located in Tomakomai center-interchange expressway slope (Hokkaido prefecture, Japan). The photograph of the testing site and its location relative to the Tomakomai expressway is shown in Fig. 1. The measurements indicated that the average gradient of the test ground was 20°. In order to make precise comparisons between test plots, the test site was chosen to have relatively uniform soil conditions. The particle size distribution curve of the soil is presented in Fig. 2A. According to the Unified Soil Classification System (USCS) [33], the slope soil can be regarded as well-graded sand (SW), having the mean particle diameter ( $D_{50}$ ) of 1.10 mm. As shown in Fig. 2B, the site consisted of both fine (passing 0.075 mm No. 200 sieve) and course (retaining on 4.75 mm No. 4 sieve) contents, at the percentages of 3 and 13 respectively. The natural water content of the ground was measured to be  $16.0 \pm 0.5\%$ .

Two test plots (TP 1 and TP 2), each with the size of 0.6 m  $\times$  0.6 m, were established at the test location (see Fig. 3A). During the preparation, the vegetation cover was removed, followed by the surfaces of the test plots were gently smoothed without affecting their original arrangement. Acrylic berms of 5.0 cm in height were embedded around the perimeter of each test plot to avoid (i) surrounding erodible soils from being transported onto the test plots, and (ii) the soils from being washed out of the plots. Thermal sensors (HOBO U12 - External Channel Outdoor, ONSET, Japan) were planted onto the surface of each plot to monitor ground temperature throughout the treatment process. Fig. 3B shows the portable plastic covers that were used to protect the plots during the curing time. It was believed that the covers would (i) prevent the excess evaporation of the treatment solutions, (ii) protect the plots from being exposed to direct sun, rainfalls and heavy winds, and (iii) safeguard from wild animals.

### 2.2. Bacterial culture inoculant

Many previous MICP studies tended to apply high performance bacteria that are commercially available, such as *Sporosarcina pasteurii* (formally known as *Bacillus pasteurii*) [5,23,31] and *Lysinibacillus sphaericus* (also known as *Bacillus sphaericus*) [34–36]. The recent articles, however, encourage the use of native bacteria over the exogenous species in the consideration of biological reliability, bio-safety and legal acceptance [37,38].

In this project, *Lysinibacillus xylanilyticus* (previously isolated from Hokkaido, Japan [28]) was the ureolytic bacteria used to induce urea hydrolysis. To grow the organism to the desired population density, ATCC specified ammonium-yeast extract (NH<sub>4</sub>-YE) media was used, which consisted yeast extract (20 g/L), ammonium sulfate (10 g/L) and tris buffer (15.7 g/L). The prepared sterile fresh media was inoculated with the *Lysinibacillus* stock culture (that is preserved in glycerol solution at  $-80$  °C) and subjected to shaking incubation at 25 °C and 160 rpm for approximately 48 h. The population of the bacteria ( $OD_{600}$ ) during the harvesting was  $4.5 \pm 0.3$ , and the urease activity at 25 °C was measured to be 1.9 U/mL. It should be noted that the indophenol spectrophotometry method was used to measure the urease activity of the bacteria culture [39].

The preparation of the bacteria culture was completed in the laboratory, and the culture solution was stored at 4 °C for not more than 24 h prior to its application. On the next day, the culture solution was transported to the testing site in the Erlenmeyer flask with the capacity of 2 L. During the transport, the culture flask was kept covered with ice packs.

### 2.3. Applied treatment scheme and procedure

Two cases were considered in this project. In the Case 1, typical analytical-grade chemicals were used to formulate the cementation media, and which was used to treat the plot TP 1. Inexpensive low-grade chemicals were used in Case 2, and the corresponding

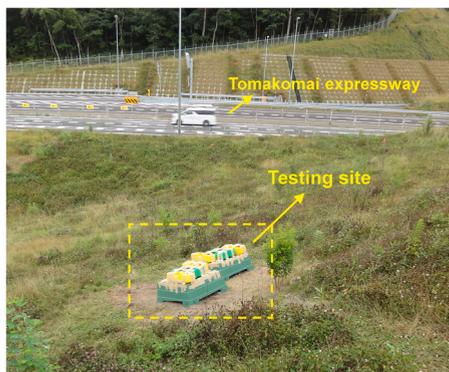


Fig. 1. Overview of the field-testing site - Tomakomai Expressway slope (the gradient of the chosen test-ground was about 20°).

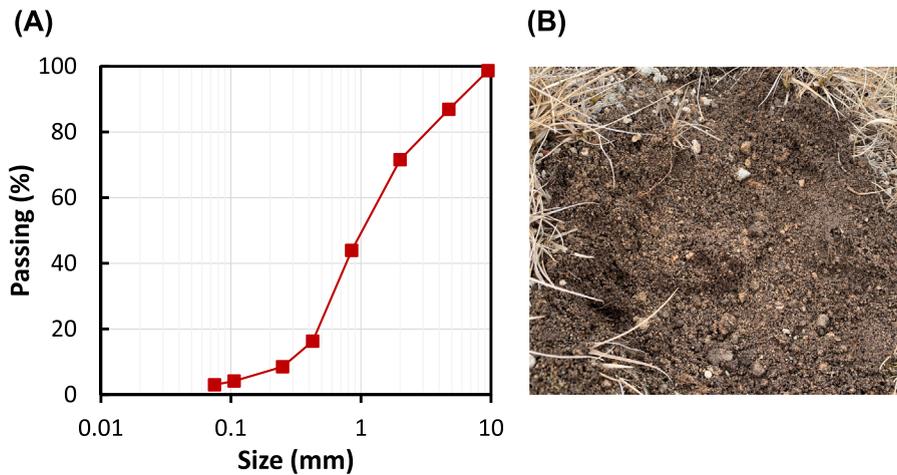


Fig. 2. (A) Grain size distribution curve and (B) physical appearance of the slope soil tested.

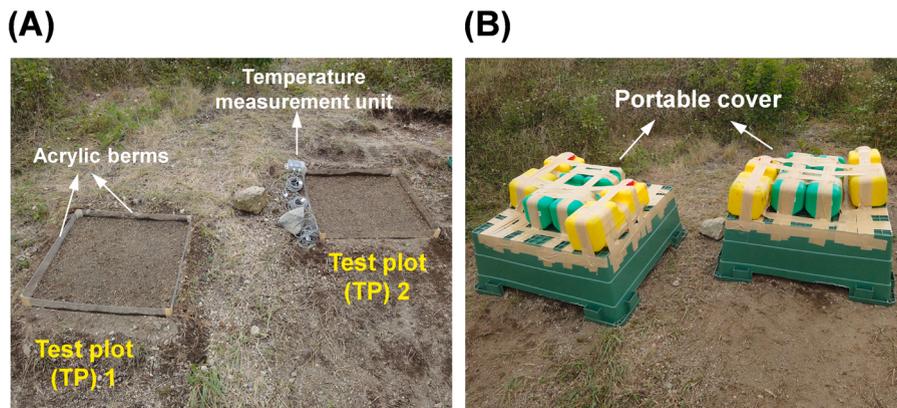


Fig. 3. (A) Established test plots [each with the size of  $0.6 \text{ m} \times 0.6 \text{ m}$ ; treatment using analytical-grade and low-grade chemicals were applied on TP 1 (also referred to as Case 1) and TP 2 (referred to as Case 2), respectively] and (B) the locally-made portable covers used to protect the test plots.

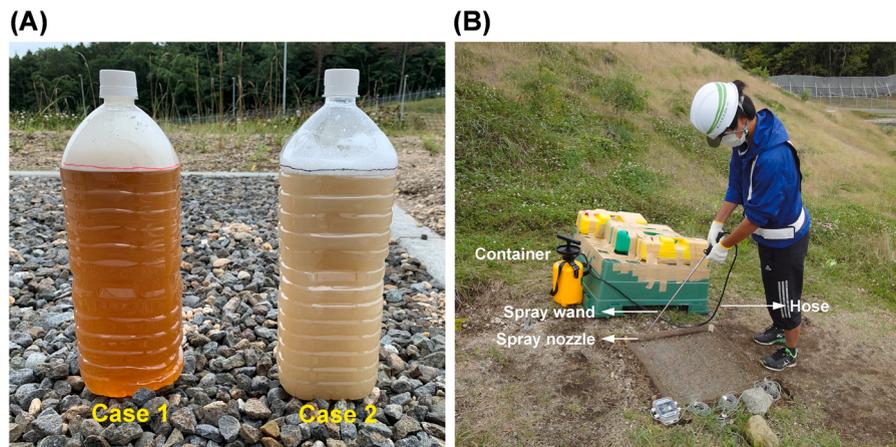
treatment plot was TP 2. The formulation of the cementation solutions used in both the cases are presented in Table 1. Concentrations of cementation media in both the cases were designed to be 1 mol/L. Since the purity of calcium chloride in snow melting agent is about 70% (as per manufacturer specifications), higher quantity of the snow melting agent was required in Case 2 (158.6 g/L) compared to the calcium chloride requirement in Case 1 (111.0 g/L). The solutions were separately prepared at the laboratory (few hours prior to the application) and transported safely to the testing site. The appearance of both the solutions can be perceived in Fig. 4A. It should be noted that the cementation solutions were prepared herein at non-sterile conditions. Many recent works disclosed that the non-sterile preparations would help to minimize the production cost of MICP by about 50% [40,41], indicated the inevitability in real-scale applications.

A two-phase spraying strategy was used to treat the plots: an initial biological spraying followed by multiple cementation spraying. During the biological phase, the bacteria culture solution was sprayed uniformly on the entire surface of the plot at relatively a constant

**Table 1**  
Formulation of cementation solutions used in the two testing cases.

Designation	Test plot	Constituents	Concentration (g/L)
Case 1	TP 1	Calcium chloride	111.00
		Urea	60.00
		Nutrient broth	6.00
Case 2	TP 2	Snow melting agent	158.60
		Fertilizer urea	60.00
		Beer-yeast	6.00

**Note:** Analytical-grade chemicals were used to formulate the cementation media of the Case 1. Low-grade inexpensive chemicals were used to formulate the cementation media of the Case 2.



**Fig. 4.** Photographs showing the (A) physical comparison of cementation medias prepared using analytical-grade chemicals (Case 1) and low-grade chemicals (Case 2) and (B) application of cementation media using sprayer device.

rate of 50 mL/min. It should be mentioned that similar spraying rate was also recommended in previous works [42,43]. One hour after the bacteria spraying, the cementation media prepared in accordance with the proportions provided in Table 1, was sprayed in the similar manner. The photograph taken during the spraying is depicted in Fig. 4B. A commercially available sprayer device (Model 7450, Furupura Co., Ltd., Japan), assembled of polyurethane container (4 L capacity), hose, regulator, spray wand and nozzle, was espoused herein to enable the spraying. Two separate containers were used to store and supply the solutions for both the cases. As suggested in a previous work [14], during the spraying, the spray nozzle was positioned at around 20 cm above the surface, in order to facilitate a gentle spraying without any turbulences.

The treatment scheme is detailed in Table 2. The treatment was designed to have a total of 3 biological spraying and 14 no. of cementation spraying. The cementation media was sprayed for every 24 h, while the biological spraying was performed once in 5 days. The interval of cementation spraying (24 h) was essentially chosen based on the effective formation of calcium carbonate crystals [44]. Since the urease activity of the bacteria begins to decrease typically after 6~8 days [5], the biological solution was introduced to the soil once in 5 days to sustain the activity. Both the volumes of bacteria culture and cementation media per spray were 1.8 L. It should be noted that the treatment programme targeted the cementation at the topmost 30 mm layer, and by considering the results from previous laboratory work [45], the scheme was formulated to achieve improvement level that would meet the goal of the project.

**Table 2**  
Treatment scheme.

Day	Preparation of biological solution	Biological spraying (Phase 1)	<sup>k</sup> Cementation spraying (Phase 2)
1	<sup>a</sup> Inoculation		
2	<sup>a</sup> Shaking incubation		
3	<sup>a</sup> Harvest & Storage		
4	<sup>a</sup> Transport	✓ (Spray No. 1)	✓ (Spray No. 1)
5			✓ (Spray No. 2)
6	<sup>b</sup> Inoculation		✓ (Spray No. 3)
7	<sup>b</sup> Shaking incubation		✓ (Spray No. 4)
8	<sup>b</sup> Harvest & Storage		✓ (Spray No. 5)
9	<sup>b</sup> Transport	✓ (Spray No. 2)	✓ (Spray No. 6)
10			✓ (Spray No. 7)
11	<sup>c</sup> Inoculation		✓ (Spray No. 8)
<sup>z</sup> 12	<sup>c</sup> Shaking incubation		
13	<sup>c</sup> Harvest & Storage		✓ (Spray No. 9)
14	<sup>c</sup> Transport	✓ (Spray No. 3)	✓ (Spray No. 10)
15			✓ (Spray No. 11)
<sup>z</sup> 16			
17			✓ (Spray No. 12)
<sup>z</sup> 18			
19			✓ (Spray No. 13)
20			✓ (Spray No. 14)

**Note:** <sup>a</sup>, <sup>b</sup>, <sup>c</sup> Preparation of biological solution for the 1st, 2nd and 3rd number of spraying, respectively; <sup>z</sup> Cementation spraying was not performed, postponed due to the unsuitable weather conditions at testing site; <sup>k</sup> Cementation media made of analytical-grade chemicals was sprayed in Case 1 (TP 1), and Cementation media made of low-grade chemicals was sprayed in Case 2 (TP 2) - refer Table 1.

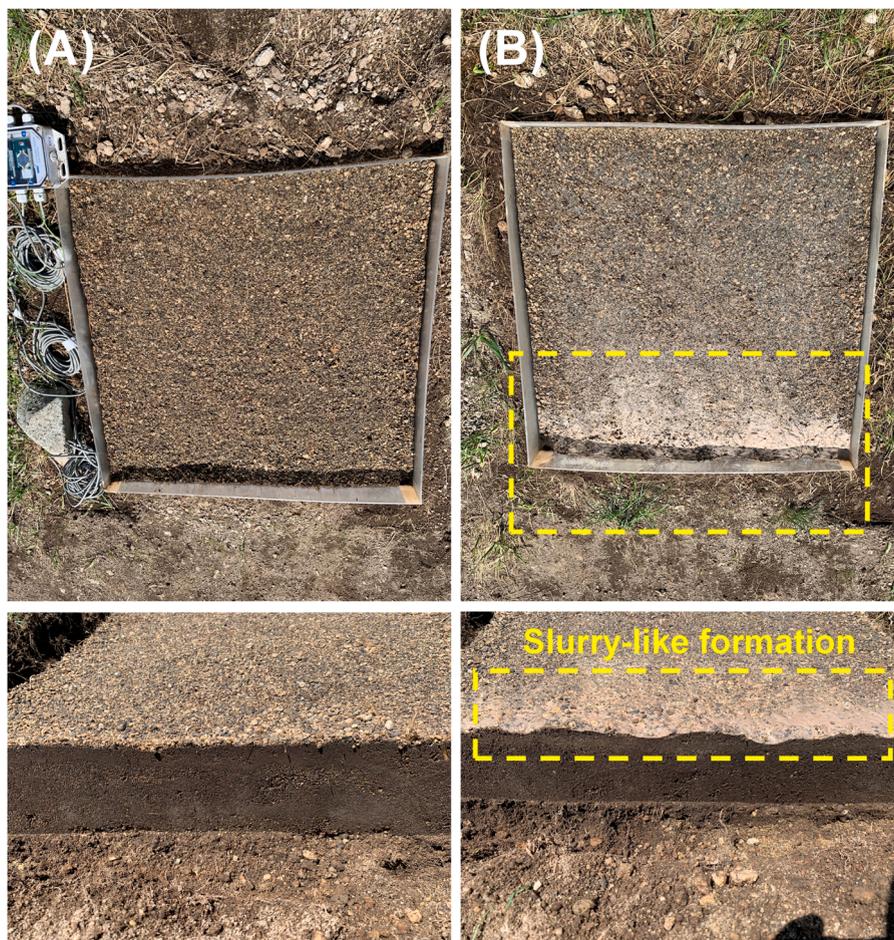
## 2.4. Evaluation program

After completing the treatment, the test plots were allowed to cure for 48 h, followed by the in-situ strength measurements. Needle penetration is one of the prevalent in-situ methods for determining unconfined compressive strength (UCS) of soft rocks and cemented soil specimens [46]. The treated slope surfaces were estranged into grids each of 10 cm × 10 cm, and the testing was performed at nodes (in total, 49 tests per Case). During the testing, the needle equipped in the device (SH-70, Maruto Testing Machine Company, Tokyo) was set perpendicular to the surface and subjected to the penetration in accordance with the standards recommended by the Japanese Geotechnical Society [47]. Readings such as penetration resistance (measured in N) and penetration depth (measured in mm) were obtained, and the UCS was determined using the developed regression relationship.

The cemented slope body was then exposed by removing the surrounded loose soil to enable visual examination. From the cemented slopes, number of samples were carefully cored, collected, and transported to the laboratory, and these were used for determining precipitated CaCO<sub>3</sub> content, SEM, EDS and XRD analysis. The precipitated CaCO<sub>3</sub> content was measured by reacting the oven-dried (60 °C for 48 h) sample with concentrated HCl in a closed chamber under constant volume and temperature conditions [48]. When the CaCO<sub>3</sub> is exposed to HCl, the dissolution occurs, resulting in the formation of CO<sub>2</sub> gas. By measuring the internal pressure variations during the reaction, mass of the existed CaCO<sub>3</sub> was estimated, hence the precipitated CaCO<sub>3</sub> content was determined using the Eq. (3).

$$\text{CaCO}_3 \text{ content (\%)} = \frac{\text{Weight of CaCO}_3}{\text{Weight of the oven dried specimen} - \text{Weight of CaCO}_3} \quad (3)$$

The SEM-EDS was performed using energy dispersive X-ray fluorescence spectrometer equipped with SEM (JSM-IT200(JEOL), Tokyo, Japan) at an accelerating voltage of 15 kV. The oven-dried (60 °C for 48 h) samples were coated using carbon coater (EC-32010CC(JEOL), Tokyo, Japan) prior to the analysis. XRD analysis was carried out to the powdered samples using the diffractometer (MultiFlex-Rigaku, Tokyo, Japan) equipped with CuK $\alpha$  X-ray source (operating at 40 kV and 40 mA). The testing was performed at a



**Fig. 5.** The physical appearance of the (A) test plot (TP 1) treated using analytical-grade chemicals and (B) test plot (TP 2) treated using low-grade chemicals after the curing.

scan rate of 6.5°/min and at angles from 5° to 70° (20).

### 3. Results

#### 3.1. Observations during the treatment process

At the early stage of the treatment, i.e., for the first few batches of spraying, the sprayed solution simply percolated into the soil. However, with the increase in number of sprayings, the percolation tended to decelerate, and the ponding over the surface became conspicuous in both the test plots. Flow lines were also sometimes evidenced over surface of the slope body. One possible reason would be the formation of calcium carbonate crystals in pore spaces, and the narrowing of the pores resulted in a decrease in percolation rate. The strong odor of ammonia was experienced every time at the removal of plot covers, which suggested that the supplied urea was being hydrolyzed.

It must be noted that a whitish slurry-like formation was observed along the surface of TP 2, the plot treated using low-grade chemicals. This formation, however, was not visible anywhere of the plot treated using analytical-grade chemicals (TP 1). The clear comparison between the plots after 20 days of treatment is presented in Fig. 5. Although the slurry-like material tended to form all over the surface of TP 2, its intensity was seemingly higher at the bottom compared to that of top. One thing is clear that there were depositions of materials in addition to the target calcium carbonate at TP 2, and higher intensity observed at the downslope might probably be due to the influence of flow lines that transported the formed material towards downwards during the sprayings.

The temperature readings obtained from the thermal sensors during the treatment process are compared in Fig. 6. The observation suggests that despite day-night fluctuations, the surface temperature of both test plots, TP 1 and TP 2, are comparable throughout the treatment period. The experimentation was essentially performed in the month of August, which falls in the summer season of Hokkaido, Japan. As expected, the ground temperature was relatively high during the daytime (up to nearly 30 °C), and that dropped by an average of 10 °C in night times (to the lowest line of nearly 15 °C). It is well-known that the temperature is one of the most important factors governing the growth and metabolism of the bacteria [2,49]. *Lysinibacillus xylanilyticus* was earlier found to be effectively performing the urea hydrolysis at the temperature ranging between 15 °C and 30 °C [45]. Since the temperature fluctuation observed herein was well in line with the performance range, the urea hydrolysis could be assured.

#### 3.2. Observations during excavation of the cemented slope body

After 20 days in which 25.2 L of cementation media and 5.4 L of bacteria culture were sprayed through in number of batches, the excavation was performed carefully around acrylic berms of the test plots. As seen in Fig. 5, the cemented slope bodies were clearly visible in both the testing cases. Following the gentle removal of loose and weakly attached soil particles using soft brush, TP 1 which was treated using analytical-grade media showed the cemented depth of about 15 cm (refer to Figs. 7A and 7B), while the cementation depth of TP 2 (treated using low-grade media) was found to be about 5–10 cm (refer to Figs. 7C and 7D). Below which, no considerable aggregation had taken place, indicating that the cementation was restricted at deeper zones of the plots. Moreover, the wedged shape was observed at the bottom of the cemented slope, which could be attributed to the induced hydrological flow. Similar wedge shape in a regular pattern parallel to the induced flow direction was also observed by van Paassen et al. [50] in their large-scale bio-cemented ground profile.

#### 3.3. Surface strength

Fig. 8A and B show the UCS results (measured in-situ) of the surfaces treated using analytical-grade and low-grade chemicals,

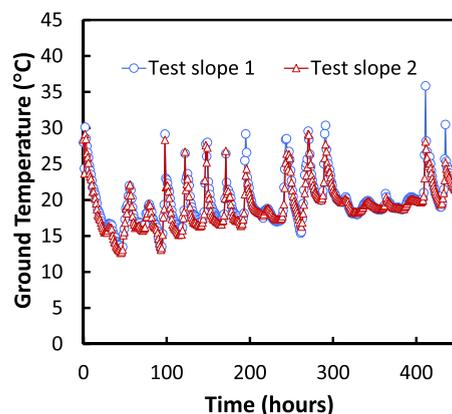
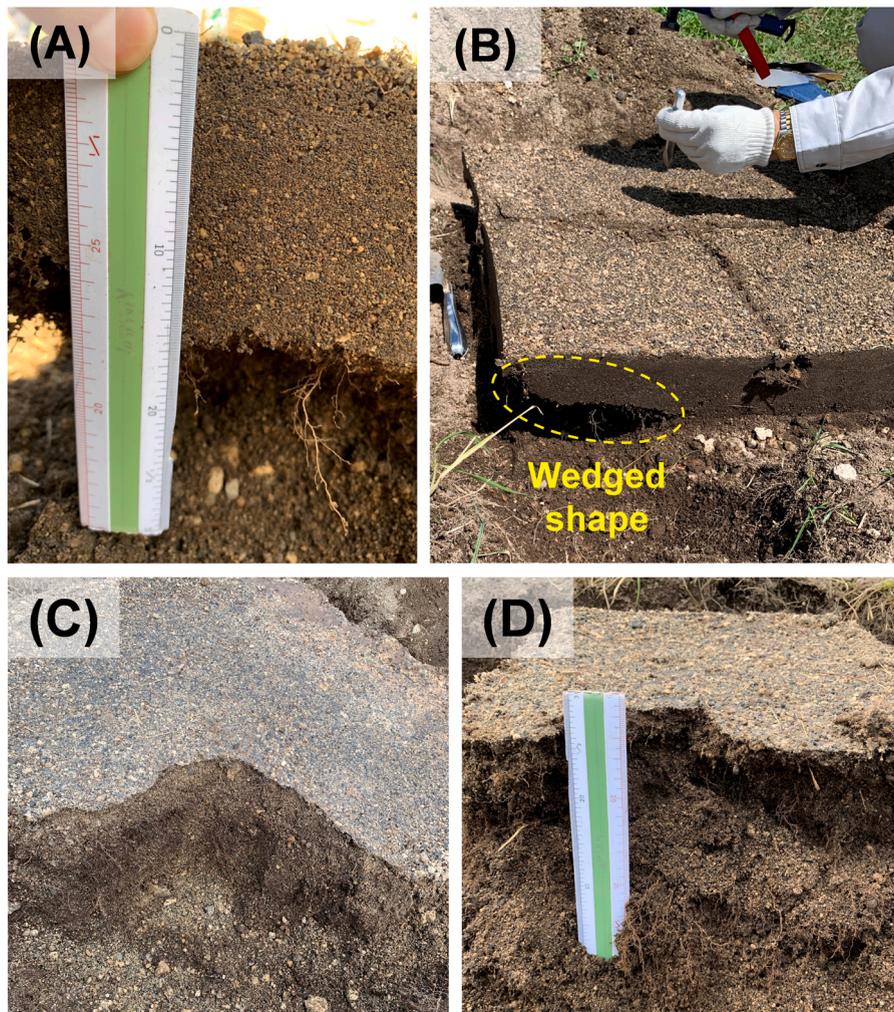


Fig. 6. The temperature readings measured at the surface of both the test plots using thermal sensors planted (readings were recorded automatically in every 4-hour interval).



**Fig. 7.** The observation of (A) depth of the cemented surface layer and (B) in-situ testing during the excavation of test plot treated using analytical-grade chemicals (Case 1); (C) excavation and (D) depth of cemented surface treated using low-grade chemicals (Case 2).

respectively – the results are presented in the form of contour distributions. The results reveal that the UCS results attained was varying between 0.14 MPa and 1.62 MPa (the UCS value of loose soil might be considered closer to zero). Relatively higher UCS was achieved in the slope treated using analytical-grade chemicals compared to that of low-grade chemicals. The highest UCS measured in analytical-grade case was 1.62 MPa, while the highest was 1.02 MPa for the case of low-grade chemicals. Despite identical treatment provided, the resulting UCS of surface treated using analytical-grade chemicals and low-grade chemicals are varying considerably, and the low-grade chemicals are likely to result in less effective cementation.

The UCS values on the surfaces were not as homogeneously distributed as preferred. It is worth noting that the heterogeneity was more pronounced in the case of low-grade chemicals, i.e., higher strengthening has taken place close to the acrylic berms and in the corners compared to that at or around the center of the surface.

### 3.4. Precipitated calcium carbonate

Measured contents of calcium carbonate along the vertical cross section through centerline of the slopes are presented in Fig. 9. The  $\text{CaCO}_3$  content of the samples collected from the cross sections varied from 0.56% to 4.23% of the total dry weight, and relatively a similar distribution pattern along vertical section was observed in both the cases. The  $\text{CaCO}_3$  content along the flow lines, i.e., parallel to the surface, was reasonably homogeneous, while the  $\text{CaCO}_3$  content in varied considerably with the depth. The results show a notable tendency that the surface contained high content of  $\text{CaCO}_3$ , and that decreased with increasing depth (which was also observed on smaller scale tests performed earlier by other researchers [51,52]). For the case of analytical-grade chemicals (TP 1), the highest amount of  $\text{CaCO}_3$  was found (up to 4.23% of dry weight) in the upper surface towards right corner of the slope body (Fig. 9A). Similarly, 3.7%  $\text{CaCO}_3$  was found in the upper middle of slope body for the case of low-grade chemicals (TP 2) (Fig. 9B). Higher

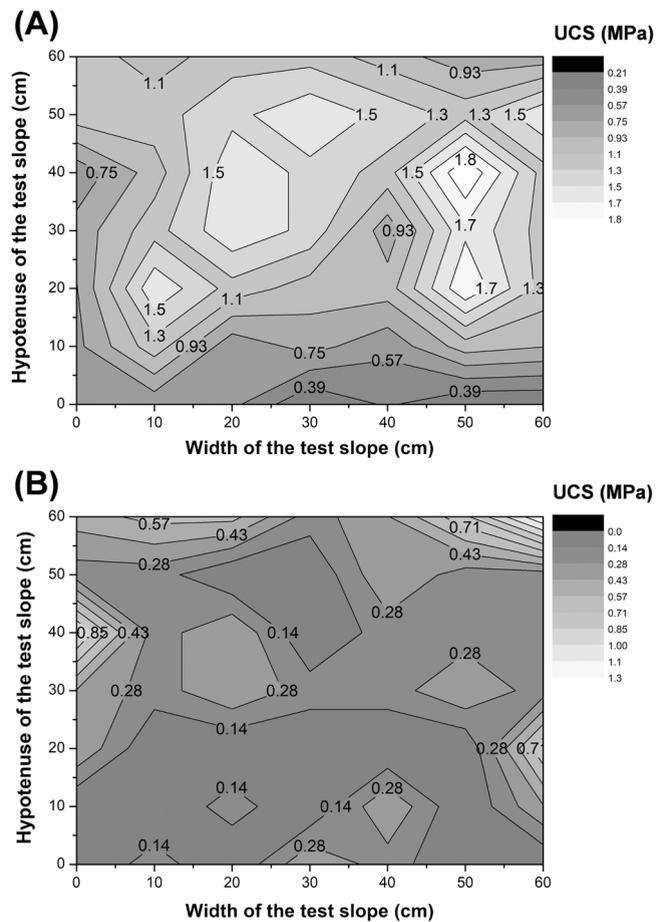


Fig. 8. Contours of UCS values measured in the surface for the test slope treated using (A) analytical-grade chemicals (Case 1) and (B) low-grade chemicals (Case 2).

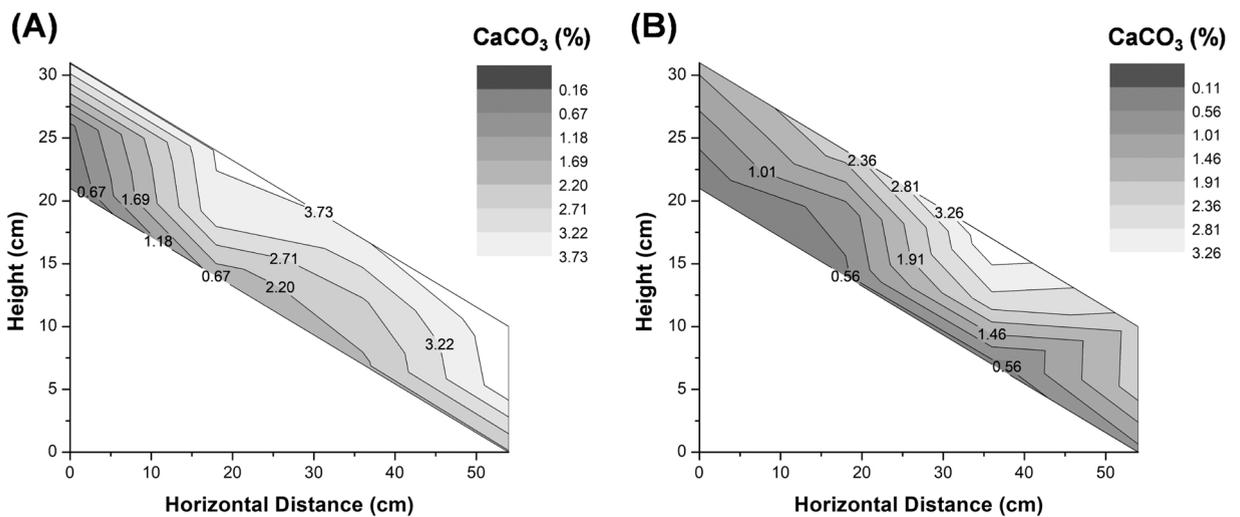


Fig. 9. Comparison of vertical distributions of calcium carbonate between (A) section of TP 1 that was treated using analytical-grade chemicals and (B) section of TP 2 that was treated using low-grade chemicals.

precipitation of  $\text{CaCO}_3$  at/near the surface (the zone immediately exposed to the spraying, seemingly with the thickness of about 5 cm) was possibly attributed to several factors including soil gradation, higher retention of bacteria cells, exposure to reactants with higher concentrations and local clogging [4,14,53].

Despite the case, the precipitated calcium carbonate content appears to be in line with the achieved strength of the surface. In another words, there was a significant improvement in strength relative to untreated sand at higher carbonate contents, while low carbonate contents did not significantly improve the strength. For instance, in the case of low-grade chemicals, the highest surface strength was 1.02 MPa, which was measured at the closer location as the maximum amount of  $\text{CaCO}_3$ . It is also professed that a minimum  $\text{CaCO}_3$  content was required for a measurable surface strength by needle-penetrometer; although not precisely figured out, the threshold value seems to be closer to 1% by weight. It should be noted that the value was in consistent with several previous reports [6,54].

## 4. Discussion

### 4.1. Feasibility of low-grade chemicals – technical perspective

Analytical-grade calcium chloride, urea and nutrient broth are the reagents being used in typical cementation media of bio-cement treatment [5,38,55]. The use of snow melting agent, fertilizer urea, and beer-yeast has been proposed instead of analytical-grade chemicals, and the field-scale test completed herein showed significant enhancement in the surface strength, proving the technical feasibility of low-grade reagents for the stabilization of slope surface. The presence of stiff crust with the thickness varying between 5 and 10 cm was confirmed during the excavation of the test plot, TP 2 (refer Figs. 7C and 7D). The results of needle penetration tests showed the UCS of the crust ranging between 0.14 and 1.02 MPa (with the mean of about 0.39 MPa). The percentage of  $\text{CaCO}_3$  measured at the vertical section varied between 0.56% and 3.7%, with the considerable decrease from 5 cm to 10 cm. The mean carbonate content found in the top 5 cm layer was about two-fold higher compared to that measured between the depth 5 – 10 cm. It is worthwhile mentioning that although the low-grade chemicals contributed to the development of surface strength through calcium carbonate cementation, the enhancement was considerably lower compared to the that achieved by analytical-grade chemicals. Particularly, two major weaknesses were noticeable: (i) the crust was formed with relatively lower thickness and (ii) measured UCS values were lower compared to those achieved in analytical-grade chemicals.

Purity of the low-grade reagents might be one major reason for the observed weaknesses. Snow-melting agent is basically the calcium chloride salt used to melt the ice deposited on roads/ pavements in seasonal winters. As per the manufacturer's specification, the purity of the agent is about 74%. Similarly, the purity of nitrogen is about 46% in fertilizer urea. As revealed from the results of X-Ray Fluorescence (XRF) spectrometry (Fig. 10), the chemicals consisted of a range of other ions and/or minerals other than their core

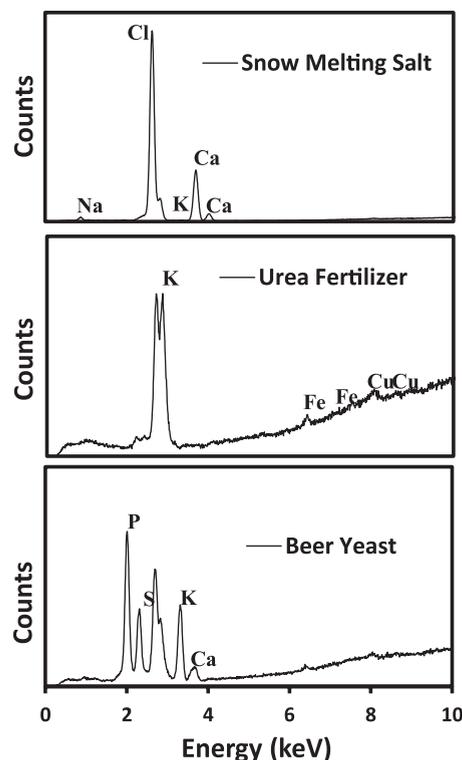


Fig. 10. The XRF energy spectrum of the low-grade chemicals used: (A) snow melting agent, (B) fertilizer urea and (C) beer-yeast.

components. For instance, snow melting agent consisted of calcium (34.87%), chloride (61.89%), sodium (1.70%) and potassium (1.53%) (Fig. 10A), and urea fertilizer was found to consist of copper, iron and potassium (45.66%, 39.86% and 14.48%, respectively) (Fig. 10B). It must be mentioned, however, the snow-melting agent and fertilizer urea could dissolve completely; a clear media was witnessed following their dissolution, which indicated the absence of any insoluble components.

In a previous study, it was reported that the highest UCS of 649.7 kPa was achieved when the calcium chloride was replaced by eggshell material, and which was about 29% higher than that treated using pure-grade calcium chloride [56]. The reason to have higher UCS for the samples treated with eggshell-derived calcium was because the calcium contents in the eggshell samples are relatively higher [30,56]. In another instance, the use of industrial-grade calcium chloride and urea resulted a lower surface UCS (1.45 MPa) compared to that treated using analytical-grade chemicals (1.79 MPa) [57]. One major reason reported by authors was that the industrial-grade calcium chloride consisted trace amounts of sodium and magnesium which were absent in the analytical-grade reagents. The presence of magnesium was earlier found to influence on  $\text{CaCO}_3$  deposition rate and polymorphs [58]. Since the urea fertilizer consisted of considerable amount of copper and iron that are prone to the formation of complex and insoluble compounds [59], the deposition rate of carbonates and polymorphs might be influenced.

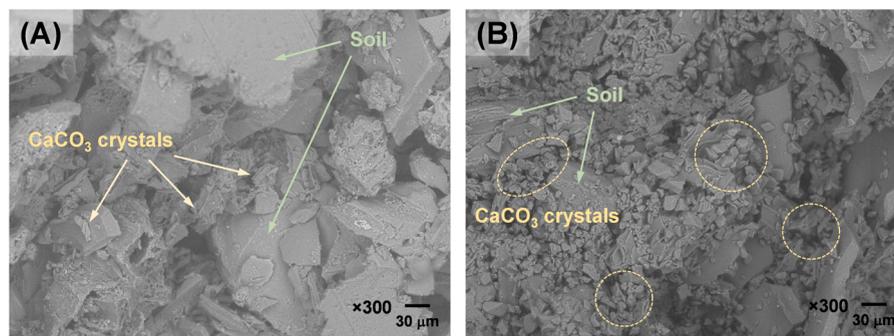
Beer yeast is a popular food industry product that is used to break down the sugar [60]. The XRF results reveals that the beer yeast consisted of potassium (38.59%), phosphorus (37.15%), sulfur (16.77%), calcium (6.82%), iron (0.45%) and copper (0.23%) (Fig. 10C). From the biological point of view, 100 g beer yeast can be reported to entail 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. It must be noted that once the beer yeast is added to the cementation solution, a milky-like turbidity was perceptible even after adequate mixing time (refer Fig. 4A – Case 2), indicated that the beer yeast is partly consisted of fine insoluble organic components. The whitish slurry-like formation observed on the surface of TP 2 (refer Fig. 5B) might be attributed to the deposition of these insoluble fines at or near the surface. Although this deposition appeared to densify the surface zone by pore filling, it was more likely to narrow down the micropores hence to restrict the transport of the bacteria cells. This led the low-grade cementation media to result in lower cementation depth and UCS values compared to those of analytical-grade media.

Some organic molecules can readily be broken down into small peptides and amino acids which the bacteria can use immediately as energy source. Tryptic soy broth medium and beef-extract medium are some previously proposed MICP growth media that facilitate the bacteria cells cultured in them to grow easier and faster [27]. Beer yeast media used herein appears to consist of the both types of organic components, (i) the molecules that are soluble and can readily be consumed by bacteria and (ii) the molecules in the form of long chain peptides which could be neither soluble nor used immediately by bacterial cells.

The use of high-purity chemicals is essential for any fundamental laboratory-scale researches [61]; however, their high cost makes the applications less feasible. It should be admitted that the purity is not the primary concern for the engineering applications; the chemical is well-thought-out to be effective as long as the crystallization process is not adversely influenced by the impurities. One best example is that the crude enzymes (which are low in purity) are increasingly used nowadays for bio-cement treatment, and the outcomes were still comparable with those of purified enzymes [62,63]. Similarly, the presence of metal ions (e.g., aluminum), phosphorus and sulfur in trace amounts was reported to favor the microbial growth and performance [57]. Nevertheless, the impact of low-purity chemical is reliant on the impurity that exists. For instance, the presence of high content of carbohydrate was reported to delay the crystallization of calcium carbonate [26]. Therefore, it is very clear that the composition analysis prior to the use of any low-grade chemicals is very essential.

#### 4.2. Micro-scale characteristics

To observe the microscopic structure of the treated soils, SEM and EDS analysis were carried out to the samples obtained from treated test plots. Figs. 11A and B illustrate the micrographs of the soils treated using analytical-grade and low-grade cementation medias, respectively, at the same magnification scale. In both the cases, abundant formation of irregular cubic and rhombohedral crystals with sharp edges could be seen. However, there was a prominent difference in the topographical feature of the crystals formed; the crystals formed in the case of analytical-grade chemicals were quite larger, and which ranged between 20 and 25  $\mu\text{m}$ , while smaller



**Fig. 11.** Micrographs of the surface treated using (A) analytical-grade chemicals and (B) low-grade chemicals (both the micrographs were captured under same magnification and scale).

crystals (size ranging between 8 and 12  $\mu\text{m}$ ) were evidenced in the case of low-grade chemicals. The result of EDS analysis depicted in Fig. 12 and Fig. 13, illustrate the distribution of calcium and silica among the treated soil matrixes. The spectrum highlights suggest that the calcium, as the proxy of calcium carbonate, was greatly found at particle-particle contacts, and a minor distribution was also seen on the surface of the soil particles. It should be noted that there were no any significant differences between the distribution patterns of both the cases. The XRD spectra compared in Fig. 14 demonstrate that irrespective of analytical-grade or low-grade chemicals, the resulted polymorph of calcium carbonate in the MICP treatment was calcite. The untreated soil majorly consisted of quartz with a minor quantity of potassium, periclase and magnesioferrite, and the calcite was the only new addition to the soil after the treatment. Although the calcite precipitates were often reported as the dominant polymorph of microbial-induced carbonate, other polymorphs were also sometimes reported in trace amounts. For instance, 94.9% calcite and 5.1% vaterite were found together in the soil treated using industrial-grade chemicals [57]. Vaterite and aragonites were the dominant precipitates in the MICP treated soil treated involving pig urine as urea source [32].

Considering the crystallization of calcium carbonate, two major observations can be reported: (i) the amount of precipitated calcite was lower (refer Fig. 9) and (ii) the size of the crystals formed was smaller (refer Fig. 11) in the treatment by low-grade cementation media than those by analytical-grade media. It is manifest that some of the impurities presenting in the low-grade chemicals were more likely to adversely influence the crystallization rate/ process of calcium carbonate. Since there are no any minerals formed other than calcite, it can be said that the influence of the salts existed in the low-grade chemicals (particularly snow-melting agent and fertilizer urea) might not be significant. This leads to another conclusion that the organic matters existed in beer yeast might be a possible reason for the hindering in crystallization process.

It is a known fact that the presence of organic matter determines the binding process in the soil, influencing the strength gain in the treated soil [64,65]. Among the types of organic compounds, humic acids are documented to be strong retarding agent making the hardening effect slower [66]. It must be noted that these organic matters, by their nature, tend to make a coating layer over the additive grains [67], finally impeding the cementation between adjacent soil grains. Furthermore, the negatively charged organic matters exhaust the supplied calcium ions that are positively charged, and restrict the availability of free  $\text{Ca}^{2+}$  ions in the solution against the favorable formation of calcium carbonate crystals [17]. Similar hindering effect of organic matters and the formation of fine crystals were also often seen in both hydration and pozzolanic reactions-based treatments [68,69]. Apart from the impurities, factors such as composition of injection interval, concentration of cementation media, nutrients, pH and salinity also might have minor influence in the precipitation process of calcium carbonate [57]. For instance, Wang et al. [44] found that the crystals formed during the short injection interval were small (5–10  $\mu\text{m}$ ), but the number of crystals was high. Similarly, the size of  $\text{CaCO}_3$  crystals formed was found to be smaller when soil samples were treated with lower concentration of cementation solution [70]. Nevertheless, large crystals that precipitated at grain contacts might be effective and can attribute to the high strength of MICP treated-soils. It must be mentioned that the organic matters, by their nature, partially exhaust the supplied  $\text{Ca}^{2+}$  ions, which leads to the hindering of the crystallization process [17,66]. Similar bulk formation fine calcite crystals (cubic and rhombohedral) was also witnessed by many previous researchers in organic matter-rich environment [65,71]. Apart from the impurities, factors such as composition of nutrients, pH and salinity also might have minor influence in the precipitation process of calcium carbonate [57].

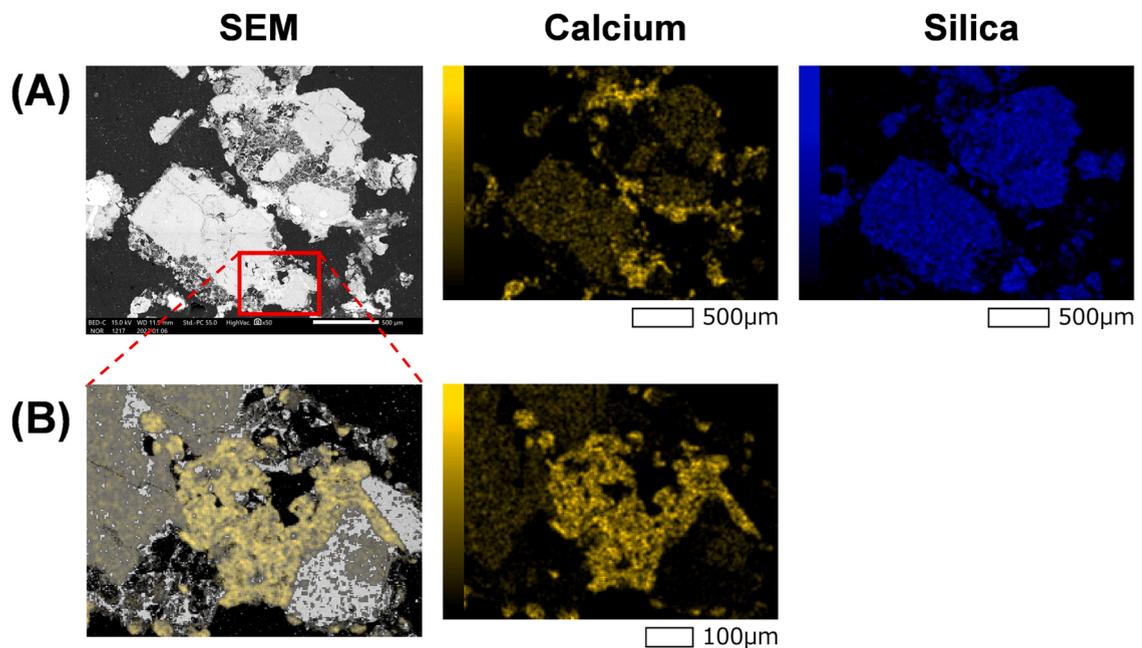


Fig. 12. The SEM-EDS analysis (for calcium and silica) for the soil treated using analytical-grade chemicals under two magnification scales (A and B).

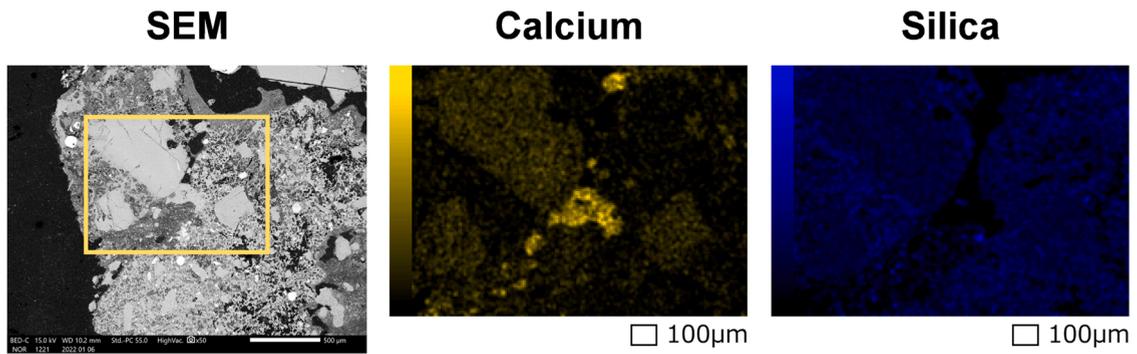


Fig. 13. The SEM-EDS analysis (for calcium and silica) for the soil treated using low-grade chemicals.

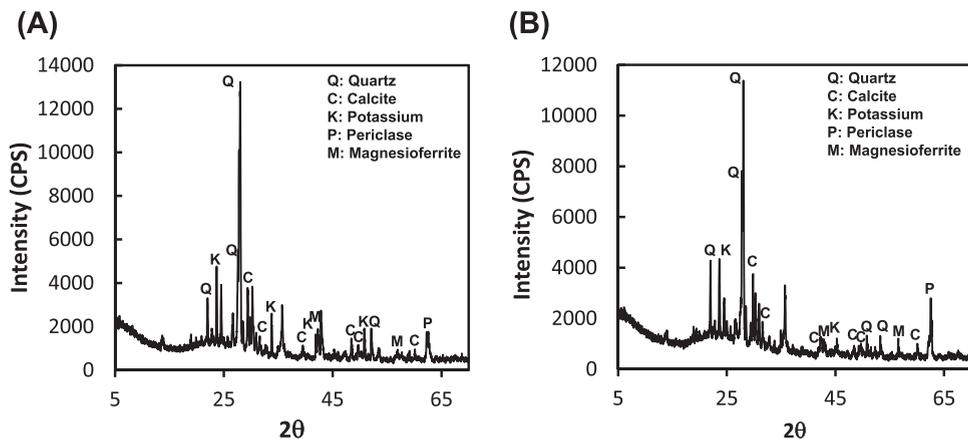


Fig. 14. The XRD analysis for the test cases of (A) analytical-grade chemicals (Case 1) and (B) low-grade chemicals (Case 2).

#### 4.3. Feasibility of low-grade chemicals - economical perspective

It is well-known that the viability of any techniques does not rely only on their technical side, but also depend on the economic aspects. Since the MICP process requires several chemical reagents in bulk quantities, the technique is often regarded to be too-expensive for field-scale applications [22,26,27]. It was also indicated that the chemical ingredients take over about 60% of the total operational cost [24]. To demonstrate the economic feasibility of the proposal, a comprehensive cost analysis was performed and compared with the typical MICP media in Table 3. The snow-melting agent was proposed and used instead of calcium chloride, and the costs of the snow-melting agent and calcium chloride are respectively 71.2 JPY/kg and 3640 JPY/kg. Although the quantity of snow-melting agent required to compromise the  $\text{Ca}^{2+}$  requirement (158.6 g/L) was quite higher than that of calcium chloride (111 g/L), the cost is still desirably lower (over 50-fold). The market cost of analytical-grade urea (5600 JPY/kg) shows about 50-fold higher compared to that of fertilizer urea (114 JPY/kg). It must be noted that microbiological ingredients are likely to be the most expensive ones among the components in cementation media, i.e., beer-yeast and nutrient broth cover about 90% cost of their media. A similar cost reduction (of about 82.8–99.8%) was also reported previously when the food-grade yeast extract was proposed in the place of conventional microbiological media [23].

As per the estimations compared (refer to Table 3), 1 L of low-grade cementation media is costing around 32.3 JPY (0.25 US\$), while about 1214 JPY (9.2 US\$) is costed for 1 L typical analytical-grade cementation media. The use of low-grade chemicals tended to decrease the material cost significantly by about 37-fold, and this 97% cost-cut clearly discloses that the proposal would be economically feasible and affordable for field-scale applications. It is also noticeable that the cost of the media can be further reduced, if the beer-yeast would be replaced by potential waste materials, for example corn steep liquor [26]. However, the following two things must be carefully considered prior to the use of waste materials: (i) availability of nutrients that can support the growth and activity of the bacteria and (ii) any adverse effects on cementing properties.

#### 4.4. Limitations and recommendations for future work

From the geotechnical perspective, adequate strengthening is essential, and which can be achieved by forming adequate  $\text{CaCO}_3$  bonds between the soil grains. The chosen inexpensive chemicals should be able to produce  $\text{CaCO}_3$  crystals with high efficacy. The

**Table 3**

The cost comparison between the cementation solutions of proposed low-cost MICP and typical MICP methods.

Proposed low-cost MICP method (low-grade chemicals)				Typical MICP method (analytical-grade chemicals)			
Required substances	<sup>a</sup> Unit price (JPY/kg)	<sup>b</sup> Content (g/L)	Cost (JPY/L)	Required substances	<sup>a</sup> Unit price (JPY/kg)	<sup>b</sup> Content (g/L)	Cost (JPY/L)
Snow melting agent	71.20	158.60	11.29	Calcium chloride	3640.00	111.00	404.04
Fertilizer urea	114.00	60.00	6.84	Urea	5600.00	60.00	336.00
Beer-yeast	2362.50	6.00	14.18	Nutrient broth	79,000.00	6.00	474.00
Material cost for producing 1 L cementation media			32.31 JPY (US\$ 0.25)	Material cost for producing 1 L cementation media			1214.04 JPY (US\$ 9.20)

<sup>a</sup> The presented costs are based on the market price of Japan;

<sup>b</sup> The quantities used to produce 1 L cementation media

outcomes of this study show that different chemicals result in different crystal precipitation patterns (at microscale), leading to varied thickness of cemented layers and varied efficacy in obtaining the strength values. This potentially has implications in terms of how the materials can be chosen and how the treatment can be applied in geotechnical practices.

One recent investigation has clearly demonstrated that the cementation of 2 cm upper surface layer (1.7% CaCO<sub>3</sub>) was adequate to prevent scour erosion under water jet and to minimize runoff erosion [54]. The use of low-grade chemicals was shown to induce the formation of surface stiff layer to the depth of several centimeters (> 5 cm), proving the applicability of MICP at low-budget for preserving the slope surface against erosion. 97% cost-cut is indeed a great achievement in MICP pathway. However, the UCS of the slope achieved by low-grade chemicals (1.02 MPa) was relatively lower compared to that of analytical-grade one (1.62 MPa), and which still remains a concern to be looked into future works. One limitation observed was related to the purity of the low-grade chemicals. The beer yeast that was added to stimulate the growth of bacteria cells was found to have insoluble fines, which resulted in localized clogging at or near the surface. Since the low-grade chemicals are poor in quality, the chemical/ bio-chemical analysis prior to their use might be essential. In the meantime, the study strongly encourages the future MICP-related works to utilize low-grade chemicals that are more promising for field applications in terms of the cost. More low-cost sources for soluble calcium, urea and nutrients must be identified and proposed. It is worthwhile mentioning that the use of (i) materials that are abundant in nature and (ii) waste materials might make the technology more sustainable [72,73].

The efficiency of crystallization might also be influenced by several other factors such as temperature, spraying rate, spraying interval, treatment duration, concentration of cementation media and particle size distribution [49,74–76]. However, the influence of those factors was not considered under the scope of this work, and which are left for future works. There is a significant possibility that the less homogeneity observed in cemented surface layer was probably due to two reasons: (i) high concentration of cementation media and (ii) clogging by impurities. Apparently, the surface closest to the spraying point was exposed to significantly more reactants and more conversion than those at depth [77], and the spraying of concentrated cementation media appears to promptly narrow the pore throats of surface soil. Spraying rate is another serious concern. For example, faster spray rates would passage the cementation media onto the column, allowing less time for reaction along the path. On the other hand, lower spray rates might leave the media in the fluid longer, resulting in deeper cementation distances. On top of the significant increase in surface strength, one merit of was attributed to remaining relative high permeability and leaving enough possibilities for further treatment or groundwater flow [78]. Therefore, a careful attention must be paid on treatment protocols to control the spatial distribution of calcium carbonate. The feasibility of ecological restoration, i.e., the vegetation cover over the MICP treated slope surface, remains as another important and interesting context and is left for future work.

## 5. Conclusions

A field-scale MICP trial was performed with the purpose of assessing the feasibility and effectiveness of using inexpensive low-grade chemicals for the in-situ stabilization of slope surface. The following key conclusions can be drawn:

- The low-grade chemicals (snow-melting agent, fertilizer urea and beer yeast) showed a potential combination to be used as cementation media. MICP treatment using the low-grade media resulted the formation of stiff surface layer to the depth of 5 – 10 cm with the surface UCS and precipitated CaCO<sub>3</sub> up to 1.02 MPa and 3.7%, respectively, which proved the feasibility of the proposal as replacement to analytical-grade cementation media.
- An attention must be paid on the purity of the low-grade chemicals to be used for cementation media. Although the observations for low-grade media and analytical-grade media are comparable, the thickness of the formed surface layer (5 – 10 cm) and UCS (~1.02 MPa) were marginally lower compared to the case of analytical-grade media (~15 cm and ~1.62 MPa, respectively), and which would be due to the presence of insoluble fines in beer-yeast that tended to result in localized clogging at the surface. For a better application, pre-treatment to the beer-yeast may be recommended.
- Microscale evaluation disclosed that the polymorph of the calcium carbonate was calcite in both the low-grade and analytical-grade cases. The calcite crystallization could form bridges between the soil particles, contributed to the cementation. However, the crystals were found to be smaller (5–10 μm), but formed in bulk, in the case of low-grade chemicals.

- Cost analysis unveiled that the material cost of the cementation media was reduced by around 37-folds (by ~97%) when the low-grade chemicals are used in the place of typical synthetic media. This eliminated one long-existed problem of MICP - unaffordable cost of chemical reagents. With these significant findings, future MICP studies are encouraged to use low-grade cementation media for economical field-scale implications.

## Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

## Data Availability

Data will be made available on request.

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