Feasibility study for slope soil stabilization by microbial induced carbonate precipitation (MICP) using indigenous bacteria isolated from cold subarctic region

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Abstract

Microbial induced carbonate precipitation (MICP) is relatively an innovative soil improvement technique, learnt from the bio-mediated geochemical reactions that naturally occur in the earth surface. During the MICP, $CaCO_3$ is metabolically precipitated in soil pores, cement the particle contacts and improves the strength and stiffness of soil. Environment temperature is one of the most key factors that determines the efficiency MICP. The purpose of this study is to investigate the feasibility of stabilizing the slope soil of cold subarctic region (Hokkaido, Japan). The implication of MICP in cold subarctic zones remains as a major challenge, as the enzymatic performance of the bacteria typically declines during lower temperatures hence insufficient formation of $CaCO_3$ in soil matrix. Therefore, as a potential approach, this study attempted to investigate the feasibility of using the bacteria which have been adapted to native cold climatic conditions. The objectives of this paper are evaluating (i) the effect of temperature in bacterial response, and (ii) the effect of grain size distribution in cementation mechanism. The observations suggest that the enzyme activity of the bacteria is negligible at and above 30°C, whereas it is significant at relatively lower temperatures. The comparison of treated soils suggests that the fine content in slope soil increased number of particle contacts, facilitated effective packing, and promoted the effectiveness of MICP compared to that of uniformly graded sands. Finally, the technical feasibility in slope soil stabilization was well demonstrated using model solidification test. The limitations in stabilizing the slope are also discussed in detail.

Key words: *Microbial Induced Carbonate Precipitation, indigenous bacteria, cold subarctic region, slope soil, temperature, grain size distribution.*

Article highlights

- Bacteria isolated from cold region were *Lysinibacillus Xylanilyticus.*
- Relatively high activity was between 15 and 25°C under weak alkali conditions.
- Slope soil showed higher UCS compared to uniform sands, which is also determined by precipitation amount.
- Slope model test demonstrates the feasibility of simple surface percolation method.

1. INTRODUCTION

MICP is an innovative biological ground improvement technique that could be applicable to many geo-engineering problems. The process utilizes the ureolytic bacteria to induce bio-cementation in a sustainable and cost-effective manner. The urease enzyme produced by the bacteria catalyses the hydrolysis of urea into ammonium and carbonic acid (Eq. 1), hence increases the alkalinity of the reaction medium. In the presence of calcium ions, the calcium carbonate cement is precipitated at the nucleation sites provided by the bacteria cells (Eq. 2) [1–3].

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

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

The precipitated carbonate crystals coat the soil particles, cement the soil matrix, fill the voids partially, hence increases the desired mechanical properties including strength and stiffness of the soil matrix [4, 5]. Despite being a relatively new technique, many MICP studies have been investigated for several geotechnical applications including liquefaction control, erosion mitigation, coastline preservation, sand dunes stabilization, borehole stabilization and settlement control [6–9]. Most of the investigations have been focused on the behavior of MICP treated sand, providing information on the strength and stiffness characteristics under different testing conditions [2, 5, 6, 9–11]. The studies to promote MICP applications need to be expanded, for soil types other than sand materials. Few researchers including van Paassen [12] and Mortensen et al. [13] have attempted the investigation on gravel and different soils ranging from very fine to very coarse and suggested that large amount of carbonate precipitation would be required to strengthen the coarse-gravel material. Also, the free passage of microorganisms through the limited pore-throat size remains a challenge in treating the very fine soils using MICP [14]. Recently, researchers have started to focus on assessing the performance of MICP in residual soils that consist fine to coarse grains distributed naturally [14–16]. However, only a very limited MICP studies on residual soils have been reported in the literature to the date, providing insufficient information on their mechanical responses.

The MICP treatment can be achieved by stimulating the growth of indigenous bacteria *in situ* (bio-stimulation) or by augmenting ureolytic bacterial culture (bio-augmentation). Bio-stimulation is a process of modifying the *in situ* environmental conditions to enrich the existing microbial community with required urease capabilities [17, 18]. Bio-augmentation largely differs from bio-stimulation and can be enabled by two ways: (i) bio-augmentation of indigenous bacteria: injecting enriched culture of indigenous bacteria isolated from native soil, and (ii) bio-augmentation using exogenous bacteria: injecting enriched culture of specialized non-native bacterial strains (e.g.,

Sporosarcina pasteurii). Bio-augmentation by exogenous bacteria has been extensively researched and demonstrated at a variety of laboratory scales [1, 5, 6, 11, 13], whereas the studies concerned on bio-augmentation using indigenous bacteria up to now are very limited [9, 19].

The augmentation of indigenous bacteria offers important environmental benefit through the elimination of nonnative bacterial supply (microbial pollution) into natural soil ecosystems [8]. Although the native species perform the urea hydrolysis at slower rates compared to that of specialized strains (e.g., *Sporosarcina pasteurii*), they would be more reliable than the exogenous strains in their native environment, resulting advantages in MICP with respect to the fitness of the strains and sustained enzymatic capabilities. At the same time, introducing non-native bacteria to a new environment may hinder the survival and enzymatic performance of the strains [20, 21], and the process may also face some ambiguities with respect to regulatory acceptance [21].

The selection of suitable ureolytic bacteria is highly important in urease-based MICP technique. The enzymes of the bacteria are thermal sensitive and can readily be denatured by even a slight change of environmental temperature [22]. Most of the MICP studies have employed *Sporosarcina pasteurrii* for urea hydrolysis due to the highly active urease enzyme [5, 6, 11, 13, 23–25]. Only a few MICP studies focused on introducing some other ureolytic strains including *Bacillus sphaercus* (MCP-11) [26], *Pararhodobactor* sp. [9, 27], *Bacillus sp.* VS1 [28, 29] and *B. megaterium* (ATCC 14581) [14]. However, most of the soil bacteria reported to date could effectively produce urease enzyme only within moderate to high temperature ranges (25°C-60°C), and within the range, the activity increased with increase in temperature [14, 30]. It must be noted that the functional temperature range of the enzymatic reaction is required to cover the temperature of the field stratum to be treated for an effective carbonate precipitation. Since the performance of the bacteria becomes negligible at low temperatures, implications of MICP in cold climatic zones remain as a huge challenge.

The study reported herein presents an initial level investigation on stabilization of a residual soil from the slope located in cold subarctic zone (Hokkaido, Japan). The novel indigenous ureolytic bacteria which had been adapted to the cold native environmental conditions were isolated from the native slope soil and investigated in detail. Effect of $CaCO_3$ precipitation on the process of strengthening in slope soil was compared with three uniformly graded soils with distinct mean particle size. The morphology and the spatial distribution of $CaCO_3$ were analysed at the microscale using scanning electron microscopy (SEM). The results obtained from this study would be beneficial to the future work that is to introduce the MICP technique as a novel candidate for surface stabilization of the slope.

2. MATERIALS AND METHOD

2.1 Soil Characteristics

The grain size distribution curves of the soils studied in this work are given in Fig. 1. Mizunami, Mikawa and Toyoura sands are standard laboratory sands, uniformly graded: SP based on Unified Soil Classification System (USCS) [31] with mean particle sizes (D_{50}) of 1.6 mm, 0.87 mm and 0.2 mm respectively. The slope soil considered in the current study is from expressway slope of Onuma, Hokkaido, Japan (Latitude 42.388532 and Longitude 140.284762). It is a well graded sand (SW based on USCS, D_{50}=0.23 mm), consisting very fine sand content (grain size < 125 μm) of 12%. pH value of the slope soil is 6.997 which is very close to that of uniformly graded soils (7.0 ± 0.1). Similar to the standard sands, the organic content in slope soil is found to be negligible. A summary of the X-Ray diffraction results of all the soils considered herein is presented in Table 1. The results showed that the silica is the dominant mineral in all the soils.

2.2 Isolation and characterization of indigenous bacteria

Isolation and characterization of bacteria was performed in accordance with the method suggested in previous studies [9, 32]. Soil sample collected from the study location (in sterile test tubes) was employed in laboratory for identifying bacteria. 5 g of soil sample was diluted to 10^1-10^5 in separate sterile test tubes with autoclaved distilled water. The solutions were applied on NH_4-YE agar plates (prepared by combining tris-buffer, ammonium sulfate, yeast extract and ager), followed by the incubation at 30°C for 72 hours. Formed colonies were then isolated and grown on separate new NH_4-YE agar plates. Each colony was employed in cresol-red test by transferring the colony into 20 mL cresol-red test solution (combined of cresol-red, urea, ethanol and distilled water), followed by the incubation at 45°C for 2 hours. The ureolytic bacteria were identified based on the pH (due to the formation of ammonium ions by hydrolyzing the urea) and color change (from yellow to purple) of the solution. The identified ureolytic strains were 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).

2.3 Reactant characteristics

The bacteria culture was enriched in sterile NH_4-YE medium consisting tris-buffer (15.7 g/L), ammonium sulfate (10 g/L) and yeast extract (20 g/L) with a neutral pH value, aerobically in a shaking incubator at 160 rpm. The

cementation reagent (1 mol/L) for the MICP treatment consisted of calcium chloride (111 g/L), urea (60 g/L) and nutrient broth (6 g/L).

2.4 Urease activity measurements

Urease activity of the bacteria was measured using spectrophotometric determination of ammonia as Indophenol [33]. 1 mL of bacterial culture was added to the 0.1 mol/L urea prepared in phosphate buffer solution. The ammonium ions produced from the urea hydrolyses could react with phenol at the presence of hypochlorite and produce the blue colour indophenol dye in the alkaline medium. The intensity of indophenol dye was measured at the wave length of 630 nm (OD_{630}) at different uniform intervals of catalysation (at every 5 minutes interval). Using the calibration curve developed between concentration of ammonium ions and intensity (OD_{630}), the rates of urea hydrolysis were estimated.

2.5 Soil column test

Soil columns (6 cm in height, 2.5 cm diameter) were prepared using 35 mL standard syringes and positioned vertically (Fig. 2). The columns of all the soils were packed to a dry density of 1.6 ± 0.1 g/cm^3 similar to that reported by Cheng and Cord-Ruwisch [3]. Reactants were injected in two subsequent phases as suggested by several MICP researchers [1, 2, 11]. In the first phase, the enriched bacteria culture was injected to the column followed by a time gap of 2 hours to immobilize the bacteria cells with soil particles. In the second phase, cementation solution was injected at a constant flow rate of 2-4 mL/min. All the solutions were simply applied to the top surface of the soil columns and allowed to percolate under gravitational and capillary forces. During the treatment, the columns were percolated with cementation solution every 24 hours (total of 14 cementation injections, 10 mL per each), whereas the columns were filled by bacteria culture once again at the middle of the fourteen days treatment period (total of 2 biological injections, 10 mL per each). The pH and Ca^{2+} concentration of the drainage were measured every 72 hours. After the treatment was completed, the samples were flushed with distilled water prior to the strength measurements.

2.6 Slope model test

The slope model (13×10×10 cm) was filled with slope soil by five layers, and each layer was compacted evenly to a similar dry density used in column test. The gradient of the slope was resolved to the standard gradient of expressway cut slope in accordance with the road earthwork guidelines of Japan [34, 35]. During the treatment, a grid-based injection method was performed. The slope surface was divided into 8×5 grids, each grid was 2 cm×2

cm, and the solutions were injected on the surface of the slope and allowed to percolate under gravitational forces. Based on the pore volume of the soil below each grid, the injection volumes were estimated, and the rate of injection was 8-10 mL/min. Similar to the column test, 2 number of biological injections and 14 number of cementation injections were performed totally during 14 days of treatment process. The experimental arrangement of slope model test is illustrated in Fig. 3.

2.7 Uniaxial compressive strength (UCS) measurement

The UCS of the cemented columns and slope were estimated using needle penetrometer (SH-70, Maruto Testing Machine Company, Tokyo, Japan) according to JGS 3431-2012 [36]. The equipment was developed in Japan for predicting the UCS of soft, weak, very weak rocks and cemented soil specimens, and recently, ISRM has recommended the method for indirect estimation of mechanical properties of the soft rock materials [37]. This penetration is a non-destructive test, and that has a great potential to be used as a sound technique for MICP field assessments. The penetration depth (mm) of the needle attached to the device and the penetration resistance (N) were measured during the test. Using the regression relationship (Eq. 3) developed by analyzing 114 natural soft rock samples and 50 cemented soil samples, the UCS of the specimen was estimated [32, 38].

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

where, x is penetration gradient (ration between penetration resistant (N) and penetration depth (mm)); y is corresponding UCS.

2.8 $CaCO_3$ content determination

Carbonate content in the treated specimen was determined by using the simplified device developed to measure the pressure of CO_2 gas released when the cemented specimen is treated with HCl in closed system under constant volume and temperature [39]. Cemented column specimen was divided into sections along the height, and the samples from the sections at the depths of 1 cm, 3 cm and 5 cm (measured from the surface) were carefully separated, ensuring an accurate representation of cemented soil at that depth. Dry mass of the separated sample was measured, and the sample was placed into the calcimeter flask. 20 mL of HCl (3 mol/L) was placed in small plastic vials and set into the calcimeter flask without spattering the specimen. Subsequently, HCl was allowed to react with specimen in the closed system until the digital manometer (connected with the system) read a constant pressure. From the calibration curve developed between the pressure and $CaCO_3$ content, the carbonate content was estimated, hence the percentage of mass of $CaCO_3$ was determined.

3. RESULTS AND DISCUSSIONS

3.1 Biological response

3.1.1 Indigenous isolates

Among the isolated colonies, only three strains showed the pH increase and colour change in cresol red indicator, suggesting the urease potential. The strains were characterized as *Lysinibacillus xylanilyticus*, *Viridibacillus arvi* and *Sporosarcina* sp. by the 16S rRNA gene sequence analysis (the authenticate phylograms of the bacteria are presented as supplementary file). From the preliminary experiment results (Fig. 4), *Lysinibacillus xylanilyticus* culture was chosen based on the highest urease production and investigated in detail for MICP application. *Lysinibacillus xylanilyticus* is a gram positive, rod shaped bacteria (average length of 8-10 μm; diameter of 0.5-0.6 μm). It is worth noting that this is the first paper reporting the urease and MICP potential of the *Lysinibacillus xylanilyticus*.

3.1.2 Effect of temperature on growth and urease performance

Biomass concentration was used as the bacteria growth indicator based on the turbidity of the bacteria culture. The optical density (OD_{600}) of *Lysinibacillus xylanilyticus* culture was measured by using the spectrophotometer at a wave length of 600 nm. The influence of temperature in bacteria growth was investigated by placing the bacteria culture in shaking incubator at temperatures ranging from 10°C to 50°C. The growth curves are presented in Fig. 5. The initial pH of the culture medium was relatively neutral (7.1~7.3). At the temperatures from 10°C to 30°C, biomass concentration increased initially up to 48-96 hours and remained relatively stable afterwards. The optimal bacteria growth decreases with increase in temperature, and the growth is negligible at the temperatures above 40°C.

Temperature and pH are the most governing factors of activity of bacterial urease [23]. Urease activities under different temperatures (10-50°C) are provided in Fig. 6. One unit of activity can be defined as the amount of urea (μmol) degraded per minute by 1 mL bacterial culture enriched under the study conditions. It should be noted that relatively a bell-shape relationship between urease activity and temperature was obtained. Urease activity of *Lysinibacillus xylanilyticus* is relatively higher within the temperature range 20-25°C (ranged between 1.5 and 3 μmol/min/mL). The urease activity reached the optimum value at 25°C (3 μmol/min/mL), above which the activity dropped significantly and became negligible. The urease activities of the bacteria at 15°C and 10°C are 0.75 μmol/min/mL and 0.2 μmol/min/mL respectively, and below 5°C, the activity becomes insignificant.

The long-term stability of bacterial urease is very essential for the adequate carbonation during treatment period under the minimal supply of bacteria cells. Urease is a molecule composed of protein subunits, that could be typically broken down into amino acids by cellular enzyme called protease (proteolysis) [40, 41]. The stability of bacterial urease with the time is shown in Fig. 7. The results indicate that the urease activity of *Lysinibacillus xylanilyticus* remains relatively stable within 15-25°C against proteolysis. However, the urease activity became negligible within the short period at higher temperature (30°C), suggesting that the bonds between protein subunits weaken at higher temperature and tended to break down quickly.

3.1.3 Effect of pH in urease performance

Effect of pH conditions in urease activity of *Lysinibacillus xylanilyticus* is illustrated in Fig. 8. The results show that the highest urease activity was found to be within the pH range from 7 to 8 (weak alkali conditions). Relatively lower urease activity was found under acidic (less than pH 6) and alkali (at pH 9) conditions, as bacterial urease underwent significant denaturing in severe acidic and alkali conditions.

3.1.4 Enzyme location

The whole cell culture medium was centrifuged (8000 rpm, 10°C, 5 mins) to separate the cell pellets from the culture supernatant. The separated cell pellets were washed and resuspended into same volume of autoclaved distilled water, followed by urease activity test was performed separately on cell medium (cells + distilled water) and supernatant. The urease activity of cell medium was very close to that of whole cell culture medium, and a negligible activity was observed in supernatant. The results suggest that the urease enzyme of *Lysinibacillus xylanilyticus* is not segregated into extracellular medium but localized completely in or on the cell membrane, indicating urease of *Lysinibacillus xylanilyticus* is cytoplasmic enzyme. This is similar to that reported to most of the urease of the species including *Sporosarcina* and *Pararhodobacter* sp. [27, 41].

3.1.5 Discussion

Numerous investigators have studied the effect of temperature on the rate of urea hydrolysis of many soil bacteria [23, 27, 30, 40]. In general, their results have indicated that the rate of urea hydrolysis increases with increasing temperature, suggesting that the urease of most of the soil bacteria is not heat inactivated (up to the temperature ranging 45-60°C). For an example, urease activity of *Sporosarcina Pasteurii* increased proportionally with temperature between 25°C and 60°C [24]. The similar tendency is reported for *Pararhodobacter* sp. [27], *Deleya venusta* [42] and *Strep-tococcus salivarius* [43]. It is worth to note that the *Lysinibacillus xylanilyticus* reported

herein exhibited a distinct behavior regarding urease activity, in which the urease enzyme is highly heat inactivated; urease is not stable even at 30°C. On the other hand, the urease enzyme is more stable and consistently performs for long period at lower temperatures below 30°C (Fig. 7). Theoretically, the activation energy for a specific enzyme-catalyzed reaction is constant [30]. Since the mean activation energy of most of the bacterial urease is high, bacteria could effectively catalyst the hydrolysis of urea at high temperatures (above 30°C), resulting negligible urease potential at lower temperatures. Since the *Lysinibacillus xylanilyticus* was isolated from subarctic cold region, it exhibited high urease potential at temperatures below 30°C, suggesting low energy requirement for enzyme-catalyzed reaction. However, urease activity of the bacteria becomes negligible below 5°C, suggesting that the supply energy below 5°C is deficient to activate the enzyme catalyzation. Therefore, application of the species reported herein is limited at the temperatures close to or below the freezing point of water.

Different soil bacteria has different tolerance towards pH conditions. Some ureolytic bacteria including *Bacillus cereus* show higher activity at around pH 4 compared to the neutral pH conditions suggesting acidic urease compatibility [44]. Also, the bacteria (for example *Helicobacter pylori*) living in harsh acidic environment produce more urease enzyme at acidic conditions [45]. In fact, urease activity of those bacteria is related to their stress response to survive at acidic condition, wherein hydrolysis of urea would neutralize the acidic environment leading to the bacterial survival. On the other hand, urease enzyme of some bacteria tends to be more active in neutral to alkaline environmental conditions. *Lysinibacillus xylanilyticus* reported in this paper showed the highest activity at weak alkali conditions. Similar phenomena could be seen on certain microbial urease like *Sporosarcina pasteurii* [23, 40], *Pararhodobacter* sp. [27] and *Klebsiella aerogenes* [44], those have the optimum activity under weak alkaline conditions.

3.2 Soil column test

3.2.1 Behavior of uniformly graded sands

The effect of MICP in slope soil was examined by comparing the behavior of three uniformly graded sands with different particle sizes. All the columns specimens were treated exactly under same physical (25°C) and chemical conditions (14 cementation injections; 2 bacterial injections) by surface percolation as explained in previous section. The entire column of all type of soils were successfully cemented, and the cemented profiles are presented in Fig. 9. Fig. 10 presents the UCS of the different soils with respect to the depth. The results indicate that the UCS values increase with the decrease in particle size in the uniformly graded sands. The average UCS estimated

close to the surface of Mizunami, Mikawa and Toyoura sands (D_{50} of 1.6 mm, 0.87 mm and 0.2 mm) are 1.82 MPa, 2.67 MPa and 3.98 MPa respectively. The UCS values and the calcium carbonate content (Fig. 11) of coarse and find sands reported herein are in a good agreement with the results reported by Cheng and Cord-Ruwisch [3]. Basically, coarse sands have high permeability that leads to the high liquid infiltration compared to the fine sands. The calcium ion concentrations of the drainage measured during the treatment (Fig. 12) suggest that more than 80% of the un-reacted cementation solutions have reached the bottom in the coarse sand columns (Mizunami and Mikawa) due to the high infiltration rate facilitated by the large pore spaces.

The UCS of all the uniformly graded sands increases with the depth of the sample. In coarse sand columns, the UCS and $CaCO_3$ content are relatively constant from the surface to the middle and then slightly increase with the depth. A similar observation in coarse sand has been reported by Cheng and Cord-Ruwisch [3]. In contrast, fine sand exhibits significant improvement in UCS with the depth, which could be due to the increased capillary effect along the sample depth. The higher capillary forces in fine sand tended to retain certain amount of cementation solutions close to particle contacts, resulting higher deposition of calcium carbonate and exhibiting higher UCS at the bottom sample compared to that at top.

3.2.2 Behavior of slope soil

The slope soil showed a distinct behavior compared to the behaviors of uniformly graded sands. The average particle size of Toyoura sand and slope soil are relatively similar (0.20 mm and 0.23 mm respectively). Also, both soils exhibited that around 80-90% of the calcium ions in cementation solution have been already utilized by the time it reached the bottom (Fig. 12). However, treated soils exhibited a significant difference regarding their UCS (Fig. 10) and $CaCO_3$ content (Fig. 11). The UCS of the slope soil estimated close to the surface is 14.98 kPa, which is around 3.75 times higher than the UCS of cemented Toyoura sand. There was no difference in the treatment process for the above specimens; therefore, the reason for the remarkable UCS achieved in slope soil could be due to its grain size distribution. Slope soil is a well graded sand consisting very fine sand content of 12%, leading to effective packing and cementation in the matrix. It was also observed that the infiltration rate of cementation solution decreased with the number of injections, which would be the result of the reduction in permeability due to the cementation at pore spaces.

In contrast to the UCS and $CaCO_3$ profile of uniformly graded sand columns, the UCS and carbonate content of slope soil decrease with the depth (Fig. 10 and Fig. 11). The higher cementation at the top of the column specimen is presumed to be due to the biological filtration. The bacteria were applied into specimen from top and allowed

to flow through the soil. As the bacteria travel through smaller pore space of the slope soil, they might be filtered through the soil with a reduction of bacteria concentration along the path. Therefore, a larger concentration of bacteria cells would retain at the top of the column compared to the bottom. The distribution of carbonate cementation observed in slope soil was similar to those reported by Cheng and Cord-Ruwisch [3], Feng and Montoya [11] and Martinez et al. [46]. Furthermore, a very less bacterial turbidity was detected at the outlet of slope soil column, suggesting that most of the cells are immobilized in the soil matrix by the filtration. It has been reported that the cementation profiles are generally determined by the bacteria distribution in the specimen [46]. The uniformity of the precipitated calcium carbonate along the soil specimens is further discussed in the subsequent section.

3.2.3 Microscale properties of MICP treated soils

The precipitation characteristics of MICP treated soils were investigated in microscale using SEM (Scanning electron microscopy). All the representative samples used for the SEM observation were obtained from 1 cm depth of the treated columns. The SEM images of uniformly graded sands are shown in Fig. 13. The images of MICP treated sands show calcium carbonate precipitated at particle contacts (contact cementing) and slightly coating particle surfaces. As the particle size of sand reduces from 1.6 mm to 0.2 mm, the images of the MICP treated sands show an increase in precipitated carbonate content (consistent with the carbonate content measurement of sands presented in Fig. 11).

The number of particles contact highly influences the effectiveness of MICP. Generally, bacteria cells prefer to position themselves in smaller surface features (such as near particle-particle contacts) compared to the particle surface, which is due to the reduced shear and a higher availability of nutrients at the particle contacts [4]. Therefore, the calcium carbonate cement preferentially precipitates at the particle contacts while coating the soil grains during the treatment. Due to the higher number of particle contacts in fine Toyoura sand, the rate of calcium carbonate precipitation was higher compared to that in coarse sands (Fig. 13). It should be noted that the matrix supporting is enabled by filling the void space with or without contacting the soil particles (i.e. grow from particle surface into pore space to create bridges between particles), when the soils are treated in saturated conditions [4, 5, 11]. Well coated grains with matrix supporting by calcite crystals are also often observed by many researchers in medium to coarse sands treated under saturated conditions [2, 5, 11, 47]. It is worth noting that the microstructure reported herein for the specimens treated under percolation condition slightly differ from that reported for saturated conditions. Since the surface percolations method is achieved with the aid of gravitational

and capillary forces, contribution of matrix supporting is limited. The growth of the crystals from the particle surface into void space is not observed in uniformly graded sands treated by surface percolation (Fig. 13).

Fig. 14 presents the SEM image of MICP treated slope soil (Fig. 14a shows the untreated soil). Based on the shape of the $CaCO_3$ crystal, the precipitated morphology in slope soil would be calcite (typical rhombohedral crystals). It can be clearly seen that the sand particles ranging from coarse to fine form an effective matrix in slope soil for the MICP (Fig. 14b). It is also worth noting that presence of fine particles could change the microstructure of sand skeleton and govern the behavior significantly. Most of the sand particles are in contact with some of the fine particles between them, and the rest filled the void spaces (Fig. 14b). Also, some of the fine particles are cemented both to each other and to the sand particles (Fig. 14c). The presence of fine content (12%) in slope soil significantly increases the number of particle contacts by bonding with the sand grains. It can be observed that the fine content plays a very important role in matrix supporting by enabling intermediate supports to form bridges among the carbonate crystals that have grown in void spaces (Fig. 14c), thus, strengthening the force chain of the treated matrix. It should also be stated here that the MICP in very fine soils are limited due to the limited rate of permeability that generally takes very longer time to infiltrate the reactants [13], and free passage of the bacteria might be inhibited due to the small pore throat size of fine soils [47].

3.3 Slope model solidification test

Slope model test is the scale-up version of soil column tests. Because the small columns are limited by confinement and boundary effects, the model test was additionally performed to demonstrate the feasibility of slope soil stabilization. After the 14 days of treatment, the slope was flushed with about 5 L of tap water to remove all soluble salts, followed by, slope specimen was carefully removed from the mold and submerged into the water bath to eliminate the uncemented aggregates. The cemented profile is presented in Fig. 15. The observation indicates that more than 80% of the soil was successfully cemented, and a stiff layer of around 3-4 cm along the entire surface was achieved. However, cementation was not observed within the zone below the top slope (Fig. 15), remained as untreated soil. The surface UCS was used as the indicator to evaluate the improvement of slope, besides the UCS estimated by the needle penetration tests are illustrated in Fig. 16. The results show that the bio-cemented slope surface had considerable UCS between 2 and 8 MPa, and relatively lower UCS values were obtained below the surface of the slope.

3.3.1 Uniformity of cementation

It is highly necessary to study uniformity of precipitated calcium carbonate along laterally (*t*-*z* axis) and vertically (*x*-*y* axis) in slope, as the MICP in soils is achieved by biochemical injections. Before the measurements, it was anticipated that relatively a homogeneous cementation could be attained along the surface (*t*-*z*) by the injection method. However, Fig. 16 suggests that the surface UCS increases along the distance (*t*) measured from the top slope, and the highest surface UCS was obtained near bottom slope. The average UCS at the bottom slope was about 7 MPa, whereas that of top is only 2 MPa. This variation along the surface could be attributed to the localization of bacteria cells. The consecutive injections of cementation solution (every 24 hours) i.e. propagated flow-lines would possibly transport the bacteria cells towards downslope, results increase in cell concentration in the direction of downslope; thus, more or less of the bacteria cells will in turn increase or decrease the cementation and UCS. The Precipitated carbonate profile is not uniform along the slope depth (*x*-*y*) similar to that observed in column specimens, suggesting higher calcium carbonate content near to the injection port. The spatial distribution of calcium carbonate generally follows the trend of microbe distributions along the specimen length/ depth [46]. As previously discussed, higher concentration of bacteria cells was filtered at the top zone, leading to higher deposition of carbonate content near the injection surface. In order to confirm this postulation, soil samples were collected from three depths of slope after performing the biological injection, and the number of existing bacteria cells were measured by similar method used for isolation of bacteria. The measured locations are indicated clearly in Fig. 3. The results (Fig. 17) suggest that the distribution of bacteria in slope soil drastically decreases with the depth of slope i.e. the number of cells per unit gram soil at 1 cm below the slope surface and at the bottom slope are 1.4×10^6 and 5×10^3 respectively. Thus, it has been confirmed that most of the bacteria cells were filtered in surface zone, resulting high cementation at the surface zone.

In addition, surface zone closest to the injection port is generally in exposure to higher concentrated reactants than the bottom zone [1]; therefore, higher carbonate content was precipitated at surface zone. It also has been reported that the amount of urease, precipitation rate and nutrient concentrations also have a great influence in carbonate distribution and must be maintained at their minimum to achieve a homogeneous cementation along the soil columns [13, 47].

The measurements indicate that the slope soil consists of very small amount (0.22-0.25%) natural carbonate content. As reported by Mortensen et al. [13], the soil particles consisted of carbonate content could provide ideal surfaces and nucleation sites for the additional precipitation of carbonate crystals. At the same time, the residual soil would consist of soil particles in broad range of shapes, irregularities and with varying surface roughness,

which could provide additional effects in precipitation of carbonate. Future works should clearly address the effect of particle shapes and surface roughness to improve the understanding on the behavior of residual soils.

The threshold calcium carbonate content required to provide measurable UCS of slope soil was found to be around 3%. The carbonate content reported herein is in a good agreement with the minimum carbonate content (3.5 %, for the sand with D_{50} of 165μm) reported by Whiffin et al. [1]. Soil cemented below the threshold carbonate content exhibited an unsolidified loose matrix which was similar to the untreated soil. However, small carbonate crystals on the particle surface were evidenced through SEM analysis. At the same time, Feng and Montoya [11] and Lin et al. [5] have reported that the minimum carbonate content required to increase the strength of sands (D_{50} of 0.22 mm and 0.33 mm respectively) was around 1 %. Actually, the threshold carbonate content of a soil is related to the number of particle contacts and grain size. Due to the increased number of particle contacts in slope soil, relatively a higher amount of threshold carbonate content (3%) (compared to that reported by Feng and Montoya [11] and Lin et al. [5]) was required to provide the measurable strength.

Fig. 18 presents a compilation of all UCS results obtained versus the measured calcium carbonate content. The UCS increases during MICP improvement as calcium carbonate precipitates and cements the soil particles, as a result of void space reduction. It is also clear that the UCS increases exponentially with the increase in calcium carbonate content, similar to that reported in previous studies [2, 6, 32].

3.3.2 Field implication and associated limitations

To date, most of the studies of MICP have been extensively investigated under saturated or water-logged environmental conditions [1, 5, 6, 10, 47], in which bacterial and cementation medias were injected to the specimen by a saturated flow with a constant flow rate using peristaltic pump. Pumping method has often resulted a heterogeneously cemented profile in soil columns, suggesting a higher precipitation level at the influent column. Recently, Li et al. [48] have proposed a rotating soak method to promote the uniformity of cementation in specimen by facilitating more nutrition supply and air replenishment within the soil for effective bacterial performance. However, in the real field, it would be a challenge to maintain the saturated or submerged flow conditions during the treatment period, which requires hydraulic injection of cementation/ biological solutions, extraction of effluent solution and heavy machinery systems. Therefore, it is an essential to introduce alternative MICP approaches to produce carbonate cement with high efficacy with desirable cost minimization. The simple surface percolation technique demonstrated in this study is more suitable for field application, which eliminates the destruction of existing soil structure and reduces the costs of required labour and machinery. However, it

would be more effective, if the surface percolation is promoted to spraying instead of injections, which is left for the future work to be performed in the subsequent phase of this study.

In civil engineering applications such as slope surface stabilizations, it is highly important to immobilize the ureolytic bacteria within the target surface zones, so that sufficient carbonate can be precipitated throughout the required zone. The experimental observations discussed in this paper suggest well immobilization of bacteria only within the surface zone, and the particle size distribution of slope soil limits the distribution of bacteria to more deeper depths. Therefore, it is appropriate to recommend the technique for slope surface stabilization i.e. stabilizing the slope by enhancing the surface cover condition of the slope and promoting high aggregate stability at the surface zone, which could be enabled against surface erosion. The results also indicate, treating the soil by surface percolation method could strengthen the soil significantly by forming more effective crystals at free-draining environmental conditions and can be highly applicable on unsaturated or partially saturated natural slopes and embankments.

In the injection method investigated herein, grain size distribution highly governs the efficiency of MICP. Fine sands, well graded soils and soils consisting of certain amount of fine particles have less permeability (compared to the coarse soils) leading to low infiltration rate, that often increases the risk regarding local clogging. In order to avoid the risk of clogging, Whiffin et al. [1] have suggested a faster injection/ flow rates of reactants by allowing less reaction time along the path. Afterwards, Cheng and Cord-Ruwisch [3] have reported the observation of non-clogged fine and coarse sand columns treated under higher infiltration rates (7-28.5 cm/min). They have also reported that severe clogging occurred in fine sand when it was treated under low infiltration rate less than 0.25 cm/min. Therefore, a moderate infiltration rate of 2-4 mL/min was maintained during the treatment period (no clogging occurred during the treatment).

4. CONCLUSIONS

The findings indicate that the bacteria (*Lysinibacillus xylanilyticus*) isolated from the subarctic cold region have a significant potential to produce urease enzyme at temperatures 15-25°C, suggesting low-temperature urease compatibility. On the other hand, the growth and the urease production of the bacteria is limited at the temperatures above 30°C. In the neutral-weak alkaline environmental conditions, urease is more stable and consistently performs. It is also found that the urease is not segregated into the extracellular medium indicating that bacterial urease is a cytoplasmic enzyme. On the whole, the novel isolate exhibits a significant impact for MICP applications in subarctic cold climatic regions.

The effect of carbonate precipitation on the process of strengthening in slope soil was investigated by comparing the behaviour of three uniformly graded sands. Microstructure of the soils treated by surface percolation shows the formation of effective crystals at particle contacts. Presence of fine content governs the behavior of residual slope soil significantly. It increases the number of particle contacts by bonding with the sand grains and participates in the force chain of the treated matrix. The fine content provides the matrix support effectively by facilitating intermediate supports to form bridges between carbonate crystals. However, grain size distribution tends to filter more bacteria at surface zone of slope, results high cementation at the surface level and relatively a long linear reduction in carbonate precipitation along the profile is observed. This preliminary investigation suggests that the technique can be implemented for stabilizing the slope by enhancing the cover condition of the slope, and the results obtained from this investigation would be highly beneficial to promote the study to the large scale.

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CONFLICT OF INTEREST

All the authors declare no conflict of interest.

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Table 1 Mineralogical composition of sands and slope soil

Oxide	Percentage (%)			
	Slope soil	Mizunami	Mikawa	Toyoura
SiO_2	57.5	97.41	97.65	92.6
Al_2O_3	25.6	1.37	0.89	3.7
Fe_2O_3	8.1	0.03	0.43	0.7
CaO	3.68	-	0.03	0.5
MgO	1.99	-	0.11	0.2
K_2O	1.05	-	0.18	-
SO_3	0.672	-	-	-
TiO_2	0.583	-	0.04	-
P_2O_5	0.302	-	-	-
MnO	0.137	-	-	-
V_2O_5	0.028	-	-	-
Na_2O	-	-	0.04	-

Figure captions

Fig. 1. Grain size distribution curves of sands and slope soil

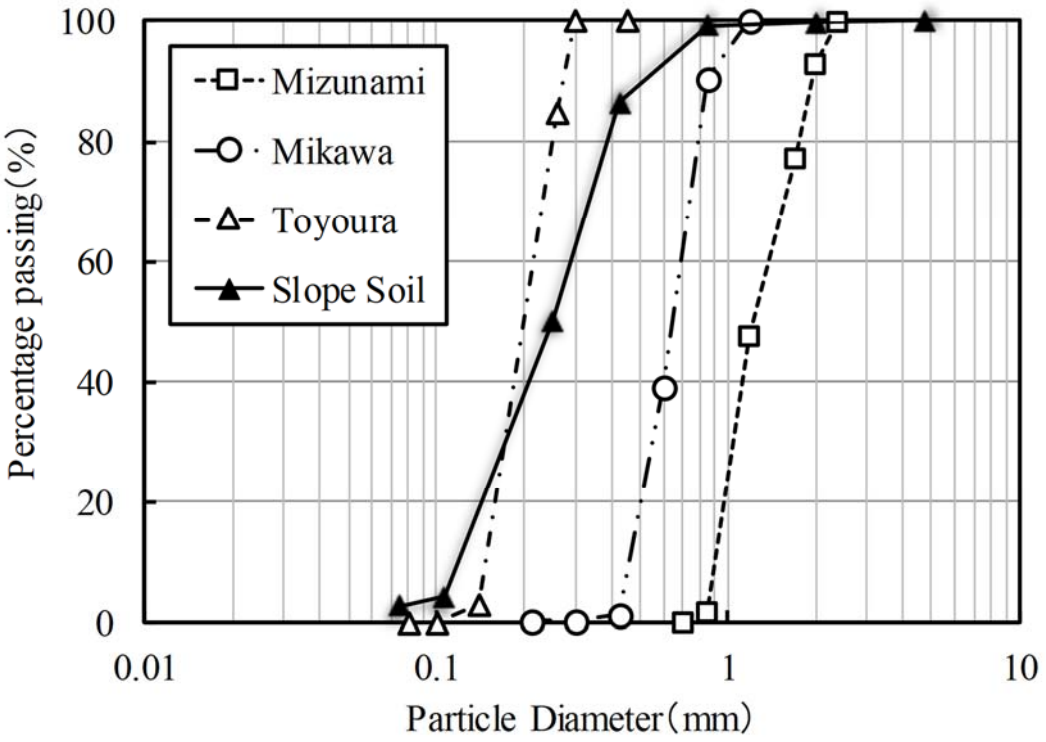

Fig. 2. Schematic diagram of soil column test (experimental set up)

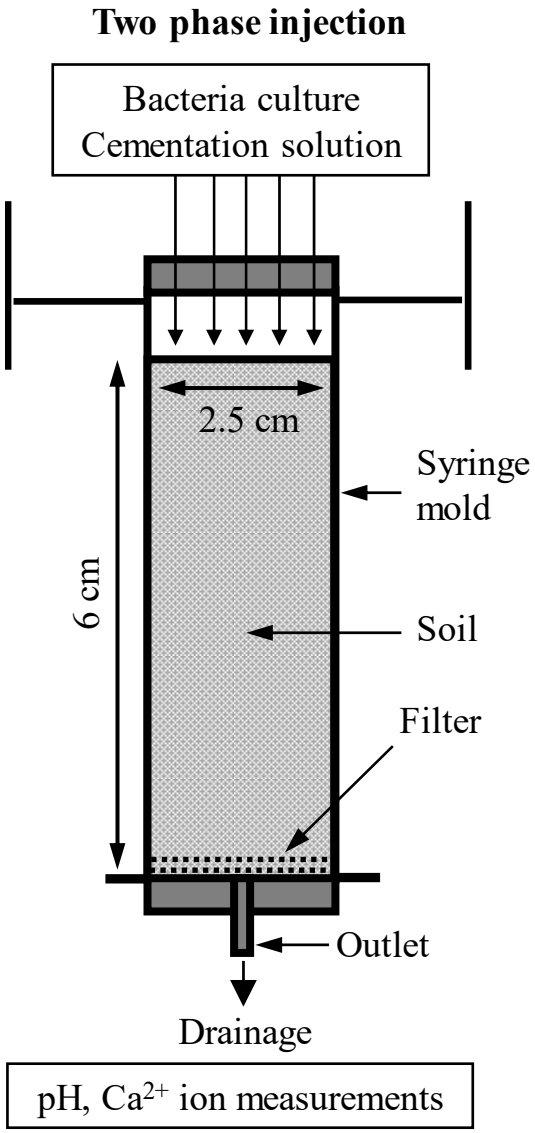

Fig. 3. Schematic diagram of the experimental arrangement of slope model

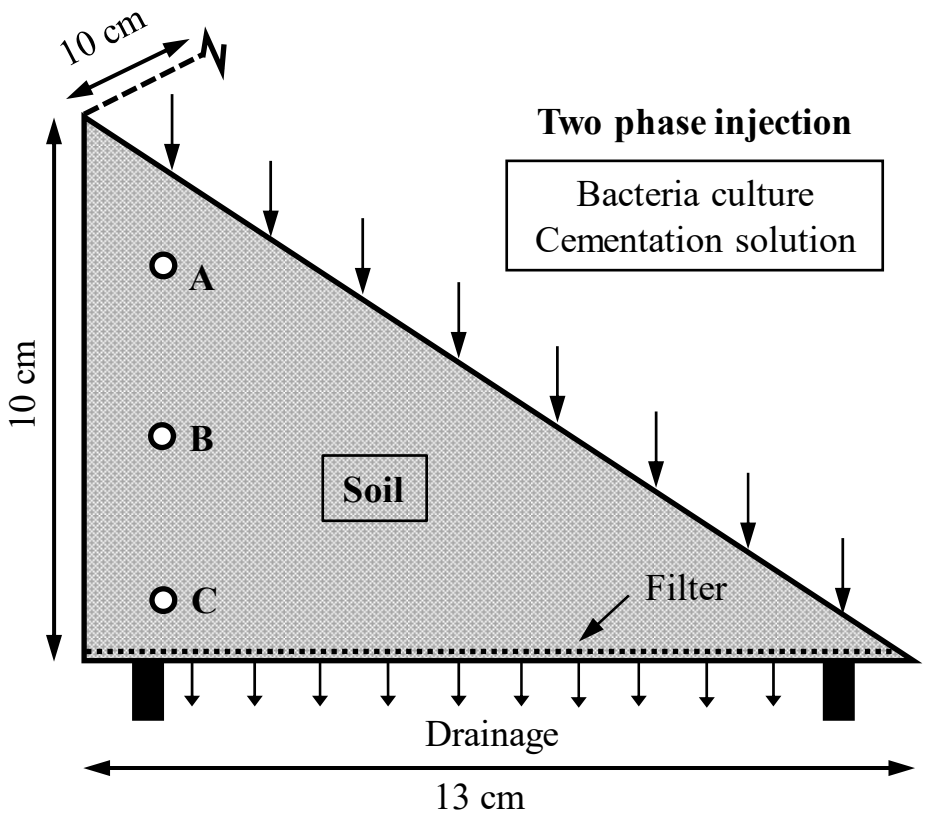

Fig. 4. Urease activity test results of all the bacteria isolated from slope soil. The error bars represent the mean ± SD (n = 3)

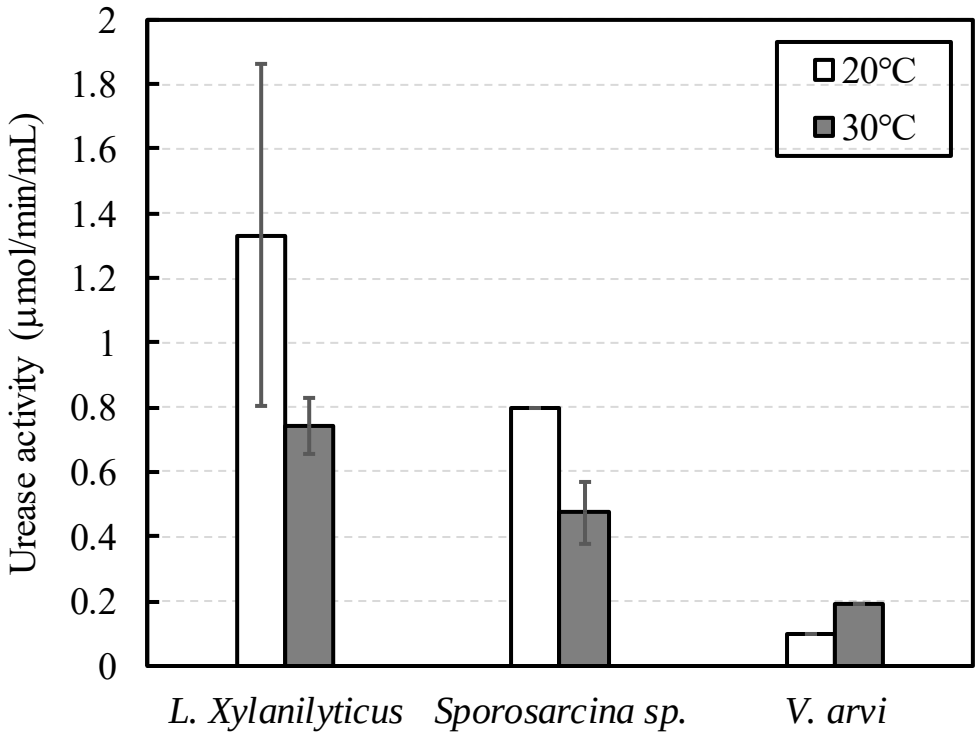

Fig. 5. Growth of *Lysinibacillus xylanilyticus* at different cultivation temperatures

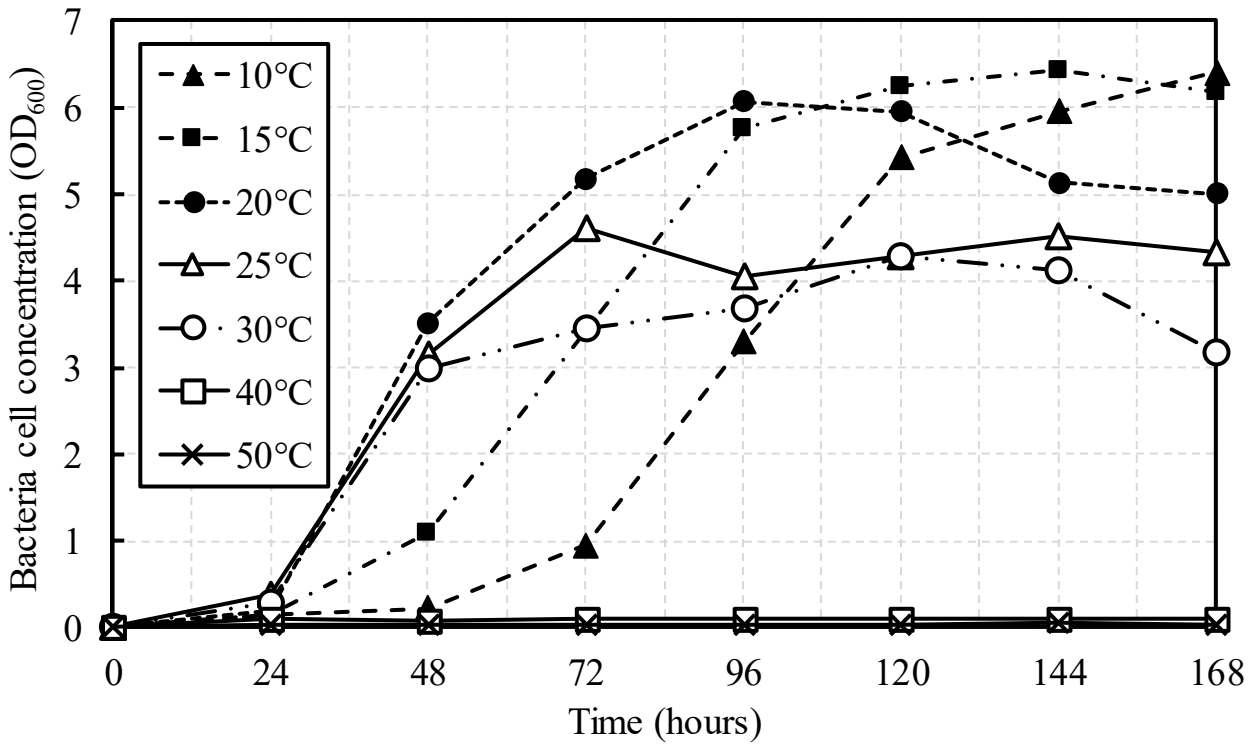

Fig. 6. Urease activity of *Lysinibacillus xylanilyticus* at different temperatures. The error bars represent the mean ± SD (n = 3)

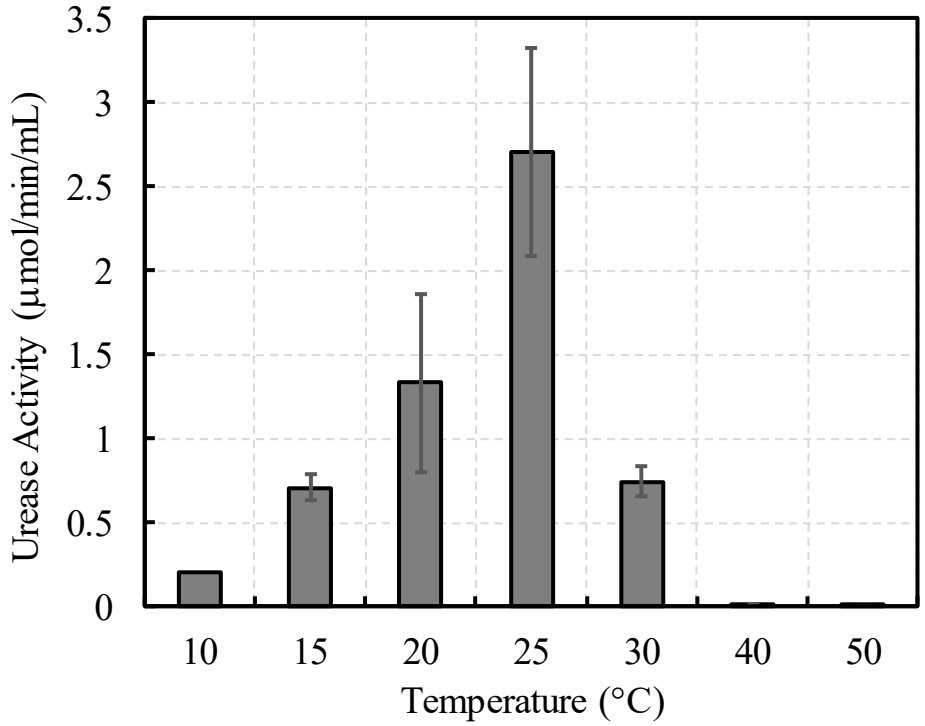

Fig. 7. Stability of the urease enzyme with the time at different cultivation temperatures

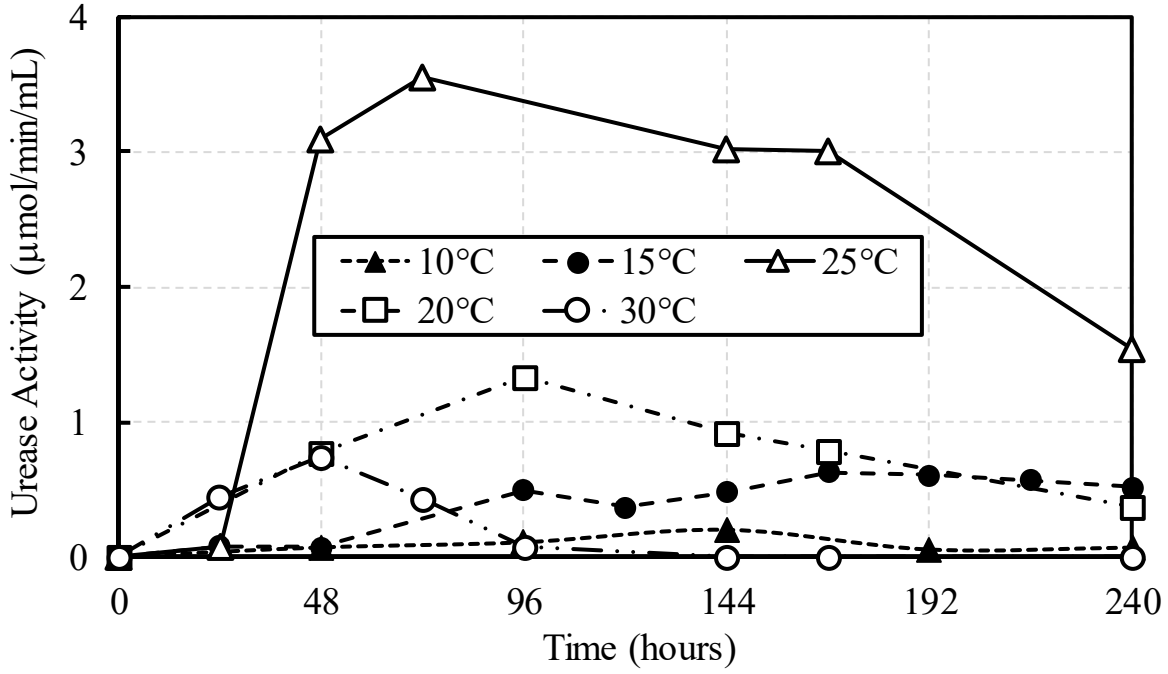

Fig. 8. Effect of pH condition of reaction medium on urease performance. The error bars represent the mean ± SD (n = 3)

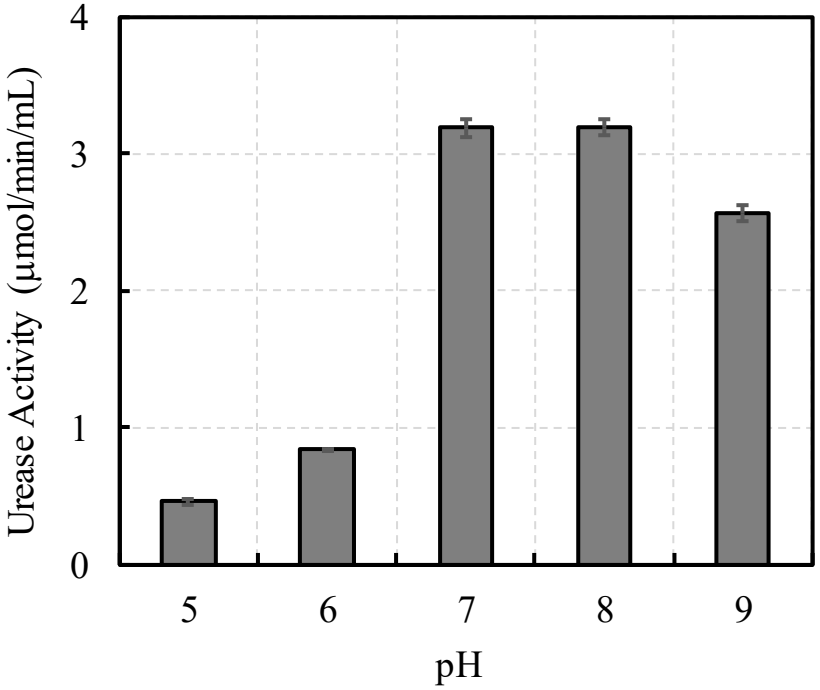

Fig. 9. Cemented columns of (a) Mizunami sand, (b) Mikawa sand, (c) Toyoura sand and (d) slope soil

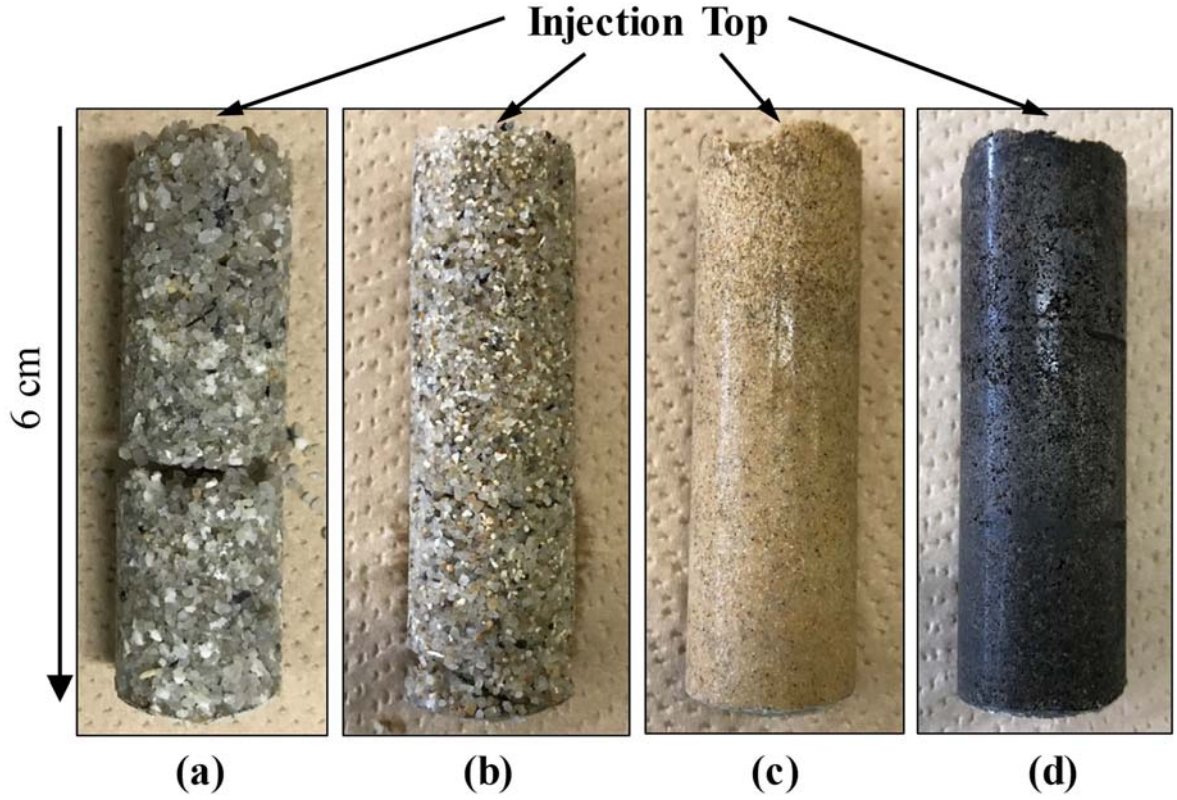

Fig. 10. UCS profiles of cemented soil columns. The error bars represent the mean ± SD (n = 3)

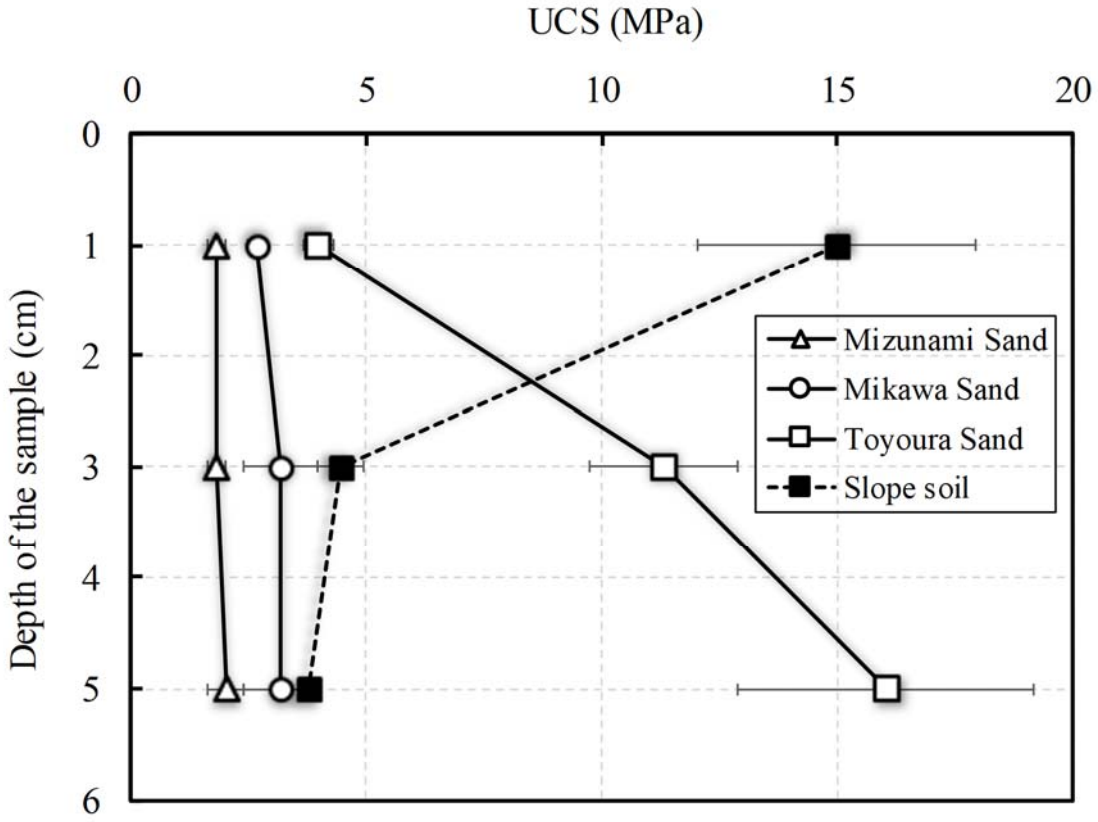

Fig. 11. Calcium carbonate content profiles of cemented soil columns. The error bars represent the mean ± SD (n = 3)

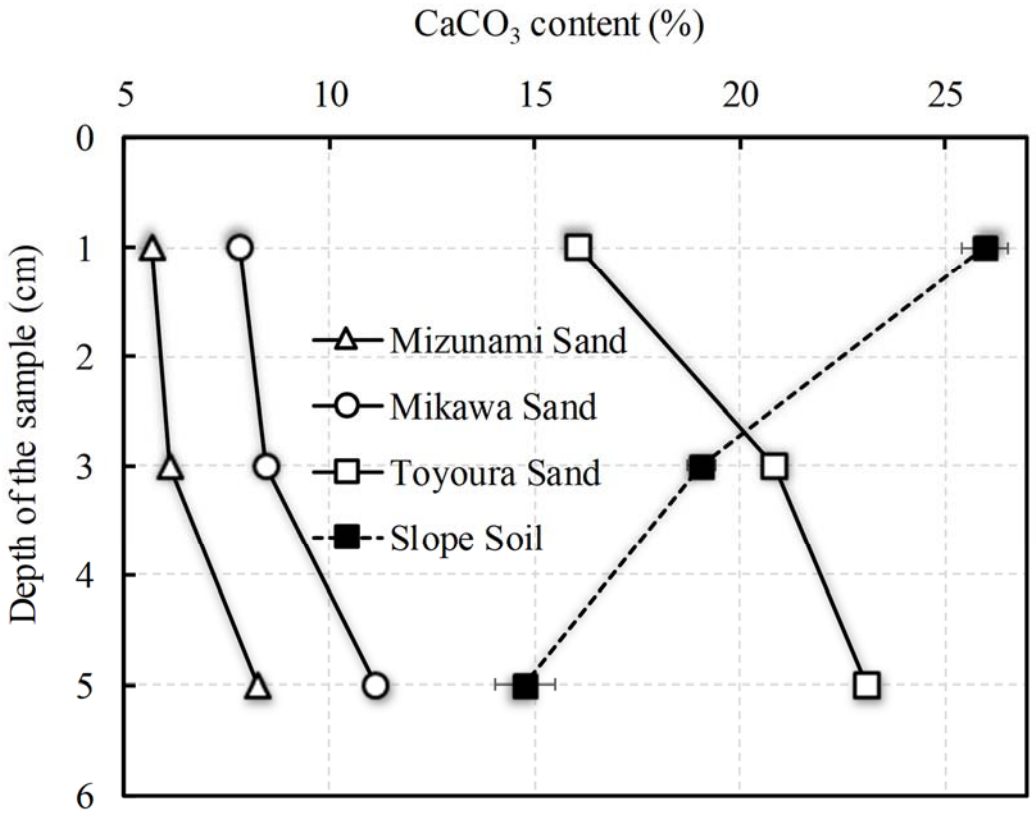

Fig. 12. Ca^{2+} concentration measured at outlet drainage of column specimens during the treatment

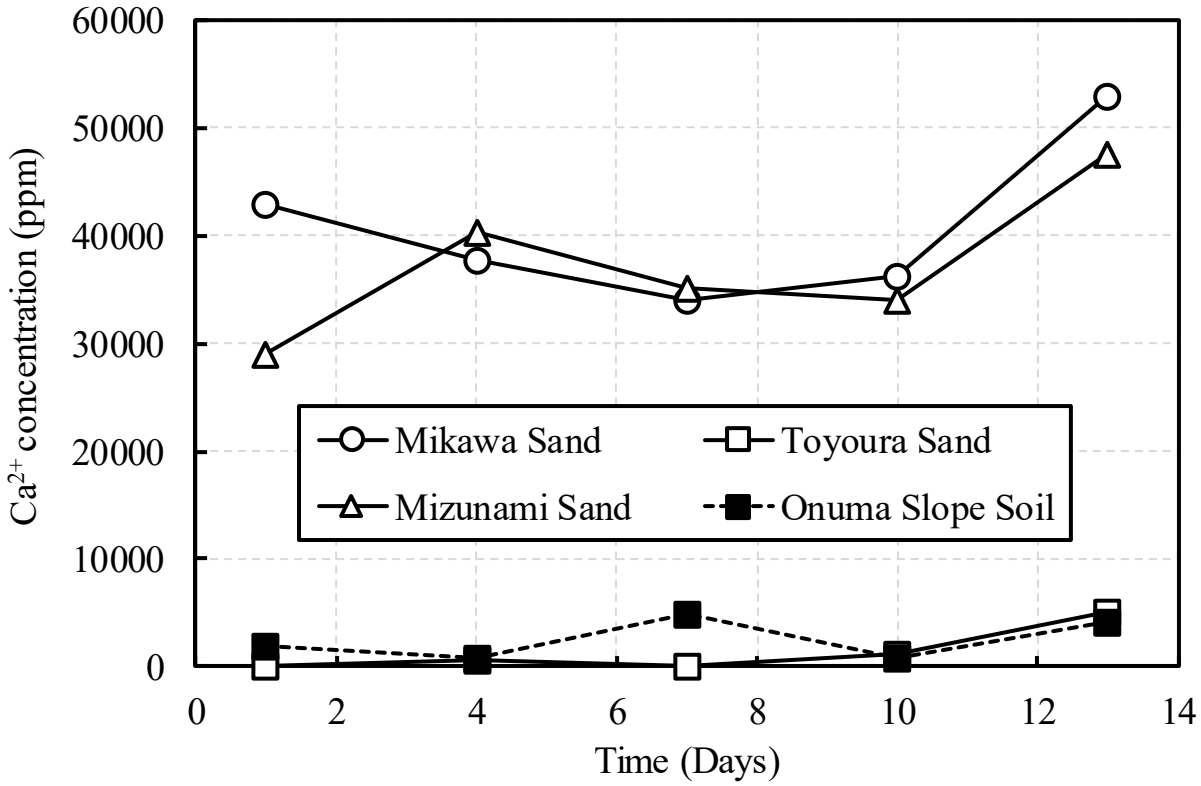

Fig. 13. Scanning electron microscopy images of uniformly graded sands (Mizunami, Mikawa and Toyoura sands)

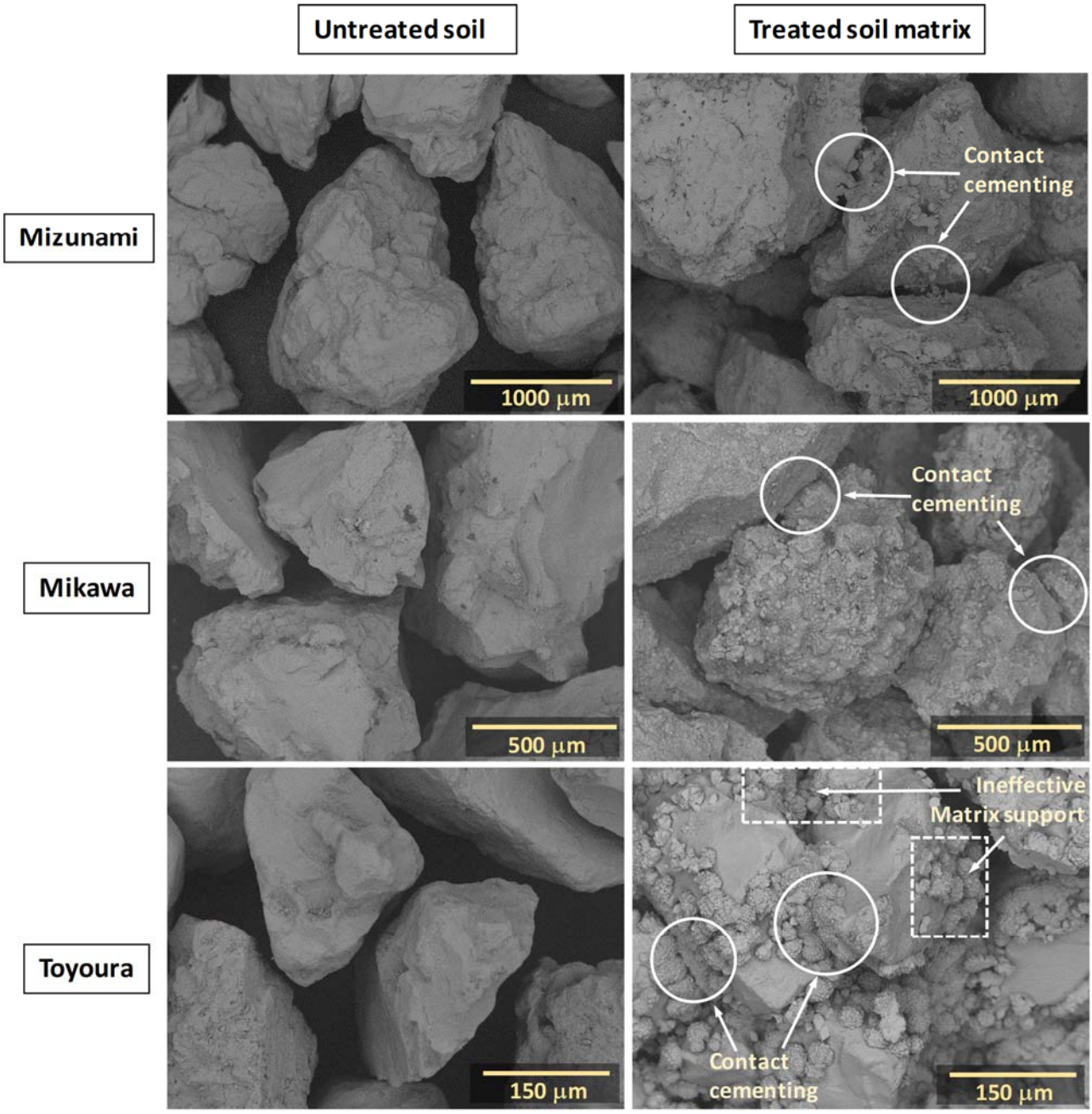

Fig. 14. Scanning electron micrographs of (a) untreated slope soil, (b) treated slope soil matrix and (c) effective matrix support by fine particles slope soil

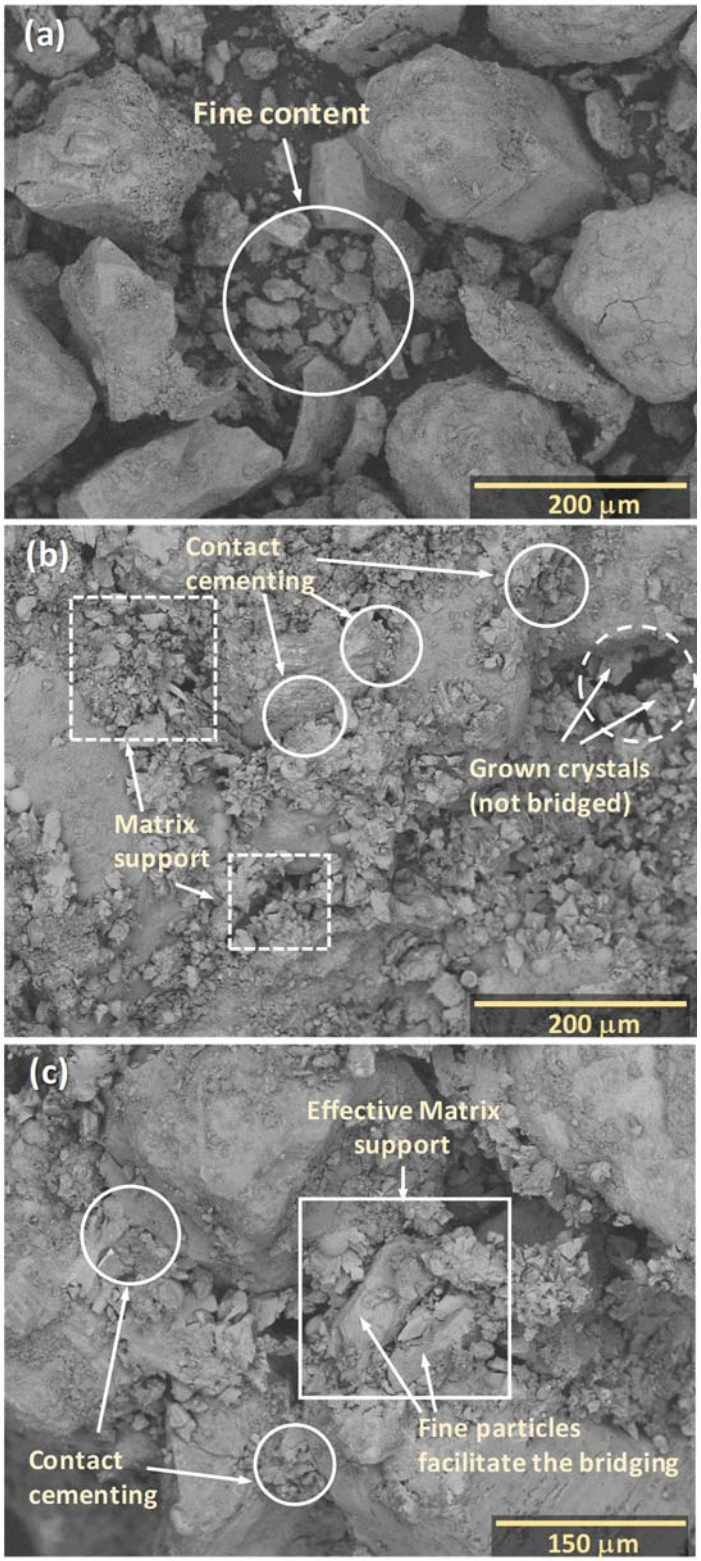

Fig. 15. Profile of the cemented slope model

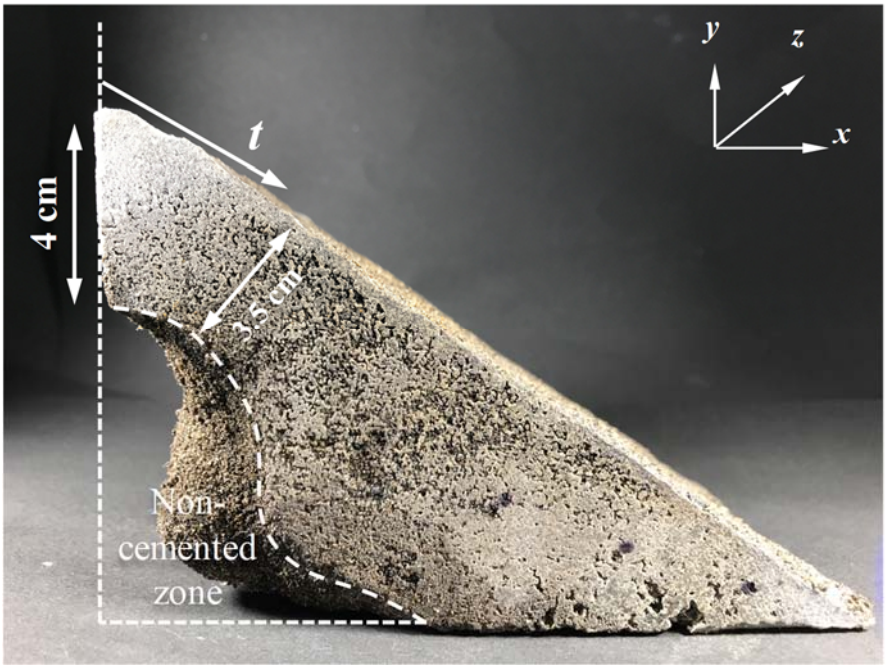

Fig. 16. UCS profile along the surface (*t*-*z* axis) of the treated slope (measured by needle penetrometer)

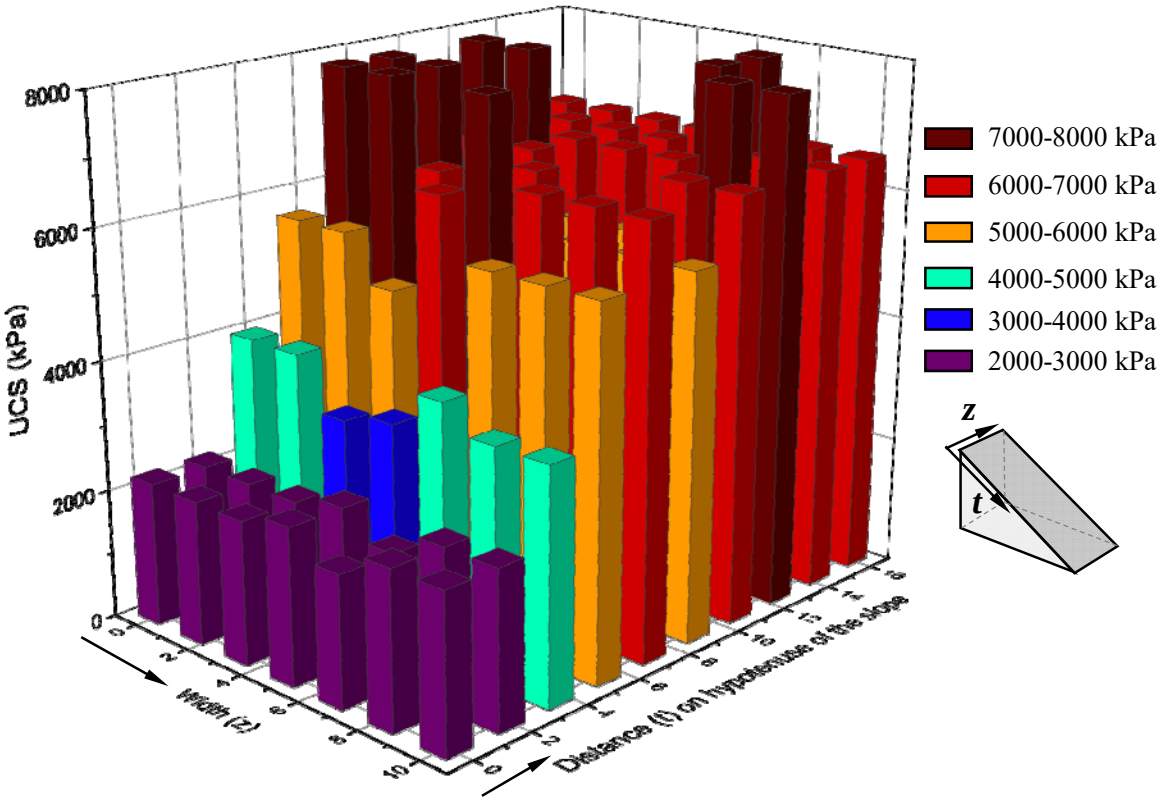

Fig. 17. Distribution of bacteria cells along the depth of the slope (locations A, B and C are indicated in Fig.3)

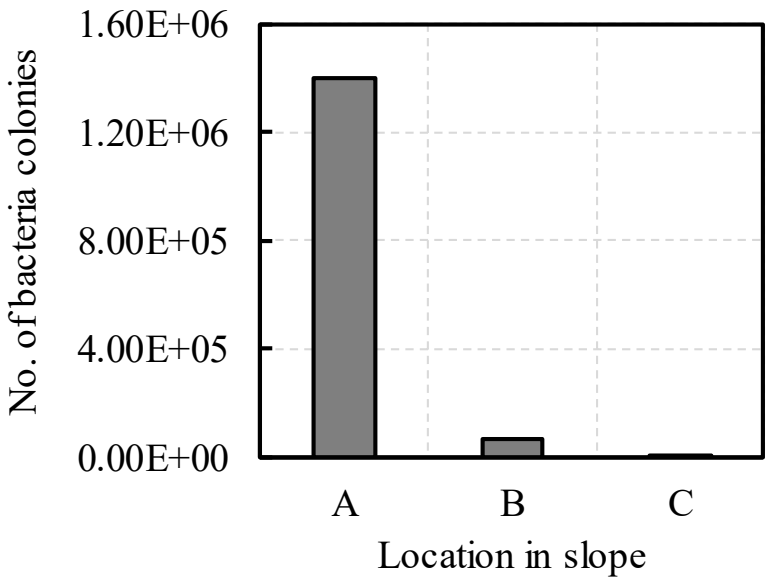

Fig. 18. Relationship between UCS verses calcium carbonate content in slope soil