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Biogeotechnical Approach for Slope Soil Stabilization Using Locally Isolated Bacteria and Inexpensive Low-grade Chemicals: A Feasibility Study on Hokkaido Expressway Soil, Japan.

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Abstract

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

Key words: *Microbial Induced Calcite Precipitation, indigenous bacteria, pure chemicals, low-grade chemicals, slope soil stabilization.*

1. INTRODUCTION

A promising soil stabilization technique that has recently gained the interests of many researchers is bio-cementation based on Microbial Induced Calcite Precipitation (MICP) (Chiet et al., 2016; DeJong et al., 2010; Ng et al., 2012; Oliveira et al., 2016; van Paassen et al., 2010). The mechanism of improvement can be described as the method of obtaining crystallization of calcium carbonate ($CaCO_3$) to cement soil particles by enzyme urease of ureolytic bacteria. Initially, enzymatic hydrolysis of urea releases ammonium and carbonate ions in the medium (Eq. 1) while increasing the pH. The produced carbonate ions precipitate in the presence of calcium ions as calcium carbonate crystals on the surface of soil (Eq. 2). The precipitated carbonate may cement the particle contacts, clad the particle surface, fill the pores with or without bridging the adjacent soil particles and eventually stiffening the soil matrix. The bacteria play another important role that the cell surfaces of bacteria are typically negatively charged, which attract the Ca^{2+} ions, and provides nucleation spots for calcium carbonate precipitation (Bao et al., 2017).

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

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

Among the wide range of applications of MICP, slope soil stabilization is getting increased attention as slopes are frequently associated with transportation systems (Bao et al., 2017; Jiang and Soga, 2017; Salifu et al., 2016). Surface erosion is one of the key challenges in stability of slope soil, which occurs due to complex interactions of sub processes between detachment and transport of surface materials (Dai et al., 2018; Zhang et al., 2018). It has been reported that the sediment yield and runoff production of a slope, are significantly impacted by soil texture, slope topography, rainfall intensity and cover condition (Fox et al., 1997; Qing-quan et al., 2001; Rieke-Zapp and Nearing, 2005; Zhang et al., 2018). Since the slopes are typically in unsaturated state, part of the flow infiltrates the slope, which may increase the pore water pressure and seepage forces within the slope. When the water table is close to the surface of the slope, rise in pore water pressure diminishes the effective stress of the slope soil, thus

reduces the soil strength and may trigger the slope failures (Harden and Scruggs, 2003; Muntohar and Liao, 2010). Also, the infiltration decreases the contribution of matric suction by lessening the negative pore-water pressure and effective stress, thus results the loss in strength of the slope soil, and the effect is crucial in slopes of fine grained soil. Eventually, the failure of embankment slopes can cause direct damages in transportation system, such as pavement drop-off, washout of expressway shoulders, failures of embankments, etc. hence increase maintenance, and risks to the travelling public (Bao et al., 2017). It is well understood that the surface treatment plays a vital role to promote the cover condition of the slope by achieving the aggregate stability and infiltration control. The conventional materials like geotextiles, wire meshes, cable nets, membranes, sheets or nails which were physically installed to promote slope enforcement, are often expensive and consume high energy (Salifu et al., 2016), whereas chemical grouting methods are reported as environmental-unfriendly and unsuitable for large-scale applications (Gomez et al., 2013). Thus, an alternative remedial action for slope soil stabilization, implementation of a bio-cement zone of MICP along slope surface has been considered in this paper.

Up to now, many studies have focused on MICP based soil stabilization in order to mitigate the potential of erodability. Most of them have been performed based on bio-augmentation strategy by introducing non-native ureolytic bacteria to the soil. In such, *Sporosarcina pasteurii* is the most researched bacterium which enables a highly active urease enzyme associated with urea hydrolysis (Gomez et al., 2013). The solidification of sand using *S. pasteurii* shows a significant control of surficial sediment erosion (Bao et al., 2017; Salifu et al., 2016), and reduces the hydraulic conductivity while increasing the confined compressive strength (Jiang and Soga, 2017; Whiffin et al., 2007). Also, *S. pasteurii* has formed an impermeable stiff crust with the thickness of 2.5 cm which exhibits increased resistance to erosion (Gomez et al., 2013). Cheng et al. (2014) have reported that *Bacillus sphaericus* can enhance the strength of silica sand with relatively retained permeability, when 10 mM urea concentrated artificial sea water was used as cementation solution. However, bio-augmentation of exogenous bacteria which have not been adapted to native environment, is associated with uncertainties in bacterial survival and performance (Sensoy et al., 2017). On the other hand, a very few studies have been attempted to investigate the feasibility of bio-augmentation using indigenous bacteria (Danjo and Kawasaki, 2016;

Khan et al., 2016; Stabnikov et al., 2011). Actually, bio-augmentation (process includes isolation of bacteria, culture enrichment, and supply back into the ground) conceptionally differs from bio-stimulation strategy in which the indigenous ureolytic bacteria are stimulated *in situ*. Danjo and Kawasaki (2016) and Khan et al. (2016) investigated the feasibility of artificial beach rock formation as the mitigation measure for coastal erosion by augmenting native *Pararhodobactor sp*. isolated from the native sand of Okinawa, Japan. Similarly, using native *Bacillus sp.* VS1, relatively impermeable biocemented crust (maximum flexural strength of 35.9 MPa; permeability of 1.6×10^{-7} m/s) has been achieved on the native sand surface for the aquaculture pond construction (Stabnikov et al., 2011). Particularly, using the natively adapted bacteria would be more effective and appropriate for the regions with fluctuating cold climatic conditions, as the urease enzymes of most of the bacteria are temperature-sensitive and can readily be denatured by changes in the environmental temperature. However, due to the uneven presence of ureolytic bacteria in natural soil, stimulating the indigenous bacteria would result diffuse zone of improvement with higher variability even within the same region (Gomez et al., 2017). At the same time, augmentation has often resulted more uniform improvement but more localized due to the reduction in soluble calcium concentrations transported to greater distances (Danjo and Kawasaki, 2016; Gomez et al., 2017). As this research aims for a uniform surface stabilization of slopes, bio-augmentation of indigenous bacteria can be the better choice among the strategies discussed above.

In fact, feasibility of MICP does not depend on technical aspects regarding conditions of treatment alone but accompanies with economical and legislative issues as well. It has been reported that discharging of vigorous chemicals and ammonium by-products to the soil-ecosystem in the MICP causes many harmful effects, and the subsequent removal of such harmful products must be taken into account (Soon et al., 2014). Cost of required substances remains another contest in assessing the complete feasibility of the process (van Paassen et al., 2010). In this research, feasibility of MICP phenomenon in slope soil stabilization has been focused at a preliminary stage using small-scale laboratory experiments. The prime objectives of this study are to investigate the efficiency of MICP process (i) by using locally isolated bacteria, and (ii) by introducing commercially available low-grade

chemicals instead of pure chemicals. The flowchart of the complete research design up to the real scale application is presented in Figure 1, and the focus of this article is clearly outlined. Laboratory experimentations of different scales listed in Figure 1, are conceptually represented in Figure 2, in which each laboratory experiment simulates the surface zone of the slope to be treated at different scales.

2. MATERIALS AND METHOD

2.1 Identification and Isolation of Ureolytic Bacterium

Three potential locations of expressway slopes (Asari, Onuma, Asahikawa) of Hokkaido, Japan were considered in this research. The samples from considered locations were collected in sterile test tubes, transported to laboratory and refrigerated at 4°C. Intrinsic properties and compositions of soils are summarized in the Table 1, and the grain size distributions of the soils are compared in Figure 3 with Standard Mikawa sand. For microbial identification, 5.0 g of the refrigerated natural soil sample was taken and mixed with 45 mL of autoclaved distilled water, followed by diluted 10^1-10^4 times using autoclaved distilled water in separate autoclaved sterile test tubes. Subsequently, 10 µL of each dilution was applied to NH_4-YE agar medium prepared by combining tris-buffer, ammonium sulfate, yeast extract, ager and distilled water. The cultured plate mediums were permitted into the incubator for 3 days at 30°C, and about 30 colonies were identified in the plate mediums at the end of the incubation.

Cresol-red test solution was prepared by combining 20 mL of cresol red solution, 0.4 g of cresol red with distilled water and 25 g of $CO(NH_2)_2$ with distilled water (for preparation of 1 L solution). Afterwards, each isolated colony was transferred to 20 mL solution, well shaken, and incubated for 2 hours at 45°C. The basis of this urease activity test is that the cresol red changes from yellow to purple when the pH changes from 7.2 to 8.8, thereby confirming the urease activity of the bacteria. Accordingly, the ureolytic bacteria were isolated, and characterized by sequencing their 16S rDNA and comparing the results to sequences available in the Apollon DB-BA 9.0 database, GenBank, DDBJ (DNA Data Bank of Japan) and EMBL (European Molecular Biology Laboratory).

2.2 Laboratory scale solidification test

Biogrouting viability of all three considered slope locations has been demonstrated by recognizing native ureolytic microbes via this research. However, detail investigations regarding MICP efficiency using solidification tests are focused only on Asari slope soil in this paper, and the similar criterion can be applicable to other slope soils which are left for the future work.

Standard syringes (1-4908-07) with diameter of 25.3 mm and height of 139.6 mm were adopted to perform the syringe solidification tests. At each syringe test, 45 g of soil was packed well into the test syringe, and experiment set up was arranged as shown in Figure 4. A two-stage injection was performed, in order to confine the bacteria for the subsequent cementation. At the first stage, bacteria culture was injected to fill the soil column, and cementation solution was injected at the second stage. All the solutions were simply applied to the top of the soil columns and allowed to percolate by gravity and capillary forces. The constituents of both culture and cementation solutions are discussed in detail below.

2.2.1 Culture solution

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

2.2.2 Cementation solution

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

At the end of the treatment, distilled water (five times of sample volume) was applied to each sample in place of bacteria-cementation solutions to rinse out the chemicals from the soil matrix. Syringes were cut carefully, and the samples were taken out from the ports followed by the draining out of solutions. Subsequently, samples were allowed to cure under atmospheric conditions which are same as the treatment conditions. The cementation strength of the specimens was examined using needle penetration device/ soft rock penetrometer (SH-70, Maruto Testing Machine Company, Tokyo, Japan). The needle penetration apparatus is a portable testing device developed in Japan, for predicting the UCS

of soft, weak to very weak rocks and cemented soil specimens. The specimen was horizontally positioned, and the needle of the device was penetrated into cylindrical surface of the specimen at three locations (at the distance of 1 cm (top), 3 cm (middle) and 5 cm (bottom) measured from the column top). The penetration resistance (N) and penetration depth (mm) were measured simultaneously. The UCS of the specimen was estimated using the regression relationship given in Eq. (3) which has been developed by analyzing 114 natural soft rock samples and 50 improved soils with cement (Amarakoon and Kawasaki, 2018; Danjo and Kawasaki, 2016; Fukue et al., 2011; Mitsuyama et al., 2017).

$$\log\ (y) = 0.978\ \log\ (x) + 2.621 \qquad \text{[Eq. 3]}$$

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

2.3 Test conditions

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

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

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

3. RESULTS AND DISCUSSIONS

3.1 Microbial Behavior

Total populations of bacteria in slope soils of Asari, Asahikawa and Onuma were found to be 10^7 cfu/g, 9×10^5 cfu/g, 16×10^5 cfu/g. However, ureolytic potential was witnessed only in few groups of bacterial strains among existed in each location, which are fractionally illustrated in Figure 5. Table 5 shows the identified ureolytic microbes with respect to their native locations. The culture was maintained in shaker at 30°C and 160 rpm, and the growth curves were obtained by monitoring the solutions' optical density at the wave length of 600 nanometers (OD_{600}) with time (Figure 6). In consideration of Asari slope (Hokkaido), *Psychrobacillus* sp. strain, at its highest performance among the identified native microbes, was incorporated for detailed experimentations in this research study. The strain has been characterized by motile rods and grew over a wide range of temperatures (-2 to 40°C) (Pham et al., 2015). Basically, the rate of urea hydrolysis has a direct relationship with the bacterial cell concentration, and high concentration of bacteria produces more urease per unit volume to commence the urea hydrolysis (Ng et al., 2012). But, a converse behavior was observed between cell concentration and the urease activity of *Psychrobacillus* sp., which are given in Figure 7 and Figure 8 respectively. The cell concentration at 20°C is significantly higher than that at 30°C, and the urease activities of strain at 20°C and 30°C are 0.10 U/ml and 0.41 U/ml respectively. Based on the observation, it can be stated that the higher temperature (30°C) inhibits the bacterial growth, whereas lower temperature (20°C) favors. However, 30°C provides more favorable condition for the bacteria to produce the protein subunits correspond to urease activity (urease enzyme) compared to that at 20°C. Similarly, the rate of urea hydrolysis of

Bacillus Megaterium and *Sporosarcina pasteurii* is marginally higher in 30 °C compared to 20 °C, and the optimum activity is found to be at around 30°C (Ng et al., 2012; Omoregie et al., 2017). At the same time, few strains including *Pararhodobacter* sp., *Deleya venusta* and *Strep-tococcus salivarius* exhibit the optimum urease activity at around 60°C (Fujita et al., 2017; Ng et al., 2012). However, it is impractical to utilize the optimum activity of bacteria in MICP treatment, as the in-situ soil temperature lies between only a certain range. Therefore, it is suggested to optimize the activity of bacteria among the feasible temperature range for MICP according to the focus zone. Thus, the solidification tests were considered in detail at the two practicable temperatures of Hokkaido.

3.2 Laboratory scale solidification tests

In order to monitor the chemical condition within the samples being treated, the continuous outlet measurements are highly important at the solidification tests. Accordingly, the Ca^{2+} concentration and pH of the Case 1 and 2 (Mikawa sand) were measured soon after 3, 6, 9 and 10 days, and are presented in Figure 9a and 9b respectively. At the same time, measurements of the cases from 3 to 8 (natural slope soil) were undertaken every 24 hours, and the results are compared in Figure 10a and 10b. pH of the drainage remains higher in Case 1 compared to Case 2 whereas, Ca^{2+} concentration of drainage for the Case 1 shows lower value than Case 2 (Figure 9). Based on the Figure 10, relatively weak alkali pH conditions were maintained during the test period, which increases with time. At the same time, Ca^{2+} concentration of drainage in all the Cases from 3 to 8 reduces with time, though there is an initial raise observed in first few days.

3.3 Effect of saturation

Figure 11 illustrates the effect of saturation in MICP efficiency. It can be observed that solidified sample under saturated condition (Case 1) exhibits higher UCS values compared to that of unsaturated case (Case 2) at all the depths (1 cm, 3 cm and 5 cm) measured from the top surface of the sample. Measurements of outlet ensures that the saturated case drains with higher pH and lower Ca^{2+} concentration compared to that of unsaturated case in most of the time (Figure 9). Sustaining the solutions entirely within soil sample tends to utilize more hydrolyzation of urea (fallouts higher pH),

hence leads to consume much Ca^{2+} ions for calcite precipitation (fallouts lower Ca^{2+} concentration). It has been reported that the highest $CaCO_3$ precipitation and highest strength is potentiated with degree of saturation (%) in sandy soils (Whiffin et al., 2007), which is in line with the results obtained in this study. However, Cheng et al. (2013) have concluded that a higher degree of saturation induces to form $CaCO_3$ crystals ineffectively in pore voids, which sometimes results lower strength. It is worth noting that treating the slope soil under saturated condition is practically impossible in the real field, thus, natural soil was treated totally under unsaturated treatment condition.

3.4 Effect of Temperature

In MICP, temperature is one of the important factors as it highly affects the growth, urease activity of microorganisms and nucleation rate of $CaCO_3$ crystals. In consideration of real field condition, MICP efficiency was investigated at two appropriate temperatures (20°C and 30°C) in this research study. The effect of temperature which is obtained at two concentrations of cementation solution (0.5 M and 1 M) are given in Figure 12. It can be seen that the treated samples exhibit highest UCS at 30°C in both cases. It is due to the higher urease performance of *Psychrobacillus* sp. at 30°C, and the performance of bacteria increases about four times when the temperature rises from 20°C to 30°C. This is consistence with previous observations that number of times strength increment has been obtained at optimal temperature of bacteria compared to that in adjacent temperature range (Amarakoon and Kawasaki, 2018; Danjo and Kawasaki, 2016). Although favorable temperature of microbial performance contributes highest strength in general, size and shape of the formed crystals which affects the strength depends on the temperature range (Mujah et al., 2017).

3.5 Effect of injection solutions

Concentration of cementation solution is also attributed as one of the controlling factors in effective $CaCO_3$ crystal formation. In this study, two concentrations (0.5 M and 1 M) were considered, and it is observed that the UCS of the soil treated using 0.5 M cementation solution were higher than that treated using 1 M solution at both temperature conditions (Figure 12). This is consistent with the previous observation by Ng et al. (2012) that the soil treated with 0.5 M cementation reagent effectively enhanced

the UCS compared to that treated with 1 M. It is clear that lower concentration results more homogeneous crystal formations at contact spots which promotes the strength, whereas high concentration hinders the establishment of effective bonding by rapid and random formation of crystals in soil voids (Mujah et al., 2017).

Further, the cementation solution was injected to the syringe every-day basis similar to that reported in previous studies (Amarakoon and Kawasaki, 2018; Danjo and Kawasaki, 2016). Moreover, culture solutions were injected only at the beginning in Case 3 and 5, whereas it was injected every-day basis in Case 4 and 6. It can be seen that the better treatment efficiency is coupled with the single injection of bacteria (Cases 3 and 5). Similar observation is reported by Amarakoon and Kawasaki (2018), in which single injection of bacteria exhibited about two times higher UCS compared to that of reinjection case.

3.6 Low-grade chemicals in MICP

Potential attempts (Case 7 and 8) were undertaken to study the solidification effect of natural soil by incorporating the commercially available low-grade chemicals. Basically, the purity is the prime difference between standard pure chemicals and low-grade chemicals. Results of elementary chemical analysis obtained from X-Ray Fluorescence (XRF) Spectrometer (JSX-3100R II JOEL, Japan) for the beer yeast, snow melting salt and urea fertilizer are provided in Figure 13-a, 13-b and 13-c respectively. The urea fertilizer is a widely used substance in agriculture industry, comprised of copper, iron and potassium (45.66%, 39.86% and 14.48% respectively). Based on the manufacturers' specifications, the purity of nitrogen in urea fertilizer is upheld at 46.0%. The snow melting agent/ de-icing salt consists of calcium (34.87%), chloride (61.89%), sodium (1.70%) and potassium (1.53%). The purity of calcium chloride is 74% in de-icing salt which is applied as bulk for melting the ice deposited on roads and pavements in winter seasons all over the world. Beer yeast is the potential substance in the food industry, which mainly functions to break down sugars, and to add alcohol to beer as a byproduct of the process. 100g of beer yeast consists of nutrients such as 48.6g of protein, 4.2g of fats, 39.4g of carbohydrate, 6.1g of sugar, 33.3g of dietary fiber and 6.2g of ash in general. Also, the XRF analysis reveals that the

beer yeast consists of potassium (38.59%), phosphorus (37.15%), sulfur (16.77%), calcium (6.82%), iron (0.45% and copper (0.23%). The attempts aimed to investigate the MICP feasibility of the above low-grade chemicals which are inexpensive and less harmful to the geo- environment compared to that of discharging pure chemicals.

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

Figure 15 shows the comparison of obtained UCS versus $CaCO_3$ content for the samples treated using pure chemicals (Case 3) and low-grade chemicals (Case 7 and 8). It is clearly perceived that the UCS value obtained by implementing low-grade chemicals at 30°C is around two times higher than that of UCS (0.417 MPa) obtained by implementing pure chemicals. As expected, precipitated $CaCO_3$ content in pure chemical case (9.5%) is marginally higher compared to the low-grade chemical case (9.3%), which is due to the difference in purity among those chemical substances. But, the precipitated carbonate contents (9.3% and 9.5%) reported herein are relatively higher than the results reported in literatures (Feng and Montoya, 2015; Lin et al., 2016a). Feng and Montoya (2015) investigated the effect of cementation level (from 1 to 3.5%) in MICP treated sand under monotonic drained conditions and reported that the heavy cementation level (above 3.5%) exhibited significant strain softening, although higher cementation level resulted higher stiffness, strength and dilative tendencies. Also, Lin et al. (2016a) and Lin et al. (2016b) have focused on drained response of MICP treated sand and improving the axial capacity of permeable piles respectively with carbonate content in the same range from 1 to 3%. The discrepancy between $CaCO_3$ content addressed in this paper and literatures can be explained by many factors such as different grain size distribution, density of the soil, particle shape, and morphology of the precipitates. The grain size distribution plays a crucial role in spatial distribution of carbonate cement in pore spaces and effective formation of bonds at particle contacts (Cheng et al.,

2014; Lin et al., 2016a; Oliveira et al., 2016). Actually, MICP is not very effective for the gravelly type of soil since the thin layer of $CaCO_3$ precipitated at the contact zone of larger particles is not sufficient to link them (Kim et al., 2014; Rebata-Landa, 2007). The soil from Asari slope (Hokkaido, Japan) is a well graded soil, consists of significant amount of coarse material including gravel content of 20-25% ranges from fine to medium (Figure 3). Thus, relatively a higher carbonate content was required to bond the large soil particles, hence to obtain the required strength in natural slope soil.

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

Moreover, for the same amount of $CaCO_3$ precipitation, UCS of soil treated using low-grade chemicals exhibits significant value compared to that of pure chemicals. This result is contrary to previous understanding that higher $CaCO_3$ content can contribute higher strength for a soil treated under same conditions (van Paassen et al., 2010; Whiffin et al., 2007). During the MICP process, precipitated carbonate crystals deposit at particle contacts, coats the exposed surface of soil particles, fills the voids and provides a matrix supporting to the soils as clearly explained by Lin et al. (2016a). The formation of crystals at contact points and their crystallographic pattern play the significant role in contributing strength to the cemented soil. Using shear wave velocity measurements, it has been proven that the distribution of $CaCO_3$ at particle contacts of MICP treated specimens can vary at the equivalent

precipitated $CaCO_3$ content (Feng and Montoya, 2017; Qabany et al., 2011; Weil et al., 2012). Thus, the strength response is governed by not the average $CaCO_3$ precipitated, but the effective $CaCO_3$ precipitated at the particle contacts (Feng and Montoya, 2017), and the amount of effective $CaCO_3$ is influenced by the number of bacteria attached at particle contact during the treatment (DeJong et al., 2010). Moreover, Cheng et al. (2014) have reported that the crystals which formed under the unfavorable bacterial condition, would be inadequate and inefficient to form the effective bridges at particle contacts. In this study, although the precipitated crystal content is low in the specimen treated using low-grade chemicals, crystals were driven to support the soil matrix effectively and contributed higher UCS compared to the soil treated using pure chemicals. However, the matrix supporting mechanism is not clear, thereby Scanning Electron Microscopy (SEM) was performed to make a clear understanding by which the soils treated under low-grade chemicals exhibited higher strength.

Moreover, as to bring out a clear economical understanding, a cost comparison is performed in detail using the market price of reagents (in Japan), and presented in Table 6. Total reagent cost of the 10 days of MICP treatment for the 1 m^3 soil using pure chemicals and low-grade chemicals are estimated as 11,972 USD and 468 USD, which is in fact 25 times advantages economically. While resulting a 96% of cost reduction, approach upsurges the strength significantly, and this would be certainly a beneficial turning point for the industrial level future MICP advancements.

3.7 Scanning Electron Microscopy Analysis

Microscopical observation using SEM was performed to study the microstructural effects of treatment and to observe cementation matrix using SuperScan SS-550 (Shimadzu Corporation, Kyoto, Japan). Samples were washed, and oven dried at 105°C for 24 hours initially, and observations were made at the solidified zone ranges up to 1 cm depth of treated samples of different cases to support the effects of treatment.

Photomicrographs of the sample treated under conditions 30°C, 0.5M (Case 1) shows the precipitation of irregular rhombohedral crystals ranges 12-20 μm (Figure 16a), which is the typical form of crystalline calcites, and is corroborated by the similar observations made by Kim et al. (2014) and Soon et al.

(2014). In contrast, crystallization obtained at the samples treated under 20°C (0.5M and 1M) (Figure 16b and 16c) shows different morphology. The particles show a spherical appearance with an average size of the spherical crystals around 15-20 μm, which are suspected to be vaterite crystals. It has been clearly reported that the vaterite crystals (can be solid or hollow) are surrounded by very abundantly accumulating nano-crystals called extracellular polymeric substances (EPS) with a large range in general (Chen et al., 2017; Sensoy et al., 2017; Tolba et al., 2016), and the similar accumulation is evidenced in Figure 16b, 16c and 16d. Generally, vaterite is the least stable form of crystalline $CaCO_3$ compared to the calcite, and can be transformed into calcite very rapidly with the time (Braissant and Verrecchia, 2002; Tolba et al., 2016). Although vaterite crystallization is promoted by kinetic effects (high supersaturation; role of organic compounds) and thermodynamic effects (energy minimizations; ionotropic effects) (Rodriguez-Navarro et al., 2007), it has been proved that vaterite precipitation is strain-specific (Braissant and Verrecchia, 2002; Sensoy et al., 2017). Based on the observations, it can be stated that calcite crystals have been precipitated under the conditions of 30°C, 0.5M urea and 0.5M $CaCl_2$, which tended to participate the contact segments, induced a strong bonding effect among soil particles, and resulted higher strength in treated sample (Case 3). At the same time, the vaterite has been resulted under conditions including 20°C, 1M urea, and 1M $CaCl_2$ (from Cases 4 to 6) hence formed weaker bonds compared to the calcite bonds. In previous observations, *Sporosarcina pasteurii* has produced vaterite and amorphous EPS at the conditions of 30°C, 0.33M urea, 1M $CaCl_2$ at the pH range of 6.5-8.17, whereas *Bacillus aerius* has resulted vaterite, calcite and EPS together at the conditions of 20°C, 0.3M urea, 1M $CaCl_2$ at the pH range of 5.5-9.28 (Sensoy et al., 2017). Moreover, Braissant and Verrecchia (2002) have concluded by considering *Xanthobacter autotrophicus* and *Alcaligenes eutrophus*, that the precipitation of vaterite instead of calcite is occurred due to the absence of exopolysaccharides produced by bacteria between pH 7 and 9.5. It can be understood that there is a great influence of physio-chemical conditions in microbial-induced different crystallizations, but exact formation mechanism of vaterite crystals still remain to be explored clearly.

On the other hand, observed morphologies of sample treated using low-grade chemicals (Case 7) are presented in Figure 16e and 16f. But, crystallization was unable to characterize or distinguish through

SEM clearly, thus, XRD analysis was performed to build an understanding on crystallization, and results are given in Figure 17. XRD results confirmed that the precipitated crystals were predominantly calcite. However, the typical crystalline form of calcite was not identified anywhere in the treated soil matrix. But instead, a well packed matrix combined of soil particles and precipitates were widely observed in the treated specimen (Figure 16e and 16f). This unusual formation of compacted matrix is suspected to be due to the presence of polymeric substances (PS) in low-grade chemicals, and the formed calcite precipitates might encapsulate with the PS, vastly filled the void spaces, bonded the soil particles in a compacted way, hence resulted the significant improvement in strength. The above statement is supported by the observation of voids of the treated matrix. For the same treatment conditions and precipitation content (9.3% and 9.5%), soil treated using low-grade chemicals exhibited significant reduction in voids compared to that of laboratory chemicals (Figure 16a and 16e respectively). This indicates that the PS from low-grade chemicals could be encapsulated with precipitated carbonate cement, which filled the void spaces largely and contributed significant matrix support. However, a detailed future investigation is required to further clarify the discrepancies in calcite crystal morphology obtained at the implementation of low-grade chemicals.

3.8 Calcium carbonate cement and strength of soil

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

$$y = 149.35\,x^2 - 7.53\,x \qquad \text{(Eq. 4)}$$

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

On the other hand, MICP treated well-graded soils exhibited higher strength compared to that of uniformly-graded soils, which is primarily due to the effect of increased number of particle contacts (Cheng et al., 2014; Oliveira et al., 2016). Effective distribution of particles (wide range of sizes) in well manner results lesser the void spaces, increases the number of contact points, and improve the strength of the soil as compare to uniformly graded soil. Although the slope soil considered in this study comprises gravel material of 20-25%, presence of large range of particles from fine to coarse compensates the MICP behavior. In fact, MICP can be significantly governed by fine content in a soil matrix. Zamani and Montoya (2018) reported that the effect of silt content is negligible if the silt content is less than 8%, and only the sand skeleton would govern the MICP behavior. Furthermore, effect of silt content is significant between 20% and 35%, resulting in a large number of contact points which supports the force chain in cemented soil matrix. In Asari slope soil, the fine content was ranged from 1-2%, which was inadequate either to fill in voids of the sand matrix effectively or to increase the number of particle contacts. It is well understood that despite having the same amount of $CaCO_3$ content, the mechanical response of MICP treated soil can vary depending on the number of particle contacts taken part in cementation.

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

4. LIMITATIONS AND FUTURE DEVELOPMENTS

In most of the considered cases of this study, solidification was detected over the length of samples, which suggested that the bacteria and reagents were utilized along the samples. However, the obtained UCS values declined with increasing sample depth in all the cases, which evidenced that relatively high amount of calcium carbonate precipitated at the injection-top zone of the sample and declined with the depth. Generally, the solutions are utilized more effectively in top zone of the sample which is closer to the injection point compared to the bottom (Whiffin et al., 2007), and diminished their performance continuously with the depth, hence reveals decreasing solidification effect with depth, which has been evidenced by the results (Figure 11, 12 and 14). Also, Martinez et al. (2013) have suggested that the distribution of bacteria is the most important factor in achieving uniform solidification. Also, *Psychrobacillus* sp., an aerobic bacterium (Pham et al., 2015), results poor performance in bottommost zone of samples due to absence of oxygen (Braissant and Verrecchia, 2002). In this study, a stiff zone of about 3-5 cm thickness was formed at favorable conditions with the average global calcite content of about 9%. However, it has been reported that a relatively shallow treatment depth of 10-20 cm is required for the stabilization of embankment/ expressway slopes for the protection from erosion (Salifu et al., 2016). Moreover, solidified sand crust of 2.5 cm in thickness (calcite content of 2.1%) exhibited a better resistance for a non-standard field erosion test (water spray for 1 minute at 22.7 liters per minute from 1 m above ground surface) (Gomez et al., 2013). It is well understood that MICP efficiency with depth of treated soil is one of the widely observed limitations, and more investigations have to be

performed to optimize the injection systems/ methods thereby to enhance the homogeneous treatment perceptions. On the other hand, an economically and environmentally beneficial approach was undertaken in this study by introducing low-grade chemicals in place of pure chemicals. However, only a limited understanding regards to the effect of low-grade chemicals in crystallization mechanism was obtained, hence further explorations should be performed in order to corroborate the deeper understanding regarding reliability and morphological effects of treatment. Moreover, scaling-up is highly required for the next steps (Figure 1 and 2) to evaluate and overcome the limitations posed by boundary effects so that to promote this approach in industrial-scale applications. Spraying method may be one of the suitable options for the large-scale applications compared to the injection method incorporated in this study. However, this small-scale investigation would be highly essential to experience the feasibility as well as to enable the optimization of treatment before scaling-up.

5. CONCLUSION

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

1. *Psychrobacillus* sp., native ureolytic bacteria found from slope soil (Asari, Hokkaido), was demonstrated for the MICP potential, and a better urease activity was found to be at 30°C compared to that at 20°C. The favorable treatment conditions for the significant strengthening of slope soil were 30°C, 0.5M concentration of cementation solution (daily injection), and injection of culture solution only at the beginning.
2. Introducing low-grade chemicals in place of laboratory-grade pure chemicals exhibited a remarkable enhancement in strength, which results the UCS value two times higher than that obtained while using pure chemicals. In economical point of view, this innovative alternation cuts down the material cost by 96%, while enthusing the focus of MICP in a way to control the leaching of harmful chemicals.

3. Surface strength of treated samples was correlated with global average calcium carbonate content precipitated in samples of slope soil. However, carbonate precipitation content is not the governing factor of strength but rather the special distribution and the effective formation of cement at the particle contacts. For the same amount of average $CaCO_3$ content, the mechanical response of MICP treated soil can vary. Moreover, particle size distribution of slope soil (particularly, presence of fine to medium gravel material) governed the number of particle contacts and carbonate content for required strength. The apparent minimum calcium carbonate content that required for a measurable increase in strength of natural soil was 5 %.
4. On the whole, MICP process consists a significant potential in improving the residual strength by solidification of soil, thereby strategy can be executed for slope soil prevention as an economically feasible alternative after the appropriate scale-up experimentations and environmental impact analysis.

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Table 1: Intrinsic properties and compositions of soils

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

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

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

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

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

Table 4: Test conditions for syringe solidification test

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

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

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

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

Reagents	**Pure Chemicals**				**Low-grade reagents**			
	Substance	Required amount (kg)	Unit price (JPY/kg)	Price (JPY)	Substance	Required amount (kg)	Unit price (JPY/kg)	Price (JPY)
Cementation Solution	Urea	64.30	1580	101,572	Urea Fertilizer	64.30	150	9,643
	$CaCl_2$	118.90	5600	666,000	Snow melting agent ($CaCl_2$)	118.90	124	14,747
	Nutrient Broth	6.40	36600	235,286	Beer yeast	6.40	2,000	12,857
Culture Solution	$(NH_4)_2SO_4$	3.57	1975	7,054	Beer Yeast	7.15	2,000	14,286
	Yeast Extract	7.14	34580	247,000				
	Tris-Buffer	5.62	10833	60,938				
Estimation	Total material cost			1,317,850 (11,972 USD)	Total material cost			51,533 (468 USD)

Figure Captions

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

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

Fig. 3: Grain size distributions of soils

Fig. 4: Syringe solidification test arrangement

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

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

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

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

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

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

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

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

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

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

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

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

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

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

Figure 1

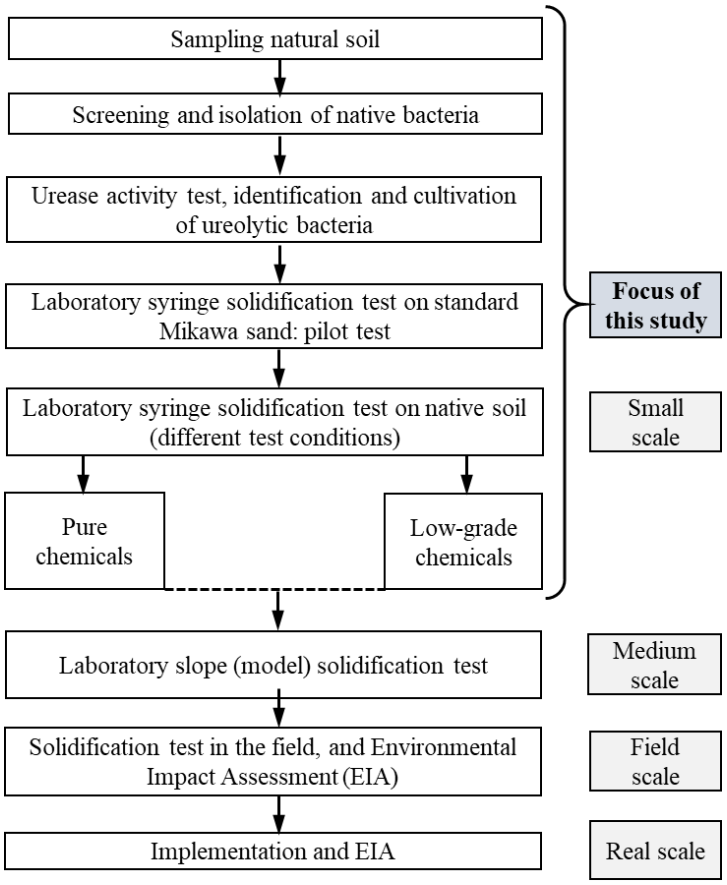

Sampling natural soil
Screening and isolation of native bacteria
Urease activity test, identification and cultivation of ureolytic bacteria
Laboratory syringe solidification test on standard Mikawa sand: pilot test
Laboratory syringe solidification test on native soil (different test conditions)
Pure chemicals
Low-grade chemicals
Laboratory slope (model) solidification test
Solidification test in the field, and Environmental Impact Assessment (EIA)
Implementation and EIA
Focus of this study
Small scale
Medium scale
Field scale
Real scale

Figure 2

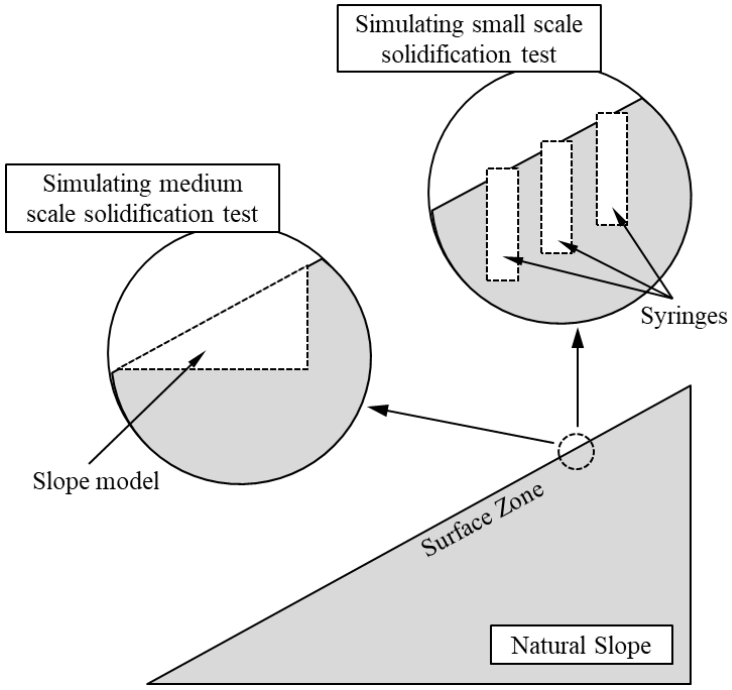

Simulating small scale solidification test
Simulating medium scale solidification test
Syringes
Slope model
Surface Zone
Natural Slope

Figure 3

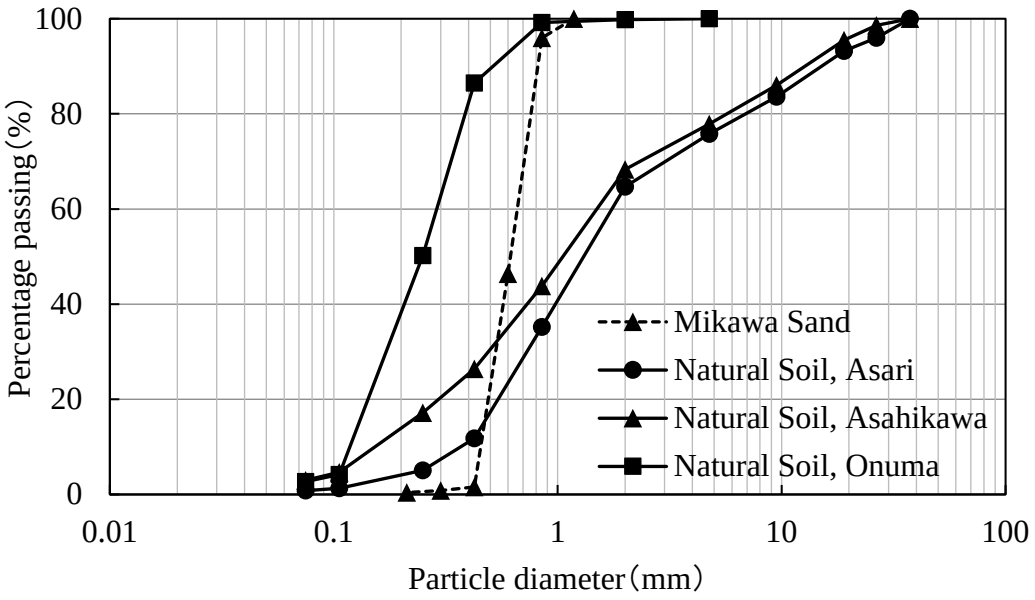

100
80
60
40
20
0
Percentage passing（%）
0.01
0.1
1
10
100
Particle diameter（mm）
Mikawa Sand
Natural Soil, Asari
Natural Soil, Asahikawa
Natural Soil, Onuma

Figure 4

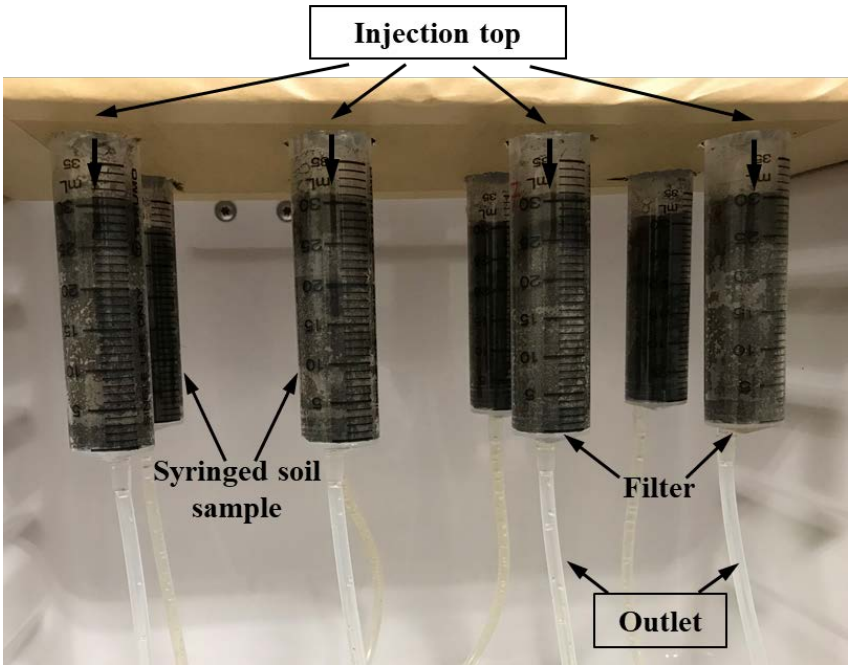


Figure 5

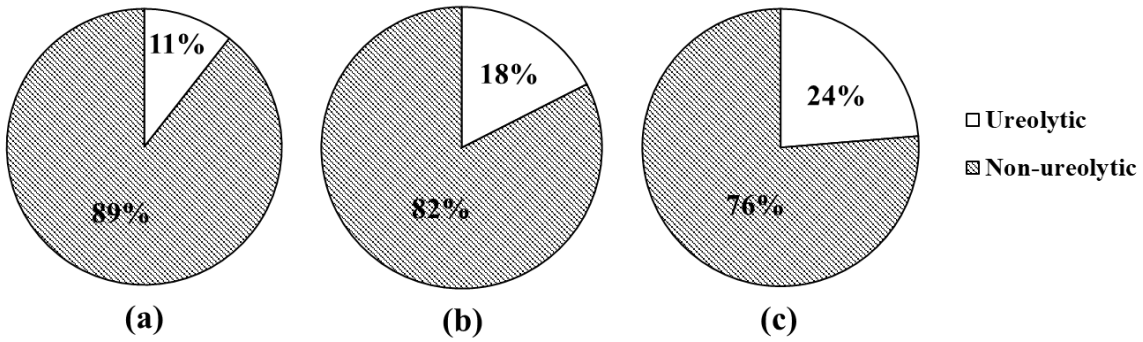


Figure 6

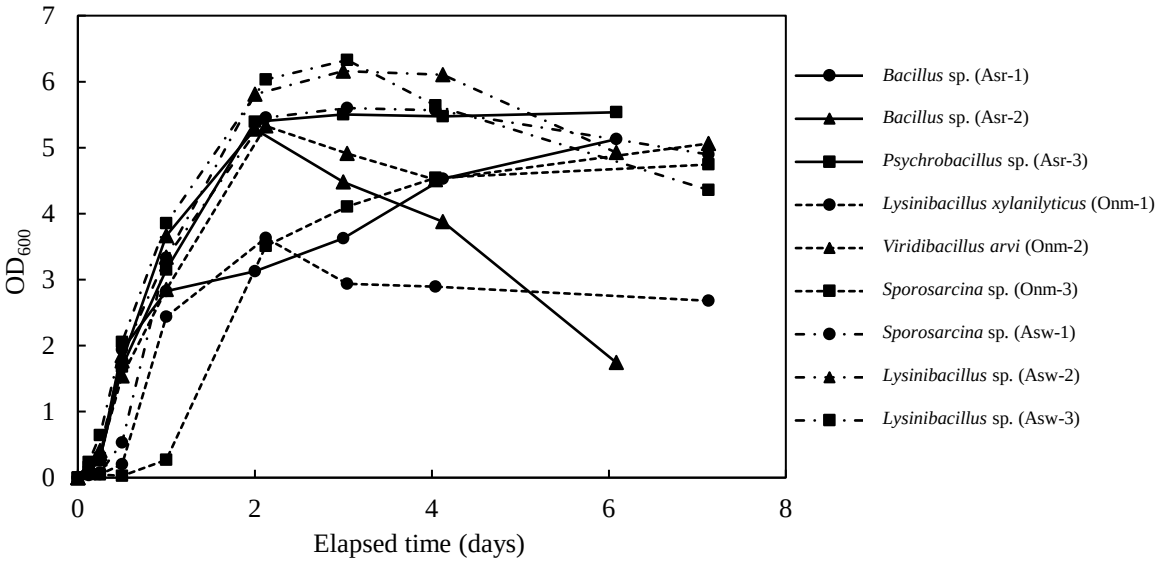

7
6
5
4
3
2
1
0
OD600
0
2
4
6
8
Elapsed time (days)
Bacillus sp. (Asr-1)
Bacillus sp. (Asr-2)
Psychrobacillus sp. (Asr-3)
Lysinibacillus xylanilyticus (Onm-1)
Viridibacillus arvi (Onm-2)
Sporosarcina sp. (Onm-3)
Sporosarcina sp. (Asw-1)
Lysinibacillus sp. (Asw-2)
Lysinibacillus sp. (Asw-3)

Figure 7

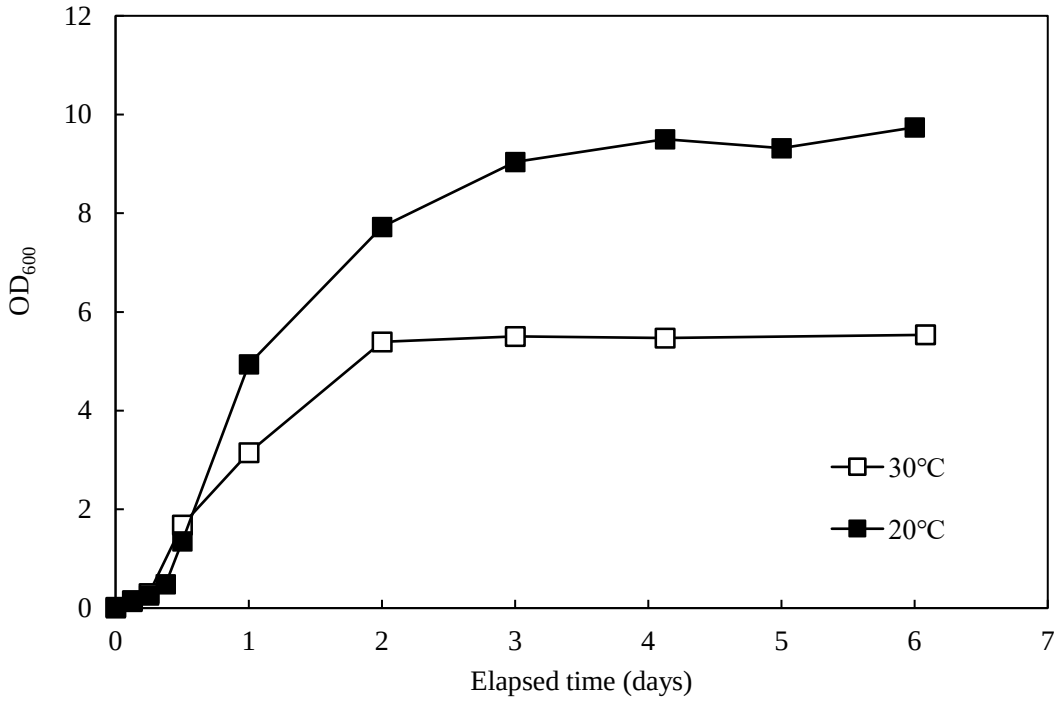


Figure 8

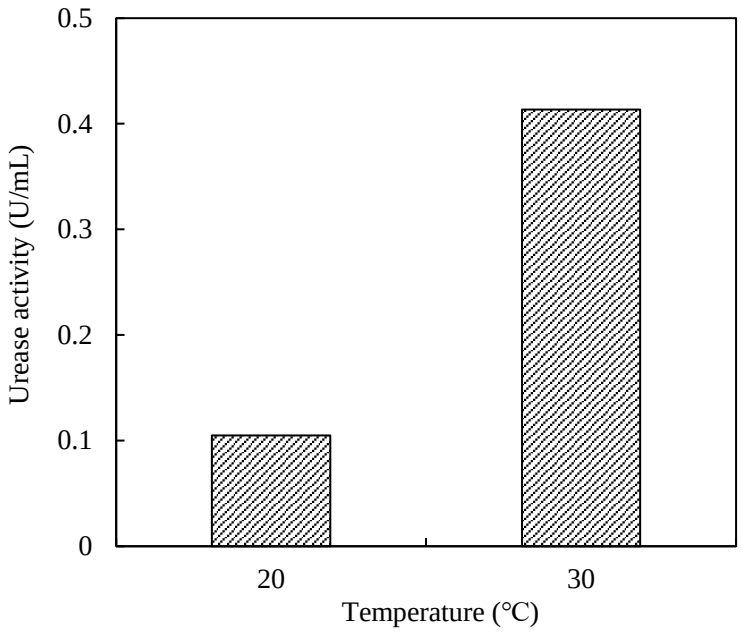


Figure 9

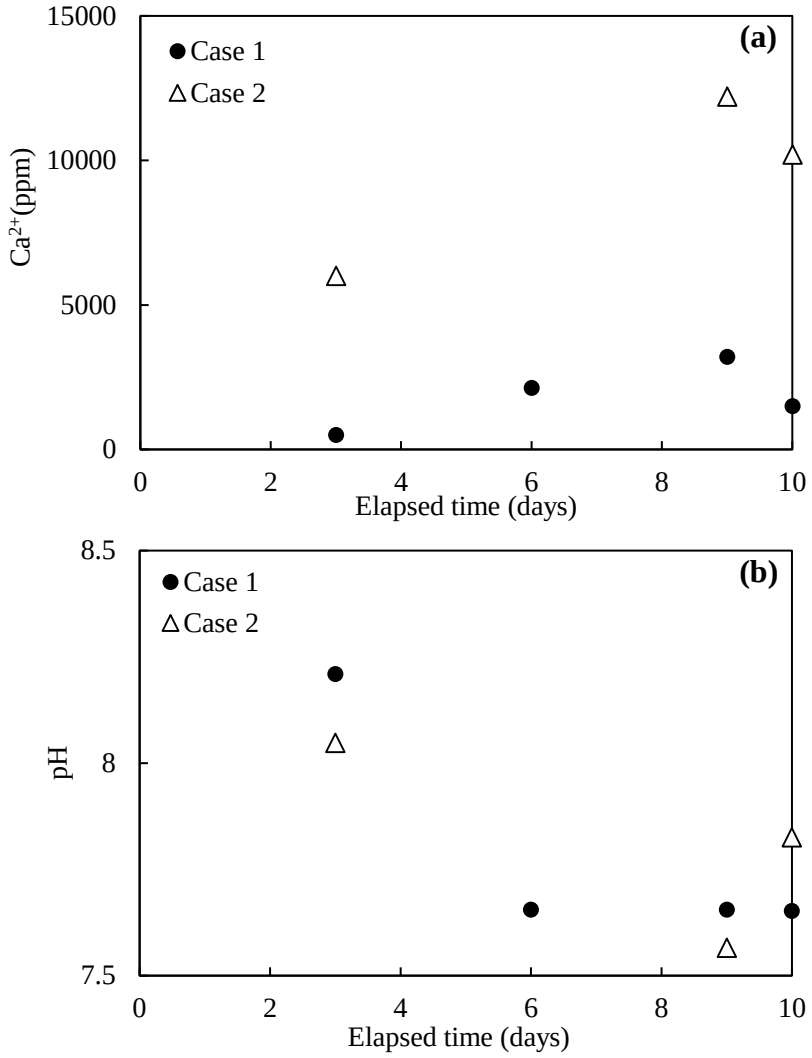

(a)
Case 1
Case 2
15000
10000
5000
0
Ca^{2+}(ppm)
0
2
4
6
8
10
Elapsed time (days)
(b)
Case 1
Case 2
8.5
8
7.5
pH
0
2
4
6
8
10
Elapsed time (days)

Figure 10

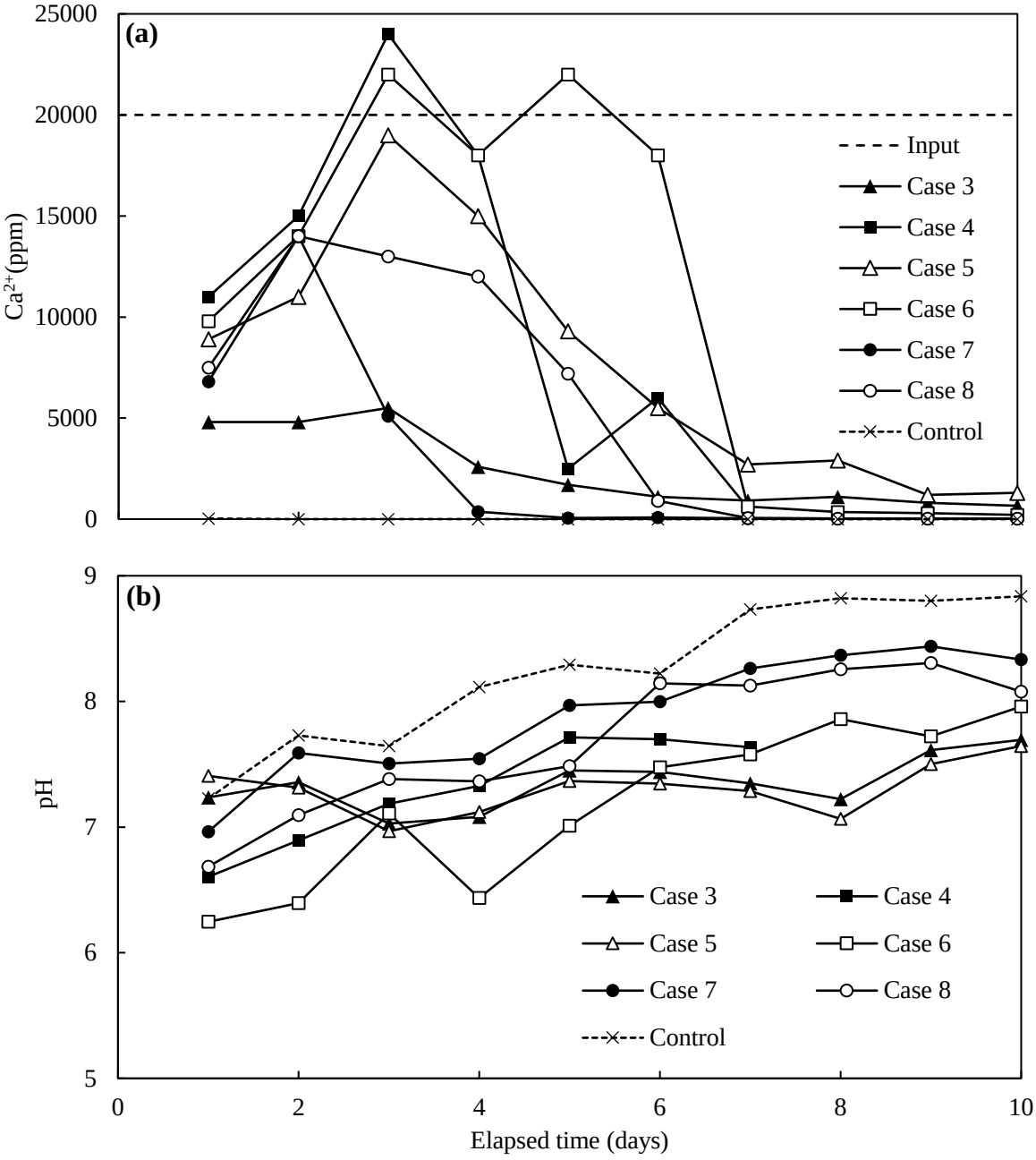

(a)
25000
20000
15000
10000
5000
0
Ca2+(ppm)
Input
Case 3
Case 4
Case 5
Case 6
Case 7
Case 8
Control
(b)
9
8
7
6
5
pH
0
2
4
6
8
10
Elapsed time (days)
Case 3
Case 4
Case 5
Case 6
Case 7
Case 8
Control

Figure 11

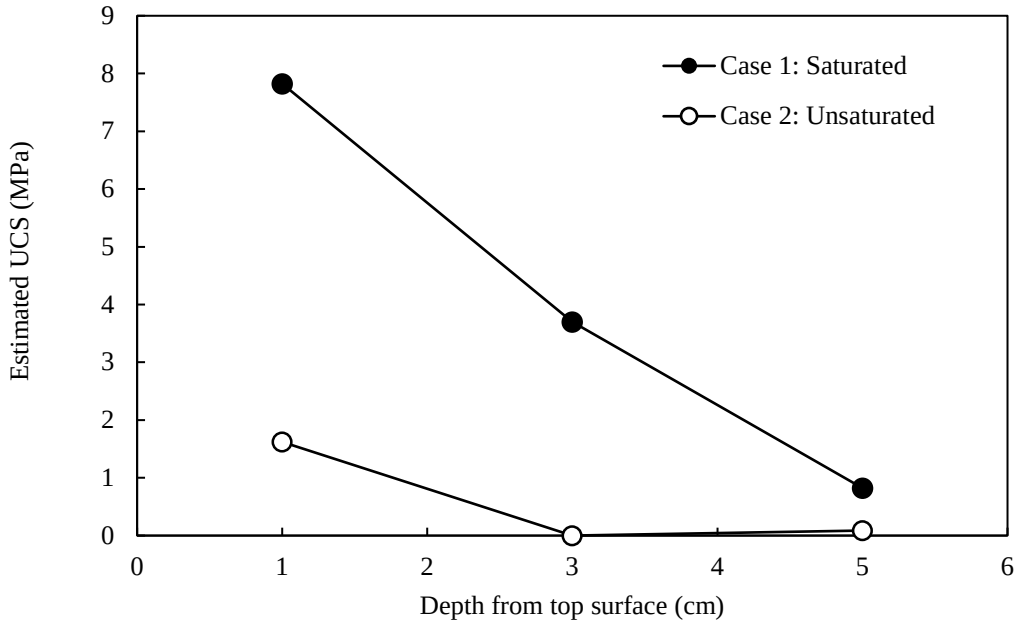

Estimated UCS (MPa)
9
8
7
6
5
4
3
2
1
0
0
1
2
3
4
5
6
Depth from top surface (cm)
Case 1: Saturated
Case 2: Unsaturated

Figure 12

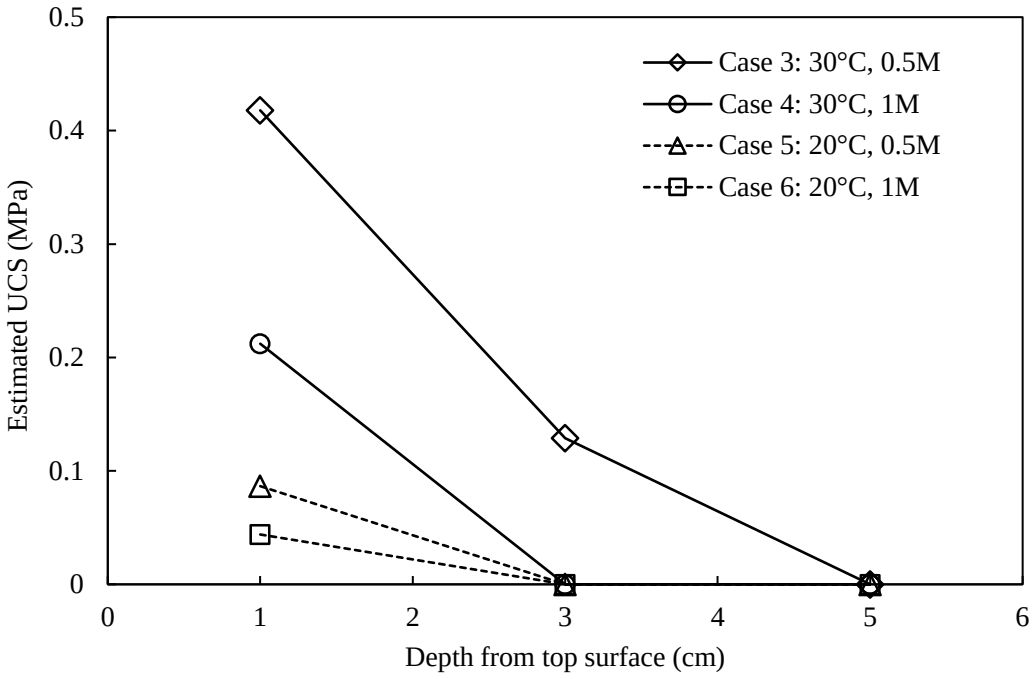

Case 3: 30°C, 0.5M
Case 4: 30°C, 1M
Case 5: 20°C, 0.5M
Case 6: 20°C, 1M
Estimated UCS (MPa)
0.5
0.4
0.3
0.2
0.1
0
0
1
2
3
4
5
6
Depth from top surface (cm)

Figure 13

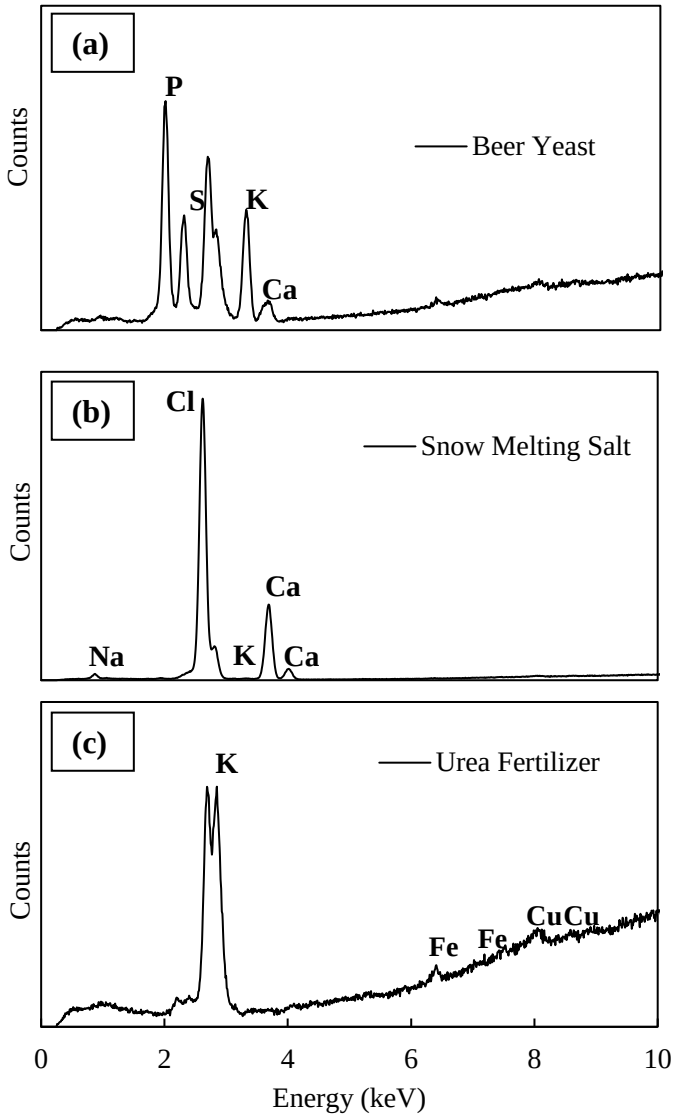

(a)
P
S
K
Ca
Beer Yeast
Counts
(b)
Cl
Ca
Na
K
Ca
Snow Melting Salt
Counts
(c)
K
Fe
Fe
CuCu
Urea Fertilizer
Counts
0
2
4
6
8
10
Energy (keV)

Figure 14

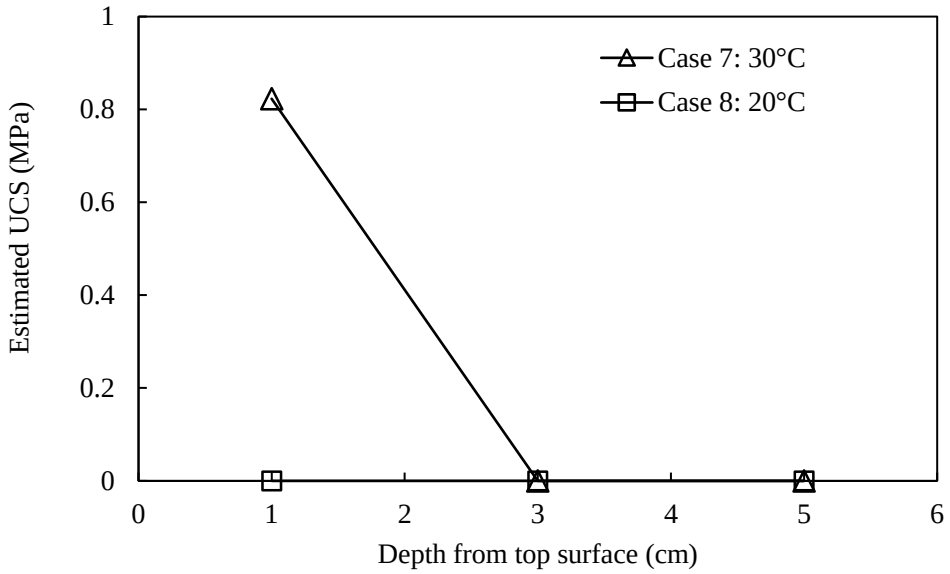

Estimated UCS (MPa)
1
0.8
0.6
0.4
0.2
0
0
1
2
3
4
5
6
Depth from top surface (cm)
Case 7: 30°C
Case 8: 20°C

Figure 15

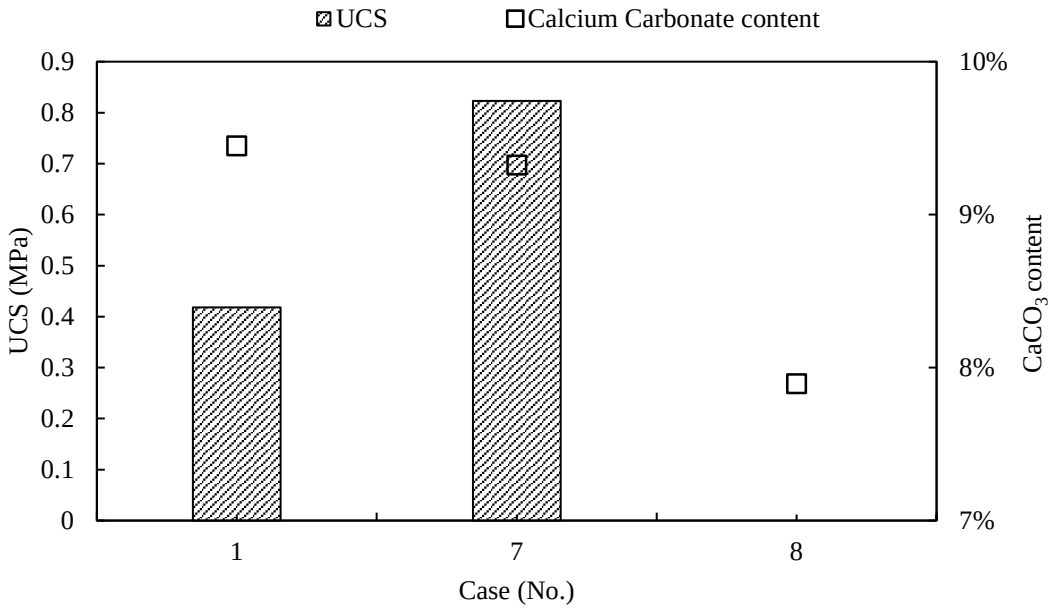

UCS
Calcium Carbonate content
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
UCS (MPa)
10%
9%
8%
7%
CaCO3 content
1
7
8
Case (No.)

Figure 16

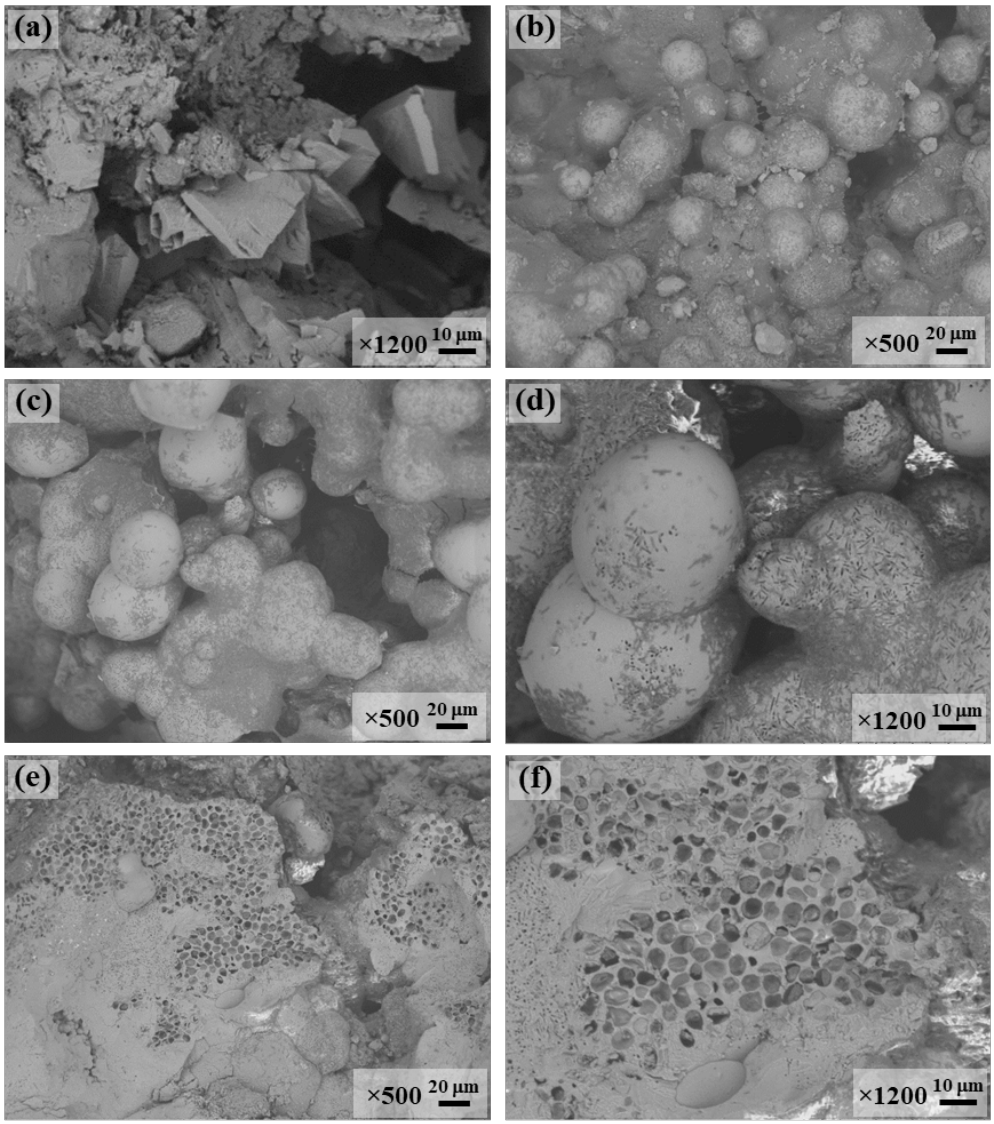

(a)
×1200 10 μm
(b)
×500 20 μm
(c)
×500 20 μm
(d)
×1200 10 μm
(e)
×500 20 μm
(f)
×1200 10 μm

Figure 17

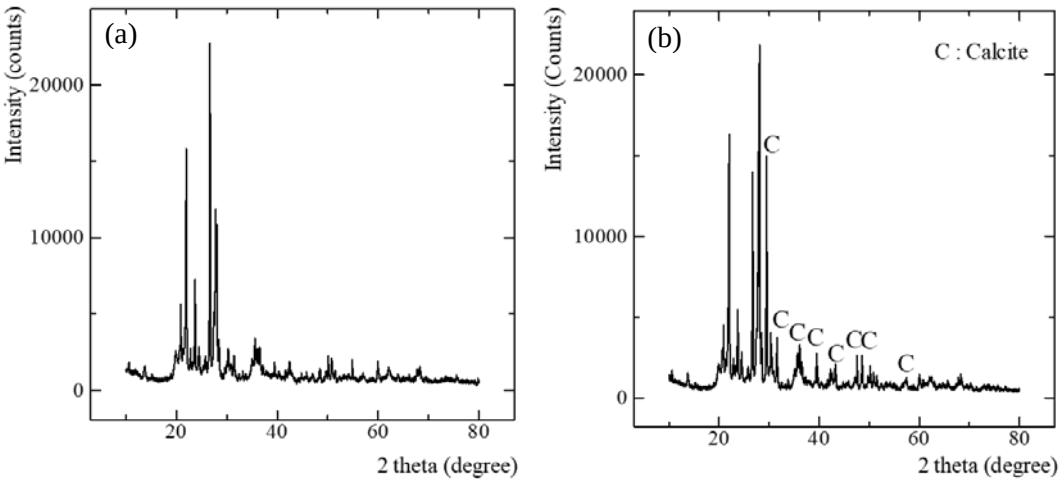
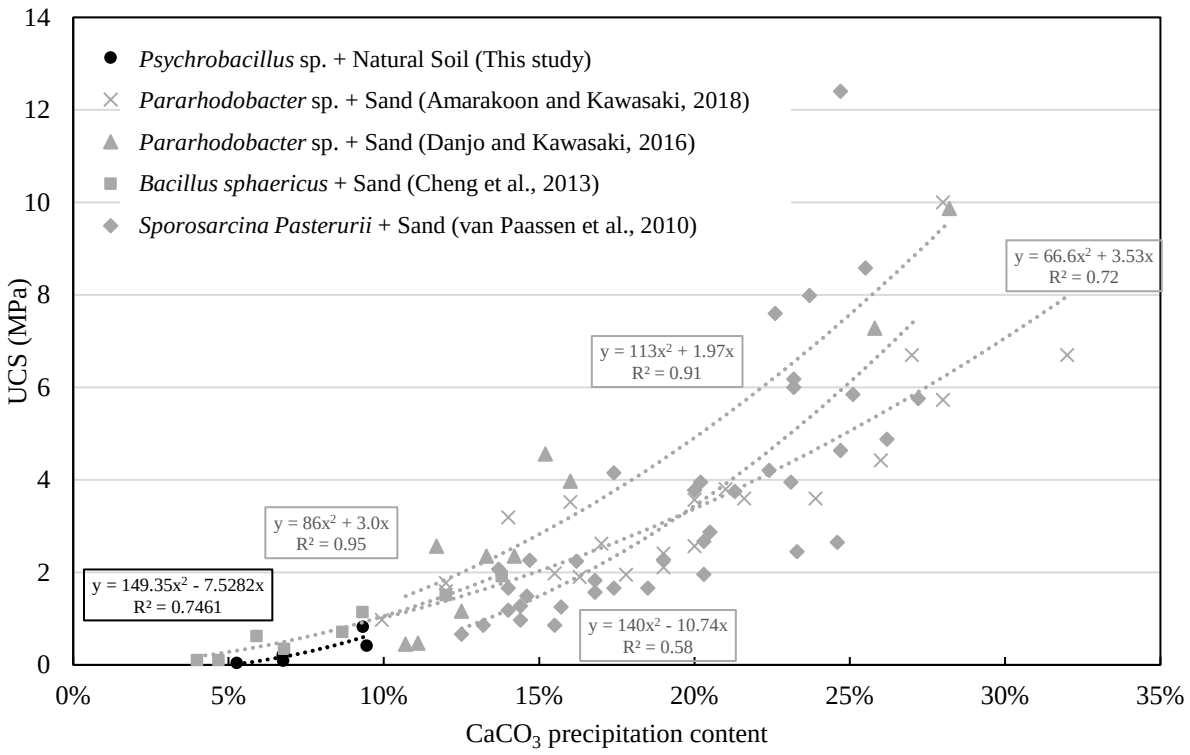

(a)
Intensity (counts)
20000
10000
0
20
40
60
80
2 theta (degree)
(b)
C : Calcite
Intensity (Counts)
20000
10000
0
C
C
C
C
C
C
C
20
40
60
80
2 theta (degree)

Figure 18


14
12
10
8
6
4
2
0
UCS (MPa)
0%
5%
10%
15%
20%
25%
30%
35%
CaCO3 precipitation content
Psychrobacillus sp. + Natural Soil (This study)
Pararhodobacter sp. + Sand (Amarakoon and Kawasaki, 2018)
Pararhodobacter sp. + Sand (Danjo and Kawasaki, 2016)
Bacillus sphaericus + Sand (Cheng et al., 2013)
Sporosarcina Pasterurii + Sand (van Paassen et al., 2010)
y = 149.35x² - 7.5282x
R² = 0.7461
y = 86x² + 3.0x
R² = 0.95
y = 113x² + 1.97x
R² = 0.91
y = 66.6x² + 3.53x
R² = 0.72
y = 140x² - 10.74x
R² = 0.58