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**EXPERIMENTAL STUDY ON
NEAR-SURFACE STABILIZATION OF SLOPES IN COLD REGION
USING BIO-MEDIATED GEOTECHNICAL ENGINEERING**

by

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DISSERTATION

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ABSTRACT

Near-surface instability poses challenges regarding the maintenance of natural and engineered slopes. The strength characteristics of the near-surface zone are progressively altered in response to climatic variations, leads the near-surface more prone to soil loss and deteriorations. High yield of sediment and near-surface failures are also often reported to be triggered during or immediately after prolonged rainfalls or snow melting (in cold regions) events. Therefore, stability of the near-surface requires more attentions in terms of eliminating risks and saving economy. Although the chemical treatments in the past were found to be reliably enhancing the near-surface conditions, most of them are reported to be toxic and hazardous, and manufacturing process of such materials causes considerable environmental damage by the release of greenhouse gases. This necessitates and urges the development of new pathways for an effective stabilization of slope near-surfaces.

As an emerging technique, microbial induced carbonate precipitation (MICP) has drawn substantial attentions in the past years. The technique has been emerged through relatively a novel interdisciplinary pitch at the confluence of microbiology, geochemistry, and geotechnical engineering. MICP persuades the calcium carbonate bio-cement within the soil matrix as a consequence of bio-chemical reactions, resulting the decrease in particle mobility and increase in strength and stiffness. The objective of the research presented in this dissertation is to assess the viability of MICP as a novel alternative for near-surface stabilization of slopes. The pathway utilizing the native bacteria has potentially been chosen in this work. From the perspective of bio-geotechnical engineering, yet significant effort has been given on understanding biological responses, mechanical responses, durability responses and application strategies to facilitate reliable treatment and to promote the technique nearly to field implementation stage.

In the first part of the research work, a fundamental scientific approach of MICP technique is presented. An erosion prone expressway slope located in Hokkaido, has been chosen as representative for the investigation. Hokkaido, the northernmost island of Japan, is located in subarctic region, experiencing a cold climate. Giving the emphasis on the intrinsic climate of the study region, a comprehensive experimental work has been performed. The ureolytic bacteria isolated from the target slope soil were characterized to be *Lysinibacillus xylanilyticus*, worth noting that these bacteria have not previously been reported for urea hydrolysis. The urease performances were evaluated under different temperature and chemical conditions. The results revealed that the bacteria could effectively mineralize the calcium carbonate between 15 °C and 25 °C, showing in more robust performance under native cold climatic conditions. The effects of various MICP recipes were also investigated to deepen the understanding on biochemical processes. The compatibility analysis on slope soil revealed that presence of silt increased the number of particle contacts, enhancing the MICP force chain. In addition, the lower bound of MICP application by surface injection was revealed in terms of silt content.

In the next part, the physical and mechanical responses of MICP treated slope soils are evaluated. Giving emphasis on comprehending desirable range of cementation level, the slope soils were treated to various levels within the possible range, and their physical states, shear-wave velocities and mechanical behaviors across the range of cementation levels were extensively studied and compared. The mechanical test program comprised of unconfined compression test, shear test and permeability test. The study demonstrated that precipitated calcium carbonate is one of the most important factors determining the responses of MICP soils.

Subsequently, a comprehensive study was conducted to investigate the influence of key climatic parameters on the long-term performance of MICP treated slope soils. As the cold regions experience periodic snowfalls and rainfalls, the cyclic freeze-thaw (FT), wet-dry (WD) and acid rain (AR) tests were chosen and investigated as potential indicators of durability. The results showed that the WD damages were not significant as those obtained under FT and AR conditions. However, level of cementation had a foremost role on long-term performance of MICP slope soil. The specimens treated to high content of calcium carbonate showed minor damage with high aggregate stability in all the durability analysis.

Finally, the work dealt with the treatment strategies in order to promote the technique towards real application and commercialization. A series of bench-scale slope models was treated by different strategies including grid-injections, single-phase and two-phase sprayings with several variables. The non-destructive needle penetration tests were used to evaluate all the treated slope surfaces. The results revealed that the two-phase technique sprayed 1 mol/L resources could be more effective and feasible for real-scale applications. Given the optimum treatment, the scaled-up slope under temperature uncontrolled conditions could achieved marked increase in near-surface strength. Based on the outcomes derived from the above works, the application guidelines are outlined regarding optimal MICP recipe, implementation strategy and conditions, along with the evaluation process that would help to ensure the durability performance of MICP treated slope near-surfaces under the effects of climate.

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CHAPTER 1

INTRODUCTION OF DISSERTATION

1.1. Research motivation

Incorporating “**Green Construction Practices (GCP)**” in Geotechnical Engineering increases day by day due to the considerations of impacts on the environment and future generations. The environmental issues associated with conventional construction materials such as chemical grouts (e.g. cement, fly-ash, epoxy, acrylamide, phenoplasts, silicates, polyurethane, etc.) and geosynthetic products necessitate alternative approaches in geotechnical applications. For example, production of Ordinary Portland Cement (OPC), the effective and widely used soil stabilizer, causes the significant release of major greenhouse gas, carbon dioxide (CO₂). According to the reports of Intergovernmental Panel on Climate Change (IPCC), the production of OPC contributes around 5-6% of global CO₂ emission. As per the prediction, if the present rate continues in cement manufacture, the emission of carbon would reach to 260% in 2050 (Cuzman et al., 2015), which would significantly impact the earth’s atmosphere and climate.

It is also apparent that most of the chemical grouts are environmentally toxic and/or hazardous (Cheng et al., 2014a; Karol, 2003); thus, these materials are extensively under the scrutiny of public policy. For instance, leaching of acrylamide grout caused severe water poisoning in Japan in 1974, leading to the subsequent ban of nearly all the chemical grouts (Karol, 2003). Since that, the initiatives are being undertaken in several countries to ban synthetic grouting materials, and to the withdrawal of most of the products in market (DeJong et al., 2010).

The conventional geosynthetic products which have been used to reinforce the slopes and weak soils are predominantly plastic composites. They are environmentally non-decomposable, manufactured from the by-products of limited petroleum resources. Worldwide capacity of such plastic composites exhibited a massive increase from 0.36 million metric tons in 2007 to 2.33 million metric tons by 2013, and is predicted to increase to 3.45 million metric tons by the end of this year, 2020 (Bordoloi et al., 2017). Similarly, implementing steel elements is also reported to be detrimental to the geo-environment at the end of its useful life due to the toxicity of corroded steel (Bordoloi et al., 2017; Gowthaman et al., 2018).

It is therefore clear that the geotechnical approaches have also a significant role in determining the environmental damage. As previously mentioned, the confluence of these impacts urges the exploration of new alternative GCP. According to the researchers and scientists (Achal and Kawasaki, 2016; DeJong et al., 2010; Ivanov and Chu, 2008), the solutions to this scenario can be potentially achieved through the multi-disciplinary research at the convergence of microbiology, geochemistry and geotechnical engineering. Hence, this work is motivated to mediate the biological processes for stabilizing the slope soils as an alternative geotechnical engineering practice, i.e. through **Bio-mediated Geotechnical Engineering**, assessing the complete feasibility towards the real practice.

1.2. General background

Since the second half of the 20th century, the urbanization has been one of the most powerful anthropogenic force in almost all over the world, which has greatly accelerated the development of infrastructures, notably the massive establishments of transportation structures (Cui and Shi, 2012). As well known, the embankment slopes are widely employed

as prime supporting structures of most of the transportation systems such as expressways, railway tracks etc., and their sturdiness is highly reliant on the stability of the slopes by which they are supported. Therefore, stabilization of slope soils, monitoring and assessments are frequently the serious concerns in the fields of Civil and Geotechnical Engineering. Human activities play many important roles in slope processes. During the construction of the transportation structures, the natural slopes are modified in a way to fit the construction and design requirements, which often causes the clearance of vegetations and topographical amendments in natural slopes (Guerra et al., 2017; Zhang et al., 2018). As the result, the near-surface substrates and indigenous characteristics of the slopes are significantly disturbed, which tend to reduce the water retention capacity of the surface. Consequently, this leads the slope near-surface more prone to degradation processes and associated failures.

As illustrated in **Figure 1.1**, the near-surface serves as a buffer-layer between atmosphere and inner substrates, significantly determining the slope responses such as infiltration, aggregate stability, degradation processes and associated failures (Dai et al., 2018; Harden and Scruggs, 2003; Muntohar and Liao, 2010; Zhang et al., 2018). As the near-surface zones are in a direct contact with the atmosphere, they become more susceptible to the climatic changes and associated deteriorations. In addition, shallow slope instabilities (**Figure 1.1**) due to heavy rainfall and/ or snowfall events poses challenges regarding maintenance of earth structures. Particularly, the near-surface stability of expressway embankments requires more attentions in terms of saving huge loss of lives and economy and has been often documented as a widespread issue all over the world.

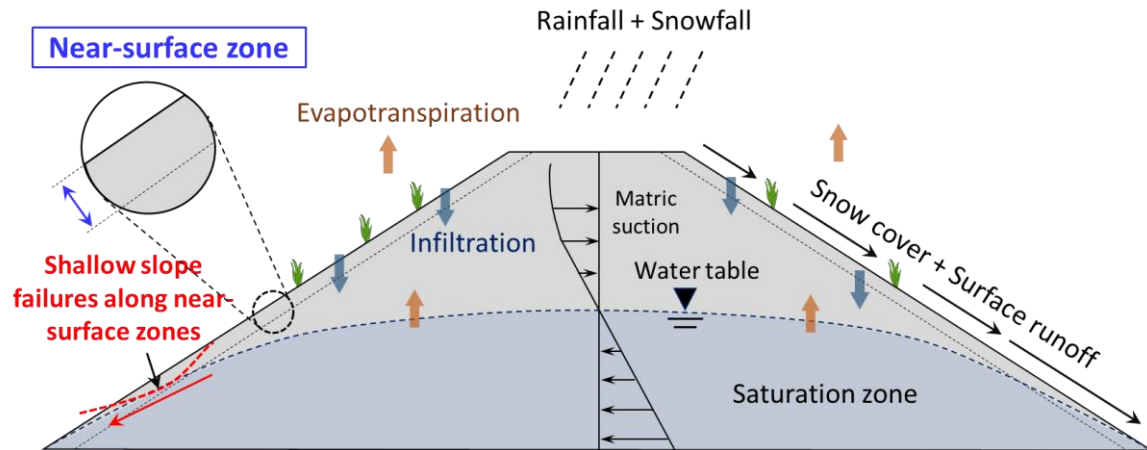


Figure 1.1: Schematic diagram of embankment slope and near-surface instability

In the past decades, numerous methods have been proposed and investigated to stabilize and enhance the near-surface conditions of the slopes. The most cost-effective and eco-friendliest method for stabilizing the slope is establishment of vegetation such as grass, weeds, plants and etc. (Chirico et al., 2013). However, to achieve the fullest benefits, it takes quite a long time, and the growth and survival of vegetation are limited to certain regions such as subarctic and arid zones (Jiang et al., 2019). Some of other methods widely in application stage are mechanical compactions, geosynthetic applications and chemical stabilizations (Kumar and Das, 2018). Majority of these stabilization techniques utilize mechanical energy and artificial materials, both of which required substantial energy for manufacture and installation. Chemical grouts are also found to be reliable, could enhance the surface by filling pore spaces and binding soil particles (Arya et al., 2018; DeJong et al., 2010). However, most of these materials are reported to be toxic and hazardous, causing pollutions and influx of dangerous substances to the geo-environment; therefore, their applications to the field-scale are extensively under the scrutiny of public policy (DeJong et al., 2010; Gowthaman et al., 2018). Confluence of these drawbacks in the conventional methods necessitates the exploration and development of new methods for a more effective stabilization.

As an emerging technique, MICP (microbial induced carbonate precipitation), sometimes referred to as bio-grouting, has drawn substantial attentions in the past decade. MICP is a bio-mediated process which persuades the calcium carbonate bio-cement within the embedded soil matrix as a consequence of the set of bio-chemical reactions (Achal and Kawasaki, 2016; DeJong et al., 2010; van Paassen et al., 2010). The precipitated calcium carbonate tends to fill the void spaces, decreasing the porosity, decreasing the mobility of particles, eventually increasing the strength and stiffness (Cheng et al., 2013; Lin et al., 2016). Previous research works demonstrated the applicability of the MICP for various geotechnical challenges including soil strengthening, liquefaction control, erosion control, fugitive dust control, and coastal protection, advancing the MICP towards field implementation. Proposal as an alternative to Portland cement is another appeal of using MICP (Dejong et al., 2013), which could sustainably reduce the emissions of CO₂ and SO₂ from cement manufacture.

1.3. Scope and objective of the research

The objective of the research presented in this dissertation is to assess the viability of using **MICP** as a novel alternative for **near-surface stabilization of slopes against sediment yield**. From the perspective of bio-geotechnical engineering, yet significant effort has been given on understanding biological responses, mechanical responses, durability responses and treatment strategies to facilitate effective and reliable stabilization process.

A representative erosion prone expressway slope located in Hokkaido, Japan, has been investigated herein. Hokkaido, the northernmost island of Japan, is located in subarctic region, experiencing a cold climate (average temperature fluctuates between 25°C ~ -15°C), in which more than hundred days is attributed to winter (Farukh and Yamada, 2018). Giving the emphasis on the intrinsic climate of the study region, a comprehensive experimental work

has been performed. The scope of the research work is summarized in **Figure 1.2**. The indigenous ureolytic bacteria were first isolated from the target slope soil, and a baseline scientific approach was taken to deepen the understanding on biochemical processes. The enzymatic responses of the bacteria were studied under various temperature and pH environments. The effects of various chemical recipes were also investigated to reliably facilitate the cementation. The physical and mechanical improvement of slope soils treated to various levels were then evaluated at the soil element scale. In order to assess the long-term performance of the MICP treatment, various durability tests such as cyclic freeze-thaw, cyclic wet-dry and acid rainfall were performed. Finally, the treatment process was implemented into slope models to assess various treatment strategies and to evaluate the enhancement of the near-surface zone of the slopes.

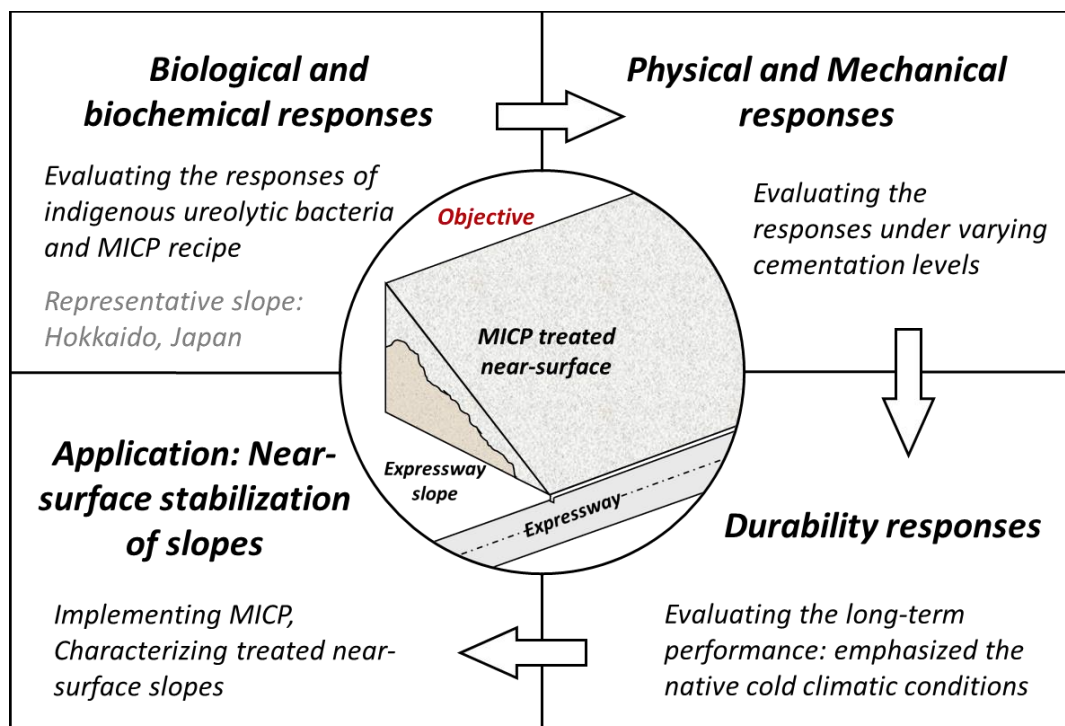


Figure 1.2: Outline of the scope of the research work

1.4. Organization of Dissertation

This dissertation is organized into a total of seven chapters.

- **Chapter 1** describes the motivation, objective, scope and significance of this study.
- **Chapter 2** presents a review of relevant literature on various aspects. Early sections of this chapter discuss the mechanism of MICP, pathways involved in MICP process and various applications from the perspective of geotechnical engineering. Latter sections discuss the near-surface stability of slopes and environmental deteriorations, followed by the review on currently available stabilization techniques. Finally, the merits on introducing MICP for near-surface stabilization of slopes are also addressed.
- **Chapter 3** describes the fundamental scientific approach of MICP technique. Major part of this chapter presents the biological responses including biomass and urease activity of the indigenous bacteria under different temperature and chemical conditions. In addition, the systematic evaluation for the optimum recipe of constituents in MICP solution, is presented. Final section of this chapter deals with the effects of particle size distribution on MICP at both micro and macro scales.
- **Chapter 4** presents the physical and mechanical behaviors of MICP treated slope soil across a range of cementation levels. Mechanical characteristics presented herein are mainly based on shear wave velocity, unconfined compression and shear tests. Shear strength parameters obtained under both peak and residual states are compared and discussed with those of untreated soil. The permeability characteristics are also presented across varying cementation levels. In addition, the homogeneity of treatment is discussed using the spatial distribution of calcium carbonate.
- **Chapter 5** presents the results of durability analysis performed to evaluate the long-term performances of MICP treatment. Cyclic freeze-thaw, cyclic wet-dry and acid rainfall tests are incorporated as credible indicators of durability, giving the emphasis on subarctic

climate. The durability responses evaluated across the range of desirable cementation levels are discussed.

- **Chapter 6** presents the results of a series of treatment process implemented into slope models at different scales. The bench-scale slope models treated essentially by different strategies under controlled conditions are characterized, and the effects on improving near-surface responses are evaluated. Finally, the chapter deals with the scaled-up slope treated under temperature-uncontrolled environment, emphasizing the effects of seasonal temperature on field implementation.
- **Chapter 7** provides the overview of the findings and conclusions drawn in this work, along with the suggestions for the future works. In addition, based on the outcomes derived from the above works, the application guidelines are outlined regarding optimal MICP recipe, implementation strategy and conditions, along with the estimation process that would help to ensure the durability performance of MICP treated slope near-surfaces under the effects of climate.

1.5. Originality and significance of the work

Currently, Ordinary Portland Cement (OPC) and vegetations are the widely used materials to stabilize the expressway slope soils in almost all the countries. This research work focus on introducing a new promising candidate (i.e. MICP) for stabilization purpose in Hokkaido, the cold region.

Most of the MICP studies reported to date augmented the soil with foreign bacteria for bio-mediated soil improvement, experienced uncertainties regarding performance and regulatory aspects. The work reported in this dissertation has chosen the pathway utilizing the native bacteria (the pathway that will be more likely to be approved by the public and regulatory).

A new bacterium which has not previously been reported for urea hydrolysis, was isolated from the cold region and investigated, showing in more robust performance under native cold climatic conditions. Notably, this finding helped to open the way for MICP applications in cold regions.

Initial as-treated characteristics of MICP slope soils are highly important for evaluating the safety and serviceability of the treated structure. The physical and mechanical responses of the cemented sands and how it varies with degree of cementation was often a question to explore. This study has systematically evaluated the MICP responses under varying degree of cementation, comprehending the desirable range for stabilization of slope near-surface.

The studies on long-term performance of MICP soils are very limited, remaining as a major research gap. As potential indicators of durability, freeze-thaw, wet-dry and acid rain simulation tests have been performed on MICP slope soils, deepening the understanding on long-term responses under various climatic environments. The findings could provide necessary information regarding monitoring, effective lifetime and requirement of re-treatment. Moreover, along with a full-scale slope model test, this study advances the MICP process nearly to field implementation and addresses several hurdles faced with during the implementation process.

CHAPTER 2

LITERATURE REVIEW

2.1. Introduction

MICP, also known as bio-cementation, is one of the popular biological processes, producing cementing agent (i.e. carbonates) in a way similar to diagenesis process. Diagenesis is a naturally occurring process in the Earth's crust, transforming the physical state of the sediments from cohesionless aggregates to cemented material due to the influence of several physical, chemical and biological factors, and the process entails a period of hundreds to thousands of years. Calcareous rocks and carbonate sands are some of the well-known examples formed by diagenesis. In addition, similar formation of cementitious material can be witnessed in the formation of animal and protozoan shells, coral reefs, etc. (Achal and Kawasaki, 2016). The above processes are found to be either biologically induced or the result of interactions between extracellular biopolymers and the geochemical environment (Decho, 2010).

With the aid of the knowledge learnt from the nature, the MICP technique has been coined in the past as the acceleration of diagenesis process for engineering purposes. Notably, the phenomenon involves the living microorganisms whose biological activity mediates the required natural reactions, proceeding the formation of minerals at accelerated rates which is faster than that predicted based on the thermo-chemical reactions (Mitchell and Santamarina, 2005). As the formation of calcite is commonly found in the nature, the calcium carbonate is more desirable for MICP in terms of cementitious material. Microorganisms, the key substance of MICP, is also found to be abundantly existing in soil environments. Therefore,

many geotechnical engineers were fascinated to this novel subject area and started to be questioned whether it is benefited as an alternative soil improvement technique. As the result, before a decade, a new multi-disciplinary pitch called bio-mediated geotechnical engineering, has been introduced by converging the fields of microbiology, chemistry, geotechnical and environmental engineering (Achal and Kawasaki, 2016; DeJong et al., 2010; Ivanov and Chu, 2008; Whiffin, 2004). Researchers found that the MICP could be a promising technique, eliminate several drawbacks experienced in conventional soil improvement techniques and be applied to the soils with minimal soil-environmental damages; MICP, therefore, experiences an increased level of interest these days and remains relatively a novel technique.

This chapter presents a comprehensive review on aspects related to this research work. Early sections of this chapter discuss the mechanism of MICP, pathways involved in MICP process and various applications from the perspective of geotechnical engineering. Latter sections discuss the near-surface stability of slopes and environmental deteriorations, followed by the review on currently available stabilization techniques. The merits on introducing MICP for near-surface stabilization of slopes are also discussed.

2.2. Urea hydrolysis and MICP reaction system

MICP technique utilizes microbial metabolic activities to produce calcium carbonate, relying on set of biochemical reactions. In fact, not all the soil bacteria could contribute to the MICP, but the urease producing bacteria (also referred as ureolytic bacteria). Urease enzymes are protein macromolecules, which can be localized in the bacterial cell membrane, cytoplasm, or excreted outside the cell (Mobley et al., 1995). The prominent feature of urease is its active site that consists of a bi-nickel center with a functional structure (Krajewska, 2018), that could exert the catalytic function.

As shown in the **Eq. 2.1**, microbial urease acts as the catalyst and contributes to the hydrolysis of urea, resulting the formation of carbonic acid and ammonia. In the aqueous medium, the products equilibrate, resulting ammonium and bicarbonate ions (**Eq. 2.2** and **Eq. 2.3** respectively). The pH increases as a result of ammonia reacting with water, producing hydroxide ions (**Eq. 2.2**), which leads to form the carbonate ions in the reaction system (**Eq. 2.4**). In the presence of suitable divalent cations such as calcium ions (Ca^{2+}), the products eventually induce the formation of calcium carbonate (CaCO_3) as per the given reaction (**Eq. 2.5**). Calcium chloride (CaCl_2) is known to be one potential source of the calcium ions.

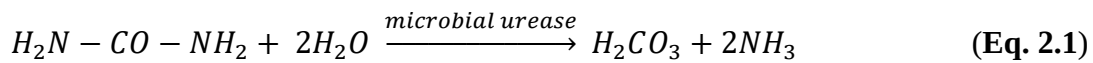


Figure 2.1 presents the relationship between pH and chemical speciation of carbonates. It can be seen that the proportion of total carbonate that exists as CO_3^{2-} in the solution is highly dependent on the pH. As mentioned earlier, the reactions increase the alkalinity (pH) of the solution. When the pH increases above 8, the bicarbonate ions (HCO_3^-) are greatly transformed to carbonate ions (CO_3^{2-}), creating an ideal condition for calcium carbonate precipitation.

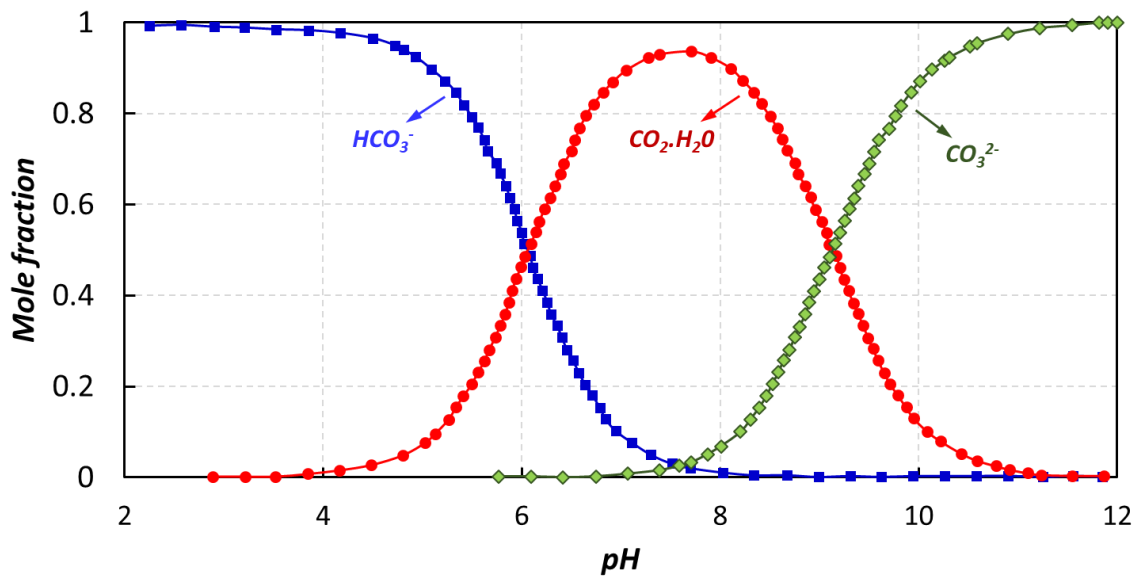


Figure 2.1: Relationship between pH and chemical speciation of carbonates (modified from Jacob, 1999)

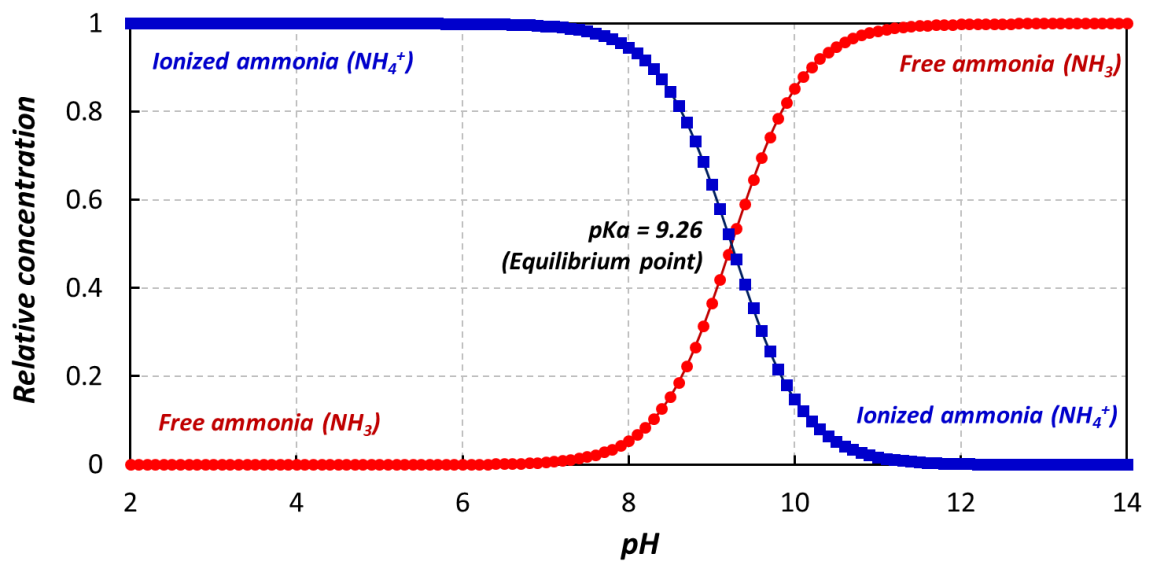


Figure 2.2: Relationship between pH and chemical speciation of ammonium ions and free ammonia (modified from Whiffin, 2004)

Urea hydrolysis, on the other hand, leads to produce undesirable by-products such as ammonia (NH_3) and ammonium ions (NH_4^+). The NH_3 is a gas and sometimes called free ammonia, and NH_4^+ is the ionized form of ammonia **Figure 2.2** shows how the ratio between NH_3 and NH_4^+ is affected by pH in a controlled sample, revealing that the ammonium

speciation in the reaction medium is highly pH dependent. The equivalence point in the NH_3 - NH_4^+ system occurs at pH of around 9.2. It can also be seen that a $\text{pH} \leq 6.90$ is required to keep all the speciation in the ionic form (NH_4^+), and worth noting that around 98.8% is found to be in the ionic form at the pH of 7.3. Conversely, in alkaline environments, as the pH increases, the NH_4^+ is liberated into gaseous NH_3 .

2.3. Different pathways of MICP

Ureolytic bacteria are generally found in the soil surface and subsurface. However, to promote the precipitation of calcium carbonate, microbial densities, fraction of attached microbes and activity level may need to be enhanced adequately.

As illustrated in **Figure 2.3**, 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 (e.g. nutrients and pH conditions) to enrich the existing microbial community with required urease capabilities for MICP purpose (Fujita et al., 2000; Gomez et al., 2015). If ureolytic bacteria are present in the subsurface, stimulating the population size and urease activity by supplying suitable nutrients is ideal, because it utilizes native organisms and reduces the engineering challenges of controlling the transport of cells in the subsurface (DeJong et al., 2009). However, there are some drawbacks reported to bio-stimulation process, which are: (i) high variability in bacterial growth, leads to the diffused zone of improvement (Gomez et al., 2018, 2017) and (ii) process might also stimulate the growth of pathogenic/ harmful strains in the soil environment, posing the risks regarding biosafety and sustainability.

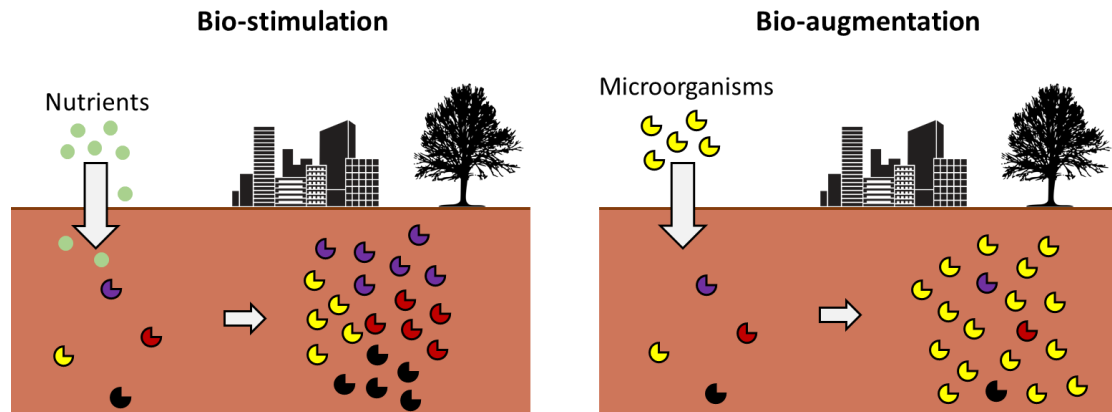


Figure 2.3: Conceptual illustration of the bio-stimulation and bio-augmentation processes

If ureolytic bacteria are not present in the subsurface or if the subsurface lends itself to augmentation (e.g. accessible, high permeability, uniform gradation), bio-augmentation of the subsurface is highly beneficial (Mortensen et al., 2011). Bio-augmentation process 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/ foreign bacterial strains. Bio-augmentation by exogenous bacteria (e.g., *Sporosarcina pasteurii*) has been extensively researched and demonstrated on sands at a variety of laboratory scales (Feng and Montoya, 2016; Lin et al., 2016; van Paassen et al., 2010; Whiffin, 2004). On the other hand, the studies concerned on bio-augmentation using indigenous bacteria up to now are limited (Danjo and Kawasaki, 2016; Gat et al., 2016; Gowthaman et al., 2019b).

Table 2.1 Summary of advantages and disadvantages of MICP processes

MICP pathways	Advantages	Disadvantages
Bio-stimulation	i. Elimination of non-native microbial supply ii. Fitness of the microbes, i.e. reliable at native environment iii. Less challenges of controlling transport of cells in subsurface iv. More possibility of approval by public and regulatory agencies	i. Applicable only if the ureolytic bacteria are present ii. Diffuse zone of improvement iii. Risks regarding the stimulation of pathogenic/ harmful strains
Bio-augmentation of exogenous bacteria	i. High initial performance ii. Possible homogeneous cementation at/ around near-surface zones	i. Microbial pollution ii. Uncertainties in long survival iii. Use of exogeneous bacteria is forbidden in some countries iv. Log-linear distribution of bacteria from supply point
Bio-augmentation of indigenous bacteria	i. Elimination of non-native microbial supply ii. Fitness of the microbes, i.e. reliable at native environment iii. Possible homogeneous cementation at/ around near-surface zones iv. More possibility of approval by public and regulatory agencies	i. Hydrolysis of urea relatively at slow rates ii. Log-linear distribution of bacteria from supply point

Similar to the bio-stimulation, the augmentation of indigenous bacteria offers important environmental benefit, eliminating the nonnative bacterial supply (microbial pollution) into natural soil ecosystems (Gomez et al., 2017). Although the native species perform the hydrolysis of urea at slower rates compared to that of specialized bacterial strains (e.g., *Sporosarcina pasteurii*), these microbes 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. The microbial enzymes are typically sensitive and can readily be denatured by even a slight change of environmental conditions (Yang et al., 2016). Introducing non-native bacteria to a new environment therefore hinders the survival and performance of the strains (DeJong et al., 2009; Sensoy et al., 2017). It is worth noting that in some countries like Japan, the augmentation of foreign bacteria for engineering applications is completely forbidden. The advantages and disadvantages of all three MICP pathways are briefly summarized in **Table 2.1**.

2.4. Compatibility of soil for MICP

The MICP, as the nondestructive soil improvement technique, the surface injection or percolation is often recommended for real-scale applications. However, as illustrated in **Figure 2.4**, the injection of MICP is limited in certain fine-grained soils (E.g. clay) due to the restrictions regarding bacterial transportation/ mobilization. Typical size of the bacteria was found to be ranged between 1 to 10 μm . The prime factor that governs the microbial transport within the soil matrix is the size of pore throats through which the cells should pass (Mitchell and Santamarina, 2005). Narrow pore throats formed by fine soil grains prevent their transport from one pore space to another, filtering the cells at surface/ near-surfaces. It has been reported that the entry of the bacteria is not possible, if the pore throat size is less than 0.4 μm (Duraismy, 2016).

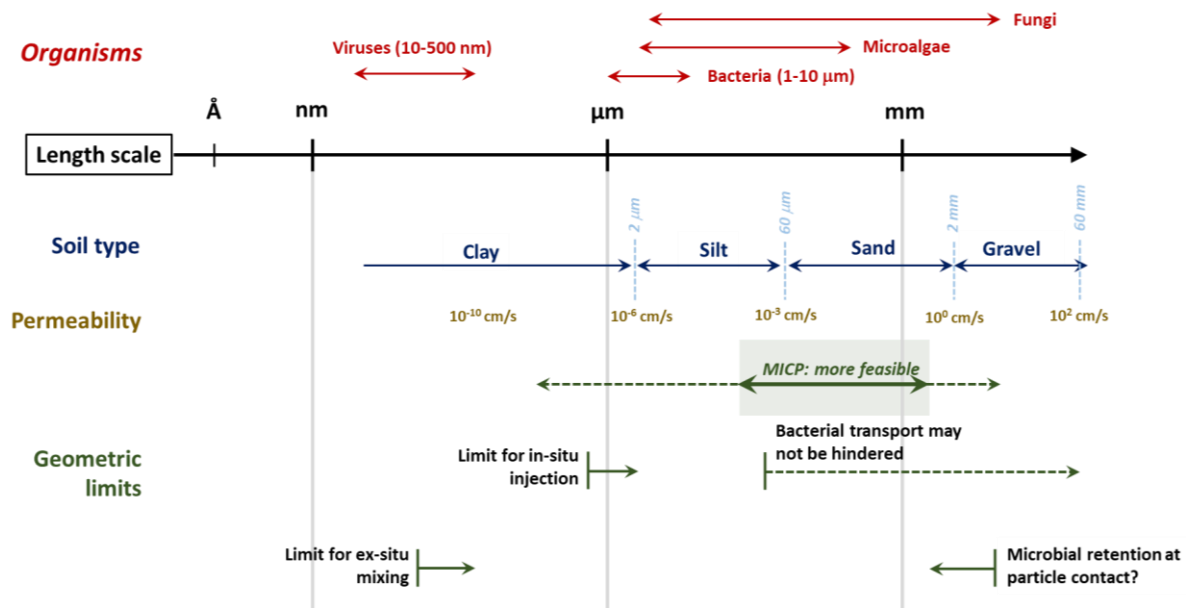


Figure 2.4: Bacteria-soil compatibility and geometric limits (modified from DeJong et al., 2010)

In fact, the size of the pore throat is related to smaller fraction of particles in soil; it can be approximately estimated as 20% of the soil particle size that corresponds to 10% passing in a mechanical sieve analysis (i.e., D_{10}) (DeJong et al., 2010). This could provide an approximate lower bound to the MICP treatment by injection/ percolation. Although the microbial transport by advection is restricted in clay material, their pore sizes are essentially adequate for the existence of bacteria cells. Therefore, *in-situ* and *ex-situ* mixing of microbes and nutrients with soil may be appropriate for silt-clay materials (**Figure 2.4**), extending the range of soils amenable to MICP applications. On the other hand, the upper bound of MICP application is reliant on the fraction of microbes acting at particle contacts. The small particle-contact points with large open pores (coarse particles, e.g. gravels and pebbles) can result less retention of bacteria, leading to ineffective MICP.

Therefore, during the site investigation program, a preliminary assessment on the compatibility of soil size is highly necessary, which would help to choose the appropriate strategy and to produce effective cementation within time-scale of engineering projects.

2.5. Geotechnical applications of MICP

2.5.1. *Effects of MICP on engineering responses of sand*

In the past decade, the effects of MICP on the mechanical characteristics of sand materials were studied by several researchers, demonstrating that the MICP responses vary depending on the complex soil properties such as mineral composition, particle size distribution and relative density. Mineral compositions were found to be altering the chemical and thermodynamic properties of the pore fluid and influencing more nucleation sites for calcium carbonate precipitation. Mortensen et al. (2011) investigated the MICP responses of different sands with different minerology such as quartz, calcite, feldspar and iron. They found that different mineral compositions had significant effects on the precipitation rate of calcium carbonate. Results also showed the fastest precipitation rate in the sand rich in calcite, revealing that existed calcium carbonate could provide ideal surfaces for the additional precipitation of calcium carbonate.

Soil particle size is another important parameter that governs the responses of MICP. In fact, the soil particle size is directly related to the pore throat size in soil matrix, controlling the free flow and distribution of the bacteria cells. In general, the size of bacteria ranges between around 2 – 10 μm (Ng et al., 2012). Therefore, soils containing pore-throat size smaller than bacterial size (for example: clay) inhibit the free flow of bacteria within the soil matrix, leading to limited and non-homogeneous precipitation of calcium carbonate (Mitchell and Santamarina, 2005). On the other hand, the larger grains (for example: gravels) have less inter-granular contacts and larger inter-granular distance, which result the formation of majority of the calcium carbonate on the grain surface rather than the contact points. Also, relatively a very high carbonate content is required to stiffen the gravel matrix, weakening the overall MICP efficiency. Rebata-Landa (2007) carried out a series of MICP column tests,

in which eleven soil materials with different particle sizes including kaolin, silt, fine sand, coarse sand and gravel were treated. The results showed that most tested soils had good MICP cementation except the following soils: kaolinite, fine sand ($D_{50} = 5000 \mu\text{m}$), coarse sand ($D_{50} = 5300 \mu\text{m}$) and fine gravel ($D_{50} = 11500 \mu\text{m}$), indicating that the soils with particle size between 10 and 1000 μm could be more effective for MICP applications.

Regarding the relationship between particle size and the soil strength after MICP treatment, Amarakoon and Kawasaki (2018) compared the UCS of two silica sands: Mikawa ($D_{50} = 600 \mu\text{m}$) and Toyoura ($D_{50} = 200 \mu\text{m}$) after MICP treatment. It was found that Mikawa sand had better cementation effect and UCS could reach 3 MPa. Mean-while, Toyoura sand showed relatively poor cementation effect with only about 1 MPa UCS. Similarly, Zhao et al. (2014) found that the strength of sand with D_{50} of 460 μm was two times higher than that of Mississippi sand with $D_{50} = 330 \mu\text{m}$ under UC tests. In contrast, Cui et al. (2016) studied the responses of MICP treated three sands with particle sizes ranging from 50-125 μm , 125-250 μm and 40-500 μm and showed the UCS values of 969 kPa, 754 kPa and 152.7 kPa, respectively, demonstrating a negative correlation between particle size and soil strength, contrary to the result of previous researchers. From the review of previous works, it could be seen that the accurate effects of soil particle size on MICP efficiency is still not clear, recommending further studies. Very recently, Gowthaman et al. (2019a) studied the MICP responses of three uniformly graded sands with different particle size (D_{50} of 1600 μm , 870 μm and 200 μm), concluding that the UCS values increase with the decrease in particle size. Using the SEM images, they explained that number of particle contacts highly influences the effectiveness of MICP; less pore spaces in fine sands were more conducive to accumulation of calcium carbonate, promoting the cementation effect. They also demonstrated that the presence of certain fine content facilitated intermediate supports to form bridges between

sand particles, enhancing the force chain of the treated matrix. On the other hand, coarse sands were shown to have less contribution to the inter-granular bond enhancement.

The relative density of soils is also important factor that determines the effectiveness of MICP. Qabany et al. (2011) studied the relationship between the relative density and strength of cemented British standard quartz sand. They found that there is a close relationship between strength and the relative density of MICP treated sand. Kim et al. (2014) applied the MICP treatment to three silty soils with 60%, 75% and 90% relative compaction, showed that the precipitated calcium carbonate content in the soil with 90% relative compaction was the highest: up to around 1.5 to 2 times higher than that with 60% and 75% relative compaction. Rowshanbakht et al. (2016) applied MICP treatment to cylindrical specimens of siliceous sand ($D_{50} = 200 \mu\text{m}$) at relative density of 40%, 70%, and 85% and tested the UCS of samples. The results revealed that the strength increased with the increase of relative density. Soon et al. (2013) carried out direct shear tests on medium-sized sand columns with relative density of 0.85, 0.90, and 0.95, confirming that the shear strength of MICP sand had a positive correlation with the relative density.

It has also been found that MICP reduces the permeability of soils, due to bio-clogging, and improves the mechanical properties of the soils such as the strength and stiffness. Feng and Montoya (2016) indicated that MICP increases the stiffness, peak strength and dilation increase, and that vary with the degree of cementation. Lin et al. (2016) performed a series of triaxial tests and showed that the increase of the soil strength could be achieved even at a threshold 1% calcium carbonate content. As a benchmark attempt, van Paassen et al. (2010) performed a large-scale test (100 m^3) test and succeeded in enhancing the soil strength near the surface using MICP. They prepared a larger concrete soil tank (8 m in length $\times$ 5.6 m in width $\times$ 2.5 m in height), laid a thin sand layer of 25 cm and installed three inlets and three

outlets to simulate a hydraulic gradient of 0.3 in the vertical direction. The results showed that the strength of the soil was significantly improved after the implementation of MICP.

Shear wave velocity measurement has been proven to be a useful nondestructive indicator for monitoring MICP process in both temporally and spatially. Shear waves velocity acts as a proxy to increases in small-strain shear stiffness as cementation occurs at particle-particle contacts (Martinez et al., 2013). Shear wave velocity was previously used by Qabany et al. (2011) to estimate the effect of CaCO_3 precipitation, showing that the shear wave velocity could increase linearly with the increasing precipitation content. Recently, Feng and Montoya (2017) found that the shear wave velocity values might vary, even to the same cementation level, thus, proposed the coupled use of shear wave velocity and calcium carbonate content for the appropriate evaluation of MICP.

2.5.2. MICP for liquefaction mitigation

Liquefaction is a phenomenon marked by a rapid and dramatic loss of soil strength, which can occur in weak deposits subjected to earthquake motions. Typically, the deposits consist of loose, saturated cohesionless soils with insufficient cyclic shear strength are often reported to be susceptible to liquefaction. In the past, several studies have demonstrated that the MICP process could offer a ground improvement method, reducing the liquefaction susceptibility and the damage due to seismic loading.

Montoya et al. (2013) conducted geotechnical centrifuge tests (1-m radius Schaevitz centrifuge) to evaluate the potential of MICP treated soil to increase soil resistance to liquefaction triggering, established the technique as a natural and sustainable solution. The responses of the soil were extensively evaluated at varying cementation levels. Depending on the level of cementation, various failure patterns were observed, when subjected to seismic loading. The failure of the heavily cemented specimens were structural fractures, whereas

the lightly and moderately cemented specimens revealed a global soil like failure. Montoya (2012) observed reduced excess pore pressure generation, reduced settlements, and greater peak surface accelerations compared to the untreated loose saturated sands under seismic loading.

Burbank et al. (2013) compared cyclic shear test results of sand treated with MICP and Portland cement, and found the cyclic stress ratio (CSR) of the MICP treated soil was higher than the cement-treated soil for similar quantities of cementing agent. Han et al. (2016) found that treating sand with MICP takes less time to improving liquefiable sand than colloidal silica grouting to achieve a similar liquefaction resistance.

Considering the dynamic loading from waves and earthquakes, Xiao et al. (2018) studied the liquefaction potential of MICP treated calcareous sands using cyclic triaxial tests. The study indicated that the increase in confining pressure leads to a decrease in the liquefaction resistance for bio-cemented specimens. In addition, they found that the number of cycles to liquefaction increased with increasing cementation content, similar to that observed by Montoya et al. (2013). Zamani et al. (2018) investigated the liquefaction resistance of three silica soils with varying grain sizes treated to moderate level of MICP cementation. The results showed that reduction in grain size and increase in angularity result in a higher resistance to liquefaction after MICP treatment.

2.5.3. MICP for dust control

Fugitive dust is often a serious environmental problem in semi-arid and arid environments. Most of the conventional methods for fugitive dust control, including the application of water, salt, or synthetic polymers, are reported to be ineffective in arid climates (Hamdan and Kavazanjian, 2016). MICP, as a promising method, has gained the attention in the recent past.

Meyer et al. (2011) studied the applicability of MICP for the dust control by examining the effects of bacterial concentration, temperature and humidity, and fines content on erosion resistance. They found that a higher fines content and higher temperature and humidity helped form the soil crust that resisted fugitive dust emission. Maleki et al. (2016) investigated the erodibility of MICP treated sand in a wind tunnel under wind velocity condition of 45 km/h and shown the maximum weight loss was around 1.29 and 0.16 % for low and high MICP soils, indicating a significant enhancement. Chen et al. (2016) tested the MICP treated desert sand under a wind velocity of 33 m/s for 20 minutes at flat and inclined (30°) conditions, observed negligible mass loss from the MICP surfaces even after the exposure of 12 freeze-thaw cycles. Similarly, Zomorodian et al. (2019) demonstrated the stable crust formation on silica and carbonate sands by spraying-MICP, which was found to be reliable at simulated 20 m/s winds.

By using large-scale wind tunnel, Naeimi and Chu (2017) concluded that the required precipitation to control the dust generation by 70% is around 15 g CaCO_3/m^2 under a simulated wind condition of 40 km/h (tested to the duration of 6 minutes). Very recently, using the digital imaging technology, Bick et al. (2019) captured the initiation of sporadic and sustained erosions in MICP treated sand and evaluated the shear strength variation timely. Overall, studies confirmed that the MICP have a significant impact on the reduction of wind erosion, reducing the number of airborne particles.

2.5.4. MICP for coastal zone preservation

Among the wide range of MICP applications, attentions have also been given on reinforcing loose sand bodies of nearshore areas and preventing cliffs scouring, thereby improving the responses against erosion from wave action without impairing the intrinsic transient characteristics. Cheng et al. (2014b) investigated the feasibility of utilizing the calcium ions

dissolved in seawater as the sole source of calcium for MICP. The results revealed that the UCS could increase up to 300 kPa with retained permeability of 30%, suggesting good drainage ability. Salifu et al. (2016) have investigated the erodibility response of the MICP treated small foreshore slopes under tidal wave effects. The results showed that a shallow treatment depth could significantly increase the stability of treated surface against the tidal effects.

Nayanthara et al. (2019) studied the effect of Mg^{2+} availability on the MICP efficacy of coast sand. The results indicated that the formation of needle shaped magnesium carbonate crystals significantly reduced the UCS at higher initial Mg^{2+} concentrations. Results also suggested that the concentrations of Mg^{2+} that naturally exists in coastal environment had negligible effects on the MICP efficiency. Recently, Imran et al. (2019) proposed a sustainable and cost-effective approach, i.e. a combination of geo-tube and MICP for preventing coastal erosion. For instance, Daryono et al. (2020) attempted to compare the geo-physical characteristics of natural and MICP induced artificial beach-rocks, revealing that the formation of natural beach-rocks are governed by several geological factors and marine activities, and are incomparable with MICP materials.

2.5.5. MICP for erosion control

Among the soil degradation processes, erosion is one of the most serious degradation processes, and which is primarily persuaded by rainfall and snow melt. Up to now, only few studies have investigated the erodibility of MICP treated deposits through various strategies. By using the simple flume tests, Bao et al. (2017) have conducted sets of flume tests to study the erodibility of MICP treated sands, demonstrated the high surface stability of MICP treated cohesionless sand against scouring. In another study, water jetting erosion test was performed to study the MICP treated sand, showing that the formation of an impermeable stiff crust with

a thickness of 2.5 cm could markedly increase resistance to erosion (Gomez et al., 2013). Similarly, shallow MICP treatment were also recommended by Salifu et al. (2016) for tidal erosion control.

Potential of MICP in controlling seepage-induced internal erosion of gravel-sand mixtures has also been examined by few researchers, suggested that the formation of calcite clusters within the soil is responsible for reduction in erosion (Jiang and Soga, 2019, 2017). Studies also showed that the increase in the predicated calcium carbonate content could increase the resistance to internal erosion. Very recently, surface erosion of sandy slope was studied under short-term simulated rainfall (5 mm/min), showed that erosion resistance was higher in slopes treated using 0.2 - 1 mol/L compared to that treated using 2 mol/L (Jiang et al., 2019). They also indicated that a shallow depth of cementation (around 2 cm) was sufficient to control the erosion significantly. It is worth noting that there are, however, no studies found in the literatures regarding the deployment of MICP to control the erosion induced by cyclic freeze-thaw effects, and that has been extensively addressed in this dissertation.

2.6. Near-surface of the slope and its stability

In this section, a literature review is made to summarize the current understanding on the responses of near-surface soils against climatic impacts and their stability in embankment slopes, providing the background information to the research work focused in this dissertation.

2.6.1. *Near-surface zone of the slope*

Near-surface zones in embankment slopes can be simply defined as shallow zone or mantle which is directly exposed and influenced by climatic effects (refer **Figure 1.1**). In fact, wetting-drying and freezing-thawing weather cycles coupled with the influence of vegetation

roots, result in the formation of the weaker zone in the order of a few meters thick (Dixon et al., 2019; Smethurst et al., 2006), and the characteristics of soil in this zone differ from its initial as-built states. For instance, Glendinning et al. (2014) observed the changes of near-surface flow characteristics up to four orders of magnitude in an aged embankment slope, and these temporal variations were found to be occurred within the depth 0-1.4 m. Recently, Dixon et al. (2019) performed 143 individual *in-situ* measurements to study the spatial variability of near-surface characteristics. They showed that for a natural embankment, hydraulic conductivity was found to vary by five orders of magnitude in the slope near-surface (0 to 0.3 meters depth), decreasing by four orders of magnitude between 0.3 - 1.2 meters depth.

2.6.2. Deterioration processes in near-surface zone

Among the slope near-surface deterioration processes, erosion is one of the most serious slope degradation process, and which is primarily persuaded by rainfall and snow melt (Fox et al., 1997; Rieke-Zapp and Nearing, 2005). As illustrated in **Figure 2.5**, it occurs due to complex interactions of subprocesses between the detachment and transport of surface materials (Dai et al., 2018; Zhang et al., 2018). The soil particle detachment is predominantly initiated by the impacts from rainfall (i.e. raindrops) and snowmelt (i.e. water build-up on/ near surface), and when their kinetic energy overcomes soil shear strength, soil particles are mobilized and dislodged from the slope surface (Fattet et al., 2011). It has been reported that the sediment yield and runoff production of a slope are significantly impacted by flow conditions (e.g. rainfall intensity, runoff rate and flow velocity) and slope surface conditions (e.g. soil texture, topography) (Fox et al., 1997; Qing-quan et al., 2001; Rieke-Zapp and Nearing, 2005). According to the literature data, the erosion leads to billions of tons of soil loss every year (Magleby, 1995). If the surface erosion is not controlled properly, it is likely to worsen other associated degradation processes such as toppling, sliding, spreading and flowing (Tang et

al., 2016). Also, the deposition of these material in drainage system often obstructs the engineering structures and systems (Omar et al., 2018).

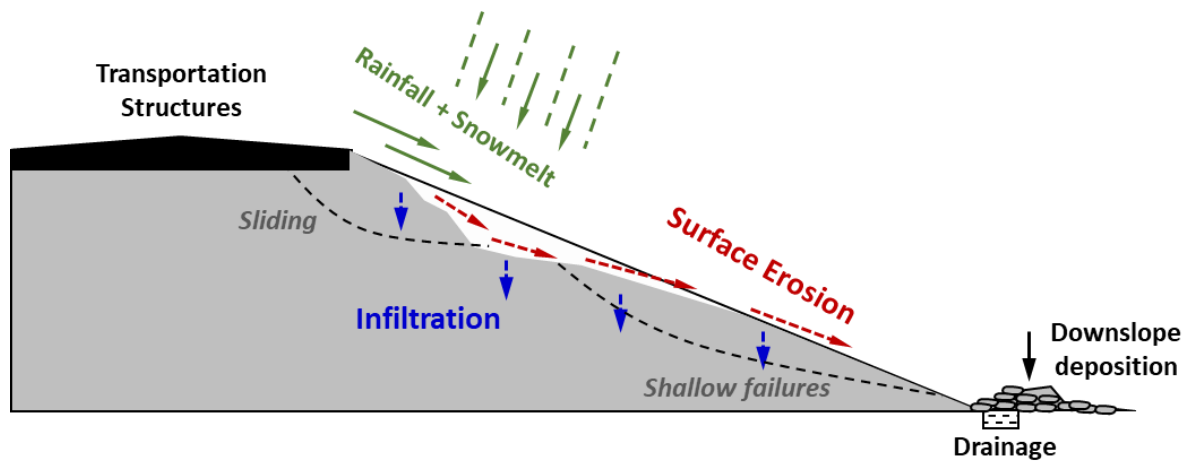


Figure 2.5: Surface erosion and interactions of subprocesses

Soils in cold regions usually experience freezing-thawing (FT) during winter seasons, resulting profound effects on stability and erodibility of soil along the slopes (Ferrick and Gatto, 2005; Gatto, 2000). The soils are covered by considerable layer of snow, keeping the soils to be frozen during winter (Farukh and Yamada, 2018). When water turns to ice within the soil pores, it increases in volume by up to 9 %, resulting in increased pressure within the water-filled pores of the matrix (Bell, 1992). This impairs the pre-existing structure of the soils. In other words, increasing the porosity has a negative effect on its strength, limiting the durability of the slope soils. However, at frozen state, the aggregate stability increases despite of the impairments caused by freezing (Dagesse, 2013; Perfect et al., 1990). When the soils experiences thawing, the aggregate stability drops significantly; as the result, soil losses are typically reported to be the highest in early spring (on thawed surfaces during snowmelt) (Oztas and Fayetorbay, 2003). The surface runoff generated from snow melt also had significant potential to detach the particles from thawed surfaces (Kværnø and Øygarden, 2006). The soil texture, organic matter content, initial aggregate size, soil water content at the time of freezing, the number of freezing and thawing cycles were found to be the factors

crucially influencing the aggregate stability under freeze-thaw actions. But, the duration and the rate of the freezing were found to have insignificant or no effect on aggregate stability (Dagesse, 2013; Li and Fan, 2014).

Moreover, near-surface of the slope is frequently exposed to rainfalls and evaporation processes. In other words, the in-situ surfaces are frequently subjected to periodical wet-dry (WD) cycles during (i) diurnal changes in rainy-sunny weathers (Tang et al., 2011) and (ii) subsequent processes of rainfall-evapotranspiration (Tang et al., 2016). Dissolution of cementing materials, constituent mineral grains, or both, sometimes in association with reprecipitation of dissolved ions as salts, are the popular chemical processes of deterioration that possibly occur during wet-dry cycles (Khanlari and Abdilor, 2015). Many previous studies shown that the presence of swelling minerals in clay and soft soils causes the temporal change in soil structure, resulting the desiccation cracking, shrinkage, porosity and volumetric changes (Albrecht and Benson, 2001; Krause et al., 2019; Pires et al., 2014; Tang et al., 2011; Zhang et al., 2013). Presence of organic content had a negative impact, found to be inducing the initiation of the microcracks during WD process (Diel et al., 2019). WD effects were also found to determine the microstructure, shear strength, hydraulic conductivity and compressibility of soil aggregates (Goh et al., 2014; Tang et al., 2016). On the other hand, fracture energy reduction and chemical corrosive actions are the most reported deterioration mechanisms for sediments. The amount of existing clay determines the quantity of mechanical degradation in tuffs, experiencing swell-shrink deformations during WD (Ulusay, 2014). Khanlari and Abdilor (2015) observed the dissolution of zeolite and carbonate fragments in sandstones under the prolonged WD cycles. Similarly, the corrosion of depositional and diagenetic carbonates resulted degradation of calcareous sediments (Ciantia et al., 2015, 2014). Consequently, WD cycles were witnessed to be resulting substantial

irreversible deformations in soil fabric, influencing the physical and mechanical responses of slope near-surfaces.

2.6.3. *Mechanism of near-surface failures*

The strength of the near-surface soil is considered to be changed after the construction of embankment slopes in response to climatic variations as discussed in the previous section (2.5.2). Typically, near-surface failures in embankments are reported to be triggered during or immediately after prolonged rainfalls or after the snow melting events, i.e. at nearly a saturated condition. The failure mechanism is typically owing to the infiltration of rainwater/snowmelt. As the slope surfaces are generally found to be in an unsaturated state, part of the flow infiltrates the slope, which increases the pore water pressure and seepage forces within/around the near-surface zone. The rise in pore water pressure diminishes the effective stress and shear strength of the slope soil, and the soil becomes heavier when wet on potential failure surface, eventually triggering the near-surface failures (Harden and Scruggs, 2003; Muntohar and Liao, 2010). In near-surface failures, the failure surface develops at shallow depth and often parallel to the slope surface. When the near-surface tends to overly the impermeable layer/ bed rock, debris avalanche landslides possibly occur, resulting the accumulation of slumps on roads and expressways (Megahan et al., 1978).

Reduction in matric suction is another crucial factor that trigger the near-surface failures. Usually, unsaturated embankment slopes experience high matric suction (i.e., negative pore-water pressure) at near-surface zones during dry periods, which significantly contributes to the shear strength of the soil. During prolonged wet periods when there is sufficient infiltration into the slope, the matric suction of the soil decreases, and this in turn results in an increase in the soil water content. As a result, the additional shear strength provided by the

matric suction can be reduced enough to trigger near-surface landslides (Fredlund and Rahardjo, 1993; Rahardjo et al., 2005, 1995).

2.6.4. *Techniques available for slope surface stabilization*

In addition to the near-surface degradations and failures reported in previous sections (2.5.2 and 2.5.3), disturbed near-surfaces are often reported to be one of the major sources of sediments, if not stabilized properly. Researches consistently indicated that construction fills such as expressway fills in mountainous environment causes severe stream sedimentation, suggesting that stabilization measures must be employed during or immediately following the construction of embankment slopes (Ice and Stednick, 2004). Sound slope surface stabilization techniques would help to keep the soils on the slopes and prevent them from ever becoming entrained in runoff or mass transport.

In fact, the engineering attentions on stabilizing slope surfaces had been a crucial concern since more than 50 years ago. From that time, the use of vegetation is known to be the simplest and most cost-effective means of stabilizing slope surfaces (Morgan and Rickson, 2003; Norris et al., 2008). The vegetation as an engineering material, serves as a cover to the surface, enduring the slope from the direct contact of climatic measures (refer **Figure 2.6(a)** as an example). Their root networks reinforce the near-surface zones and contributes to the suction regime, enhancing and guaranteeing the stability of slope substrates (Chirico et al., 2013). The appropriate choice of the plant material is a difficult part, as sound knowledge is required to determine the capability of the plant to grow on a particular site. Native plants are generally preferred, requiring less expense and maintenance, as well as being visually harmonious with the natural landscape. Many exotic species (herbaceous species, grass, weeds, plants etc.) have also been cultivated specifically for erosion protection, showing viability. However, to achieve the desired level of protection and benefits, the vegetation technique takes quite a

long time (Jiang et al., 2019). The applicability of vegetation is limited to certain regions such as subarctic and arid zones, as the growth and survival are inhibited by the intrinsic climatic conditions.



Figure 2.6: Some techniques currently in use to stabilize the slope surface

Use of geotextiles such as nonwoven, open weave textiles (OWT), erosion control blankets (ECB) and base mulches (BM) are some conventional techniques, used as cover to stabilize the slope surfaces against sediment run-off (refer **Figure 2.6(b)** as an example: photograph taken at Noveribetsu, Hokkaido, Japan). In most of the cases, they were developed by using either natural fibers such as jute, coir, and sisal or synthetic fibers such as polyester and polypropylene (Kumar and Das, 2018; Mwasha, 2009; Vishnudas et al., 2006). OWT contains large open spaces and are commonly comprised of jute or coir reinforcement mats and polymer yarns woven into a matrix. In ECB, curled and barbed excelsior fibers were engineered into layers of net-like structure to provide erosion control and vegetation establishment capabilities. For instance, after the failure of railway embankment occurred in United Kingdom in 2013, multifunctional geotextiles were potentially applied in Germany, along the railroads (Fuggini et al., 2016). BM, since early years, was a popular technique used for surface stabilization purposes. However, with the advancement, the technique was

promoted to hydraulically applied erosion control products, i.e. pre-packaged fibrous material that was mixed with water and hydraulically applied as a slurry designed to reduce surface erosion and assist in the establishment of vegetation (Kelsey, 2014; Lipscomb et al., 2006). Although the above techniques are locally abundant and preferable, as a notable drawback, their durability is quite limited due to their rapid bio-degradable characteristic.

In the recent years, bituminous and chemical-based methods have drawn considerable attention and broadly applied for near-surface stabilization of slopes and erosion control. For instance, grout materials such as polyacrylamide and phosphor-gypsum were applied to bind surface substrates to improve erosion resistance through agglomeration and reduced dispersion (Cochrane et al., 2005; Yu et al., 2003). The use of Portland cement is the mostly used slope sealing method all over the world, as it could provide rapid benefits over other conventional methods (refer **Figures 2.6(c)** and **(d)** as examples: photographs were from Hokkaido-Japan and Hatton-Sri Lanka, respectively). For instance, embankment slopes of highways in Iraq was stabilized by Portland cement (6%), resulted two times increase of UCS and reduced swelling potential by 90% (Daraei et al., 2018). In another case, sandy slopes in Indonesia were stabilized by injecting the Portland cement, which could increase the friction angle up to 47.57° and increased the FOS from 0.78 to 1.17 (Arya et al., 2018). However, as indicated earlier, the environmental issues on Portland cement manufacture remain a huge barrier to move forward. At the same time, surface sealing by cement grouts inhibit the growth of vegetation, impeding natural slope processes. Recently in Australia, Tang et al. (2018) proposed and investigated the porous concrete for stabilizing slope surface, successfully integrated the vegetation and root networks within the concrete pores.

2.6.5. Merits on introducing MICP for near-surface stabilization of slopes

In this dissertation, the MICP is proposed as a novel promising alternative for stabilizing the near-surface of embankment slopes. From the above comprehensive review, it is clearly perceived that MICP technique has several unique merits over currently available methods, and those are listed and briefed below.

- **Non-destructiveness:** Construction of slopes and near-surface treatments were often come across as challenging practices. Therefore, non-destructive methods are expected to be convincing best practices and are preferable in Geotechnical Engineering, which could eliminate the disturbance of substrates, preserving energy, environment and cost. MICP treatment can be implemented non-destructively and easily, through surface, requiring very less energy and cost.
- **Levels of treatment:** MICP technique has a uniqueness that the degree of cementation can be controllable. Therefore, depending on the requirement, the treatment can be performed to varying levels. However, comprehending the required level is a mandatory process prior to the applications.
- **Retained permeability:** One of the issues in the currently existing techniques (bituminous and cement-based sealing) is complete loss in permeability of treated surface, which interrupts the infiltration flow lines, leading to the increase in water-buildup and surface scouring. MICP treatment preferentially results the formation of crystals at particle contacts, holding the soil pores relatively open with retained permeability. The permeability retention leads to less/no excess water build-up on treated surface during runoff and snowmelt and eliminates the cost regarding construction and maintenance of additional drainage systems.
- **Can easily be healed:** The cemented bonds can be easily healed once the MICP matrix or cementation is found to be degraded. Resupplying the necessary MICP resources by injections could heal the cementation bonds, and the mechanical responses of the healed specimen could essentially be equivalent to that of the initial as-built responses.

CHAPTER 3

INDIGENOUS BACTERIA AND BASELINE INVESTIGATIONS

3.1. Introduction

MICP is a biochemical process which is highly reliant on the performance of the bacteria chosen and associated limitations. Therefore, for a successful application, the selection of suitable ureolytic bacteria is the most important factor in this urease-based technique. Temperature is one of the most important factors to bacteria growth and their metabolism. A typical response of bacterial urease at the exposure to various temperature conditions is presented in **Figure 3.1**. As shown, the optimal urease activity of the bacteria can be achieved above 30°C. Numerous previous studies evidenced that most of the soil bacteria could effectively produce urease enzyme within moderate to high temperature ranges (30°C-70°C). For example, Whiffin (2004) studied the enzymatic response of *Sporosarcina pasteurii* (also known as *Bacillus Pasteurii*) with temperature between 15-80°C, suggesting that the optimum temperature for the enzyme activity is 70°C. A similar tendency has also been reported to *Pararhodobacter* sp. by Fujita et al. (2017). Omoregie et al. (2017) concluded that around 30°C provides best environment for the optimum bacterial performance. Activity of the soil urease has also been demonstrated to be the highest at 50°C (Moyo et al., 1989).

On the other hand, urease activity is extremely impaired at low temperatures; the activity rapidly drops with the decrease in temperature notably below 20°C (trivial zone, as shown in **Figure 3.1**). It can also be seen that the urease activity is almost completely inhibited at the

temperature below 10°C (inactive zone). For instance, *Sporosarcina pasteurii*, the popular MICP bacteria were applied at low temperature and observed. Due to the very weak activity of the bacteria at low temperature, it was very difficult to bind sand particles effectively (Sun et al., 2019). Moreover, at low temperatures, the reproduction of bacterial cells also substantially decreases (Keykha et al., 2012). The coupled effect of both insufficient enzymatic activity and growth eventually affects the calcium carbonate precipitation at lower temperatures, restricting the wide range of applications.

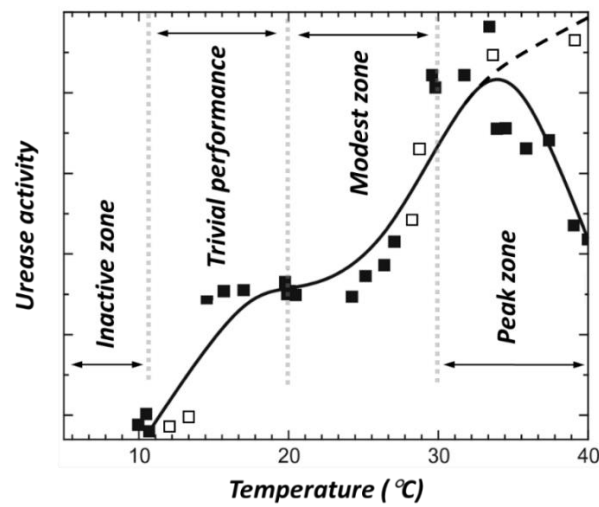


Figure 3.1: Typical bacterial urease activity with the temperature (modified from Wang et al., 2019)

In most regions of the world, the peak value of the soil temperature is not greater than 30°C; the soil temperature typically ranges from 10°C to 30°C, excluding the winter seasons (Wang et al., 2019). The soil temperature (exclusive of winter season) in Hokkaido also ranges between 5°C to 25°C with considerable day-night fluctuations (Farukh and Yamada, 2018). Apparently, the low temperature climate poses uncertainties on the metabolic performance of most of the previously reported bacteria, restricting the application of MICP in Hokkaido and

other cold regions. This necessitates the exploration of potential ureolytic bacteria which can induce the calcium carbonate formation under wide range of soil temperatures.

This chapter presents a baseline study performed on bacterial and MICP responses. The main objective of this work is to study the urease performance of the bacteria isolated from the native cold region, under different temperature and chemical conditions. For this purpose, series of urease activity tests and test tube tests were carried out to determine the optimum recipe for the MICP treatment. Next, the effect of particle size distribution on the effectiveness of MICP was evaluated by comparing the responses of sands with different grain distributions. The micro-structure of the treated soils was also well examined.

3.2. Representative expressway slope of Hokkaido

One of the erosion prone expressway slopes located in cold region (Hokkaido, Japan) has been chosen for the investigation as the representative. As indicated in **Figure 3.2**, the study location is in the Do-O expressway line, positioning within the range of Onuma, Hakodate (latitude of 42.388532 and longitude of 140.284762). The *in-situ* view of the slope and the sampling photographs are presented in **Figure 3.3**.

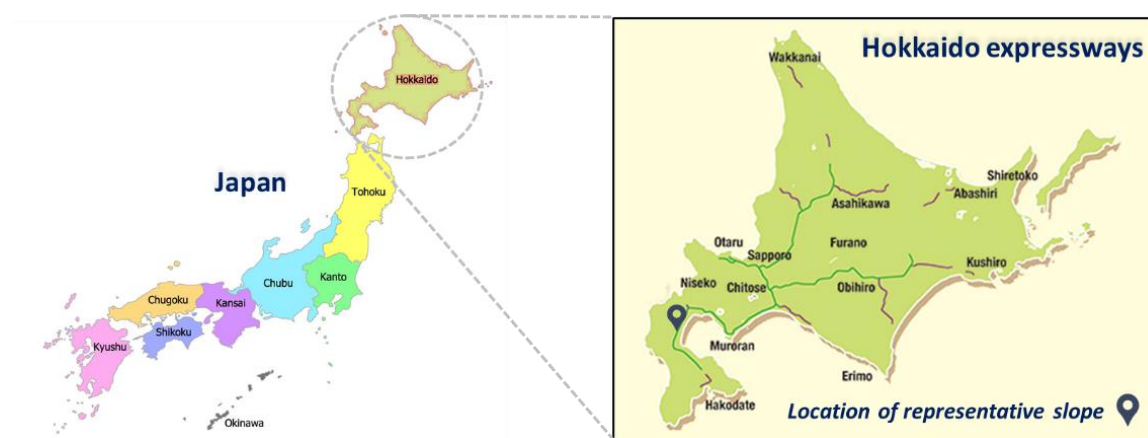


Figure 3.2: The location of the representative slope in Hokkaido, Japan

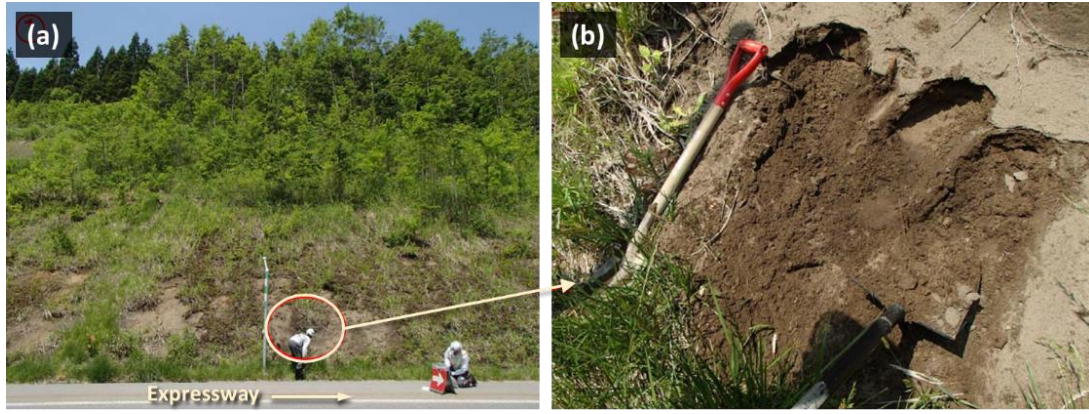


Figure 3.3: (a) Field survey on the slope and (b) sample collection

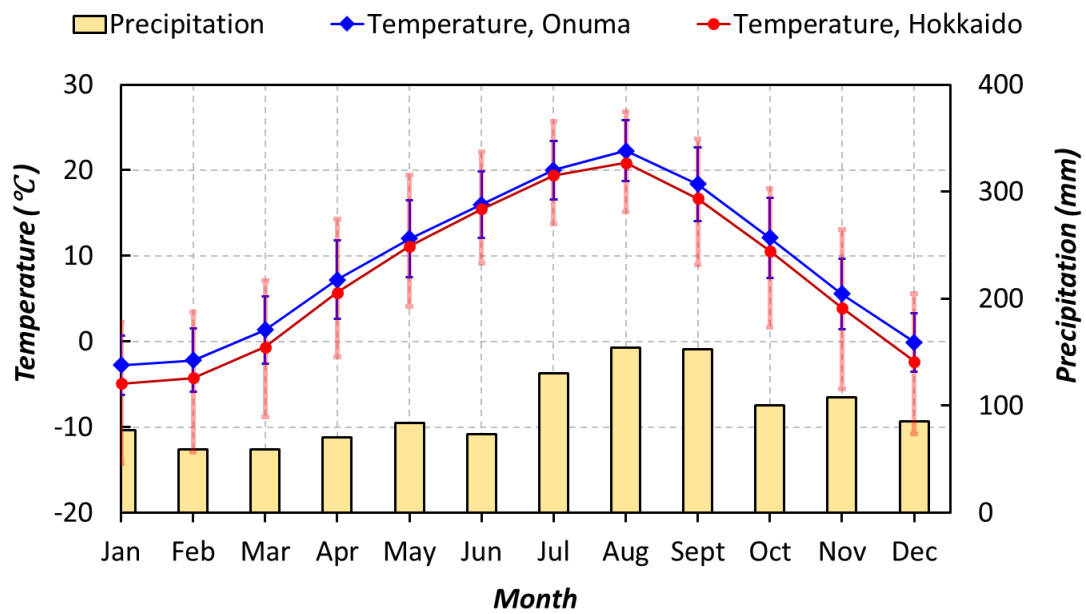


Figure 3.4: The temperature and precipitation data of the study location

As mentioned earlier, the study location is famous for tourism, experiencing a cold sub-arctic climate with around 100 days of snowfall per winter. **Figure 3.4** shows the monthly variations in mean atmospheric temperature of Hokkaido island and study location (Onuma, Hakodate), along with the average precipitation data (Onuma, Hakodate) based on the means of 1981-2010 meteorological data, Japan Meteorological Agency. The other climatological data can be found in **Appendix A**. It can be seen that the maximum average temperature ranges

between 20-25°C, achieved during August (summer season). The annual average rainfall is reported to be 1211 mm (47.7 inch).

3.3. Materials and methods

3.3.1. Isolation and characterization of indigenous ureolytic bacteria

Set of soil samples collected from the above location (in sterile test tubes) were 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 (50 μ L) were applied on NH_4 -YE agar plates (prepared by combining tris-buffer, ammonium sulfate, yeast extract and agar), followed by the incubation at 30 °C for 72 h. 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 h. 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 finally 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).

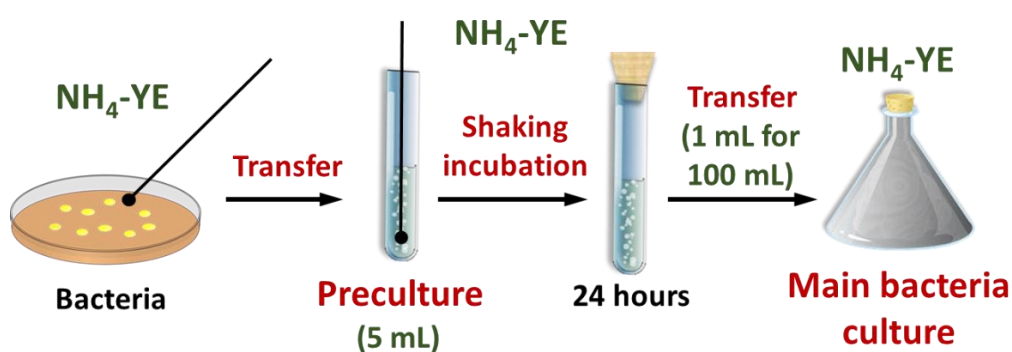


Figure 3.5: Bacteria cultivation process

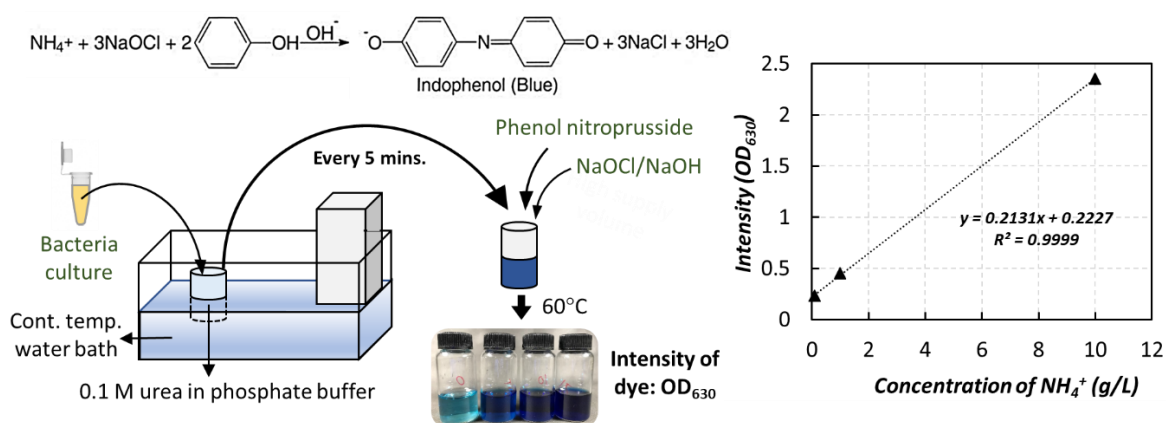


Figure 3.6: Indophenol spectrophotometry and calibration curve

3.3.2. Growth media and culturing method

To grow the bacteria to the desired population density, an ammonium-yeast extract media (NH₄-YE, ATCC 1376) was used, which consists of 20 g/L of yeast extract, 10 g/L of ammonium sulfate and 15.7 g/L of tris buffer. Initially, the bacteria were grown in agar plates (solid medium) at desired temperature. When the adequate growth was attained, a preculture (5 mL of NH₄-YE) was used to enrich the bacteria (liquid medium) under shaking incubation (160 rpm). After 24 hours, the preculture was suspended to the main culture, wherein 1 mL preculture was used to prepare 100 mL of main culture. **Figure 3.5** explains the process graphically. Finally, the bacteria were harvested from main culture when the desired growth was achieved.

3.3.3. Urease activity test

Urease activity of the bacteria was measured using spectrophotometric determination of ammonia as Indophenol (Bolleter et al., 1961), and the methodology is illustrated in **Figure 3.6**. 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 wavelength of 630 nm (OD₆₃₀)

at different uniform intervals of catalysation (at every 5 minutes interval). Using the calibration curve developed (presented in **Figure 3.6**) between concentration of ammonium ions and intensity (OD_{630}), the rates of urea hydrolysis were estimated. One unit of activity (U) can be defined as the amount of urea (mmol) degraded per minute.

3.3.4. Test tube test

$CaCO_3$ precipitation tests were carried out in aqueous media using test tubes containing 10 mL MICP solution. The MICP solutions were prepared at different physical and chemical conditions to study different factors governing the MICP reactions. **Table 3.1** presents the information of 16 different cases studied in these tests. The prepared test tubes specimens were incubated at corresponding temperature at 160 rpm for 48 hours using shaking incubator. By the end of the test, the precipitates in each tube were collected using filter paper, washed well using distilled water and dried at $60^\circ C$ until constant mass was achieved. Finally, the retained mass of the precipitates was measured. For the cases investigated the effect of initial concentration of resources ($CaCl_2$, urea and biomass) (Case 1 – 10), the utilization ratios were evaluated and discussed. The ratio between precipitated mass and the theoretical maximum, estimated based on the $CaCl_2$ concentration, has been defined as the utilization ratio. The cases 11 – 16 were studied up to 72 hours to correlate the precipitation rates at different temperature, and the average precipitation rates were correlated with the average urease activity.

Table 3.1: Test cases of test the tube test

Case No.	[CaCl ₂] (mol/L)	[Urea] (mol/L)	Bacterial inoculation (OD ₆₀₀)	Temperature (°C)	Shaking incubation time (h)	Purpose
1	0.25	0.25	0.31	25	48	<i>To study the effect of initial concentration of resources</i>
2	0.50	0.50	0.31	25	48	
3	0.75	0.75	0.31	25	48	
4	1.0	1.0	0.31	25	48	
5	1.50	1.50	0.31	25	48	
6	2.0	2.0	0.31	25	48	
7	1.0	1.0	0.15	25	48	<i>To study the effect of biomass concentration</i>
8	1.0	1.0	0.31	25	48	
9	1.0	1.0	0.46	25	48	
10	1.0	1.0	0.62	25	48	
11	1.0	1.0	0.31	5	72	<i>To study the effect of temperature</i>
12	1.0	1.0	0.31	10	72	
13	1.0	1.0	0.31	15	72	
14	1.0	1.0	0.31	20	72	
15	1.0	1.0	0.31	25	72	
16	1.0	1.0	0.31	30	72	

3.3.5. Characteristics of soils used

Figure 3.7 presents the grain size distribution curves of the expressway slope soil and sands used in this investigation. Based on Unified Soil Classification System (USCS) (ASTM D2487-17, 2017), the slope soil can be categorized as poorly graded sand (SP), consisting fine content (< 0.1 mm) of 5 % (in which, silt content (< 0.063 mm) is 3 %). The other

standard sands (Mizunami, Mikawa and Toyoura sands) used herein are also poorly graded (SP). The intrinsic parameters of the sands are given in **Table 3.2**. Also, a summary of the chemical composition obtained from X-Ray fluorescence (XRF) analysis for all the sands is presented in **Table 3.3**. The results show that the silica is the dominant mineral in all the sands.

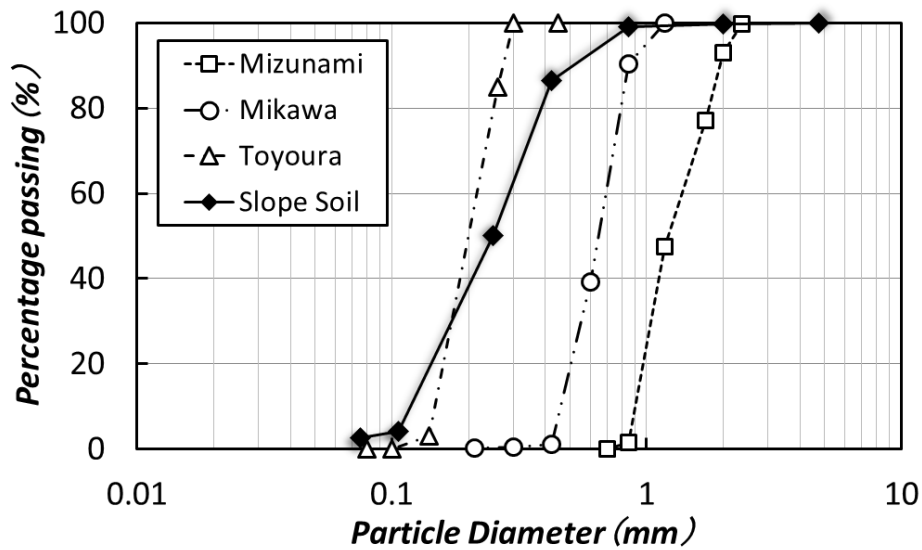


Figure 3.7: Particle size distribution curves of soils studied in this work

Table 3.2: Intrinsic characteristics of soils studied in this work

Soils	USCS	pH	D_{50} (mm)	C_u	C_c	G_s
Slope soil	SP	6.997	0.23	2.5	0.8	2.71
Mizunami	SP	7.0 ± 0.1	1.60	1.3	0.97	-
Mikawa	SP	7.0 ± 0.1	0.87	1.5	0.92	2.66
Toyoura	SP	7.0 ± 0.1	0.20	1.5	1.0	2.64

(D_{50} : mean particle diameter; C_u : coefficient of uniformity; C_c : coefficient of curvature; G_s : specific gravity)

Table 3.3: Mineralogical composition of soils studied in this work

Soils	Oxides											
	SiO ₂	Al ₂ O ₃	Fe ₂ O ₃	CaO	MgO	K ₂ O	SO ₃	TiO ₂	P ₂ O ₅	MnO	V ₂ O ₅	Na ₂ O
Slope soil	57.5	25.6	8.1	3.68	1.99	1.05	0.67	0.58	0.3	0.14	0.03	-
Mizunami	97.4	1.37	0.03	-	-	-	-	-	-	-	-	-
Mikawa	97.7	0.89	0.43	0.03	0.11	0.18	-	-	-	-	-	0.04
Toyoura	92.6	3.7	0.7	0.5	0.2	-	-	-	-	-	-	-

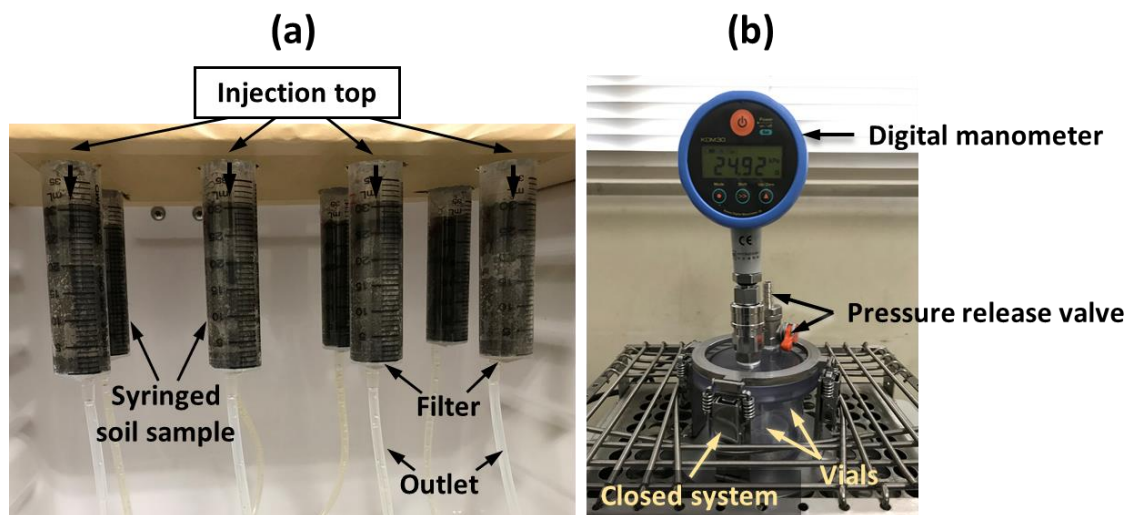


Figure 3.8: The images of **(a)** soil columns test setup and **(b)** calcium carbonate measurement device

3.3.6. Soil column tests

Soil columns (6 cm in height, 2.5 cm diameter) were prepared using 35 mL syringes and positioned vertically (**Figure 3.8(a)**). The soils in columns were prepared to an initial dry density of 1.6 ± 0.1 g/cm³. Reactants were injected in two subsequent phases as suggested by several MICP researchers (Amarakoon and Kawasaki, 2018; Feng and Montoya, 2016; Whiffin et al., 2007). In the first phase, the bacteria culture was injected to the column at the

population (OD_{600}) of 4 – 4.5, 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. The cementation solution (1 mol/L) consisted of calcium chloride (110.9 g/L), urea (60 g/L) and nutrient broth (6 g/L). All the solutions were simply applied to the top surface of the soil columns and allowed to percolate under gravitational and capillary forces. The cementation solution was percolated every 24 hours (total of 14 cementation injections, 10 mL per each), and the bacteria culture was once again injected at the middle of the 14 days treatment (total of 2 biological injections, 10 mL per each). The pH and Ca^{2+} concentration of the outlet were measured once in every 72 hours. After the treatment was completed, the samples were flushed with distilled water prior to the UCS measurements.

3.3.7. Measurement of calcium carbonate content

Carbonate content was determined by the simplified device (**Figure 3.8(b)**) 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 (Fukue et al., 2001). Mass of the oven dried ($105^\circ C$ for 48 h) sample was measured, and the sample was placed into the flask. HCl (2 mol/L) was placed in small plastic vials and set into the 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 (**Figure 3.9**), the carbonate content was estimated, hence the percentage of mass of $CaCO_3$ was determined using the following equation (**Eq. 3.1**).

$$CaCO_3 \text{ content (\%)} = \frac{\text{Weight of } CaCO_3 \text{ (in the specimen)}}{\text{Weight of the oven dried specimen} - \text{Weight of } CaCO_3} \quad \text{Eq. (3.1)}$$

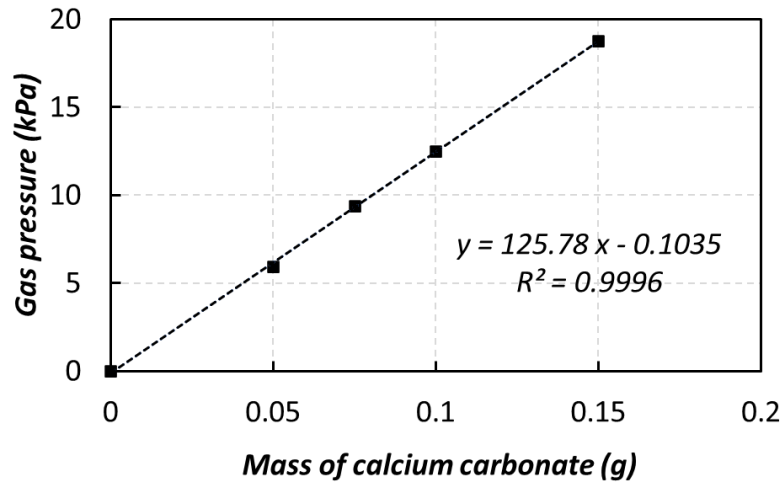


Figure 3.9: The calibration curve: gas pressure versus mass of calcium carbonate

3.3.8. Needle penetration test for estimation of UCS

The unconfined compressive strength (UCS) of the cemented specimens were estimated using needle penetrometer (SH-70, Maruto Testing Machine Company, Tokyo, Japan, shown in **Figure 3.10**) according to JGS 3431-2012 (JGS 3431-2012, 2012). 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 (Ulusay, 2014). 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. 3.2**) developed by analyzing 114 natural soft rock samples and 50 cemented soil samples, the UCS of the specimen was estimated.

$$\log (y) = 0.978 \log (x) + 2.621 \quad \text{Eq. (3.2)}$$

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

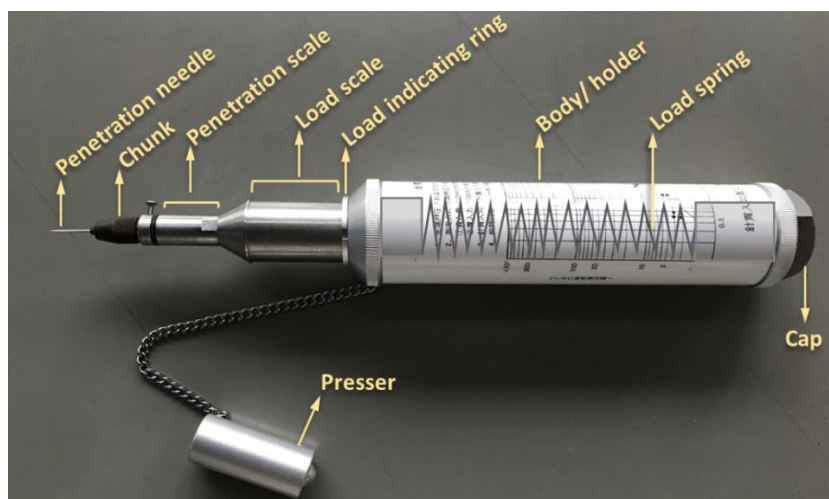


Figure 3.10: Needle penetration test device

3.3.9. Scanning electron microscopy (SEM) analysis

To study the microstructural changes in the specimens, SEM analysis was performed using Miniscope TM 3000 (Hitachi, Tokyo, Japan). The representative samples were carefully taken from the samples and observed through SEM after the oven dry at 60°C.

3.3.10. Fluorescence microscopy analysis

To observe the bacteria cells clearly in the liquid media, the fluorescence microscopy analysis was performed. First, the bacteria solution was centrifuged at 8000 rpm for 5 minutes, and the cell pellets were resuspended in 10 mmol/L concentrated HEPES buffer (pH 7.4), followed by the staining. 3 μ L of the dye (combination of fluorescence dye A and B) was added to the bacteria suspension prepared, mixed thoroughly and incubated at room temperature in the dark for 15 minutes. Finally, 5 μ L of the stained bacteria suspension was dropped in a 35 mm dish, subjected to fluorescence microscopy analysis.

3.4. Results and Discussion

3.4.1. Indigenous isolates and selection

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* (*L. xylanilyticus*), *Sporosarcina* sp. and *Viridibacillus arvi* (*V. arvi*) by the 16S rRNA gene sequence analysis (the authenticate phylograms of the bacteria are presented in **Appendix B**). From the preliminary experiment results (**Figure 3.11**), *L. xylanilyticus* was chosen based on the highest urease production and investigated in detail for MICP application. *L. xylanilyticus* is a gram positive, rod shaped bacteria (average length of 8-10 μm ; diameter of 0.5-0.6 μm). The digital microscope image of the strain is also presented in **Figure 3.11**. It is worth noting that this is the first work reporting the urease and MICP potential of the *L. xylanilyticus*.

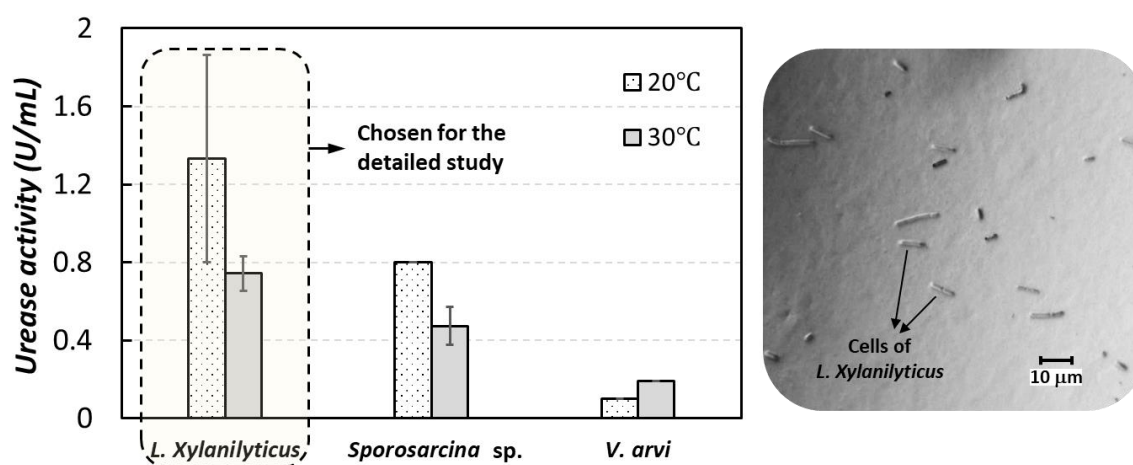


Figure 3.11: Preliminary activity test results of the isolates and the microscopical image

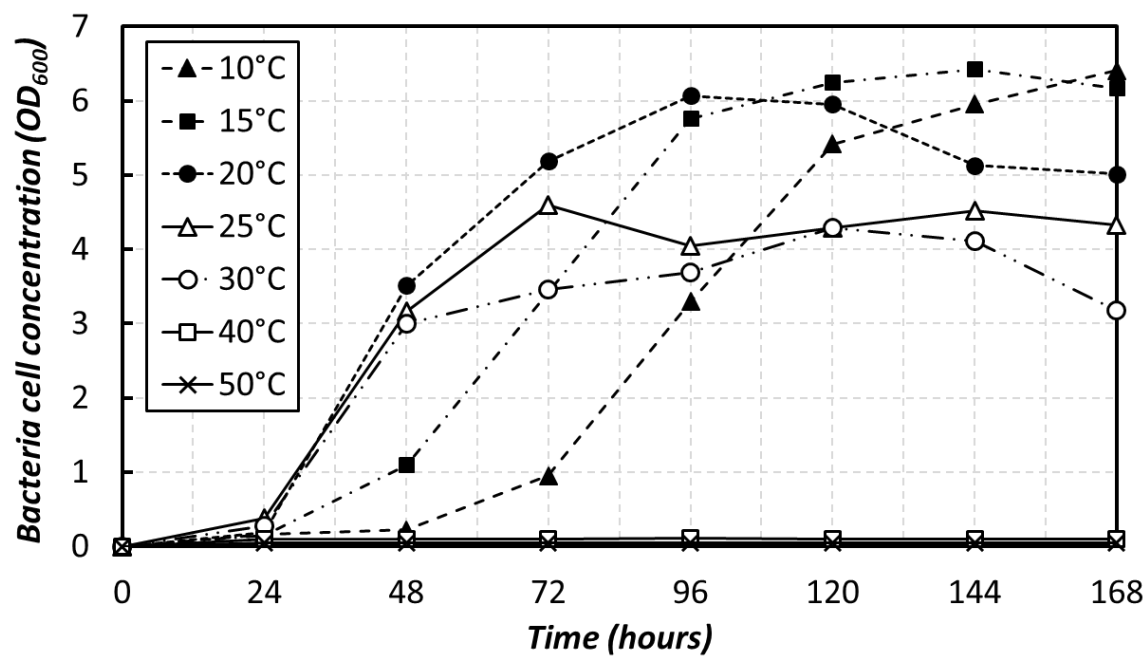


Figure 3.12: Growth of *L. xylanilyticus* at different temperature conditions

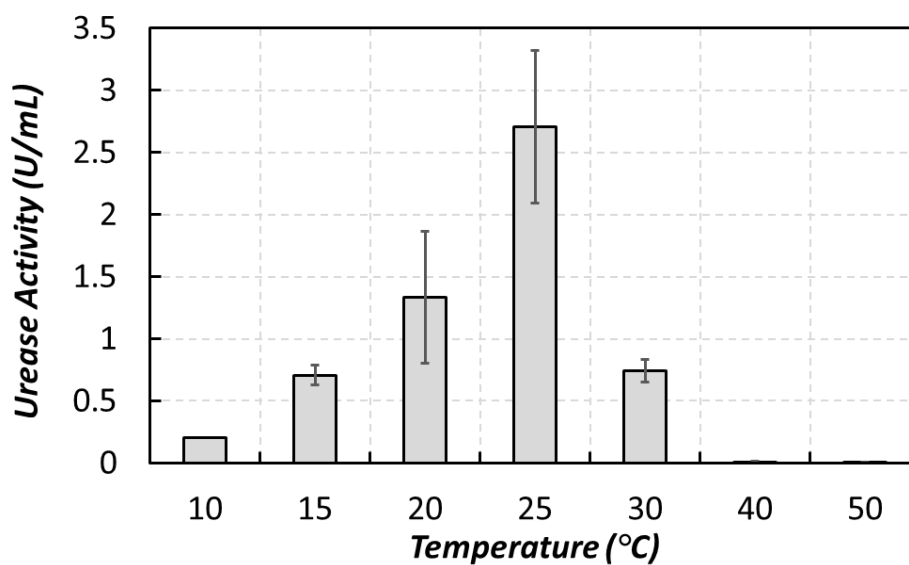


Figure 3.13: Urease activity of *L. xylanilyticus* at different temperature conditions

3.4.2. *Biological responses of bacteria*

3.4.2.1. *Effect of temperature on growth and urease performance*

The influence of temperature on growth of *L. xylanilyticus* 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 **Figure 3.12**. 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 (Omorieg et al., 2017). **Figure 3.13** presents the urease activities under different temperatures. It should be noted that relatively a bell-shape relationship between urease activity and temperature was obtained. Urease activity of *L. xylanilyticus* is relatively higher within the temperature range 20-25°C (ranged between 1.5 and 3 U/mL). The urease activity reached the optimum value at 25°C, above which the activity dropped significantly and became negligible. Considerable activities are evidenced at 15°C and 10°C (0.75 U/mL and 0.2 U/mL respectively), but the activity becomes insignificant below 5°C.

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) (Ciurli et al., 1996; Mobley et al., 1995). The stability of bacterial urease with the time is shown in **Figure 3.14**. The results indicate that the urease activity of *L. 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 proteolyze quickly.

3.4.2.2. Effect of pH in urease performance

Figure 3.15 presents the effect of pH conditions on urease activity of *L. xylanilyticus*. 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.

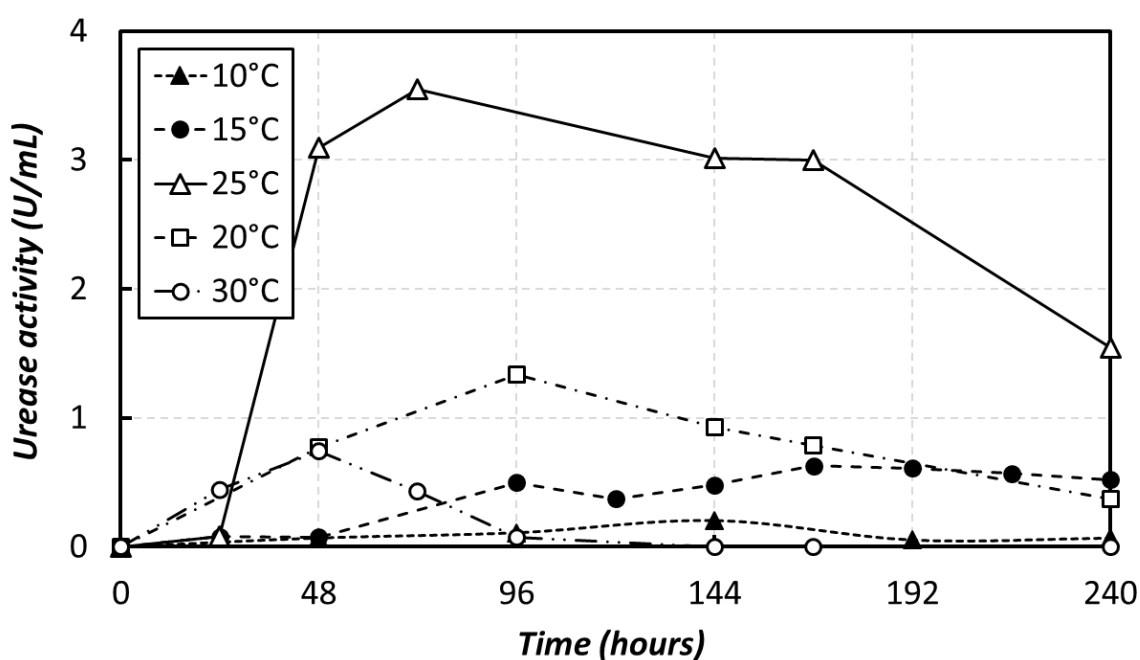


Figure 3.14: Performance of the urease enzyme with time under different temperatures

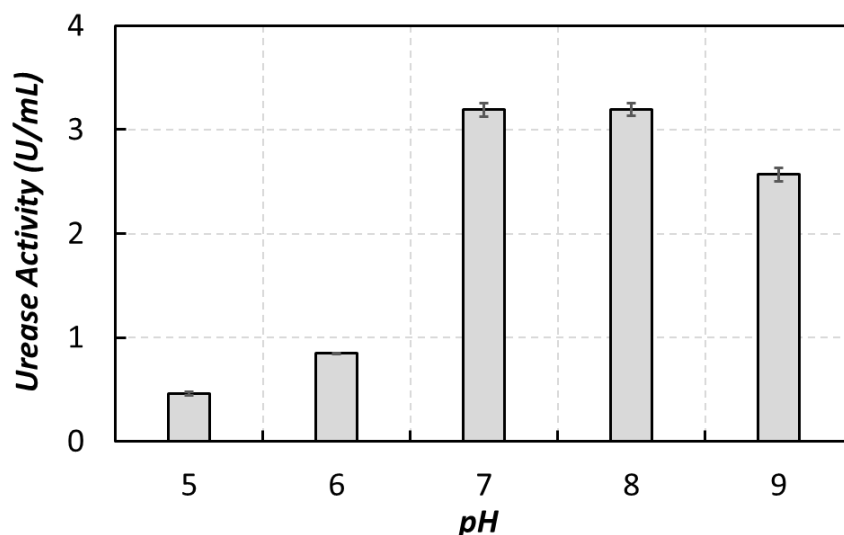


Figure 3.15: Urease activity of *L. xylanilyticus* under different pH conditions

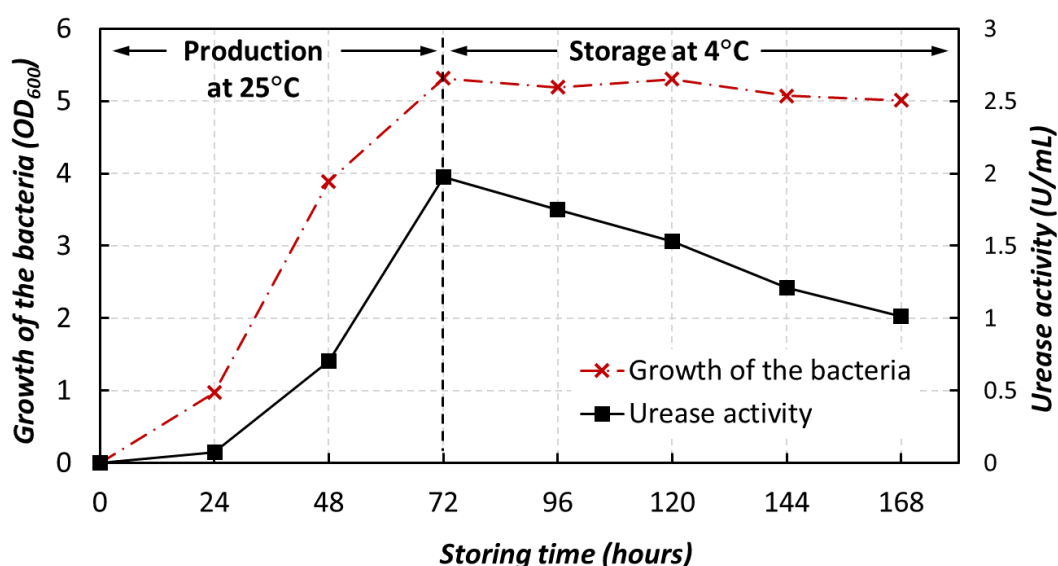


Figure 3.16: The variation of urease performance during the storage at 4°C

3.4.2.3. 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 separate urease activity tests 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 *L. xylanilyticus* is not segregated into extracellular medium but localized completely in or on the cell membrane, indicating cytoplasmic enzyme. This is similar to that reported to most of the urease of the species including *Sporosarcina* and *Pararhodobacter* sp. (Fujita et al., 2017; Mobley et al., 1995).

3.4.2.4. *Effects on storing at 4 °C*

Storing of the bacteria culture is also an important process in MICP. If the characteristics of the bacteria culture could be preserved for a certain term, the storing could be effective, helping to reduce the use of energy, effort and cost. Particularly, during the field implementation of MICP, storing can help to avoid multiple productions of the bacteria for treatments. Therefore, the feasibility of storing the bacteria at low temperature (4°C) was studied. After producing the bacteria at 25°C in NH₄-YE medium, the culture medium was placed in refrigerator (4°C) for storing. Every 24 hours of storing, the samples were collected, and the enzyme performance was evaluated at 25°C (allowed several hours in room temperature before testing). The results (**Figure 3.16**) reveal that there are no observable changes occurred in the population of the bacteria; however, the urease activity considerably dropped with the storing time. The activity dropped by 50% by the end of 168 hours. It could be seen that in almost all the temperatures, the similar drop in urease activity occurred (refer **Figure 3.14**), suggesting that this could be attributed to the proteolysis. Even at 4°C, the production of protease (cellular enzyme) was high, leading to the denaturation of urease enzyme within a short time.

3.4.2.5. *Discussion*

Previous studies indicated that the rate of urea hydrolysis increases with increasing temperature, suggesting that the urease is not heat inactivated. For example, urease activity of *Sporosarcina Pasteurii* increased proportionally with the increasing temperature (25°C-

60°C) (Whiffin, 2004). The similar tendency was also reported to *Pararhodobacter* sp. (Fujita et al., 2017), *Deleya venusta* (Jahns, 1992) and *Streptococcus salivarius* (Chen et al., 1996). It is worth to note that the *L. xylanilyticus* studied herein exhibited a distinct behavior regarding activity: the urease enzyme is highly heat inactivated. On the other hand, the urease enzyme is more stable and consistently performs for long period at lower temperatures (**Figure 3.14**).

Theoretically, the activation energy for a specific enzyme-catalyzed reaction is constant (Moyo et al., 1989). 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 *L. xylanilyticus* was isolated from subarctic cold region, it exhibited high urease potential at low temperatures (below 25°C), suggesting low energy requirement for enzyme-catalyzed reaction. Meanwhile, the activity becomes negligible below 5°C, suggesting that the supply energy below 5°C is deficient to activate the 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 (Phang et al., 2018). Also, the bacteria (for example *Helicobacter pylori*) living in harsh acidic environment produce more urease enzyme at acidic conditions (P et al., 1997). 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. *L.*

xylanilyticus reported in this work showed the highest activity at weak alkali conditions. Similar phenomena could be seen on certain microbial urease like *Sporosarcina pasteurii* (Ciurli et al., 1996; Omoregie et al., 2017), *Pararhodobacter* sp. (Fujita et al., 2017) and *Klebsiella aerogenes* (Phang et al., 2018), those have the optimum activity under weak alkaline conditions.

3.4.3. Test tube test results

Precipitation tests using test tubes were carried out to find the optimum recipe for MICP solutions. The objective was to optimize the MICP ingredients to be the best initial concentrations resulting in both high precipitation content and utilization ratio. **Table 3.4** presents the obtained precipitation mass and utilization ratio of each test cases.

3.4.3.1. Optimizing initial concentrations of Ca, urea

Figure 3.17 presents the effect of initial concentration of calcium and urea on CaCO_3 precipitation. It was previously expected that the increase in initial resource concentrations (urea and calcium chloride) could increase the precipitation content of calcium carbonate. However, the results reveal that high initial concentrations (above 1 mol/L) significantly suppressed the formation of CaCO_3 . At the same time, between the initial concentrations 0.25 – 1 mol/L, the resources were found to be completely utilized (utilization ratio almost 100%). The discrepancy can be explained by the denaturation of the urease enzyme. Urea is a well-known chaotropic agent (protein destabilizer), which would denature the protein at its high concentrations (Cabrita and Bottomley, 2004; Yamaguchi and Miyazaki, 2014). As conceptually illustrated in **Figure 3.18**, urea disrupts the hydrophobic interactions (primarily by increasing the solubility of nonpolar substances in water) in proteins when the concentration is increased, leading to protein folding. As the result, the functionality of the urease enzyme becomes less, posing ineffective MICP.

Table 3.4: Test cases of test the tube test

Case No.	Theoretical maximum CaCO ₃ (g/L)	Precipitated CaCO ₃ (g/L)	Utilization ratio (%)
1	25	24.8	99.2
2	50	50.1	100
3	75	73.2	97.6
4	100	98.8	98.8
5	150	94.6	63.1
6	200	36.4	18.2
7	100	67	67
8	100	92.2	92.2
9	100	100.4	100
10	100	101.2	101

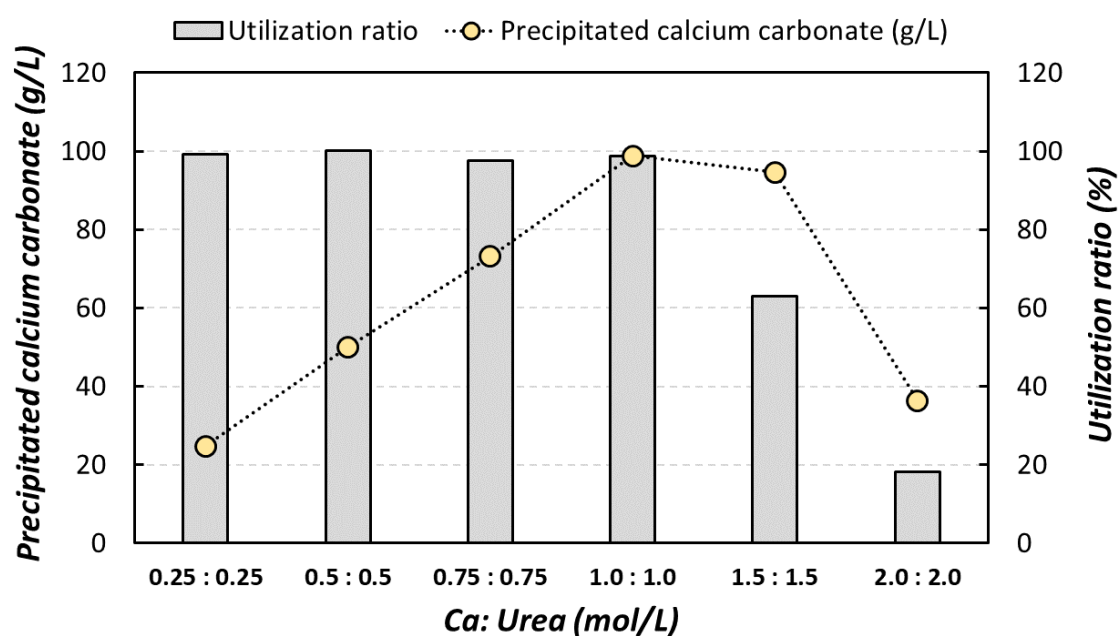


Figure 3.17: Precipitated calcium carbonate and utilization ratio with different initial concentration of resources

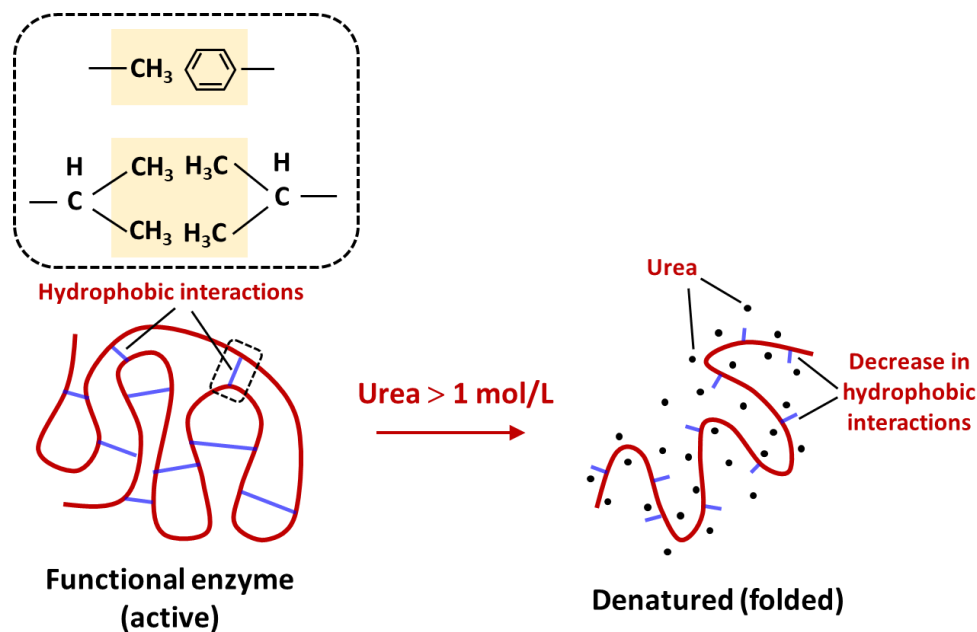


Figure 3.18: Conceptual illustration of denaturing of the urease enzyme at high urea concentrations

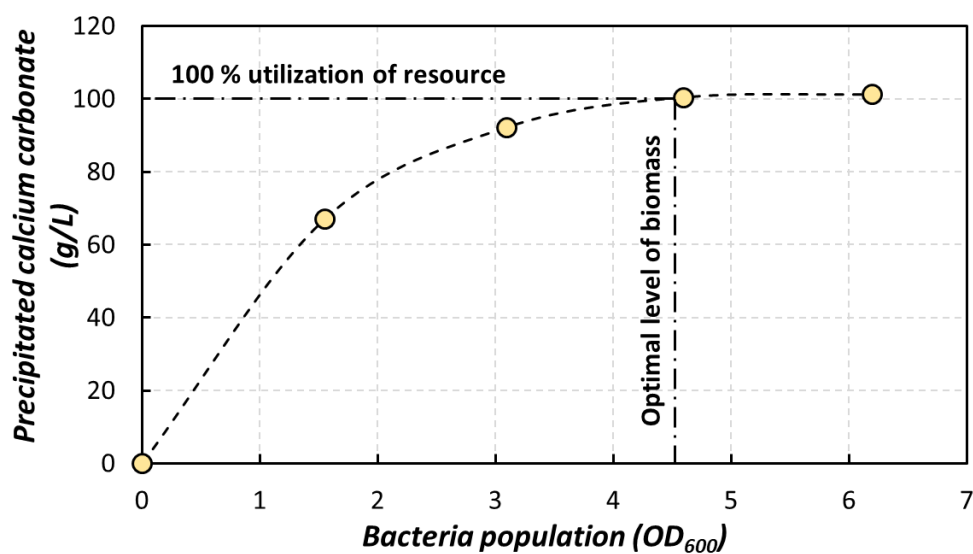


Figure 3.19: The effect of initial concentration of biomass on carbonate precipitation

3.4.3.2. Effect of initial biomass concentration

Figure 3.19 shows the effect of biomass on the CaCO_3 precipitation. The results demonstrate that the precipitation increased by increasing the amount of biomass for a given concentration of calcium and urea (1 mol/L each). The 100% utilization ratio was achieved almost in all the

cases of above 4.6 (OD₆₀₀). It should be noted that high initial concentration of biomass did not affect the CaCO₃ precipitation, which is in contrast with the previous study reporting a reduction in precipitation at high initial enzyme concentrations (Neupane et al., 2013).

3.4.3.3. *Effect of temperature in calcium carbonate formation*

Figure 3.20 illustrates the amount and rate of precipitation of CaCO₃ at different temperatures. The results show that during early stage of the tests (first 20 - 30 hours), higher precipitation rates were witnessed regardless of temperatures, and subsequently, the precipitation rate decreased in all the cases relatively at a similar trend. This could possibly be attributed to the denaturation of bacterial urease, i.e. the ability of the bacteria to hydrolyze urea reduced with the reaction time. It can also be seen that the trend of bacterial urease activity at different temperatures (**Figure 3.13**) shows fairly a good agreement with the trend of CaCO₃ precipitation rate in aqueous solution (**Figure 3.20**). However, as demonstrated in **Figure 3.21**, the rate of precipitation and urease activity are not in a linear relationship, varying logarithmically. For example, the urase activity at 25°C and 20°C are 2.6 and 1.3 respectively; but, the rates of precipitation are almost similar. This confirms that the formation of calcium carbonate is not completely reliant on bacterial activity, but also reliant on environmental conditions. Also, within the temperature 15 – 25°C, there is no significant difference in the formation of calcium carbonate, demonstrating that the bacteria (*L. xylanilyticus*) has a high potential for the MICP applications in cold regions.

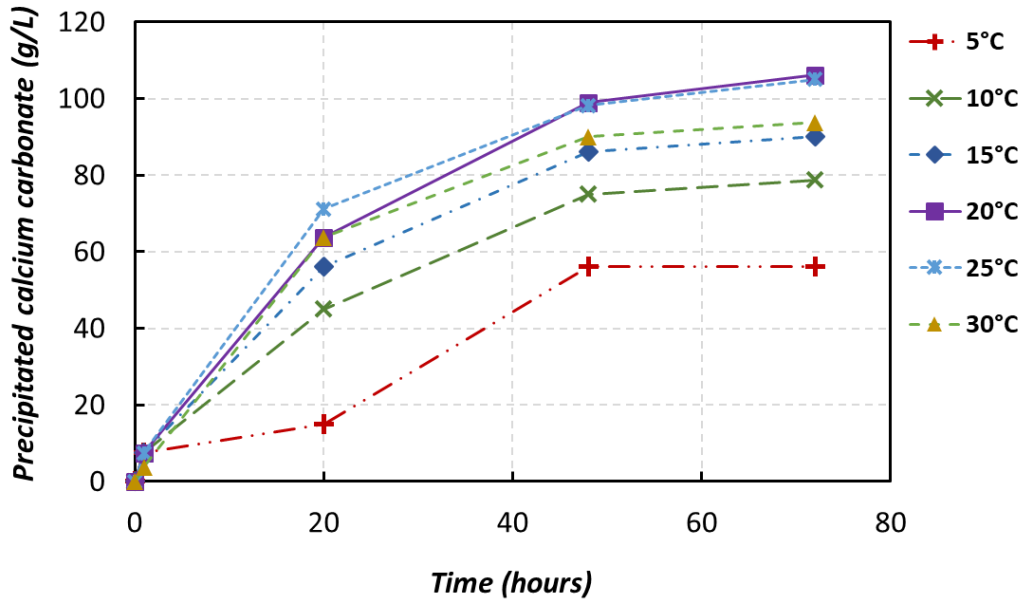


Figure 3.20: The effect of temperature on carbonate formation

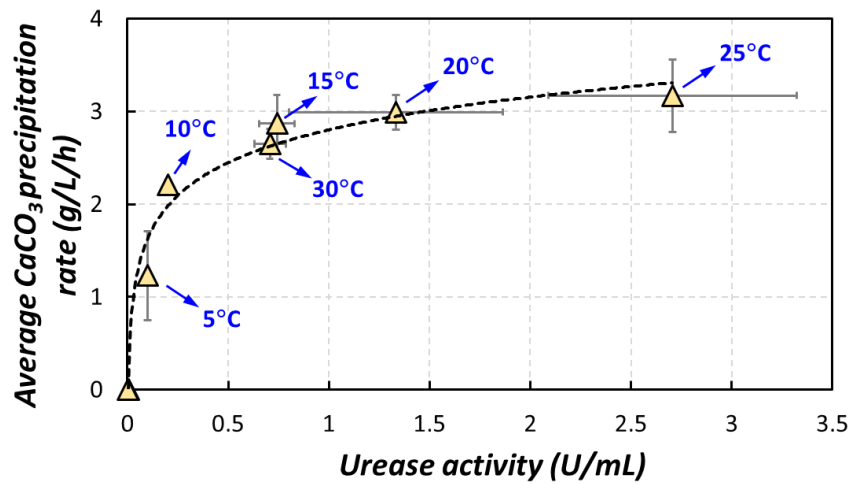


Figure 3.21: Rate of calcium carbonate precipitation versus urease activity

3.4.3.4. SEM analysis of precipitates

Figure 3.22 presents the microstructure of the crystals formed under different temperatures in test tube tests. The formation of calcium carbonate at all the temperatures were obviously found to be rhombohedral calcite crystals, demonstrating that the temperature did not affect the crystal type, but only the rate of formation. At 5°C, the formed calcium carbonate crystals were appeared to be poorly crystallized. A large amount of amorphous were formed and

aggregated with calcite, and the formation of calcite could be seen from small to moderate sizes (2-15 μm). When the temperature was 10°C, the formation of amorphous was found to be small. At 15°C, a better formation of calcite crystals could be seen compared to those at 5 and 10°C, and there was no formation of amorphous found. The average crystal size also was likely to be increased with the increase in temperature. The crystal size at 15°C ranged between 10-25 μm . Within the temperature 20-30°C, the formation uniform and well-crystallized calcite could be seen. The crystal size was found to be between 20-30 μm . On the other hand, the formation of calcium carbonate above 40°C was negligible.

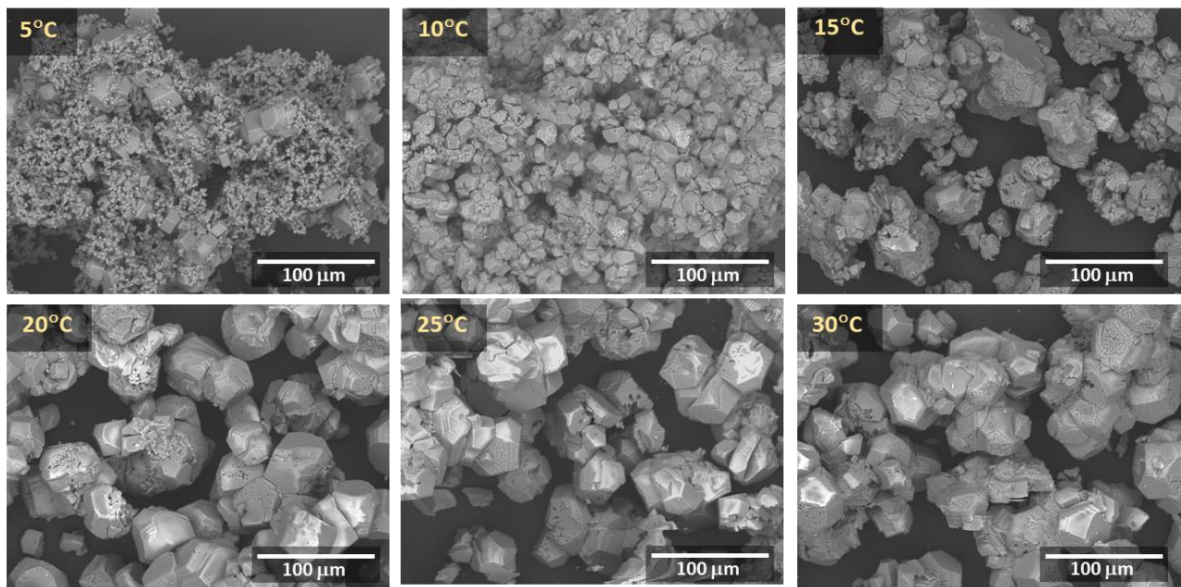


Figure 3.22: The micrographs of the crystals formed under different temperatures

In the process of MICP crystallization, the effect of temperature involves microbial metabolism and the activity of enzymes. The effects influence the crystallization rate, morphology and crystal size, consequently altering the engineering properties of MICP treated soils. Previous studies revealed that no precipitation could be induced by MICP at or below 10°C (Yan et al., 2009). Several studies also showed that the precipitation content increased with increasing temperatures; for instance, precipitated content of calcium

carbonate at 50°C is several times higher than that at 25°C (Cheng et al., 2014a). Thus, it was believed that the applicability of MICP in cold regions would not be feasible. However, this study has proven that the crystallization in MICP is highly strain dependent. It is worth noting that the perfect crystallization structure observed herein was within 15-30°C, and the crystallization was considerably occurred even at 5°C, demonstrating that relatively low temperatures favors the MICP process of *L. xylanilyticus*.

3.4.4. Soil column test results: Compatibility of expressway slope soil

The objective of this experiment is to study the compatibility of the expressway slope soil for MICP treatment. The sand columns used to study here simulate the near-surface zone of the slope as graphically illustrated in **Figure 3.23**. The enhancement and microstructure of treated slope soil were well examined by comparing the responses of uniformly graded sands (as references).

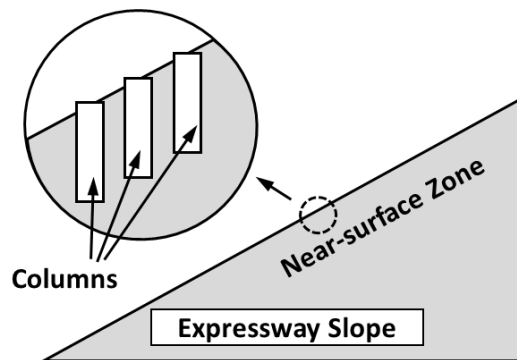


Figure 3.23: Soil column tests representing slope near-surface

3.4.4.1. Behavior of uniformly graded sands

All the columns specimens were treated exactly under same physical and chemical conditions by surface percolation. By the end of the treatment, the entire columns of all the soils were successfully cemented. The cemented profiles are depicted in **Figure 3.24**. **Figure 3.25(a)** and **(b)** present the UCS and precipitated CaCO_3 content respectively with respect to the depth of the columns. The observation suggests that the particle size distribution greatly influences

the MICP responses of the soils. In the cases of uniformly graded sands, the UCS increases with the decrease in mean particle size (**Figure 3.25(a)**). For example, the average UCS estimated close to the surface of Mizunami, Mikawa and Toyoura sands are 1.82 MPa, 2.67 MPa and 3.98 MPa respectively. The similar tendency can also be observed in the measurements of calcium carbonate content (**Figure 3.25(b)**). Basically, coarse sands have larger pore throat size and high permeability compared to that of fine sands, influencing bacteria retention and liquid infiltration. In order to study the effects of particle size distribution on bacteria retention, the outlet solutions were collected during the injection of bacteria culture, and the bacteria concentrations were observed using fluorescence microscopy. **Figure 3.26** presents the fluorescence microscopy images of all the soils treated. From the observation, it can be clearly perceived that higher bacteria cells were retained in fine sand (Toyoura) compared to that of coarse sand (Mizunami), due to the smaller pore throat size and higher particle contact points.

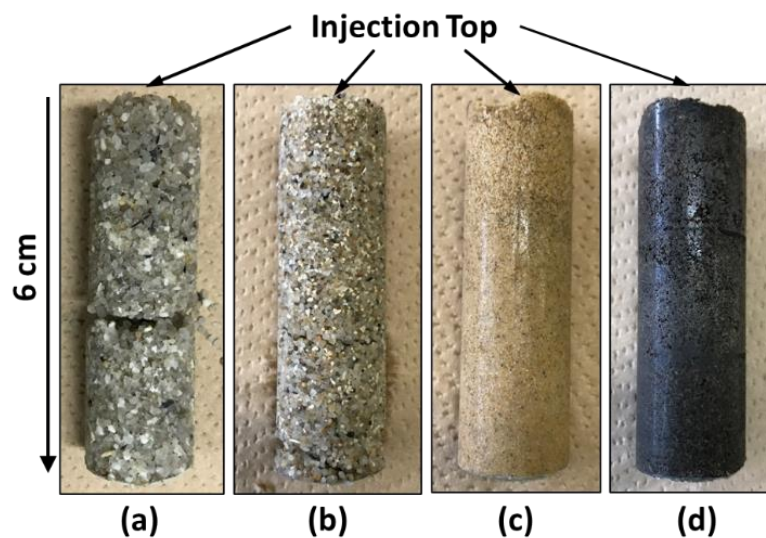


Figure 3.24: The cemented profiles of (a) Mizunami, (b) Mikawa, (c) Toyoura and (d) slope soil columns.

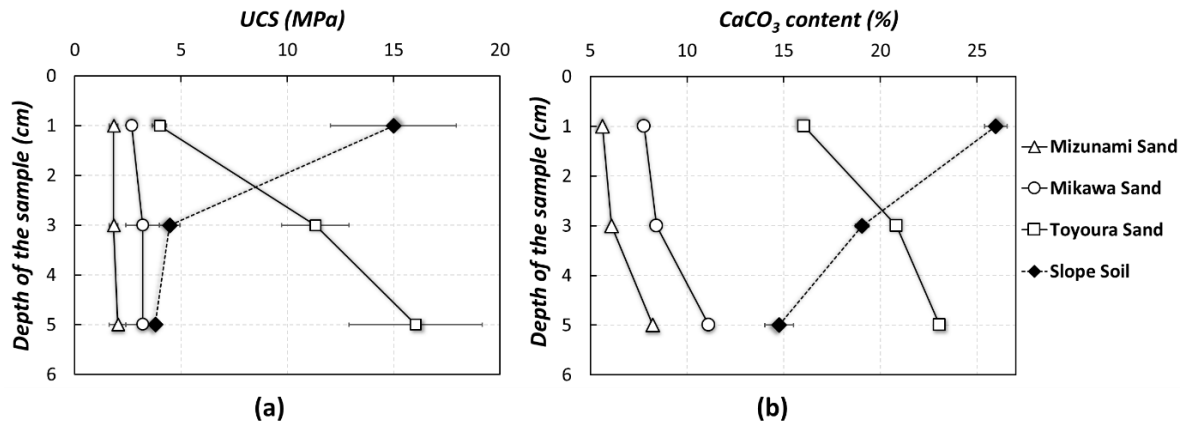


Figure 3.25: (a) UCS and (b) calcium carbonate profiles with respect to depth

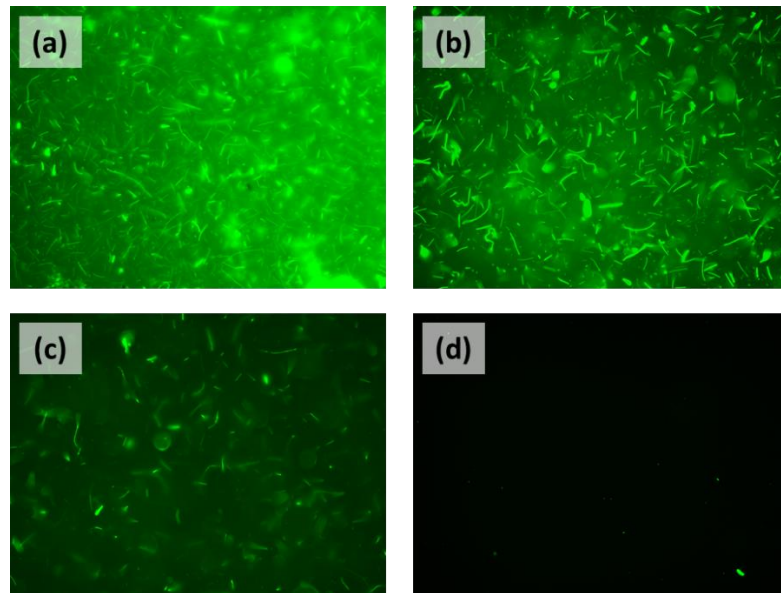


Figure 3.26: Fluorescence microscopy analysis images of outlet solutions from (a) Mizunami, (b) Mikawa, (c) Toyoura and (d) slope soil

In addition, high infiltration rate in coarse sand is found to affect the resource utilization. The measurements of calcium ion concentrations at the outlet (**Figure 3.27**) revealed, in the coarse sand columns (Mizunami and Mikawa), more than 80% of the resources have reached the bottom before utilized. In the Toyoura sand, on the other hand, low infiltration rate facilitated by the smaller pores resulted high resource utilization. As the result, high precipitation of calcium carbonate and UCS was achieved in fine Toyoura sand (**Figure 3.25**).

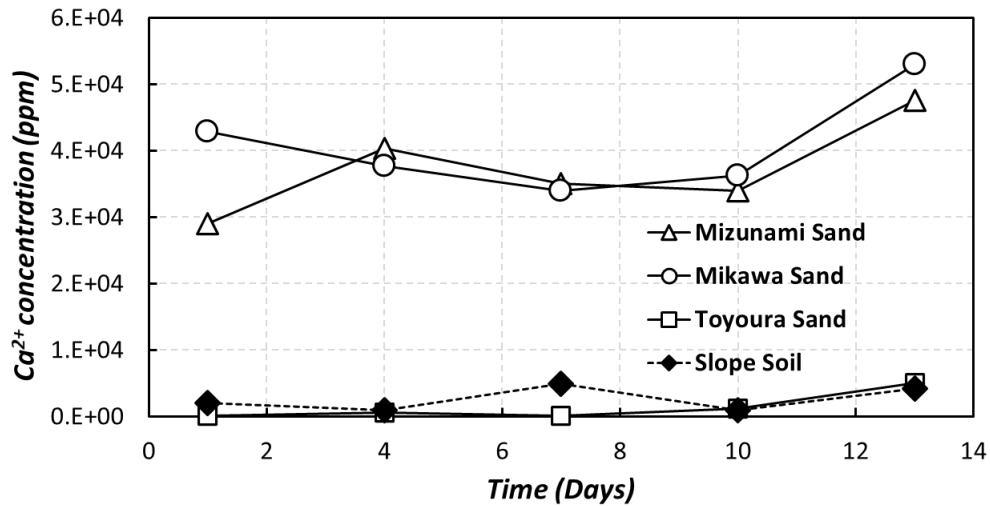


Figure 3.27: The outlet measurement of calcium ions

The UCS and precipitated CaCO_3 of all the uniformly graded sands increases with the depth of the sample (**Figure 3.25**). In coarse sand columns (Mizunami and Mikawa), only a slight variation occurred with the depth. A similar observation was also reported by Cheng and Cord-Ruwisch (2014) to coarse sand. On the other hand, fine sand (Toyouira) exhibited significant increase in UCS and precipitated CaCO_3 with the depth, which could be attributed 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 at the bottom sample compared to that at top.

3.4.4.2. 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, the results confirmed that around 80-90% of the calcium ions in cementation solution have been already utilized by the time it reached the bottom (**Figure 3.27**). However, the slope soil and Toyoura sand exhibited a significant difference regarding their UCS and CaCO_3 profiles (**Figure 3.25**). For instance, the UCS of

the slope soil estimated close to the surface is 14.98 kPa, which is around 3.75 times higher than that of 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 fine sand consisting silt content of around 3%, leading to effective packing and cementation in the matrix.

In contrast to the profiles of uniformly graded sands, the UCS and carbonate content of slope soil decrease with the depth (**Figure 3.25**). The higher cementation at the top of the column specimen was presumed to be due to the biological filtration (as demonstrated by fluorescence microscopy, refer **Figure 3.26**). 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 were 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. It is worth to note that negligible number of bacteria cells were detected at the outlet solution of slope soil column (**Figure 3.26(d)**), suggesting that almost all the cells are retained in the soil.

3.4.4.3. *Microscale properties of MICP treated soils*

The SEM images of uniformly graded sands are shown in **Figure 3.28**. The images demonstrate that the calcium carbonate preferentially precipitated at particle contacts (contact cementing). Generally, bacteria cells prefer to position themselves in smaller surface features (such as near particle-particle contacts) compared to the particle surface due to the reduced shear and a higher availability of nutrients at the particle contacts (DeJong et al., 2010), which leads to the precipitates at the particle contacts. Also, the matrix support (i.e. grow from particle surface into pore space to create bridges between particles) is barely observed in sands treated herein by percolation. As the particle size of sand reduces from 1.6 mm to 0.2 mm,

the SEM images show an increase in precipitated carbonate content (consistent with the carbonate content measurement of sands presented in **Figure 3.25**). Due to the high retention of bacteria cells and less permeability, the crystallization was higher in Toyoura sand compared to that in coarse sands.

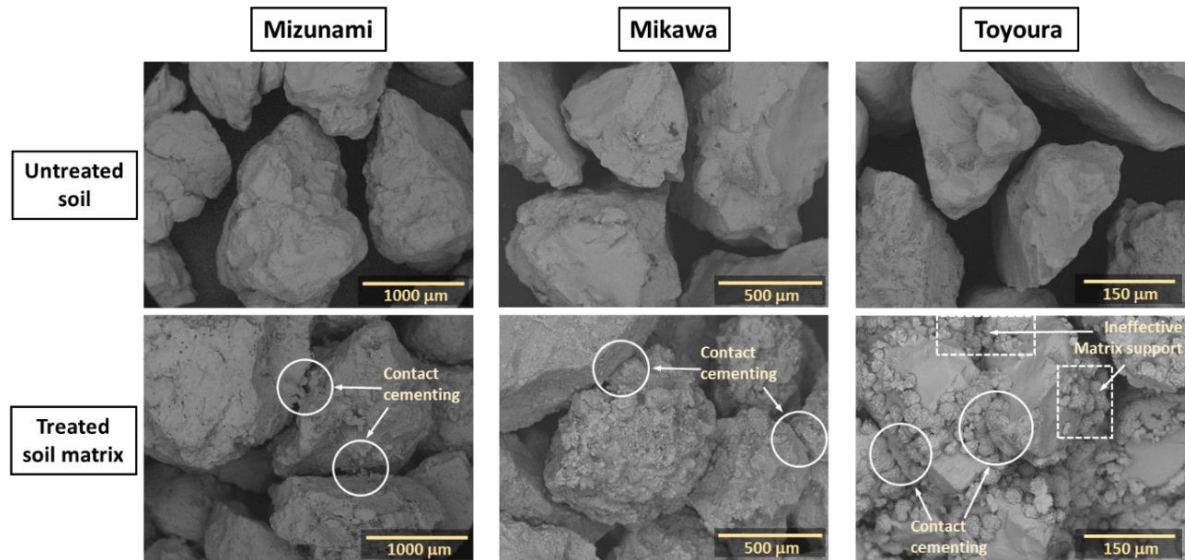


Figure 3.28: SEM images of uniformly graded sands

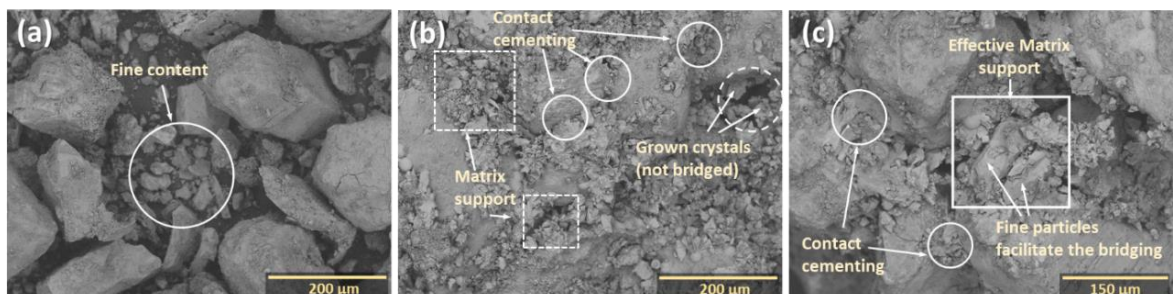


Figure 3.29: SEM images of slope soil

Figure 3.29 presents the SEM image of MICP treated slope soil. **Figure 3.29(a)** shows the micrograph of untreated slope soil. The micrographs reveal that the grains ranging from coarse to fine tended to form an effective matrix of slope soil (**Figure 3.29(b)** and **(c)**). The presence of fine particles could alter the microstructure of soil skeleton, governing the responses significantly. Most of the soil particles are in contact with some of the fine particles between them, and the rest filled the void spaces. Also, some of the fine particles are cemented

both to each other and to the sand particles. The fine content in slope soil increased the number of particle contacts by bonding with the sand grains. As observed, the fine content played a very important role in matrix supporting by enabling intermediate supports to form bridges among the carbonate crystals that have grown in void spaces (**Figure 3.29(c)**), thus, strengthening the force chain of the treated matrix.

The applications of MICP on very fine soils are typically limited due to the limited rate of permeability, that generally takes very longer time to infiltrate the reactants (Mortensen et al., 2011), and free passage of the bacteria might be inhibited due to the small pore throat size of fine soils (Cheng et al., 2016). However, an existence of certain quantity of fine particles is likely to be enhancing the MICP responses.

3.4.5. Soil column test results: Effect of silt content on MICP responses

Typically, the in-land residual soils are not uniformly distributed in grain size, consisting the fine particles in considerable quantity. For a soil, the size of the pore throat (i.e., the narrowest radius of a circle drawn perpendicular to fluid flow through pore space) is highly dependent on the smaller fraction of particles existing in soil, and that is believed to have a great impact on microbial passage. Therefore, to deepen the understanding on lower bound limit of MICP application by surface percolation, an additional work has been carried out to study the effect of fine content (i.e., silt) in fine sand.

The expressway slope soil considered in this dissertation is fine sand, and the existence of silt content ($0.002 < D < 0.063$ mm) was found to be around 3%. In this work, the silt content of slope soil was adjusted by sieving and mixing to different silt contents (0, 5, 10, 15, 20, 30 and 50 % by weight), and the soil column tests were performed (column size: 30 mm in diameter and 70 mm in depth) to evaluate the MICP responses. The grain size distribution of each case is presented in **Figure 3.30**.

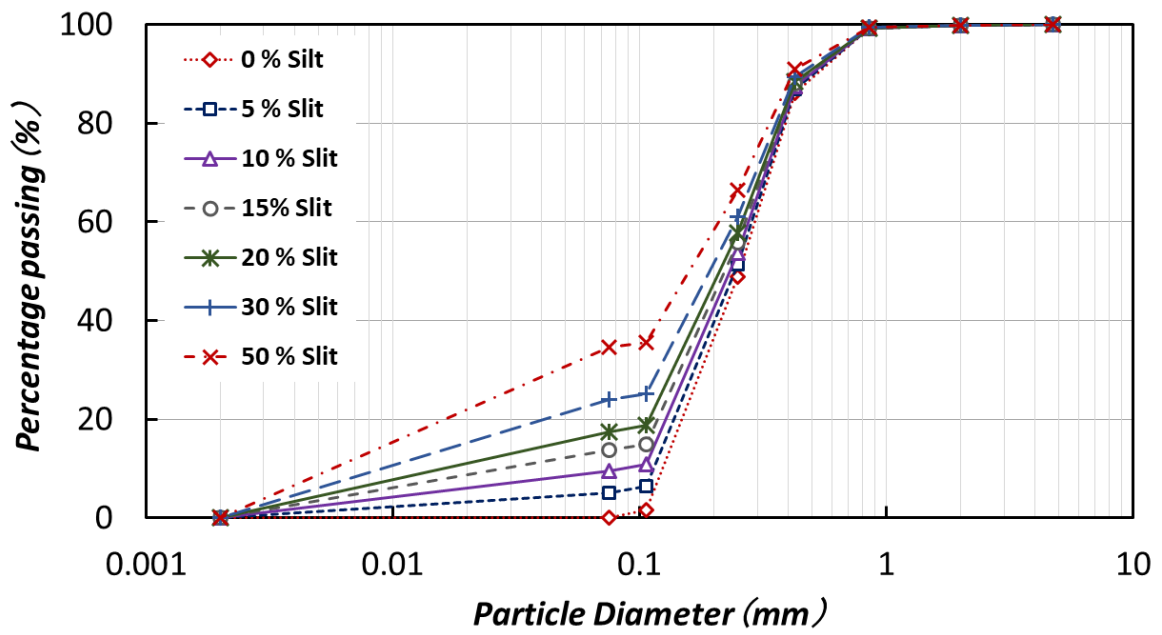


Figure 3.30: Grain size distribution of soils with different silt content

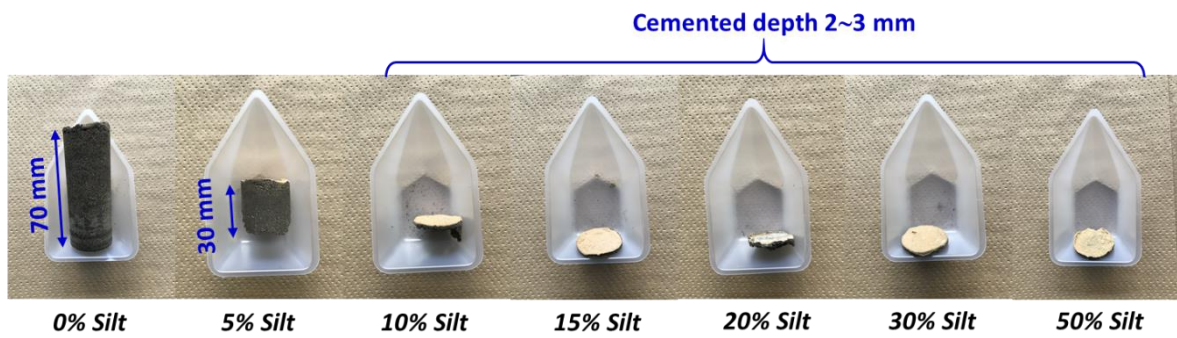


Figure 3.31: Cemented profiles of soil columns with different silt content

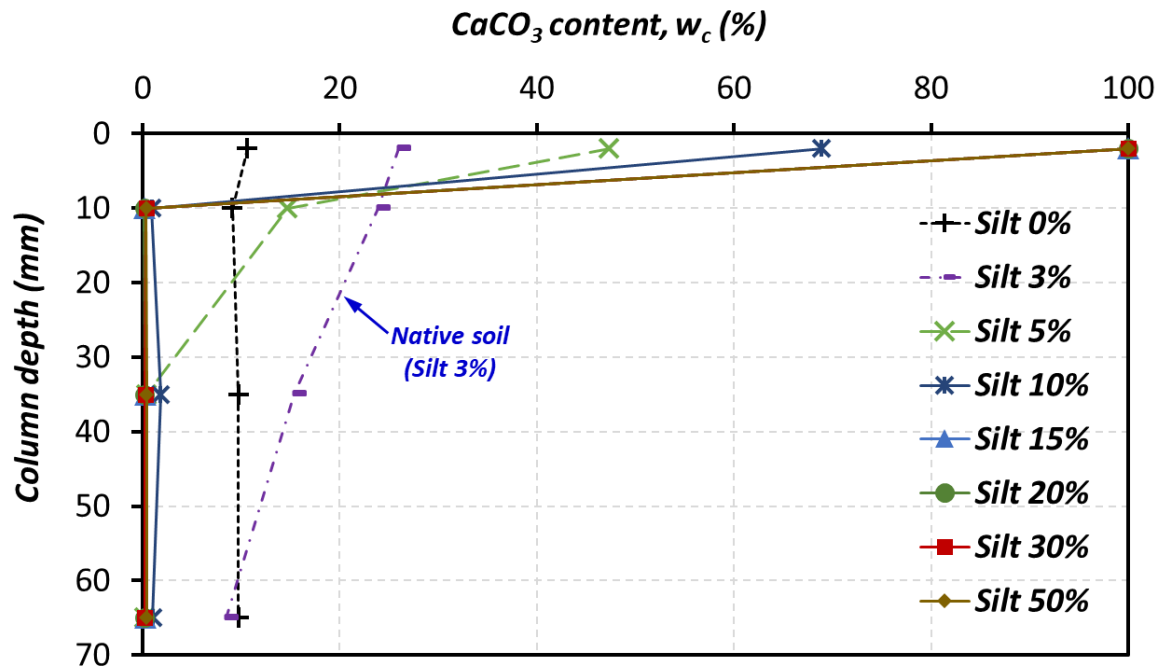


Figure 3.32: Distribution of calcium carbonate in the soils with varying silt content

The soil columns were identically packed and treated, followed by the evaluation. **Figure 3.31** presents the solidified profiles of specimens. The observation suggests that the silt content greatly influenced the depth of the treatment. When there was no silt content (0%) in the soil, the entire column (70 mm) was cemented, revealing that the bacteria cells could be easily transported through pore throats, throughout the column. On the other hand, when the silt content of the soil was increased, the transportation of bacteria cells has been found to be inhibited. For the soil with 5% silt, the maximum depth that the bacteria could be transported by advection, appears to be around 30 mm. For the soils with high silt content (> 10%), the penetration of bacteria cells through pore throats were completely inhibited. The measurement of calcium carbonate content (**Figure 3.32**) further confirmed that the crystallization of CaCO_3 occurred above the column surface (not within the soil matrix) for the soils with silt content over 10%.

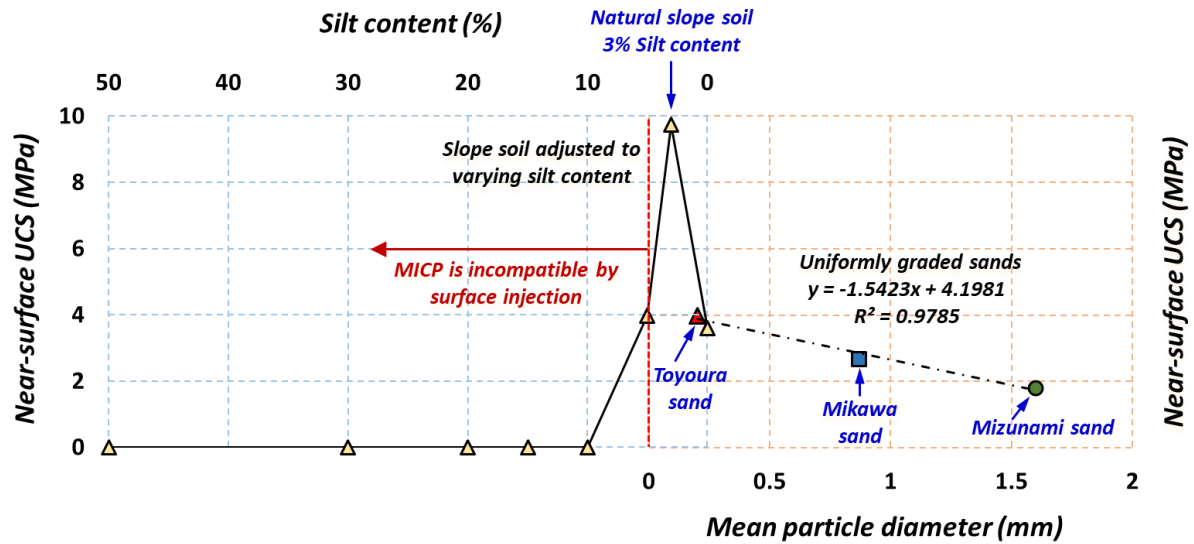


Figure 3.33: Effect of grain size distribution on near-surface UCS of MICP treated soils

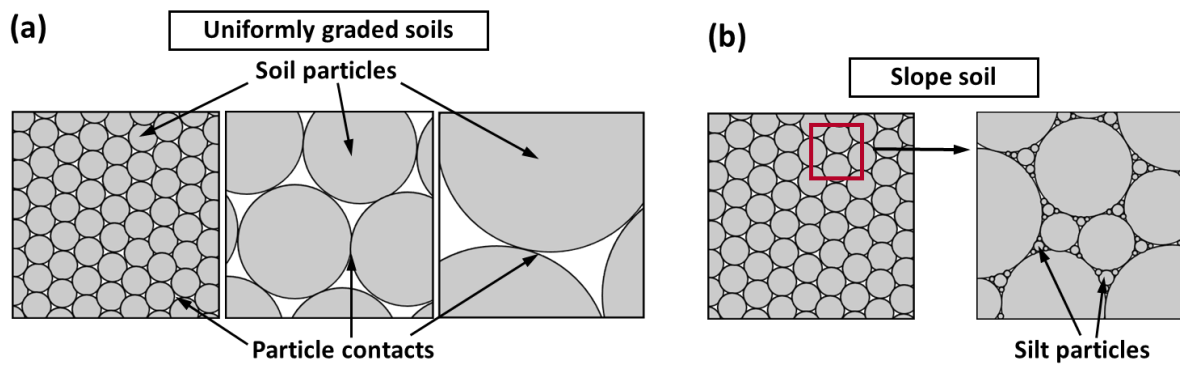


Figure 3.34: Conceptual illustration of the effect of grain size

Figure 3.33 presents the near-surface UCS of the MICP columns treated identically. It can be seen that the near-surface UCS of the uniformly graded sands show a linear increase with the decrease in mean particle diameter. As mentioned earlier, although the mean particle diameter of slope soil and Toyoura sand are almost similar (0.23 and 0.2 mm, respectively), the slope soil exhibited a marked difference in UCS. It is worth noting, when the 3% silt was eliminated from the slope soil, the UCS value dropped to the value very closer to that of

Toyoura sand (**Figure 3.33**), demonstrating that the 3% silt was the factor responsible for such marked difference.

The effectiveness of MICP on grain size distribution can be explained by the number of particle contacts. For the three types of uniformly graded sand tested herein, the crystals were more effective in improving the strength of the sand of smaller grain size. This could be attributed to the increase in number of contact points (as depicted in **Figure 3.34(a)**), which provides higher bacteria retention, better location for crystals to precipitate and decrease the stress acting per particle. On the other hand, the greater the percentage of the coarser fraction, the smaller the number of particle contacts within soil matrix. As the average number of contacts per sphere increases with the decrease in particle diameter, the UCS showed a linear increase (**Figure 3.33**).

In the case of slope soil, the presence of silt content (3%) could further increase the number of particle contents compared to that of uniformly graded sand with same mean particle diameter (as illustrated in **Figure 3.34(b)**). This larger number of interparticle contact points favored more bridging crystals formed at the contact points, and hence led to achieve a remarkable near-surface UCS in slope soil (**Figure 3.33**). However, further increase in silt content decrease the average pore throat size, restricts the transportation of bacteria cells onto deeper soils, and found to be affecting the depth of MICP cementation and UCS significantly. Considering the depth of the treatment (**Figure 3.32**) and UCS improvement (**Figure 3.33**), presence of 5% silt content appears to be the lower bound for MICP improvement of fine sands by surface injection; above 5%, the soil skeleton is likely to be ruled by silt particles, revealing treatment incompatibility.

3.5. Conclusions

This chapter consisted of a fundamental scientific program of MICP. The indigenous ureolytic bacteria were isolated from the target expressway slope (Hokkaido, Japan) and characterized for MICP application. The framework of this chapter includes the evaluation of biological, biochemical responses and the effects of particle size distribution on the MICP treatment. Based on the outcomes, the following conclusions are drawn.

- The ureolytic bacteria isolated from the expressway slope soil were characterized to be *Lysinibacillus xylanilyticus*. The urease activity results revealed that the bacteria could effectively hydrolyze the urea between the temperatures 15-25°C, showing in more robust performance under native cold climatic conditions. However, the growth and the urease production of the bacteria was limited at the temperatures above 30°C. In the neutral-weak alkaline environmental conditions, urease was more stable and consistently performed.
- Test tube test results demonstrated that the initial concentration of urea should not be more than 1 mol/L for an efficient and reliable precipitation of calcium carbonate. Further increase in urea concentration was found to be denaturing the urease enzyme, leading to ineffective MICP.
- Study also found that the temperature does not affect the crystal type, but the crystallization rate and the average size of the crystals. Formation of well-crystallized rhombohedral clusters were obtained at temperatures 15-30°C. The calcium carbonate was considerably precipitated even at 5°C; however, at 5°C relatively smaller crystals were formed, along with the accumulation of amorphous.
- Soil column tests suggest that grain size distribution of the soil influences the bacteria retention capacity and resource utilization in MICP by surface injection. The decrease

in mean particle diameter of sand results the increase in bacteria retention, number of particle contacts, providing better location for crystals to precipitate and decreasing the stress acting per soil particle. In the expressway slope soil, presence of certain quantity of silt (3%) further increased the bacteria retention, number of particle contacts, participated in the force chain of treated matrix and provided effective matrix support by facilitating intermediate supports to form bridges between carbonate crystals. Study also found that 5% silt content can be the lower bound for MICP application in fine sands by surface injection; above 5%, the soil skeleton is likely to be ruled by silt particles, restricting the bacterial transport through pore throats, revealing treatment incompatibility.

CHAPTER 4

PHYSICAL AND MECHANICAL CHARACTERISTICS OF MICP TREATED SLOPE SOIL

4.1. Introduction

This chapter presents the experimental works performed to study physical and mechanical characteristics of MICP treated slope soils. The near-surface zones are considered to be the weakest zone in a slope, which frequently losses its inherent properties due to climatic influences and transient nature, thus, the shallow slope failures are typically triggered at near-surface zones (Harden and Scruggs, 2003; Tang et al., 2016). It is therefore very important to understand the initial as-treated characteristics of MICP treated slope soils to ensure the safety and serviceability of slopes.

Precipitated content of CaCO_3 has a great influence on determining physical and mechanical responses of MICP treated soils, could be controlled at different levels from light to heavy, transforming the state of the soil respectively from loose to rock-like material. The improvement in strength is basically due to the combination of reduction in porosity (i.e. increase in density from the precipitated calcium carbonate) and particle bonding. However, the increase in cementation level above desirable ranges would adversely impact the MICP application with respect to overburden pressure and treatment cost. Therefore, in this study, the emphasis is given on comprehending suitable range of cementation level for near-surface stabilization.

The slope soils were treated to various levels within the possible range, and their physical states, shear-wave velocities and behaviors across the chosen range of cementation levels were studied and compared, which also supported to percept the mechanical test program. Within the desirable range, the mechanical responses of MICP slope soils were characterized using unconfined compression tests, shear tests and permeability tests. Applying the Mohr-Coulomb criterion, the shear strength envelopes were developed, and shear strength parameters were derived at both peak and critical states. The permeability characteristics were also evaluated with varying cementation levels.

4.2. Materials and Methods

4.2.1. *Sample preparation*

The columns specimens were prepared using vertically positioned syringe columns (30 mm in diameter) to a density of $1.6 \pm 0.1 \text{ g/cm}^3$ (the corresponding relative density was around 60%). The methods of culturing bacteria and treatment are similar to that reported in the previous chapter (Refer **Sections 3.3.2** and **3.3.6**).

Different durations of treatment were undertaken to obtain different levels of cementation. The number of cementation injections ranging from 1 to 14 were considered in this work, leading the specimens to fall under light, moderate and heavy cementation categories as defined in the previous work (Montoya and DeJong, 2015). After the treatment, samples were rinsed, then removed carefully from the syringe columns and finally trimmed to required testing height. The calcium carbonate content was determined using the method described in the **Section 3.3.7**. To estimate the spatial distribution of calcium carbonate, the MICP specimens were carefully cut into three sections along the vertical height and separately tested.

4.2.2. Ultrasonic measurements

S-wave and P-wave velocities of the specimens were determined using a high voltage pulse receiver (SonicViewer-SX: 5251, Japan). After the preparation of specimens (3 cm in diameter, 3 cm in length), the ultrasonic measurements were performed using transducers (transmitter and receiver) located at top and bottom of the specimens as shown in **Figure 4.1**. Signals from the benders were captured using the two-channel digital oscilloscope at frequency bandwidth of 150 MHz. The elapsed time (digital oscilloscope, determined from the output waveforms) and the wave travel distance (sample height, measured by Vernier caliber) were used to compute the shear wave velocity, as shown in Equation 4.1.

$$V_s = \frac{L}{t - \delta t} \quad \text{Eq. (4.1)}$$

where L is the distance between the transmitter and receiver (i.e., specimen height), and t is the effective travel time of the signal from the transmitter to the receiver. δt is the delay of the wave when there is no specimen placed, measured by assembling only transmitter and receiver.

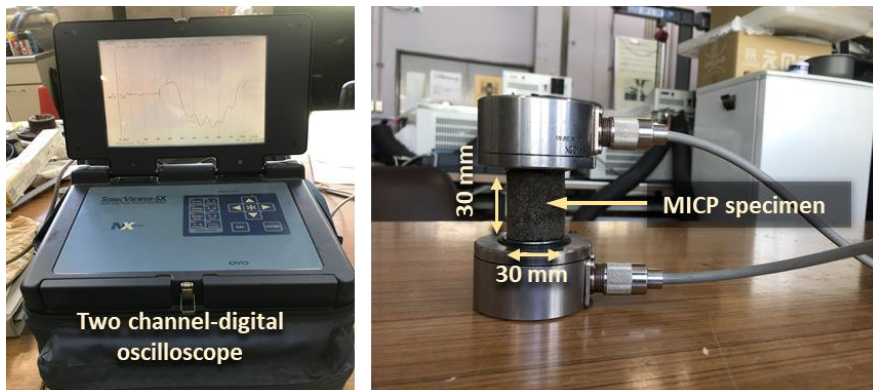


Figure 4.1: Ultrasonic measurement on MICP specimen

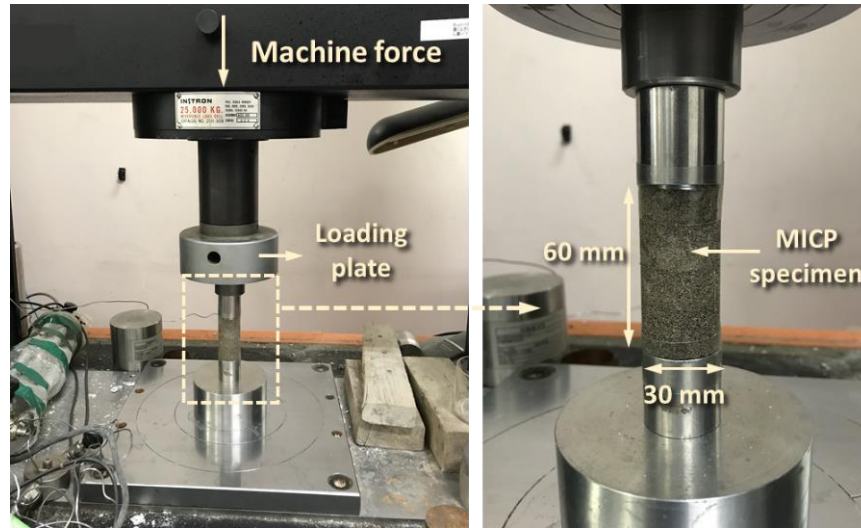


Figure 4.2: The UCS load cell and test arrangement

4.2.3. Unconfined compression test and equipment

To study the strength characteristics, the unconfined compressive strength (UCS) tests using automated INSTRON 2511-308 load cell (USA) (**Figure 4.2**), were performed on a set of specimens (3 cm in diameter, 6 cm in height) cemented to various levels, in accordance with the ASTM D7012 (2014). The samples were compressed at the rate of 0.036 mm/min until the critical state was achieved.

4.2.4. Shear test and equipment

Shear tests were used to investigate the mechanical behavior of heavily cemented MICP treated slope soil. **Figure 4.3(a)** presents the arrangement of shear test equipment used herein. It is a widely used method to determine the shear strength of soft to hard rocks and cemented specimens by uniaxial compression, previously suggested by Protodyakonov (1969). The equipment is made of stainless steel, and number of rollers are placed between pressure plate and upper die to eliminate the horizontal displacement of upper die. The cylindrical specimen is placed between two inclined dies attached to the testing machine (**Figure 4.3(b)** and **4.3(c)**) and sheared along the pre-determined surface (**Figure 4.3(d)**). The inclination of the shear

plane is determined by the angle (α) of the die is set. There are set of wedges that can adjust the dies and specimen to different angles (**Figure 4.3(e)**), hence, to vary the vertical and tangential components of applied forces and stresses.

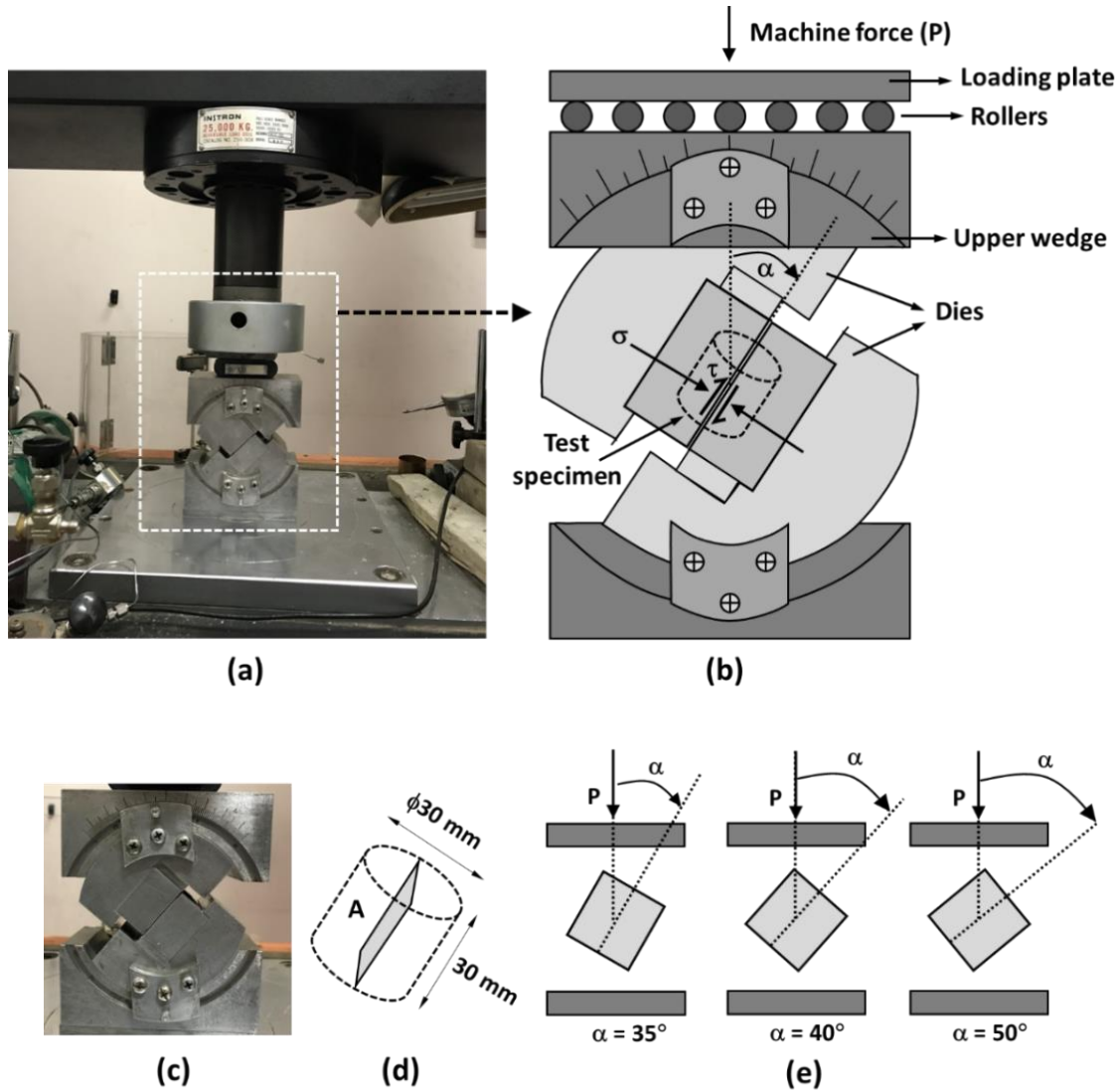


Figure 4.3: Shear test equipment used in this study

In this study, for each test condition, three identically treated samples were tested at different tilted angles (α of 35° , 40° and 50°) as shown in **Figure 4.3(e)**. The axis of the cylindrical sample installed is the same angle as the shearing plane. The machine force (P) is divided into normal force (vertical component to the shear plane) and shear force (along the shear plane) based on the tilted angle of specimen used to shear, and the shear stress (τ) and normal

stress (σ) were calculated using the following equations (**Eqs. 4.2** and **4.3**) respectively. The summary of treated specimens used for the shear test is given in **Table 4.1**.

$$\tau = \frac{P}{A} \cos \alpha \quad \text{Eq. (4.2)}$$

$$\sigma = \frac{P}{A} \sin \alpha \quad \text{Eq. (4.3)}$$

Table 4.1: Summary of the specimens used for the shear test

Level of cementation	No. of cementation injections	Average CaCO ₃ content (%)	Inclination angle (α) of specimen tested (°)	Specimen No.
C-1	7	12.51	35	7-35
	7	12.87	40	7-40
	7	12.76	50	7-50
C-2	10	16.65	35	10-35
	10	17.11	40	10-40
	10	16.91	50	10-50
C-3	14	21.27	35	14-35
	14	21.41	40	14-40
	14	22.47	50	14-50

4.2.5. Permeability test

Permeability is one of the most important geotechnical parameters, which determines the flow performance of the porous media. The effect of cementation level on permeability characteristics were studied by falling head permeability tests using DIK 4000 system (Daiki Rika Kogyo Co., Ltd., Saitama, Japan), in accordance with the ASTM D5084-03 (2003). As shown in **Figure 4.4**, the specimens treated to different cementation levels were placed in the

testing unit after the saturated for 48 h. The standpipe was then filled with de-aired water to a given level. The test was started by allowing water to flow through the sample until the water in the standpipe reaches a given lower limit. The time required for the water in the standpipe to drop from the upper level ($H1$) to the lower level ($H2$) were recorded. The hydraulic conductivity was calculated using the following equation (Eq. 4.4).

$$K = \frac{2.3}{A \Delta t} \frac{a L}{\log_{10} \left(\frac{H1}{H2} \right)} \quad \text{Eq. (4.4)}$$

where L is the height of the soil sample column. A is the sample cross section (19.6 cm^2). a is the cross-sectional area of the standpipe (0.503 cm^2). Δt is the recorded time. $H1$ and $H2$ are respectively the upper and lower water level in the standpipe measured using the same water head reference.

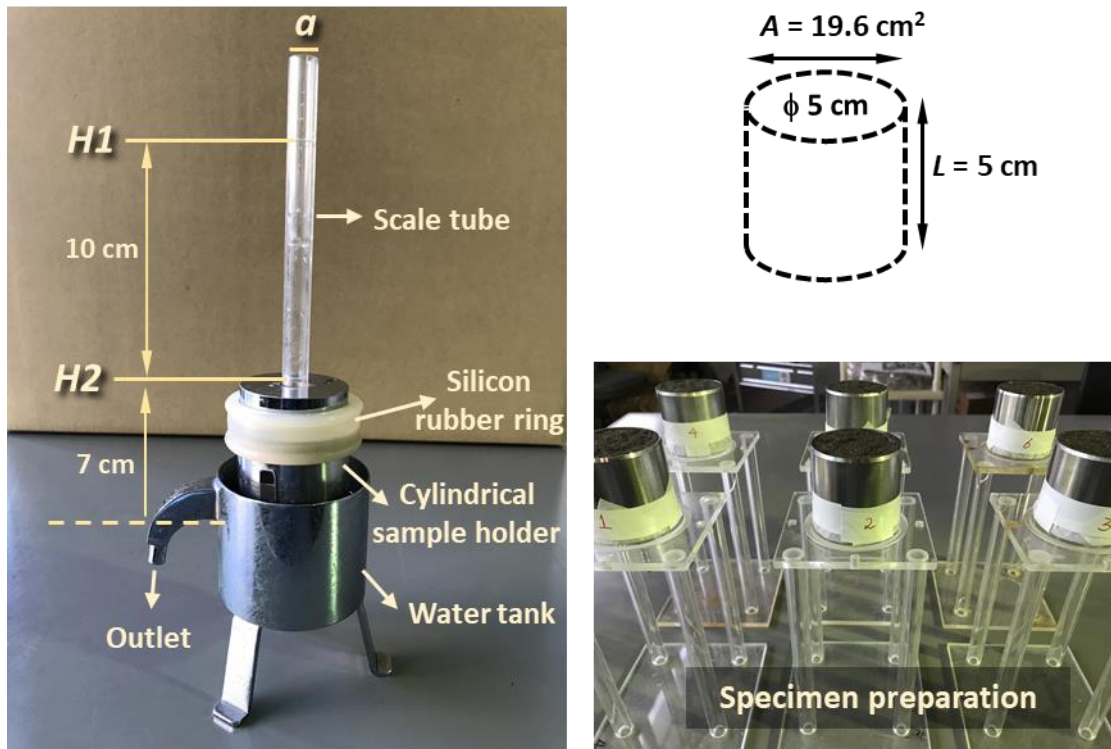


Figure 4.4: Permeability test device and specimen preparation

4.3. Results and Discussion on physical characteristics

4.3.1. *Physical state of soil with varying cementation levels*

The changes in the physical state of the soil were continuously monitored with the treatment quantity, i.e. across the varying cementation levels. Initially, the untreated slope soil (referred as $N=0$) was in loose state as young and fresh. When the treatment was applied, the CaCO_3 crystals formed within the soil matrix, cementing the particle contacts, eventually converting the separate soil particles to aggregated larger clusters as depicted in **Figure 4.5**. At the low treatment levels ($N=1$ and $N=3$), the particles were not well aggregated; lightly cemented clusters were clearly observed together with loose soil material (**Figure 4.5**), suggesting that the quantity of precipitated carbonate was not adequate to provide strong connections. When the number of treatment (N) increased to 5, a moderately cemented soil structure was achieved, and the precipitated carbonate could be able to retain its initial shape at its self-weight. However, the sample bottom was not stably cemented as top, and the soil particles were easily detachable from the bottom.

As presented in **Figure 4.5**, samples became more stable and stiffer, when the number of treatments is increased further ($N=7$). Within this heavy treatment zone, the quantity of precipitated carbonate facilitates strong bridges among particles, leading to a stiff soil skeleton. It should be noted that aggregate stability is the most serious concern in slope soils and related treatments, and the physical states at these heavy cementation levels are likely to be more suitable and effective.

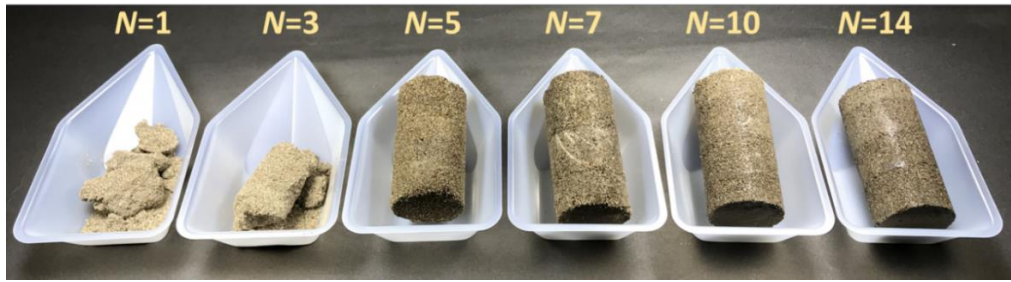


Figure 4.5: States of the specimens at different treatment levels

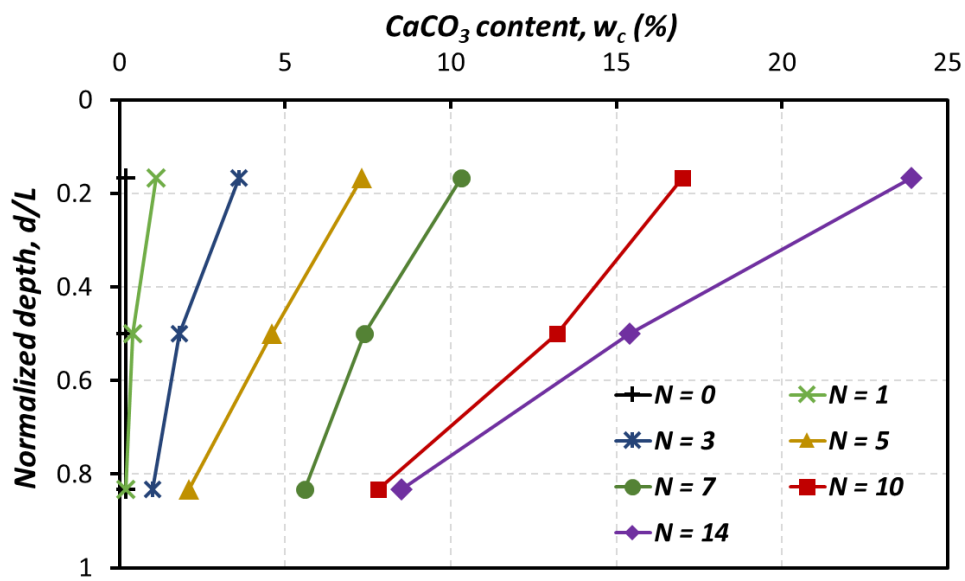


Figure 4.6: Distribution of calcium carbonate in specimens

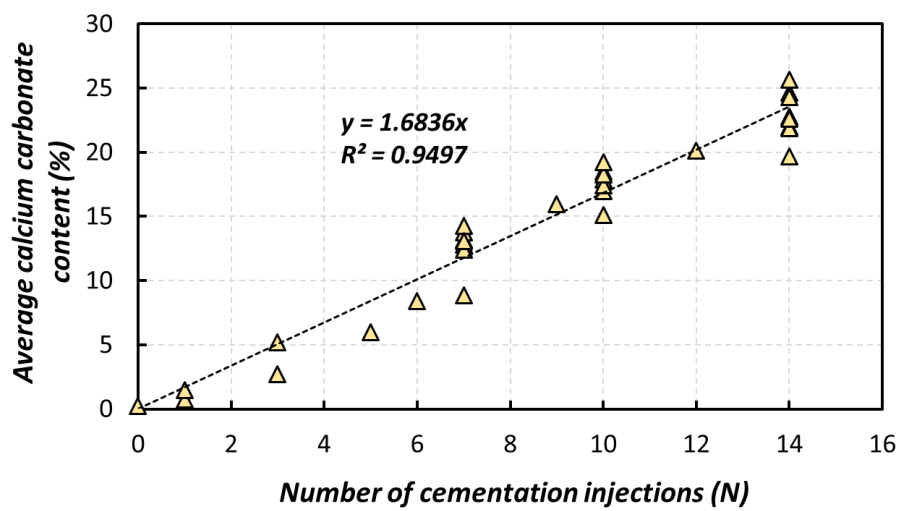


Figure 4.7: The variation of average calcium carbonate content with the number of cementation injections

4.3.2. *Spatial distribution of calcium carbonate in specimens*

As the bio-cementation is achieved by the surface percolation method, it is very necessary to assess the distribution of the calcium carbonate and the homogeneity of treatment with the depth. **Figure 4.6** presents the distribution of calcium carbonate in specimens cemented to different levels, plotted against with the normalized height (d/L ; d is the depth measured from surface; L is the total length of the specimen). The measured carbonate content in the untreated slope soil was 0.22-0.25% by mass, and this could be due to the natural presence of calcite, dolomite and siderite. The residual carbonate content of slope soil studied herein was relatively negligible compared to that of residual soils reported previously (around 1.35-6.5% by mass) (Lee et al., 2013; Ng et al., 2012).

The results reveal that the average calcium carbonate content increases gradually with the increase in number of cementation injections. The results also show relatively a gradient (i.e. non-uniformity) in the distribution of CaCO_3 along the height at all the treatment levels. The variation in the precipitation along the depth could be attributed to the distribution of bacteria and pore space distribution (Martinez et al., 2013). During the surface injection, more bacteria cells are filtered near the injection point due to the less pores in slope soil. As the liquid resources are supplied to the soil, reactions are induced rapidly at the zones with high bacteria concentration, leading to form the carbonates at high rates. At the time the solutions reach the specimen bottom, both the resource and the bacteria concentration would be less, resulting a less formation of calcium carbonate.

Considering the target near surface zone, the average calcium carbonate within the top 3 cm layer was evaluated and plotted against the number of cementation injections (**Figure 4.7**). The plot is based on the compilation of 19 number of specimen tests. The results revealed that the average calcium carbonate content is likely to be increasing linearly with the increase

in number of cementation injections. It can be seen that by the end of 14 injections, the average calcium carbonate content ranges between 20-25%.

4.3.3. Density of MICP soils with varying cementation levels

Figure 4.8 presents the variation of dry and saturated densities of the specimens treated to varying cementation levels. As the MICP treatment fills the void spaces by calcium carbonate cement, both dry and saturated densities show increasing tendency with treatment numbers. However, the dry density shows higher gradient in increase compared to that of saturated density. As the pore filling (by calcium carbonate) decreased the pore void which had been previously occupied by water during saturation, the increase in saturated density would not be as high as that of dry density with number of injections.

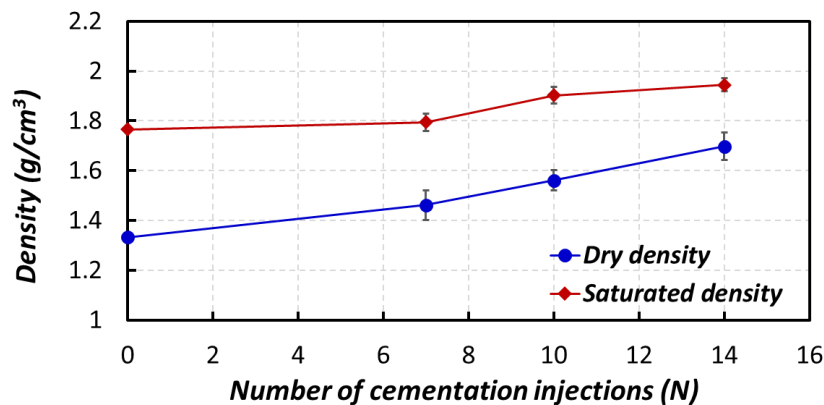


Figure 4.8: Variation of dry and saturated density with increasing treatment numbers

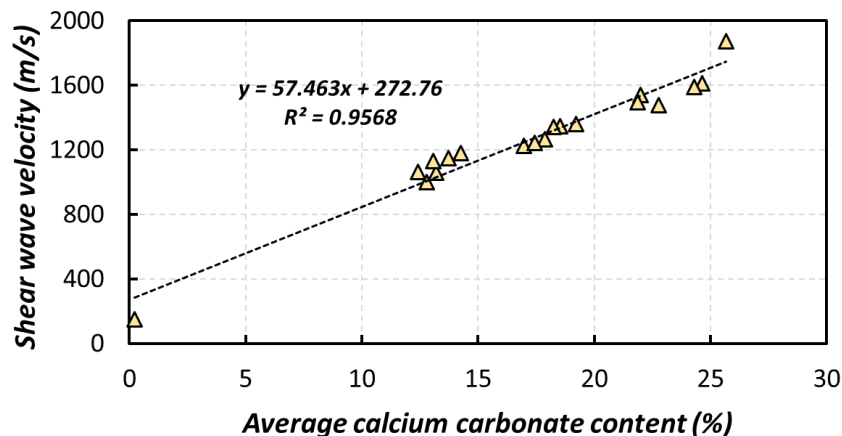


Figure 4.9: Relationship between shear wave velocity and calcium carbonate content

4.4. Results and Discussion on mechanical characteristics

4.4.1. *S-wave velocity measurements and correlations*

Shear wave velocity measurement is a widely used technique to characterize the mechanical properties of *in-situ* soils. In this study, the S-wave velocity was proposed as a good indicator for precipitated CaCO_3 (as cementation index), correlating more directly the stiffness. Typically, untreated soils may have a shear wave velocity ranging between 100-200 m/s depending on the level of stress.

Figure 4.9 presents the variation of shear wave velocity with average calcium carbonate content. The results fairly reveal a linear increase. The S-wave velocities were unable to measure to the specimens treated by less than 5 number of cementation injections (N), as they were in partial loose state, hence unable to withstand their state during the measurement. The shear wave velocities of C-1, C-2 and C-3 specimens (**Table 4.1**) averagely range between 1000-1100 m/s, 1200-1300 m/s and 1450-1550 m/s, respectively, falling under heavy cementation category. It is worth noting that the specimens were unable to withstand their shape under own self-weight below the shear wave velocity value of around 900-1000 m/s.

4.4.2. *Unconfined compressive strength (UCS) results*

A series of unconfined compression tests were performed to the samples treated to varying cementation levels. As shown in **Figure 4.10**, specimens cemented to higher levels exhibit higher strength and stiffness compared to that of low and uncemented soils. The response of untreated specimen is plotted as reference to compare the behavior of cemented slope soils. The peak and residual strengths increase with the increase in cementation level. However, the increase in the peak and residual strengths are not very significant up to the cementation level of 4.7% (up to around 1.4 times), particularly the residual strength is close to that of untreated soil. It is clearly understood that at lower cementation levels, the stress-strain behavior of

slope soils is quite similar to the uncemented specimens. But, when the average cementation is increased to 7.8%, 12.7% and 16%, the peak strength increases respectively by around 5.2, 17.6 and 58.5 times higher than that of uncemented one. Also, the higher the cementation level, the larger the initial modulus of the stress-strain response is observed. At higher cementation levels, there is an increase in the residual strength also. The increase in the critical state stress is likely to be due to the increase in surface roughness of soil particles from the coated calcium carbonate.

The cementation appears to have a large influence not only the peak and residual strength but also the localization of corresponding axial strain (i.e. failure strain). The samples cemented to 7.8%, 12.7% and 16%, the peak was reached at the axial strains of 2.0%, 1.3% and 0.9% respectively (Fig.), perceiving that the peak strength reaches at low axial strains as the cementation increases. This behavior is likely due to the stiffening effect i.e. the densified initial state of the specimen corresponding to the level of cementation. The stress-strain response of uncemented slope soil exhibits the strain-hardening behavior. Worth noting that as the cementation level increases, the behavior of slope soil transitioned from strain-hardening to strain-softening.

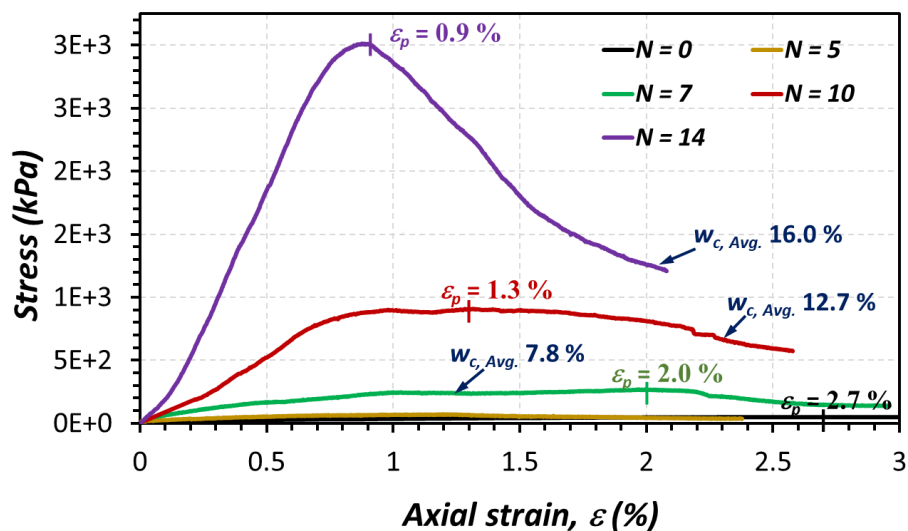


Figure 4.10: UCS results of specimens treated to varying cementation levels

4.4.3. Shear strength characteristics

A set of shear tests were performed on slope soils treated to different cementation levels (**Table 4.1**). The responses of the MICP specimens cemented to C-1, C-2 and C-3 levels are illustrated in **Figures 4.11(a), (b) and (c)** respectively, where the shear and corresponding normal stresses are provided against shear strain. It can be seen that the distribution of shear and normal stresses are dependent on the angle held during the shearing. The normal stress changes together with the shear stress (based on α), and peak of the sample would occur when the shear-normal stress point reaches the failure envelope. For the comparison purpose, set of direct shear tests were performed to untreated slope soils under same initial density, and the results are presented in **Figure 4.12**.

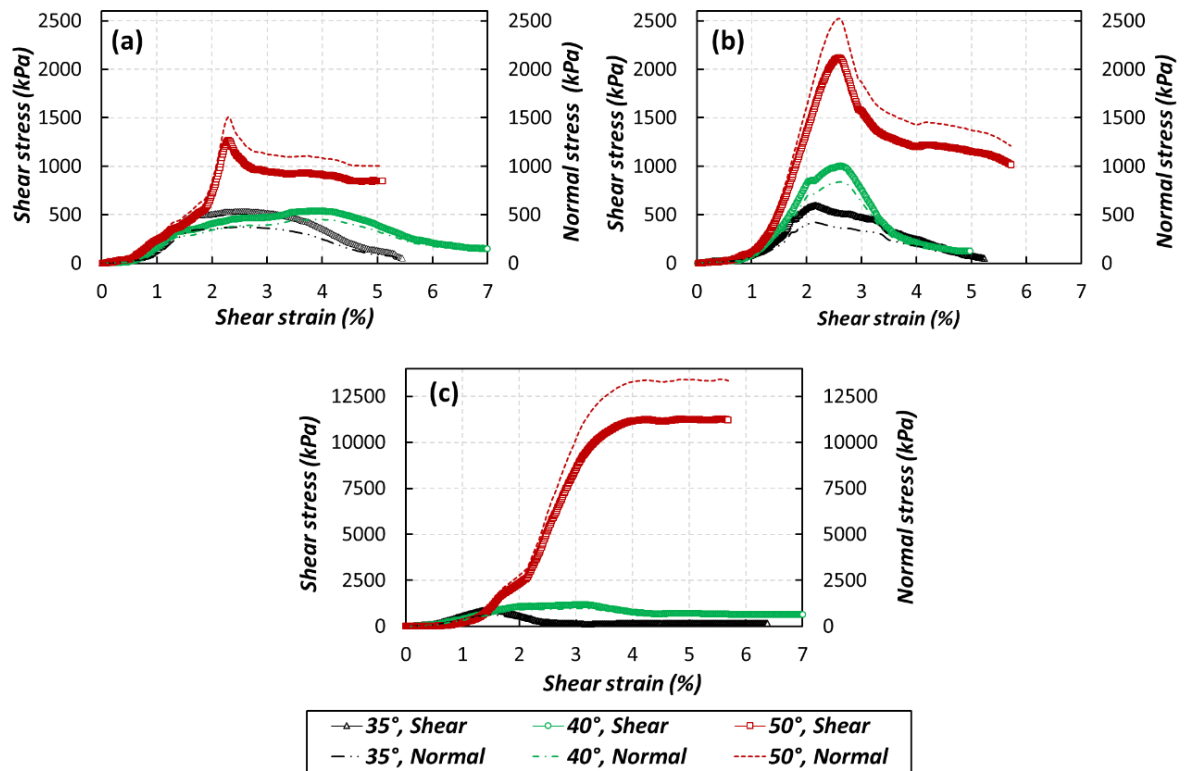


Figure 4.11: Shear test results of MICP treated slope soils

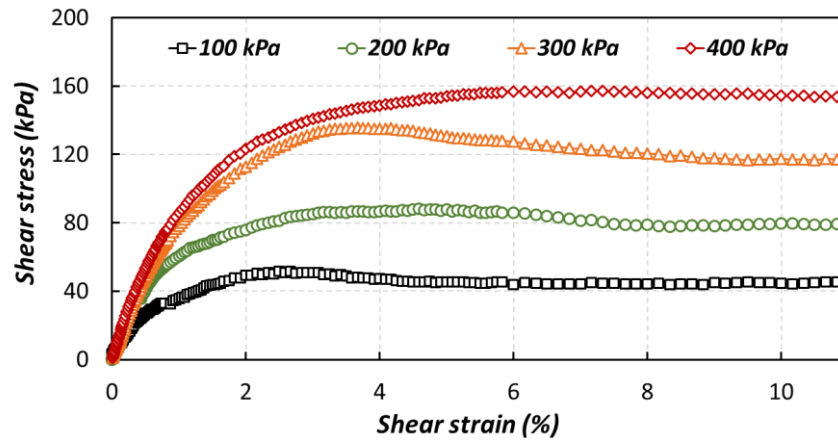


Figure 4.12: The shear test results of untreated slope soils under different normal stresses

Based on the stress-strain results (**Figures 4.11 and 4.12**), the failure envelopes of MICP cemented specimens corresponding to both peak state and residual state conditions were obtained. The failure envelopes were derived by plotting the shear stresses against the normal stresses of the specimens sheared at different inclined angles, and then by connecting all the peak or residual state points of tests. **Figures 4.13 and 4.14** present the peak and residual strength envelopes of all three cementation levels, respectively. It can be seen that relatively linear failure envelopes are obtained for all the cementation levels.

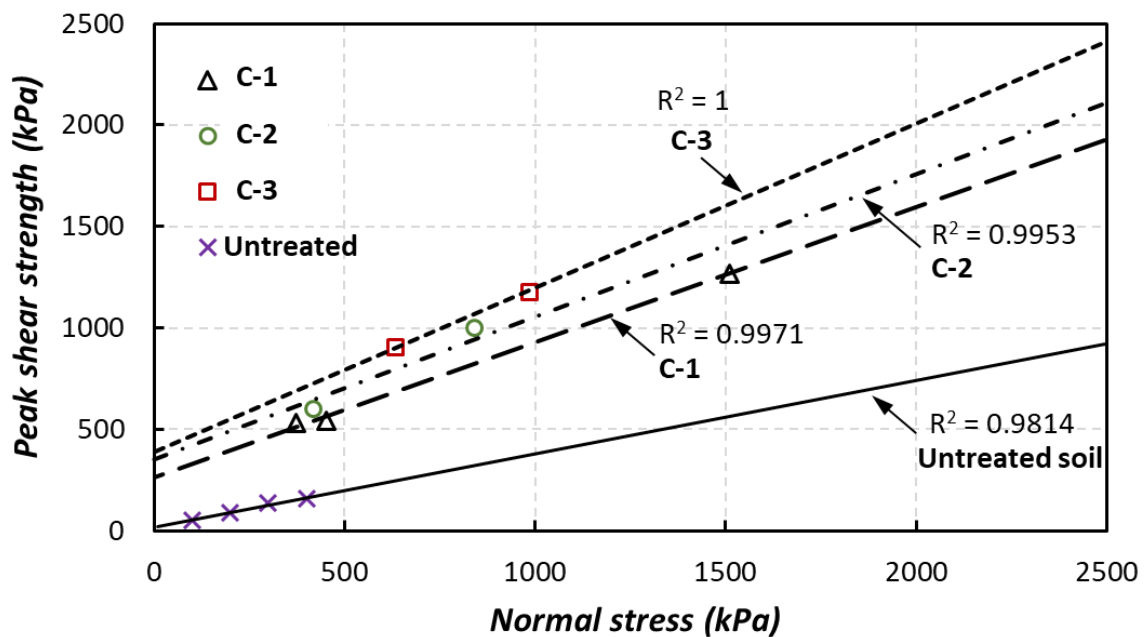


Figure 4.13: The peak shear strength envelopes of slope soil at varying cementations

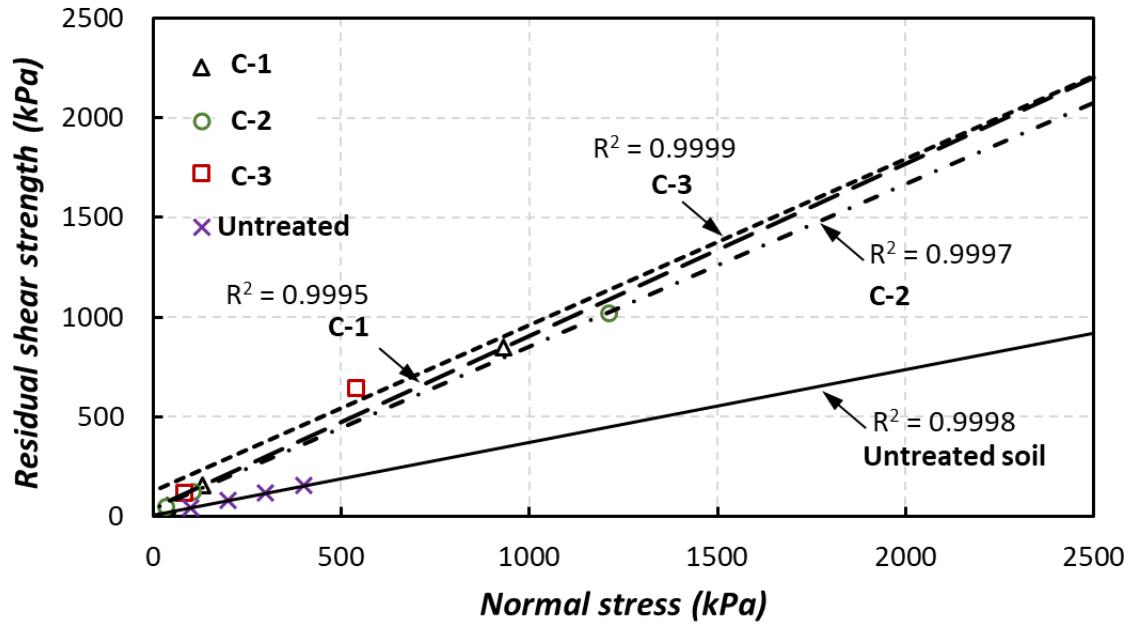


Figure 4.14: The residual shear strength envelopes of slope soil at varying cementations

4.4.3.1. Peak and residual shear strengths

Figure 4.13 presents the peak shear strengths (τ_{peak}) of the cemented specimens at different cementation levels. As expected, the τ_{peak} values of the cemented specimens increase with the increase in normal stress (i.e. vertical confining pressure), regardless of cementation level. It also can be seen that the specimens exhibit high τ_{peak} values compared to that of untreated soil of similar initial relative density. A comparison of untreated and cemented specimen indicates that the τ_{peak} value increased by an average of 520% for the C-1 cementation, 695% for the C-2 and 780% for the C-3 cementation compared with the τ_{peak} value of untreated specimen at a normal pressure of 100 kPa. It is worth noting, the failure envelopes clearly reveal that the peak shear strength is attributed to both friction and cohesion components at these levels of MICP cementation.

The residual shear strengths (τ_{res}) of the cemented specimens against normal confining stress are shown in **Figure 4.14**. MICP treatment has increased τ_{res} values of residual slope soil, similar to that observed for τ_{peak} . However, under similar normal stress, the increase in τ_{res}

with the increase in cementation levels is not high as observed in behavior of τ_{peak} (**Figure 4.13**). The results further demonstrate that the residual state envelope is also a straight line with relatively low cohesion which is very close to that of untreated soil, suggesting minimal portion of strength increase attributed to cohesion.

During the shearing, the samples showed a loss of strength after the peak stress, until residual state is reached. The cemented specimens showed relatively a sharp loss of stress upon reaching the peak point, but this summit was not observed in shear stress-strain response of untreated specimen (results are not presented in this paper). This post-peak strength loss in untreated soil is around 9.5 kPa at 100 kPa normal confining pressure. At the same normal stress, the losses are around 210, 251 and 315 kPa for the samples cemented to C-1, C-2 and C-3, respectively. It is clear that the loss of post-peak strength increases with the increase in cementation level, suggesting the increase in brittleness.

4.4.3.2. *Shear strength parameters*

To further understand the improvement in shear strength of cemented specimens, effective friction angle (ϕ') and cohesion (c') were calculated from the shear test results based on the Mohr-Coulomb failure criterion. The variations of friction angles and cohesions with average carbonate contents are presented in **Figures 4.15** and **4.16**, respectively. The peak (ϕ'_p) and residual friction (ϕ'_r) angles for the untreated soils are 21° and 20° respectively, and the associated cohesions are 16 and 7 kPa (c'_p and c'_r respectively). **Figure 4.15** clearly illustrates that the increase in cementation results in an increase in ϕ'_p of all tested levels. The peak friction angles range around 33.5° , 35° and 39° for C-1, C-2 and C-3 specimens, respectively, depicting relatively a linear increase. Cementation has also resulted a remarkable increase in c'_p (**Figure 4.16**).

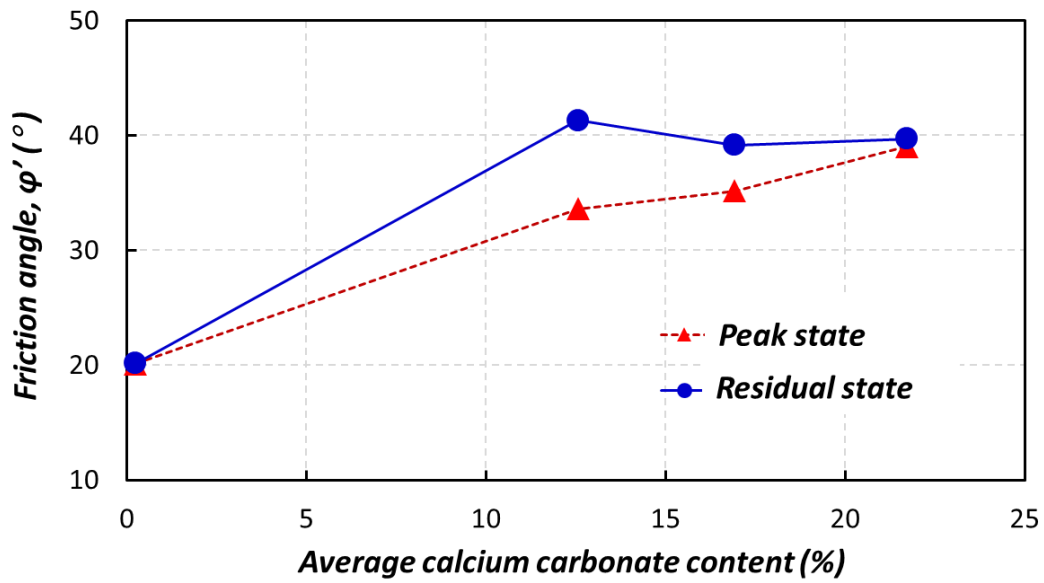


Figure 4.15: Variation of peak and residual friction angle with the average calcium carbonate content

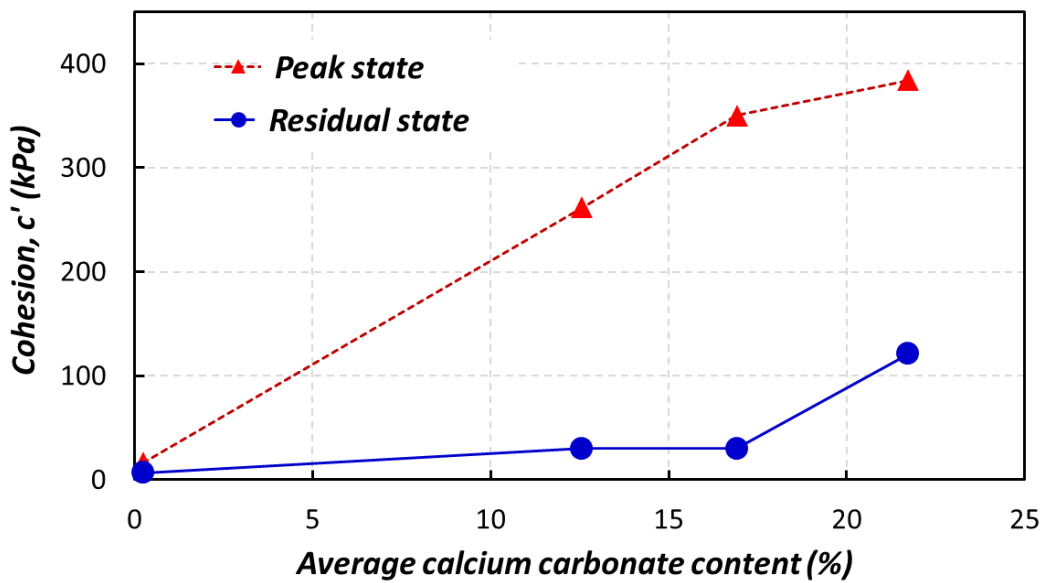


Figure 4.16: Variation of peak and residual cohesion with the average calcium carbonate content

It is also found that the trend of ϕ'_r is similar to that of ϕ'_p ; however, the cemented soils demonstrate larger ϕ'_r than the ϕ'_p at all levels of cementation. On the other hand, the cohesion parameters at residual state is significantly lower than that at peak state (**Figure 4.16**). For example, c'_p of C-1 cemented soil was 261 kPa, and that dropped drastically by an average

factor of 8.5 at residual state, resulting small amount c'_r . Lin et al. (2016) have reported that the c'_r of MICP cemented sand was similar to the c'_r of untreated sand. A similar residual cohesion also was reported to the sands treated by Portland cement (Clough et al., 1981). These results suggest that the residual shear strength of slope soil is predominantly frictional with a minimum portion of the strength attributed by cohesion at these cementation levels.

4.4.3.3. Discussion

Based on the results, MICP appears to have influence on both friction angle and cohesion strength parameters for soils at heavy cementation levels. Previously, Feng and Montoya (2016) have demonstrated that the effect of MICP on the cohesion strength parameter is limited in lightly and moderately cemented sands. Following the investigation, this study has demonstrated that the above conclusion is not applicable for soils cemented heavily (above 12% CaCO_3). During the MICP treatment process, the CaCO_3 crystals coat the soil particles and cement them at contact points (DeJong et al., 2010). At low cementation levels (0-3.5%), precipitated carbonate crystals are insufficient to provide effective bonding at particle contacts, and the crystals could randomly precipitate within the soil matrix while increasing the dry density, leading to increase only the friction angle parameters significantly (Cheng et al., 2013; Feng and Montoya, 2016). On the other hand, higher calcium carbonate content could develop strong interparticle connections, thereby enhancing both the friction angle and cohesion parameters (**Figures 4.15 and 4.16**).

Once the maximum shear resistance is reached, localized shearing occurs with the breakage of inter particle connections (initiate with the yielding of crystals), eventually resulting the loss of effective cementation and associated peak strength. However, the specimens exhibited higher residual strength compared to that of untreated slope soil at the cementation levels investigated herein (12-23%), which is primarily due to the frictional parameter. **Figure 4.17**

confirms that high cementations increase the particle roughness significantly by coating the soils. The representative samples taken from the middle of the sheared plane (after the shearing test) were observed through SEM. The samples cemented to C-1, C-2 and C-3 are illustrated in **Figures 4.17(a), 4.17(b)** and **4.17(c)**, respectively. The observation suggests that the high cementation leads more crystal formation on particle surface in addition to the contact cementations. During the residual shearing, these crystals localized on the surface have provided shear resistance by interlockings, and that would possibly be the reason for the increase in residual friction angle. Friction at residual state is also contributed by degraded calcium carbonate crystals produced from the shearing process (Montoya and DeJong, 2015). Overall, it can be concluded that the cementation at particle contact attributes to the peak shear strength, whereas the precipitation occurred on particle surfaces attributes to the residual strength.

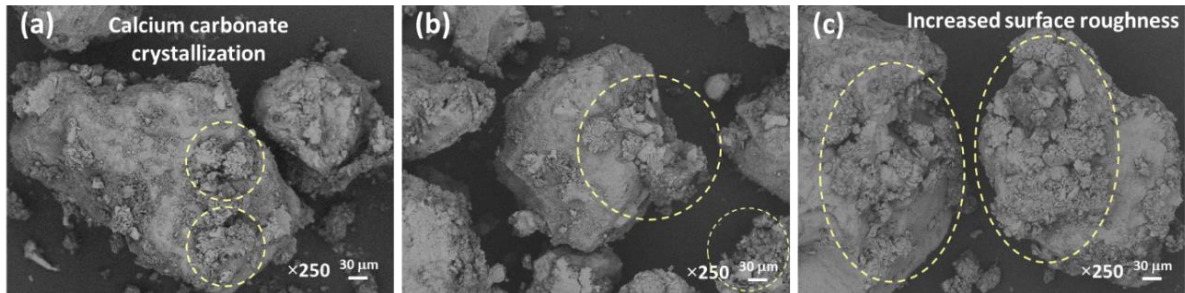


Figure 4.17: SEM images of representative samples collected from the sheared plane of **(a)** C-1, **(b)** C-2 and **(c)** C-3 specimens

4.5. Results and Discussion on permeability characteristics

The hydraulic conductivity of the untreated specimen is around 2×10^{-2} cm/s, which falls under the category of the clean sand (Craig, 1983). **Figure 4.18** presents the variation of hydraulic conductivity with the magnitude of maximum and average precipitated calcium carbonate contents ($w_{c,max}$ and $w_{c,avg}$ respectively). It can be seen that the hydraulic conductivity of the

slope soil linearly decreases (in log scale) with the increase in the precipitated carbonate content. Basically, the hydraulic conductivity of soil is largely determined by its void space (Martinez et al., 2013); the precipitated carbonate content increases as the number of treatment increases, resulting the void filling. When the soil is treated slightly ($w_{c,avg}$ of 0-3%), the reduction in hydraulic conductivity is negligible ($1-2 \times 10^{-2}$ cm/s). A similar observation is reported by Do et al. (2017) for lightly cemented sand material.

When the soils are heavily treated ($w_{c,avg}$ of above 5%), reduction in hydraulic conductivity is significant; for example, the samples cemented to around 8% ($N=7$) and 13% ($N=10$), the reduction of hydraulic conductivity is about 80% and 85%, respectively. Also, up to around 90% of permeability reduction is observed when the sample is cemented to $w_{c,avg}$ of about 16%.

As explained in the previous section, there is a non-uniformity of carbonate distribution in the treated slope soil. It is worth noting that in one-dimensional column specimen, the bulk hydraulic conductivity is controlled by the zone where the deposition of calcium carbonate is the highest, and therefore, the hydraulic conductivity is also plotted against $w_{c,max}$ (**Figure 4.18**). When the permeability of the specimens reaches 10^{-3} cm/s, the flow of the resource liquids is significantly inhibited, leading to the retainment of the solutions on the specimens for many hours, sometimes to bio-clogging stage.

When the samples are treated by more than 15 injections of cementation solution, bio-clogging is frequently experienced during the experiment. It should be noted that bio-clogging does not literally means that the entire voids in soil are filled by calcium carbonate. As observed earlier, the surface zone of the treated soil shows high carbonate content, revealing as the probable zone of bio-clogging. The continuous deposition in the above zone results the

inhibition of flow paths, leading to undesirable stage called bio-clogging. However, clogging is favorably induced in sealant and water-proof applications.

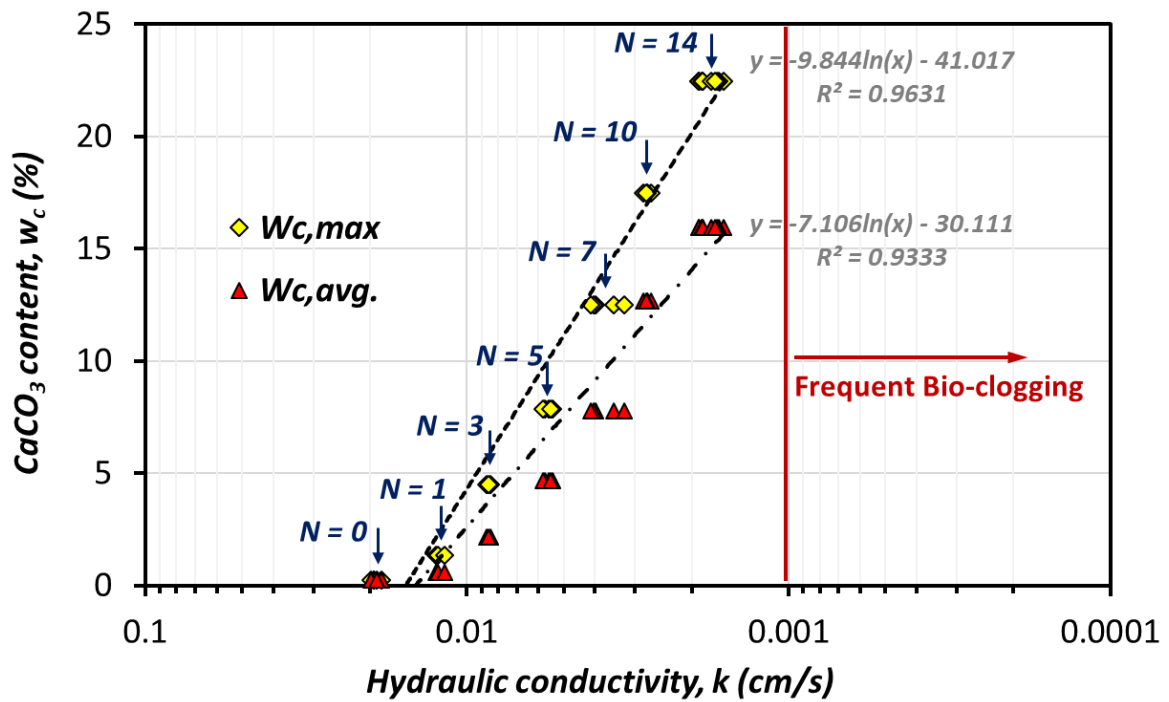


Figure 4.18: Variation of hydraulic conductivity with maximum and average precipitated calcium carbonate content

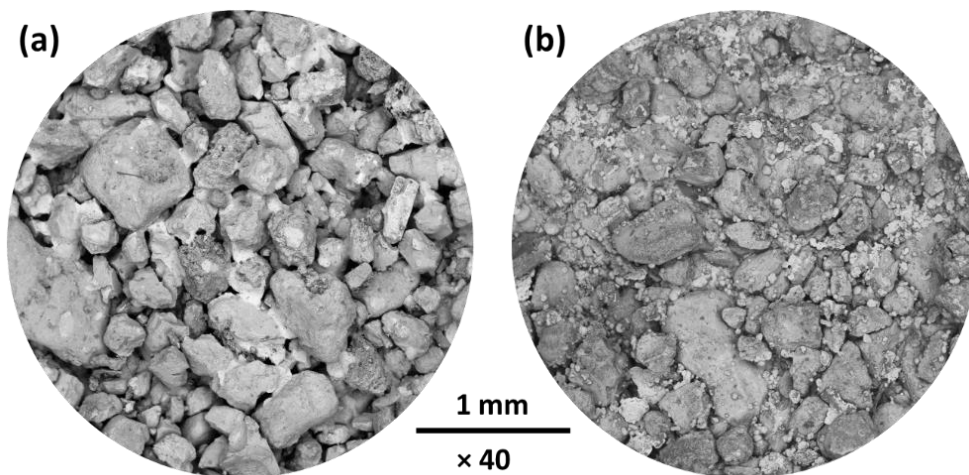


Figure 4.19: MICP with (a) retained permeability and (b) bio-clogged surface

Figure 4.19 presents the SEM images of MICP specimens' surfaces. It is clearly perceived that MICP has the unique merit, the retained permeability (**Figure 4.19(a)**) even at heavy cementation levels (**Figure 4.19(a)** corresponds to $N=14$). In the surface injection method, filtration of bacteria at particle contacts leads to the preferential deposition of calcium carbonate at contact points. Unlike the sealings by conventional grouts like OPC, this mechanism typically holds the soil pores relatively open, and which are clearly visible at micro-scale. On the other hand, over treatment results bio-clogging. The image presented in **Figure 4.19(b)** was of surface of the bio-clogged specimen (when $N>16$), in which the flow through of the injected solution became extremely slow, retaining on the specimen surface more than 24 hours. It can be seen that the surface pores are more clogged by carbonate bio-cement (compared to **Figure 4.19(a)**). In this case, the further infiltration was impossible, acting as an impermeable bio-crust.

4.6. Conclusions

This chapter has demonstrated that the MICP technique has a large influence on improving the physical and mechanical characteristics. The behaviors of slope soil treated to varying cementation levels were tested, and the following conclusions are drawn based on the outcomes.

- As the cementation level increases from light to heavy, the state of the soil desirably transitions from loose to dense (i.e. soft-rocklike material). However, the precipitated calcium carbonate revealed a heterogeneity in the vertical distribution, and the highest mass of calcium carbonate is observed closer to the injection top.
- Unconfined compression tests revealed that the increase in level of MICP in slope soil results in a higher stiffness, peak and residual strength. As the cementation increases,

the peak stress is reached at lower axial strains. The strain-hardening behavior was observed during the compression of uncemented slope soil, and as the cementation increases, the strain-hardening behavior transitions to strain-softening.

- Shear test results shown that the cementation level has a great influence in shear response, increasing the peak and residual strengths with the increase in average carbonate content. The results also revealed that the cementation at particle contact attributed to the peak strength, increasing both friction angle and cohesion strength parameters. However, particle roughness i.e. crystals precipitated on soil surface attributes to the residual strength.
- The increase in cementation, the decrease in the permeability was observed. However, in surface injection method, bio-cement is preferentially deposited at particle contacts, leading to retain certain permeability level (even at heavy cementation levels), which was one of the unique merits demonstrated to MICP.

CHAPTER 5

DURABILITY CHARACTERISTICS OF MICP TREATED SLOPE SOIL

5.1. Introduction

Selection of an appropriate biogeochemical treatment for a specific application requires compatibility of its permanence and durability with service life requirements. If the longevity of MICP treated soils is expected to be consistent with that of natural calcium carbonate-rich geomaterials, they can be expected to be stable for more than 50 years, provided alkaline conditions persist (Dejong et al., 2013). A time frame of fifty years is compatible with the expected service life of many treatments, and occasional retreatment can also be implemented to extend the service life. However, the mechanical and chemical characteristics of natural calcium carbonate-rich geomaterials and artificial MICP materials are reported to be considerably varying from each, depending on their mineralogy and geo-physicochemical conditions they formed (Daryono et al., 2020). Therefore, a performance-assessment with respect to treatment longevity and performance-monitoring are necessary and recommended prior to the field applications.

The slopes located in subarctic regions typically exposed to several climatic changes such as seasonal frosts and rainfall-evaporations, experiencing cyclic effects of freeze-thaw and wet-dry at the near-surface. Due to the porous structure of MICP soils (Cheng and Cord-Ruwisch, 2014; Whiffin et al., 2007), it is anticipated that the above processes would strongly interact along with the infiltration, causing progressive degradations and instabilities. Therefore, the durability analysis becomes even more important as MICP to be undergone several decades

of prolonged exposure to the atmosphere. It should also be noted that the studies assessing the deterioration of MICP soils found in the scientific literature so far are very limited.

In this chapter, a comprehensive study conducted to investigate the influence of key climatic parameters on the long-term performance of MICP treated slope soils, is presented. As the cold regions experience periodic snowfalls and rainfalls, the cyclic freeze-thaw (FT), wet-dry (WD) and acid rain (AR) tests were chosen and investigated as potential indicators of durability. The specimens were treated to three desirable MICP cementation levels, and their longevities were evaluated and compared. With the aid of SEM and XRD analysis, the deterioration mechanisms were also clearly explained under corresponding test conditions.

5.2. Materials and Methods

5.2.1. *Specimens preparation*

The columns specimens (30 mm in diameter and 30 mm in height) were used in this work. The methods of culturing bacteria and treatment are similar to that reported in the previous chapter (Refer **Sections 3.3.2** and **3.3.6**). Different durations of treatment were undertaken to obtain different levels of cementation. Based on the outcomes of previous chapter (**Chapter 4**), three heavy cementation levels (C-1, C-2 and C-3) were potentially and desirably chosen for this analysis.

5.2.2. *Cyclic freeze-thaw test*

For the freeze-thaw (FT) test, MICP samples were prepared at three different desirable levels of cementation (**Table 5.1**). The FT tests were performed in accordance with the methodology suggested in ASTM D 5312-04 (1997). Each FT cycle was subjected to a 12 h of freezing at $-20 \pm 1^{\circ}\text{C}$ followed by a 12 h of thawing at room $25 \pm 1^{\circ}\text{C}$. First, samples were placed in scarp carpeted container, and enough water was added to the container so that the water

completely covered the samples (kept for 12 h). Before subjecting to freezing, enough water was decanted so that the scarp carpet was just immersed. Relatively a fully saturated condition was maintained in the samples throughout the FT cycles. Experiments were performed in triplicate. The effect of FT cycles on cemented samples was evaluated by measuring the mass loss, P-wave and S-wave velocities (SonicViewer-SX: 5251, Japan) during the FT test. UCS and SEM analysis (refer **Sections 3.3.8** and **3.3.9**) were also performed after the FT test.

5.2.3. *Cyclic wet-dry test*

The wet-dry (WD) cycles were performed in accordance with the method suggested in ASTM D559-03 (2003). During the wetting process, specimens were submerged completely in distilled water at room temperature (25 ± 1 °C) for 6 hours. The saturated specimens were then placed in the oven at 70 ± 1 °C for at least 42 hours, and when the mass of the specimen became constant, their dry mass was recorded prior to the next WD cycle. Specimens were subjected to maximum of continuous 50 WD cycles, and the effect of WD cycles were evaluated using the following measurements. Scaled-off mass and S-wave velocity of the specimens were determined with number of cycles. The porosity of the specimens were measured before, after 12 and 50 WD cycles using the saturation method (Mahawish et al., 2019). SEM analysis and UCS measurements were also performed. The detail of the specimens used in wet-dry analysis are presented in **Table 5.2**.

Table 5.1: Detail of the specimens used for FT tests

Level of cementation	No. of cementation injections	Initial V_s (km/S)	Average CaCO_3 content (%)	Specimen No.
C-1	7	1.00	12.80	FT-7-1
	7	1.05	13.18	FT-7-2
	7	1.06	12.42	FT-7-3
C-2	10	1.27	17.88	FT-10-1
	10	1.22	16.95	FT-10-2
	10	1.24	17.43	FT-10-3
C-3	14	1.48	22.76	FT-14-1
	14	1.54	22.01	FT-14-2
	14	1.49	21.86	FT-14-3

Table 5.2: Detail of the specimens used for WD tests

Level of cementation	No. of cementation injections	Initial V_s (km/S)	Specimen No.
C-1	7	1.15	WD-7-1
	7	1.17	WD-7-2
	7	1.13	WD-7-3
C-2	10	1.35	WD-10-1
	10	1.34	WD-10-2
	10	1.36	WD-10-3
C-3	14	1.61	WD-14-1
	14	1.87	WD-14-2
	14	1.59	WD-14-3

5.2.4. Acid rain simulation test

In this laboratory study, accelerated test was applied in acid rain (AR) simulation. In real situations, AR could potentially be in contact with MICP treated slope soils at two possible conditions: (i) during infiltration and/ or (ii) submerged, and both conditions were investigated in this study. In order to reproduce the process of AR interaction, an infiltration setup was arranged as shown in **Figure 5.1**. Effects of various parameters such as level of cementation, pH level of AR and intensity of AR were evaluated, and the conditions are presented clearly in **Table 5.3**.

The main causes of AR and acid snowfall are sulfur oxides and nitrogen oxides. Therefore, AR solution was artificially prepared by dropping 1 mol/L nitric acid (HNO_3) and sulfuric acid (H_2SO_4) to deionized distilled water, and the pH levels were adjusted to 3.0 ± 0.1 , 4.5 ± 0.1 and 6.0 ± 0.1 . The infiltration quantity of the AR was estimated based on the average annual precipitation in study location (Onuma, Hokkaido), as per the given equation (**Eq. 5.1**). Based on the previous reports, it was assumed that one-third of the total precipitation infiltrates in to the slope and two-third would be surface-runoff (Bakhshipour et al., 2016; Kamon and Ying, 1997).

$$\text{Infiltration quantity of AR} = \frac{1}{3} P_{avg} A \quad (\text{Eq. 5.1})$$

where, the P_{avg} stands for the annual average precipitation of study location (1210 mm), and A is the cross-sectional area of the specimen ($\phi = 3$ cm). Accordingly, specimens were infiltrated by AR solution equivalent to the precipitation of 15 years. The AR solution was delivered to the specimens at two rates, and the rates were chosen on the basis of typical high and low rain fall intensities (around 20 and 100 mm/h, respectively). It should be noted that

the average high intensity of Hokkaido was recorded to be around 50 mm/hour (Yamada et al., 2012), covered with in the range studied herein.

For the submerged condition, specimens were soaked completely into corrosion-resisting cylindrical molds containing AR solution. As the pH values of the AR solutions were changing due to the consumption of protons (H^+) by the reaction with $CaCO_3$, the solution was replaced every 24 hours by newly prepared AR (to the total volume equivalent to the precipitation of 15 years). At the same time, the concentration of leached calcium ions was measured in the reacted solution. In addition, with the different immersion times, the weight loss was measured. Finally, all the treated specimens were oven-dried for 48 hours, followed by the estimation of the UCS and SEM analysis.

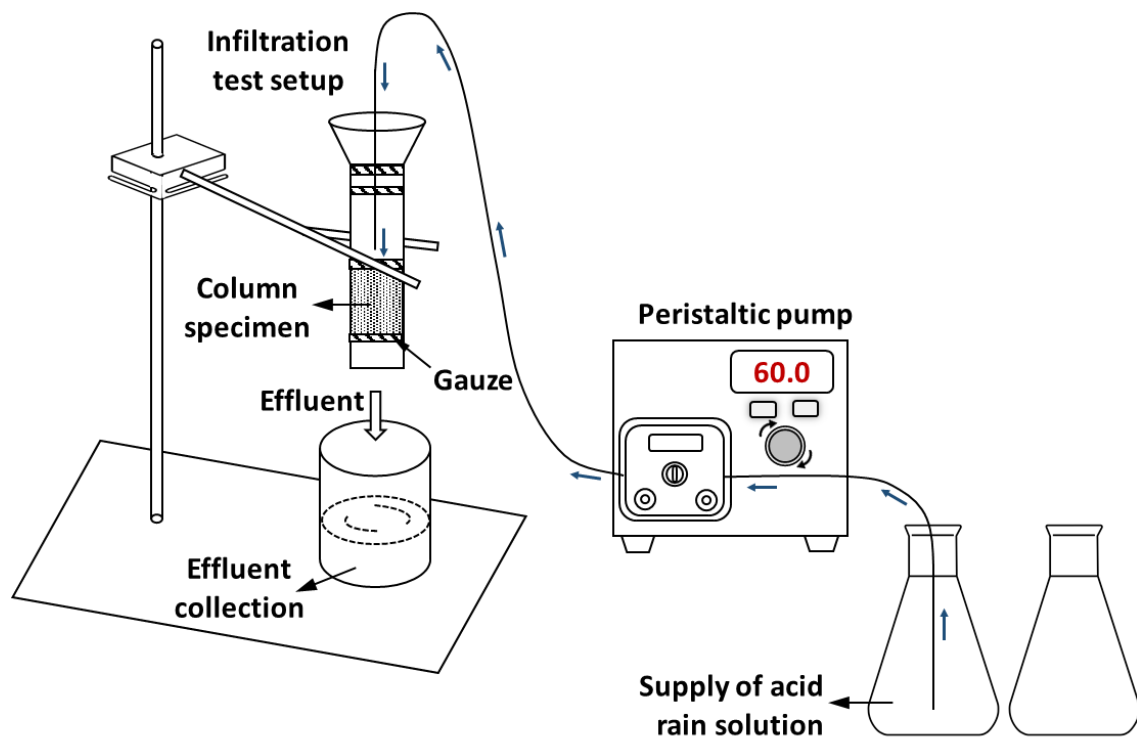


Figure 5.1: Acid rain (AR) infiltration test set up

Table 5.3: The conditions and specimens used for AR simulation test

pH of AR solution	Level of cementation	AR delivery condition	AR intensity	Specimen No.
6.0 ± 0.1	C-1	Infiltration	High	AR6-7-1
			Low	AR6-7-2
		Submerged	-	AR6-7-3
	C-2	Infiltration	High	AR6-10-1
			Low	AR6-10-2
		Submerged	-	AR6-10-3
	C-3	Infiltration	High	AR6-14-1
			Low	AR6-14-2
		Submerged	-	AR6-14-3
4.5 ± 0.1	C-1	Infiltration	High	AR4.5-7-1
			Low	AR4.5-7-2
		Submerged	-	AR4.5-7-3
	C-2	Infiltration	High	AR4.5-10-1
			Low	AR4.5-10-2
		Submerged	-	AR4.5-10-3
	C-3	Infiltration	High	AR4.5-14-1
			Low	AR4.5-14-2
		Submerged	-	AR4.5-14-3
3.0 ± 0.1	C-1	Infiltration	High	AR3-7-1
			Low	AR3-7-2
		Submerged	-	AR3-7-3
	C-2	Infiltration	High	AR3-10-1
			Low	AR3-10-2
		Submerged	-	AR3-10-3
	C-3	Infiltration	High	AR3-14-1
			Low	AR3-14-2
		Submerged	-	AR3-14-3

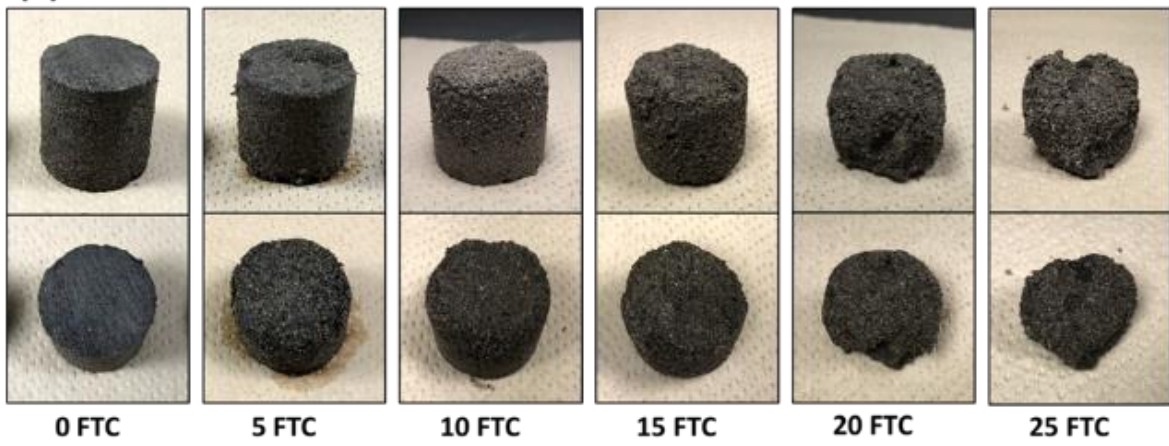
(AR intensities high and low represents the intensities of around 20 and 100 mm/h, respectively)

5.3. Results and Discussion on freeze-thaw (FT) action

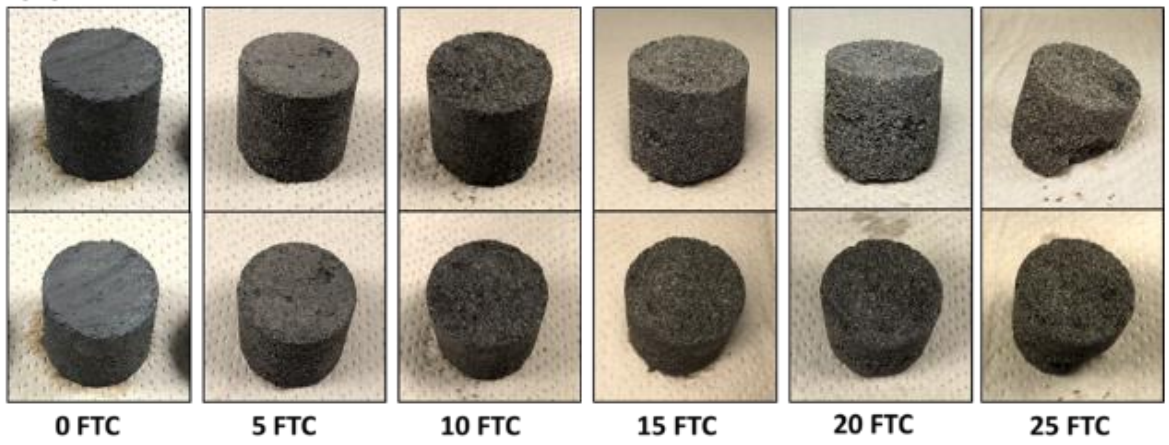
The regions of relatively high latitude (i.e. cold regions) such as Hokkaido, Japan, usually exposed to freeze-thaw (FT) cycles during winter seasons. It is anticipated that similar to soft rocks, FT cycles may induce uneven stresses due to the progressive expansion of the pore water, resulting structural damages and decrease in soil stability. In this research work, slope soil samples treated to three different cementation levels (**Table 5.1**) were subjected to continuous FT cycles; assuming one FT cycle equals to one seasonal frost (one year), the response of MICP specimens were studied for 25 seasonal frosts (i.e. 25 FT cycles).

Hokkaido experiences more than 100 days winter, and soils are covered by considerable layer of snow, keeping the soils continuously to be frozen (temperature ranging from 0°C to -15°C) during entire winter (Farukh and Yamada, 2018). Despite of the impairments caused by freezing, the aggregate stability increases at frozen state (Dagesse, 2013; Perfect et al., 1990). When the soils experiences thawing, the aggregate stability drops significantly; as the result, soil losses are typically reported in early spring (on thawed surfaces during snowmelt) (Oztas and Fayetorbay, 2003). On the above basis, therefore, the assumption that the effect of one FT cycle is comparable with one seasonal frost (i.e. long freeze in winter, followed by thawing in early spring), is made. The assumption is further fortified, as the duration and rate of the freezing were found to have less effect on aggregate stability (Dagesse, 2013; Li and Fan, 2014).

(a)



(b)



(c)

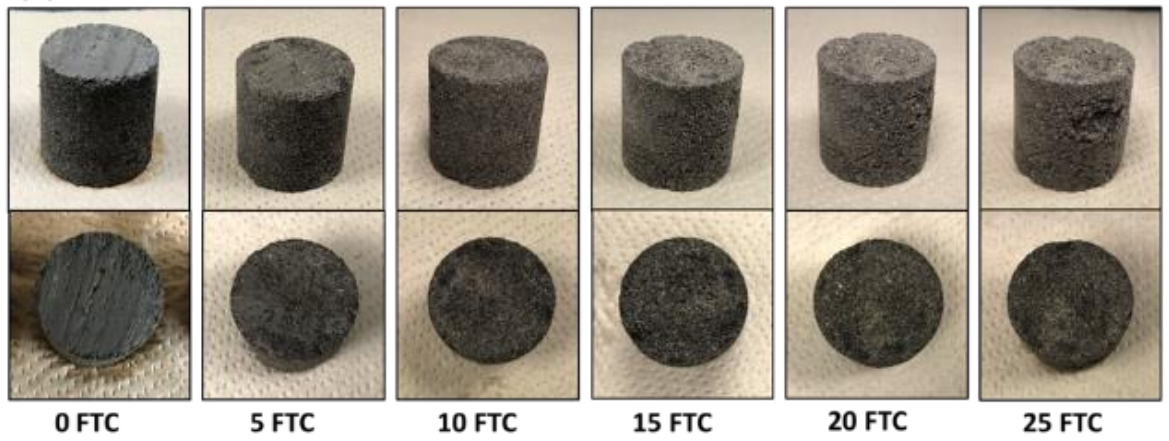


Figure 5.2: Appearance of (a) C-1, (b) C-2 and (c) C-3 specimens subjected to FT cycles

5.3.1. *Visual observations and loss in mass*

Fig. 5 presents the appearance of the cemented samples after subjecting repeated FT cycles. The damages in C-1 cemented specimens could be seen after 5 cycles (**Figure 5.2(a)**). The signs of damages in C-2 and C-3 specimens could be able to see after 15 and 25 cycles respectively. By the end of 25 cycles, the C-1 specimens experienced severe damage with separation of cemented pieces (less than 1 cm) and loose soil particles (similar to that of untreated). Relatively a moderate level of damage was observed in C-2 specimens after 25 cycles (**Figure 5.2(b)**). Level 3 specimens (**Figure 5.2(c)**), on the other hand, showed only a minor level of surface damage, indicating higher resistance to continuous FT damages compared to that of C-1 which is inherently susceptible to severe FT damage.

Figure 5.3 compares the cumulative mass loss from the specimens after exposure to FT cycles. The mass loss is severe in C-1 cemented samples, which exponentially increases with increasing number of FT cycles. The C-2 specimens experienced relatively a moderate mass loss: the loss was around 3% after 15 cycles, and thereafter, that increased dramatically to around 20% by the end of 25 cycles, whereas the loss was above 50% in C-1 cemented specimens. Conversely, at high CaCO_3 content (C-3), loss of mass was negligible (less than 2%), and this response is similar to that observed in ordinary Portland cement and alkali aluminosilicate cement specimens subjected to repeated FT cycles (Matalkah and Soroushian, 2018).

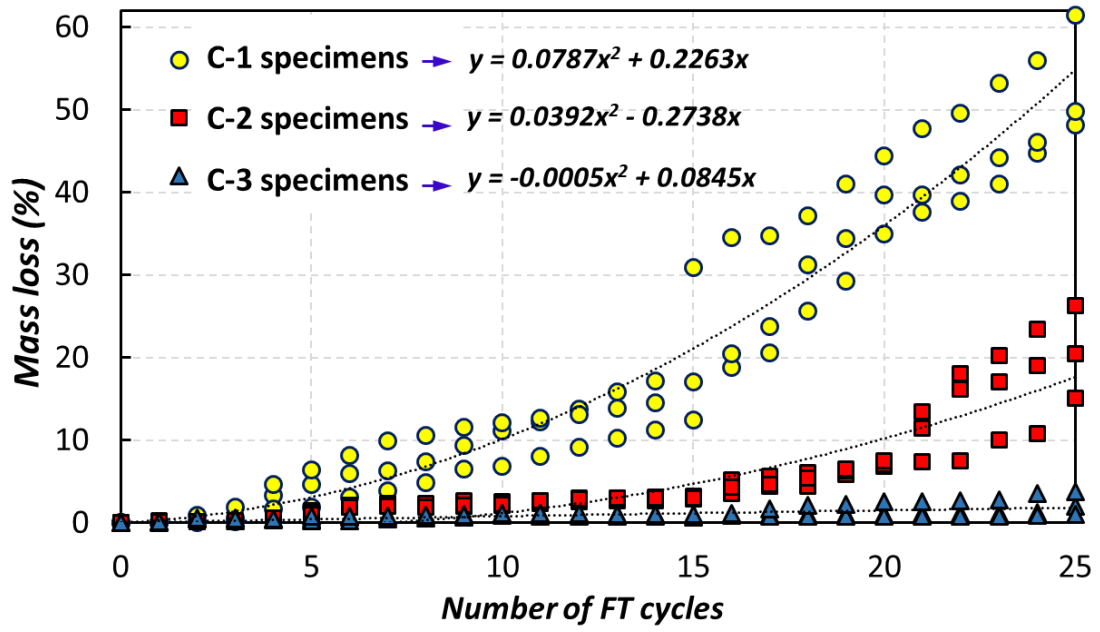


Figure 5.3: Mass loss versus number of FT cycles exposed

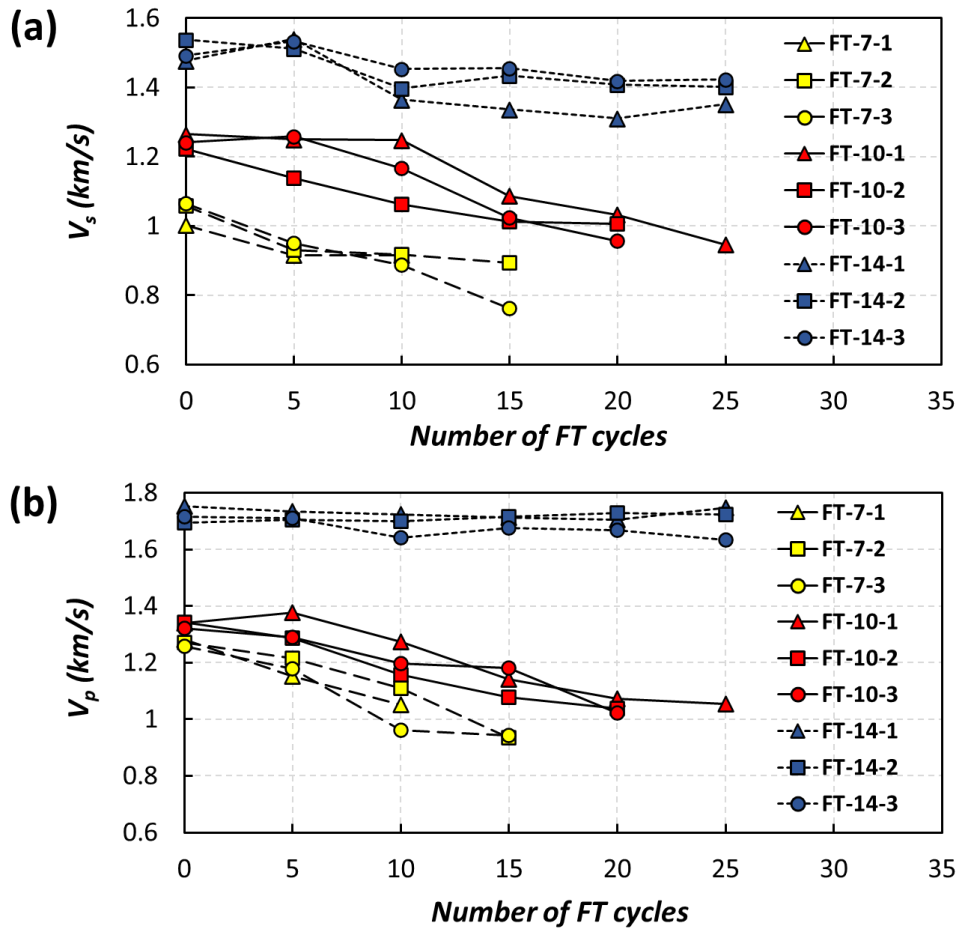


Figure 5.4: Variation of (a) S-wave and (b) P-wave velocities of specimens under FT cycles

5.3.2. Ultrasonic measurements

Figures 5.4(a) and **5.4(b)** shows the variation of S-wave and P-wave velocities (V_s and V_p , respectively) versus number of cycles during FT test. It can be seen that the V_p measurements are slightly higher than the V_s measurements for all the conditions, and that might be attributed to the dominant influence of the porewater on the bulk modulus of saturated specimens. Similar observations were also reported to the saturated sand specimens (Lin et al., 2016). Both S-wave and P-wave velocities show a gradual decrease following FT cycles in both specimens cemented to C-1 and C-2. By the end of 15 cycles, S-wave velocities decreased by 20% for C-1 specimens. As mentioned early, C-1 specimens exhibited severe damages after 15 cycles, and their velocities were unable to be measured precisely. For the C-2 specimens, they were measurable until 25 FT cycles, and the decrease in V_s was 24% after exposure of complete 25 cycles. On the other hand, the loss in V_s and V_p in C-3 cemented specimens were only 7.3% and 1.12%. These decrease in both V_s and V_p with increasing FT cycles is attributed to the formation and evolution of microcracks due to the stresses developed around the soil particles during the freezing of porewater within soil matrix. Although V_s and V_p have shown the decrease, evolution of these microcracks could significantly affect the travel path of S-waves compared to P-waves, resulted high loss in V_s .

These results further reveal that the FT response is highly influenced by the amount of precipitated CaCO_3 . In fact, the precipitated CaCO_3 at particle contacts has the greatest effect on the V_s (Qabany et al., 2011), thereby, more or less of the precipitation content at particle contact would in turn increase or decrease the V_s respectively. At the end of the test, C-3 cemented specimens experienced only a minor decrease in V_s (7.3%) where the loss is high for lower cementation levels (above 20%), suggesting high resistive stresses are attributed to strong contact cementations. Treating the samples to high cementation (E.g. C-3) would lead more carbonate precipitation at particle contacts, strengthening the connective bonds (i.e.

bridging adjacent particles in voids; strengthening the previously formed bonds). This would contribute to increased resistance for FT effects.

5.3.3. UCS measurements

Figure 5.5 presents the UCS of the specimens before and after subjecting to FT cycles. It can be seen that the UCS of the specimens significantly dropped by the end of the test. In order to better understand the effect of precipitated calcium carbonate content against strength degradation, SDR values under cyclic treatments are computed by using the following relationship (**Eq. 5.2**) and indicated in **Figure 5.5**.

$$SDR = 1 - \frac{UCS_{(f)}}{UCS_{(i)}} \quad (\text{Eq. 5.2})$$

SDR is the simplified measure, typically used to explain the strength deterioration of specimens exposed to cyclic treatments and had been broadly used for soft to hard rock materials (Hale and Shakoor, 2003; Khanlari and Abdilor, 2015). The $UCS_{(f)}$ represents the compressive strength at the end of the cyclic treatment and $UCS_{(i)}$ represents the compressive strength of virgin MICP specimens. The *SDR* varies between “0” and “1”. A rating of “1” is allotted to the weakest specimen, reveals complete degradation at the end of the cyclic treatment. For the strongest specimens, which do not show any damages after the treatment, a rating value of “0” is allotted.

The SDR_{FT} values of sandstones materials are found to be between around 0.25 and 0.6 (Hale and Shakoor, 2003; Khanlari and Abdilor, 2015), perceiving that the MICP specimens are more vulnerable to the FT actions i.e. the values range between 0.73 – 1.0. It should be noted, as the UCS of the C-1 specimens were unable to measure, the SDR_{FT} values are assumed to be 1.0. It can also be seen that the SDR_{FT} values decrease with the increase in cementation

level, demonstrating that precipitated calcium carbonate content has a vital role against FT damage.

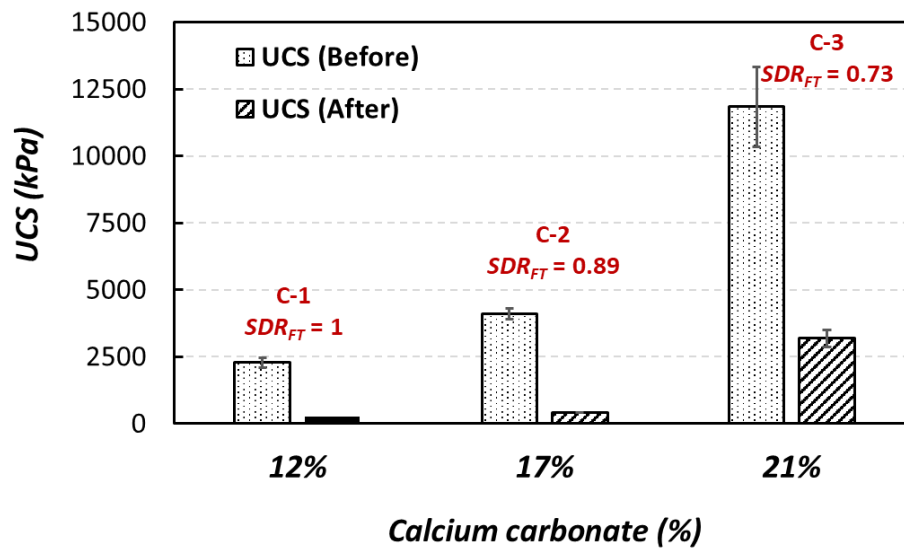


Figure 5.5: The variation of UCS before and after cyclic FT tests

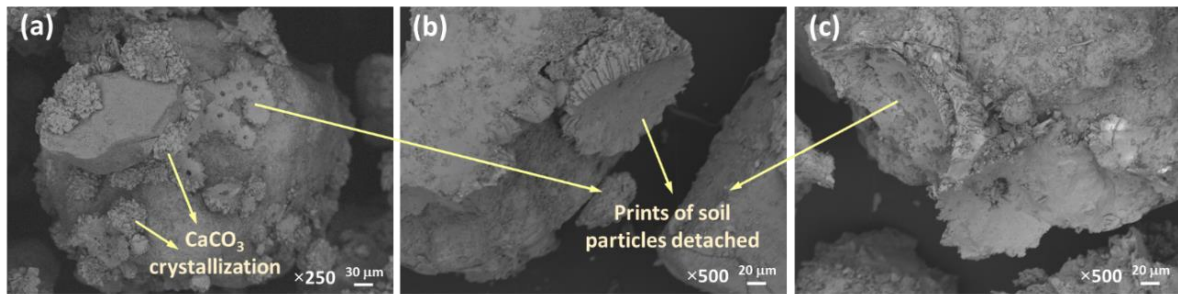


Figure 5.6: Micrographs of footprints of crystal-soil detachment under FT effects

5.3.4. Discussion

Previous studies have demonstrated that seasonal freezing-thawing plays a fundamental role in slope erosion during run-off events that follows thawing (Ferrick and Gatto, 2005; Gatto, 2000; Sadeghi et al., 2018). Typically, the FT cycles largely affects the aggregate stability, which scales-off the residue from the soil matrix, increasing the erodibility potential. MICP treatment enhances the aggregate stability by cementing the particle contacts and sometimes bridging the particles in void spaces, and this bonding facilitates additional resistive forces

against the FT effects. The results have demonstrated that level of cementation has a great role in FT response of MICP treated specimens.

The SEM analysis performed by the end of 25 cycles demonstrates that the degradation mode occurred during FT cycles is dominantly crystal-soil detachment (**Figure 5.6**), compared to the localized cracking of crystals which was barely observed in the soil matrix. Micrographs depicts the prints of the soil particles detached from the cementation by the uneven forces developed during the freezing. Fundamentally, the detachment of soil particles from the cementation is controlled by the calcium carbonate precipitation content at the contact points. Higher precipitation content (C-3) could facilitate the formation of larger and stronger clusters with large contact area at particle contacts, thus reducing the likelihood of crystal-soil detachment. In the case of C-1, the particle bonds were comparatively weak, and that could easily be ruptured or detached during FT cycles.

There are many factors that control the FT response of soil matrix, including particle size distribution, water content, soil texture, number of cycles and freezing temperature (Behzadfar et al., 2017; Cheng et al., 2016; Oztas and Fayetorbay, 2003). Among them, particle size distribution has a major role in determining freezing-thawing behavior of MICP treated specimens, as the MICP behaviors significantly vary with different grain sizes (Dhami et al., 2016; Oliveira et al., 2016). Previously, many studies have confirmed that soils dominated by large aggregates could withstand more against cyclic FT, as their high porosity led to the rapid water flows and movements (Oztas and Fayetorbay, 2003; Viklander, 2011). However, recently, Cheng et al. (2016) have demonstrated that the FT response of MICP cemented sands differ from typical response of untreated sands, in which the number of contacts per soil particles governs the behavior. The number of particle contacts per soil sphere increases with the decrease in porosity, and thereby, contact points per particle

increases when the average grain size decreases from coarse to fine. Comparing the behavior of MICP treated fine sand (D_{50} , 0.15 mm) with coarse sand (D_{50} , 1.18 mm), the coarse sand exposed to FT cycles shown significant drop in UCS compared to that of fine sand (Cheng et al., 2016). Larger particle contacts in fine sand could decrease the resultant tensile stress (aroused from freezing) acted per particle and exhibited more durability. For the well-graded sand, further enhancement in FT resistance was reported due to the presence of both coarse and fines contributing high permeability and high particle contacts respectively.

This study has verified that MICP technique with the required level of cementation can be used to stabilize the slope near-surfaces against freezing-thawing that affect the aggregate stability and erodibility of soil deposits. The adequate treatment could cement the particle contacts sufficiently, enhancing the resistance to the formation of ruptures and crystal-soil detachments due to uneven tensile stresses developed during the frost of porewater. The design life of the treatment can also be approximately determined based on the results presented (i.e. relating number of seasonal frosts with number of FT cycles). For example, it can be roughly said that soils treated to the average CaCO_3 content of C-1, C-2 and C-3 by mass can effectively withstand against freeze-thaw erosions for 5, 15 and 25 years of service.

5.4. Results and Discussion on the effects of wet-dry (WD) action

5.4.1. *Physical changes*

Figure 5.7 shows the impact of WD cycles on the physical appearance of MICP specimens. In all the specimens, observable changes in the appearance could be slightly seen after 30 WD cycles. By the end of 50 WD cycles, C-1 specimens showed severely damaged surface with large open pores. C-2 and C-3 specimens, on the other hand, exhibited relatively minor surface damage, suggesting higher resistance to the WD damages compared to C-1.

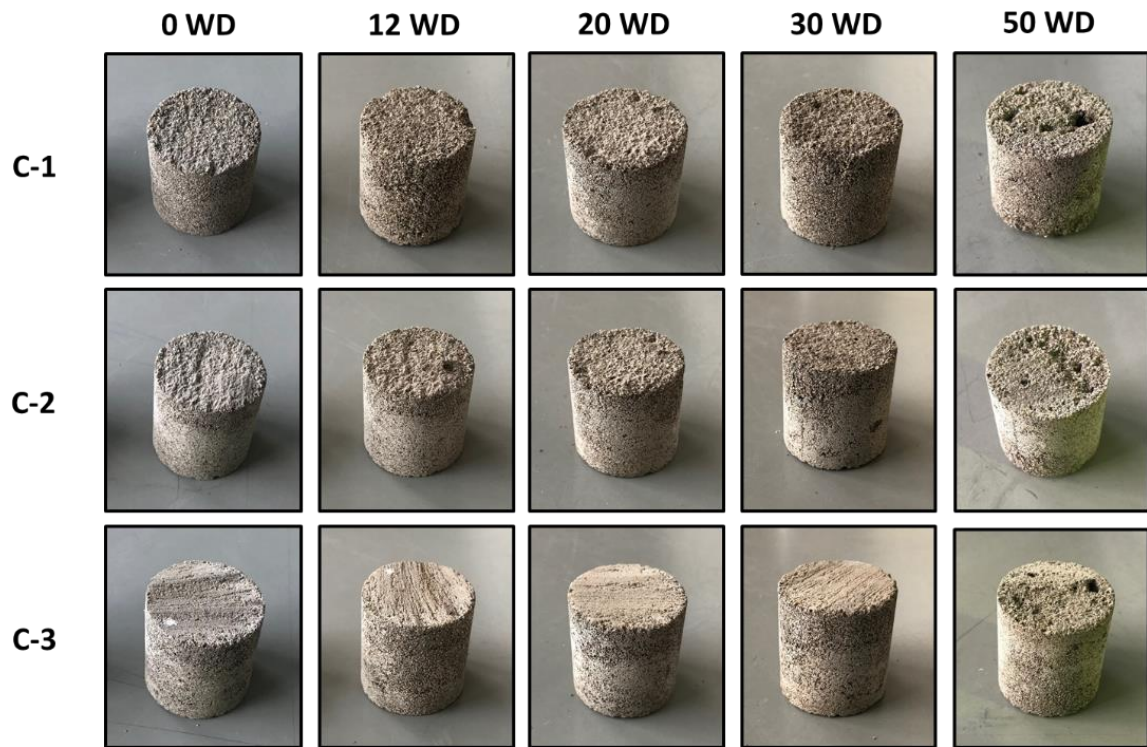


Figure 5.7: Appearance of cemented specimens subjected to WD cycles

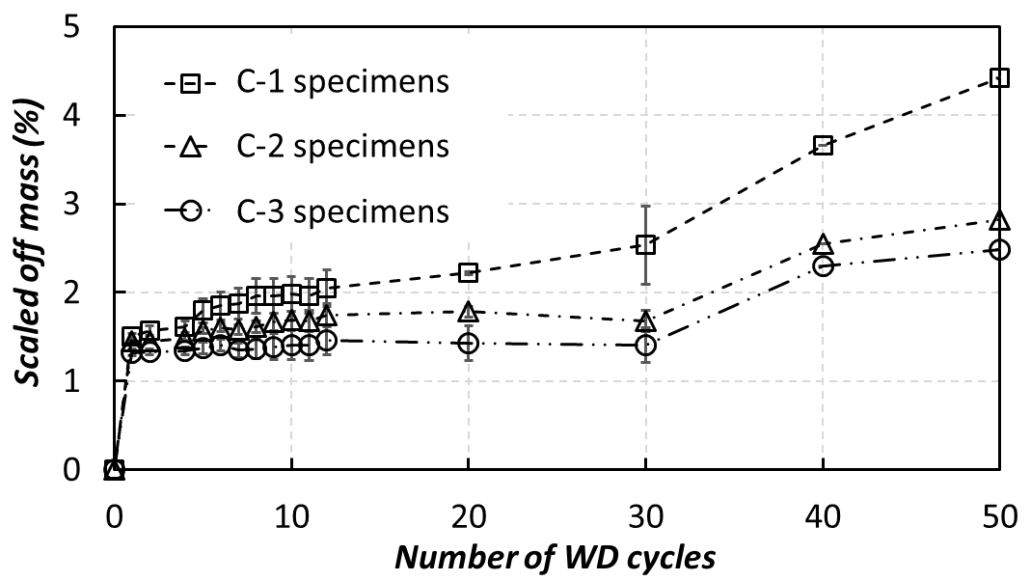


Figure 5.8: Mass loss of specimens versus number of WD cycles exposed

Figure 5.8 presents the cumulative mass loss of the specimens with increasing number of WD cycles. The results show that MICP specimens underwent considerable corrosion during WD cycles, resulting an average mass loss ranging between 2 - 5 % by the end of 50 WD

cycles. The results show that the level of cementation influenced the corrosion resistance. By the end of the 50 WD cycles, the scaled-off mass of the specimens cemented to C-1, C-2 and C-3 levels are 4.4, 2.8 and 2.4 % respectively, suggesting more or less of the level of cementation would in turn increase or decrease the corrosion resistance of specimens respectively.

It can also be observed that the end of first WD cycle, all the specimens exhibit an abrupt increase of mass loss (over around 1.2 %) regardless to the level of cementation. A similar observation is also reported by Ciantia et al. (2015) to the calcarenite soft rock material. Within another few more WD cycles, the rate of mass loss decreases gradually and tends to become to the equilibrium state. Up to around 30 number of WD cycles, relatively flat graphs are obtained in all the specimens, suggesting that the loss of mass are not significant within that duration. However, when the specimens are subjected to further WD cycles (above 30), mass loss becomes significant once again. At this later period, high loss rate is observed for the C-1 specimens, whereas the loss rates experienced in C-2 and C-3 specimens are moderate.

The porosity measurements (**Figure 5.9**) indicate that the average porosity of the specimens before the WD process are 0.31, 0.29 and 0.26 (C-1, C-2 and C-3, respectively). As expected, longer treatment caused more deposition of calcium carbonate in C-3 specimens, leading to a lower value of porosity compared to that of other cases (C-1 and C-2). The observation indicated that the porosity increased significantly by the end of 12 cycles. The increase could possibly be attributed to the loss of material which creates additional pore spaces during WD cycles. However, the porosity change between 12 and 50 WD cycles is likely to be insignificant.

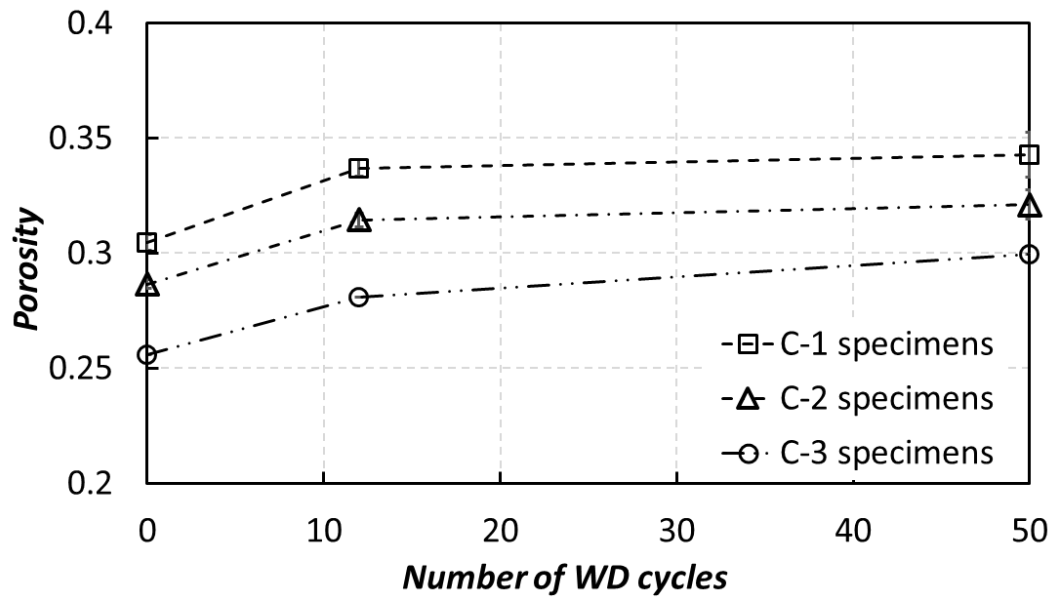


Figure 5.9: Average porosity of the specimens with number of WD cycles

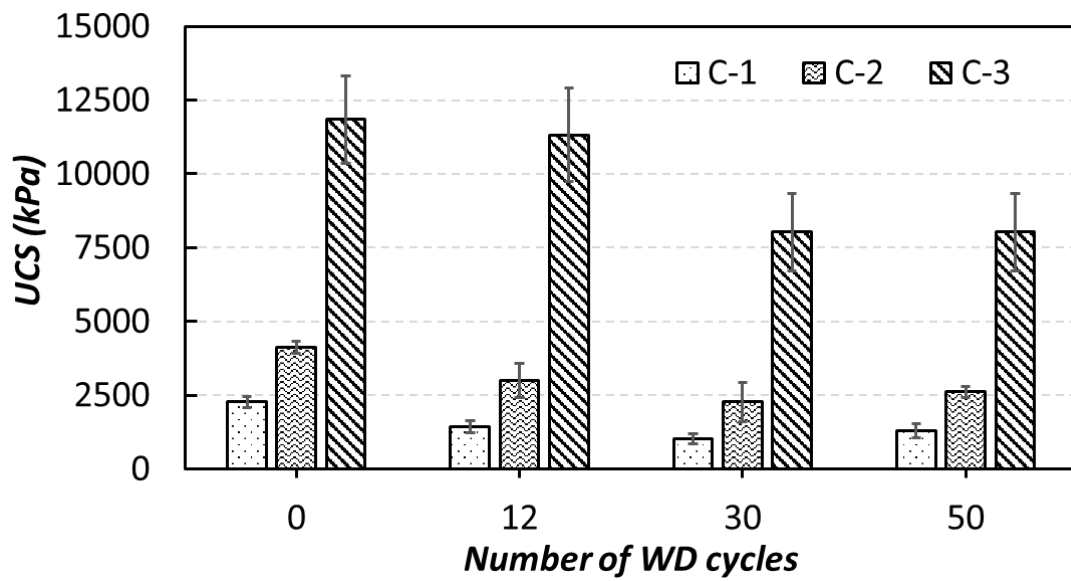


Figure 5.10: Variation of UCS with number of WD cycles

5.4.2. UCS measurements

Figure 5.10 shows the variation in UCS of MICP specimens after the complete exposure of 0, 12, 30 and 50 WD cycles. The significant reductions are observed to the specimens treated to C-1 and C-2 within the first 12 cycles. However, within the first 12 cycles, the specimens cemented to C-3 showed insignificant change in UCS. By the end of 30 cycles, all the

specimens showed a marked drop in UCS. On the other hand, between 30 and 50 WD cycles, almost no UCS reduction is observed in all the cases. This overall observation suggests that the mechanical degradation conspicuously occurs at the early stage of the WD process, compared to that occurred at the later stage.

5.4.3. *S-wave velocity measurements*

For better understanding the changes in the mechanical behavior, the specimens were monitored using S-wave velocity, and the results are provided in **Figure 5.11**. The precipitated calcium carbonate at particle contact has the utmost effect of S-wave velocity, because the S-waves can only be transmitted through solids (not through soil pores) (Al Qabany et al., 2011). The results reveal that irrespective of the cementation levels, variation in velocity measurements follows relatively a similar pattern with increasing WD cycles: rapid drop is observed within first few cycles (up to 5 WD cycles), followed by a smooth and gradual reduction. These rapid reductions in S-wave velocity are consistent with those of observed in mass loss (**Figure 5.8**), and this could possibly be attributed to the weakening of particle connections due to the corrosion induced by WD process. On the other hand, the average velocity reduction occurred between 5 – 50 WD cycles ranges only between 0.06 km/s and 0.1 km/s, suggesting the minor damage in the later stage. This observation is in a very good agreement with that observed in UCS results (**Figure 5.10**).

5.4.4. *XRD results*

Figure 5.12 shows the results obtained from the XRD analysis. The analysis was performed to verify the occurrence of phase transformation of carbonate deposits, precipitation of new minerals and dissolving of existing minerals. The XRD results confirm that there are no observable changes in the morphology of minerals throughout the WD process. The observed

peaks are identical with the increase in number of cycles, suggesting neither formation of new minerals nor phase changes were induced.

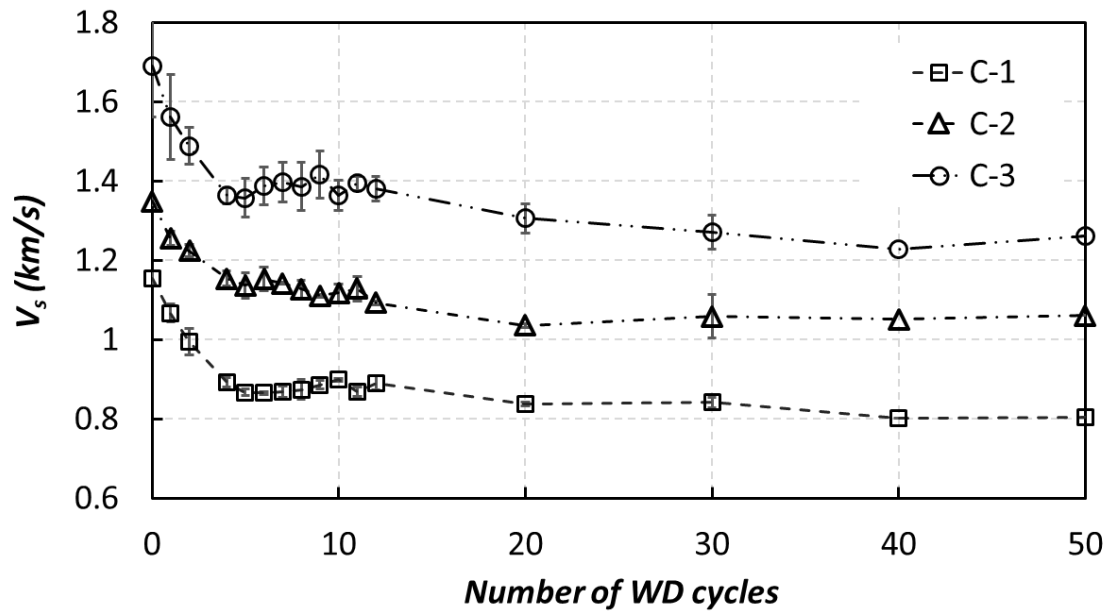


Figure 5.11: Changes in the S-wave velocity with the number of WD cycles

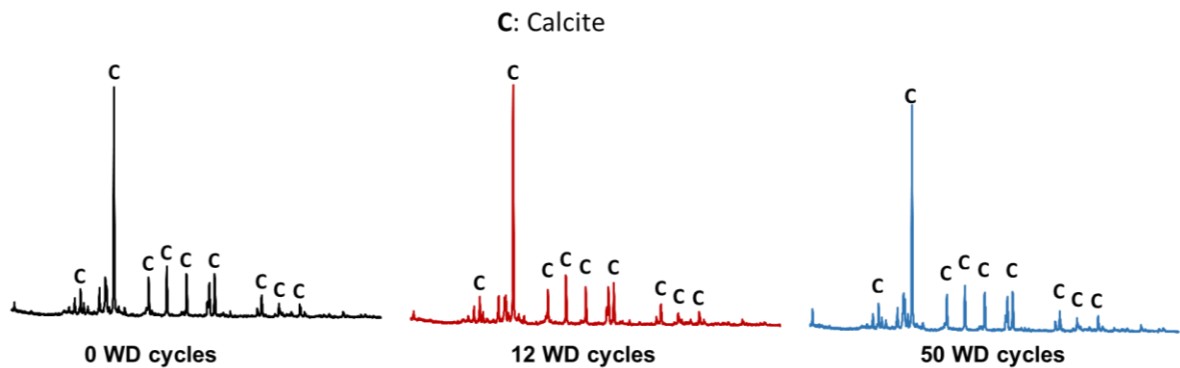


Figure 5.12: XRD results of specimens subjected to WD cycles

5.4.5. Discussion

It is clearly perceived that the physical and mechanical behaviours are significantly influenced by the WD cycles. Based on the observations (**Figures 5.8** and **5.11**), the performance deterioration of specimens can be considered into two stages. In the first stage (up to 5 WD cycles), severe degradation was witnessed, hereafter referred to as STD (Short

term deterioration). A gradual degradation was observed in second stage (30 – 50 WD cycles), hereafter referred to as LTD (Long term deterioration).

5.4.5.1. *Short-term deterioration*

The STD mechanism can be explained from the evolution of calcium carbonate precipitates during MICP. As demonstrated by Wang et al. (2019), during the injection of cementation solution, the calcium carbonates are initially induced as irregular fines (amorphous), and they continue to grow with the time and eventually form the regular calcium carbonate crystals. Such precipitates, in the reaction system, often randomly stabilize on soil surfaces as powdery deposits before transforming to crystalline structure. Micrographs depicted in **Figure 5.13** illustrate different forms of carbonate deposits. As shown, the secondary formations of the calcium carbonate could embed as supporting clusters, individual deposits and powdery deposits. Similar powdery deposits of calcites were observed in the calcarenite rocks, in which they were sedimented upon porewater drying (Ciantia et al., 2015). It is very clear that not all the precipitated carbonates contribute to the contact cementation (i.e. primary bonds); a considerable portion of carbonates are found to be either crystalized randomly on the soil grains or set on the already formed carbonate bridges.

Figure 5.14 shows the serial progression of water inundation during the initial wetting process. When the samples are initially subjected to the wetting, water tends to penetrate through the porous system and inundate the material, resulting the powdery deposits and their accumulations to fall into suspension, followed by the corrosion. This could attribute to the leap increase in mass loss observed after first WD cycle (**Figure 5.8**). By the end of the first cycle, layer of powdery formations was evidenced at the bottom of the container wherein the samples were subjected to wetting, confirming the mechanism suggested herein. The rate of loss decreased with few more WD cycles (3, 4 and 5) and afterwards, tended to become

relatively stable, suggesting that the powdery deposits have totally been corroded. The rapid drop observed in S-wave velocity within first 5 WD cycles (**Figure 5.11**) could also be attributed to the disintegration of powdery and weak deposits. As explained before, there are carbonate clusters which are feebly bonded, and those weak attachments had a substantial contribution in particle connections, i.e. supporting primary bonds. The saturation effect that accompanied with internal fatigue stresses (Li et al., 2019) developed at the early stage, could disintegrate the weak deposits and weakened the particle connections, leading to the loss in S-wave velocity within the short-term. This STD is likely to be affecting the UCS also (**Figure 5.10**). However, the specimens treated to C-3 level did not show any significant loss in UCS even after 12 WD cycles. But, the C-1 and C-3 specimens showed significant UCS loss. C-3 specimens, due to the strong primary connection, could be able to withstand its UCS, even if the weaker deposits were disintegrated and corroded.

It is clear that MICP soil structure has been significantly affected within first few WD cycles (STD), and then tended to be relatively stable. For a similar instance, Ciantia et al. (2015) studied the deterioration of carbonatic rocks and observed the occurrence of instantaneous debonding of depositional bonds (menisci shaped calcite ensembles) when subjected to saturation. The researchers also found that single wetting process caused a considerable loss in compressive strength, and the loss was more pronounced for high porosity calcarenites (Ciantia et al., 2014).



Figure 5.13: Illustration of types of crystallizations formed in MICP treated soil

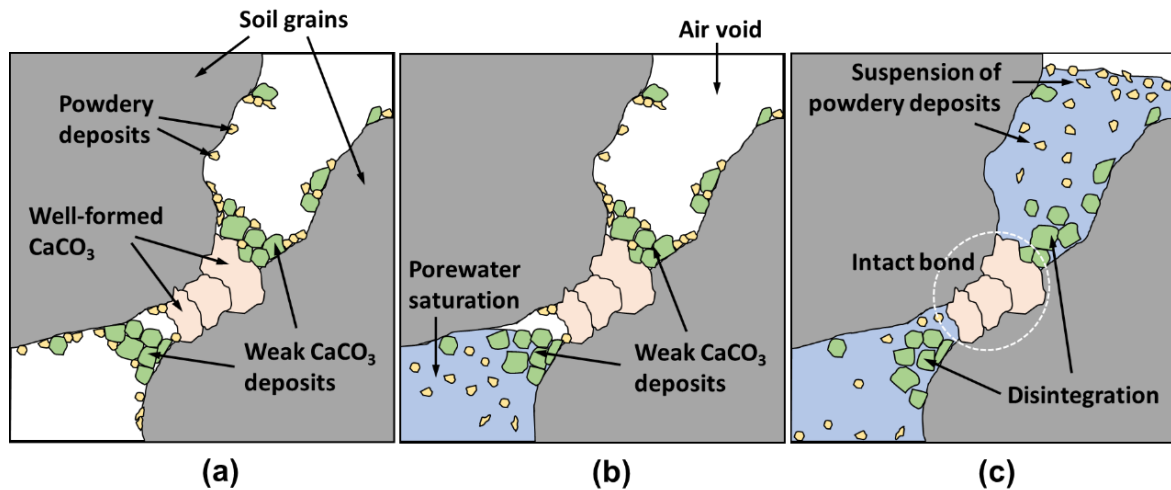


Figure 5.14: Schematic illustration of (a) MICP soil before subjected to wetting, (b) wetting: penetration of water into pores and (c) wetting: almost saturated stage

5.4.5.2. Long-term deterioration

When the specimens are subjected to further WD cycles, the observed degradations were not as rapid as that observed in the STD. After the corrosion of powdery carbonates, the well-formed carbonates, i.e. intact bonds were continuously exposed to the subsequent cycles. As per the results, the considerable degradation was once again observed during 30 – 50 WD cycles (**Figure 5.8**).

To deepen the understanding on LTD mechanism, SEM analysis was performed. **Figure 5.15** and **5.16** present the SEM images captured respectively before and after the cyclic WD process. All the micrographs presented herein were captured at the same magnification and scale. A significant change in the microstructure is observed after subjected to WD test (**Figure 5.16**). Before the test, presence of large carbonate clusters is evidenced in SEM, and their shape and structure could also be easily detectable (**Figure 5.15**). However, by the end of WD process, a complex shrunken microstructure is observed for carbonate deposits, and the morphology could not be easily distinguished by the observation. However, the XRD analysis confirmed that this deformation is not related to the chemical changes or phase

transformations, therefore, this might be possibly due to the effects from the development of internal stresses during cyclic WD process as reported by Li et al. (2019).

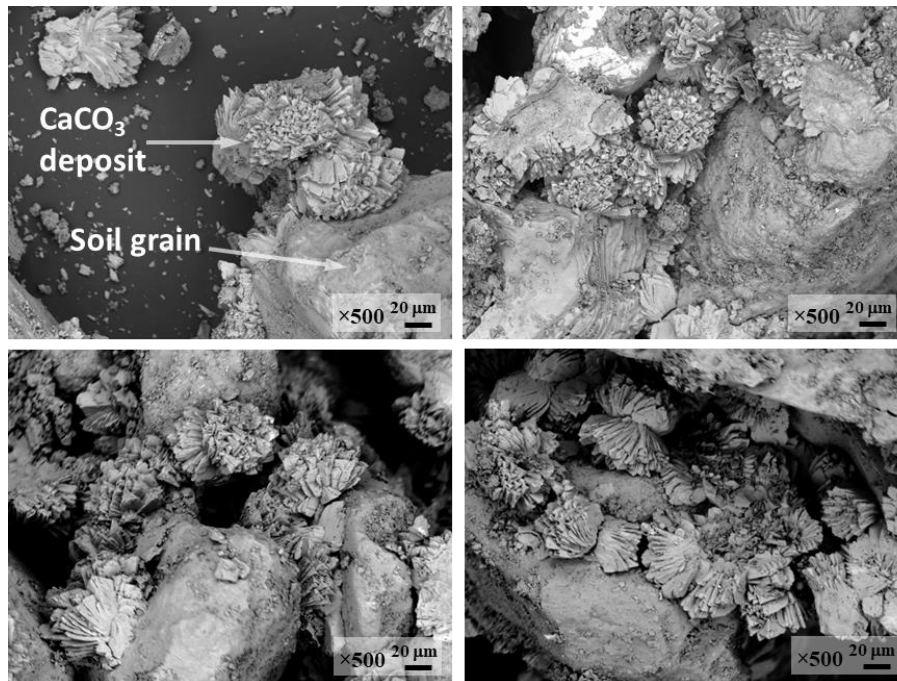


Figure 5.15: The micrographs of calcium carbonate deposits before subjected to WD cycles

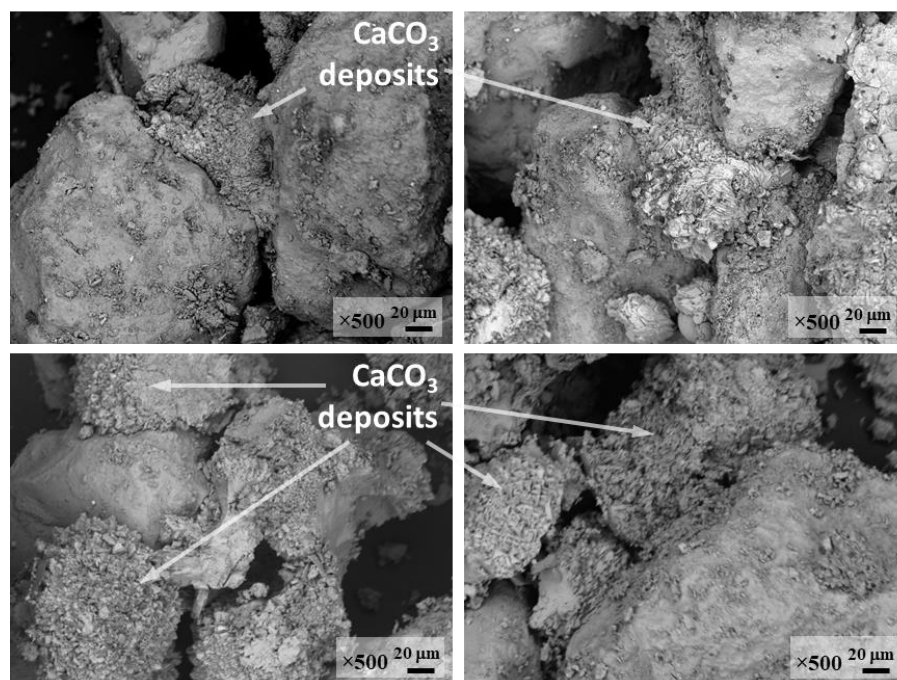


Figure 5.16: The micrographs of calcium carbonate deposits after subjected to 50 WD cycles

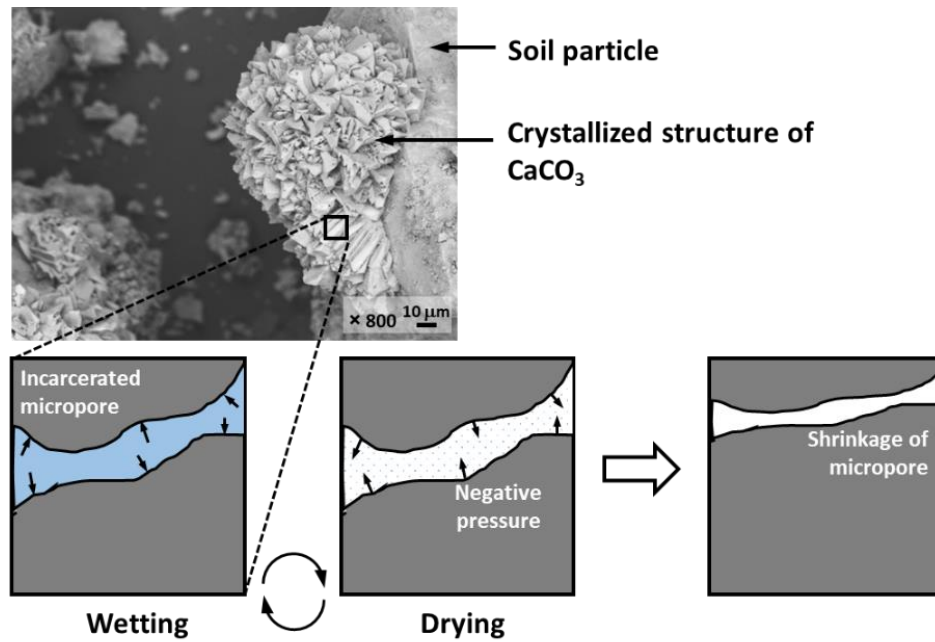


Figure 5.17: Micropores in crystallized calcium carbonate deposit and schematic illustration of deformation during WD process

When the specimens are treated to high cementation levels, assemblages of rhombohedral calcite clusters are produced by bacteria, which incarcerate number of micropores (**Figure 5.17**). Similar accumulates were also evidenced in the micrographs of Omoregie et al. (2019). When the specimens were submerged during wetting (see **Figure 5.17**), the micropores confined within carbonate deposits were also saturated, followed by rapid evaporation during the drying process at 70°C. As shown, this resulted the development of fatigue negative stresses in the micropores, led to the shrinkage in the microstructure of well-formed carbonates (as witnessed in **Figure 5.16**). This deformation has probably initiated from the early cycles, but the changes could be observable only at the later stage. During this stage, however, no significant change in the mechanical properties are observed (**Figures 5.10** and **5.11**). Significant mass loss, on the other hand, was continuously experienced between 30 – 50 WD cycles (**Figure 5.8**). Rapid progression of macropores was also observed on the

surfaces of all the specimens within the above cycles (**Figure 5.7**). This can be explained by the gradual degradation from the surface to the core of the specimens.

Figure 5.18 presents the microstructures of specimen surface (after 50 WD cycles). Higher degradation is evidenced on the surface compared to that observed at core of the specimens. The surface carbonate deposits were found to be shrunken and degraded. Microcracks were also observable at and around the intact bonds (**Figure 5.18**). On the other hand, these impairments were not evidenced in SEM performed on the core specimens. When specimens are subjected to drying, the interior relative humidity does not drop immediately but decreases in a gradual manner (Zhang et al., 2012), whereas the surface undergoes rapid evaporations, experiencing high negative stresses. Also, the core zone of the sample is confined stiffly, the developed fatigue stresses therefore caused more damages at outer surface of the column, attributing to the progression of macropores. Similar initial surface degradations due to WD stresses were also often reported to concrete specimens (Gao et al., 2014; Zhang et al., 2012).

Furthermore, the difference in thermal expansion coefficient of calcium carbonate and soil material could be another possible factor that could result the development of internal fatigue stresses under temperature change, inducing the degradation process. The above mechanism has also been reported to magnesium potassium phosphate cement, in which the internal fatigue stresses resulted pores and microcracks (Li et al., 2019). When the calcites are flooded by water for a long time, a minor portion would possibly undergo chemical dissolution (Ciantia et al., 2014; Khanlari and Abdilor, 2015). Ciantia et al. (2015) have confirmed that the long-term weathering of carbonatic rocks is due to the dissolution of diagenetic calcite bonds. In this study, the effect of dissolution is assumed to be negligible under the standard conditions.

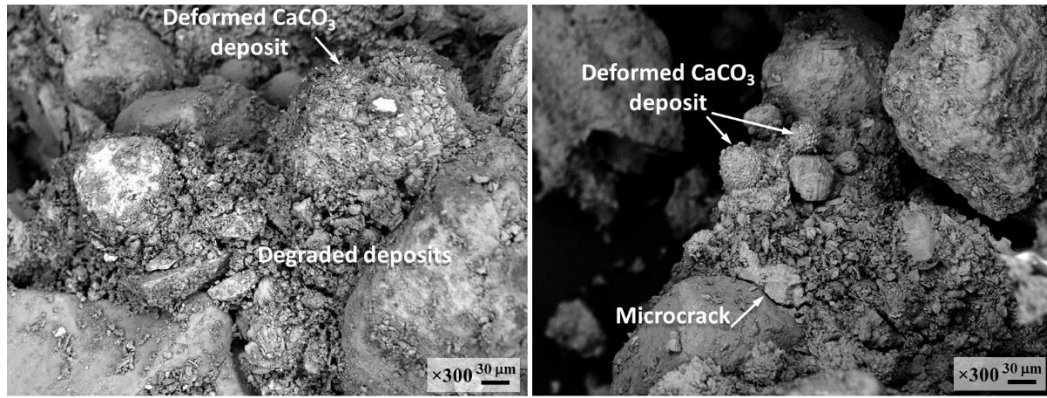


Figure 5.18: Degraded microstructure observed at surface of specimens after 50 WD cycles

5.4.6. *Effect of cementation level on WD cycles*

Regardless to the level of cementation, all the specimens experienced considerable deterioration under the exposure of continuous WD cycles. To understand the effect on cementation level on mechanical damage, strength deterioration ratios (*SDR*) were calculated using **Eq. 5.2** and compared in **Table 5.4**.

Table 5.4: Comparison of strength deterioration ratio (*SDR*) of MICP specimens under cyclic actions

Specimens		SDR_{WD}	SDR_{FT}
MICP specimens	C-1	0.43	1
	C-2	0.37	0.89
	C-3	0.32	0.73
Sandstone		0.1 – 0.2	0.25 - 0.6

(The SDR_{WD} and SDR_{FT} values of sandstones were from the literatures (Hale and Shakoor, 2003; Khanlari and Abdilor, 2015), and they correspond to the exposure 40 WD cycles and 30 FT cycles)

The SDR_{WD} values considerably decrease with the increase in calcium carbonate content (**Table 5.4**). The tendency demonstrates that the high deposition of carbonates likely to be preserving the MICP responses against WD actions. Longer MICP treatments achieve high

precipitation content, which could strengthen the primary connections, providing high resistive forces during the development of fatigue stresses. Also, these strong connections could withstand against suspension and corrosion induced by WD process.

MICP specimens show higher SDR_{WD} values compared to those of sand stones (**Table 5.4**), suggesting that MICP cemented soils are more susceptible than sandstones. The tendency observed to SDR_{WD} is quite similar to the SDR_{FT} . However, comparing the SDR_{WD} and SDR_{FT} values, it can be precisely concluded that the MICP specimens underwent severe mechanical degradation under FT cycles (over 70%), and the degradation was minor under WD cycles (between around 30 – 45%).

5.5. Results and Discussion on the effects of acid rain

5.5.1. Corrosion of calcium carbonate

The tested conditions are clearly tabulated in **Table 5.3**. The chemical reaction between AR and calcium carbonate could result in the dissolution of CaCO_3 that precipitated in MICP specimens. During the test, leached calcium ions was continuously measured from the effluent of the specimens. From the Ca^{2+} concentrations, the corroded calcium carbonate was theoretically estimated in each case, and the results are plotted and compared in **Figure 5.19**. From the plots, it can be clearly observed that regardless of specimen cementation level, the corrosion of calcium carbonate was highly reliant on the pH condition of AR solution. Minor corrosion rates of calcium carbonate were observed to the specimens exposed to AR of pH 6.0, and the corrosion rate showed an increasing tendency with the decrease in pH. For instance, when the pH of AR decreased from 6.0 to 4.5, the corrosion rate increased by around 10 times, and that rate further increased at pH of 3.0.

The corrosion rate of calcium carbonate was also considerably influenced by the intensity of AR (**Figure 5.19**). The low intensity of AR (20 mm/h) resulted higher corrosion rate compared to that under high intensity (100 mm/h). For example, by the end of the test (at pH 3.0), the specimens showed the corrosion of around 0.7 g CaCO_3 under low intensity of AR, which was around 1.75 times higher than the corrosion experienced under high intensity of AR. This could be attributed to the contact time of AR within MICP specimens. During the high intensity of AR, certain hydraulic pressure was developed on the surface (due to ponding), which tended to infiltrate the AR at high rates. The less contact time of AR with soil MICP matrix resulted the less reaction with CaCO_3 . On the other hand, the low intensity facilitated relatively slow infiltration, allowing sufficient time to the reaction with CaCO_3 during the percolation.

Figure 5.19 further demonstrates that the corrosion of CaCO_3 under low intensity and fully submerged conditions are likely to be similar in all the cementation levels. As sufficient reaction time was given in both the test conditions, relatively similar dissolution rate of bio-cement could be resulted. Experimental data confirmed that the pH value of the reacted/effluent was above 7.5, indicating that the protons (H^+) in the acid solution were almost fully consumed by reacting with CaCO_3 .

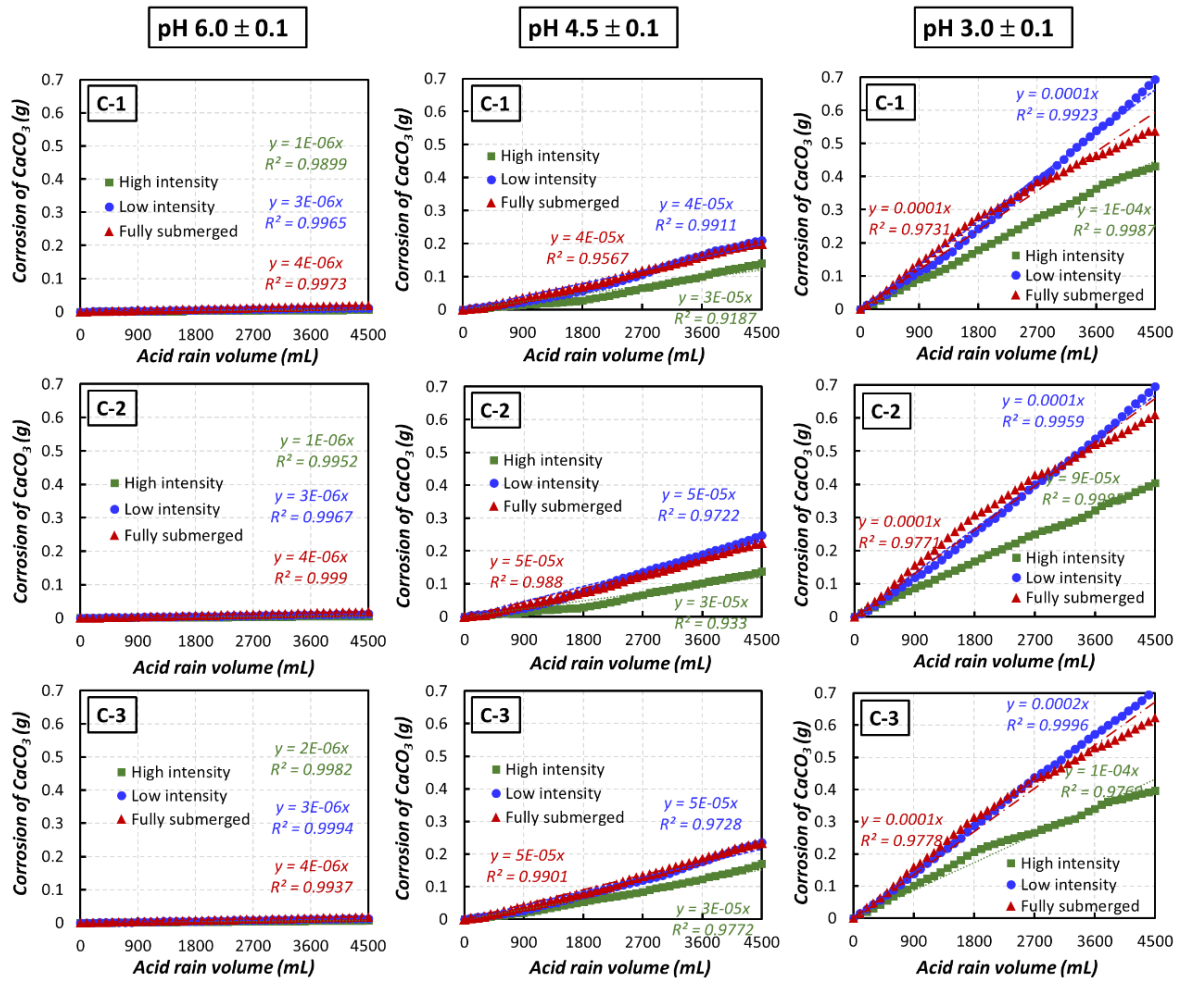


Figure 5.19: The corrosion of calcium carbonate during the AR simulation test

5.5.2. Physical changes

The physical changes of the MICP specimens before and after subjecting to AR simulation tests ($\text{pH } 3.0$) are shown in **Figure 5.20**. It can be seen that the specimens treated to high cementation level (C-3) showed lower physical impairments than those of low cementation levels (C-1). The specimens subjected to low intensity of AR supply showed higher surficial damage compared to that of high intensity of AR. As the AR was delivered to the specimens from the top, the proton concentration of AR was high and effective at the supply point, resulted high dissolution of CaCO_3 at surface. When the solution reached specimen depths, the certain quantity of protons has already been reacted and neutralized, which leads to the less reaction at bottom specimens. At the same time, the specimens tested under submerged

conditions showed clear damage structure on cylindrical surfaces as well (**Figure 5.20**), as the entire surface of the specimens was directly exposed to AR solution when submerged. Relatively low physical damages were seen in specimens subjected to AR of pH 4.5. It also should be noted that specimens subjected to AR of pH 6.0 did not show any observable changes.

Figure 5.21 shows the mass loss of MICP samples as a function of delivered volume of acid rain at different pH conditions. As shown, the mass loss rate of MICP treated specimens increased with increasing delivered volume, and that increases with the increase in pH of the AR. The tendency of mass loss rate (**Figure 5.21**) is in a good agreement with the corrosion rate of calcium carbonate (**Figure 5.19**). However, the cementation level highly governed the rate of mass loss. For example, by the end of the test (at pH 3.0), all the specimens (C-1, C-2 and C-3, i.e. irrespective of the level of cementation) showed the same corrosion rate of calcium carbonate. The soil losses, on the other hand, were up to around 20%, 9% and 5% for C-1, C-2 and C-3 specimens, respectively, suggesting that high cementation level attributes to high durability of MICP treatment against AR induced erosion.

5.5.3. *UCS measurements*

Figure 5.22 presents the UCS of the MICP specimens subjected to AR with different pH levels (6.0, 4.5 and 3.0). In each case, the UCS values before the AR test are also compared to clearly understand the effect of AR in UCS.

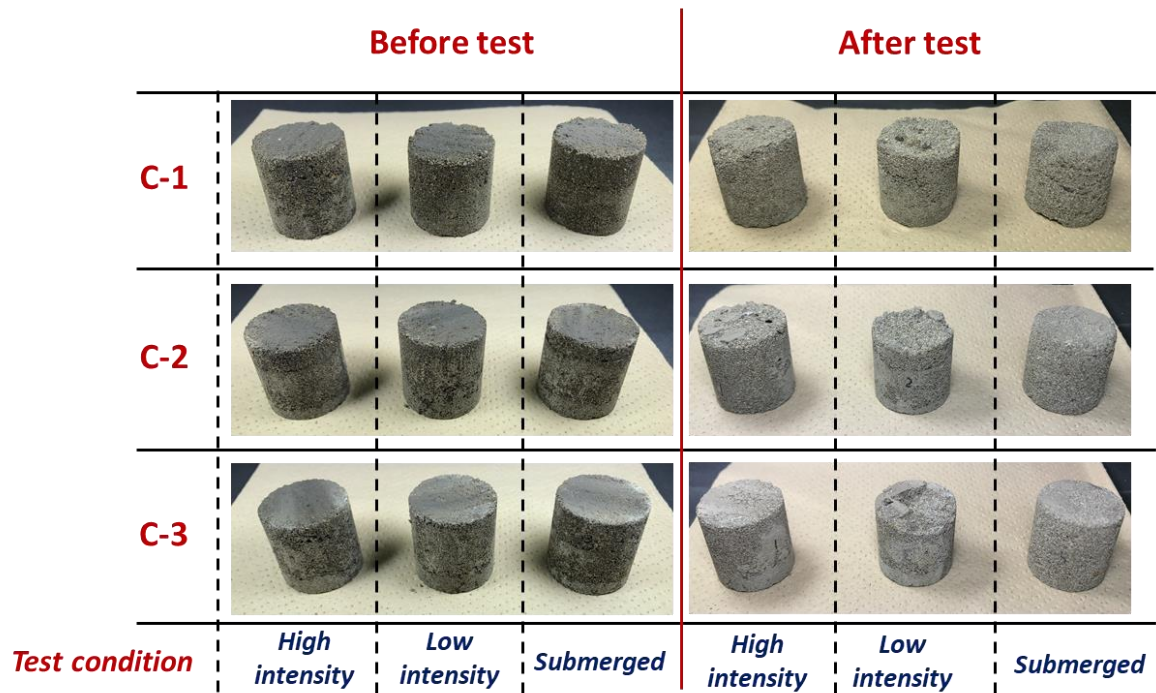


Figure 5.20: The physical appearance of the specimens before and after the exposure to AR
(pH 3.0)

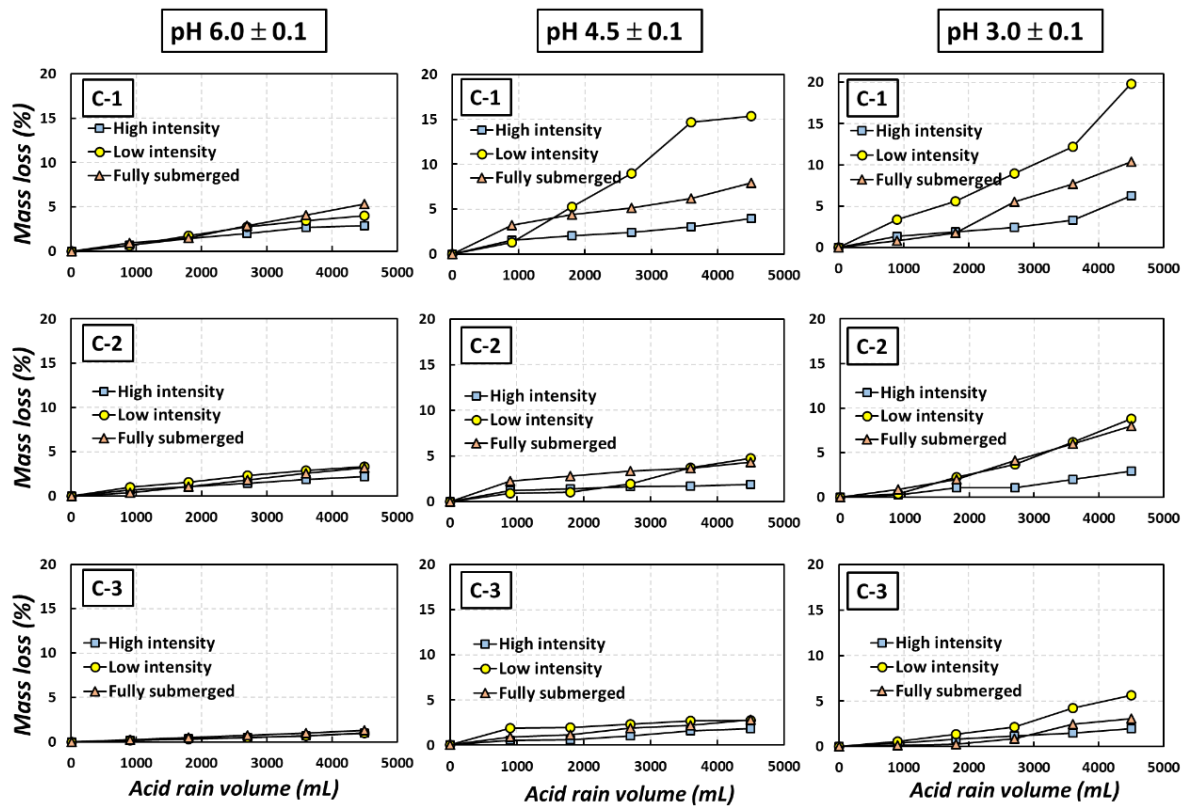


Figure 5.21: Mass loss of the specimens subjected to AR

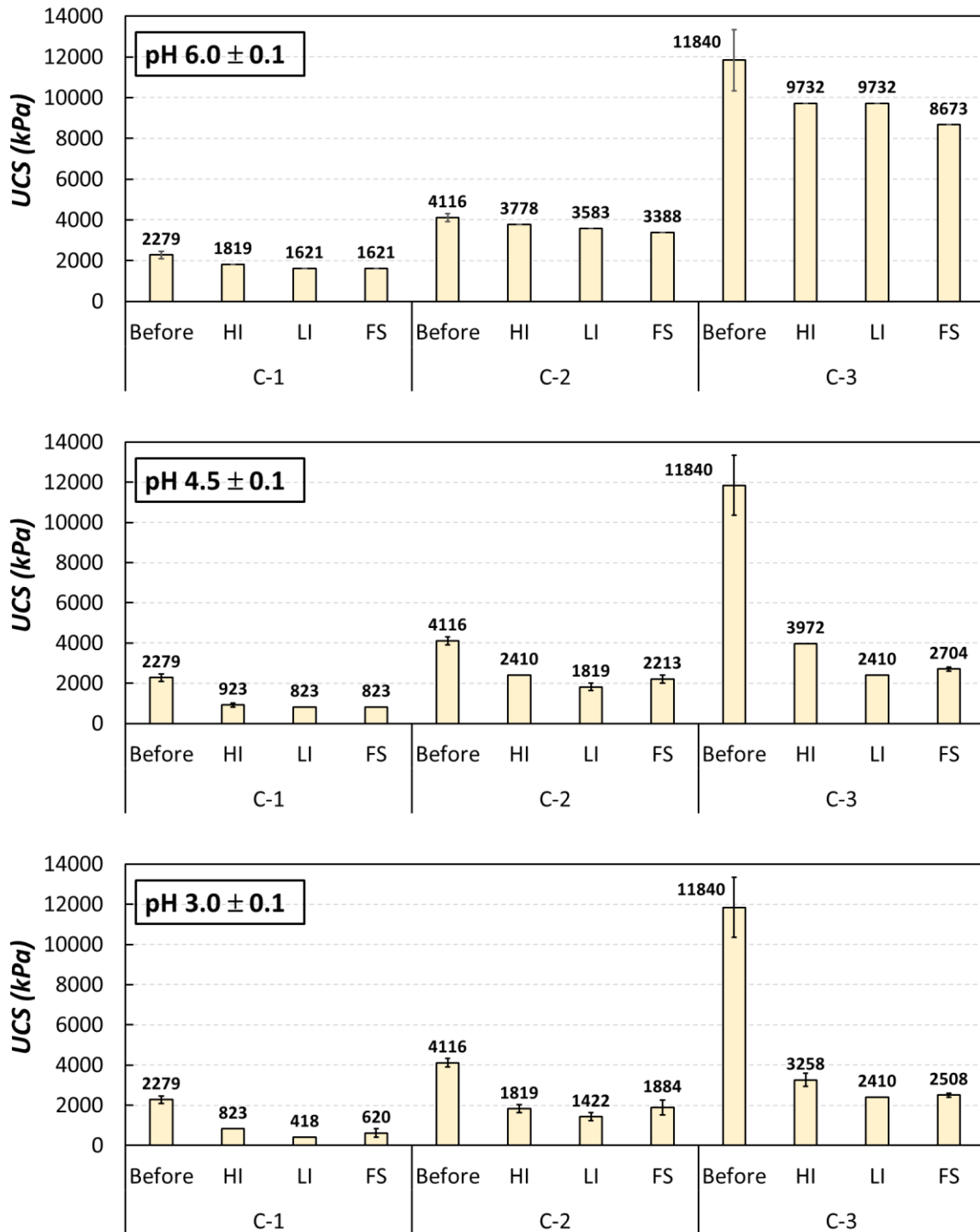


Figure 5.22: UCS of the MICP specimens subjected to AR

The comparison in **Figure 5.22** reveals that the corrosion of calcium carbonate during AR supply degraded the strength (UCS) of the MICP specimens. HI, LI and FS refer to high intensity, low intensity and fully saturated, respectively. Similar to that observed in corrosion

and mass loss (**Figures 5.19 and 5.21**), the decrease in pH increased the drop in UCS. The intensity of supply was also likely to be influencing the mechanical damage. The higher the intensity the lower the UCS loss, which is consistent with the results observed in mass loss (**Figure 5.21**).

5.5.4. Discussion

The corrosion of calcium carbonate bio-cement is the degradation mode of MICP under the exposure of AR environment. Under the high intensity AR supply, the corrosion of calcium carbonate was low. In contrast, when the AR is delivered at low infiltration or fully submerged conditions, the rate of corrosion was high, which is due to the sufficient reaction time given. Regardless of the conditions tested, the ultimate factor that determines the responses of the specimens exposed to AR, is the corroded quantity of calcium carbonate. **Figure 5.23** presents the compilation of mass loss data versus corresponding corrosion of calcium carbonate (i.e., the data from all the test conditions). The figure suggests that the mass loss, with the corrosion of CaCO_3 , can be fairly evaluated by linear relationships.

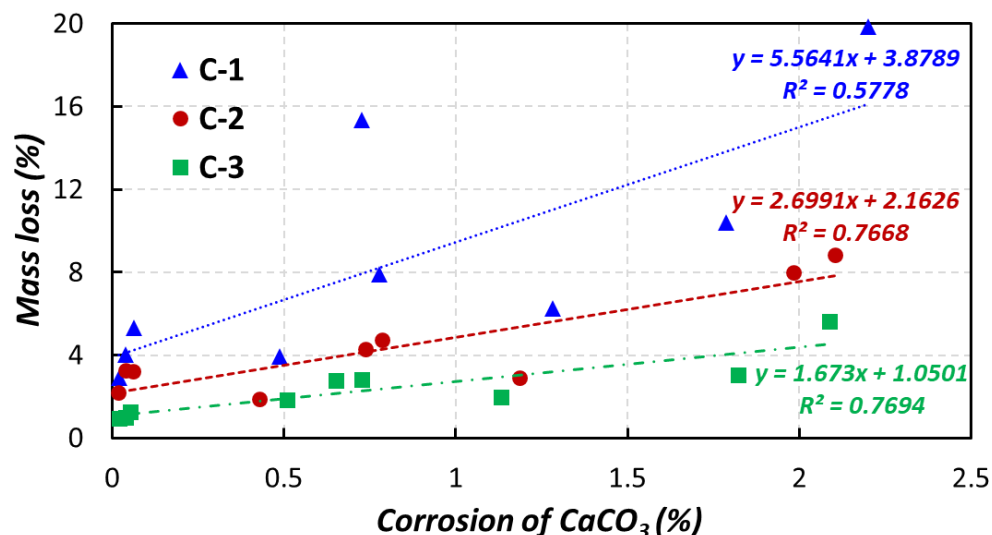


Figure 5.23: The relationships between corroded calcium carbonate and mass loss at different cementation levels

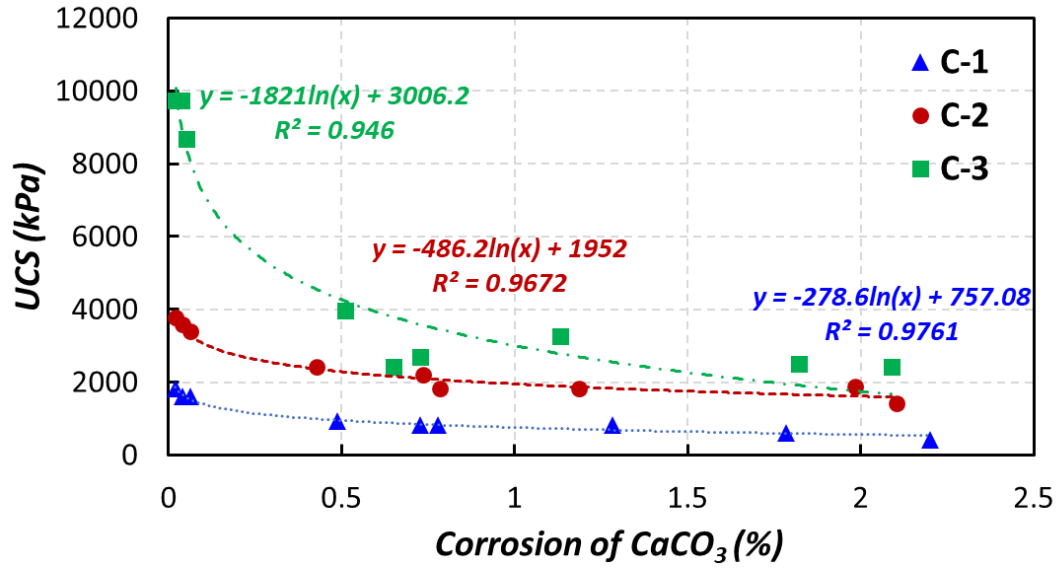


Figure 5.24: Variation of UCS with the corrosion of calcium carbonate content at different cementation levels

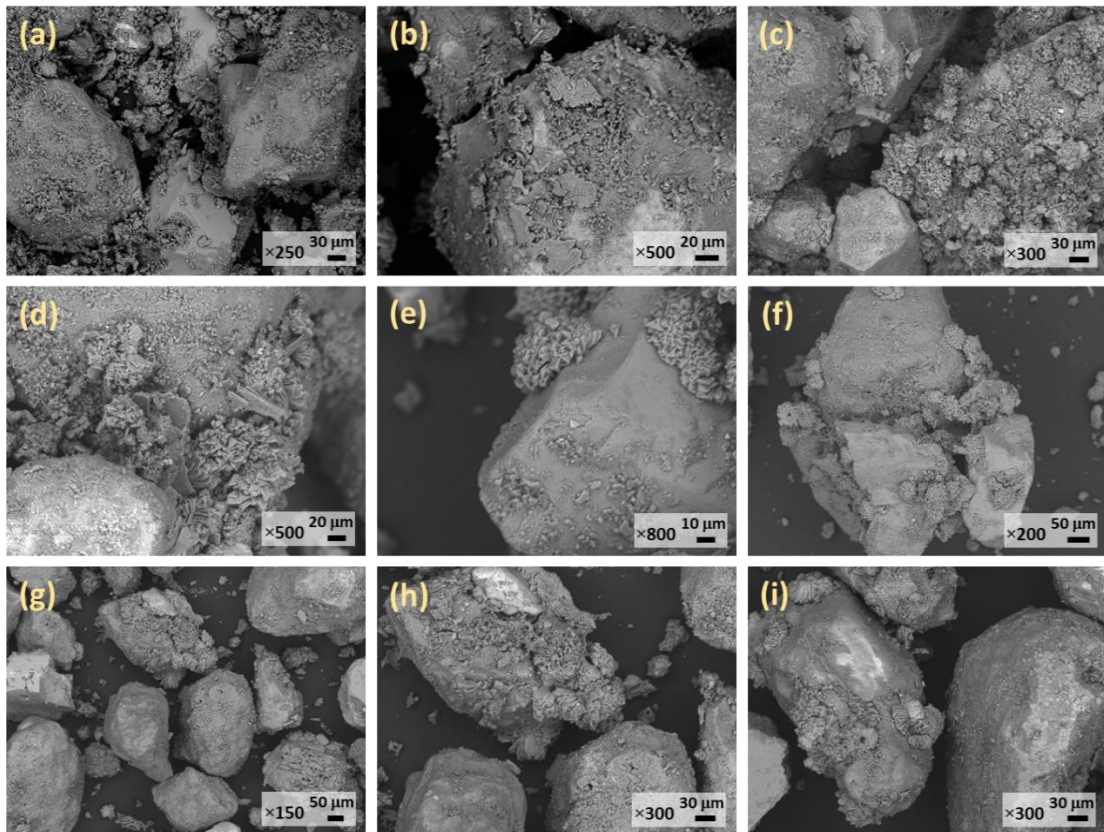


Figure 5.25: SEM images of MICP specimens of AR tests of pH 3.0 (a-b) surface (infiltration condition), (c) core (infiltration condition), (d-c) submerged specimen and (g-i) debonded soil particles

Figure 5.24 presents the compilation of test data, i.e., UCS plotted against the corrosion of calcium carbonate for all the test conditions. The results indicate that the UCS of the specimens exponentially decrease with the increase in corrosion of CaCO_3 for all the levels of cementation. The exponential tendency in decrease can be explained by the decay of matrix support. During the MICP treatment, the cementation begins at particle contact, increasing the strength gradually (progression of contact cementation); with the increasing treatment, the crystals start to grow within the pore spaces, sometimes bridging the particles (Lin et al., 2016), leading to rapid increase in strength at latter stage. The exponential progression of UCS in MICP soils was evidenced in previous studies (Gowthaman et al., 2019a; van Paassen et al., 2010). The results presented herein (**Figure 5.24**) demonstrate that the UCS follows relatively the same path for the decrease during corrosion.

The results (**Figures 5.23 and 5.24**) prove that the treatment durability essentially depends on high cementation levels. At high cementation level (e.g. C-3), particle contact points are cemented stiffly by higher quantity of calcium carbonate compared that in lower levels (e.g. C-1). For the same supply of AR, the corrosion of calcium carbonate might be same, irrespective of the level of cementation; however, the connections points could more quickly be debonded/ weakened in low cementation levels, showing high susceptibility to AR. On the other hand, for debonding/ weakening the particle contacts in high cementation levels, more calcium carbonates are required to be corroded, suggesting high longevity in performance.

Figure 5.25 presents the SEM images of the specimens subjected to AR tests (pH 3.0). From the infiltration case (low intensity AR), representative samples were collected from the surface (**Figures 5.25(a-b)**) and core (**Figure 5.25(c)**). The surface SEM images showed that almost no bridging of CaCO_3 was left by the end of the AR test under pH 3.5. However, at around the specimen core, the calcium carbonate bridges could be observable. In the

submerged case (**Figures 5.25(d-f)**), partially degraded/ corroded crystals were seen in the soil matrix, and considerable cementation at particle contacts were observable. In addition, the debonded soil particles were collected from the bottom container and subjected to SEM analysis. Smaller crystals (i.e., partially dissolved) were found on the surface of the detached soil particles, suggesting that the soil particles were detached when the crystals weaker than the strength required to bear the particle. The strength of MICP treated slope soils is related to the crystallized CaCO_3 that bonds soil particles together. The corrosion of CaCO_3 led the connections between soil particles to partially/ fully degrade, resulting loss of mass and strength. Theoretically, the responses of MICP treated soils against AR conditions were anticipated to be poor. However, this study has demonstrated that the durability and longevity against AR could be considerably enhanced by increasing the level of cementation.

AR and acid-snowfall are international environmental problems and are also serious problems in Hokkaido, Japan. In fact, the pH value of the clean water of well dissolved carbon dioxide in atmosphere is 5.6. Therefore, the pH value of 5.6 is frequently used as the guideline value for acid rain. However, as per the reports, the damages start to appear once the pH value falls below 6.5. **Figure 5.26** presents the average pH conditions of rainfall/ snowfall in Hokkaido from 1990 to 2019. It can be seen that the acidification is significant in almost everywhere in Hokkaido since many decades ago, although there are slight differences in the degree of acidification from place to place and with the time. Within the past ten years, the minimum recorded pH is 4.7 in Hokkaido.

For the performance analysis and predictions of MICP in Hokkaido, the responses reported herein to pH 4.5 can be more appropriate. For a fifteen years of AR/ acid-snowfall of pH 4.5, the erosion (mass loss) was observed up to 15% (C-1); however, it could be resulted less than 5% erosion for the high levels of cementation (C-2 and C-3), although similar content of

CaCO_3 has been corroded. The results clearly demonstrate that the MICP treatment could withstand the AR/ acid-snowfall for several decades depending on the level of cementation, and an occasional MICP retreatment to the slope near-surface might endure the prolonged performance and increase the longevity.

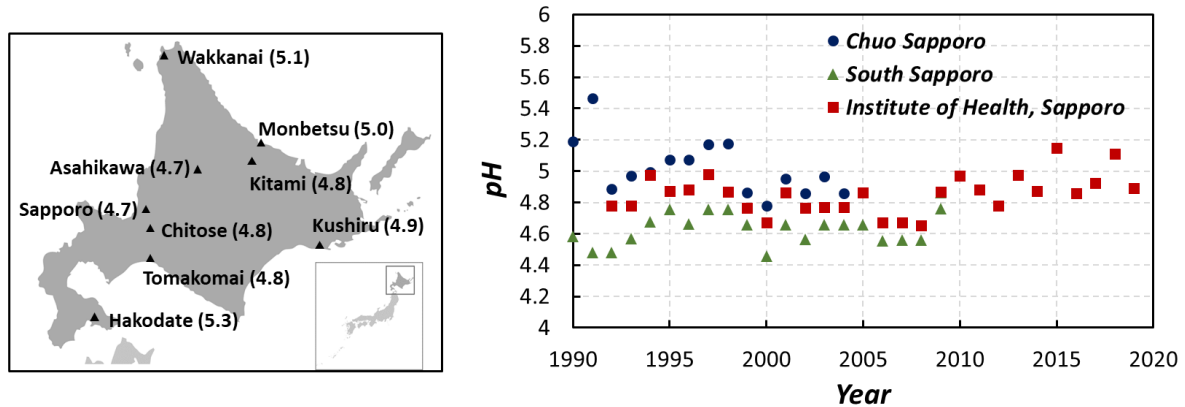


Figure 5.26: pH values of precipitation in Hokkaido

(https://www.city.sapporo.jp/kankyo/taiki_osen/kekka/sanseiu/index.html)

5.6. Conclusions

In this chapter, the effects of climatic factors on the mechanical responses of MICP treated slope soil were extensively studied through potential indicators related to the study location, such as freeze-thaw, wet-dry and acid rain simulation tests. Based on the outcomes, the following conclusions are drawn.

- The results from freeze-thaw (FT) tests revealed that the MICP treated slope soils are durable to the freeze-thaw induced erosion, and however, the level of cementation highly determines the aggregate stability. The specimens cemented to an average of 11-13% (CaCO_3 by mass) results high erodibility (50% mass loss), whereas the 20-23% cemented specimens show only a minor erosion (2% mass loss), when they are subjected to 25 cyclic frosts. The similar tendency is also observed in ultrasonic measurements. The high durability of MICP treated soils is primarily attributed to

adequate cementation level which facilitates effective particle contacts that could resist the evolution of ruptures hence crystal-soil detachments when the uneven tensile stresses develop around the cemented soil particles during frosting process of pore water.

- Cyclic wet-dry (WD) effects appears to have adverse effects on the physical and mechanical characteristics of MICP slope soils. The deterioration mechanism of MICP soils due to WD cycles is related to the degradation of calcium carbonates, and that is found to be occurring at two stages: short-term and long-term. The short-term deterioration rapidly occurs within the first few WD cycles due to the suspension and corrosion of powdery carbonate deposits. The long-term deterioration could be observable after 30 WD cycles. The fatigue stresses developed during WD process could deform the primary bonds, and particularly impairing the surface of the specimens. However, the increase in precipitated calcium carbonate content increased the aggregate stability and mechanical durability. The results also suggested that the MICP specimens are less susceptible to WD actions, compared to that observed under FT effects.
- Hypothetically, it was believed that the MICP treated soils are susceptible to acid rain (AR) conditions. However, this study has demonstrated that the durability and longevity can be considerably enhanced by the levels of cementation. The tests suggest that the corrosion rate of calcium carbonate depends on the pH of AR and intensity. However, the loss of soil particles and strength due to corrosion of CaCO_3 is highly governed by the level of cementation. In another words, for the same quantity of corrosion, the higher the cementation level the lower the loss of mass and UCS, enhancing the longevity of the treatment.

CHAPTER 6

TREATMENT STRATEGIES AND CHARACTERIZATION OF TREATED SLOPE NEAR-SURFACE

6.1. Introduction

Treatment technique is critical to the in-situ applications of MICP, as the effectiveness is highly related to the delivery and utilization efficiency of resources (i.e., method of supply, supply quantity and rate). The heterogeneity in spatial distribution of MICP is one of the most challenging factors that limits the field implementation and commercialization of the MICP. Conventionally, Portland cement-soil mixtures were often prepared using a mechanical mixing method (Ismail et al., 2002), which was believed to give a relatively uniform distribution of the cementing agent along the soil depth. In the MICP treatment, the resources are essentially introduced as solutions, thereby, simple surficial injections are preferably investigated. Accordingly, surface injection method is popular, that uses cylindrical columns and syringes with a peristaltic pump to inject the bacteria and cementation resources (DeJong et al., 2010; Martinez et al., 2013; Whiffin et al., 2007). Surface-spraying is another emerging method (Gu et al., 2018; Zomorodian et al., 2019), that simply introduces the MICP solutions to the surface in a scattered way, allowing the percolation of solutions naturally under the gravitational effects. The grouting pressure is sometimes a requirement to ensure sufficient flowability of solutions into deeper zones, and that can possibly be controllable in injection methods with additional instrumentation (Tang et al., 2020).

For the near-surface stabilization of slopes, the non-destructive surficial treatments appear to be more appropriate compared to the mechanical mixing from a sustainability perspective.

Due to the inclined nature, the effects of flow lines and gravity are diverse in slopes, which would more possibly impact the treatment efficiency (compared to the treatment on flat grounds). The slopes with fine soils have high potential to the development of surface sheet-flow during the treatment, which could lead the supply to step down without being utilized. Therefore, more attentions should be given on method of supply, supply quantity and rate, to implement an effective MICP treatment with enhanced distribution and homogeneity of calcium carbonate within the target depths.

This chapter presents a series of treatment process implemented into slope models at different scales. A series of slope models treated essentially by different strategies including grid-injections, single-phase and two-phase sprayings with several variables, are characterized, and the effects on improving near-surface responses are evaluated using non-destructive needle penetration tests. Finally, the chapter deals with the scaled-up slope treated under temperature-uncontrolled environment, emphasizing the effects of seasonal temperature on field implementation.

6.2. Slope models and Scales

Slope models used in this study were essentially chosen to two different scales: small-scale and bench-scale, shown in **Figures 6.1** and **6.2**, respectively. For the small-scale slopes, triangular face dimensions were 12 cm and 10 cm (horizontal and vertical, respectively), and the width space was designed to be 10 cm (refer **Figure 6.1**). For the bench-scale slope, the dimensions were selected to be three times of the measurements of small-scale models, i.e. 36 cm, 30 cm and 30 cm (refer **Figure 6.2**). In another terms, design volume of the soil to be filled in bench-scale slope is around 25 times higher than that to be filled in small-scale model.

The slope molds were made of transparent stiff acrylic material, and the molds can be assembled and dismantled from all vertical sides (screw-fixed). The bottom of the slope contained a large number of small holes to facilitate the drain out of the solutions during treatment. The gradient of the slope was resolved to the gradient of cut slope (1:1.2) in accordance with the road earthwork guidelines, Japan (*Japan Road Association*, 2009), considering that cut slopes are generally critical in stability compared to that of standard filling/ embankment slope (1: 1.8) (Gowthaman et al., 2019c).

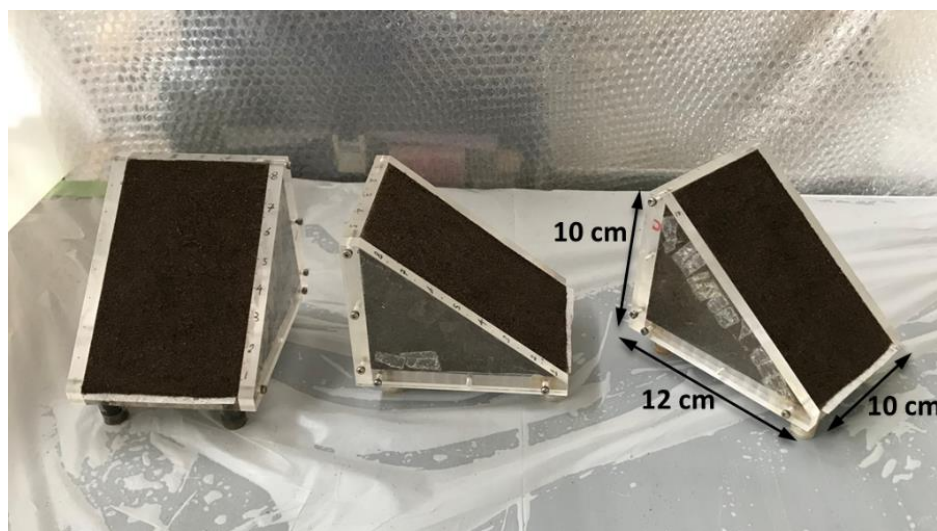


Figure 6.1: Small-scale slope models

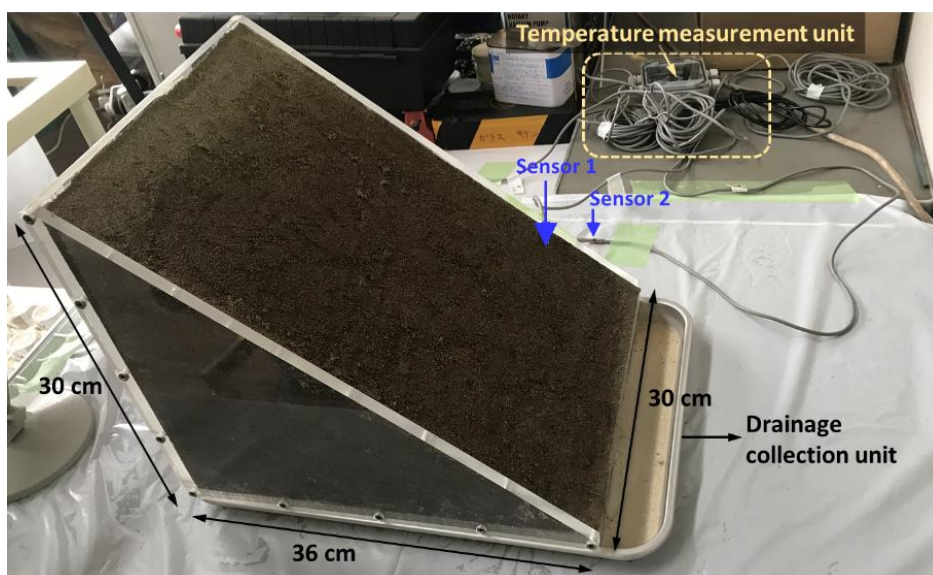


Figure 6.2: A full bench-scale slope model

6.3. Treatment methods

During the preparation of slope models, filter sheet was placed at the bottom of the slope to prevent the fine soil particles from being flushed out during treatment, followed by the filling of soil. The soil was used at its native conditions, i.e. neither oven dried nor chemically treated. For the small-scale model, around 910 – 920 g soil was packed into the mold in five consecutive layers (layer by layer), ensuring that each layer was compacted evenly using blowing rod, thereby to maintain consistency of experiments. The initial density of the fill was maintained within the range between 1.5 – 1.6 g/cm³.

Table 6.1 presents the detail of the model tests performed. In total, eight number of model tests were performed, which included seven number of small-scale models and finally a full bench-scale model. Small scale models were used to assess the feasibility of various treatment strategies and conditions, and based on the outcomes, the optimum treatment was finally implemented to the bench-scale slope under an open environment conditions with real-time monitoring to demonstrate the complete feasibility of the method.

6.3.1. *Grid-injection method*

In grid-injection method (**Figure 6.3**), the slope surface was divided into 8×5 grids (eight slots along the longer side, and each slot was divided horizontally into five grids), each grid was 2 cm $\times$ 2 cm. 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 are presented in **Figure 6.3**. Similar to the column test (explained in **Section 3.3.6**), 2 number of biological injections and 14 number of cementation injections were performed during 14 days of treatment process. Both the bacteria culture and cementation solution were injected at same rate of around 10 mL/min. During

every injection, treatment was started from the bottom slope (bottom grids) and completed at top slope (top grids).

Table 6.1: Test cases and the detail of the model tests performed

Case	Slope scale	Initial average density (g/cm ³)	Method of treatment	Elaboration		
				Cementation resource concentration	Supply rate of cementation resource (every 24 hours)	Purpose
1	Small	1.522	Grid-injection (2-Phase)	1 mol/L	1.15 mL/cm ²	To study (i) feasibility of injection method (ii) effect of segregation
2	Small	1.577	Grid-injection (2-Phase)	1 mol/L	1.15 mL/cm ²	
3	Small	1.610	Spraying (1-Phase)	1 mol/L	0.32 mL/cm ²	To study the feasibility of two types of the spraying methods
4	Small	1.584	Spraying (2-Phase)	1 mol/L	0.32 mL/cm ²	
5	Small	1.521	Spraying (2-Phase)	1 mol/L	0.63 mL/cm ²	To study the effect of the quantity of supply
6	Small	1.518	Spraying (2-Phase)	1 mol/L	1.25 mL/cm ²	
7	Small	1.514	Spraying (2-Phase)	0.5 mol/L	0.63 mL/cm ²	To study the effect of the concentration of resources
8	Bench-scale	1.512	Spraying (2-Phase)	1 mol/L	0.63 mL/cm ²	Feasibility study under temperature uncontrolled (open) environment

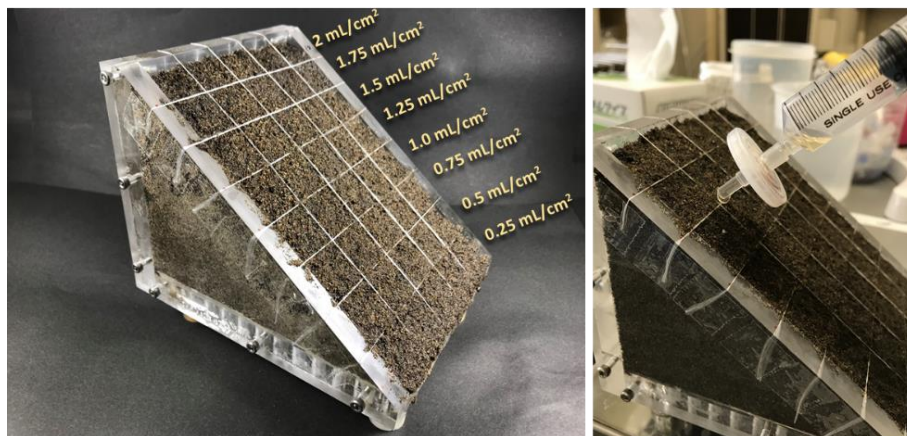


Figure 6.3: Grid-injection experimental setup

Two slope models were treated using grid-injection technique (Cases 1 and 2, **Table 6.1**). In Case 1, the typical bacteria culture was inoculated to the soil. In case 2, the slope was treated using segregated cell pellets in the place of bacteria culture. The segregation process was the same as explained by Fujita et al. (2017). The bacteria culture medium was centrifuged at 10°C, 8000 rpm for 5 minutes using centrifuge tubes. The supernatant solution was then removed from the tubes, and cell pellets were washed few times by distilled water. Subsequently, the cell pellets were resuspended in distilled water to the same previous optical density, followed by the inoculation.

6.3.2. *Spraying method*

In spraying technique, bacteria culture and cementation solutions were uniformly sprayed onto the exposed top surfaces of the slope specimens using sprayer device. Feasibility of two types of spraying methods were potentially investigated: (i) single-phase (1-phase) spraying and (ii) two-phase (2-phase spraying). For 1-phase spraying, both the bacteria culture and cementation solutions in equal quantity were mixed together and sprayed onto the slopes. In total, 14 number of sprays were introduced on every 24-hour basis. On the other hand, in 2-phase spraying technique, the solutions (bacteria and cementation solutions) were separately sprayed as two subsequent phases with a certain time interval between the phases. In this case, the bacteria culture was sprayed twice among 14 number of sprays.

Spraying rate was chosen on the basis of practical and possible field application and was around 40-50 mL/ min. Closely a similar spraying rate was also used in surface treatments (Gu et al., 2018). The spraying quantity and concentration of resources were based on the test purpose (refer **Table 6.1**).

After the 14 days of treatment, all the treated slopes were rinsed with tap water to remove all soluble salts, followed by, slope specimens were carefully removed from the mold and submerged into the water bath to eliminate the uncemented aggregates.

6.4. Results and Discussion: Small-scale slopes

6.4.1. Grid-injection technique and segregation

The near-surface UCS was used as the indicator to evaluate the MICP improvement, besides the UCS was estimated by the needle penetration tests (refer **Section 3.3.8** for the method). **Figure 6.4** presents the variation of near-surface UCS of slopes treated by grid-injection. The solidified profile and the UCS with respect to the depth are shown in **Figure 6.5**. 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 by grid-injection. However, the cementation was not achieved within the zone below the top slope (**Figure 6.5**), remained as untreated soil. The results also show that the MICP treated slope near-surface had considerable UCS between 2 and 8 MPa, and relatively lower UCS values were obtained below the surface of the slopes.

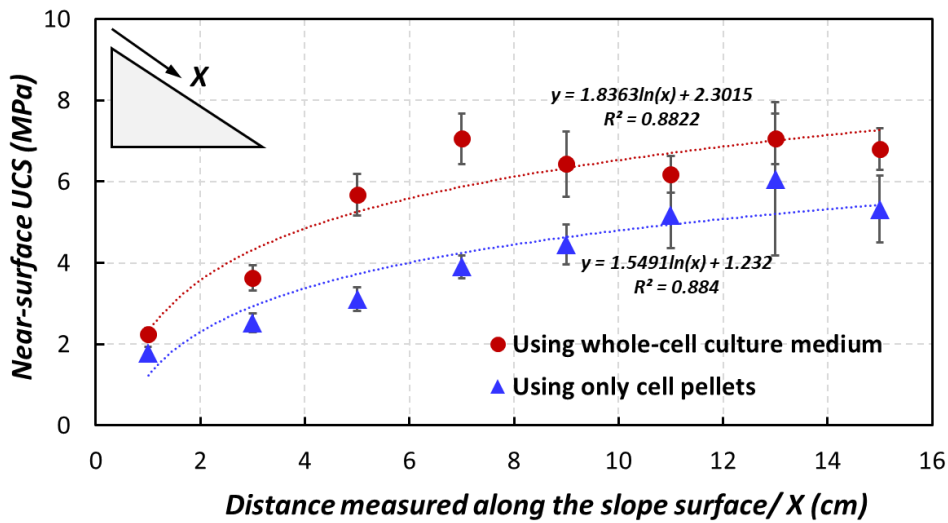


Figure 6.4: Near-surface UCS of the slopes treated using grid-injection

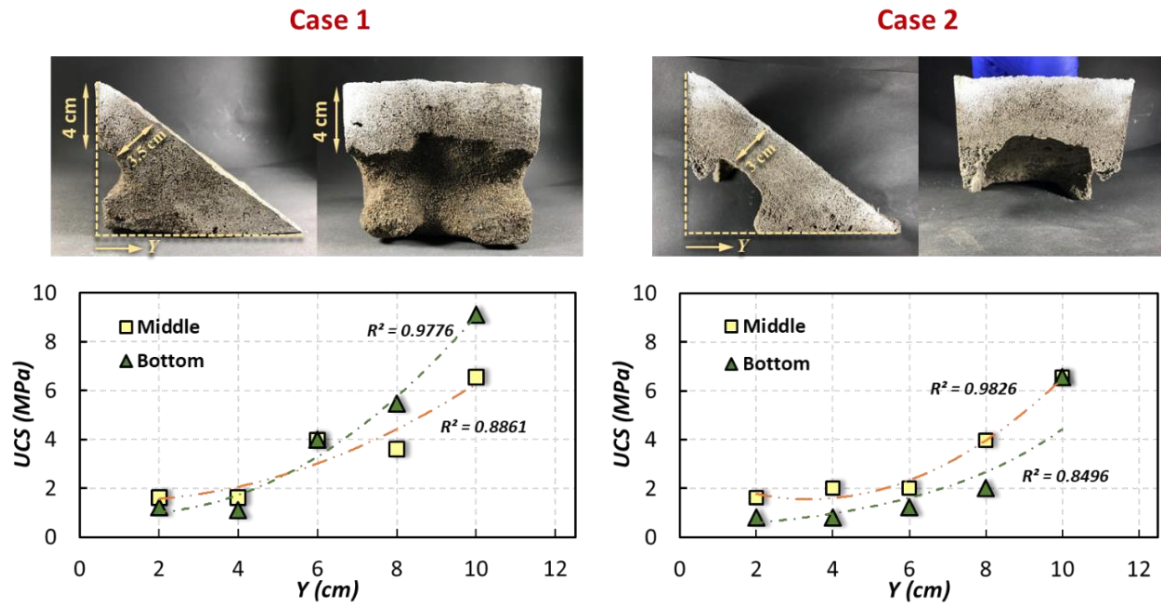


Figure 6.5: Cemented slope profiled and variation of UCS with depth

Before the measurements, it was anticipated that relatively a homogeneous cementation could be attained along the near-surfaces by the grid-injection method. However, **Figure 6.4** suggests that the surface UCS increases along the distance (X) measured from the top slope, and the highest surface UCS was obtained near bottom slope. The average UCS at the bottom slope was around 7 MPa, whereas that of top was only 2 MPa. The UCS values reported herein are relatively closer to the values reported by van Paassen et al. (2010), ranged between 0.7 and 12.4 MPa in their large-scale experiment, and the corresponding CaCO_3 contents were reported to be between 12.6 and 27.3%. In fact, this variation along the surface could be attributed to the localization of bacteria cells. The consecutive injections of cementation solution (every 24 h) 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 slope treated using segregated cell pellets (Case 2) shows a similar tendency in near-surface strength and cemented profile (**Figures 6.4 and 6.5**). However, the achieved UCS

values ranged between 0.8 and 6.5 MPa, which is considerably lower than the slope treated using regular whole-cell culture medium (Case 1) where the UCS ranged between 2 and 8 MPa. The comparison of the near-surface UCS values of the slopes treated using regular culture and segregated cell pellets shows that relatively higher CaCO_3 could precipitate in the slope treated using regular culture, resulted higher UCS. Also, the large uncemented zone evidenced that a low carbonate content was precipitated while using segregated cell pellets. The major reason is that the removal of the supernatant leads to the removal of the extracellular polymeric substances (EPS) matrix that typically protects the bacteria from various environmental stresses and provides mechanical integrity (Davey and Toole, 2000; Jayathilake et al., 2017), affecting the performance and survival of the bacteria. Another possible reason is that the urease enzyme of the bacteria would be somewhat secreted into the extracellular medium, that contributed to the increase of UCS while using regular whole-cell culture medium. However, a very low urease activity was observed in culture supernatant.

6.4.2. *Effect of spraying strategy and phases*

The feasibility of spraying techniques on near-surface stabilization was studied through 1-phase and 2-phase spraying (Case 3 and Case 4, respectively, refer **Table 6.1**). The near-surface UCS of the slopes are presented in **Figure 6.6**. As mentioned earlier, the bacteria culture and cementation solutions (CaCl_2 and urea) were mixed and sprayed in 1-phase method. In 2-phase method, they were sprayed separately, and the bacteria culture was introduced only on day 1 and day 7 during 14 days of treatment. In both cases, the quantity of sprayed cementation solutions was maintained to be same (0.32 mL/ cm^2). **Figure 6.6** reveals that the 1 phase spraying resulted higher near-surface UCS compared to the 2-phase spraying. The average near-surface UCS achieved by 1-phase and 2-phase spraying techniques were around 3 and 1.5 MPa.

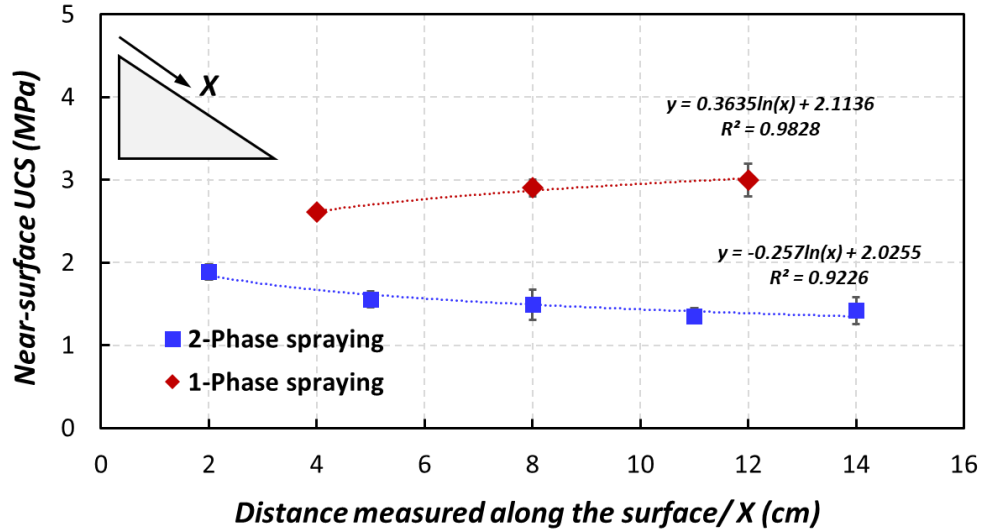


Figure 6.6: Near-surface UCS of slopes treated by 1-phase and 2-phase spraying methods

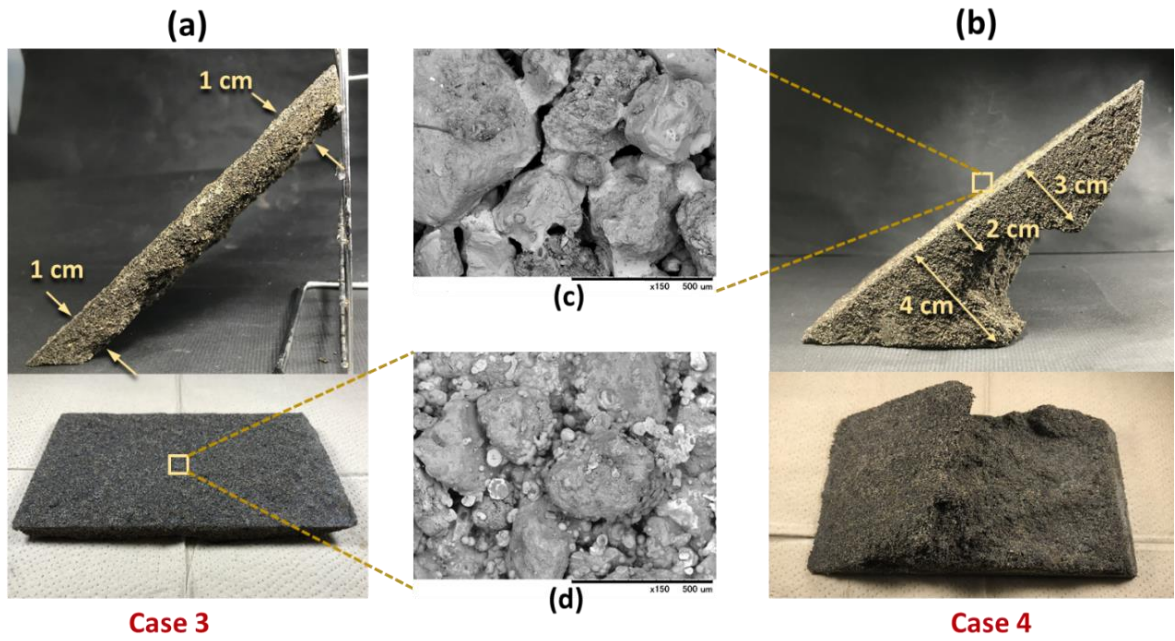


Figure 6.7: The cemented profiles and micro-scale structure of slopes treated by 1-phase and 2-phase spraying methods

Figure 6.7 presents the cemented profiles and microstructure of the slopes treated by spraying. The cemented profiles show that 1-phase spraying led to the formation of thin cemented layer (with the average thickness of 1 cm), whereas the 2-phase spraying resulted relatively a thick layer with the thinness ranging between 2 – 3 cm. In fact, the supply of the

bacteria cells was high in 1-phase spraying (as bacteria cells were mixed and sprayed every 24 hours), expected more deeper cementation. However, the 2-phase spraying evidenced more deeper cementations, in which the bacteria supply was only twice during treatment. The difference in cementation profile and UCS could be explained by the early crystallization process in mixed 1-phase spraying.

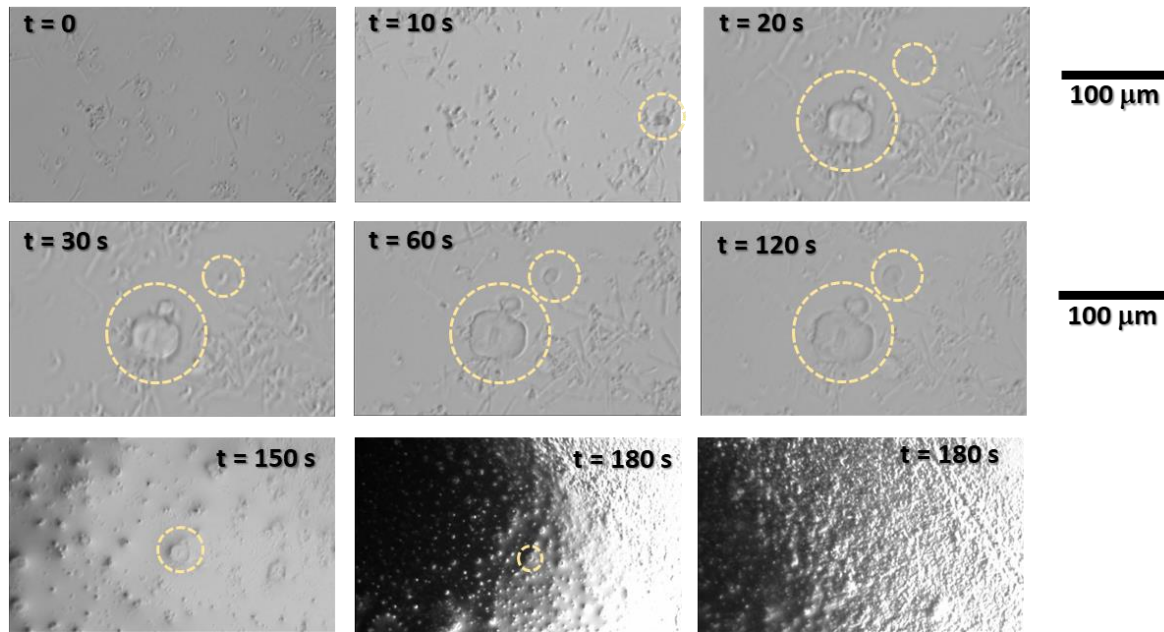


Figure 6.8: Digital microscopy image of evolution of calcium carbonate crystals in the solution right after mixing the bacteria and cementation solutions (1-phase)

In order to understand the crystallization and its evolution in liquid media, the solutions were mixed (bacteria and cementation solution, same as of 1-phase), and a real-time digital microscopy was performed. Results presented in **Figure 6.8** demonstrates that the crystallization of CaCO_3 occurs right immediately after the mixing of bacteria culture and cementation solution consisting urea and CaCl_2 . Within a short time, calcium carbonate crystals achieved considerable size; for instance, within 60 seconds, the crystals have grown to be an average size of 20 – 40 μm. After around 150 seconds, the solution was found to be consisting of numerous micro crystals of calcium carbonate in the solution to be sprayed. The

process time between the mixing and spraying had been essentially around 3 – 5 minutes, suggesting that the sprayed solution (in 1-phase) was not completely in a liquid form, but likely to be in a slurry state. In this partial slurry state, layer of crystals could possibly exist around the bacteria cells, which lead to the reduction in mobility and diffusion rate of the bacteria. It should also be noted that the aperture clogging was often encountered in spraying device during 1-phase spraying treatment.

In order to confirm the effects of bacteria transportation, the soil samples were collected from three depth locations of slope (indicated clearly in **Figure 6.9**) after performing the first bacteria spraying, and the number of existing bacteria cells were measured by the method used for isolation of bacteria (refer **Section 3.3.1**). The results (**Figure 6.9**) suggest that the distributions of bacteria in 1-phase and 2-phase spraying had a significant difference. For instance, the samples collected from the location A (1.5-2 cm depth) showed the number of bacterial colonies of around 4×10^5 and 1.4×10^6 for the 1-phase and 2-phase, respectively. This confirms that the bacteria mobility in 2-phase spraying is 2.5 times higher compared to that of 1-phase spraying. However, only a very small number of bacteria colonies were found at locations B and C for both cases.

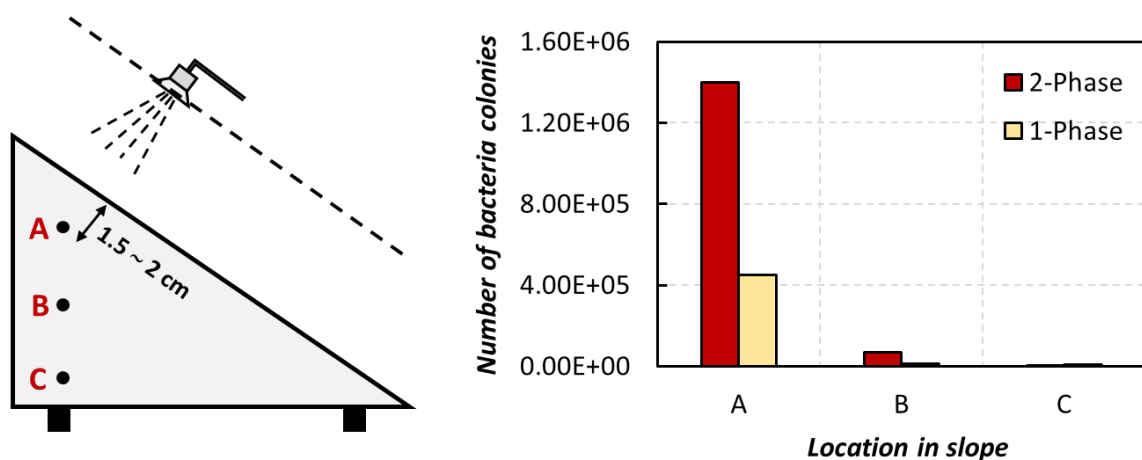


Figure 6.9: Distributions of bacteria cells in slopes treated by 1-phase and 2-phase spray

Typical size of the bacteria ranges between 0.5 – 12 μm , transporting across the soil through the pore throat between soil particles, either by self-propelled movement or by passive diffusion (Ng et al., 2012). But, when the bacteria cells are encapsulated with/ within the formed crystals, geometric compatibility becomes unfit, i.e. the soil pore throat size would limit their free passage into the soil at the present encapsulated state. This leads the bacteria cells to be entrapped at pore throats and immobilized within the shallow near-surface zones. The SEM image of the slope near-surface treated by 2-phase spraying reveals the clear cementation at particle contacts, along with the retained pore structure (**Figure 6.7(c)**). On the other hand, the SEM image of the near-surface treated by 1-phase spraying shows a condensed microstructure of near-surface soils consisting of many attached spherical crystals at various sizes (ranging between 1 – 20 μm), appeared to be nearly a clogged near-surface (**Figure 6.7(d)**). It should be noted that in previous studies, a similar morphology was reported to pure bio-slurry, i.e. spherical crystals with the size ranging between 0.2 – 5 μm (Cheng and Shahin, 2016). As indicated earlier, the already formed microcrystals are more likely to be entrapped at pore throats, therefore enabling more crystal formation at that particular position, contributing to more strength development. As the result, the near-surface could achieve relatively a high strength up to 3 MPa in 1-phase treatment compared to that achieved in 2-phase technique (**Figure 6.6**).

Another disadvantage in 1-phase method is that the urea hydrolysis of the bacteria would significantly be inhibited when their cells got captured within the slurry or crystals (Cuthbert et al., 2012). As the progression of microcrystals i.e. slurry was studied only within the short duration, the above effect was not observable in this research work. However, as evidenced by bacteria distributions (**Figure 6.9**) and cementation profiles (**Figures 6.7(a)** and **(b)**), the 1-phase approach has been found to be significantly restricting the distance/ depth of the

treatment. Therefore, the 1-phase technique can be more effective for sealant applications or very shallow surficial treatments.

6.4.3. *Effect of supply quantity on near-surface treatment*

For an effective and economic MICP application, optimum quantity of resources should be supplied in a proper way. In order to study the effect of supply quantity on MICP treatment, three cases were incorporated with different supply quantities (Cases 4, 5 and 6, refer **Table 6.1**). Considering a target near-surface treatment depth (D_T), the supply quantity of bacteria and cementation solutions were determined based on the pore volume of target near-surface zone (V_P), and the calculations are shown below.

Consider that L and W are slope length and considered slope width, respectively.

The surface area of the considered slope (A) $= L \times W$.

Target near-surface treatment depth $= D_T$

Volume of the target-near surface zone (V_T) $= D_T \times A$

For the slope soil compacted to a relative density of R_D ,

The pore volume of the target near-surface zone (V_P) $= V_T \times R_D$

For this study, the supply volumes were chosen to be 0.25, 0.5 and 1 times of pore volume of the target near-surface zone (V_P), i.e. $0.25 \times V_P$, $0.5 \times V_P$ and $1 \times V_P$, and the detail of the studied cases are summarized in **Table 6.2**. It is worth noting that in this study, the treatment was designed to target the topmost 30 mm of near-surface (based on results of **Section 3.4.5**).

Table 6.2: Detail of cases with different supply quantities

Case No.	Bacteria culture solution		Cementation solution	
	Supply volume	Total number of sprays	Supply volume	Total number of sprays
4	$0.25 \times V_P = 0.32 \text{ mL/cm}^2$	2	$0.25 \times V_P = 0.32 \text{ mL/cm}^2$	14
5	$0.5 \times V_P = 0.63 \text{ mL/cm}^2$	2	$0.5 \times V_P = 0.63 \text{ mL/cm}^2$	14
6	$1 \times V_P = 1.25 \text{ mL/cm}^2$	2	$1 \times V_P = 1.25 \text{ mL/cm}^2$	14

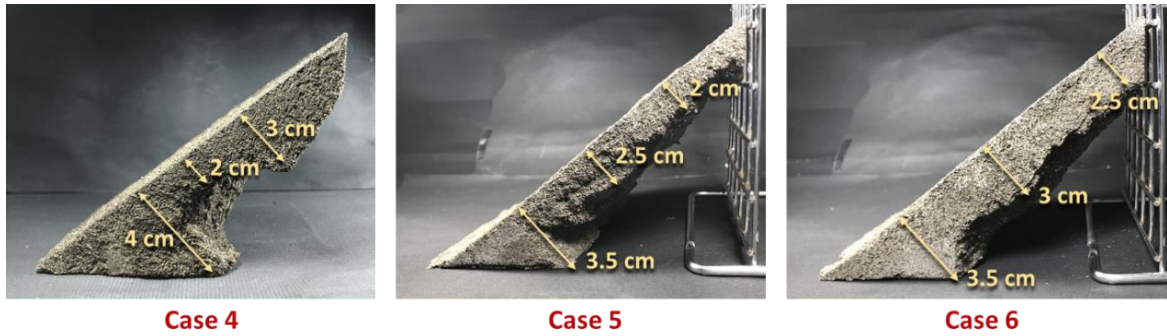


Figure 6.10: The appearance of the cemented slope specimens treated using different supply volumes of bacteria and resource solutions

Figure 6.10 presents the physical appearance of the cemented slopes (Cases 4, 5, and 6) treated using $0.25 \times V_P$, $0.5 \times V_P$ and $1 \times V_P$, respectively. It can be seen that all the supply volumes could nearly achieve the target treatment depth of near-surface. The observation also reveals that the supply volume is likely to be influencing the depth of treatment. The case 6 was treated using the supply volume equal to pore volume, exhibiting an average treatment depth of 25-30 mm. At the same time, the cases treated using lower supply volumes (half and one-fourth) showed an average treatment depth of 20-25 mm.

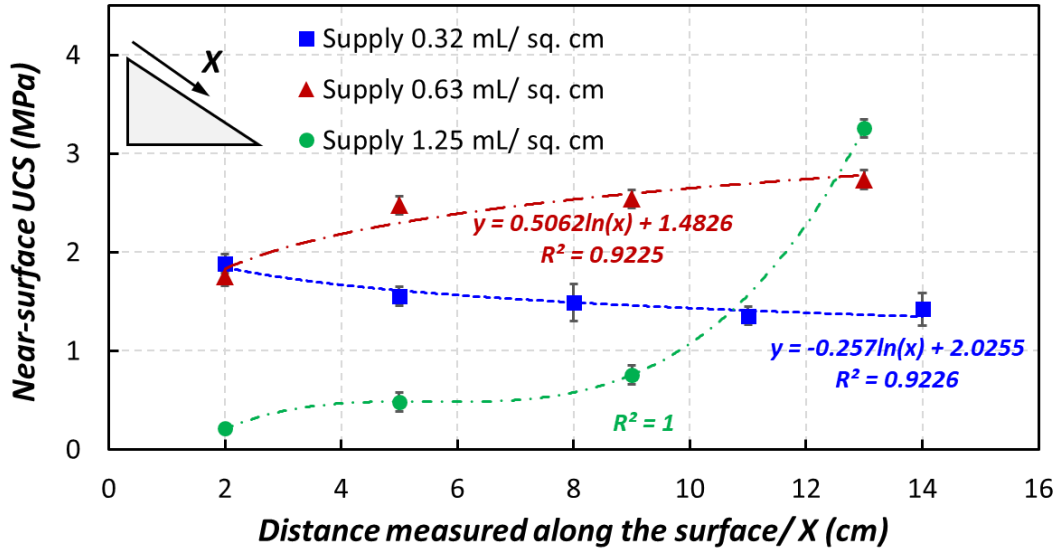


Figure 6.11: The near-surface UCS of the slopes treated using different supply volumes of bacteria culture and cementation solutions

Figure 6.11 shows the near-surface UCS of the slopes treated using the supply of $0.25 \times V_P$, $0.5 \times V_P$ and $1 \times V_P$. Before the treatment, it was expected that increase in supply volume can increase the near-surface strength. However, the observation has disproven the hypothesis, suggesting that the slope treated using a high supply volume (equals to V_P , Case 6) showed relatively a non-uniform near-surface, the UCS ranging between very low to high (200 kPa – above 3 MPa). On the other hand, the slopes treated with lower supply quantities resulted nearly a uniform cementation within the target near-surface zone. The average UCS of the slopes (Case 5 and Case 6) were around 1.6 MPa and 2.4 MPa, respectively.

The increase in treatment depth in Case 6 (**Figure 6.10**) could be attributed to the supply of high volume of bacteria culture, which led the bacteria cells to transport deeper near-surface zones. However, spraying high quantity of cementation solutions in subsequent phases could possibly detach the bacteria cells from surface soils and transported to downslope. As the result, the down-slope could be able to achieve remarkable UCS for Case 6, but the top slope exhibited low UCS values. This suggests that the supply quantity has a very important role in

determining MICP responses, and that should be chosen appropriately for an effective treatment.

During every spraying process, the solution that has reached the downslope as sheet-flow, was collected and quantified. **Figure 6.12** shows the volume of sheet-flow and infiltrated volume of all three cases. It can be observed that in all the cases, with the increase in number of sprays, the generation of sheet-flow increased, and the infiltration decreased. This could be explained by the occurrence of crystallization. The progression of calcium carbonate crystals decreased the permeability of the near-surface, which led to the reduction in infiltration and increased the sheet-flow generation. Measurement of Ca^{2+} in collected sheet-flow confirmed that the flow has been developed before the resources were utilized for MICP reactions. As said before, the cementation solutions were sprayed every 24 hours. During every spray, at the beginning the sprayed solutions easily infiltrated into the soil; when the near-surfaces reaches nearly the saturation state, further spraying would partially turn into sheet-flow, leaving the system without being utilized. The infiltration, with the time, further decelerated by the crystallization process (refer **Figure 6.12**). Nevertheless, the quantity that infiltrated into the surface is considered to be contributing to the MICP, determining the efficiency of supply.

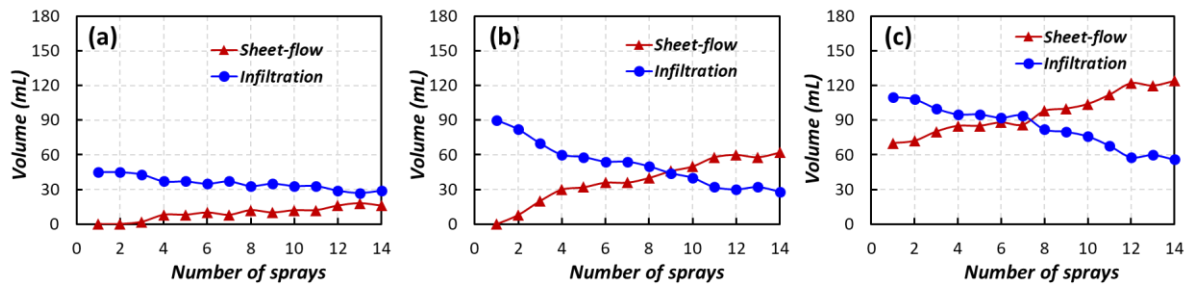


Figure 6.12: The volumes converted to sheet-flow and infiltration in slope (a) Case 4, (b) Case 5 and (c) Case 6

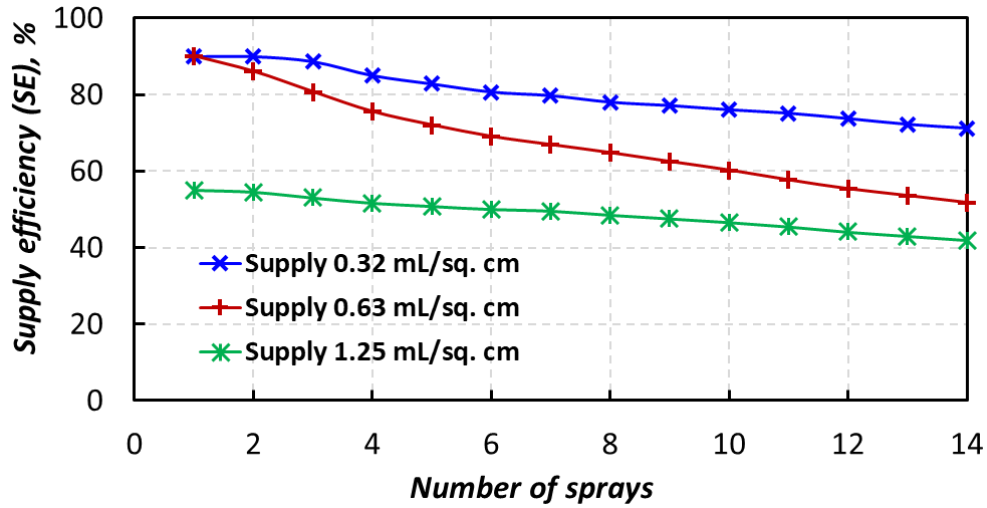


Figure 6.13: The supply efficiency in cases treated using different quantities of cementation solution

In Case 4, low quantity of cementation solution was sprayed every 24 hours, which resulted less generation of sheet flow (**Figure 6.12(a)**). On the other hand, in Case 3, high quantity was sprayed every 24 hours, resulted the development of high sheet-flow during the treatment (**Figure 6.12(c)**). For instant, on 14th spraying, the sheet-flow was around 120 mL among total effective supply of 180 mL. In fact, the generation of sheet-flow represents the leftover of non-utilized resources, which directly affects the economy and efficiency of the treatment. In order to relate the supply quantity and efficiency of the treatment, the “**supply efficiency (SE)**” has been used as an index parameter (**Eq. 6.1**). Simply, the *SE* can be defined as the effective supply that contributes to the MICP among total supply. Herein, the infiltrated quantity was considered as contributor. Accordingly, at any (n^{th}) number of supply,

$$\text{Supply efficiency (SE)} = \frac{\text{Cumulative of infiltration quantity}}{\text{Cumulative of the supply quantity}} \times 100 \% \quad (\text{Eq. 6.1})$$

$$\text{Infiltration quantity} = \text{Supply quantity} - \text{sheetflow quantity} - \text{loss} \quad (\text{Eq. 6.2})$$

The spray loss was found to be around 10 % of the supply volume. **Figure 6.13** presents the comparison of supply efficiencies of the considered cases, suggesting that high supply lessens the supply efficiency. The supply efficiencies by the end of the treatment were around 71 %, 52 % and 42 % for Case 4, Case 5 and Case 6, respectively.

6.4.4. *Effect of concentration of resources on near-surface strengthening*

Figure 6.14 presents the cemented near-surface profiles treated using 1 and 0.5 mol/L concentrated cementation solution (Case 5 and Case 7, **Table 6.1**). The observation reveals that the treatments using both 1 and 0.5 mol/L were able to improve surface; however, cementation layer thickness is smaller for the slopes treated using 0.5 mol/L solutions. The average thickness of cemented near surface (0.5 mol/L) was around 1 cm; less than 1 cm also witnessed in some spots. The average thickness for 1 mol/L was around 2.5 cm.

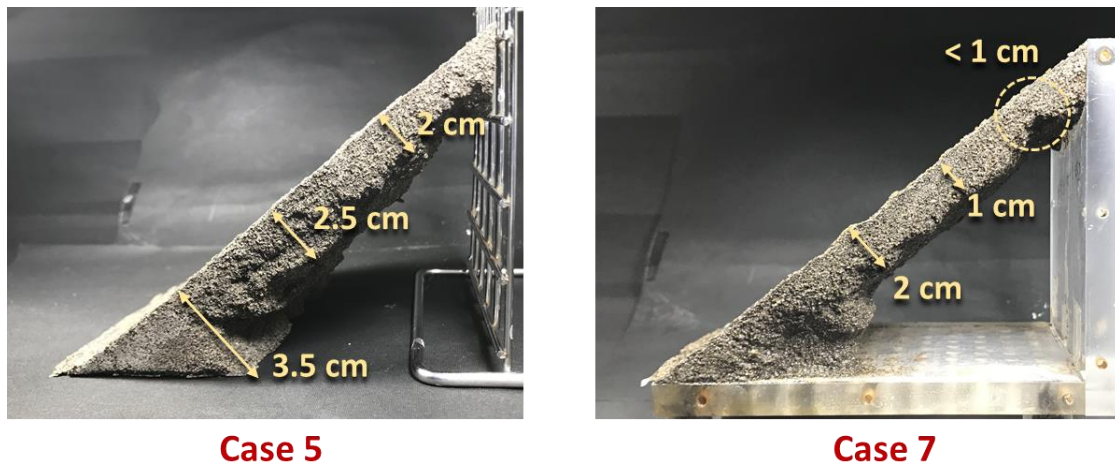


Figure 6.14: Cemented profiles of the slopes treated using 1 and 0.5 mol/L cementation solutions

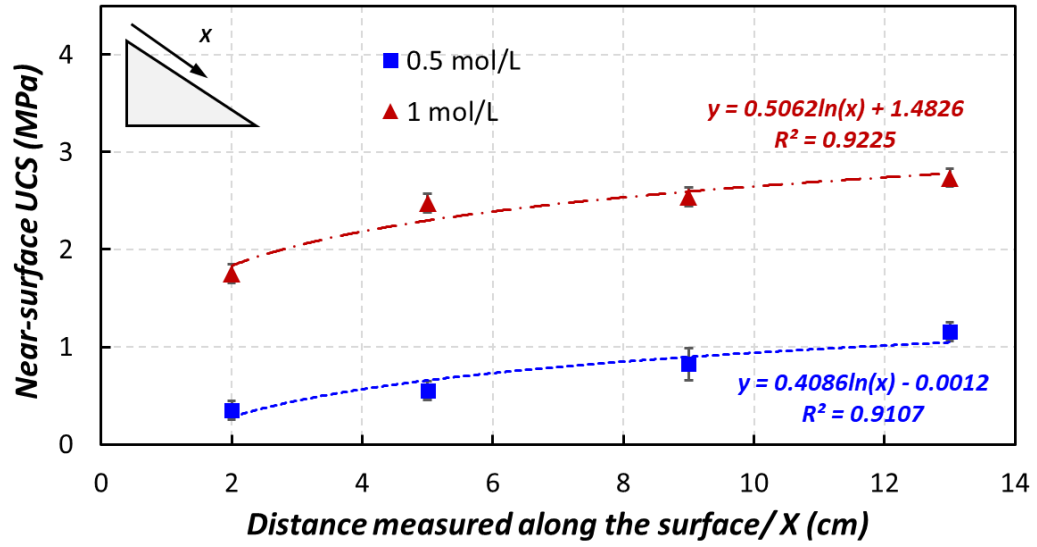


Figure 6.15: Near-surface UCS of the slopes treated using 1 and 0.5 mol/L cementation solutions

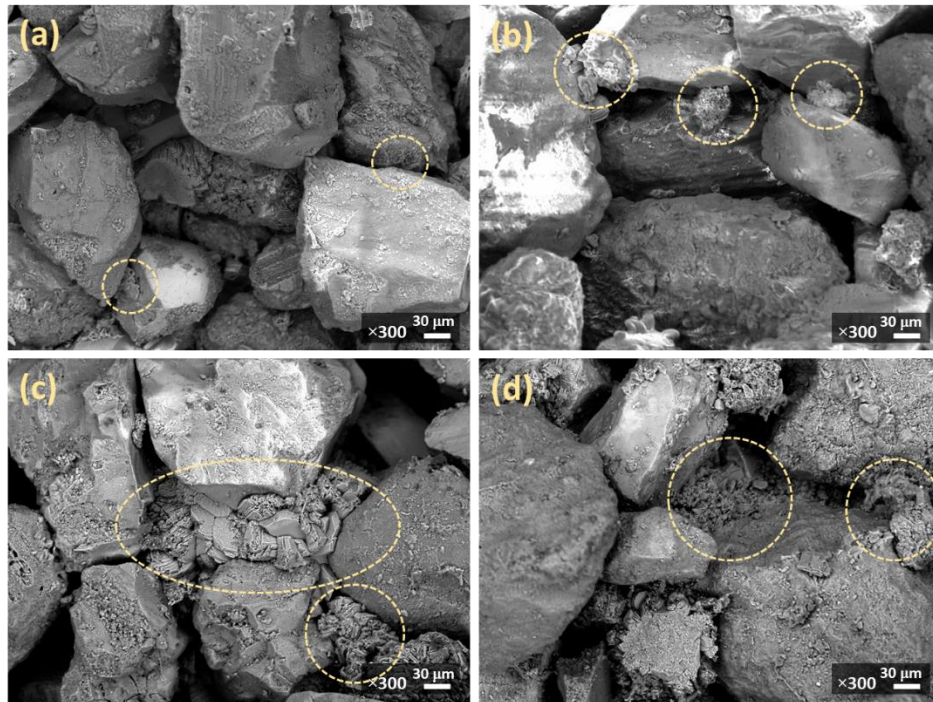


Figure 6.16: The SEM images of slopes soils treated by (a-b) 0.5 mol/L and (c-d) 1 mol/L cementation solutions

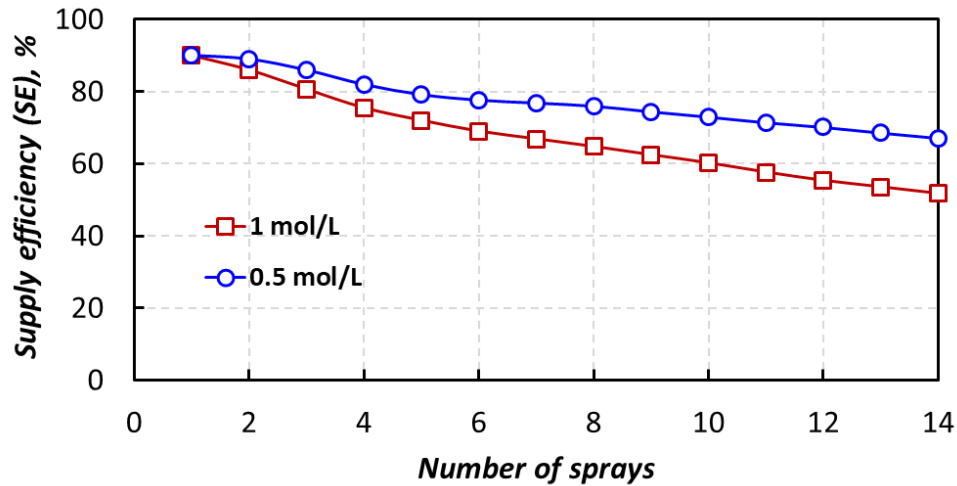


Figure 6.17: Supply efficiency of the slopes treated using 1 and 0.5 mol/L cementation solutions

Figure 6.15 presents the near-surface strength of the slopes treated by 1 and 0.5 mol/L cementation solutions. It is perceived that the concentration of the solutions significantly governs the MICP improvement of near-surface. The near-surface treated by 1 mol/L shows the UCS values between 1.8 and 2.8 MPa. On the other hand, 0.5 mol/L resulted relatively low strength, UCS between 200 kPa and 1 MPa. As demonstrated in previous chapter (**Chapter 4**), the precipitation content of calcium carbonate governs the mechanical response of the MICP soil. When the slope is treated using 0.5 mol/L, relatively lower precipitation of CaCO_3 occurs, leading to the weaker particle connections (**Figures 6.16(a-b)**); on the other hand, stronger intergranular bridging could be observed in treatment using 1 mol/L (**Figures 6.16(c-d)**), which can be considered to be the major contributor to erosion resistance. In addition, the layer thickness was found to be smaller in 0.5 mol/L compared to that attained in 1 mol/L, led to the low UCS values.

Figure 6.17 compares the effect of concentration on supply efficiency. The results reveal that treatment using 0.5 mol/L had slightly a higher supply efficiency than the 1 mol/L. When 0.5 mol/L was used to treat, the progression of crystallization was slow, which could retain the

infiltration capacity of near-surface soils compared to that of 1 mol/L, allowing more infiltration among the supply. However, as mentioned above, relatively lower strength (**Figure 6.15**) and weaker cementation (**Figure 6.16**) were evidenced in 0.5 mol/L, which is not really desirable for the stabilization of slope near-surfaces. Previously investigation has also concluded that surfaces treated using 1 mol/L could show higher resistance to rainfall induced erosion compared to lower concentrations (Jiang et al., 2019).

6.5. Full bench-scale slope in temperature-uncontrolled environment

6.5.1. *Treatment and monitoring process*

Based on the outcomes of the series of small-scale slope model solidification tests, the optimum treatment conditions were derived and implemented to a full bench-scale slope model in an open (temperature uncontrolled) environment in order to demonstrate the applicability in real-field, promoting the technique. The slope-model is shown in **Figure 6.2**. As shown, two temperature sensors were used to monitor the diurnal temperature fluctuations: one was installed on the slope surface, and the other one was established at little far from the slope model. Around 24.5 kg of soil was filled in several layers to the average density of 1.5 g/cm³. The 2-phase spraying was performed (refer **Section 6.3.2**), and the supply quantity was effectively chosen to be $0.5 \times V_P$. The infiltrated liquid that had drained out was collected at the foot of the slope and subjected to the measurements of pH and Ca²⁺ concentration.

6.5.2. *Temperature measurement*

Figure 6.18 presents the real-time temperature fluctuations obtained from the sensors. The both sensors show relatively similar reading, suggesting that there were no significant differences between atmospheric temperature and that of slope surface. The results clearly indicate the day-night variation in temperature. The temperature was relatively high during

the daytime, and that dropped by an average of 5 – 6 °C in night times. It can also be seen that the average temperature was around 15°C within the 216 hours, but the temperature dropped to the lowest value of 5°C for the next 48 hours, and then once again that increased at latter periods. As per the biological response of the bacteria (**Chapter 3**), the bacteria could effectively precipitate CaCO_3 within the temperature of 15-25°C, and that is relatively low below 10°C. Accordingly, it can be understood that the bacteria were effectively performing during around 70% of the treatment process.

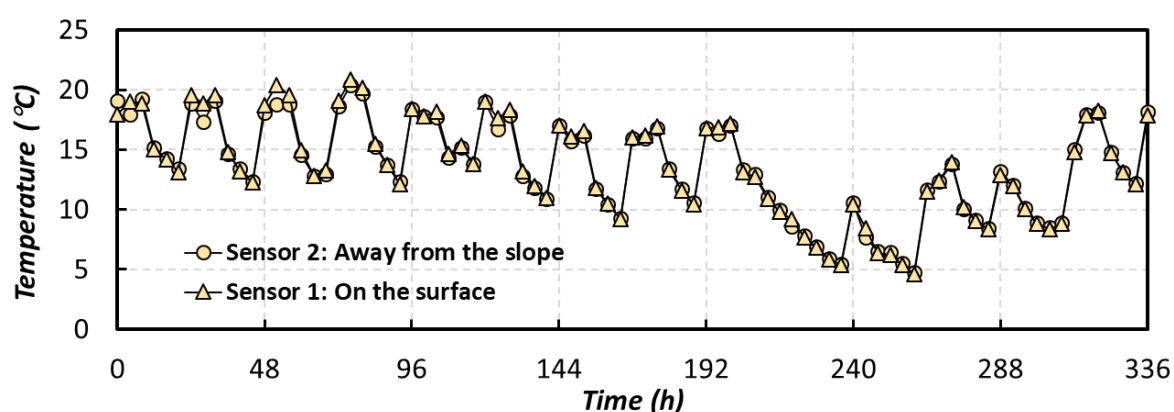


Figure 6.18: The real-time temperature measurement from sensors

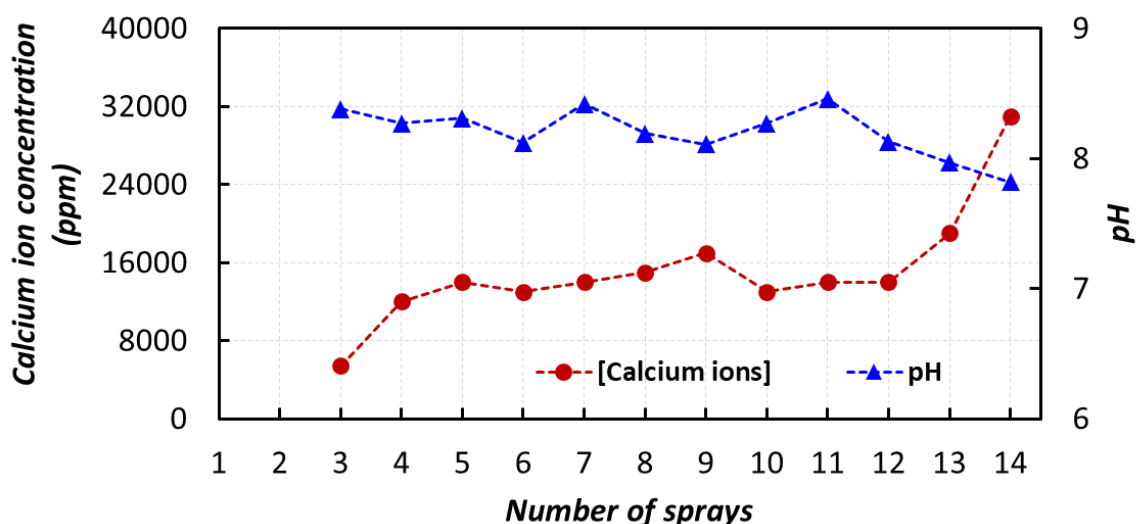


Figure 6.19: Concentration of calcium ions and pH of the outflow solution

6.5.3. *Monitoring outflow solution*

The infiltrated solutions were collected at the foot of the slope (worth noting that the sheet-slow was carefully managed not to mix with the infiltrated solutions). The pH and Ca^{2+} concentration of the outflow solution can be the indicators of the MICP process, representing the bio-chemical conditions of the slope soil, thus, the monitoring was mandatory. **Figure 6.19** presents the Ca^{2+} and pH of the outflow solution with the treatment times. It can be found that the pH of the outflow solutions was between 8.0 and 8.5 in most of the times, suggesting the occurrence of MICP reactions that turned the solutions into alkaline condition. The Ca^{2+} values also indicate that the resources were utilized up to around 68 %. Until the 11th number of treatments, the pH was found to be between 8.0 – 8.5; however, afterwards, a drop was seen, led the pH value to around 7.8. Similar tendency was also observed in Ca^{2+} measurements, i.e. rapid increase was observed in Ca^{2+} readings after 12th treatment. This could be probably attributed to the low temperature effect. When the temperature decreased below 10°C, the MICP reactions were likely to be trivial within soil, resulted the decrease in pH and increase in leached Ca^{2+} (**Figure 6.19**).

6.5.4. *Evaluation of treated slope and calcium carbonate distribution*

After the 14 number sprays, the slope was allowed to cure in the same environment for 72 hours, followed by the evaluations. The UCS measurements were performed using needle penetration tests, and a total number of 54 points were tested on the slope surface. **Figure 6.20** presents the variation of near-surface UCS of the slope in contour format. It can be seen from the results that relatively a marked improvement was achieved, and that ranged between 1.6 – 2.8 MPa. Similar to small-scale slopes, higher UCS was observed at bottom slopes, which could probably be attributed to the flow-lines and transportation of bacteria cells as explained previously.

To prevent the soil particles from flowing, at least a strength up to 150 kPa is required. It has also been suggested that the sand should be treated up to a UCS value of around 1.5 MPa to prevent the dynamic and liquefaction damage (Duraismy, 2016; Shibata and Teparaksa, 1988). Therefore, the near-surface UCS achieved here is considered to be sufficient enough to withstand against the surface erosion and to provide a reliable cover condition to the expressway slopes.

Subsequently, the mold was dismantled at vertical assembly, and the cementation was assessed along the vertical direction. To evaluate the spatial distribution of calcium carbonate, samples were collected from different depth positions of the slope. The precipitated calcium carbonate content was evaluated in accordance with the method described in **Section 3.3.7**. **Figure 6.21** presents the distribution of calcium carbonate along the depth as linearly interpolated contours. The measurements showed that the calcium carbonate was distributed up to around 12%, and the maximum distribution of calcium carbonate was found to be at around bottom surface. The distribution of calcium carbonate (**Figure 6.21**) was in consistent with the UCS measurements (**Figure 6.20**).

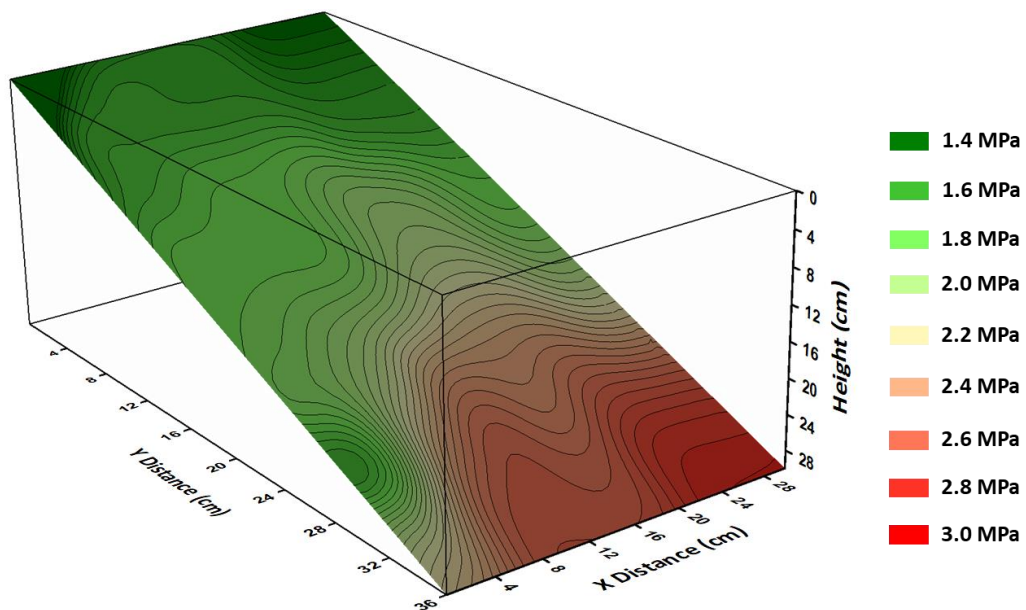


Figure 6.20: The variation of near-surface UCS of full-scale slope

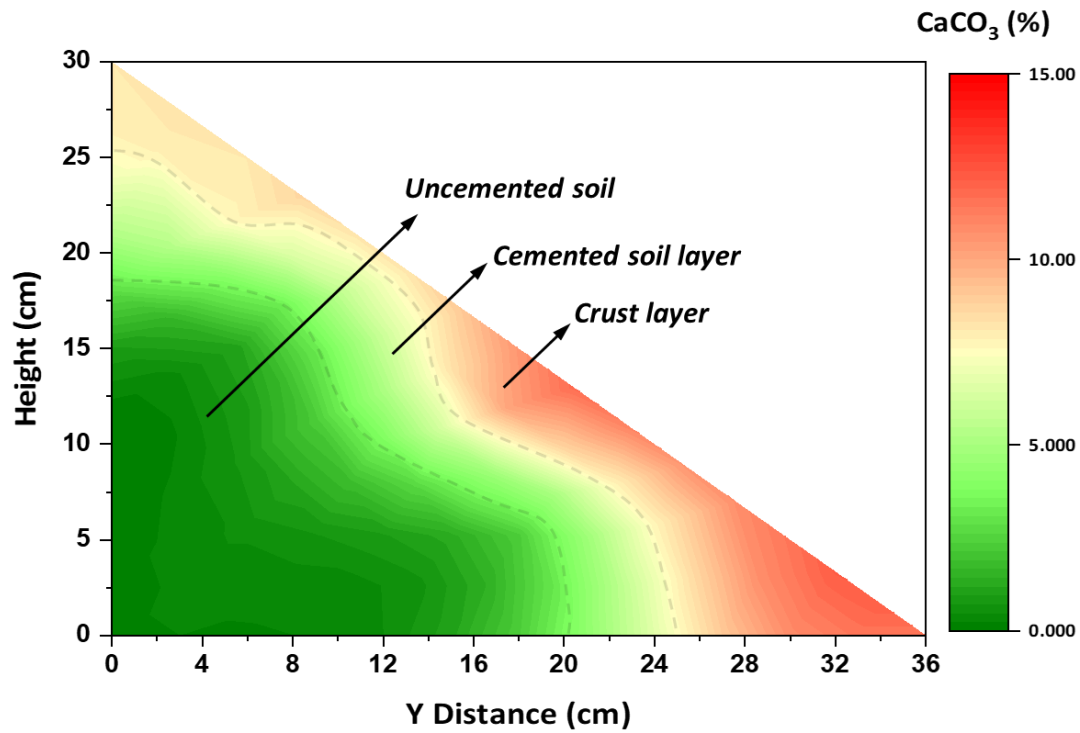


Figure 6.21: Spatial distribution of calcium carbonate in full-scale slope

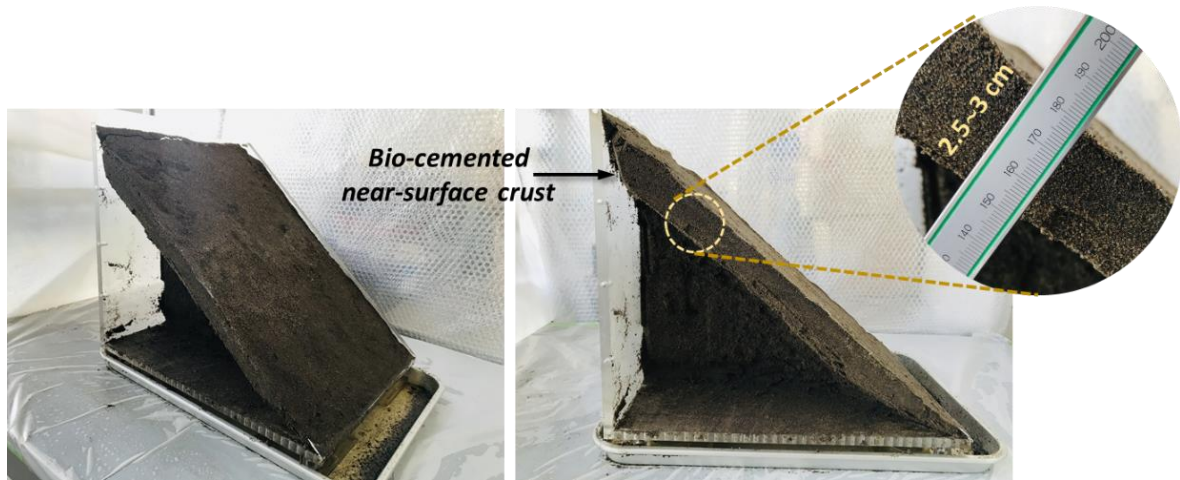


Figure 6.22: Well cemented crust layer of near-surface

The assessment clearly demonstrated that the MICP treated slope near-surface can be considered namely into three categories/ layers: (i) well cemented surface crust, (ii) cemented soil layer and (iii) uncemented soil (as indicated in **Figure 6.21**). **Figure 6.22** clearly shows the surface crust, that formed to a thickness ranging between 2.5 – 3.0 cm and primarily comprised of well-developed calcium carbonate bonds. This near-surface crust was underlain

by a cemented soil layer, and that was underlain by uncemented soils. The cemented soil layer was not be able to withstand the existed shape, as the formed calcium carbonate content was found to be low and was unable to facilitate strong connections. The thickness of this cemented soil layer was unable to measure exactly; but the measurement of calcium carbonate content (**Figure 6.21**) of the soil sampled at various locations revealed that the thickness of cemented soil layer could be nearly around 4-6 cm. Below that, no considerable calcium carbonate deposits were found, remaining as same as untreated soil.

6.5.5. *Micro-scale analysis (SEM)*

To understand the microstructural changes, SEM analysis were performed to the samples collected from the above three zones: surface crust, cemented soil layer and uncemented zone; the micrographs are presented in **Figures 6.23(a), (b) and (c)**, respectively. A well-connected soil matrix could be seen in all over the crust layer, i.e., the precipitated calcium carbonate could sufficiently cement the particles in topmost crust layer (**Figure 6.23(a)**). As shown in **Figure 6.23(b)**, the precipitated calcium carbonates were evidenced in the microstructure of the cemented soil layer; however, there were no considerable particle connections observed. The formed crystals were mostly found to be located on the surface of the grains. On the other hand, as expected, there were no calcium carbonate crystals were observed in the bottom uncemented soil layer (**Figure 6.23(c)**).

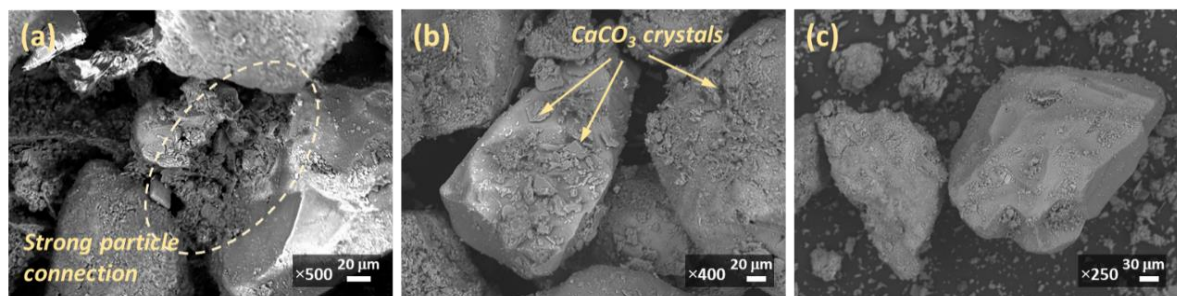


Figure 6.23: The SEM images of the samples collected from (a) surface crust, (b) cemented soil layer and (c) far below the surface

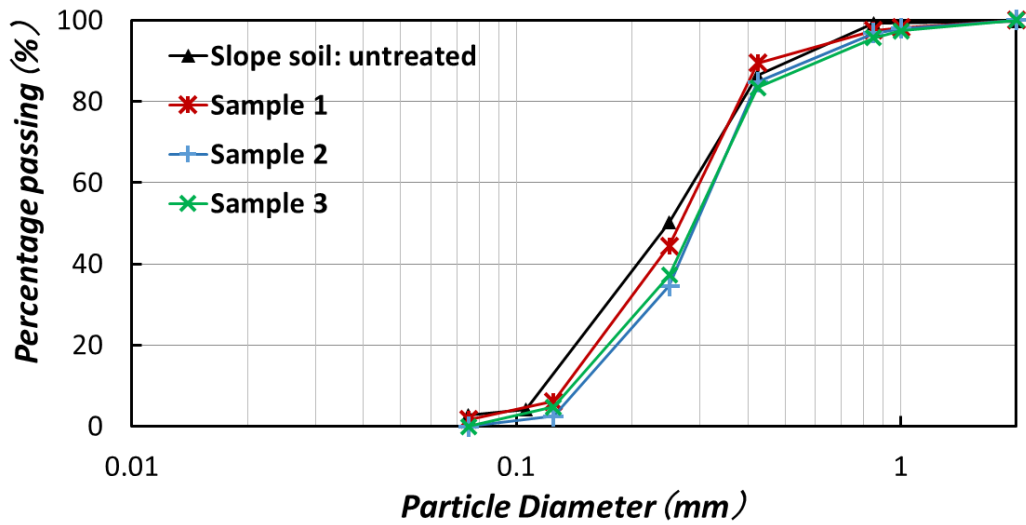


Figure 6.24: Particle size distributions of cemented slope soil samples

6.5.6. Sieve analysis of cemented soil

A set of samples were collected from cemented soil layer (i.e., below the surface crust layer), and sieve analysis were performed. **Figure 6.24** presents the comparison of particle size distribution between cemented soil samples and uncemented (virgin) slope soil. The results reveal that the grain size distribution is observably altered by crystallization, although there are no strong intergranular connections achieved. The grain sizes appear to be increased, which could be probably due to the CaCO_3 precipitates on grain surface. For instance, the untreated soil had a mean particle diameter of 0.23 mm, and the treated soil yielded a mean particle diameter of 0.3 mm.

6.5.7. Interpretation on erodibility

Near-surface erosion-resistance can be considered in terms of critical shear stress (τ_c); τ_c can be simply defined as the minimum shear stress required for incipient motion of the near-surface substrate. The soil particle undergoes erosion when the strength of the flow (i.e., the bed stress, τ_b) exceeds the critical shear stress (Crowley et al., 2019). As per Einstein and Krone (1962), the erodibility of the substrates (E) can be simply defined as,

$$E \propto (\tau_b - \tau_c) \quad (\text{Eq. 6.3})$$

τ_b depends on several factors such as flow conditions, slope of the surface, viscosity and the turbulence of the flow, and the increase in τ_b increases the erosion. Conversely, the increase in τ_c decreases the erodibility of the surface, and which can theoretically be defined as,

$$\tau_c = \theta(\rho_s - \rho_w) \cdot g \cdot D_{50} \quad (\text{Eq. 6.4})$$

where, θ is shields stress parameter (Crone, 2004); ρ_s is density of the bed material; ρ_w is density of the runoff water; D_{50} is the mean particle diameter.

From the **Eq. 6.4**, it is very clear that the grain size of the bed material greatly influences the erodibility. In the MICP treatment, the particle contacts are cemented by calcium carbonate, which leads to the aggregation of soil particles, resulting the increase in mean size of substrate. The surface crust layer achieved herein is a well-aggregated matrix and is unlikely to be considered as soil grains, hence the probable mean particle size may be huge. Therefore, as shown in **Figure 6.25**, the crust formed on the surface can be expected to provide a protection through sort of armoring/ shielding mechanism and can be primarily responsible for surface erosion resistance. The thicker the crust layer enable the better the cover condition, preserving the slope near-surface against the degradation processes including erosion.

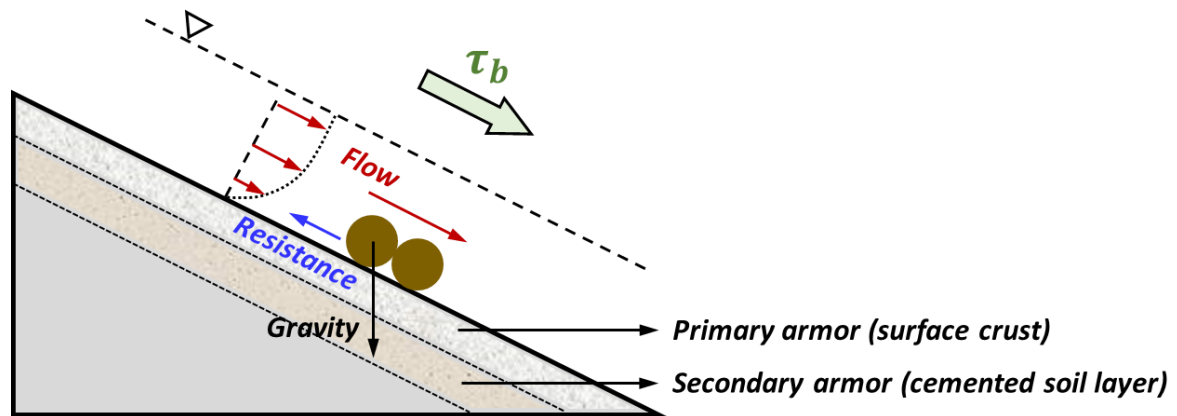


Figure 6.25: Conceptual interpretation of armoring by MICP technique against erosion

As demonstrated by sieve analysis in **Section 6.5.6**, the cemented soils found below the surface crust shows up to 30% increase in mean particle diameter due to the calcium carbonate crystals formed on grain surface. This suggests that the cemented soil layer (even slight crystallization on grain surface) can also be attributed to the increase in both internal and external erosion-resistances, as per the **Eq. 6.4**. Therefore, the cemented soil layer can be potentially considered as secondary shield/ armor (**Figure 6.25**) against erosion process. Moreover, the critical shear stress directly proportional to the tolerable wave height; therefore, the MICP treatment can be expected to withstand against increased surficial run-offs. In the near-surface stabilization process, the deeper slopes are not necessarily to be cemented, as the deeper zones do not pose any advantages towards this application.

6.6. Conclusions

This chapter dealt with MICP treatment to a series of slope models, promoted the technique from elementary scale to bench-scale slopes. Several treatment techniques were investigated with several variables to optimize the treatment. Near-surface of the treated slopes were also evaluated. From the outcomes of the study, the following conclusions can be drawn.

- Both the surface injection and spraying techniques were found to be feasible for near-surface stabilization of slopes. Among the spraying techniques, 2-phase spraying showed better response compared to the 1-phase spraying. 1-phase spraying resulted the entrapping of bacteria cells at surface pore throats along with/ within the formed crystals, led to the formation of relatively a thin stiff surface layer (10 mm). In 2-phase treatment, the bacteria cells were found to penetrate more deeply (20-40 mm).
- The MICP responses have been found to be varying depending on the pore volume supplied. High supply of cementation solution develops a highly nonuniform profile compared with low supplies, where the MICP treated near-surface profiles are relatively

uniform. For the near-surface stabilization of slope considered in this study, 0.5 times the pore volume of target near-surface zone was found to be the optimum, and the corresponding supply efficiency appears to be acceptable.

- Treatment using 1 mol/L cementation solutions showed better response than that of 0.5 mol/L. When the slope is treated using 0.5 mol/L, relatively lower precipitation of CaCO_3 occurred (led to 1.8-2.8 MPa) compared to that achieved in treatment using 1 mol/L (led to 200 kPa-1 MPa).
- The full bench-scale slope model results revealed that the MICP treatment in an open environment, i.e. day-night temperature fluctuations within 15°C-25°C, insignificantly affected the microbial performance, demonstrating the feasibility for the applications in cold regions. The near-surface achieved the UCS of 1.6-2.8 MPa with acceptable uniformity. The spatial distribution of calcium carbonate showed the treated slope can be considered into three layers: surface-crust layer (25-30 mm in thickness), cemented soil layer (40-60 mm in thickness) and uncemented soil, suggesting that the application technique may provide erosion protection via two armoring mechanisms: (i) the crust formed along the outer surface of the slope (primary armor) and (ii) cemented soil material formed on the interior of the slope (secondary armor).

CHAPTER 7

CONCLUSIONS, RECOMMENDATIONS AND APPLICATION GUIDELINES

7.1. Introduction

This dissertation presents the results of a study performed on bio-mediated near-surface stabilization of slopes. The objective of this research work was to evaluate the feasibility of microbial induced carbonate precipitation (MICP) technique using indigenous ureolytic isolate, to be used *in-situ* as a novel candidate for near-surface stabilization of expressway slopes (Hokkaido, Japan) and to deepen the understanding on biological, mechanical, durability responses and treatment techniques to facilitate effective stabilization process.

The research work consists of the following major components: (i) an overview on MICP technique, pathways and possible applications (described in **Chapter 2**), (ii) an overview on near-surface of slopes, deterioration processes, failure mechanisms and currently available mitigation measures (described in **Chapter 2**), (iii) evaluation of the fundamental factors of MICP process considering biological, biochemical, grain-size parameters and associated effects (described in **Chapter 3**), (iv) evaluation of physical and mechanical properties of MICP treated slope soils (described in **Chapter 4**), (v) evaluation of long-term performance of MICP treated slope soils (described in **Chapter 5**), and (vi) evaluation of treatment strategies and treated slope near-surfaces (described in **Chapter 6**).

This chapter presents the summary of conclusions of each component of the study and outlines the application guidelines based on the findings. Finally, the chapter provides the suggestions for the future research work.

7.2. Summary of conclusions

7.2.1. *Biological, bio-chemical and soil compatibility responses*

MICP is a bio-mediated soil stabilization technique, and the effectiveness of the treatment greatly rely on biological and biochemical factors. A potential pathway through augmentation by native bacteria, has been studied herein to stabilize the near-surface of slopes located in cold region. The native ureolytic bacteria, *Lysinibacillus xylanilyticus* (newly introduced herein for urea hydrolysis), could effectively hydrolyze the urea between the temperatures 15-25°C, showing in more robust performance under native cold climatic conditions. The urease was found to be more stable and consistently performed under the neutral-weak alkaline environmental conditions. For an effective and reliable MICP, the maximum initial concentrations of calcium and urea resources could be 1 mol/L. When the initial concentration of urea was increased above 1 mol/L, the urease enzyme was found to be denatured by the urea.

Efficiency of MICP was found to depend on grain-size distribution of soil which determines the bacteria retention capacity and resource utilization during surface treatment. The column tests suggested that the increase in number of particle contacts (i.e., decrease in mean particle diameter of the soils) increase the effectiveness of near-surface MICP, providing better location for crystals to precipitate and decreasing the stress acting per soil particle. In slope soils, presence of certain silt content (3%) increased the particle contacts and enhanced the force chain. It has also been found that 5% silt content can be the lower bound for MICP

application in fine sands by surface injection; above 5%, the soil skeleton is likely to be ruled by silt particles, restricting the bacterial transport through pore throats, revealing treatment incompatibility. It can therefore be suggested that during the site characterization, a preliminary analysis needs to be performed to confirm the compatibility of soil grain size distribution for MICP application.

7.2.2. *Physical and mechanical responses of MICP slope soil*

Physical and mechanical characteristics of MICP treated soils were found to be increased dramatically with an increase in precipitation content of calcium carbonate. As the cementation level increased from light to heavy, the state of the soil desirably transitions from loose to dense (i.e. rocklike material). The increase in cementation level resulted in a higher stiffness, peak and residual strength. As the cementation increased, the peak stress was reached at lower axial strains. The strain-hardening behavior was observed during the compression of uncemented slope soil, and as the cementation increases, the strain-hardening behavior transitions to strain-softening.

Shear test results shown that the cementation level had a great influence in shear response, increasing the peak and residual strengths with the increase in average carbonate content. The results also revealed that the cementation at particle contact attributed to the peak strength, increasing both friction angle and cohesion strength parameters. However, particle roughness i.e. crystals precipitated on soil surface attributed to the residual strength. The increase in cementation, the decrease in the permeability was observed. However, bio-cement was preferentially deposited at particle contacts, leading to retain certain permeability level (even at heavy cementation levels), which was one of the unique merits demonstrated to MICP.

7.2.3. Evaluation of long-term performance of MICP slope soil

Cyclic freeze-thaw (FT) test results revealed that the MICP treated slope soils were durable to the freeze-thaw induced erosion; however, the level of cementation highly determined the aggregate stability. The specimens cemented to an average of 11-13% (CaCO_3 by mass) resulted high erodibility (50% mass loss), whereas the 20-23% cemented specimens showed only a minor erosion (2% mass loss), when they were exposed to 25 cyclic frosts. The similar tendency is also observed in ultrasonic measurements. The high durability of MICP treated soils was primarily attributed to adequate cementation level which facilitates effective particle contacts that could resist the evolution of ruptures hence crystal-soil detachments when the uneven tensile stresses develop around the cemented soil particles during frosting process of pore water.

Cyclic wet-dry (WD) effects appeared to have adverse effects on the physical and mechanical characteristics of MICP slope soils. The deterioration mechanism of MICP soils due to WD cycles was related to the degradation of calcium carbonates, and that was found to be occurring at two stages: short-term and long-term. The short-term deterioration rapidly occurred within the first few WD cycles due to the suspension and corrosion of powdery carbonate deposits. The long-term deterioration could be observable after 30 WD cycles. The fatigue stresses developed during WD process could deform the primary bonds, and particularly impairing the surface of the specimens. However, the increase in precipitated calcium carbonate content increased the aggregate stability and mechanical durability. It was also found that the MICP specimens were less susceptible to WD actions, compared to that observed under FT effects.

Previously, it had been believed that the MICP treated soils are susceptible to acid rain (AR) conditions. However, this study demonstrated that the longevity of MICP soils under AR

conditions could be considerably enhanced by increasing the level of cementation. The corrosion rate of calcium carbonate was reliant on the pH of AR and its intensity. However, the loss of soil particles and strength were governed by the level of cementation. In another words, for the same quantity of corrosion, the higher the cementation level the lower the loss of mass and UCS were found.

7.2.4. *Response to treatment techniques and treated slope near-surface*

Treatment technique is critical to the *in-situ* applications of MICP, as the effectiveness is related to the delivery, utilization and spatial distribution of resources. Both the surface injection and spraying techniques were found to be feasible for near-surface stabilization of slopes. Among the techniques, 2-phase spraying showed better response compared to the 1-phase spraying. 1-phase spraying resulted the entrapping of bacteria cells at surface pore throats with/ within the formed crystals, led to the formation of relatively a thin crust layer (10 mm). In 2-phase treatment, the bacteria cells were found to penetrate more deeply, forming thick stable crust (20-40 mm).

The MICP responses were found to be varying depending on the pore volume supplied. High supply of cementation solution developed a highly nonuniform profile compared with low supplies, where the MICP treated near-surface profiles were relatively uniform. For the slope considered in this study (fine sandy soil), 0.5 times the pore volume of target near-surface zone was found to be the optimum, and the corresponding supply efficiency appears to be acceptable. Treatment using 1 mol/L cementation solutions showed better response than that of 0.5 mol/L.

The full bench-scale slope model results revealed that the MICP treatment in open environment, i.e. day-night temperature fluctuations within 15°C-25°C, affected negligibly the microbial performance, demonstrating the feasibility for the applications in cold regions.

The slope near-surface achieved the UCS of 1.6-2.8 MPa with acceptable uniformity. The spatial distribution of calcium carbonate showed the treated slope could be considered as surface-crust layer (25-30 mm in thickness), cemented soil layer (40-60 mm in thickness) and uncemented soil, suggesting that the application technique may provide erosion protection via two sorts of armoring/ shielding mechanisms (i) the crust formed along the outer surface of the slope (primary armor) and (ii) cemented soil material formed on the interior of the slope (secondary armor).

With all the above conclusions, it is well demonstrated that the newly emerged MICP technique can be a viable alternative for near-surface stabilization of slopes in cold regions as well.

7.3. Application guidelines

Based on the findings of this dissertation, the application guidelines are outlined in this section for effective and reliable MICP treatment. The guidelines include the information regarding optimal conditions for bacteria production (**Table 7.1**), optimal MICP recipe (**Table 7.2**), desirable level of treatment (**Table 7.3**), treatment protocols (**Table 7.4**) and performance-estimation under specific climatic exposures (**Figures 7.1, 7.2 and 7.3**). The proposed guidelines would turn into fully applicable after the verification on *in situ* trial tests which is to be performed in the subsequent phase of this work. It should be noted that these findings and guidelines can be applicable to the soil studied herein, and the responses may vary to the soils with different grain size distribution and mineralogy (unless verified).

Table 7.1: Optimal production and harvesting of bacteria

Bacteria and culture medium	Culturing conditions	Harvesting	Storage
Strain: <i>Lysinibacillus xylanilyticus</i> Culture medium: NH ₄ -YE (ATCC 1376) Method: Preculture to main culture <i>(refer Section 3.3.2)</i>	Temperature: 25°C Shaking incubation: 160 rpm	Desirable growth: OD ₆₀₀ of 4.0 – 4.5 <i>(harvestable after 48-72 hours of growth time)</i> <i>* expected urease activity 2-3 U/mL.</i>	Can be stored at 4°C <i>(20-25% expected loss in urease activity after 48 hours of storing)</i> <i>* newly prepared culture is recommended.</i>

Table 7.2: Optimum cementation recipe

Concentration	Resources
Quantity of CaCO₃ precipitate: <i>0.25 < 0.5 < 0.75 < 1 > 1.5 > 2 (mol/L)</i> Utilization ratio: <i>0.25 = 0.5 = 0.75 = 1 > 1.5 > 2 (mol/L)</i> Optimum concentration: 1 mol/L <i>(refer Section 3.4.3)</i>	For 1 mol/L concentration, CaCl₂: 111 g/L Urea: 60 g/L Nutrient broth: 6 g/L <i>* newly prepared solution is recommended.</i>

Table 7.3: Transition of soil and desirable cementation levels for near-surface stabilization

Location: Hakodate, Hokkaido (42.388532, 140.284762)

Slope soil: Poorly graded fine sand (SP, USCS); silt content of 3%

Treatment type: 2-Phase treatment (refer **Section 3.3.6**), augmentation using native strain under optimum conditions (refer **Tables 7.1 and 7.2**)

Test scale: Column element

Cementation injection (N)	Soil state	CaCO ₃ % (within top 30 mm)	CaCO ₃ % (average in 60 mm)	Cementation category	S-wave velocity (m/s)	Info.
0	Loose soil	0.2	0.2	untreated	100-150	Untreated
1	Dense soil	0.4-1.1	0.6	Light	-	-
3	Dense soil	1.8-3.6	2.1	Moderate	-	-
5	Dense soil - Soft rock	4.6-7.3	4.6	Moderate - Heavy	-	-
7	Soft rock	8.9-13.2	7.8	Heavy	1000-1100	Desirable levels for stable aggregation
10	Soft rock	15.1-17.9	12.7	Heavy	1200-1300	
14	Soft rock	19.7-22.8	15.9	Heavy	1450-1550	

** Three cementation categories can be considered: light cementation (below 1.5%), moderate cementation (1.5-5%) and heavy cementation (above 5%).*

Table 7.4: Treatment technique in technical terms

Treatment type	Treatment procedure
2-phase spraying (biological spraying and cementation spraying in two subsequent phases) <i>* A moderate and practical spraying rate of around 40-50 mL/min is recommended.</i>	Site-specific investigation - Compatibility of grain size distribution and intrinsic characteristics - <i>In-situ</i> ground water characteristics Preliminary field-works - Clearance of obstacles from the surface of the slope to be treated, and surface smoothening (if required) Treatment - Spraying bacteria culture (<i>phase 1</i>) - 2-3 hours of retention time - Spraying cementation solution (<i>phase-2</i>)
Desirable conditions	
Atmospheric temperature: 15 – 25°C <i>* July, August and September are recommended for field implementation in Hokkaido.</i>	- Cementation spraying: Every 24 hours basis (<i>until desirable level is achieved, refer Table 7.3</i>) - Bacteria spraying: Once in a week (i.e. once in seven cementation sprayings) basis - Treatment monitoring: <i>groundwater-ammonium ions monitoring, ultrasonic waves, pH, atmosphere</i> Posttreatment - Performance-monitoring/ assessment: ultrasonic waves and visual monitoring (<i>once in a while, after prolonged rainfalls, after seasonal winters</i>) - Occasional retreatments (<i>if required</i>)
<i>* For soil compatibility analysis (i.e., effect of grain size distribution) refer Sections 3.4.4 and 3.4.5.</i> <i>*For the supply volume (of each spraying) refer Section 6.4.3</i> <i>* Climatological data of the study location can be found in Appendix A.</i>	

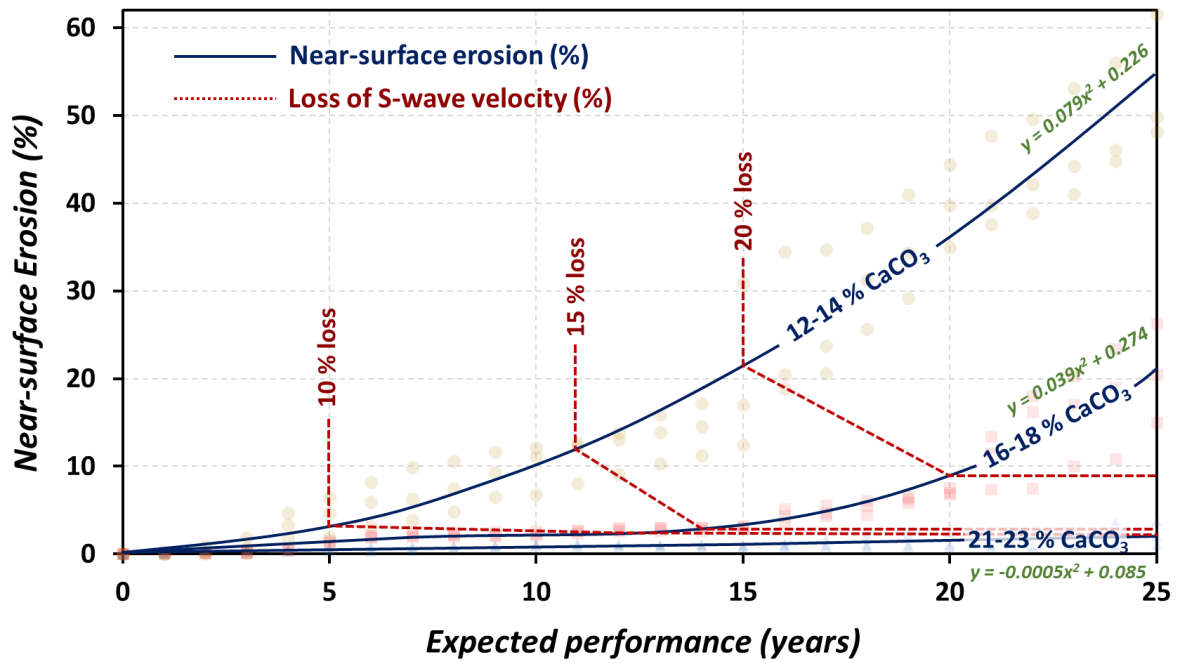


Figure 7.1: Performance-estimation under seasonal freeze-thaw

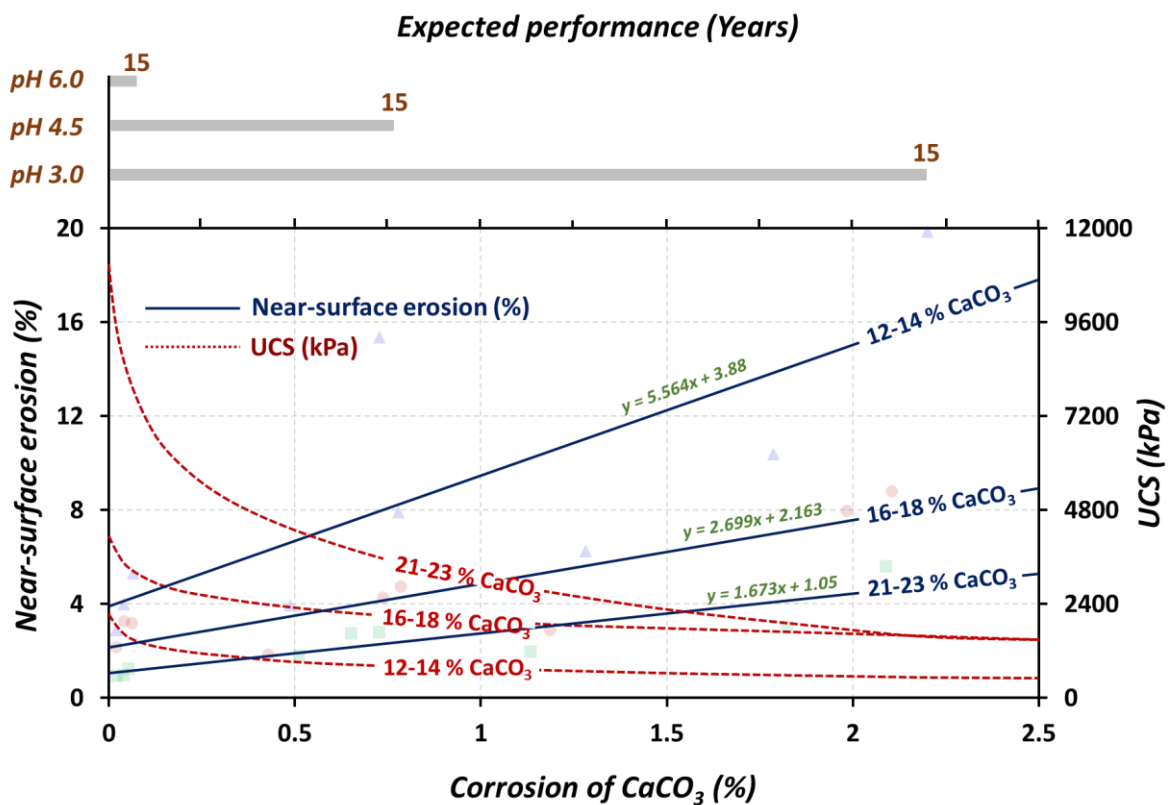


Figure 7.2: Performance-estimation under acid rainfall

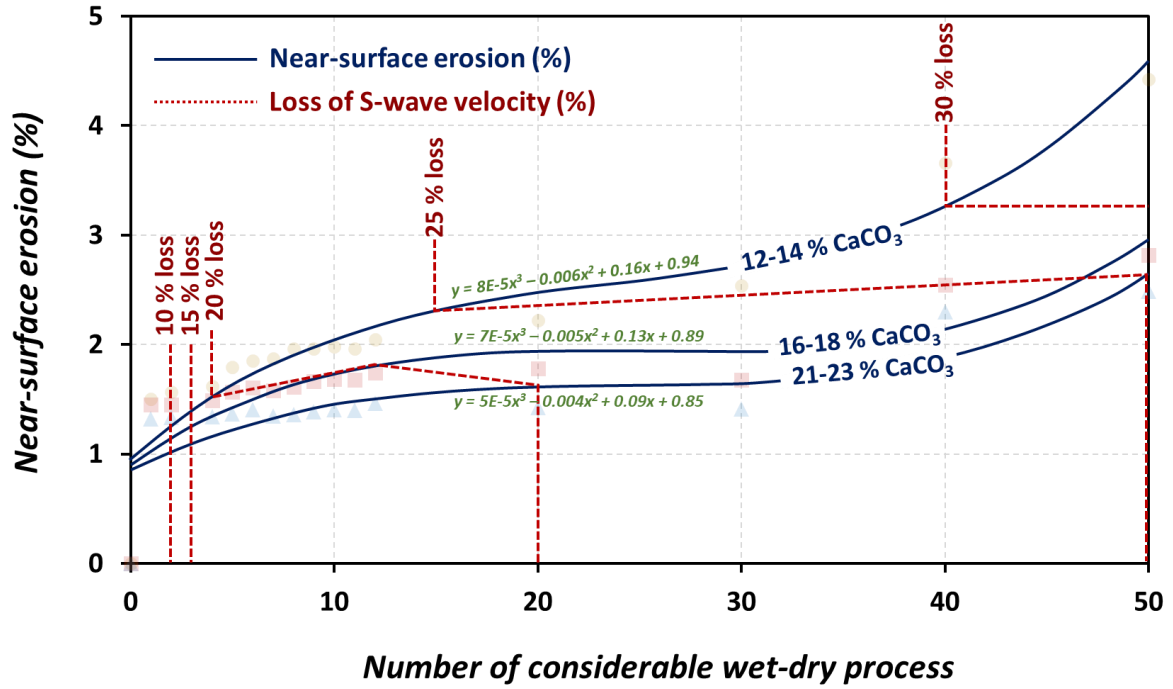


Figure 7.3: Performance-estimation under cyclic wet-dry process

7.4. Suggestions for the future work

This research work targeted to introduce the MICP technique in promoting the cover condition of the expressway slope which attributes to the near-surface stability. In this work, the viability has been extensively demonstrated, and the application guidelines for reliable MICP have also been proposed based on the findings, promoting the MICP nearly to the real-scale application stage. However, prior to the industrial use, test trials need to be performed *in situ*, and the limitations posed by the boundary effect need to be evaluated, as the subsequent phase of this work. The test-trials should incorporate (i) the assessment of groundwater-precipitate interactions using test-wells, (ii) use of ultrasonic waves, (iii) pH monitoring, and (iv) atmospheric monitoring (temperature and precipitation) during the entire treatment. The durability evaluation of treated near-surface also needs to be performed subsequently, and that can be performed either mechanically or visually (e.g. physical damages, appearance changes, crack formations) with the time (like once in a few months;

particularly after seasonal winters and prolonged rainfalls). Occasionally, retreatment may need to be performed occasionally, if major deteriorations are observed, and meanwhile studies should focus on further possible enhancements in treatment mechanisms. During the test trials, monitoring a control test field (with no MICP treatment applied) would be beneficial for comparison purpose.

Feasibility of MICP retreatment and its efficiency needs to be studied in the future, and that would be beneficial during the maintenance. It is found that the seasonal frost highly deteriorates the MICP treated slope near-surface (Gowthaman et al., 2020). Even though the high level of cementation results higher aggregate stability, the strength of the material is found to decrease significantly after decades of exposure due to the formation of microcracks in calcium carbonate bonds. Therefore, laboratory investigations are recommended to focus on the retreatment efficiency and revivability (of deteriorated MICP slopes) on as-treated characteristics.

Urea hydrolysis results the undesirable byproducts of free ammonia and ammonium ions depending on the pH level, and the removal of ammonium byproducts from the system is one of the major concerns for the future work. If the MICP reactions could be controlled at low pH levels (below 7.5), around 98% of ammonia could be formed as ions, inhibiting the release of ammonia gas, and future work should focus on low pH MICP. The ammonium ions can be possibly eliminated by collecting and treated the drained solutions, that could help to avoid the contamination with ground water. Immobilization of ammonium ions within the target near-surface/ treatment zones can also be a feasible pathway to avoid ground water contaminations. Therefore, future works are highly recommended to couple the MICP technique with possible ion exchange methods, adsorption methods and precipitation methods

and to propose new environmental-friendly ammonium-free MICP methods; on the environmental perspective, this research area appears to be the future of MICP research field.

There are also possible bio-mediated geotechnical methods (less investigated) such as denitrification, sulfate reduction, iron reduction and hydroxyapatite mineralization, which can also induce the bio-cement similar to the MICP. These pathways should also be studied as possible alternatives, as those processes can eliminate the formation of ammonium byproducts.

Cost of the material, i.e. chemicals (**Appendix C**) used for the MICP is also found to be another great hurdle to MICP applications. Studies need to propose suitable cheaper alternatives for bacteria growth medium, calcium sources, urea sources and nutrients and address the positive and negative sides of such alternatives on MICP. Obviously, MICP treatments often encounters non-uniform cementations along the depth. Although the vertical heterogeneity in cementation is not a serious concern for near-surface stabilizations, it would be beneficial if the cementation depth could be increased and achieved uniformly. The surface treatment revealed limitations in transporting bacteria cells to deeper zones. Therefore, researches on developing more effective delivery methods are advantageous.

In this dissertation, the durability analysis focused on freeze-thaw, wet-dry and acid solution as potential indicators. The impact of rainfall on the yield-response of MICP treated near-surface and how it impacts with degree of cementation is also a very important subject to explore, and which is also a recommendation for the future work.

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APPENDIX A: Meteorological data of the study location

Table A.1: Meteorological data means of 30 years (1981-2010) (sourced from Japan Meteorological Agency)

Month	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec
Avg. Mean Temperature (°C)	-2.6	-2.1	1.4	7.2	11.9	15.8	19.7	22	18.3	12.2	5.7	0
Avg. High Temperature (°C)	0.7	1.5	5.3	11.8	16.5	19.9	23.4	25.8	22.7	16.8	9.7	3.3
Avg. Low Temperature (°C)	-6.2	-5.9	-2.6	2.6	7.5	12.1	16.6	18.7	14.1	7.4	1.4	-3.5
Snow fall (mm)	118	90	53	4	0	0	0	0	0	0	27	86
Average wind speed (m/s)	3.9	3.9	4.2	4.0	3.7	3.3	2.9	3.1	3.5	3.5	4.0	3.9
Rainfall (mm)	77.2	59.3	59.3	70.1	83.6	72.9	130.3	153.8	152.5	100	108.2	84.7
Relative Humidity (%)	73	71	68	67	73	79	82	81	76	72	71	72

(<https://www.city.hakodate.hokkaido.jp/docs/2014013100471/>)

APPENDIX B: Authenticate phylograms of the native isolates

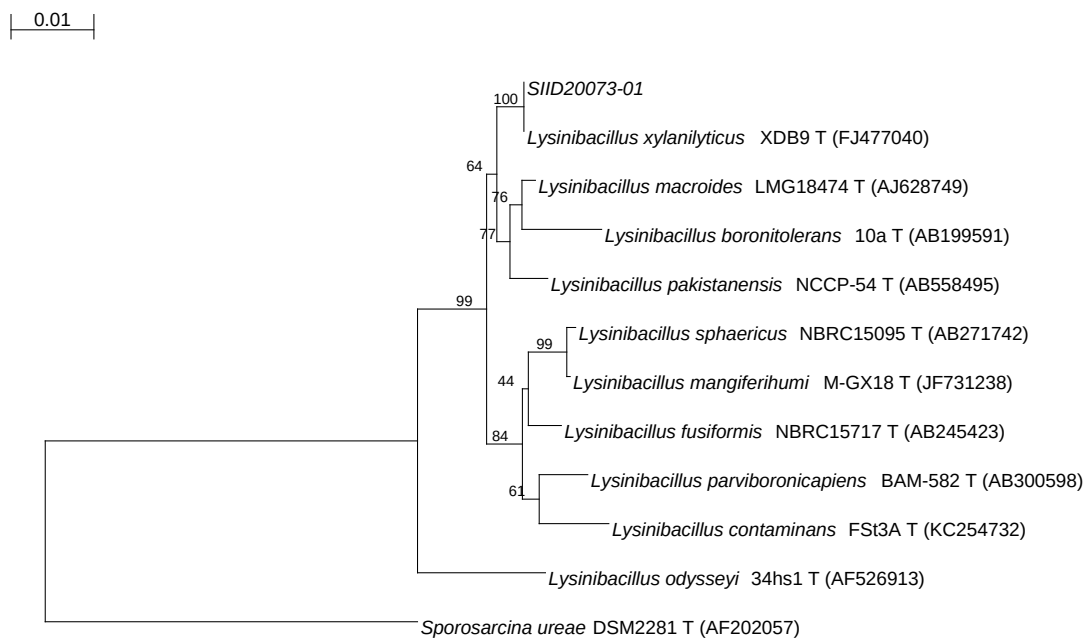


Figure B.1: Phylogenetic tree based on 16S rDNA nucleotide sequence of *Lysinibacillus xylanilyticus*.

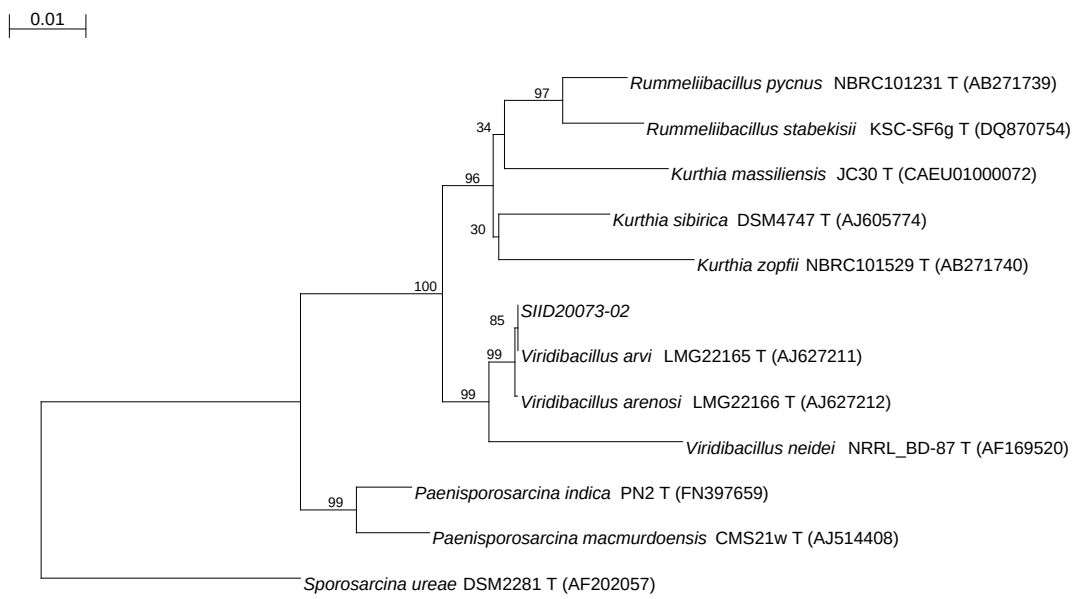


Figure B.2: Phylogenetic tree based on 16S rDNA nucleotide sequence of *Viridibacillus arvi*.

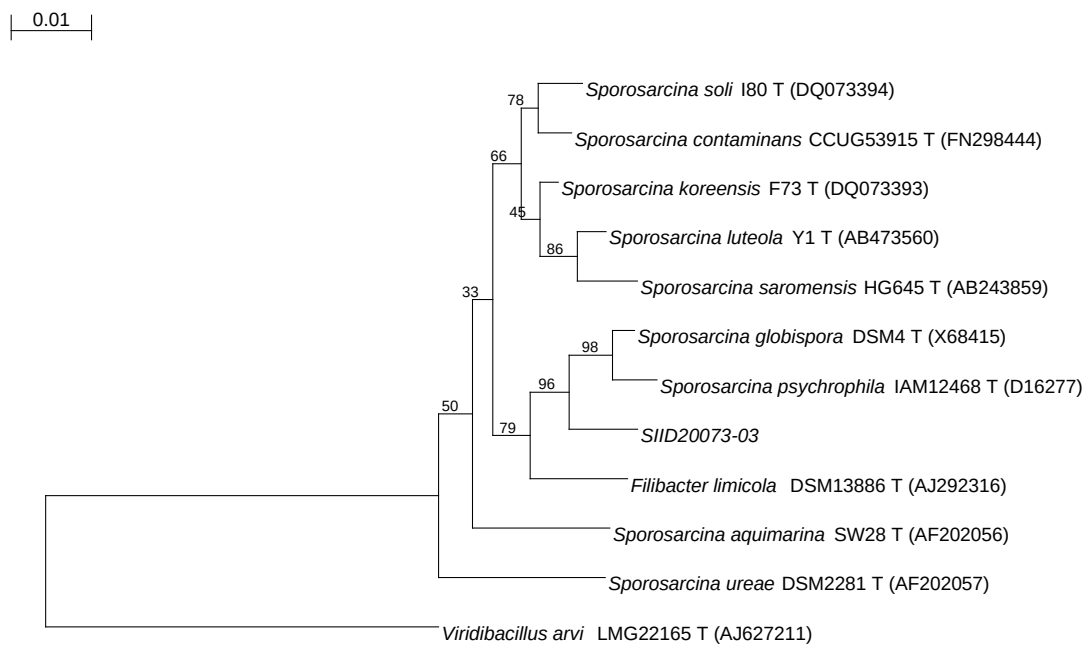


Figure B.3: Phylogenetic tree based on 16S rDNA nucleotide sequence of *Sporosarcina* sp.

APPENDIX C: Cost of the MICP chemicals

Table C.1 presents the cost comparison between laboratory-grade (pure) reagents and low-grade (commercial) chemicals. The prices are based on the market price of Japan (2019-2020). For the real-field applications, using low-grade chemicals provides remarkable benefits in terms of economy. Gowthaman et al. (2019b) demonstrated that using low-grade chemicals (refer **Table C.1**) for MICP treatment could result up to 96% cost reduction compared to the use of laboratory-grade chemicals, enabling economic pathway for future industrial level MICP advancements. Additional details such as applicability of low-grade chemicals and their effects on MICP can also be found in Gowthaman et al. (2019b).

Table C.1: Unit cost of the MICP chemicals

Chemicals	Pure Chemicals		Low-grade Chemicals	
	Substance	Unit price (JPY/kg)	Substance	Unit price (JPY/kg)
<i>Cementation Solution</i>	Urea	1,580	Urea Fertilizer	150
	CaCl ₂	5,600	Snow melting agent (CaCl ₂)	124
	Nutrient Broth	36,600	Beer yeast	2,000
<i>Culture Solution</i>	(NH ₄) ₂ SO ₄	1,975	Beer Yeast	2,000
	Yeast Extract	34,580		
	Tris-Buffer	10,833		