Influence of key environmental conditions on microbially induced cementation for soil stabilization

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ABSTRACT: Microbially induced calcite precipitation (MICP) is a sustainable biological ground improvement technique that is capable of altering and improving soil mechanical and geotechnical engineering properties. In this paper, laboratory studies were carried out to examine the effects of some key environmental parameters on ureolytic MICP mediated soils, including the impact of urease concentrations, temperature, rainwater flushing, oil contamination and freeze-thaw cycling. The results indicate that effective crystals precipitation pattern can be obtained at low urease activity and ambient temperature, resulting in high improvement in soil unconfined compressive strength (UCS). The microstructural images of such crystals showed agglomerated large clusters filling the gaps between the soil grains, leading to effective crystals formation. The rainwater flushing was detrimental to the bio-cementation process. The results also indicate that although traditional MICP treatment by the two-phase injection method did not succeed in treatment of oil contaminated soils, and the proposed premixing of bio-flocs with soil can significantly improve UCS and stiffness of oil contaminated soils. Finally, MICP treated soils showed a high durability to the freeze-thaw erosion, which is attributed to the inter-particle contact points and bridging of crystals formation.

Author Keywords: Microbially induced calcite precipitation (MICP); bio-cemented sand; ground improvement; microstructural analysis; unconfined compressive strength (UCS).

Introduction

Due to its versatility and sustainable application, microbially induced calcite precipitation (MICP) has recently gained much attention from the geotechnical engineering researchers worldwide. MICP is a naturally driven biological technique that harnesses the metabolism of bacteria to create an in-situ cementation agent known as calcium carbonate or calcite. Although there are many biological processes that can lead to MICP, the use of urea hydrolysis by ureolytic bacteria is gaining more interest, and the current study will thus focus on MICP via urea hydrolysis pathway. In this technique, aerobically cultivated bacteria with highly active urease enzyme (*Sporosarcina pasteurii*, formerly known as *Bacillus pasteurii*) are introduced to a targeted soil. The urea hydrolysis takes place when the active ureolytic bacteria catalyze the reaction of urea hydrolysis to produce ammonium (NH_4^+) and carbonate ions (CO_3^{2-}). In the presence of calcium from a supplied calcium source (i.e. $CaCl_2$), crystals of calcium carbonate (i.e. calcite) form inside the soil matrix. The chemical reactions involved in this technique are as follows:

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

$$Ca^{2+} + CO_3^{2-} \rightarrow CaCO_3\ (s) \downarrow \quad (2)$$

The bonding of soil particles by calcite provides an improvement to the mechanical and geotechnical engineering properties of bio-treated soils (DeJong et al. 2010). For example, bio-cemented sand treated by MICP has been reported to provide a reduction in soil settlement (DeJong et al. 2006; van Paassen et al. 2010a), an increase in soil shear strength (Ismail et al. 2002a; DeJong et al. 2006; Chou et al. 2011), and an improvement in soil stiffness (Montoya and DeJong 2015; Feng and Montoya 2015; Lin et al. 2015). In an attempt to advance the MICP technique for field implementation, van Paassen et al. (2010b) applied MICP to treat 100 m^3 of sand in a large-scale experiment, and the results demonstrated that

MICP was able to improve large spatial areas of treated sand. DeJong et al. (2014) developed a repeated five-spot treatment model to simulate a more realistic soil volume treatment in the field, and it was found that the proposed model was able to show spatial and temporal trends in the microbial content, which indicates that the model has the means to capture the complex treatment scenario involving MICP.

Mortensen et al. (2011) studied the environmental factors affecting MICP, namely the aqueous environmental conditions, various soil particle sizes and soil gradation. The results showed that the natural aqueous environments (freshwater and seawater) and myriad soil particle sizes and gradation (gravel, well-graded sand, poorly-graded sand and silt) were equally favorable to calcite precipitation. Mortensen et al. (2011) also proposed that the calcite precipitation rate and nutrient concentrations must be kept to their minimum in order to achieve treatment homogeneity across the bio-cemented soil columns. Similar findings were reported by Al Qabany and Soga (2013). However, the correlation between the mechanical and geotechnical behavior and amount of calcite formed under the above conditions has not been yet investigated. Earlier studies on the correlation between the shear strength of bio-cemented sand and amount of calcite content enveloping the sand particles have been investigated by Fujita et al. (2000) and Okwadha and Li (2010), showing that the shear strength of sand may not be directly proportional to the amount of calcite content (Whiffin et al. 2007). This was confirmed and explained by Cheng et al. (2013) who observed deposition of calcite crystals in the contact points between the sand grains, forming effective bridges that contribute to the shear strength improvement of bio-cemented soil.

The calcite precipitation pattern is another factor that greatly influences the target application of bio-cementation in the field as it influences the flow properties of porous media, which may lead to treatment homogeneity by shaping the preferential flow path according to the size, shape, and structure of the pore throats affected by the accumulation of calcite crystals (Al Qabany et al. 2012). The calcite precipitation pattern also affects the load transfer mechanism between the soil particles, through the area of contact points developed by the precipitated calcite, leading to variation in strength and stiffness of bio-cemented soils (Ismail et al. 2002b).

This paper aims to investigate the impact of some key environmental parameters on the efficacy of MICP for its in-situ implementation and optimization process. The parameters studied include the effects of urease enzyme concentration, degree of temperature, rainwater flushing, oil contamination, and freeze-thaw weathering in winter alpine regions. The impact of these parameters on the performance and effectiveness of MICP technique was related to both the shear strength improvement of bio-treated soil and $CaCO_3$ precipitation pattern at the micro-scale level. In this study, the introduction of urease enzyme to the soil samples was achieved by the injection of cultivated exogenous pure ureolytic bacteria, which can also be achieved by other methods, such as enrichment of indigenous ureolytic active microorganisms (Burbank et al. 2011) or premixing ureolytic bacteria with soil (Duraisamy and Airey 2015). The aim of the current study is to understand the effect of micro-scale level behavior of MICP (crystal pattern, crystal bonding, bacteria attachment, etc.) under various environmental conditions on the macro-scale mechanical behavior of soil. This is applicable to the MICP process carried out through various introduction methods of urease active bacteria including injection, pre-mixing, and in-situ enrichment. Therefore, the current study is crucial as it provides a deeper understanding of MICP treatment for site conditions, which is crucial before the technique can be applied successfully in the field.

Materials and Methods

Soil used

The soil used in the current study was silica sand obtained from Cook Industrial Minerals Pty Ltd, Western Australia. A number of four different particle size distributions, i.e. three poorly-graded and one well-graded, were utilized (Fig. s1). The characteristics of the sand used are listed in Table 1. Sand-1 (uniform sand with 99.6% less than 0.425 mm) was used to investigate the influence of urease enzyme concentration, degree of temperature, rainwater flushing, and oil contamination. Sand-2 (uniform sand with 99.6% less than 0.15 mm), Sand-3 (uniform sand with 99.9% less than 1.18 mm), and Sand-4 (well-graded sand with grain size ranges from 0.053 mm to 2.36 mm) were used to study the impact of freeze-thaw weathering.

Laboratory set-up

The laboratory setup comprised of polyvinyl chloride (PVC) column (45 mm in diameter and 180 mm in length) which was cut into half vertically and then glued together using a silicon glue with an inlet (top) connected to a peristaltic pump to allow injection of the reagents into the PVC column and an outlet (bottom) affixed to a U-type tubing. Scour pads and 10 mm thick gravel were placed at the top and bottom of the column, acting as filters to facilitate the spread of the solution during treatment and to prevent the fine sand particles from being flushed out during the treatment cycles. The sand was packed into the column in three consecutive layers, ensuring that each layer was compacted evenly to achieve at least 95% of the maximum dry density (Table 1) so as to maintain consistency of experiments.

Bacterial suspension and cementation solution

The urease active bacteria used in the current experiments were *Bacillus sp.* isolated from a previous work carried out by Al-Thawadi and Cord-Ruwisch (2012). The bacteria were cultivated in a sterile aerobic batch growth medium consisting of 20 g/L yeast extract, 0.17 M ammonia sulphate, and 0.1 mM $NiCl_2$, with a pH value of 9.25. The cultivated bacteria were collected at the stationary phase of the culture growth after 24 hours of incubation at 28°C. The optical density (OD_{600}) of the harvested culture varied between 2–2.5, and the urease activity was approximately 10 U/mL (1 U = 1 μmol urea hydrolyzed per minute). The culture was stored at 4°C refrigerator for no longer than one week prior to use. The cementation solution used in the present study consisted of 1 M anhydrous calcium chloride (110.98 g/L) and 1 M urea (60.06 g/L).

MICP treatment process

Except elsewhere stated, the process of MICP treatment was as follows. The PVC column was fully saturated with water at 100% degree of saturation prior to MICP treatment to ensure a relatively controlled flow field during the treatment injections. This was carried out using the upward flow method that facilitates the removal of the air voids from inside of the soil sample. Then, the MICP treatment was employed using the down flow injection method. In order to keep the soil sample fully saturated throughout the treatment, the water level in the external U-type tubing attached to the bottom of the PVC column was maintained equally to the top of the PVC column.

A two-phase injection scheme was employed for the MICP treatment (except elsewhere stated) by injecting a half void volume of bacteria culture followed by injecting a half void volume of cementation solution (bacterial placement phase), as recommended by Martinez et al. (2013) and Cheng and Cord-Ruwisch (2014). Then the column was cured for 24 hours to allow for bonding of bacteria with the sand particles. Full void volume of cementation solution was then injected into the sand column and was left to cure for 24 hours to allow for calcite precipitation. For each individual sand column, treatment through the cementation solution was repeated several times to gain different degrees of cementation, and the ammonium content and bacterial activity in the effluent of each treatment was monitored.

Determination of $CaCO_3$ content

The calcium carbonate content of treated soil samples was determined by adding solution of 2 mL of 2M hydrochloric acid (HCl) to 1–2 g of dry crushed cemented sample, and the volume of CO_2 gas was then measured using a U-tube manometer under standard conditions of 25°C at 1 atm (Whiffin et al. 2007). The actual amount of $CaCO_3$ was calculated based on an established relationship between the volume of CO_2 gas and amount of pure analytical grade $CaCO_3$ powder.

Soil Properties Investigated

Permeability

The permeability tests were carried out using the constant head permeability method in accordance with the Australian Standards (2001). The tests were conducted inside the PVC column, where the treated soil samples were still intact, instead of using the standard mold specified in the guidelines. The permeability of soil samples was taken before and after treatment.

Unconfined compressive strength (UCS)

After MICP treatment, the treated sand samples were flushed with at least five void volume of tap water to wash away all excess soluble salts prior to UCS experiments. Before the tests were carried out, the sand samples were dried at 105ºC for at least 24 hours. The UCS tests were conducted on specimens with selected aspect diameter-to-height ratios of 1:1.5 to 1:2. The axial load was applied at a constant rate of 1.0 mm/min.

Microstructure analysis

To characterize the shapes and location of precipitated $CaCO_3$ and to investigate the bonding behavior between the grain hosts and bio-cementation agent, microscopy analysis was conducted on the bio-treated soil samples. Before conducting the microscopy investigation, all bio-treated samples were flushed with tap water and dried at 60°C for 24 hours, and were then crashed to small pieces and coated with gold under vacuum conditions. The microscopy investigation was carried out using the available scanning electron microscopy (SEM), PHILIPS XL20 scanning electron microscope (Eindhoven, The Netherlands).

Environmental Parameters Investigated

In order to investigate the influence of various environmental conditions on the mechanical and geotechnical response of bio-cemented soils, some key parameters including the urease enzyme concentration (i.e. bacterial cell concentration), degree of temperature, rainwater flushing, oil contamination, and freeze-thaw cycles were examined and procedure of each parameter is explained below.

Urease enzyme concentrations

Hammad et al. (2013) observed that more rapid $CaCO_3$ crystals were formed under higher urease activity in agar plates. Earlier study carried out by Nemati et al. (2003) indicated that an increase in the urease concentration enhances the extent of $CaCO_3$ precipitation. However, no study is available in the literature directly linking the effect of different urease enzyme concentrations on the effective crystal bridging formation and the final strength of bio-cemented soils. In the present study, by either diluting the raw bacterial culture (10 U/mL) with deionized (DI) water or concentrating it via centrifuge, a series of bacterial culture with different urease enzyme concentrations (indicated by various levels of urease activity of 5, 10 and 50 U/mL) was obtained and utilized for bio-cementation of Sand-1. The treatment process followed the same procedure of that used for the raw bacterial culture, as stated earlier. The urease activity of bacterial culture was determined by measuring the urea hydrolysis rate of the tested bacterial culture. This was made by recording the change in the ammonium concentration of mixture over 30 mins of reaction period, which consisted of 10 ml of 3 M urea solution, 2 ml of bacterial culture, and 8 ml of DI water. The ammonium concentration was determined using the Nessler method (Greenburg et al. 1992), and the correlation between the UCS and $CaCO_3$ content of bio-cemented soils was examined using different urease enzyme concentrations (i.e. urease activities).

Temperature

The subsurface temperature surrounding MICP treated soils was previously investigated by Ng et al. (2012). It was found that the urease activity, which leads to the rate of $CaCO_3$ crystal precipitation, was increased with the increase in temperature from 10°C to 60°C. Nemati et al. (2003) demonstrated that an increase in temperature (from 20ºC to 50ºC) enhanced both the production rate of $CaCO_3$ and extent of the cementation solution conversion in a batch system. However, the effectiveness of crystals formed at different degrees of temperature was not investigated. In the current study, three different temperature scenarios were examined for Sand-1. The temperature values were selected to simulate the subsurface soil temperature in cold regions (4°C), tropical regions (25°C), and arid regions (50°C). During the process of MICP treatment, the sand samples used were placed as follows: (1) inside 4°C refrigerator; (2) room temperature of 25°C; and (3) inside 50ºC oven, and the UCS and SEM analysis were conducted for the bio-treated samples.

Rainwater flushing

In the current study, tap water (pH ranges from 6.8-7.2) was used to simulate the effect of heavy rain with rainfall intensity of 50 mm/h (National Meteorological Library 2015) on the

MICP process. Sand columns made from Sand-1 were subjected to tap water flushing for about 12 hours. The tap water flushing was conducted immediately or 24 hours after the bacterial placement, which was consisted of sequential injections of a half void volume of bacterial culture (45 mL) and a half void volume of cementation solution (45 mL), as stated earlier. All sand columns were then treated with cementation solution (full void volume) for two times. After treatment, the sand samples were extracted from the PVC columns (by gently cutting the silicon glue and opening the PVC column for sand column extraction) for final UCS (if applicable) and $CaCO_3$ content measurements.

Oil contamination

Hydrocarbon contaminants from oil exploration, transportation, production, and processing can change the behavior of soil and may alter the soil engineering properties, resulting in safety issues in civil engineering structures (Nicholson and Tsugawa 1997; Shroff et al. 1998). In the current study, for the first time, the stabilization of oil-contaminated soil using MICP technology was investigated and evaluated through measurements of UCS and $CaCO_3$ content.

Dried Sand-1 was contaminated with 5% diesel engine oil (w/w), which obtained a maximum dry density of 17.8 kN/m^3 and corresponding optimum moisture content of 7%. The oil-contaminated soil was treated using two procedures, including the two-phase injection method as described above and the premixing method in which bacterial culture was premixed with sand prior to the application of cementation solution. The latter process will be further explained later.

Freeze-thaw cycles

Destruction of porous materials caused by freezing and thawing has been of a great concern to engineers for more than 200 years (Johnson 1952). As suggested by Litvan (1980), soil grain size distribution and permeability affect soil durability against the freeze-thaw (FT) cycles. Therefore, in the present study, sand samples with three different grain size distributions (i.e. Sand-2 to Sand-4, see Fig. 1 and Table 1), and different permeability values (stated in the result section) were examined. Each bio-cemented sand column was cut into half to obtain diameter-to-height aspect ratios of 1:1.5 to 1:2, followed by exposure to 4-FT and 10-FT cycles prior to UCS experiments. Each procedure of FT cycle was subjected to a 12h freeze at –14°C followed by a 12h thaw at the room temperature (25±1°C). To ensure that full saturation was achieved, all treated samples were completely immersed in water throughout the FT cycles. The effect of FT cycles on the bio-cemented samples was evaluated by comparing the UCS values before and after the FT cycles.

Results and Discussion

Effect of urease enzyme concentrations

The influence of different urease concentrations on UCS of bio-cemented soil samples was examined by plotting the measured UCS values against $CaCO_3$ contents (Fig. 1a). It can be seen that for all urease activities, the UCS increases exponentially with the increase of the $CaCO_3$ content, which is in line with previous results reported by van Paassen et al. (2010b) and Cheng et al. (2013). It can also be seen that for the same amount of $CaCO_3$ precipitation, the UCS of bio-cemented soil treated with higher urease activity is less than that of the samples treated with lower activity. For instance, at an amount of 0.04 g/g $CaCO_3$ precipitation, the sand sample treated with 50 U/ml urease activity achieved UCS value of around 250 kPa, whereas the sand samples treated with 10 and 5 U/ml urease activity achieved higher UCS values of around 500 and 850 kPa, respectively. This suggests that the

$CaCO_3$ formed slowly, which is due to the slower hydrolysis of urea caused by the lower urease activity, are more effective to form "bridges" that bond the sand grains together in a more effective way. It should be noted that the efficiency in UCS improvement was only slightly improved using the 2 U/ml activity compared to the 5 U/ml (data not shown), and the use of such low urease activity required a significant extension of treatment period to allow for completion of the MICP reaction.

In Fig. 1b, the measured permeability values of soil samples after MICP treatment were normalized with the initial permeability values and plotted against the $CaCO_3$ content. As can be seen, all samples show a reduction in permeability with the increase in the amount of $CaCO_3$ precipitation. Fig. 1b also indicates that the reduction in permeability of bio-cemented samples was mainly controlled by the amount of $CaCO_3$ precipitation, whereas the level of urease activity used in treatment has a minor impact.

The micro-feature of precipitated crystals within the sand matrix was examined using SEM (Fig. 2). It can be seen in Fig. 2(a-c) that the $CaCO_3$ produced at high urease activity formed small crystals of a typical crystal size of 2-5 μm. The agglomerated small crystal precipitates formed thin coating layers ($CaCO_3$ envelope) with a thickness of around 5 μm covering the individual sand grain surface and bridging the adjacent sand grains. This type of crystal precipitation might not be strong enough to bear high shear strength, which is probably due to the thin and weak "bridging layer" and remaining large gaps between the sand grains. In contrast, the crystals produced at low urease activity were agglomerated and formed large clusters of size of around 20-50 μm [Fig. 2(d-f)]. One important feature in this regard is that although large crystal clusters precipitated on the sand grain surface, the gaps between the sand grains were almost completely filled with crystals (Fig. 2d). It should be noted that the samples presented in Fig. 2 demonstrate similar UCS values but have different $CaCO_3$

contents, suggesting that the crystals pattern formed at lower urease activity is more effective in gaining strength.

It has been reported in the literature that there are two mechanisms for crystal precipitation in MICP (Stocks-Fischer et al. 1999; DeJong et al. 2006). The first mechanism is that bacterial cells act as nucleation sites for $CaCO_3$ precipitation. The second mechanism is that the urea hydrolysis produces CO_3^{2-} ions and raises the pH around the cells, favoring the precipitation of $CaCO_3$ by lowering the solubility. In the current study, it is apparent that development of $CaCO_3$ is vastly different under different urease activities, in terms of location, size, and shape of crystals. Because the same cementation solution was applied to all samples, it was expected that the bacterial urease activity would be the only factor that can have an impact on the precipitation patterns.

An explanation of the noted difference in the precipitation patterns may be attributed to the competition between the crystal growth and crystal nucleation. Gandhi et al. (1995) reported that the nucleation of new crystals would compete with the process of crystal growth if nucleation of new crystals prevails over the growth of those exist. In case of high urease enzyme concentration, a high number of bacterial cells were introduced to the soil samples, which were accumulated at the pore “throat” (contact points) and also attached to the sand grain surface. With the presence of abundant bacterial cells acting as nucleation site, the produced carbonate ions may be consumed mainly by nucleation of new $CaCO_3$ crystals rather than growth of existing crystals, resulting in an abundance of small crystals. These numerous small crystals would develop to form dense crystal layers with continuous supply of cementation solution. In contrast, in case of low urease enzyme concentration (i.e. low urease activity/bacteria concentration), the nucleation site was limited by the small number of bacterial cells. Otterstedt and Brandreth (2013) stated that the final crystal size is inversely related to the number of nuclei. For low urease activity, the presence of high concentration of calcium (i.e. 1 M in the current study) and increased super-saturation in the solution due to urea hydrolysis would result in accumulating of precipitation over the initial small crystals, leading to formation of larger crystal sizes rather than new small crystals. It is worthwhile noting that using different ureolytic bacteria, with either higher or lower specific urease activity (urase activity per cell) compared to the current strain, might lead to different results to those presented herein. This is because in order to obtain the same urease activity as obtained in the current study, different amount of biomass might be required, leading to different number of nucleation site and hence, affecting the patterns of precipitated crystals potentially.

The findings obtained from this study indicate that the efficacy (strength per mass of calcite) of larger calcite crystals precipitated at the gaps between the sand grains is higher than that obtained from smaller crystals randomly precipitated throughout the sand matrix. This is consistent with the described phenomena in the literature. For example, Cheng et al. (2013) found that bridging crystals were formed by restricting the pore water at the contact points between the sand grains, showing more efficiency in terms of strength improvement compared to crystals randomly precipitated. Al Qabany and Soga (2013) demonstrated that using cementation solution of low concentration may yield to higher sample strengths, which may be attributed to the larger amount of precipitation at the particle contacts, similar to the findings of Okwadha and Li (2010) and Cheng et al. (2014). In particular, the significance of calcium carbonate precipitation pattern on properties of MICP treated soils was highlighted by many researchers (e.g., Al Qabany and Soga 2013; Okwadha and Li 2010; Cheng et al. 2013). Martinez and DeJong (2009) reported that the degradation of bio-cemented sand particles at the micro scale level was governed by the calcite bonds between the silica sand grains. Accordingly, it was expected that the shear strength of sand may not be directly proportional to the amount of calcite content. Those crystals filling the gaps between the sand grains and forming effective bridges contribute to the pathway of load transferred between the soil particles; thus, improve the soil stiffness and strength.

Effect of degree of temperature

The effect of various degrees of temperature equal to 4, 25 and 50°C on strength improvement of bio-cemented soil was examined and presented in Fig. 3. It can be seen that all UCS values of bio-treated sand samples were exponentially increased with the increase in $CaCO_3$ content, irrespective of the applied temperature. However, the strength improvement as per the amount of crystals produced was higher at 25°C compared to that at temperature of either extremely low (4ºC) or high (50ºC). The crystals formed at the highest temperature of 50ºC was the least efficient to gain strength improvement.

The crystals microstructure analysis indicates that MICP treatment at 50ºC results in crystal distribution over the entire sand grain surface, with a typical individual crystal size of 2-5 µm [Fig. 4(e-f)]. For such samples, the individual $CaCO_3$ crystals possessed similar size and were well-distributed spatially and also covered the surface of the sand grains as a coating-like layer. However, the sand grains were not effectively connected because of the remaining large gaps [Fig. 4(e-f)]. For samples treated at the ambient temperature, it was found that the average crystal size increased by 10 times (individual crystals size between 20 to 50 µm) compared to that formed at 50ºC. These large crystals were found to precipitate on the grain surface, covering the contact areas of the sand grains [Fig. 4(c-d)]. This type of crystals distribution pattern was also found in the samples treated at 4ºC, and small crystal size was observed [Fig. 4(a-b)].

The kinetics of crystallization indicates that activated energy, which is a function of temperature and relative supersaturation degree, has a strong impact on the rate of nucleation and crystal growth. It is the relationship between the competing kinetic rates of nucleation (birth of new crystal nuclei) and crystal growth (increase in size of crystals) that determines the size distribution of crystals. The higher the temperature the lower the activation energy barrier as well as the faster the rate of nucleation of $CaCO_3$ precipitation (Wojtowicz 1998). The faster nucleation rate may induce excess nucleation sites, which would cause smaller average crystal size as it was found in the samples treated at 50ºC. It is worthwhile noting that although the nucleation rate was low at low temperature of 4ºC, small size of crystals was also observed. This is possibly due to the slow crystal growth at low temperature due to the low relative supersaturation degree as a result of the low urease activity at low temperature (Sahrawat 1984). A decrease in the relative supersaturation led to a decrease in both the crystal nucleation rate and growth rate. As stated earlier, the final crystal size distribution is dependent on the competition between these two rates.

The precipitation of $CaCO_3$ in MICP is a very complex process because of the involvement of bacteria acting as nucleation site and CO_3^{2-} ion producer. Therefore, if different amount of bacteria is applied, the precipitation pattern can be different from what was presented in the current study. It is evident from Fig. 2(a-c) that large amount of bacteria (high activity 50 U/ml) at the ambient temperature would result in abundant nucleation sites (numerous bacterial cells) and high relative supersaturation degree (high urease activity). As a result, the precipitation pattern obtained under this environmental condition could be similar to that obtained at 50ºC with low amount of bacteria (10 U/ml) [Fig. 4(e-f)]. Generally, temperature can affect many physical, chemical, and biological properties of MICP system. For example, temperature can affect the urease activity, which in turn influences the urea hydrolysis rate, CO_3^{2-} production rate, and consequent crystal growth rate. The solubility of $CaCO_3$ crystals also varies with temperature. Therefore, further investigation on this complex process is worthwhile to carry out in future development of the current study. Overall, based on the results obtained from the current study of different urease concentrations and temperatures, it can be concluded that large size crystals located at the gaps between the sand grains are deemed to be effective crystals, which contribute the most to the strength gain of bio-cemented soils.

Effect of rainwater flushing

In the current study, tap water flushing was made to determine the impact of intensive rainfall towards $CaCO_3$ precipitation and corresponding soil stabilization. The control sample (i.e. no water flushing) was fully cemented (Fig. 5a). In contrast, the samples encountered with water flushing during the treatment process were partly cemented or completely non-cemented [Fig. 5(b-c)]. For the control sample, the chemical conversion efficiency, which is defined as the percentage of injected urea and calcium chloride that precipitate as $CaCO_3$, was found to reduce from about 95% to 50% throughout the MICP process (Fig. 6a). The decrease in chemical conversion efficiency was probably explained by the loss of urease activity due to the encapsulation of the bacterial cells by precipitated crystals. The water flushing caused significant decrease in the chemical conversion efficiency down to less than 5%, irrespective of the waiting period applied to the bacterial attachment. The negative impact of water flushing on bio-cementation was also demonstrated by the low degree of cementation and final $CaCO_3$ content measurements. Less than 0.3% (0.003 g/g sand) of crystals content was detected in the samples subjected to water flushing, whereas 10 times more (0.043 g/g sand) crystals were found in the control sample with a detectable UCS of about 260 kPa (Fig. 6b).

The low chemical conversion efficiency and $CaCO_3$ precipitation after water flushing may be due to the washout of bacteria and substrate. For successful bio-cementation, it is necessary that the bacteria are introduced into the soil first and immobilized, then followed by application of cementation solution that contains urea and calcium chloride. Many authors have shown that the adsorption of bacteria increases with increasing the salinity of bacterial suspension (Scholl et al. 1990; Torkzaban et al. 2008). By flushing low salinity solutions after the bacterial suspension, a large part of the adsorbed bacteria can be remobilized from the solid surface into the liquid phase (Harkes et al. 2010). This suggests that in real application the rainwater flushing during MICP bio-cementation can impact the effectiveness of MICP treatment by washing out the immobilized bacterial cells and reducing the chemical conversion efficiency. In order to overcome this problem, a new way of bacterial fixation, which can prevent bacterial cells from being washed out by the low salinity solution (i.e. rainwater) will be investigated in future work.

Effect of oil contamination

Oil contamination can alter the soil engineering properties, resulting in safety issues in relation to civil engineering infrastructures. Also, oil-contaminated soil represents the worst case scenario of hydrophobic soil particle surface property, which might have a negative impact on bacteria immobilization. As expected, the initial trial of stabilization of oil contaminated soils using MICP via the normal two-phase injection method did not succeed (data not shown). This suggests that the traditional bacterial immobilization method does not work effectively for oil-contaminated soils due to the hydrophobic surface of oil-contaminated soil particles, resulting in poor bacterial attachment. In fact, the impact of hydrophobic oil on bacteria attachment is a function of the bacterial culture properties. The bacterial cells (i.e. oil-degrading strain), which are hydrophobic can attach to the hydrophobic surfaces (Rosenberg 2006); however, bacteria with hydrophilic characteristics prefer hydrophilic surface (An and Friedman 1998). In order to improve the bacterial retention, premixing of bacteria with soil in the presence of 100 mM $CaCl_2$ was applied. The presence of $CaCl_2$ acted as flocculent and induced cells coagulation. This coagulation of bacterial cells was found not to significantly lower the urease activity, as reported by Al-Thawadi (2008). To provide a sufficient urease activity, 180 mL of bacterial culture containing 100 mM $CaCl_2$ was used and concentrated into 25 mL volume using centrifuge. The concentrated bacterial flocs were then mixed with the oil-contaminated soil to reach the optimum moisture content. The mixture of bacterial flocs and soil was compacted thoroughly in the PVC column to gain the maximum dry density, which was then repeatedly treated 5 times by the cementation solution.

The UCS results indicated that by increasing the $CaCO_3$ content from 0.035 to 0.054 g/g sand, strength of oil contaminated soil was improved significantly from about 150 kPa to 400 kPa (see Fig. s2). Accordingly, soil stiffness was also improved from 10.7 MPa to 68.3 MPa. The failure mechanisms of bio-treated oil contaminated soil were consistent with previous observations on cemented pure silica sand (Cheng et al. 2013). For the weak sample of UCS = 150 kPa, the broken cores completely lost strength at the grain scale around the failure plane. On the other hand, the strong sample of UCS = 400 kPa, longitudinal tensile cracks along the sample were clearly observed.

Effect of freeze-thaw cycles

Litvan (1980) found that porous solids with high porosity or permeability usually have a good service record after FT action. Cheng et al. (2013) found that microbially induced $CaCO_3$ crystals covered the connecting points between the sand grains and thus provided key bonding force within the soil matrix against the FT cycles. This bonding behavior might be different for different PSD sand samples. Therefore, before carrying out the FT cycles, it was necessary to investigate the mechanical behavior of different PSD sand samples after MICP treatment.

Fig. 7 shows that the UCS increases exponentially with the increase in the $CaCO_3$ content. At low level of $CaCO_3$ content (below 0.04 g/g sand), similar UCS improvement was obtained for all different PSD samples; however, the improvement varied at high level of crystal content (above 0.05 g/g sand). At high $CaCO_3$ content, Sand-3 showed the least effective strength improvement because it required the highest amount of crystals to gain strength similar to that of the other PSD samples. For the two types of uniform sand tested herein, the crystals were more effective in improving the strength of soil of smaller grain size, which is consistent with previous findings obtained by Ismail et al. (2002b). This is probably due to the increase in number of contact points, which provide better location for crystals to precipitate, and the decrease in stress acting per particle contact. For Sand-4 (well-graded), the effectiveness of crystal precipitation in terms of strength improvement was greater than that of the uniform coarse sand (i.e. Sand-3) but less than the uniform fine sand (i.e. Sand-2). According to Schiffman and Wilson (1958), the greater the percentage of the coarser fraction, the smaller the available grain surfaces for grout adhesion and the lower the internal tension in the grouted mass. Although the used well-graded sand has the highest density compared to the fine sand, it possesses less contact points and grain surface due to its large percentage of coarse particles. Therefore, the crystals formed for well-graded sand were less effective compared to those formed for fine sand.

The effect of FT cycles on the mechanical performance of different PSD sand samples treated with MICP is illustrated in Fig. 8. Generally speaking, it can be observed that an increase in the number of FT cycles is associated with a decrease in the compressive strength, for all uniform sand specimens (Fig. 8 (a-b)). The main reason for the strength decrease after FT cycles is due to the formation and growth of micro-cracks generated due to the tensile stresses around the soil particles when the pore water turns into ice during the freezing process. In theory, higher porosity and permeability allow more rapid water mass transfer in the sand matrix, which can increase the FT resistance. However, in the current study, the fine uniform sand (0.15 mm in size) of less porosity and smaller pore size was more durable against the FT cycles compared to the coarse sand (1.18 mm in size) of higher porosity and larger pore size. Smith et al. (1929) found that the average number of contacts per sphere increases with the decrease in porosity. Therefore, for finer sand, the larger number of inter-particle contact points favored more bridging crystals formed at the contact points, which reduced the acting tensile stress per particle contact; hence, resulted in higher durability. Fig. 8c shows that the FT cycles have a minor impact on the well-graded sand. The main reason for the high durability of MICP well-graded sand is probably due to the unique characteristics of having high number of inter-particle contact points (attributed to the presence of fine sand) and high permeability, as well as large pore size (attributed to the presence of coarse sand). Overall, it can be concluded that the influence of FT cycles on soil strength and durability depends on the soil porosity, pore size, and bonding behavior of MICP in the soil matrix.

Implication for civil engineering applications

In civil engineering applications such as soil stabilization for transportation subgrades and embankments, adequate MICP strength improvement can be achieved by forming sufficient $CaCO_3$ bonds between the soil grains. The ability to produce $CaCO_3$ crystals with high efficacy is thus highly desirable for cost minimization. The findings obtained from the current study show that different urease activities can result in different micro-scale crystal precipitation patterns, leading to varied efficacy in obtaining the macro-scale strength values. This potentially has implications in terms of how MICP treatment can be applied more economically in engineering practice. Low level of urease activity associated with an optimum operating temperature could result in larger calcium carbonate crystals precipitated in the voids of soil grains, which can lead to a more effective strength improvement with less consumed chemicals.

In other civil engineering applications such as control of seepage-induced internal erosion of dams, it is important to immobilize the urease active bacteria and urease activity within the target zones so that sufficient $CaCO_3$ can be produced throughout the entire treated soil. The experimental observations shown in this paper indicated poor immobilization through the two-phase injection method after tap water flushing, which implies that the current bacteria fixation method might not be applicable to field application of MICP during rainy days. It is thus considered essential to develop a new bacteria fixation method, which can further extend MICP application to continuous water flow regions.

Soils at high latitude or elevation usually freeze during winter. Under this environmental condition, geotechnical engineering foundations that are exposed to FT cycles can be subject to significant structure damages. In such a case, FT cycles induce uneven stresses within the soil, resulting in a decrease in soil stability. Given the improvement in UCS values of the sand tested in the current study, it can be confirmed that MICP can be used as a viable solution to improve the properties of uncemented soils by creating cemented soil bodies that have high durability against FT cycles. The high durability of MICP treated soils against FT cycles is attributed to the sufficient contact points in the soil matrix and large pore size and permeability of soil. This characteristic enhances the efficacy of MICP cementing agents in bridging the particle-to-particle contacts, and in the meanwhile allows a rapid water mass transfer in the sand matrix.

Conclusions

This paper presented a series of experimental results in relation to the mechanical behavior of silica sand stabilized using microbially induced calcite precipitation (MICP) under different environmental conditions. The formation of large agglomerated crystal clusters filling the gaps between the sand grains showed high efficiency in improving the compressive strength of bio-treated soils. Such optimum crystal precipitation pattern was found to be obtained at low urease concentration and ambient temperature of 25°C. Trials of "rain" flushing and oil contaminated soil stabilization have clearly shown the bacterial attachment using the two-phase injection method can be deteriorated by flushing of low ionic strength water (i.e. tap water, rain water) or hydrophobic surface of sand grain particles (i.e. oil-contaminated soils). Alternatively, premixing of bio-flocs with oil-contaminated soil was able to achieve sufficient retention of bacteria and urease activity, leading to successful soil stabilization of significant UCS improvement. The FT cycle tests have shown that the durability of bio-cemented soils was varied depending on the particle size distribution. The soil matrix of well-graded sand allowed sufficient amount of contact points for effective crystals that create strong bonding, large pore size, and high permeability, leading to a rapid water mass transfer in the sand matrix and contributing to the resistance of the FT cycles.

Acknowledgement

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Supplemental Data

Figs. s1-s2 are available online in the ASCE library (ascelibrary.org)

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Captions:

Fig. 1. Effect of the different urease activity on (a) UCS and (b) permeability of the bio-cemented samples

Fig. 2. SEM images of bio-cemented samples treated with different urease activities (a-c: 50 U/mL, UCS = 713 kPa, and $CaCO_3$ content = 0.061 g/g sand; d-f: 5 U/mL, UCS=709 kPa, and $CaCO_3$ content = 0.039 g/g sand)

Fig. 3. Effect of the temperature on the UCS of the bio-cemented samples

Fig. 4. SEM images of bio-cemented samples treated at different temperatures (a-b: 4ºC, UCS = 108 kPa and $CaCO_3$ content = 0.021 g/g sand; c-d: 25ºC, UCS=245 kPa and $CaCO_3$ content = 0.028 g/g sand; e-f: 50ºC, UCS=121 kPa and $CaCO_3$ content = 0.034 g/g sand)

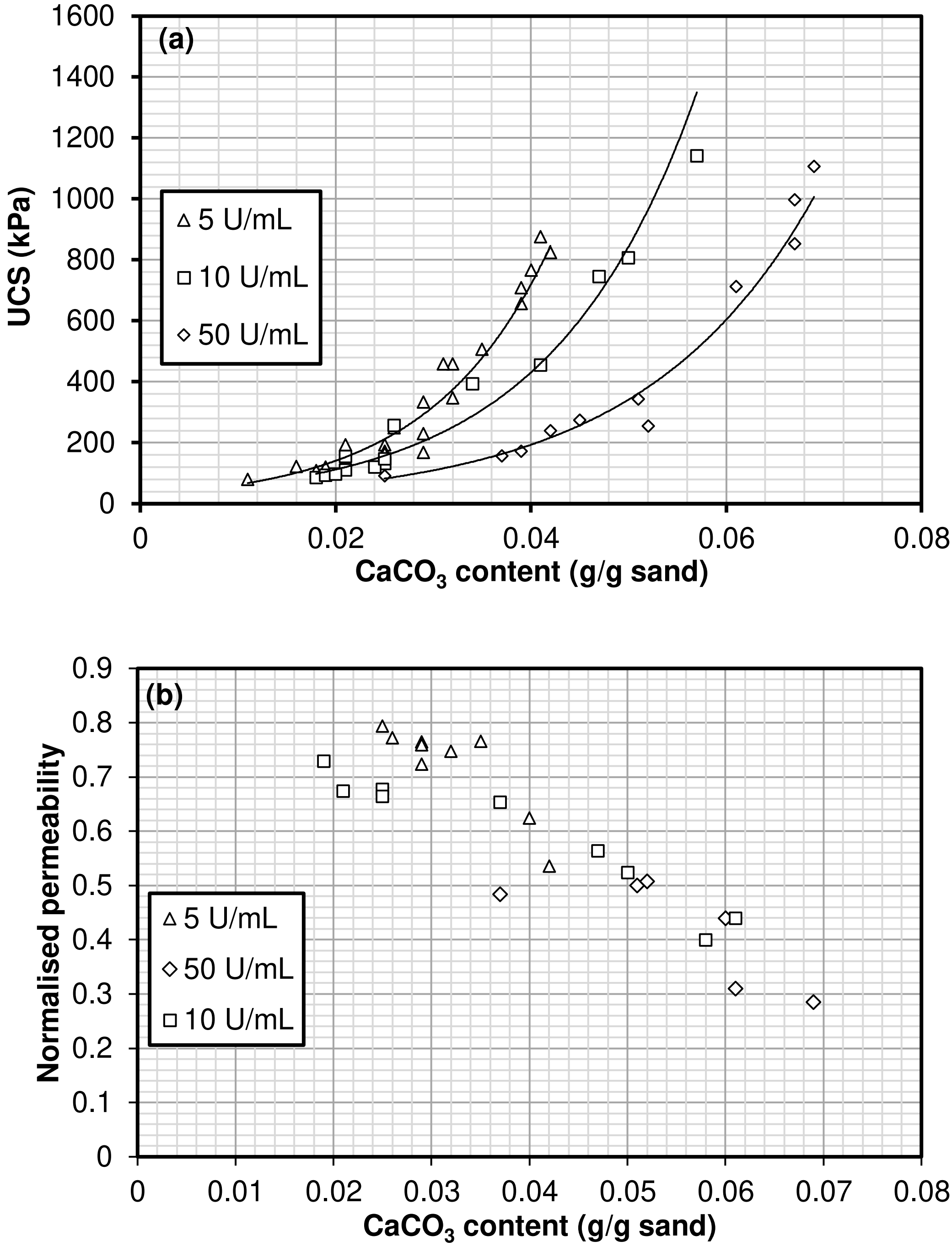
Fig. 5. Images of MICP treated samples (removed from the PCV columns prior to UCS test) subjected to water flushing during the treatment process: (a) control with no water flushing; (b) water flushing after 24 hours of bacterial placement; and (c) water flushing immediately after bacterial placement

Fig. 6. MICP treated samples subject to water flushing during the treatment process: (a) chemical conversion efficiency; and (b) UCS and $CaCO_3$ content

Fig. 7. UCS of different PSD sand samples treated with different amount of MICP

Fig. 8. Effect of FT cycles on UCS of different PSD sands treated with different amount of MICP: (a) 0.15 mm uniform sand; (b) 1.18 mm uniform sand; and (c) well-graded sand

Fig. 1.



(a)
UCS (kPa)
1600
1400
1200
1000
800
600
400
200
0
0
0.02
0.04
0.06
0.08
$CaCO_3$ content (g/g sand)
5 U/mL
10 U/mL
50 U/mL
(b)
Normalised permeability
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
0
0.01
0.02
0.03
0.04
0.05
0.06
0.07
0.08
$CaCO_3$ content (g/g sand)
5 U/mL
50 U/mL
10 U/mL

Fig. 2

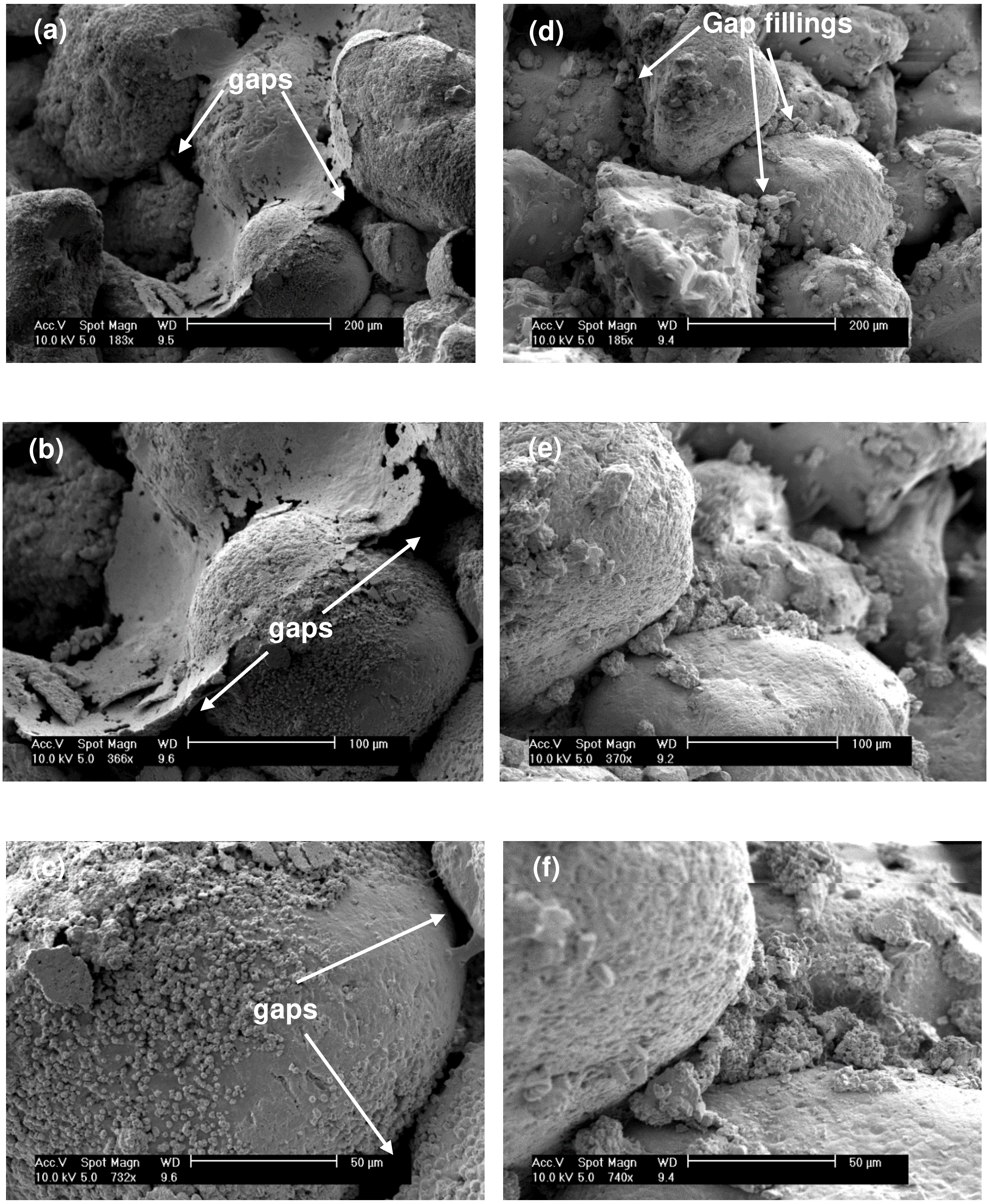
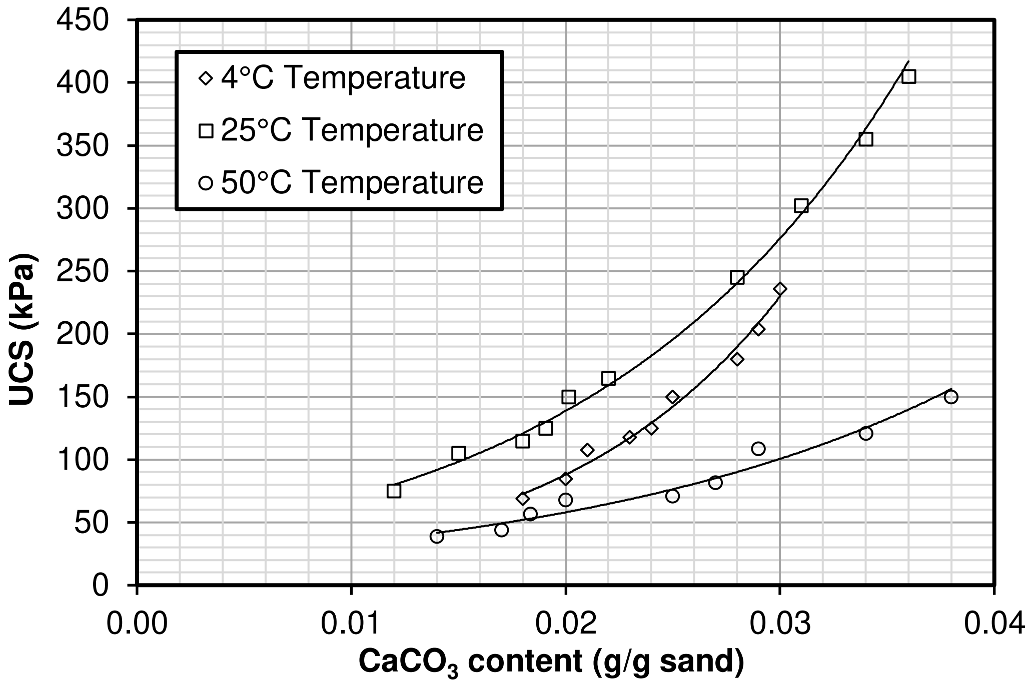

(a)
gaps
Acc.V Spot Magn WD 200 µm
10.0 kV 5.0 183x 9.5
(d)
Gap fillings
Acc.V Spot Magn WD 200 µm
10.0 kV 5.0 185x 9.4
(b)
gaps
Acc.V Spot Magn WD 100 µm
10.0 kV 5.0 366x 9.6
(e)
Acc.V Spot Magn WD 100 µm
10.0 kV 5.0 370x 9.2
(c)
gaps
Acc.V Spot Magn WD 50 µm
10.0 kV 5.0 732x 9.6
(f)
Acc.V Spot Magn WD 50 µm
10.0 kV 5.0 740x 9.4

Fig. 3.



4°C Temperature
25°C Temperature
50°C Temperature
UCS (kPa)
0
50
100
150
200
250
300
350
400
450
0.00
0.01
0.02
0.03
0.04
$CaCO_3$ content (g/g sand)

Fig. 4.

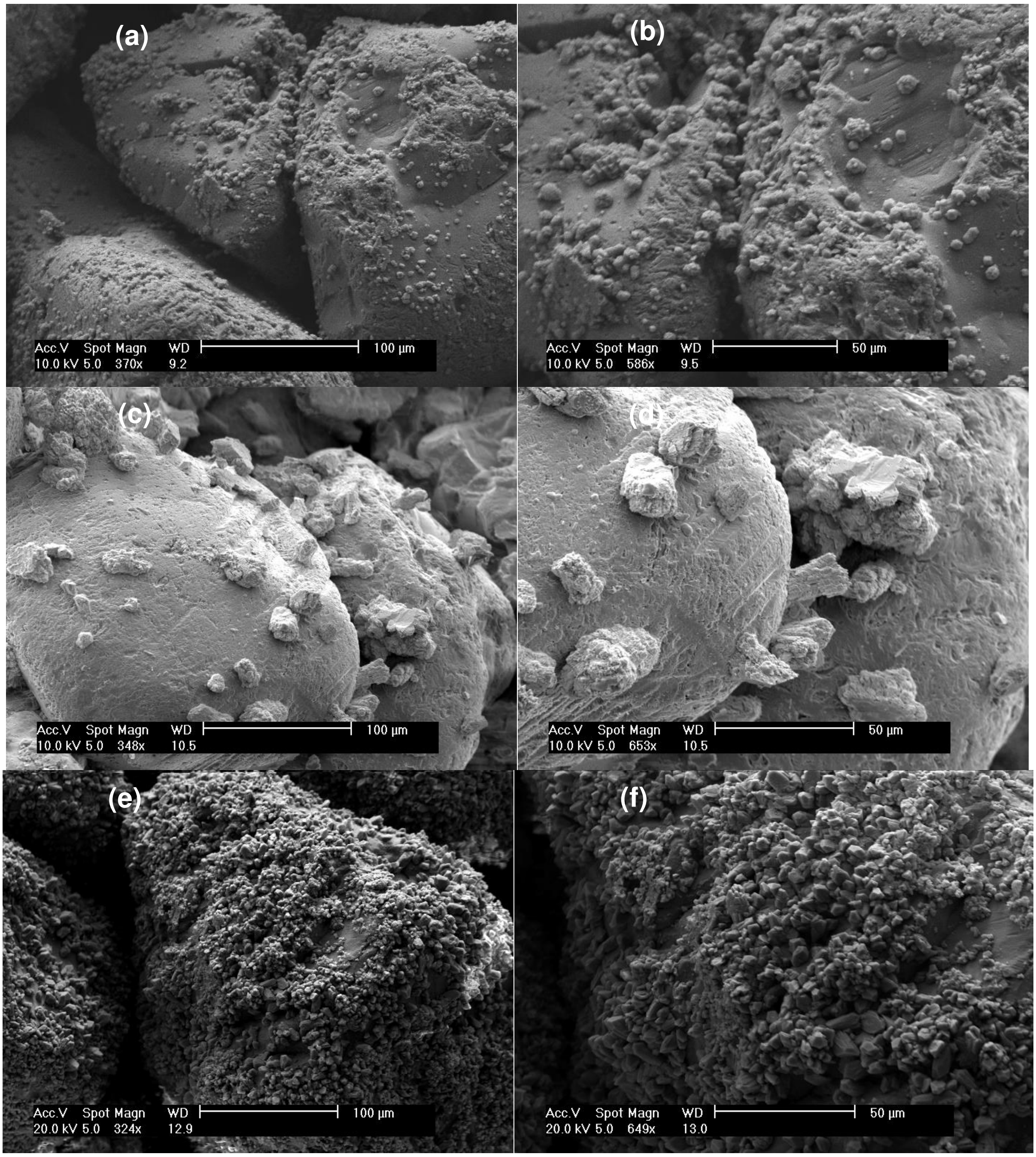

(a)
Acc.V Spot Magn WD 100 µm
10.0 kV 5.0 370x 9.2
(b)
Acc.V Spot Magn WD 50 µm
10.0 kV 5.0 586x 9.5
(c)
Acc.V Spot Magn WD 100 µm
10.0 kV 5.0 348x 10.5
(d)
Acc.V Spot Magn WD 50 µm
10.0 kV 5.0 653x 10.5
(e)
Acc.V Spot Magn WD 100 µm
20.0 kV 5.0 324x 12.9
(f)
Acc.V Spot Magn WD 50 µm
20.0 kV 5.0 649x 13.0

Fig. 5

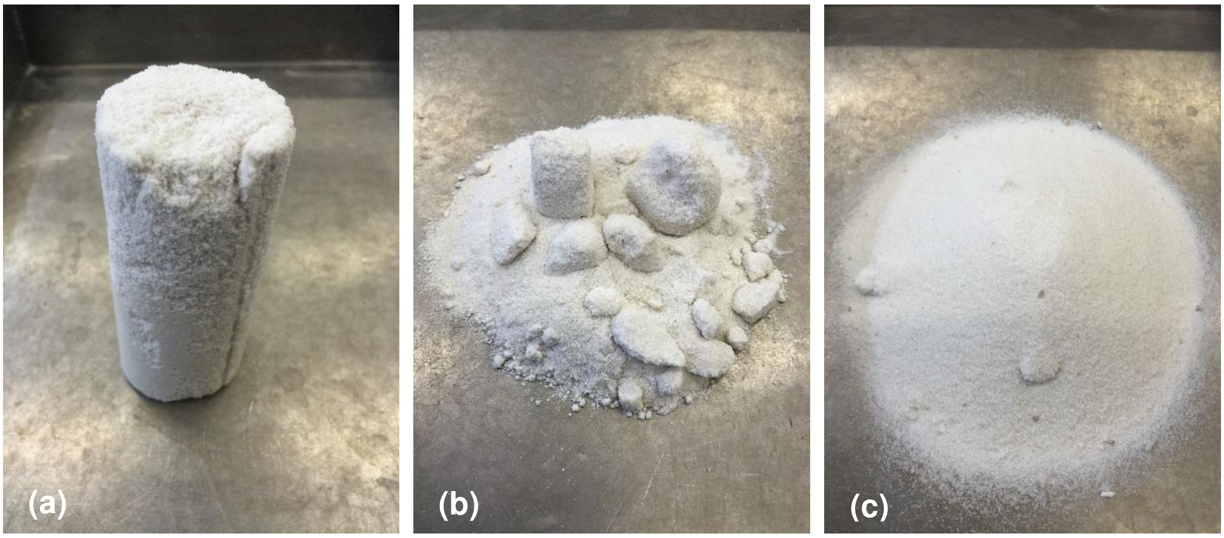
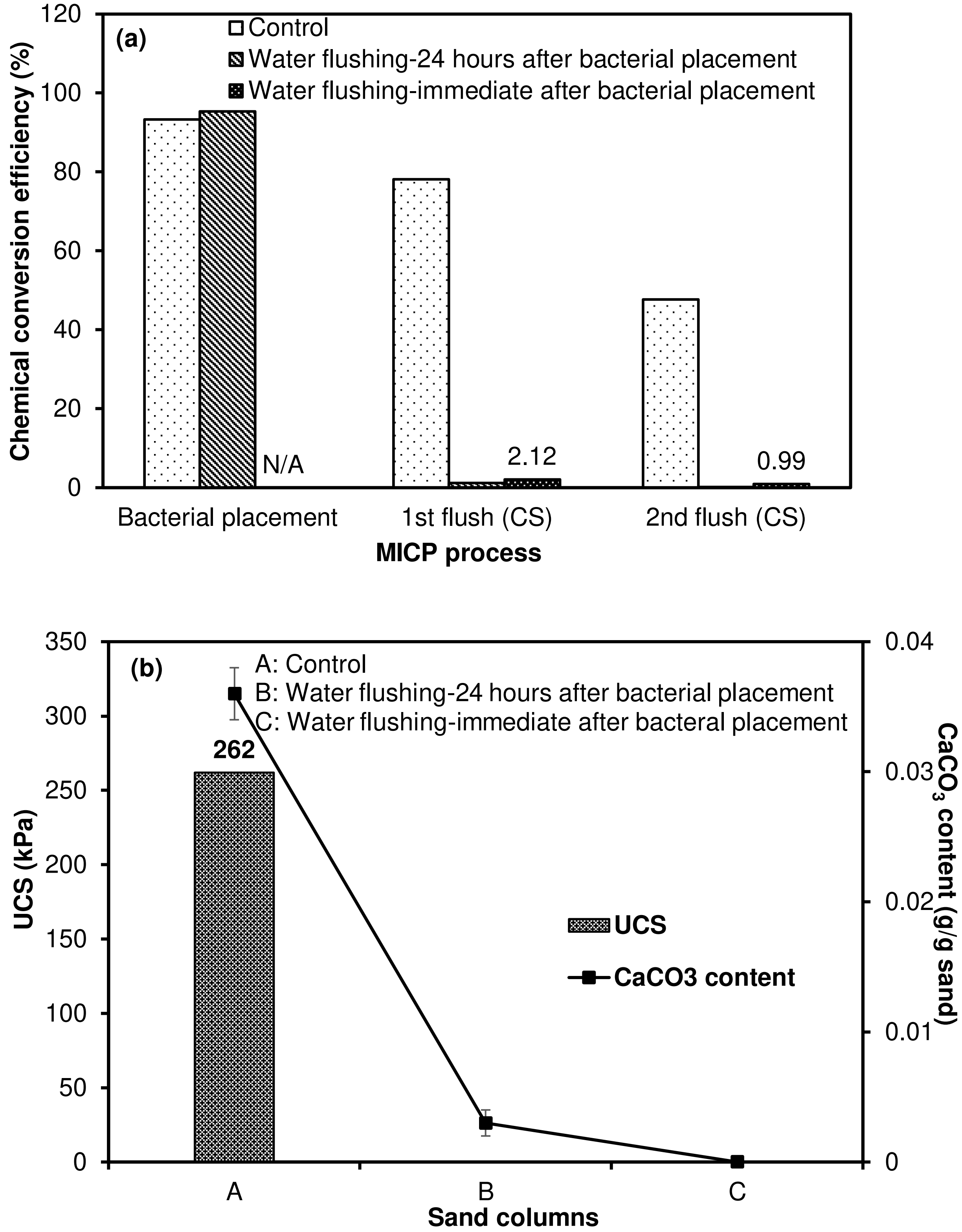
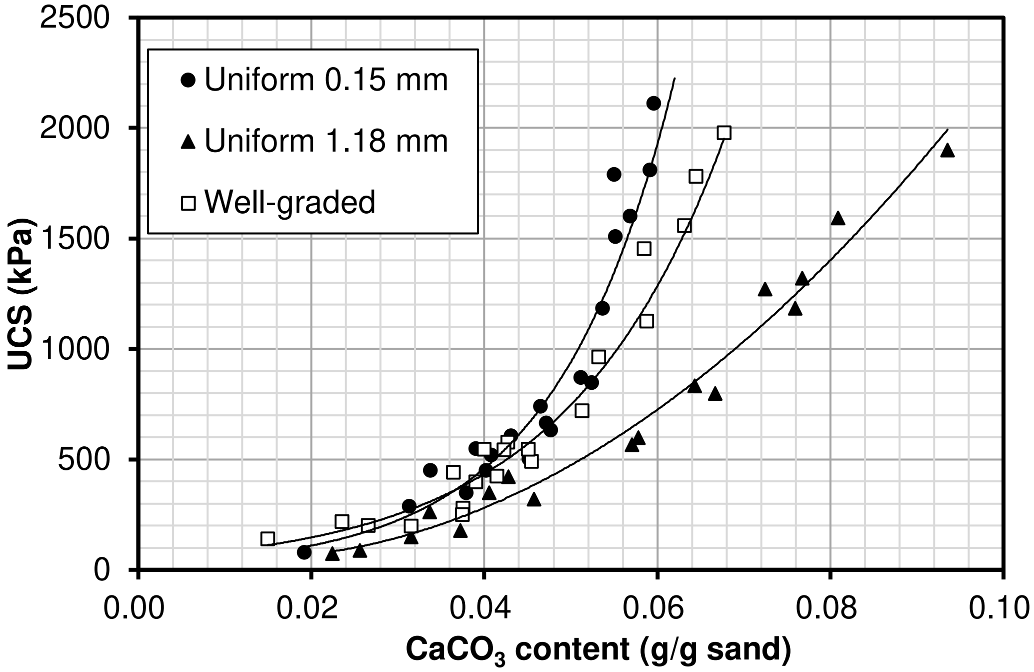


Fig. 6



(a)
Control
Water flushing-24 hours after bacterial placement
Water flushing-immediate after bacterial placement
Chemical conversion efficiency (%)
120
100
80
60
40
20
0
N/A
2.12
0.99
Bacterial placement
1st flush (CS)
2nd flush (CS)
MICP process
(b)
A: Control
B: Water flushing-24 hours after bacterial placement
C: Water flushing-immediate after bacteral placement
262
UCS
CaCO3 content
UCS (kPa)
350
300
250
200
150
100
50
0
0.04
0.03
0.02
0.01
0
CaCO3 content (g/g sand)
A
B
C
Sand columns

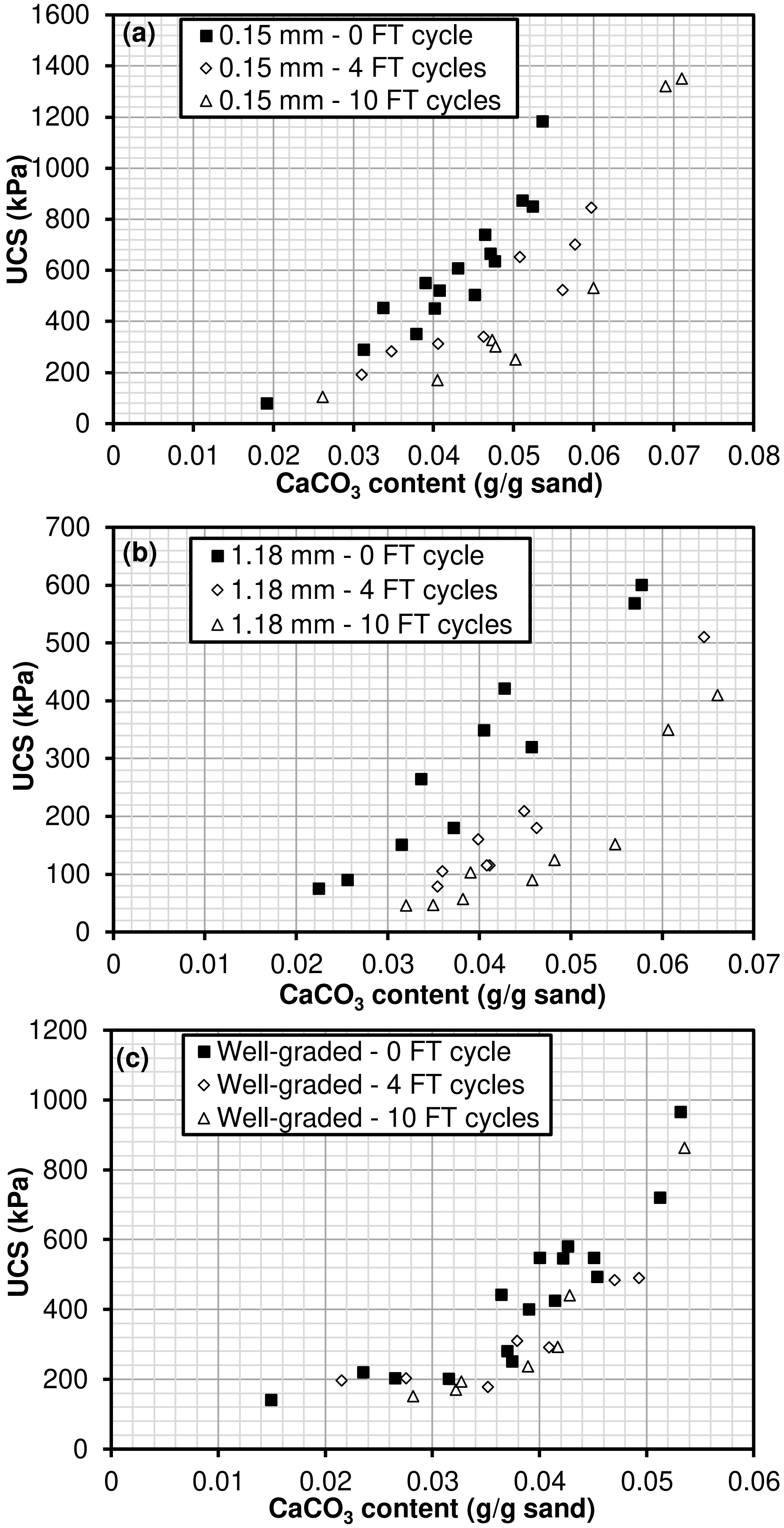
782 Fig. 7



783

784

Fig. 8


(a)
0.15 mm - 0 FT cycle
0.15 mm - 4 FT cycles
0.15 mm - 10 FT cycles
UCS (kPa)
0
200
400
600
800
1000
1200
1400
1600
0
0.01
0.02
0.03
0.04
0.05
0.06
0.07
0.08
CaCO3 content (g/g sand)
(b)
1.18 mm - 0 FT cycle
1.18 mm - 4 FT cycles
1.18 mm - 10 FT cycles
UCS (kPa)
0
100
200
300
400
500
600
700
0
0.01
0.02
0.03
0.04
0.05
0.06
0.07
CaCO3 content (g/g sand)
(c)
Well-graded - 0 FT cycle
Well-graded - 4 FT cycles
Well-graded - 10 FT cycles
UCS (kPa)
0
200
400
600
800
1000
1200
0
0.01
0.02
0.03
0.04
0.05
0.06
CaCO3 content (g/g sand)