Journal of GeoEngineering, Vol. 15, No. 2, pp. 79-88, June 2020
http://dx.doi.org/10.6310/jog.202006_15(2).2

STRENGTH IMPROVEMENT OF A SILTY CLAY WITH MICROBIOLOGICALLY INDUCED PROCESS AND COIR FIBER

Fuchen Teng [1*], Colette Ouedraogo [2], and Yong-Cheng Sie [2]

ABSTRACT

Microbiologically induced calcite precipitation (MICP), a multidisciplinary technique involving biology, chemistry, and soil mechanics, is used for improving the engineering properties of soils. This technique involves the hydrolysis of urea by urease (a bacterial enzyme) into carbonate and ammonium ions, which precipitate in the form of calcite in the presence of a calcium source. The pore space of soil varies with the soil type. The pore space of coarse-grained soil is greater than that of fine-grained soil. Therefore, application of MICP on fine-grained soil is limited and was investigated here using Taipei silty clay. MICP was successfully applied on Taipei silty clay with the mixing method. Factors affecting MICP, such as concentration of bacterial/cementation solution, curing time, and pH of soil, were investigated. A two-fold increase in the shear strength of Taipei silty clay was achieved. An appropriate configuration on the test, such as concentration of bacterial and cementation solutions, was proposed. In addition to MICP, soil improvement by a combination of natural fiber and MICP was also studied. This shows that soil strength increased with the addition of up to 1% fiber. However, pure MICP gave a better result in terms of soil improvement than natural fiber. The results showed that MICP is effective in reducing the amount of fibers added to the soil while yielding the same improvement ratio.

Key words: MICP, urease activity, *Sporosarcina pasteurii,* coir fiber.

1. INTRODUCTION

Soil improvement is vital for constructions in soft ground, where failure or instability may be an issue. Several methods have been developed to achieve soil improvement (for example, compaction, chemical grouting, and electro-kinematic chemical treatment). However, most of these methods consume considerable energy and involve toxic chemical solutions. Thus, a promising soil improvement method using a biological process has been proposed and applied to various soils recently.

Bio-mediated soil improvement is an innovative and environment-friendly soil improvement technique. Among the various bio-mediated soil improvement methods developed so far, microbiologically induced calcite precipitation (MICP), which involves urea decomposition, is a commonly used one. Microbial urease catalyzes the hydrolysis of urea [$CO(NH_2)_2$] and produces ammonium and carbonate ions Eq. (1), thus raising pH and creating an alkaline environment for calcium precipitation.

$$CO(NH_2)_2 + 3H_2O \rightarrow 2NH_4^+ + HCO_3^- + OH^- \quad (1)$$

Then, in the presence of calcium ions, the produced carbonate ions precipitate in the form of calcium carbonate crystals Eq. (2), where pH tends to lower back to neutral (Al Qabany *et al.* 2012).

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

MICP has been proven effective on sands and residual soils and has been used extensively to enhance soil strength and reduce permeability (Al Qabany *et al.* 2012; Soon *et al.* 2014; Pan *et al.* 2019). In addition, factors affecting soil improvement, such as pH, temperature, and concentrations of calcium ions and dissolved inorganic carbon, have been studied for granular materials (Kile *et al.* 2000; Soon *et al.* 2014; Cheng *et al.* 2019; Sun *et al.* 2019).

Soil improvement involving fibers has also been extensively used in geotechnical engineering. Among the fibers, natural fiber is more environment-friendly and can be decomposed by bacteria in soils. Lingo-cellulosic fibers are one of the natural fibers, such as rice, jute, palm, coir, sisal, and hemp. They are used as soil reinforcement material because of their matrix structure and chemical composition as shown in Fig. 1. The cellulose content in natural fiber controls the tensile strength of the material. While the lignin content is essential to the protection and resistance of the internal structure by microorganisms. Hemicellulose secures the cellulose polymers and controls the moisture content of the structure (Bordoloi *et al.* 2017). The California bearing ratio and unconfined compressive strength of the soil mixed with lingo-cellulosic fibers are found to be higher (Gowthaman *et al.* 2018).

Coconut (coir) fibers are among the widely used natural fibers for soil engineering improvement purpose (Lekha 2004) and was used in this study. They are generally extracted from the husk of outer shell of the coconut (Maurya 2015). Coconut fibers have cellulose (32% to 43%) hemicellulose (approximate 21%), and a high content of lignin ranging between 40% to 45%, which is responsible for the strength of coconut fiber (Gowthaman *et al.* 2018; Lekha 2004). Coconut fibers have high initial modulus, consistency in tenacity, high torsional rigidity, and low percentage of elongation during breakage, which lead to their use in soil improvement.

Manuscript received August 2, 2019; revised November 6, 2019; accepted February 12, 2020.

[1*] Assistant professor (corresponding author), Department of Civil and Construction Engineering, National Taiwan University of Science and Technology, No. 43 Keelung Rd. Sec.4, Taipei, 10607, Taiwan (e-mail: fteng@mail.ntust.edu.tw)

[2] Graduate student, Department of Civil and Construction Engineering, National Taiwan University of Science and Technology.

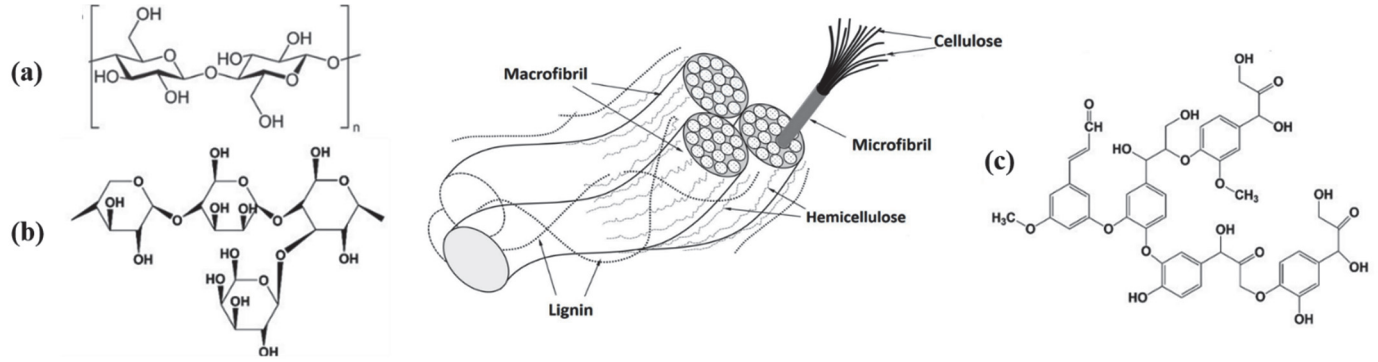


Fig. 1 Fibril matrix structure of plant fiber and the chemical composition of (a) cellulose, (b) hemicellulose, and (c) lignin (Gowthaman *et al.* 2018)

Soft clays are usually soils that need improvement in terms of both strength and stiffness for most constructions. However, MICP is not well studied on clays, that is, in terms of factors and performance. In this study, a series of tests using MICP on soft clays under different conditions, such as bacterial solution concentration, cementation solution concentration, pH, and curing time, were conducted. The bacteria used in MICP were grown in the authors' laboratory, and thus, this paper also provides a detailed procedure for bacterial growth. Soil improvement with a combination of natural coir fibers and MICP was studied by adding different fiber content at an optimum bacterial solution concentration.

2. PREPARATION OF MICROBIAL CULTURE

The bacteria used in this study was *Sporosarcina pasteurii* (*S. pasteurii*) because of its high urease activity and good performance in the MICP reaction (Whiffin 2004). The cells (number 11596) were obtained from the Bio Resources Collection and Research Center in Taiwan and grown at 30°C in a nutrient medium containing 5 g/L of peptone, 5 g/L of NaCl, 3 g/L of beef extract, and 500 mM of urea.

A detailed procedure for bacterial growth is presented in Fig. 2. All ingredients, except urea, were mixed in an autoclave at 121°C and cooled down at room temperature (WHO Laboratory Biosafety Manual 2004). Then, concentrated filter-sterilized urea was aseptically added to the mixture. A freshly washed bacterial pellet was inoculated in the urea medium and incubated at 30°C on a hot-stir plate for 9 hours; and the plate was used to maintain the homogeneity of the mixture during growth. A sterile two-port connection cover was used to provide filtered oxygen to the cells. Different amounts of bacterial pellets were transferred to the solution to study the effect of bacterial concentration on urease activity. At least 3 mL of the bacterial solution was filtered using a 0.22-μm filter at 30 min and 2, 5, 7, and 9 hours after inoculation of the bacterial pellets in the urea medium and used for measuring pH and electrical conductivity. In addition, using a spectrophotometer, the optical density (OD) was measured at 2, 5, 7, and 9 hours after transferring the bacterial pellets into the growth medium. The urease activity of the solution was estimated using the correlation proposed by Whiffin (2004) Eq. (3):

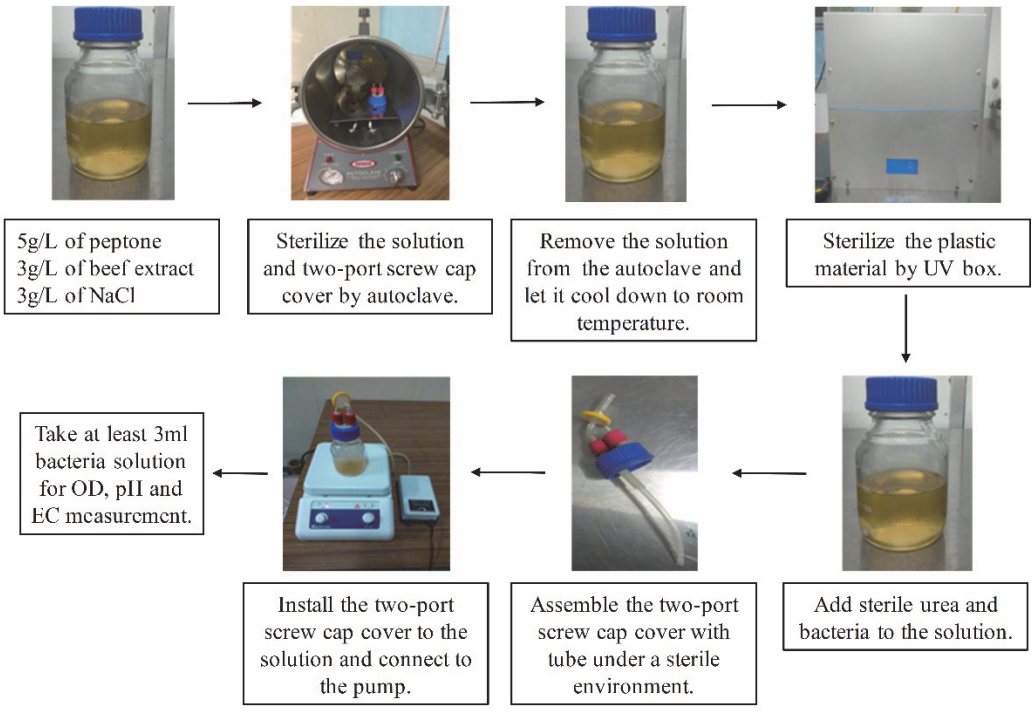


Fig. 2 Bacterial growth procedure

Urease activity (mM/min)
= (ΔConductivity (ms/cm) × 11.11)/ΔTime (min) (3)

A higher urease activity in the bacterial solution led to a larger increase in electrical conductivity because more ammonium and carbonate ions were generated.

The serial dilution method was employed for determining the bacterial density during growth. The plates were drawn at a known OD_{600} (OD with a wavelength of 600 nm) and incubated at 30°C for 36 hours to establish a correlation between OD_{600} and the bacterial density (number of cells per milliliter). The OD value is used to represent the bacterial solution concentration herein.

3. TEST MATERIAL, PLAN, AND METHOD

3.1 Test Material

Table 1 summarizes the basic information and properties of the soil used in this study. The soil was retrieved from a construction site in Shilin, Taipei City. It had a plasticity index of 21 and was classified as low-plasticity clay (CL) in Unified Soil Classification System (USCS). It is a typical Taipei silty clay with more than 60% silts. The main clay mineral of the silty clay is Illite. The XRF result also showed some trace of calcium in the soil.

The Taipei silty clay has a pH of 2.75 and may influence the MICP reaction within the soil sample. Krishnamurthy *et al.* (1998) and Madiva *et al.* (2017) studied the ability of urease bacterium to survive in an acid environment. Cheng (2014) demonstrated that calcite can be formed in acid soil, but its contribution to the increase in soil shear strength is less significant.

3.2 Test Plan

In this study, the effects of three factors (cementation solution concentration, bacterial solution concentration, and curing time) on MICP were studied using the mixing method (Table 2). The influence of cementation solution was determined using 1-day curing samples with different cementation and bacterial solution concentrations. The influence of bacterial solution concentration was determined using 3-day curing samples with different bacterial solutions and 0.5 M of urea/$CaCl_2$ solution. The influence of curing time was determined using a sample with 0.8 OD and 0.5 M of urea/$CaCl_2$ solution with 1-, 3-, 5-, and 7-day curing. All combination samples were tested in duplicates for a consistent result, and strength improvement was evaluated on the basis of unconfined compressive strength.

Coir fibers with a diameter of 0.2 ~ 0.45 mm and a length of 3 mm were used in this study. Samples with different fiber content in weight (no bacteria added) were used to investigate the influence of the fibers on the silty clay. The clay samples with both coir fibers and bacterial solution were used to study the effect of the MICP plus natural fiber (MICP+fiber). All tests are listed in Table 3. The MICP+fiber sample was prepared with 0.8 OD_{600} bacterial solution concentration and 0.5 M of cementation solution. The mechanical properties were also determined on the basis of unconfined compressive strength.

In addition, the effect of initial pH of soils on MICP was studied by adjusting the soil pH with the addition of KOH. Cementation solution of 0.25 M and bacterial solution with 0.175 OD_{600} were used. The test combinations are listed in Table 4.

Table 1 Basic properties of the tested soil

Source information	
Origin	Shilin, Taipei
USCS	CL
Physical properties	
Liquid Limit	49
Plastic Limit	28
Plasticity index	21
Maximum dry density (kg/m^3)	1492
Optimum moisture content (%)	27.04
Specific gravity	2.51
Silt (%)	68.7
Clay (%)	21.1
Permeability (m/sec)	7×10^{-8}
pH	2.75
Chemical compositions	
SiO_2 (%)	48.83
Al_2O_3 (%)	30.85
Fe_2O_3 (%)	8.73
CaO (%)	1.43
Major clay mineral	Illite

Table 2 MICP-treated sample combinations by mixing method

Curing period	Bacterial solution concentration, OD_{600}	Urea/$CaCl_2$ concentration (M)	Purpose
1 day	0.16	0.25	Influence of cementation concentration
	0.35	0.25	
		0.5	
		1	
	0.8	0.25	
		0.5	
		1	
3 days	0.35	0.5	Influence of bacterial solution concentration
	0.8		
	1.45		
1 day	0.8	0.5	Influence of curing time
3 days			
5 days			
7 days			

* All the aforementioned samples were tested in duplicates or triplicates for consistent result.

Table 3 Sample combination generated using the mixing method and containing fiber

Curing time	Fiber content (%)	Bacterial solution concentration, OD_{600}	Urea/$CaCl_2$ concentration (M)	Purpose
No curing time	0.25	No bacteria	No cementation solution	Influence of Fiber content in soil
	0.50			
	1.00			
3 days	0.25	0.8	0.5	Influence of Fiber on MICP
	0.50			

* All the aforementioned samples were duplicated or triplicated for consistent results.

Table 4 Sample combination for the influence of initial pH on MICP

Bacterial solution concentration, OD_{600}	Urea/$CaCl_2$ concentration (M)	KOH concentration (M)	Initial pH	Purpose
0.175	0.25	2.5	7.1	Influence of initial pH
		3.75	9.6	
		5	12.0	

3.3 Test Method

MICP is limited to coarse-grained soils. For clayey soils, the lack of pore space may hinder bacterial movement and activity. Therefore, a direct mixing method was used to enable MICP application on fine-grained soils. This method can be used to improve the subgrade soil of road embankments or any construction that needs soil compensation by MICP. The amount of water needed to compact the soil was replaced by the bacterial solution and an equimolar urea/$CaCl_2$ solution. Samples were sheared by the unconfined compressive test. For the test of adjusting the initial pH of the soils, the bacterial solution, KOH solution, and an equimolar urea/$CaCl_2$ solution substituted the amount of water required to compact the soil. Samples were sheared by the unconfined compressive test.

3.4 Sample Preparation

The silty clay was first washed with No. 60 sieve, then air dried and crushed. It was sieved with No. 60 sieve again before proceeding to the experiment. The MICP soil sample was mixed manually with a mixture of cementation solution and bacterial solution that was equal to the amount of water needed to meet 85% maximum dry density on the wet side of the optimal moisture content (OMC). The original samples and fiber samples were mixed with de-ionized (DI) water. The fiber content used in this study was 0.25%, 0.5%, and 1% by weight of solid, as studied by other researchers (Bordoloi *et al.* 2017; Maurya *et al.* 2015; Gowthaman *et al.* 2018). The fiber was mixed with the soil prior to being mixed with the liquid (same water amount corresponding to 85% maximum dry density at the wet side). After mixing, the soil was kept in plastic bags in a humidity chamber at 27°C ~ 30°C for 24 hours (Fig. 3). After stabilization, the soil was compacted 20 blow/layer in five layers using the special designed tools shown in Fig. 3. For all tested samples (both MICP and fiber test), the water content and dry density of samples were controlled as 38% and 1.27 g/cm^3 (85% of OMC). Then, the samples were cured in the humidity chamber.

For studying the influence of initial pH, the samples were mixed with a mixture of cementation solution, KOH solution, and bacterial solution, which was equal to the amount of water needed to meet 85% maximum dry density on the wet side of OMC. The KOH solution amounted to 40% of the mixed solution and had concentration of 2.5 M, 3.75 M, and 5 M in this study. It was first mixed with the soil and then kept in plastic bags in a humidity chamber at 27 ~ 30°C for 24 hours to measure the initial pH of the soil. Then, the soil was mixed with the cementation solution and bacterial solution. After stabilization, it was compacted by 5 layers by using a static compaction machine. Although the compaction tool is different, the water content and dry density of samples were still controlled as 38% and 1.27 g/cm^3 (85% of OMC). The samples compacted by the static compaction machine were used in studying pH effect only.

4. TEST RESULTS AND DISCUSSION

4.1 Microbial Growth Result

The correlation of OD_{600} and bacterial density (cells/mL) showed that the bacterial solution of 0.2 ~ 0.5 OD_{600} contained approximately 10^6 cells/mL, 0.5 ~ 1.2 OD_{600} comprised approximately 10^7 cells/mL, and 1.2 ~ 1.6 OD_{600} comprised approximately 10^8 cells/mL (Fig. 4). The result shows the correlation between bacterial density and OD_{600} values, which can be easily measured using a spectrophotometer.

Urea hydrolysis by urease leads to a high rise in the pH of the nutrient medium within the first hours of bacterial growth (Fig. 5(a)) and an increase in the electrical conductivity of the bacterial solution (Fig. 5(b)). These results show the ability of the cells used in this study to hydrolyze urea. The high initial inoculated concentration of bacteria leads to higher urease activity than an initial low concentration (Fig. 6).

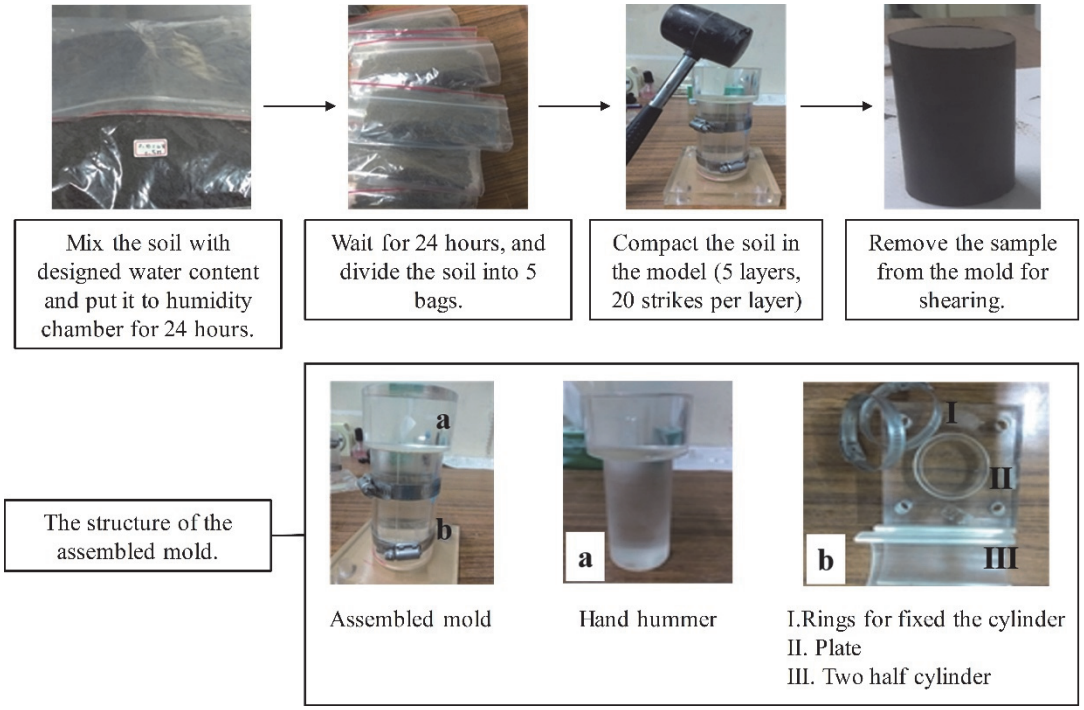


Fig. 3 Sample preparation process

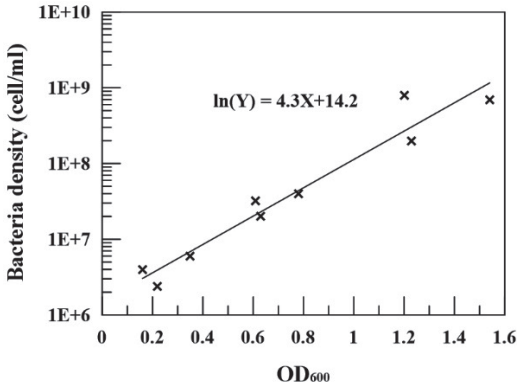
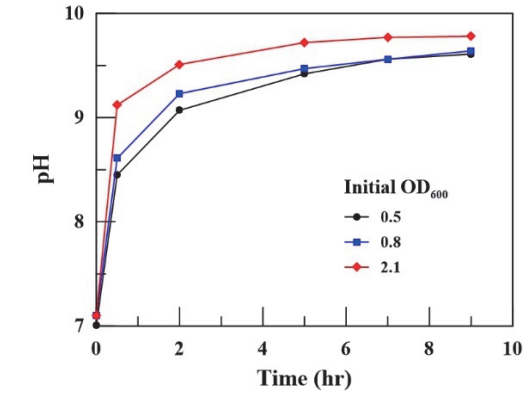
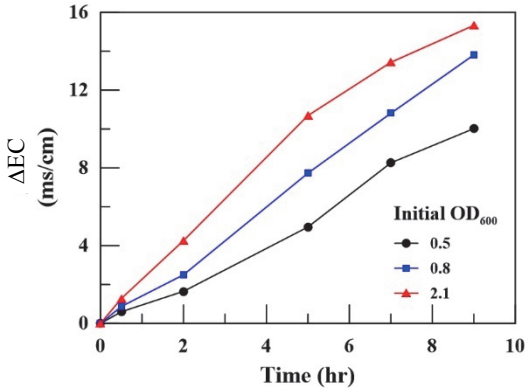


Fig. 4 Correlation between OD_{600} and bacteria density



(a) pH value



(b) Electrical conductivity value

Fig. 5 Condition of nutrient solution during bacterial growth

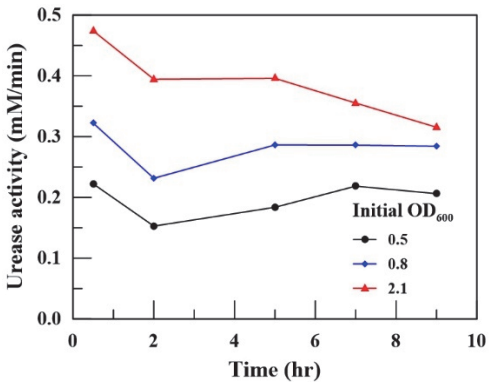


Fig. 6 Urease activity during bacterial growth

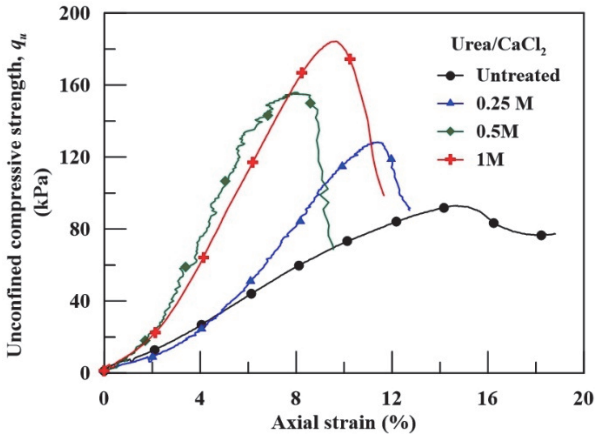
4.2 Pure MICP Test Results

Influence of Cementation Solution

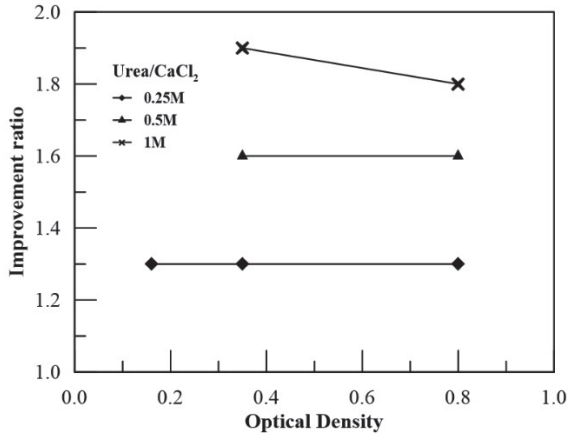
Figure 7(a) shows the influence of cementation solution (urea/$CaCl_2$) on the unconfined compressive strength of treated samples having an OD_{600} of 0.35 for a curing period of 1 day. The unconfined compressive strength increased with the concentration of an equimolar solution of urea/$CaCl_2$. The same trend was observed with different bacterial concentrations. Thus, it can be concluded that a high cementation solution concentration promotes better improvement in soil engineering properties for short-term curing. However, the improvement ratios of samples treated with different bacterial concentrations and the same cementation solution are approximately the same (Fig. 7(b)). The improvement ratio is defined as follows:

$$\text{Improvement ratio (IR)} = \frac{q_u \text{ of treated sample}}{q_u \text{ untreated sample}} \tag{4}$$

It implies that the improvement in soil strength for a short curing period mainly depends on the concentration of the cementation solution.



(a) OD = 0.35



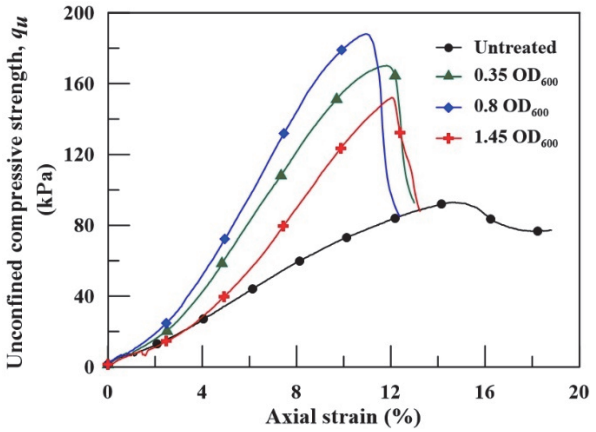
(b) Different OD_{600}

Fig. 7 Effect of urea/$CaCl_2$ concentration on MICP-treated samples

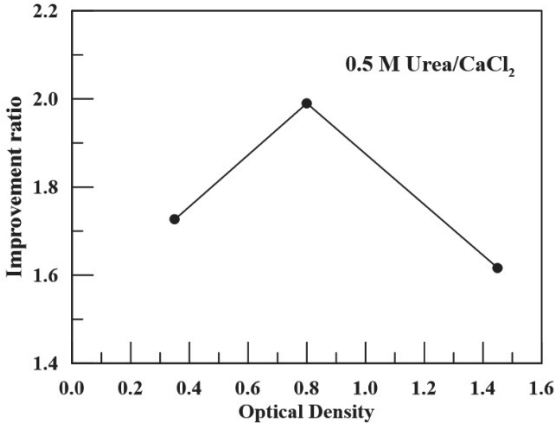
Influence of Bacterial Concentration

The high salinity of the nutrient solution inhibits the urease activity of the bacteria (Rivadeneyra *et al.* 1998). Therefore, a moderate-low concentration of cementation solution has been used by many researchers for MICP (Soon *et al.* 2014; Lee *et al.* 2014) with a 48-hour treatment duration. An MICP-treated sample having a different OD_{600} and containing 0.5 M cementation solution was cured for 3 days before the unconfined compressive strength test. It was presumed that the improvement in soil strength will be affected by the concentration of the bacterial solution for a relatively long curing period.

Figure 8 shows the influence of bacterial concentration on the unconfined compressive strength of the MICP-treated sample with 3-day curing. The unconfined compressive strength increased with the bacterial concentration from 0.35 OD_{600} to 0.8 OD_{600}. However, a decrease in the strength was observed at 1.45 OD_{600}, which is attributable to the dimensions of *S. pasteurii* and pore space in fine-grained soils. Sufficient pore space might not be available for the distribution of cells within the soil. Therefore, in the absence of oxygen, *S. pasteurii* cannot produce urease after a limiting time. Dejong *et al.* (2010) stated that *S. pasteurii* can survive in an anaerobic environment; however, there might be a limiting time for the production of urease in the absence of oxygen. In conclusion, the concentration of bacteria is a limiting factor in the MICP process compared with the concentration of the cementation solution.



(a) Unconfined compressive strength of MICP-treated samples with 0.5 M of urea/$CaCl_2$



(b) Improvement ratio of MICP-treated samples with 0.5 M of urea/$CaCl_2$

Fig. 8 Effect of bacteria concentration on soil strength improvement

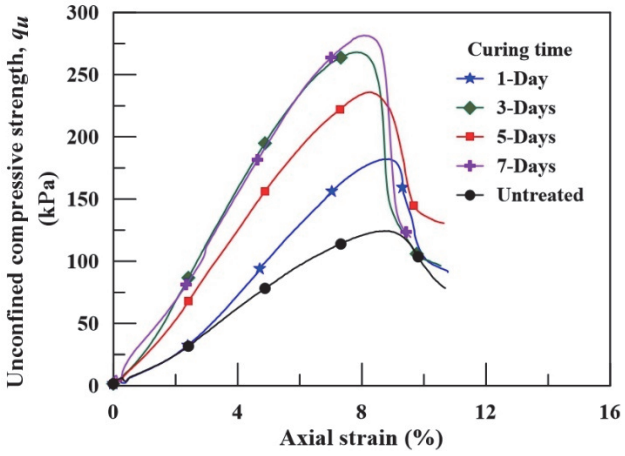
Influence of Curing Time

MICP samples were treated for more than 3 days to study the influence of curing time on strength improvement and the activity of *S. pasteurii* within the sample after 3 days.

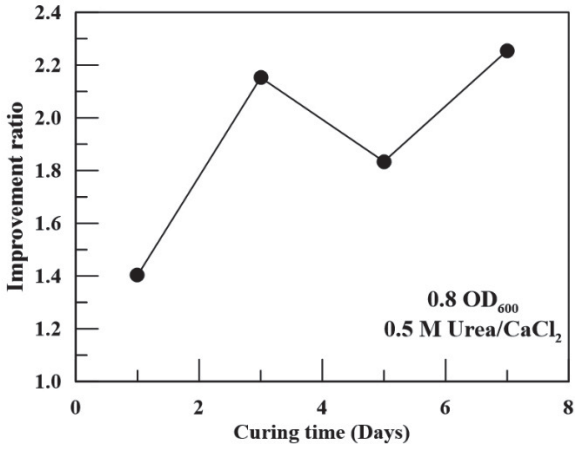
Figure 9(a) shows the influence of curing time of the MICP-treated sample obtained by the mixing method on the unconfined compressive strength. The strength increases with the curing time, except for a slight decrease for the 5-day curing sample. This result indicates that bacterial activity within each sample was slightly different, although all samples were prepared using identical parameters.

Figure 9(b) shows the influence of curing time on the improvement ratio. The optimum time for the MICP-treated sample was determined as 3 days in this study, because beyond this duration, the improvement ratio did not increase significantly. For 1-day curing, the strength can still be controlled by the concentration of the cementation solution, as stated previously. Therefore, a minimum curing time of 3 days should be adopted for MICP-treated samples for obtaining a reasonable result.

The pH of the sample after the unconfined compressive strength test remained low, varied between 3 and 4, which shows a slight increase from the original sample. This indicates that there was urease activity within the soil from the day of mixing to a limited time. Because of the acid environment within the sample, the urease bacteria could not survive after a few days. Thus, a decrease



(a) Unconfined compressive strength



(b) Improvement ratio

Fig. 9 Effect of curing time on soil strength improvement

in urease activity after 3 days is attributable to the inhibition caused by the pH environment and shortage of oxygen. Cheng (2014) demonstrated that calcite bonds can be formed in acid pH soil; however, the improvement ratio is lower than those for alkaline and neutral pH soils. Therefore, if the pH of the soil used in this study was neutral, a greater improvement ratio may be obtained. To support this inference, the initial pH of the soils was increased to study the influence of the initial pH of the soil on MICP. The results are presented in the next section.

Influence of Soil pH

Figure 10 shows the influence of the initial pH of the MICP-treated sample on the unconfined compressive strength. Different concentrations of KOH were added to the samples to adjust the initial pH of the soils. Although the alkaline environment is more conducive to calcium precipitation, the optimum strength was still obtained in a neutral environment. The improvement ratio under the acid condition was close to that under the alkaline condition. This result does not exactly agree with the conclusion obtained by Cheng *et al.* (2014) that indicated that MICP is less significant under the acid condition. The difference is attributable to the different soil types, and thus, the position where MICP occurs is different. In conclusion, the mechanism of MICP increasing the soil strength in clays was presumably different for granular materials. The MICP process still occurred and resulted in a satisfactory improvement in soil strength, even at a low initial pH, that is, an acid condition.

Summary

Table 5 listed all improvement ratios on MICP-treated samples. The shear strength of the soil using MICP with mixing was double that of the soil without MICP despite its low pH. Almost the same improvement was achieved with different batches of the soil, which shows that MICP can be used as a potential strength improvement method for silty clays. The improvement is not always increased with the concentration of bacterial solution, because of the limitation of pore space, such as crowded pore space if too many bacteria cells were added into the soil. However, more urea and calcium sources are beneficial for the strength improvement under a reasonable bacterial solution concentration, *e.g.,* $OD_{600} = 0.8$.

The curing time has a significant impact on the improvement in the soil sample with the mixing method. One-day curing did not show a significant increase in the shear strength of the soil. Three-day curing led to a significant increase in the shear strength of the soil, which remained stable with a further increase in the curing time. Therefore, 1-day curing is not sufficient for the complete MICP reaction within the soil samples, while 3-day curing is the most appropriate curing time for the MICP sample by the mixing method. A combination of 0.8 OD_{600} bacterial concentration and 0.5 M of urea/$CaCl_2$ solution has proved suitable for MICP on soft silty clays. MICP may be applied to similar silty clays without adjusting the pH.

Figure 11 shows the SEM photos on untreated and MICP-treated samples. Due to the clay structure and size, it was difficult to compare soil particles and calcites. However, comparing the SEM photos in Fig. 11, the MICP treated sample seems to be coated by a layer of crystal material and the gap between soil particles were filled. This is presumably the source of strength improvement in the results.

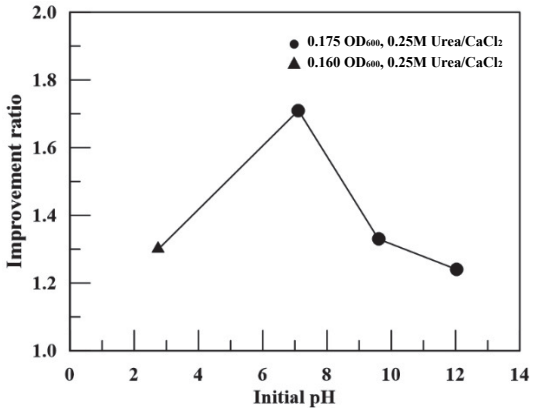
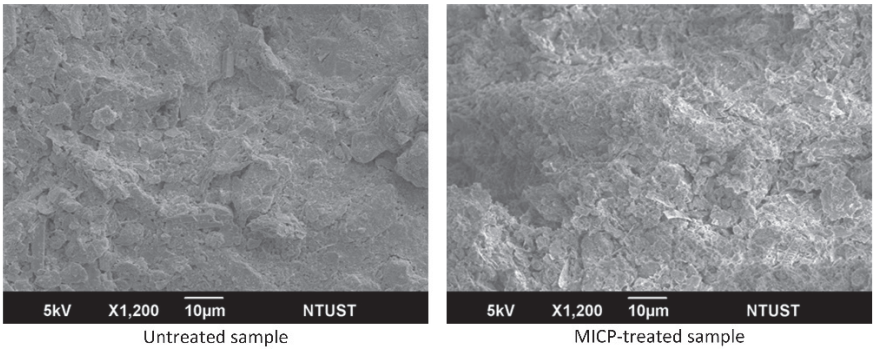


Fig. 10 Influence of initial pH on MICP-treated samples

Table 5 Improvement result summary for MICP-treated samples

Curing period	Bacterial concentration, OD_{600}	Urea/$CaCl_2$ concentration (M)	$q_{u,treated} / q_{u,un\text{-}treated}$	
			Mean	Standard deviation
1 day	0.16	0.25	1.29	0.006
	0.35	0.25	1.29	0.055
		0.5	1.65	0.030
		1	1.94	0.038
	0.8	0.25	1.29	0.055
		0.5	1.58	0.031
		1	1.84	0.020
3 days	0.35	0.5	1.73	0.105
	0.8		1.99	0.036
	1.45		1.62	0.018
1 day	0.8	0.5	1.43	0.057
3 days			2.20	0.015
5 days			1.88	0.046
7 days			2.28	0.010



Untreated sample MICP-treated sample

Fig. 11 SEM photos of untreated and MICP-treated samples

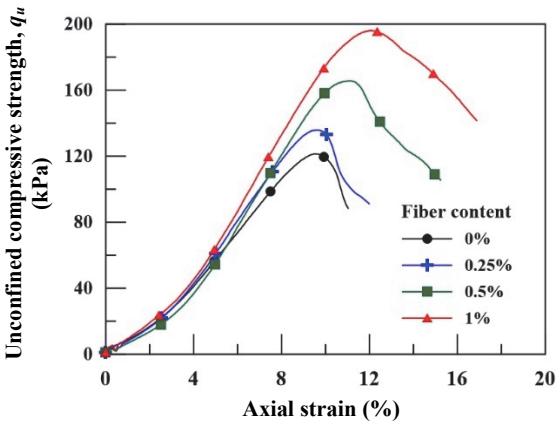
5. MICP WITH COIR FIBERS

5.1 Influence of Fiber Content on Soil Shear Strength

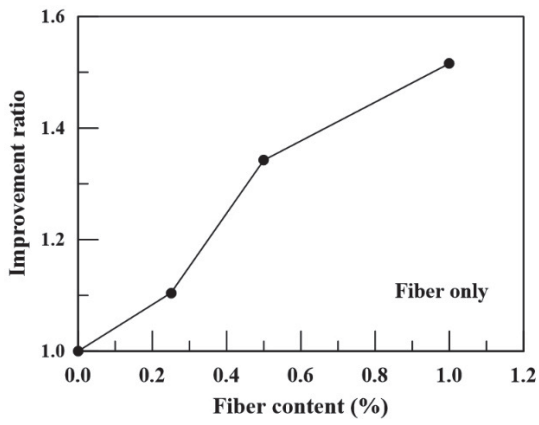
Figure 12(a) shows the effect of fiber content on soil strength improvement. The strength increases with the fiber content. These results are consistent with those of Muthu *et al.* (2018). Figure 12(b) shows the improvement ratio indicating the influence of fiber content on soil strength improvement. Addition of 0.25% fiber content does not show a significant increase in the soil strength (IR = 1.1); therefore, an improvement in soil obtained by adding 0.25% of fiber can be neglected. A relatively high improvement was achieved with the addition of 0.5% and 1% natural fibers, that is, improvement ratios were 1.35 and 1.5, respectively. For an MICP test with 0.8 OD_{600} bacterial concentration and 0.5 M cementation solution, an improvement ratio higher than 2.0 was achieved. Thus, an improvement method that combines MICP and natural fibers was proposed hereinafter.

5.2 Influence of Fiber Content on MICP Process

To study the influence of natural fibers on the MICP process, 0.25% and 0.5% fibers were included in the soil. The soil samples were prepared with coir fibers and a mixed solution containing 0.8 OD_{600} bacterial concentration and 0.5 M of urea/calcium chloride solution, which is exactly the same as that used for the mixing method.



(a) Unconfined compressive strength

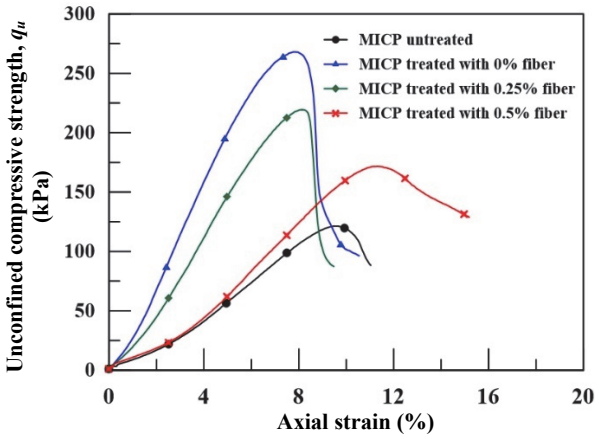


(b) Improvement ratio

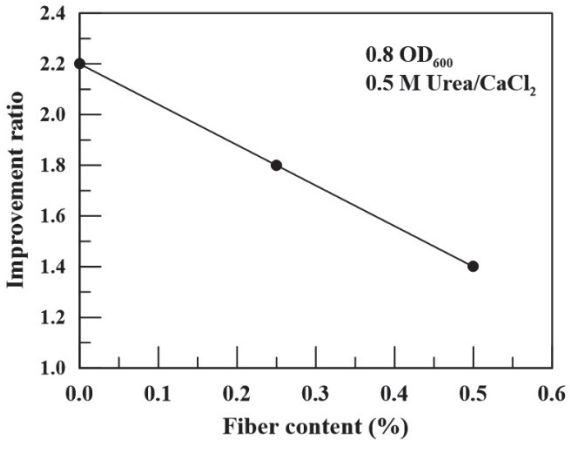
Fig. 12 Improvement of soil strength by coir fibers

Figure 13 shows the influence of fiber content on the MICP process. A value of 0% in the figure represents a pure MICP sample, which yielded an improvement ratio of 2.2. The unconfined compressive strength/improvement ratios decreased with an increase in the fiber content. This may be due to the presence of cellulose, which results in inappropriate precipitation positions of calcites. *S. pasteurii* can produce urease enzyme (Whiffin 2004) as well as cellulose enzyme (Horikoshi *et al.* 1984). The bacteria uses fiber cellulose as its source of energy; therefore, they tend to stick on the fibers instead of creating bonds within the soil particles.

Figure 14 shows the SEM images of natural fiber and natural fiber treated with 0.5 M cementation solution and bacterial solution for 5 days. The surface of the natural fiber is smoother compared to the treated fiber which is cover by particles identified as precipitated calcite. However, as can be seen in Fig. 14(b), the calcite is not perfectly bonding with the fiber. It is because that the bacteria tend to stick on the fiber and calcite will be surrounding the bacteria. After the bacteria died, there is a gap between the formed calcite and the fiber. Thus, precipitated calcite around the fibers do not contribute much on the strength. In addition, in the presence of natural fibers in MICP process, the calcite precipitated more on the fiber surface rather than the contact points between the soil particles. Thus, the improvement ratios decreased with a higher fiber content in the MICP soil samples.



(a) Unconfined compressive strength



(b) Improvement ratio

Fig. 13 Improvement of soil strength by coir fibers and MICP

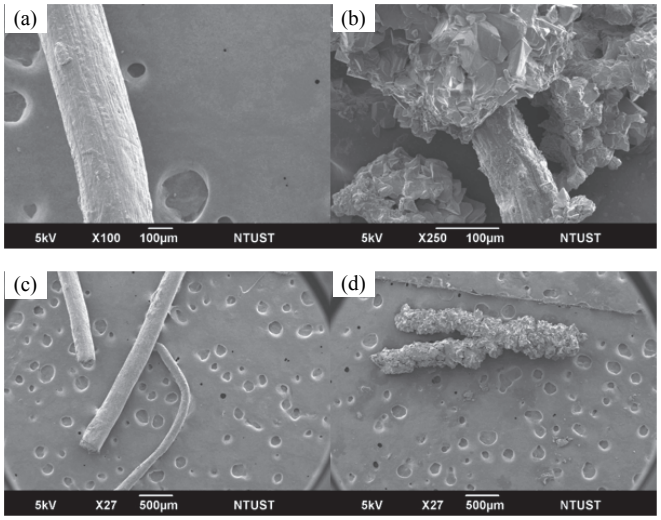


Fig. 14 SEM of (a) natural coir fiber, (b) natural fiber covered by calcite crystal, (c) natural coir fiber, and (d) natural fiber covered by calcite crystal

5.3 Summary

Table 6 listed all improvement ratios on fiber-treated samples. The addition of fiber to the MICP sample is not favorable for soil strength improvement because of the presence of cellulose. However, MICP+fiber can still help in reducing the fiber content used in soil strength improvement. The mixed solution (bacterial and cementation solutions) containing 0.25% fiber content increases the strength compared with 1% fiber only (Fig. 15). Therefore, it can be deduced that the percentage of fiber can be reduced using the MICP process.

Table 6 Improvement result summary for MICP-treated samples with fibers

Curing time	Fiber content (%)	Bacterial solution concentration, OD_{600}	Urea/$CaCl_2$ concentration (M)	$q_{u,treated}/q_{u,un\text{-}treated}$	
				Mean	Standard deviation
No curing time	0.25	No bacteria	No cementation solution	1.10	0.002
	0.50			1.34	0.006
	1.00			1.51	0.066
3Days	0.25	0.8	0.5	1.76	0.021
	0.50			1.40	0.003

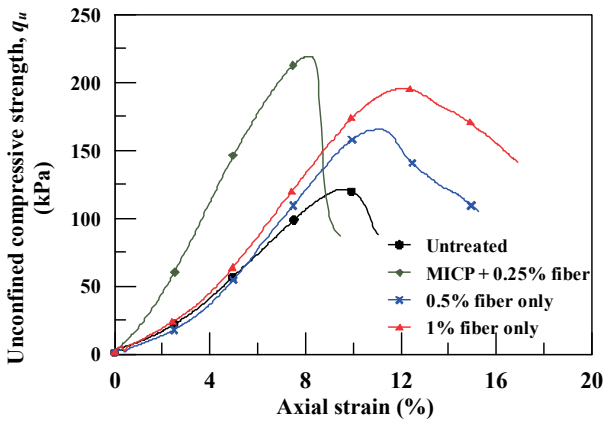


Fig. 15 Comparison of soil improvement by fiber only and MICP+fiber

6. CONCLUSIONS

In this study, the application of MICP on fine-grained soils was investigated using Taipei silty clay and *S. pasteurii*. The following conclusions were drawn:

1. The application of MICP by using the mixing method had a positive effect on soil strength improvement. Despite the low pH of the soil, its shear strength was double after 3-day curing. A combination of the bacterial solution with 0.8 OD_{600} and 0.5 M of urea/$CaCl_2$ solution yielded an optimum improvement in soil strength.
2. A high concentration of *S. pasteurii* led to a high urease activity in the growth medium but did not promote much MICP reaction in clayey soil. The bacterial concentration was a relative limiting factor for MICP on silty clay compared with the concentration of cementation solution.
3. One-day curing time was controlled by the concentration of cementation solution used for mixing the soil sample. A curing time of at least 3 days was recommended for MICP application by the mixing method.
4. An addition of fibers to the samples with bacterial solution affected the calcite precipitation position, that is, on the fiber surface rather than the contact-to-contact points of the soil particles. This was not favorable for MICP treatment. Pure MICP is more recommended than a combination of MICP and natural fibers based on the results.
5. It should be stated that the results presented in this study only applicable to the similar silty clays under similar conditions, such as bacteria type, bacterial solution concentration, cementation solution concentration, fiber contents, *etc.*

FUNDING

Financial support for this work provided by the Ministry of Science and Technology (Grant No. 106-2221-E-011-168) in Taiwan is appreciated.

ACKNOWLEDGEMENTS

Knowledge and assistance for bacteria growing from Prof. S.L. Tsai in Dept. of Chemical Engineering of National Taiwan University of Science and Technology is greatly appreciated.

DATA AVAILABILITY

Data for figures in this paper are available from the corresponding author upon reasonable request.

REFERENCES

Al Qabany, A., Soga, K., and Santamarina, C. (2012). "Factors affecting efficiency of microbially induced calcite precipitation." *Journal of Geotechnical and Geoenvironmental Engineering*, ASCE, **138**, 992-1001. https://doi.org/10.1061/(ASCE)GT.1943-5606.0000666

Bordoloi, S., Hussain, R., Garg, A., Sreedeep, S., and Zhou, W.-H. (2017). "Infiltration characteristics of natural fiber reinforced soil." *Transportation Geotechnics*, **12**, 37-44. https://doi.org/10.1016/j.trgeo.2017.08.007

Cheng, L., Shahin, M.A., Cord-Ruwisch, R., Addis, M., Hartanto, T., and Elms, C. (2014). "Soil stabilisation by microbial-induced calcite precipitation (MICP): Investigation into some physical and environmental aspects." *7th International Congress on Environmental Geotechnics: ICEG2014,* Engineers Australia, 1105-1112.

Cheng, L., Shahin, M.A., and Chu, J. (2019). "Soil bio-cementation using a new one-phase low-pH injection method." *Acta Geotech*., **14**(3), 615-626. https://doi.org/10.1007/s11440-018-0738-2

DeJong, J.T., Mortensen, B.M., Martinez, B.C., and Nelson, D.C. (2010). "Bio-mediated soil improvement." *Ecological Engineering*, **36**, 197-210. https://doi.org/10.1016/j.ecoleng.2008.12.029

Gowthaman, S., Nakashima, K., and Kawasaki, S. (2018). "A state-of-the-art review on soil reinforcement technology using natural plant fiber materials: past findings, present trends and future directions." *Materials*, **11**(4), 553. https://doi.org/10.3390/ma11040553

Horikoshi, K., Nakao, M., Kurono, Y., and Sashiha, N. (1984). "Cellulases of an alkalophilic Bacillus strain isolated from soil." *Canadian Journal of Microbiology*, **30**, 774-779. https://doi.org/10.1139/m84-118

Kile, D.E., Eberl, D.D., Hoch, A.R., and Reddy, M.M. (2000). "An assessment of calcite crystal growth mechanisms based on crystal size distributions." *Geochimica et Cosmochimica Acta*, **64**, 2937-2950. https://doi.org/10.1016/S0016-7037(00)00394-X

Lekha, K.R. (2004). "Field instrumentation and monitoring of soil erosion in coir geotextile stabilised slopes — A case study." *Geotextiles and Geomembranes*, **22**, 399-413. https://doi.org/10.1016/j.geotexmem.2003.12.003

Mahdavia, A., Sajedib, R.H., and Rassac, M. (2017). "Investigation of acid-neutralizing property of bacillus cereus GUF8." *Biomacromolecular Journal*, **3**, 18-25.

Muthu Lakshmi, S., Sasikala, S., Padmavathi, V., Priya, S., and Saranya, V. (2018). "Utilization of coconut coir fiber for improving subgrade strength characteristics of clayey sand." *International Research Journal of Engineering and Technology* (IRJET), **5**.

Pan, X., Chu, J., Yang, Y., and Cheng, L. (2019). "A new biogrouting method for fine to coarse sand." *Acta Geotechica*, 15, 1-16. https://doi.org/10.1007/s11440-019-00872-0

Krishnamurthy, P., Parlow, M., Zitzer, J.B., Phadnis, S.H., Vakil, N.B., Dunn, B.E., Parlow, M., and Mobley, H.L.T. (1998). "Helicobacter pylori containing only cytoplasmic urease is susceptible to acid." *Infection and Immunity*, **66**, 5060-5066. https://doi.org/10.1128/IAI.66.11.5060-5066.1998

Soon, N.W., Lee, L.M., Khun, T.C., and Ling, H.S. (2014). "Factors affecting improvement in engineering properties of residual soil through microbial-induced calcite precipitation." *Journal of Geotechnical and Geoenvironmental Engineering*, ASCE, **140**(5), 04014006. https://doi.org/10.1061/(ASCE)GT.1943-5606.0001089

Maurya, S., Sharma, A.K., Jainc, P.K., and Kumard, R. (2015). "Review on stabilization of soil using coir fiber." *International Journal of Engineering Research*, **4**(6), 296-299. https://doi.org/10.17950/ijer/v4s6/605

Sun, X., Miao, L., Tong, T., and Wang, C. (2019). *Acta Geotechnica*, **14**, 627-638. https://doi.org/10.1007/s11440-018-0758-y

Whiffin, V.S. (2004). *Microbial $CaCO_3$ Precipitation for the Production of Biocement*. Murdoch University, Ph.D. Dissertaion, Western Australia.

World Health Organization (2004). *Laboratory Biosafety Manual*. 3rd Edition, Geneva.