Microbially induced carbonate precipitations to improve residual soil at various temperatures

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Abstract: Microbial induced carbonate precipitation (MICP) has emerged recently as a new approach of green and sustainable soil improvement technique. The main aim of this study was to determine the most suitable temperature for MICP to improve tropical residual soil. The physical and engineering properties of residual soil used in the study were determined through several laboratory experiments. A urease active strain of *Sporosarcinapasteurii* obtained from American Type Culture Collection (ATCC) was used to trigger the carbonate precipitation. Experimental parameters such as curing temperature, treatment duration and bacteria to cementation reagents ratios were evaluated. The results show that the optimum temperature for MICP treatment of residual soil is 55°C. It was also found that at this optimum temperature, specimens treated with bacteria and cementation reagents in proportion of 2:1 produces the highest strength improvement ratio of 1.27 relative to untreated sample and calcite content of 1.09% after 7-day curing. The shear strength of the treated soil also increases with the increase in treatment durations as the 7 days curing produces higher strength improvement for all the experimental conditions. The specimens cured under the atmospheric temperature recorded the lowest calcite content and hence the lower shear strength improvement ratio. Moreover, the experimental results obtained from this study also can be used as a guide in the future bio-geotechnology researches and lead to further scope in geotechnical applications.

Keywords: Microbial induced carbonate precipitation, MICP, shear strength, temperature, residual soil

INTRODUCTION

Many civil infrastructural expansion and development which are prompted by the rapid global urbanization and industrialization are sometimes limited by the inadequate soil condition and geographical boundaries (DeJong *et al*., 2010); this has subsequently forces geotechnical engineers to build some infrastructures on problematic soil. These problematic soils are characterized as weak in shear strength and having high compressibility properties. Therefore, buildings and other civil infrastructures founded on these loose, weak or soft sediments requires some precautionary measures to avoid structural or foundation failure; these may include but not limited to soil stabilization, pile foundations, large embankments or continuous maintenance. In Malaysia, tropical residual soils are found in abundance, but the intense rate of infiltration as a result of continues heavy downpour subjected the residual soils to further softening. Similarly, presence of excess water in the soil could lead to further softening and subsequent geo-hazards such as slope failure, ground settlement, soil erosion, landslides, etc and consequently increases the risk of failure of the existing structures.

Traditional methods of soil improvement in geotechnical engineering such as the use of chemical grouting techniques to improve the engineering properties of soil have become very common practice in civil engineering construction. On the other hand, application of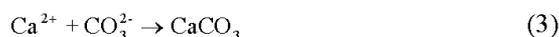 some of these methods usually require high amounts of energy, costs, have limitations with regards to treatment range and require materials which have considerable impact on the environment (Karol, 2003). Though many of the additives used in chemical grouting were found to be successful in improving the engineering properties of the soils, they might also contaminate the soil and groundwater (Karol, 2003; DeJong *et al.*, 2006). In fact, the use of chemical grouts in soil improvement create environmental concerns over their field application; as it was found that excessive use of chemicals grouts except sodium silicate is toxic and/or hazardous to the environment (DeJong *et al*., 2010; Chu *et al*., 2012; Soon *et al*., 2014).

Recently, the use of microbial geotechnology to improve the engineering properties of soils through biomineralization process for engineering applications has emerged (Umar *et al.,* 2016). Biomineralization is referred to as the process by which living organisms produce minerals. It was documented in many literatures reported by (Lian *et al.,* 2006) that biomineralization is an active process in almost every environment on earth; with much of the microbial activity leading to the formation of carbonate minerals happening at near surface of the earth. Formation of soil carbonate deposits, sediments and minerals have been largely attributed to the microbial activities. Therefore, inducement of calcium carbonate precipitates either in natural or experimental settings are predominantly attributed to the native microorganisms from soils and some aqueous

media. Though, carbonates are the most obvious minerals emanating from biomineralization process; microbially induced calcium carbonates are largely considered in the field of biotechnology, geotechnology and civil engineering applications (Dhami *et al.*, 2013). Hence, numerous bacteria species have been identified and widely linked with the natural carbonate precipitation from diverse environments. Micro-organisms containing the enzyme urease are utilized in the process and their main role in the precipitation process has been strongly ascribed to their ability to create an alkaline environment through various physiological activities (Hammes *et al.*, 2002).

The major expected advantage of using microorganisms in soil improvement process was based on the fact that the microbes are native to the soil; therefore, are likely will not pose any danger to the environmental safety. These ureolytic bacteria such as *Sporosarcinapasteurii* are capable of producing urease enzyme through their metabolic activity that facilitates the rate of urea hydrolysis to form ammonium and bicarbonate ions. Urea hydrolysis generally follows a series of chemical reactions that leads to the formation of ammonia ions (NH_4^+) and carbonate ions (CO_3^{2-}).

The production of ammonium ions increases the in-situ soil pH and create an ideal circumstance for bicarbonate ions to react with calcium ions (from the supplied calcium chloride) to form calcite precipitates. The end-product of $CaCO_3$ subsequently binds the soil particles together thereby improving the strength and stiffness of the treated soil. The chemical reactions are presented in Equations 1 to 3.

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

$$CaCl_2 \rightarrow Ca^{2+} + Cl^{2-} \qquad (2)$$

$$Ca^{2+} + CO_3^{2-} \rightarrow CaCO_3 \qquad (3)$$

In this study, MICP treatment was performed on tropical residual soil at laboratory scale. Some experimental parameters such as curing temperature, treatment duration, in order to assess the performance of the MICP treatment in tropical residual soil using urease active strain *Sporosarcinapasteurii*. Hence, some parameters such as unconfined compressive strength and calcite contents were directly measured after the treatment to evaluate the performance and effectiveness of the MICP process in improving the strength of tropical residual soil.

MATERIALS AND METHODS
Urease producing bacteria and cementation reagent concentrations

The urease producing bacteria namely *Sporosarcinapasteurii* was obtained from American Type Culture Collection (ATCC) and cultivated under aerobic condition in a sterile yeast extract-based medium containing 20 g yeast extract, 10 g ammonium sulphate $(NH_4)_2SO_4$ in 1 litre of 0.13M Tris buffer solution at a pH of 9.0. The bacterium was grown to its exponential growth phase in a shaker incubator using 140 revolutions per minutes at 30°C for 14 hours. Hence, serial dilution and spectrophotometer were used to obtain concentrations of 1.6×10^6 cfu/ml at 600 nm optical density (OD_{600}) in the study.

The cementation reagents are very important ingredient for MICP treatment process. This is because the carbonate and calcium ions that form the calcite precipitates are obtained from the decomposition of urea and supplied calcium chloride respectively. The reagents consist of 3 g nutrient broth, urea and calcium chloride solution at 0.5 M concentrations. The 0.5 M reagents concentration was obtained by dissolving 36.8 g of urea, 15.02 g of calcium chloride and 3 g of nutrient broth in a 500 ml of deionised water. Cementation reagents concentrations of 0.5M were used throughout the MICP treatment as it was found to be the most suitable concentration for efficient biocementation of residual soils (Soon *et al.*, 2014; Sharma & Ramkrishman, 2016; Umar *et al.*, 2016).

Preparation of soil specimens and MICP treatment

Stepped injection method was adopted to allow for fixation and even distribution of cementation reagents and bacterial activity in the soil as suggested by Harkes *et al.* (2010). In the first step; air dried soil passing 2 mm sieve was mixed properly with the liquid medium containing the microorganisms (*Sporosarcinapasteurii*) at a concentration of 1.6×10^6 cfu/ml based on the designed bacteria-reagents ratio. The cementation reagents (urea, calcium chloride and nutrient broth) were sprayed to the soil-bacteria mixture at 6 hours interval for 48 hours. The bacteria solution and cementation reagents used in the bio-treatment were prepared in proportions of 1:2, 1:1 and 2:1 respectively. This is aimed at determining the best bacteria/reagents combinations for efficient MICP treatment. The soil-bacteria-reagents mixture was then compacted into 38 mm diameter and 76 mm height stainless steel mould to a density corresponding to 95% of the maximum dry density obtained from compaction curve. The specimens were then extruded and cured at atmospheric temperatures (22°C to 34°C), 45°C, 55°C and 65°C for 1, 3 and 7 days respectively.

Calcite contents determination

The calcium carbonate contents in the treated soils were determined using gravimetric analysis of acidified samples. After MICP treatment and compressive strength test, part of the failed specimens was prepared into powder and 20 g of the powdered samples were oven dried. 2 M Hydrochloric acid was added to the prepared powdered sample and carbon dioxide was liberated due to the reaction between calcite and hydrochloric acid. The residue was collected and oven dried again and the loss in weight was used to estimate the percentage of calcite contents in the specimen. The method was based on the assumption that the increment of carbonate content in the soil after the MICP treatment was purely caused by the formation of calcium carbonate. This method was also adopted by Soon *et al.* (2014).

RESULTS AND DISCUSSIONS
Residual soil sample

Tropical residual soil used in this study was collected at P18, Faculty of Electrical Engineering, Universiti Teknologi Malaysia (UTM), Johor campus. The physical and engineering properties tests were performed on the representative soil sample for classification purpose. Figure 1 shows the particle size distribution curve of the soil and Table 1 summarized the index and engineering properties of the soil. The soil was classified as Gravelly Clay of very high plasticity based on the British Soil Classification System (BSCS). The MICP treatment was performed on the residual soil that passes 2 mm diameter British Standard Sieves; this was to provide reasonable nucleation sites for the bacteria to mediate between the soils individual particles and precipitates the required calcites.

Effects of curing time on shear strength improvement

Undrained shear strength, which is equal to one-half of the unconfined compressive strength, q_u was used to evaluate the shear strength improvement of the MICP treated soil in this study. Figure 2 presents the shear strength improvement of the treated sample using bacteria cementation reagents ratio of 2:1 at different curing periods. The shear strength of the untreated residual soil was initially 40.5 kPa which was used as control. It was observed that the strength of treated soil increases with the increase in the curing period for all the curing temperatures. For instance,
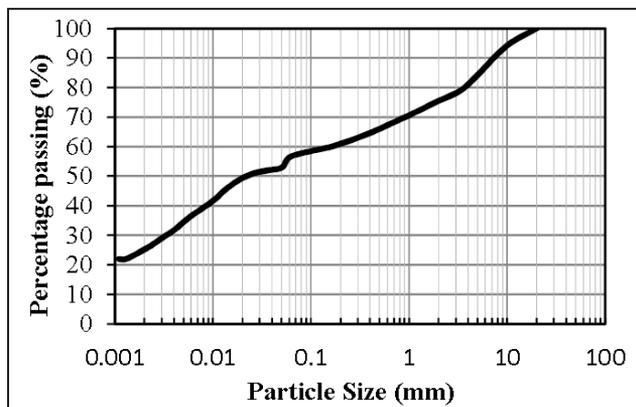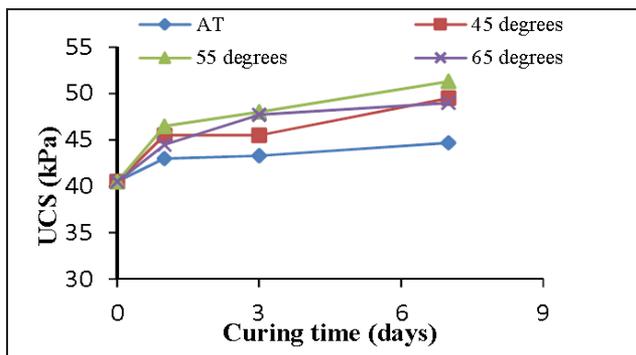
shear strength improvement of 6% relative to untreated sample was recorded at 1-day curing at atmospheric temperatures; while 10% improvement was recorded at 7 days under the same condition (Figure 1). Similarly, at 55°C curing temperature, strength improvement of 15% and 27% relative to untreated sample were observed for 1 and 7 days respectively.

The continuous increase in strength of the biotreated soil may be attributed to the continuous bacterial activity utilizing the remaining nutrients and cementation reagents to precipitates more calcites after the treatment period. Similar observations were made by Umar & Kassim (2017), the author reported peak strength improvement of 28.6% when the MICP treated residual soil was cured for 14 days. Likewise, Moravej *et al.* (2018) reported the peak strength improvement of MICP treated soil on the third day of curing. Thereafter, no further improvement was observed for samples cured at 7 and 14 days. The summary of the test results of MICP treated soils under different experimental conditions are presented in Table 2.

Effects of curing temperatures on shear strength improvement

From the graphical representations shown in Figures 3, 4, 5 and 6, it can be explicitly seen that the shear strength of the MICP treated soil increases with increasing curing temperatures, curing period and the amount of bacteria added into the soil. It was observed that the shear strength of the bio-treated soils continues to improve as the curing temperatures were increases up to 55°C. However, beyond 55°C curing temperature the strength declined. This may be attributed to the fact that long term curing of biotreated soil under high temperature would have a negative effect on the microbial activity. As revealed by Whiffin (2004) that extremely high temperatures weakens the hydrolysing potential of the microorganism and

Figure 1: Particle size distribution curve of the soil sample.

Figure 2: Relationship between UCS improvement and curing period.

Table 1: Physical and engineering properties of tropical residual soil used in the study.

Physical properties	Symbol	Index
Gravel composition	G	24.5%
Sand composition	S	19%
Silt composition	M	31%
Clay composition	C	25.5%
Soil Classification (BSCS)	-	CVG
Natural Moisture Content	W	31.9 %
Liquid Limit	w_L	71.5%
Plastic Limit	w_P	36.1%
Plasticity Index	Ip	35.4%
Specify Gravity	Gs	2.67
Maximum Dry Density	$\gamma_{d\,max}$	1420 kg/m³
Optimum Moisture Content	OMC	28.3%
Unconfined Compressive Strength (UCS)	q_u	72.7 kPa

facilitates cell lysis. Though, most of the microorganisms utilized in the MICP process are capable of surviving harsh environmental conditions without nutrients; they tend to

Table 2: Summary of shear strength test results at different curing periods, curing temperatures and bacteria and cementation reagents ratios.

Temperature	Bacteria : Cementation reagent Ratio	Shear strength (kPa)		
		1 day curing	3 days curing	7 days curing
22°C - 34°C	1:2	40.5	42.0	43.5
(Atmospheric	1:1	42.0	42.0	43.7
Temperature)	2:1	43.0	43.3	44.7
	1:2	41.0	42.5	45.0
45°C	1:1	41.5	43.5	45.0
	2:1	45.5	45.5	49.5
	1:2	43.5	45.0	47.0
55°C	1:1	43.5	46.0	50.3
	2:1	46.5	48.0	51.3
	1:2	41.0	43.0	43.3
65°C	1:1	43.0	43.3	44.0
	2:1	44.5	47.7	49.0

excrete their enzymes which degrade more easily outside than inside the cells (Mujah *et al.*, 2017; Gadd, 2010). The soil specimens cured under atmospheric temperature exhibited insignificant improvement in shear strength with the maximum increment of only 10% after 7 days of curing (Figure 3). However, at 55°C, the shear strength improvement reached 27% which was notable when the specimens were treated with bacteria and cementation reagents ratio of 2:1 and cured for 7 days.

It can be deduced that the urease activity of the microorganism was most active at 55°C as more calcite were precipitated at that temperature thereby recording the highest strength improvement. The results are in good agreement with the findings of other studies. It was reported by Nemati & Voordouw (2003) that the optimum temperature for urease activity was 50°C. Similarly, Cheng *et al.* (2014) reported peak urease activity at 60°C. Likewise, Whiffin (2004) also studied the effect of temperature on urease enzyme activity. The author found that the activity of urease enzyme in *Sporosarcinapasteurii* increased with increasing temperature from 25°C to 60°C and had an optimum at 70°C. However, it was believed that presence of clay and organic matter in the soil protects the urease enzyme from degradation at high temperature (Zantua & Bremer, 1977).
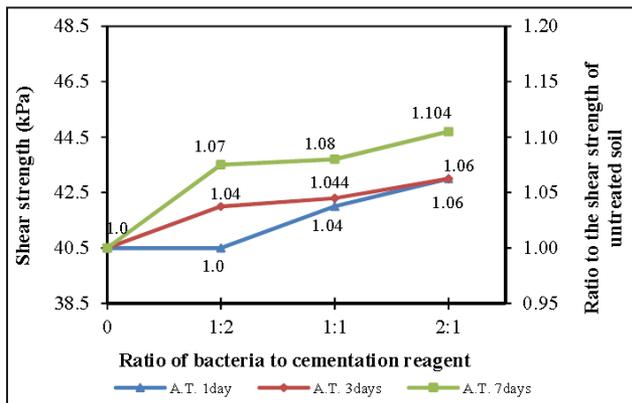

Figure 3: Relationship between shear strength and bacteria and cementation reagents ratios at atmospheric temperatures curing.
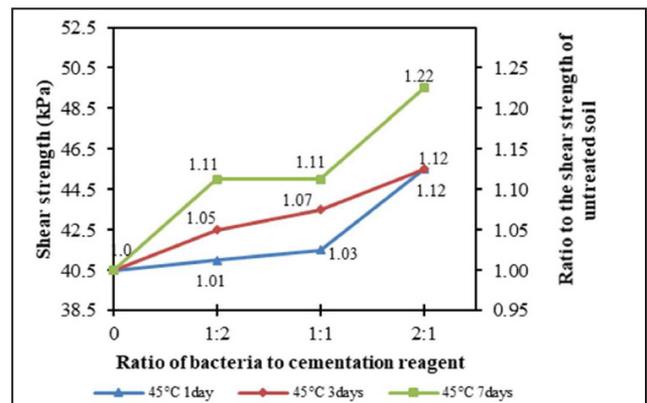

Figure 4: Relationship between shear strength and bacteria and cementation reagents ratios at 45 degrees curing.
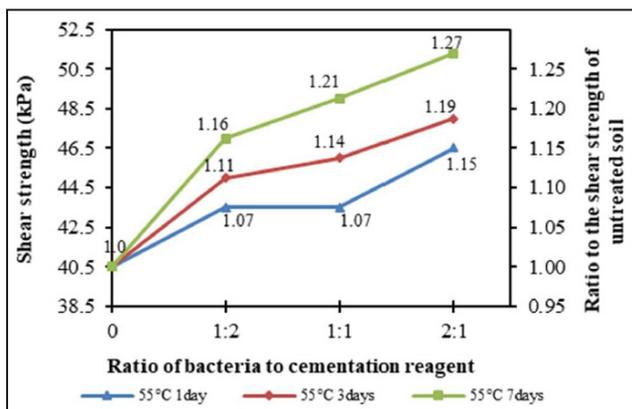

Figure 5: Relationship between shear strength and bacteria and cementation reagents ratios at 55 degrees curing.
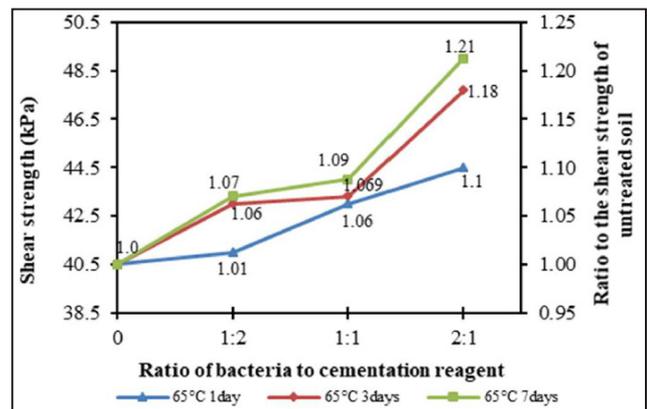

Figure 6: Relationship between shear strength and bacteria and cementation reagents ratios at 65 degrees curing.

Effects of bacteria and cementation reagents ratios on the strength improvement

The basic parameters required for MICP treatment are the urease producing bacteria and the cementation reagents (urea and calcium chloride). However, for efficient and economic biocementation of residual soil, proper combination of bacteria solution and the cementation reagents need to be determined. Therefore, three different combinations of bacteria and reagents were considered in this study. Bacteria/reagents proportion of 1:2, 1:1 and 2:1 used revealed that using bacteria solution twice more than the reagents solution (2:1) in the treatment process provided better strength improvement for all the treatment conditions. Therefore, it can be deduced that concentration of bacteria in the MICP treatment plays a dominant role in the calcite precipitation process when there is sufficient cementation reagent supplied into the soil medium. It can also be inferred that the higher the amount of bacteria added into the soil, the greater the urease enzyme would be produced per unit volume to hydrolyse the available urea and provide the required carbonate ions for the calcite precipitation. It was stated by Warren *et al*. (2001) that no precipitation occurs when MICP was conducted with no bacteria present. Similarly, Stocks-Fischer *et al*. (1999) found that during microbial calcite precipitation, identified bacteria were found in the middle of the calcite crystals which inferred that bacteria themselves serve as nucleation site for calcite precipitation. Likewise, Okwadha & Li (2010) revealed that the rate of ureolysis is dependent more on the concentration of bacteria cell rather than urea concentration as long as there is enough urea to sustain the bacteria. The authors also stated that the calcite is not likely to be utilized during bacterial metabolism but would accumulate on the cell surfaces when they are readily available for calcite precipitation.

Calcite precipitation and shear strength improvement

To assess the performance of the MICP process in improving the soil strength; amount of calcite content precipitated in the treated soil specimens were determined using gravimetric analysis of acidified samples. Figures 7 and 8 present the relationship between calcite content and shear strength improvement of the MICP treated soil at atmospheric temperature and 55°C curing. It was observed that the increment in shear strength was purely caused by the formation of the calcium carbonate in the treated soil. The soil specimens cured under atmospheric temperature recorded the lowest calcite content which is an indication of very low bacterial activity and hence resulted in the lower shear strength improvement.

On the other hand, the highest calcite content was obtained from the specimens that were cured at 55°C with the highest value of 1.09% obtained at 7 days curing using bacteria and cementation reagents ratio of 2:1. The calcite crystals formed bind the soils particles together and subsequently resulted in the shear strength improvement of the treated soil (Sharma & Ramakrishnan, 2016). It can
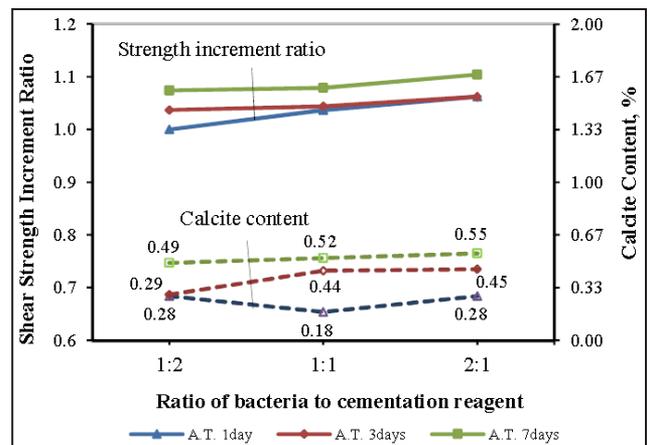

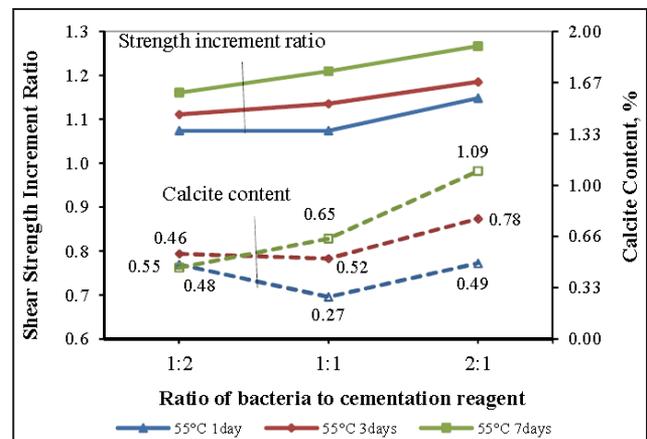**Figure 7:** Relationship between shear strength improvement and calcite content.

Figure 8: Relationship between shear strength improvement and calcite content.

also be seen from Figure 8, that the calcite content does not increase consistently with the increase in curing period but the overall trend shows reasonably comparisons with the trend of the strength improvement.

According to Sari (2015) soil microbe sizes vary from 0.5 to 3 μm. Therefore, bacterial movement through the pore throats of soils which are smaller than 0.4 μm may be restricted (Mitchell & Santamarina, 2005). The residual soil used in the present study offered optimum pore space for microbes to move freely throughout the soil composite. Pore throat sizes in soil affect the effectiveness of MICP. The smaller the pore throat, the denser the soil particles arrangement and the higher the concentration of particle-particle contacts per unit volume. Hence, it promoted better calcite bonding between particle-particle contacts and resulted higher improvement in soil strength (Lee *et al.,* 2012; Sharma & Ramkrishnan, 2016).

CONCLUSION

The shear strength of the MICP treated soil increased with increasing curing temperature, treatment duration and the amount of bacteria added into the specimens. The MICP treated soil showed the highest shear strength

improvement ratio with solutions of bacteria and cementation reagent in proportion of 2:1 added into the specimens. It can be explained that the bacteria play a dominant role in $CaCO_3$ precipitation. The soil specimens treated and cured under the atmospheric temperature exhibited insignificant improvement in shear strength with increment ratio of only 1.1 (10%). However, at 55°C, it gave the optimum results; with the shear strength increment ratio reached to 1.27 (27%), which was notable when the specimens were treated with solutions of bacteria and cementation reagent of 2:1 proportions in 7-day curing period. It was believed that the urease was the most active at 55°C which further enhanced the production rate of $CaCO_3$. The soil specimens that cured under the atmospheric temperature recorded the lowest of calcite content and hence contributed to lower shear strength improvement ratio. The highest calcite content was measured in the specimens cured at 55°C. However, calcite contents do not show direct proportionality to the shear strength. It was assumed that the microbial slime produced by the microorganism also help in bonding the soil particles together and increase the soil shear strength. Hence, the optimum temperature for microbial induced carbonate precipitation to improve residual soil is about 55°C.

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