Effect of Reagents Concentration on Biocementation of Tropical Residual Soil

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Abstract. This study explores the feasibility of Bacillus subtilis and optimum reagents concentration used in Microbial-Induced Calcite Precipitation (MICP) treatment of tropical residual soil. Experiment was conducted to investigate the effect of cementation reagents concentration toward MICP treatment. The performance of MICP treatment was assessed by measurement of the soil shear strength and calcite content. Based on the experimental results, it is discovered that the cementation reagent concentration has significantly affected on the performance of MICP treatment. The results suggested that the most preferable MICP treatment reagents concentration is 0.25M with the presence of Bacillus subtilis; using these treatment parameters, both UCS value and calcite content of treated soil had increased about 38% and 65.6% respectively. However, the reduction in UCS value was manifested for those samples treated at higher reagents concentration (0.35M); this phenomenon is attributed to the salinity of reagents where high salinity is not favourable to the bacteria growth and microbial activity; subsequently, this resulted in a consequential decrease in shear strength of the treated soil.

Keywords: Microbial induced calcite precipitation (MICP), tropical residual soil, biocementation.

1. Introduction
Microbial-Induced Calcite Precipitation (MICP) method is relatively green, sustainable technique and environmental friendly technique in soil stabilization, which able to alter and improve the ground condition [1]. Soil stabilization using MICP technique can meet the green construction requirement as this treatment causes minimal disturbance to soil environment [2]. Bacteria and cementation reagents were percolated into the soil to form calcite precipitation which improve the engineering properties of soil [3]. In addition, many studies have been conducted to explore the potential and feasibility of the MICP technique in different kind of civil engineering applications such as: improved concrete strength and durability [4, 5]; improve mortar and brick durability [6]; increased soil strength [7,8]; and sand impermeability [9].

In the past few years before introducing MICP technique, chemical grouting method has been applied for soil stabilization in civil engineering. The process of chemical grouting is achieved by adding different additives, some of the additives are very toxic and hazardous to the environment such as lime, asphalt, sodium silicate, acrylate, lignin, urethane, and resin [10]; these additives usually modify the soil pH, and contaminate the groundwater and soil [11-14].

In this case, MICP technique seems to be a more promising green and sustainable alternative as it utilizes biochemical process to improve the engineering properties of soil [15]. However, most of the MICP studies were conducted on the sandy soil rather than fine grained soils; the MICP studies on fine grain soils are limited [15, 16]. Due to the complexity in soil microbiology and chemical components, the MICP application on fine grain soils remains uncertain and challenging; more attempt
and studies are needed to provide more understanding and information on the MICP in fine grain soils. Hence, the objective of the paper is to provide better understanding of this technique by investigating the effect of various concentrations of cementation reagents toward MICP treatment; as well as to determine the effectiveness and feasibility of \textit{Bacillus subtilis} in this MICP treatment for tropical residual soil; Moreover, this research was designed to address the question arose in previous study [16].

The application of Microbial induced calcite precipitation (MICP) in this study focused on the bio-cementation. Bio-cementation is a process that produces binding materials in form of calcite within the soil particles through biochemical reaction between the introduced bacteria and cementation reagents into the soil [15]. Bio-cementation results in the improvement of shear strength and stiffness of the soil. At the initial stages of the process, bacteria perform urea hydrolysis in which the supplied urea is decomposed into ammonium ion by its urease enzyme (Eq. 1); and provide an alkaline environment for the precipitation of calcium carbonate [17]. The formation of calcite precipitation causes the pH of the medium back to neutral and the final pH of the medium depending on the rates of reaction and substrate concentration [18]. The negative charged bacteria cells attract the carbonate ion (the by product from urea hydrolysis) and combine with the supplied calcium ions to form calcite as shown in Eq. 2 and Eq. 3.

2. Material and Methodology

2.1 Type of Bacteria

The urease producing bacteria used in the current study was \textit{Bacillus subtilis} (ATCC 55422) which is a type of facultative anaerobe bacteria. \textit{Bacillus subtilis} is a gram positive and rod shaped bacterium with diameter about 2\(\mu\)m.

2.2 Cementation reagent

Cementation reagents are the essential ingredients to generate calcite precipitation in microbial processes. The cementation reagents comprise of urea, calcium chloride and 3g/L of nutrient broth as tabulated in Table 1. The nutrient broth was added to provide additional nutrients for bacterial growth.

| Chemical | Concentration of Cementation Reagent (M) |
|----------|-----------------------------------------|
|          | 0.15 | 0.25 | 0.35  |
| Urea, CH\(_4\)N\(_2\)O (g) | 9.0  | 15.0 | 21.0  |
| Nutrient broth (g)  | 3.0  | 3.0  | 3.0   |
| Calcium chloride, CaCl\(_2\) 2H\(_2\)O (g) | 22.0 | 36.8 | 51.5  |

2.3 Laboratory Setup/Placement of microorganism

Fig. 1 illustrates the laboratory setup of the experiment. The setup comprises of the soil moulds, pressure tank, pneumatic compressor, effluent pipes, and effluent collector. The cementation reagents supplied continuously throughout the treatment. Corrosion resistance material, stainless steel was used to fabricate the sample mould of 50 mm in diameter and 200 mm in height (Fig. 2a). Before the soil sample was loaded into the stainless steel mould, a thin layer of lubricant applied at the inner surface of the mould to ease the sample extrusion process. All connections were checked to ensure no leakage of air in pressure tank and cementation reagents in the sample mould.
**Figure 1.** Schematic diagram of the laboratory set up

**Figure 2a.** Stainless steel mould

**Figure 2b.** Breakdown diagram of MICP sample and parts inside the mould
2.4 Soil Specimen
The soil material used in current study is tropical residual soil obtained from a site near Faculty of Electrical Engineering, Universiti Teknologi Malaysia. Table 1 tabulates the physical and index properties of the residual soil, which were determined in accordance to British standard soil properties test.

| Soil Index Properties | Description                  |
|-----------------------|------------------------------|
| Particle Size         | 15.3 % sand, 50.7 % silt, 33.6 % clay |
| Plastic Limit         | 48.95 %                      |
| Liquid Limit          | 80.80 %                      |
| Plasticity Index      | 31.85 %                      |
| Maximum Dry Density   | 1.322 /m³                    |

2.5 Soil Treatment Procedures
The bacteria were incubated at 30˚C with 200 rpm for 24 hours. The bacteria were cultivated to a concentration of $1 \times 10^8$ cfu/ml. In prior MICP treatment, the desired density was achieved by mixing the soil specimen with the cultivated bacteria solution (about 87 ml) which is equivalent to the optimum moisture content of the soil. The soil sample was sprayed and mixed evenly with distilled water (control) or cultivated bacteria (MICP sample). After that, the mixed soil was poured and hand tamped into the sample mould. The soil specimen was compressed by compressor until it achieved the desired length of 100 mm. As shown in Fig 2b, the soil specimen was sandwiched between two layers of sand material to avoid turbulent flow and clogging at inlet. The plastic nettings were placed to separate each layer and as protection sheet for the top and bottom surface of the soil specimen. Then, the soil specimen was treated for 2 days with supply continuous flow of cementation reagents at a flow pressure of 0.2 bars. After the completion of MICP treatment, the treated soil samples were extruded from the moulds for shear strength testing by using unconfined compression strength test (UCS).

2.6 Experimental Variables
There different reagents concentration were used in this study (0.15 M, 0.25 M and 0.35 M). A control soil specimen without bacteria was prepared at the same time and supply with continuous flow of cementation reagent; the control sample was used to make comparison with result of treated soil specimen. All soil specimens treated for 2 days with cementation reagents flow pressure of 0.2 bars.

3.0 Result and Discussions

3.1 Shear Strength and Calcite Content Improvement
Fig. 3 shows the result of the effect of cementation reagents concentration on UCS and calcite content of the MICP treated sample. As seen in Figure 3, the optimum concentration was found to be 0.25M with the highest improvement of about 38% in UCS. While, the sample treated at 0.15M recorded improvements of about 23.7% and 19.4 % in UCS value and calcite content, respectively. The results shows that the UCS increased with an increased in the reagents concentration for both the samples treated at 0.15M and 0.25M; however, reduction in UCS value was observed for the sample treated at 0.35M. The UCS value for the sample treated at 0.35M was 94 kPa which is slightly lower than the control counterpart; a possible explanation for this observation is that, the improvement of UCS and calcite content could be affected as a result of the decrease in bacteria microbial activity due to high salinity of reagents; as high salinity impairs the nutrient and water availability for microorganism [19, 20]. As a result of that, the change of nutrient and water availability with the increase of the salinity might consequently affected on the growth and microbial activity of the bacteria [21]. Therefore, this could explain that the use of 0.35M reagents concentration in this study may have inhibited the
growth of the bacteria. Since, bacteria act as the nucleon site for calcite precipitation [22]; the reduction in bacteria number might subsequently reduce the calcite precipitation and resulted in lower UCS value. Excessive calcium chloride or further increase in the salt/reagents concentration limits the enzyme activity, and results in slow production of urease enzyme from ureolytic bacteria [15].

![Figure 3. UCS and calcite content for various concentration of cementation reagent.](image)

Furthermore, the use of calcium chloride in this MICP treatment might not be suitable, which impaired the enzyme produced by the bacteria and affected on the MICP treatment. Esawy et al. [23] reported that the performance of enzyme produced by *Bacillus subtilis* in calcium chloride was very low. Other alternative to replace the calcium chloride might produce better outcome and performance. It is worth noting that calcite content of the soil sample treated at 0.35M (6.25%) was almost identical as compared with the control specimen (6.2%). This observation suggests no apparent calcite precipitation was formed as a result of the impaired microbial activity as mentioned earlier. The result in this study also supports and in agreement with Qabany et al. 2013 [24] that the lower concentration (equal and lower than 0.25M) resulted in higher UCS value; this may associated with uniformity of the calcite precipitation in the treated sample. More investigation has to conduct to verify the uniformity of the calcite precipitation.

3.2 Relationship between Shear Strength and Calcite Content Improvement

As seen in Fig. 3, the shear strength of treated soil increased with the increase in calcite content. Similar observation was reported by Hammes et al. [25] in which the shear strength and calcite content tend to increase with the increase of reagents concentration during MICP treatment. This improvement in UCS is most probably due to the presence of calcite generated from MICP process, the formation
of calcite tend to bind the soil particles together and subsequently improved the shear strength of soil. Calcite precipitation act as a bridges to bind the soil particles, which increase the shear strength of the treated soil. [26].

However, it is found The calcite precipitated become less effective in strength improvement when the calcite content was less than 8%; greater UCS improvement was manifested at those treated samples with a calcite content higher than 7.4%. This observation implies that more effective calcite bonding between the soils particles could be achieved when the total calcite content reached 7.4% in this study. Nonetheless, this may not be applicable to other MICP treatment which involve other type of soil and bacteria; as different bacteria and soil may require different quantity of calcite to achieve the same level of improvement. In addition, bacteria are living organism that subjected to uncertainties; other environmental factors such as temperature, and soil pH value might also affected on the MICP treatment. More investigations are needed to verify this point.

No correlation and relationship were found between the calcite precipitation and the reagents concentration; the calcite precipitation did not increase with increase of the reagents concentration. This observation implies that the calcite precipitation is governed and it is limited by the microbial activity and the urease enzyme production as discuss earlier.

4. Conclusion

From this study, the utilization of Bacillus subtilis in MICP treatment has shown improvement in UCS of tropical residual soil. This positive improvement has also proven the feasibility of Bacillus subtilis in MICP treatment for tropical residual soil. The results show that the UCS of the treated soil improved with increased concentration of cementation reagent and reached optimum at 0.25M of cementation reagent. However, no improvement was observable for those sample treated at 0.35M; This might associated with high salinity of the reagents concentration which might cause inhibitory effect to bacteria microbial activity, and subsequently, limit the enzyme activity and the production of urease enzyme. Furthermore, it is essential to determine the optimum reagents concentration as it may be vary with soil type and bacteria used. Nonetheless, more studies and experiment have to be conducted to confirm the points that arose in this paper.

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