Factors Affecting Improvement in Engineering Properties of Residual Soil through Microbial-Induced Calcite Precipitation

Ng Wei Soon[1]; Lee Min Lee[2]; Tan Chew Khun[3]; and Hii Siew Ling[4]

Abstract: Studies of soil improvement by microbial-induced calcite precipitation (MICP) have focused primarily on fine sand. This paper explores the viability of the MICP technique for improving the engineering properties of a typical tropical residual soil. A species of *Bacillus*, *B. megaterium*, was used to trigger calcite precipitation. Four variables were considered in this study: the concentration of *B. megaterium*, the concentration of the cementation reagent, the treatment duration, and the flow pressure of the cementation reagent. The results show that the improvement in the engineering properties of the MICP-treated residual soils is comparable to those of treated fine sands. The preferable treatment conditions for the soil studied are *B. megaterium* concentration of 1×10^8 cfu/mL, cementation reagent concentration of 0.5 M, flow pressure of 1.1 bar of the cementation reagent, and treatment duration of 48 h. Using this combination of parameters, the obtained shear strength increase and hydraulic conductivity reduction are 69 and 90%, respectively. A minimum calcite content of 1.0% ($15\,kg/m^3$) is required to provide measurable improvement in the engineering properties of the soil. **DOI: 10.1061/(ASCE)GT.1943-5606.0001089.**

Author keywords: Microbial-induced calcite precipitation; Soil improvement; Shear strength; Hydraulic conductivity; Tropical residual soil.

Introduction

Microbial-induced calcite precipitation (MICP) is a relatively new and innovative soil improvement technique. Existing soil improvement techniques, such as chemical grouting (except sodium silicate) are mostly toxic and hazardous (Karol 2003; DeJong et al. 2010). Therefore, there are expressed environmental concerns over their field applications (DeJong et al. 2010). Soil improvement by MICP can meet the requirement of green construction because the process exerts minimal disturbance to the soil and environment. It involves the natural process of in situ urease hydrolysis by selected microorganisms that can be found in abundance in soil and groundwater (Lloyd and Sheaffe 1973; Fujita et al. 2000). For the purpose of soil improvement, the MICP process is intensified technologically. Nevertheless, the MICP technique may not be entirely environmentally friendly. The process may still generate by-products, i.e., ammonium and its oxidized by-product nitrate, which can be hazardous to human health and soil organisms, particularly at high concentrations (van Paassen et al. 2010).

[1]Postgraduate Candidate, Faculty of Engineering and Science, Univ. Tunku Abdul Rahman, Kuala Lumpur, Malaysia 53300. E-mail: just_ws@hotmail.com

[2]Assistant Professor, Faculty of Engineering and Science, Univ. Tunku Abdul Rahman, Kuala Lumpur, Malaysia 53300 (corresponding author). E-mail: mllee@utar.edu.my

[3]Assistant Professor, Faculty of Engineering and Green Technology, Univ. Tunku Abdul Rahman, Perak Campus, Malaysia 31900. E-mail: tckhun@utar.edu.my

[4]Associate Professor, Faculty of Engineering and Science, Univ. Tunku Abdul Rahman, Kuala Lumpur, Malaysia 53300. E-mail: hiisl@utar.edu.my

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Factors Affecting the Efficiency of the Microbial-Induced Calcite Precipitation Treatment

The MICP process is regulated mainly by four key factors: (1) concentration of calcium ion; (2) concentration of dissolved inorganic carbon; (3) pH; and (4) availability of nucleation sites (Kile et al. 2000). In addition, several environmental parameters such as salinity, temperature, geometric compatibility of bacteria, etc., may also govern the performance of calcite precipitation (Nemati et al. 2005; Maier et al. 2009; De Muynck et al. 2010).

Temperature has a significant influence on urease activity, and hence on the rate of MICP. Below 5°C, urease activity is negligible (van Paassen 2009). Between 25 and 60°C, Whiffin (2004) found that the urease activity in *Sporosarcina pasteurii* increased proportionally with temperature. The urease activity reached an optimum value at 70°C, above which the activity dropped significantly. It was almost half the optimum value at 80°C.

High concentrations (0.5–1.0 M) of urea and calcium chloride can generate a significant amount of calcite; however, the efficiency is low (Nemati et al. 2005; Okwadha and Li 2010). The formation of calcite at lower concentrations (0.05–0.25 M) is more efficient. De Muynck et al. (2010) found that the efficiency of calcite formation at 0.5-M urea and calcium chloride was almost half that at 0.25 M.

Geometric compatibility of urease-producing microorganisms is vital for soil treatment by injection of the microorganisms. Bacteria size typically ranges between 0.5 and 3.0 μm (Mitchell and Santamarina 2005). Pore-throat size that is smaller than the microorganism size can limit the free passage of the infiltrating microorganism (Rebata-Landa and Santamarina 2006). For example, bacteria with a size range of 0.3−2 μm can move freely within sandy soil with a particle size range of 0.05–2.0 mm (Maier et al. 2009). The existence of a considerable amount of fine grains, i.e., silt and clay (size $< 2\,\mu$m), would inhibit the movement of these bacteria. Furthermore, sediment-cell interaction may also result in puncture or tensile failure of the cell membrane (Rebata-Landa and Santamarina 2006).

With the exception of a small group of acid ureases, the optimum pH of microbial ureases is generally near neutral (Mobley et al. 1995).

Urease activity of alkalo-tolerant bacteria, such as *S. pasteurii* is optimum at pH 8 (Ciurli et al. 1996; Stocks-Fischer et al. 1999). At pH < 5, microbial ureases could be irreversibly denatured (Mobley et al. 1995). With respect to the relationship between calcite precipitation and pH, numerous studies performed using *S. pasteurii* found that the MICP reached a plateau at pH 8.7–9.5—i.e., pH 8.7–9.5 in Dupraz et al. (2009); pH 9.1 in Fujita et al. (2004); pH 9.3 in Ferris et al. (2004); and pH 9.5 in Stocks-Fischer et al. (1999). Arunachalam et al. (2010), who performed the MICP treatment using *Bacillus sphaericus*, reported that the calcite precipitation peaked at pH 8. Khan (2011) reported that urease activity peaked at pH 7 for *Bacillus megaterium*. Production of ammonia from urea hydrolysis will increase with pH during the MICP process. On the other hand, bicarbonate from urea hydrolysis and microbial respiration acts to buffer the pH rise.

Al Qabany et al. (2012) investigated the effect of various treatment parameters (reagent concentration, retention time, and reagent input rate) on the efficiency of calcite formation in sand by *S. pasteurii*. The calcite formation efficiency was examined based on the reaction stoichiometry. Al Qabany et al. (2012) found that high chemical efficiency of more than 90% can be achieved by applying a reagent input lower than $0.042\,\mathrm{M/h^{-1}}$, regardless of the reagent concentrations (up to 1.0 M). The results were consistent with the findings of Rebata-Landa (2007), Whiffin et al. (2007), and DeJong et al. (2006), who used the same urease-producing bacterium for the MICP treatment.

State of the Current Research

Despite being a relatively new technique, many studies of soil improvement using MICP have been reported. DeJong et al. (2006) treated loose and collapsible sand specimens and found that MICP improved the soil strength by enhancing the shear stiffness and shear capacity. Harkes et al. (2010) and Martinez et al. (2011) attempted to formulate appropriate procedures to distribute and fix urease-producing bacteria homogeneously in soil to promote effective MICP.

Ivanov and Chu (2008) presented a detailed review on the applications of MICP for soil improvement. Presently, promising applications focus primarily on biocementation, bioclogging, and biogas. Biocementation improves soil strength by formation of cementation materials through microbial means. Bioclogging reduces hydraulic conductivity of soil by generating pore-filling materials from microbial processes. Biogas refers to gas bubbles produced by microbial processes to reduce the degree of saturation in soil. Recently, a comprehensive review on the state of research on biomediated soil has been reported by a group of experts in the area (DeJong et al. 2013).

Most studies of MICP treatment have been performed on a laboratory scale (DeJong et al. 2010; Whiffin et al. 2007; DeJong et al. 2006). van Paassen (2011) provided an overview of the latest research development in Netherlands using scale-up laboratory tests and field-scale experiments. The MICP technique has been applied successfully in the field to strengthen the walls of boreholes from collapsing during the drilling process.

Issues Addressed by the Current Study

The current research into MICP soil improvement focuses primarily on fine sand. Very little studies have been done on other soil types. Fine soils, where the pore-throat size is sufficiently small to limit the free passage of bacteria, is not favorable. Coarse soils would require a large amount of calcite for effective improvement. Nevertheless, it is of particular interest to many geotechnical engineers to assess the performance of MICP in natural soils that contain fine and coarse grains. van Paassen (2011) attempted the MICP technique on gravels. Mortensen et al. (2011) tested it on a wide range of grain sizes comprised of sand, silty sand, and silt and concluded that the MICP treatment was equally robust for these soils.

Most *Bacillus* species can trigger urea hydrolysis by producing urease enzymes (Hammes et al. 2003). The reported studies have mainly adopted *S. pasteurii* as the urease-producing microorganism (Martinez et al. 2011; Harkes et al. 2010; DeJong et al. 2006; Stocks-Fischer et al. 1999). However, studies on alternative species are still very limited.

Several studies evaluated the effectiveness of MICP in sand using calcite content measurement (Okwadha and Li 2010; Martinez et al. 2011). However, improvement in the shear strength of sand may not be directly proportional to the calcite content (Whiffin et al. 2007). Improvement in the shear strength of soil is not measurable for calcite content below a 3.5% weight-to-weight ratio or $60\,\mathrm{kg/m^3}$ because a sufficient amount of calcite needs to be formed at the particle contact points to promote effective soil improvement. Al Qabany et al. (2011) and DeJong et al. (2006) used shear-wave velocity as an indirect and nondestructive indicator of calcite precipitation and stiffness improvement in soil specimens. Martinez et al. (2011) and Weil et al. (2012) used this nondestructive technique to monitor the calcite precipitation process in soils. Overall, these indirect measurements have shown good correlations with stiffness, dry density, and porosity of soil. However, they may not be appropriate indicators of the shear strength and hydraulic conductivity of soil, which are more complex in behavioral phenomena. Direct measurement of shear strength using unconfined compression or direct shear tests, and hydraulic conductivity using constant head or falling head permeability tests, are still preferred for assessing the effectiveness of MICP in improving the soil engineering properties in geotechnical applications.

From the aforementioned literature, it can be seen that the procedures and materials required for performing MICP soil improvement have been well studied (Al Qabany et al. 2011; Martinez et al. 2011; De Muynck et al. 2010; Harkes et al. 2010). The preferable conditions to promote MICP for soil improvement need to be studied, particularly for soil types other than sand. This research gap forms the basis for the initiation of the current study. The main objective of this paper is to investigate the preferable conditions for MICP treatment to improve the engineering properties of a typical tropical residual soil (silt). The preferable treatment configuration is determined by taking into account factors that include improved engineering properties, economic practicality, and field applicability. The urease enzyme was produced by *B. megaterium* (strain ATCC 14581). The treatment conditions studied included concentrations of *B. megaterium* and cementation reagent, the flow pressure of the cementation reagent, and the treatment duration. The effectiveness of MICP was evaluated by direct measurements of the unconfined compressive strength and saturated hydraulic conductivity of the soil specimens.

Materials and Methods

Laboratory Setup

Fig. 1 shows the schematic diagram of the experimental setup for the MICP treatment. The apparatus consisted of a steel mold (50 mm in diameter and 170 mm in length), an air compressor, a pressure tank, and an effluent collector. The steel mold was coated with anticorrosion paint to prevent the potential formation of rust during the

course of testing. In addition, the inner surface of the steel mold was coated with grease before each soil specimen was compacted to provide additional rust protection, and to function as a lubricant in the specimen extrusion process. To prepare the soil specimens, air-dried residual soil was first mixed with a culture medium containing the urease-producing microorganism. Sufficient water was added to attain a moisture content of 16.6% (consistent with the optimum moisture content determined from the compaction test). The soil specimen was then compacted into the prefabricated steel mold to a dry density of 1,519 kg/m^3 (which was 90% of the maximum dry density). The soil specimen was sandwiched between two filter layers (gravel), each 10 mm thick, to avoid turbulent inflow and clogging at the inlet.

The specimen mold was clamped vertically on a retort stand. The mold inlet was connected to the pressure tank with the cementation reagent solution. The reagent solution was supplied into the specimen mold at a desired flow pressure by regulating the air compressor. All treatments were performed at room temperature (22–27°C). The pH and ammonium content were monitored by sampling the effluent from the specimen mold at 12-h intervals.

Residual Soil Specimen

The tropical residual soil tested in the current study was taken from a site at the Universiti Tunku Abdul Rahman, Kuala Lumpur campus. Table 1 tabulates the values of the physical indices of the soil specimen obtained from the standard soil properties tests. Based on the Unified Soil Classification System (USCS), the soil was classified as silt (ML), with 32% of the particle grains being 50−400 μm, the ideal size range for MICP (Rebata-Landa 2007).

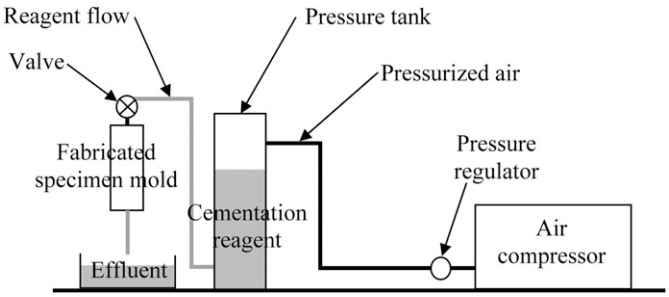

Fig. 1. Schematic diagram of the laboratory setup

Table 1. Properties of the Residual Soil

Property	Value/index
Gravel composition (percentage)	0
Sand composition (percentage)	38
Silt composition (percentage)	43
Clay composition (percentage)	19
Liquid limit (percentage)	40.4
Plastic limit (percentage)	25.9
Plasticity index	14.5
Soil classification (USCS)	ML
Maximum dry density (kg/m^3)	1,688.5
Optimum moisture content (percentage)	16.6
Unconfined compressive strength, q_u (kPa)	76
Saturated hydraulic conductivity, k_{sat} (m/s)	5.4×10^{-8}
Carbonate content (percentage)	0.7

Note: ML = silt; USCS = Unified Soil Classification System.

Microorganism and Growth Conditions

The urease-producing microorganism used in the current study was *B. megaterium* (strain ATCC 14581). *B. megaterium* is a Gram-positive bacterium that can be found in a broad habitat range; however, it is mainly found in soil (Vary 1994). *B. megaterium* has been proven to have the ability to induce calcite precipitation in natural soils (Lian et al. 2006; Cacchio et al. 2003). *B. megaterium* is one of the largest (2−5 × 1.2−1.5 μm) eubacteria known, and has a relatively low urease enzyme activity compared with *S. pasteurii* (Whiffin 2004; Bachmeier et al. 2002; Kaltwasser et al. 1972). The selection of *B. megaterium* as the urease-producing microorganism in the current study was based on three considerations: (1) *B. megaterium* can be found in abundance in natural tropical soils (Lian et al. 2006); (2) the large and elongated rod-shaped *B. megaterium* cell may provide the advantage of avoiding being flushed out during injection of the cementation reagent or by intense tropical rain; and (3) *B. megaterium* can form endospores that are highly resistant to extreme environmental conditions. These characteristics of *B. megaterium* provide enormous advantages for field implementations of the MICP technique in tropical regions that are commonly characterized by high heat and intense rainfall.

The *B. megaterium* was cultivated at pH 7 under aerobic batch conditions in a sterile culture medium consisting of 5-g/L peptone, 5-g/L sodium chloride, 2-g/L yeast extract, and 1-g/L beef extract. Incubation was performed in a shaking incubator at 200 revolutions per minute and constant temperature of 37°C. The *B. megaterium* was grown to an early stationary phase before harvesting at a concentration of approximately 1×10^8 cfu/mL (optical density of 3.3). Other desired concentrations (i.e., 1×10^6 and 1×10^7 cfu/mL) were obtained by dilution with sterile sodium chloride solution (9-g/L NaCl). Viable cell concentration was determined by direct plate counting.

Cementation Reagent

The cementation reagent for the MICP treatment consisted of equimolar concentrations of urea and calcium chloride at varying concentrations (Table 2), and 3 g/L of nutrient broth supplement. The urea and calcium chloride served as important ingredients for promoting calcite precipitation.

Soil Engineering Properties Tests

After the MICP treatment, the specimen mold was attached to a standard falling head permeability test setup to measure the saturated hydraulic conductivity (k_{sat}). Subsequently, the soil specimen was extruded from the mold and trimmed to a height of 100 mm and diameter of 50 mm for the unconfined compression test in accordance with ASTM D2166 (ASTM 2006). It was important to use a specimen mold with an identical diameter (i.e., 50 mm) during the MICP treatment to avoid unnecessarily disturbing the soil when preparing the specimen for the unconfined compression test.

Table 2. MICP Treatment Variables

B. megaterium concentration (cfu/mL)	Reagent concentration (M)	Treatment duration (h)	Reagent flow pressure (bar)
1×10^6	0.25	24	0.2
1×10^7	0.50	48	1.1
1×10^8	1.00	72	2.0

Ammonium Concentration Determination

The ammonium concentration of the effluent was determined by the phenate method [American Public Health Association/American Water Works Association/Water Environment Federation (APHA/AWWA/WEF) 2005]. This method is accurate for measuring ammonium over the range of 0.02−2-mg NH_4^+/1. Samples of higher concentrations were diluted with distilled water to within this range. A sample of 10 mL was mixed with 1 mL of oxidizing agent, 0.4 mL of sodium nitroprusside, and 0.4 mL of phenol solution in a universal bottle. The oxidizing agent was prepared by mixing 100 mL of alkaline citrate solution with 25 mL of sodium hypochlorite (5%). The alkaline citrate solution was prepared by dissolving 200 g of trisodium citrate and 10 g of sodium hydroxide in 1,000 mL of deionized water. The mixture was allowed to react for 1 h at room temperature (22–27°C), under subdued light. The sample was then analyzed using an ultraviolet-visible spectrophotometer (Varian-Cary 100, Varian Medical Systems, Palo Alto, California). The resulting peak's absorbance was 640 nm. The area under the base peak was calibrated with several NH_4Cl standards measured under the same conditions.

Calcite Content Determination

Calcite content in the MICP-treated soil was determined by gravimetric analysis of acidified samples. An oven-dried (at 105°C) soil sample of 20 g was prepared for the test. Carbon dioxide was liberated from calcite by reaction with hydrochloric acid (2 M) as indicated by effervescence. The residue was collected on a filter paper and oven dried at 105°C. The weight loss was used to estimate the percentage of calcite content in the soil specimen. The calcite content was expressed on a dry-weight basis (percentage of the dry weight of soil, i.e., 20 g). It was assumed that the increment of carbonate content in the soil after the MICP treatment was purely caused by the formation of calcium carbonate (calcite).

Treatment Variables

Four treatment variables were considered in this study: (1) concentration of *B. megaterium*, (2) concentration of cementation reagent; (3) treatment duration; and (4) flow pressure of the cementation reagent. The values of these variables are tabulated in Table 2. In total, there were 81 combinations. In addition, seven controls were included to investigate the isolated effect of the *B. megaterium* cell and reagent flow on the engineering properties of the soil. All experiments were done in triplicate and only the average readings are reported because the measurements obtained from the sample replicates were reasonably consistent.

Results

Control Tests

Effective MICP treatment requires the supply of both urease-producing microorganisms and cementation reagents into the soil. Besides calcite formation, resting and dead cells may also improve the shear strength of the soil (Chou et al. 2011). For that reason, seven control tests (Specimens C1–C7) were carried out prior to the main experimental tests: (1) the original soil specimen (C1); (2) a soil specimen with inclusion of only *B. megaterium* (1×10^8 cfu/mL) (C2) to study the effect of the microorganism cell; (3) a soil specimen treated only with cementation reagent (0.5 M) for a duration of 48 h at the low flow pressure of 0.2 bar (C3); (4) three soil specimens treated only with cementation reagent (0.5 M) for 24, 48, and 72 h, respectively, at the intermediate flow pressure of 1.1 bar (C4–C6); and (5) a soil specimen treated with only cementation reagent (0.5 M) for 48 h at the high flow pressure of 2.0 bar (C7). Specimens C3, C5, and C7 were used to compare the influence of the reagent flow pressure, while Specimens C4–C6 were used to study the effect of the treatment duration.

Figs. 2 and 3 show the isolated effects of the *B. megaterium* cell and cementation reagent fluid on the shear strength and hydraulic conductivity of the soil, respectively. Alteration in the soil properties of all control specimens are presented as ratios to the properties of the original control specimen (C1). Treatments with the inclusion of *B. megaterium* or the cementation reagent only were not effective. The shear strengths of all control specimens were reduced (Fig. 2). In another study reported by Ng et al. (2013), it was demonstrated that the biocementation process using both *B. megaterium* and cementation reagent was successful in both sand and residual soil. It was anticipated that Specimen C2 (inclusion of *B. megaterium* only) in the current study could not promote calcite precipitation

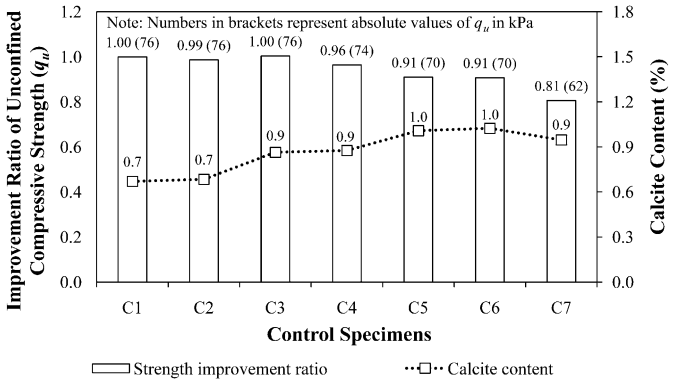

Fig. 2. Unconfined compressive strength (q_u) and calcite content of the control specimens: C1, original soil; C2, with the inclusion of *B. megaterium* (1×10^8 cfu/mL) only; C3, treated with cementation reagent only (0.5 M) for 48 h at 0.2-bar flow pressure; C4–C6, treated with cementation reagent only (0.5 M) for 24, 48, and 72 h, respectively, at 1.1-bar flow pressure; C7, treated with cementation reagent only (0.5 M) for 48 h at 2.0-bar flow pressure

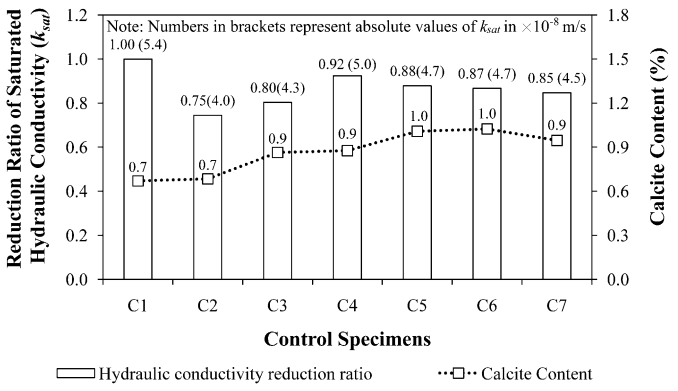

Fig. 3. Saturated hydraulic conductivity (k_{sat}) and calcite content of control specimens: C1, original soil; C2, with the inclusion of *B. megaterium* (1×10^8 cfu/mL) only; C3, treated with cementation reagent only (0.5 M) for 48 h at 0.2-bar flow pressure; C4–C6, treated with cementation reagent only (0.5 M) for 24, 48, and 72 h, respectively, at 1.1-bar flow pressure; C7, treated with cementation reagent only (0.5 M) for 48 h at 2.0-bar flow pressure

without the presence of the cementation reagent. The slight reduction in shear strength (about 1%) of Specimen C2 compared with the original soil (C1) was likely caused by an inconsistency in sample preparation. The reduction in shear strength of the specimen (C7) treated with 2.0-bar reagent pressure was particularly significant, i.e., 19%. This was probably caused by a buildup of pore-water pressure in the soil, which will be discussed in a subsequent section. All control specimens treated with cementation reagent only (C3–C6) exhibited slight decreases (0–9%) in shear strength. This can be explained by the hygroscopic behavior of the reagent as suggested by Lu et al. (2010). The plasticity index and shear strength of soil can be improved by the addition of salt such as calcium chloride or sodium chloride. However, a reverse effect may be encountered when these salts are added in an excessive amount, i.e., >4% (Naeini and Jahanfar 2011).

The inclusion of *B. megaterium* only (C2) significantly reduced the hydraulic conductivity by 25%. This reduction was caused by the physical plugging of soil pores with *B. megaterium* cells. The control specimen (C3) treated with 0.2-bar reagent pressure experienced greater reduction (20%) in hydraulic conductivity than those treated with higher flow pressures, i.e., 1.1 bar (C5) and 2.0 bar (C7) (12 and 15%, respectively). This was because the low flow pressure (0.2 bar) encouraged the growth of indigenous bacteria and prevented the bacterial cells from being flushed out of the soil.

The calcite content in the original residual soil specimen (C1) was 0.7%. Inclusion of *B. megaterium* only (C2) could not promote calcite precipitation. A slight increment (0.2–0.3%) in calcite content was observed in the control specimens treated with only cementation reagent. These observations could be attributed to two factors: (1) the presence of indigenous urease-producing bacteria in the soil; and (2) an oversaturation state of the reagent in the soil leading to chemical precipitation of calcium carbonate or other carbonate minerals.

Effect of Cementation Reagent Flow Pressure

The typical reagent flow pressure for fine sand ranging from 0.1 to 0.3 bar was used, as deduced from flow rates reported in previous studies (Martinez et al. 2011; Nemati et al. 2005; Whiffin et al. 2007). Martinez et al. (2011) performed MICP treatments on a sand column, compacted to 80% relative density. A vertical pressure of 100 kPa was applied on the top of the sand column to normalize the pressure of the reagent injected into the column from the bottom to the top. In the current study, no vertical pressure was applied to the column because the cementation reagent was supplied to the column from the top to the bottom. Higher pressure should be adopted to maintain an equivalent flow rate in residual soil that has a lower hydraulic conductivity than fine sand. Three cementation reagent flow pressures were considered in this study, i.e., 0.2, 1.1, and 2.0 bar.

Fig. 4 compares the shear strength of the soil specimens treated with the three flow pressures. The concentration of *B. megaterium*, cementation reagent, and treatment duration were kept at 1×10^8 cfu/mL, 0.5 M, and 48 h, respectively. The shear strength improvements of these MICP-treated soils were computed as ratios of the shear strength to the original soil control specimen (C1). More calcite content was produced by treatment at 1.1 bar (2.6%) than at 0.2 bar (2.3%). However, the increment in shear strength for treatment at 0.2 bar (100%) was higher than at 1.1 bar (69%). This implied that a low flow pressure (i.e., 0.2 bar) encouraged calcite cementation at the particle contact points. At an excessively high flow pressure (i.e., 2.0 bar), the shear strength was reduced by 13% despite a considerable amount of calcite precipitated (1.4%). A plausible explanation for this observation is that high flow pressure may lead to a buildup of pore-water pressure in the soil as a result of clogging of the soil body and mold outlet, and eventually result in a decrease in the effective stress. A high hydraulic gradient may also result in detachment of soil particles or disturbance of soil structures, and hence a reduction in soil strength, as demonstrated in the results of the control tests.

Fig. 5 shows the variation of hydraulic conductivity with flow pressure. The soil specimens experienced significant reduction in hydraulic conductivity at 0.2 and 1.1 bar. Opposing trends were observed between hydraulic conductivity and shear strength. The hydraulic conductivity reduction was slightly lower at 1.1 bar than at 0.2 bar, whereas the shear strength improvement was significantly higher at 0.2 bar than at 1.1 bar. The contrary trends implied that hydraulic conductivity reduction has a different mechanism than the shear strength improvement. The reduction of hydraulic conductivity in the soil matrix is mainly attributed to calcite clogging in the pore spaces or pore throats. The formation of calcite in pore spaces would be suspended in pore fluid, and eventually filtered by the soil pore throat as the fluid flows through the soil. This filtering phenomenon is controlled by the size ratio of the precipitated calcite particle to the pore throat (Valdes and Santamarina 2006). The larger the size of the precipitated calcite particle relative to pore throat, the more significant is the filtering phenomenon. No specific binding of soil particles is required to obstruct the water flow. Therefore, the

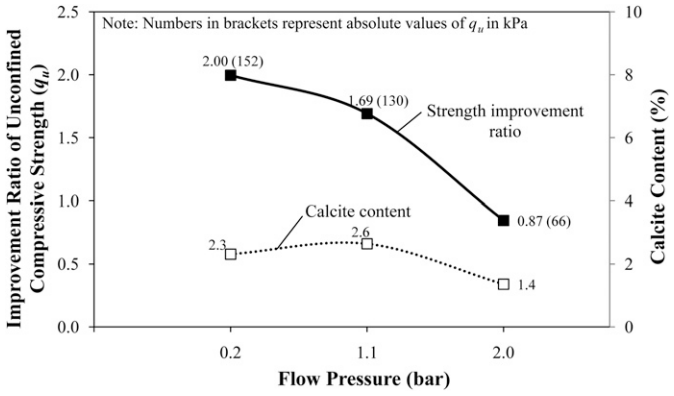

Fig. 4. Effect of the cementation reagent pressure (0.2, 1.1, and 2.0 bar) on the unconfined compressive strength (q_u) and calcite content of the MICP-treated residual soils

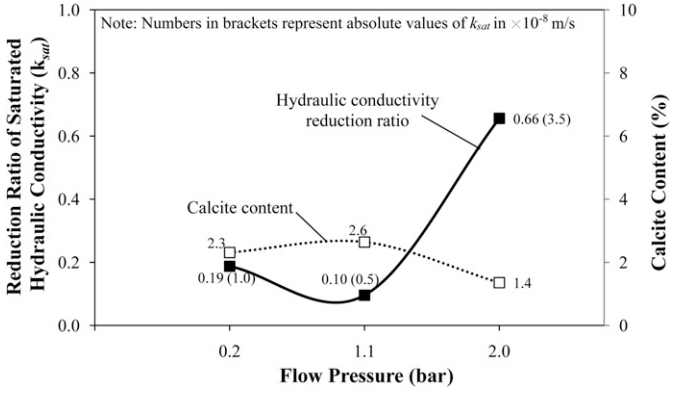

Fig. 5. Effect of the cementation reagent pressure (0.2, 1.1, and 2.0 bar) on the saturated hydraulic conductivity (k_{sat}) and calcite content of the MICP-treated residual soils

reduction in soil hydraulic conductivity tends to be proportional to the amount of calcite precipitated. In the current study, the highest calcite content was measured in the 1.1-bar specimen that contributed to the greatest reduction in hydraulic conductivity. The excessively high flow pressure (i.e., 2.0 bar) flushed out the bacteria in the soil, resulting in a low calcite precipitation, and hence a low reduction in hydraulic conductivity.

Effect of Treatment Duration

Fig. 6 shows the shear strength improvement with treatment duration. To enable indisputable comparison, the *B. megaterium* concentration and cementation reagent flow pressure were maintained at 1×10^8 cfu/mL and 1.1 bar, respectively. The effects of treatment duration at two cementation reagent concentrations, i.e., 0.25 and 0.5 M, are presented.

Treatment with cementation reagent at concentrations of 0.25 and 0.5 M indicated that longer treatment duration produced greater shear strength improvement. The improvement with 0.25 M (34–70%) was slightly lower than with 0.5 M (47–82%) for all treatment durations studied. This is because under the same experimental condition, the 0.5-M reagent provided a greater amount of ingredients (urea and ammonium) per unit of time for promoting the MICP process compared with 0.25 M.

The shear strength results suggested that the improvement (34–47%) primarily developed within the first 24 h of treatment. The second 24 h of treatment contributed to an additional improvement of 23%. The contribution from the third 24 h (12–13%) was the lowest. The trend of shear strength improvement was consistent with the amount of calcite precipitated. The calcite production between 48 and 72 h was insignificant. The results implied that MICP was most effective within 48 h.

Fig. 7 shows the reduction in hydraulic conductivity with treatment duration. The results are comparable with the shear strength results. The reduction in hydraulic conductivity decreased with treatment duration. The reduction for the treatment of the cementation reagent at a concentration of 0.5 M (78–91%) was marginally higher than the treatment with 0.25 M (68–82%).

Effects of Concentration of Bacillus Megaterium and Cementation Reagent

An increase in the concentration of cementation reagent should be complemented by an increase in urease enzyme, produced by the *B. megaterium*, and vice versa. The results of shear strength and hydraulic conductivity with concentrations of cementation reagent and *B. megaterium* at flow pressure of 1.1 bar and treatment duration of 48 h are presented in Figs. 8 and 9, respectively. Treatment with 1.0 M of cementation reagent did not show any measureable change in shear strength and hydraulic conductivity. The measurement of ammonium content and pH further confirmed no detectable urease activity. Kunst and Rapoport (1995) reported that microbial growth under salt-stressed conditions has an adverse impact on enzyme production. High salinity (i.e., 1.0 M) would strongly retard the growth of *B. megaterium* (Nekolny and Chaloupka 2000); however, other species of bacteria may adapt differently to changes in salinity.

For the specimens treated with the cementation reagent at concentrations of 0.25 and 0.5 M, the soil improvement increased with increased *B. megaterium* concentration. The shear strength of the specimens treated with 0.25-M cementation reagent improved by 26–57%, while the 0.5-M reagent recorded improvements of 25–69%. The reduction in hydraulic conductivity for the cementation reagent at concentrations of 0.25 and 0.5 M ranged from 16 to 73 and 22 to 90%, respectively.

At low concentrations of *B. megaterium* (i.e., 1×10^6 and 1×10^7 cfu/mL), the increase in the reagent concentration from 0.25 to 0.5 M did not promote any measurable alterations in the soil

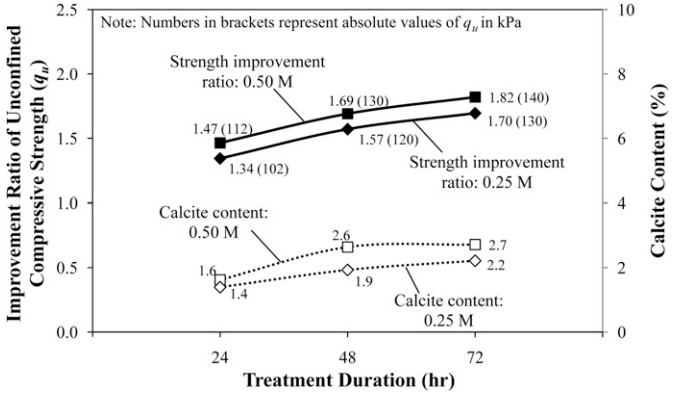

Fig. 6. Effect of the treatment duration (24, 48, and 72 h) on the unconfined compressive strength (q_u) and calcite content of the MICP-treated residual soils

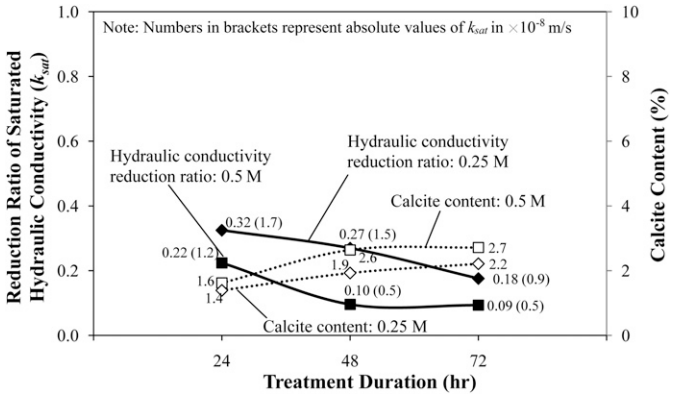

Fig. 7. Effect of the treatment duration (24, 48, and 72 h) on the saturated hydraulic conductivity (k_{sat}) and calcite content of the MICP-treated residual soils

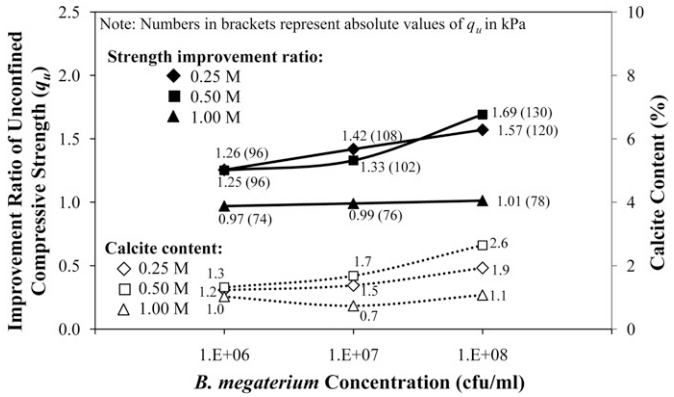

Fig. 8. Effects of the concentration of *B. megaterium* (1×10^6, 1×10^7, and 1×10^8 cfu/mL) and reagent (0.25, 0.5, and 1 M) on the unconfined compressive strength (q_u) and calcite content of the MICP-treated residual soils

engineering properties or calcite content. It was deduced that the concentration of *B. megaterium* was the limiting factor for the MICP. The cementation reagent supplied (i.e., urea and calcium chloride) was in excess of the urease enzyme produced by *B. megaterium*. At a concentration of 1×10^8 cfu/mL *B. megaterium*, there was sufficient urease enzyme produced such that the calcite content increased with increased cementation reagent concentration from 0.25 to 0.5 M, resulting in significantly improved soil engineering properties. The optimum concentration of the cementation reagent should lie between 0.5 and 1 M. Al Qabany et al. (2012) also obtained a similar optimum concentration of cementation reagent, i.e., 0.66 M, despite using a different bacterium (*S. pasteurii*) in their study.

Correlations between Calcite Content, Shear Strength, and Hydraulic Conductivity

Fig. 10 shows the correlations between calcite content, shear strength, and hydraulic conductivity. No improvement in shear strength or hydraulic conductivity was observable for calcite content below 1.0%. Between 1.0 and 2.5% calcite content, good correlations were observed with shear strength improvement ($R^2 = 0.87$) and hydraulic conductivity reduction ($R^2 = 0.65$). At about 2.5% calcite content, maximum enhancement in shear strength was achieved, while hydraulic conductivity still exhibited a steady reduction. This can be attributed to the different mechanisms of shear strength improvement and hydraulic conductivity reduction, as explained previously.

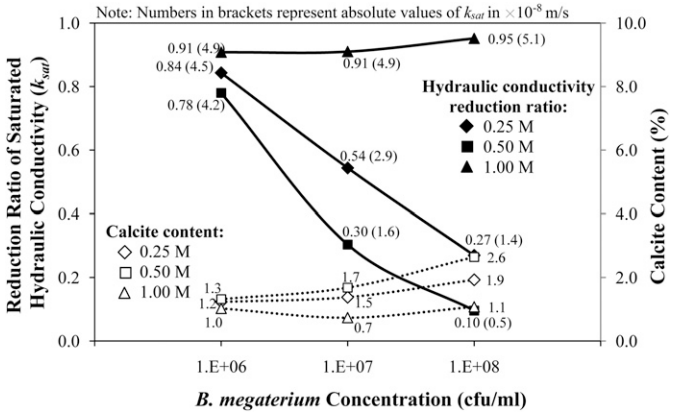

Fig. 9. Effects of the concentration of *B. megaterium* (1×10^6, 1×10^7, and 1×10^8 cfu/mL) and reagent (0.25, 0.5, and 1 M) on the saturated hydraulic conductivity (k_{sat}) and calcite content of the MICP-treated residual soils

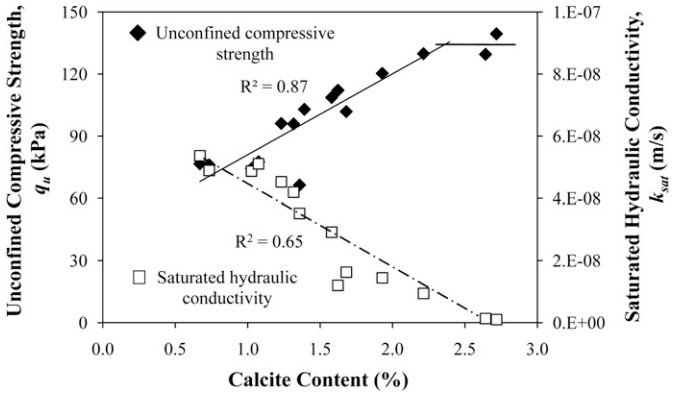

Fig. 10. Correlations between the unconfined compressive strength (q_u), saturated hydraulic conductivity (k_{sat}), and calcite content

Variation in Ammonium Concentration and pH

Figs. 11 and 12 present the variations in ammonium concentration and pH of effluent over treatment time, respectively, for the cementation reagent at concentrations of 0.25, 0.5, and 1.0 M, and a control treatment with a cementation reagent at a concentration of 0.5 M (C5). Except for the control (C5) that was not supplied with *B. megaterium*, all treatments had identical *B. megaterium* concentration (1×10^8 cfu/mL), treatment duration (48 h), and flow pressure (1.1 bar). Both treatments with the cementation reagent at concentrations of 0.25 and 0.5 M showed dramatic increments in ammonium content after 10 h of treatment. The ammonium content of the treatment at 0.25 M peaked after about 24 h. Longer treatment duration did not promote further urea hydrolysis. This could be attributed to insufficient cementation reagent supplied to the soil. The ammonium content of the treatment at 0.5 M peaked after about

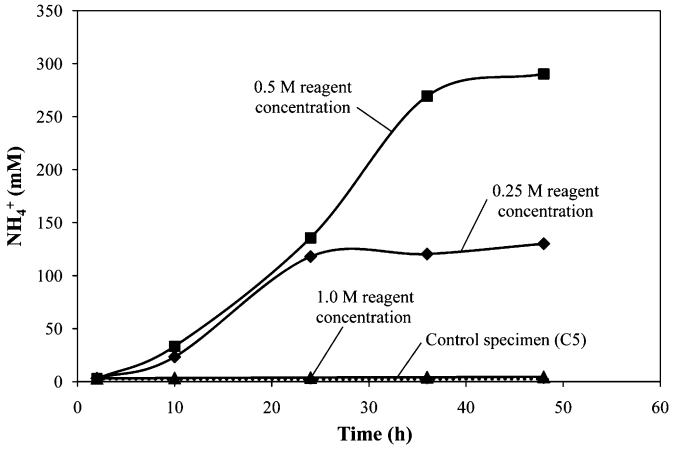

Fig. 11. Variation of the ammonium concentration over time during the MICP treatment of an original control specimen (C1) and three MICP-treated cementation reagent specimens (0.25, 0.5, and 1.0 M)

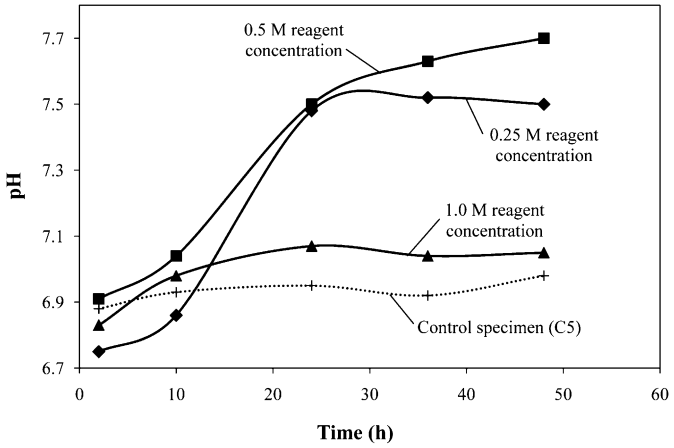

Fig. 12. Variation of the mean pH over time during the MICP treatment of an original control specimen (C1) and three MICP-treated cementation reagent specimens (0.25, 0.5, and 1.0 M)

40 h. The peak ammonium concentration in the effluent was about 2.5 times higher for the treatment at 0.5 M than for the treatment at 0.25 M. A similar trend was observed for pH. The ammonium content and pH for the treatment at 1.1 M were almost identical to that of the control (C5), and this was consistent with the previous findings in the current study. These results showed that ammonium content or pH can be a good indicator of urease activity in MICP treatment.

The starting pH of the effluents was slightly acidic (lower than pH 7), which was attributed to the acidic nature of the residual soil being studied. The production of ammonium ions from urea hydrolysis, induced by the microbes, gradually increased the pH of the soil environment. The pH increase further improved the rate of urea hydrolysis because the optimum pH for urease enzyme is in the range of pH 7–8 (Khan 2011; Stocks-Fischer et al. 1999; Ciurli et al. 1996). This repetitive cycle continued until the pH was no longer optimum (excessively alkaline) for the urease enzyme or survival of *B. megaterium*. The results of the ammonium concentration and pH showed reasonably good agreement with the results of the calcite content and soil engineering properties.

Scanning Electron Microscopy Analyses

Scanning electron microscopy (SEM) analyses were carried out on selected samples to visualize qualitatively the calcite bonds and their distribution in the soil. Figs. 13(a–c) show the SEM images for the original control specimen (C1) and the specimens treated with the cementation reagent at concentrations of 0.25 and 0.5 M, respectively. The particles of the original control specimen (C1) have a smooth surface [Fig. 13(a)]. Both treatments at 0.25 and 0.5 M showed abundant calcite crystals formed at the contact points and on the surfaces of the soil particles. Comparatively, the distribution of calcite crystals was denser for the higher concentration treatment, i.e., 0.5 M [Fig. 13(c)] was denser than 0.25 M [Fig. 13(b)]. The precipitated calcite generally formed prismatic crystals in a bladelike form. The morphology of the calcite crystal was somewhat different from those reported by Al Qabany et al. (2012) and Chou et al. (2011), which had smoother and rounder edges. The morphology of calcite crystals could be governed by numerous factors including CO_2 concentration, the pH of the soil, rate of carbonation, etc. (Cizer et al. 2008). A detailed future investigation of the surrounding environment of the cementation process is required to further clarify the factors affecting the discrepancy in calcite crystal morphology.

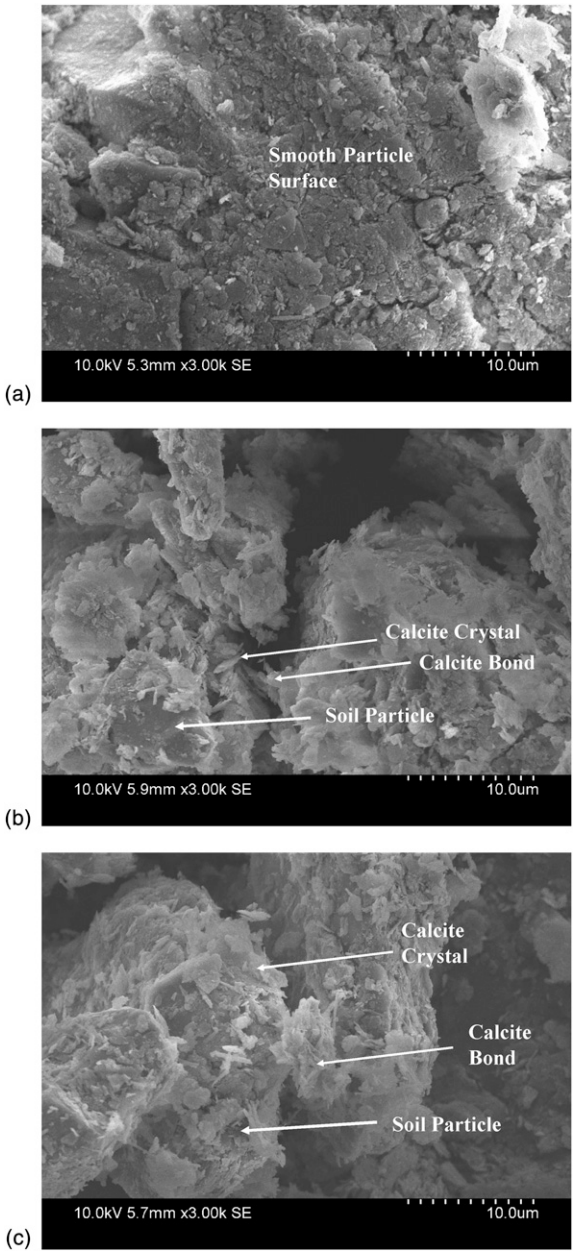

Fig. 13. SEM images of the (a) control specimen (C1); (b) specimen treated with 0.25-M cementation reagent; and (c) specimen treated with 0.5-M cementation reagent

Discussion

From the present experimental study, it can be concluded that MICP treatment has contributed to considerable improvement in the engineering properties of residual soil. However, its applicability to soils other than sand is still very limited; successful attempts using the MICP treatment on residual soil will broaden the applications of MICP soil improvement.

The flow pressure of cementation reagent is an important controlling factor for the MICP treatment. The present laboratory results showed that the high reagent pressure (i.e., 2 bar) has led to an excessive development of pore-water pressure, eventually reducing the shear strength of the soil. In the actual field, it is expected that such an effect may only be critical at the cementation reagent injection point. The effect of excess pore-water pressure tends to be temporary because pressurized fluids eventually drain away, leading to gradual dissipation of the pore-water pressure. However, the disturbance of in situ soil structures could be permanent. The soil particles could be detached by the high-pressure flowing fluid. Despite low flow pressure encouraging formations of effective calcite bonds (as indicated from the present results), the reagent pressure cannot be too low because sufficient pressure is required to offer an acceptable injection distance in field soils. A long injection distance would minimize the required number of injection wells, and hence reduce the cost of treatment. From the calcite content results, 0.2-bar reagent flow pressure produced slightly lower calcite content than did 1.1 bar. A low reagent pressure (i.e., 0.2 bar) can cause bioclogging near the inlet of the specimen, and eventually retard the flowing through of the cementation reagent into the soil specimen. Somehow, this mechanism was not obvious in the current study, which was attributed to the small specimen used. For field residual soil treatment, a cementation reagent pressure in between 0.2 and 2 bar (i.e., 1.1 bar) is recommended.

The amount of calcite precipitated increased with the treatment duration. However, from an economical point of view, the soil improvement needs to be completed within as short a time period of time as possible to minimize the cost of treatment. Therefore, it is important to determine the peak rate of calcite precipitation and the effective calcite content needed to promote soil improvement. At the initial stage of the MICP treatment, the calcite precipitation rate increased with the treatment time. The production of ammonium ions during urea hydrolysis increased the pH and provided a favorable environment to further promote urea hydrolysis. In the current study, calcite precipitation using *B. megaterium* reached a plateau between pH 7.5 and 7.7 (Fig. 12). Numerous studies performed using the more alkalo-tolerant *S. pasteurii* reported a plateau at pH 8.7–9.5 (Martinez et al. 2011; Dupraz et al. 2009; Fujita et al. 2004; Ferris et al. 2004; Stocks-Fischer et al. 1999). Longer treatment duration would further increase the pH to create an excessively alkaline environment, which is not favorable for *Bacillus* survival and urea degradation. De Muynck et al. (2010), and Hammes and Verstraete (2002) suggested that long treatment duration in the presence of calcium ions may also result in local supersaturation and heterogeneous calcite precipitation on the bacteria cell wall. This would eventually lead to cell death and impair the efficiency of MICP. The effective calcite content for promoting measurable improvement in soil engineering properties is in the range of 1.0–2.5% (Fig. 10). From the results, it is justified to suggest that the preferable treatment duration for the residual soil is 48 h. By adopting a treatment duration of 48 h with *B. megaterium* concentration of 1×10^8 cfu/mL, cementation reagent concentration of 0.5 M, and flow pressure of 1.1 bar, the pH increased to pH 7.7 and the calcite precipitated was about 2.6% (marginally exceeding the effective calcite range of 1.0–2.5%).

The soil improvement in the first 24 h of treatment was greater than the second 24 h, despite the almost identical calcite content precipitated. The initial bonding formed between soil particles is crucial for shear strength improvement (DeJong et al. 2010). As treatment continues, most of the particle contact points would have been bonded and the calcite precipitated thereafter is deemed to be less effective in improving the shear strength.

Both the concentration of *B. megaterium* and the concentration of cementation reagent are interdependent factors in the MICP treatment. *B. megaterium* produces the urease enzyme required in urea hydrolysis, and itself acts as a nucleation site for calcite precipitation. The amount of calcite precipitated would increase with increased concentration of *B. megaterium*, provided sufficient cementation reagent is supplied. The cementation reagent that contains urea and calcium chloride should be provided in equimolar concentrations.

Despite the fact that less calcite can be precipitated in the residual soil, the improvement in shear strength of the MICP-treated residual soil (25–100%) was comparable to the results in reported studies on fine sands, i.e., 25–120% of improvements by Lu et al. (2010) and Whiffin et al. (2007). The minimum effective calcite content (1.0%) identified in this study was significantly lower than that reported by Whiffin et al. (2007), i.e., 3.5% for fine sand. This discrepancy of lower effective calcite for improved shear strength in residual tropical soil can be explained by the higher particle-particle contacts per unit volume in residual soil compared with fine sand. The residual soil used in the current study consisted of a mixture of coarse and fine grains. The pores between the coarse grains were filled with the finer grains, and thus resulted in greater particle-particle contacts. This created a favorable environment for the formation of calcite bonds at the particle-particle contacts to improve the shear strength of the soil. With respect to the reduction in hydraulic conductivity, it was comparatively less effective for residual soil (reduction range of 0.3–0.9) than for fine sand (about 1.0 reduction) (Nemati et al. 2005). Ng et al. (2013) claimed that sand, which has a higher porosity, can provide a larger volume of pore spaces for calcite deposition than in the case of residual soil, and hence a greater reduction in hydraulic conductivity.

The MICP process generates ammonium ions as a by-product of urea hydrolysis. Ammonium ions are essential to increase the pH and accelerate the urea degradation rate. However, the toxicity of ammonium ions may cause soil contamination. Ammonia comes in two forms: the ionized form (NH_4^+) and the ammonium salt form (NH_3), while the toxicity is mainly contributed by the latter form. Most ammonium produced in urea hydrolysis would be converted to the ammonium salt form when the pH exceeds 9.5. Furthermore, a fraction of the ammonium may be converted to nitrate (NO_3^-) through bacterial denitrification (Hamdan et al. 2011).

Conclusions

This paper reports a series of experimental tests conducted to investigate the viability of the MICP technique for improving the engineering properties of a typical tropical residual soil. The following conclusions can be drawn from this study:

1. The greatest improvements in shear strength and reduction in hydraulic conductivity achieved are 100 and 90%, respectively. The treatment parameters controlling the improvement considered in this study included *B. megaterium* concentration, cementation reagent concentration, flow pressure of the cementation reagent, and treatment duration.
2. Excessively high cementation reagent flow pressure (i.e., 2 bar) may lead to a buildup of pore-water pressure and disturbance of soil structure, and hence adversely impact the soil improvement. To the other extreme, excessively low flow pressure (i.e., 0.2 bar) may precipitate calcite close to the inlet to prohibit the flow of reagent through the soil specimen. A moderate flow pressure (i.e., 1 bar) is recommended to maintain an adequate injection distance of the cementation reagent while avoiding the potential development of excess pore-water pressure.
3. The preferable treatment conditions for residual soil are *B. megaterium* concentration of 1×10^8 cfu/mL, cementation reagent concentration of 0.5 M, and flow pressure of 1.1 bar for a treatment duration of 48 h. The shear strength improvement and hydraulic conductivity reduction obtained from this combination of treatment parameters are 69 and 90%, respectively.
4. A minimum calcite content of 1.0% (15 kg/m^3) is required for a measurable improvement in shear strength or reduction in hydraulic conductivity of residual soil. The shear strength improvement and hydraulic conductivity reduction are linearly proportional with calcite content between 1.0 and 2.5%. Above 2.5% calcite content, the shear strength improvement becomes less because almost all available particle-particle contact points are bonded by calcite. The hydraulic conductivity reduction does not exhibit this effect.
5. The improvement in the soil engineering properties obtained from the control specimens treated with cementation reagent only is negligible. The inclusion of *B. megaterium* only reduces the soil hydraulic conductivity by about 26% when the soil pores are plugged by the microorganism cells, which is deemed to be a temporary effect.

Acknowledgments

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