IMPROVEMENTS IN ENGINEERING PROPERTIES OF TROPICAL RESIDUAL SOIL BY MICROBIALLY-INDUCED CALCITE PRECIPITATION

NG WEI SOON

MASTER OF ENGINEERING SCIENCE

FACULTY OF ENGINEERING AND SCIENCE
UNIVERSITI TUNKU ABDUL RAHMAN
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IMPROVEMENTS IN ENGINEERING PROPERTIES OF TROPICAL RESIDUAL SOIL BY MICROBIALLY-INDUCED CALCITE PRECIPITATION

By

NG WEI SOON

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ABSTRACT

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NG WEI SOON

Microbially-induced calcite precipitation (MICP) is a relatively new and sustainable soil improvement technique. This technique utilizes bio-activity to precipitate calcite, and to improve engineering properties of soil through formations of coating and bonds between soil particles. Preliminary results have proved the feasibility of MICP in improvement of residual soil. The main objective of this study is to determine the preference conditions for effective MICP treatment in improving the soil engineering properties (shear strength and hydraulic conductivity) of a typical residual soil. Four variables were considered in the MICP treatment; they were reagent flow pressure (0.2, 1.1, and 2.0 bars), treatment duration (24, 48, and 72 hours), reagent concentration (0.25, 0.5, and 1.0 M), and *B. megaterium* concentration (1×10^6, 1×10^7, and 1×10^8 cfu/ml). The results suggested that the preference treatment conditions are 1.1 bars reagent flow pressure, 48 hours treatment duration, 0.5 M reagent concentration, and 1×10^8 cfu/ml *B. megaterium* concentration. The corresponding alteration recorded were 69% increment for shear strength and 90% reduction for hydraulic conductivity. The calcite content showed reasonably good comparison with the improvements in the soil engineering properties. Ammonium concentration and pH of effluent increased during MICP treatment indicating the presence of urease bio-activity. Control specimens (original untreated soil, inclusion of *B. megaterium* only, flowing of reagent only) did not show any sizeable alterations to soil engineering properties. There was an exception where the growth of biomass (inclusion *B. megaterium* only) clogged the soil pores and reduced the hydraulic conductivity of soil by about 25.5%. Calcite formation at the surface of residual soil particles was further verified by the Scanning Electron Microscope (SEM) imaging.

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APPROVAL SHEET

This dissertation entitled "**IMPROVEMENTS IN ENGINEERING PROPERTIES OF TROPICAL RESIDUAL SOIL BY MICROBIALLY-INDUCED CALCITE PRECIPITATION"** was prepared by NG WEI SOON and submitted as partial fulfilment of the requirements for the degree of Master of Engineering Science at Universiti Tunku Abdul Rahman.

Approved by:

____
(Dr. Lee Min Lee)   Date:…………………
Assistant Professor/ Supervisor
Department of Civil Engineering
Faculty of Engineering Science
Universiti Tunku Abdul Rahman

____
(Dr. Tan Chew Khun)   Date:…………………
Assistant Professor/ Co-supervisor
Department of Petrochemical Engineering
Faculty of Engineering and Green Technology
Universiti Tunku Abdul Rahman

FACULTY OF ENGINEERING SCIENCE

UNIVERSITI TUNKU ABDUL RAHMAN

Date: ______________

SUBMISSION OF FINAL YEAR PROJECT /DISSERTATION/THESIS

It is hereby certified that NG WEI SOON (ID No: 11UEM06216) has completed this final year project/ dissertation/ thesis* entitled "IMPROVEMENTS IN ENGINEERING PROPERTIES OF TROPICAL RESIDUAL SOIL BY MICROBIALLY-INDUCED CALCITE PRECIPITATION" under the supervision of Dr. Lee Min Lee (Supervisor) from the Department of Civil Engineering, Faculty of Engineering Science, and Dr. Tan Chew Khun (Co-Supervisor)* from the Department of Petrochemical Engineering, Faculty of Engineering and Green Technology.

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DECLARATION

I NG WEI SOON hereby declare that the dissertation is based on my original work except for quotations and citations which have been duly acknowledged. I also declare that it has not been previously and concurrently submitted for any other degree at UTAR or other institutions.

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(NG WEI SOON)

Date ______________________

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LIST OF SYMBOLS / ABBREVIATIONS

A	Area of cross section, mm^2
c_u	Shear strength of soil, kPa
k_{sat}	Saturated hydraulic conductivity, m/s
q_u	Unconfined compressive strength, kPa

ρ	Density, kg/m^3
ρ_{max}	Maximum index density
ρ_{min}	Minimum index density
ϕ'	Effective internal friction angle

AR	Analytical reagent
BHI	Brain heart infusion
BS	British Standard
cfu	Colony forming unit
EDS	Energy dispersion spectroscopy
MDD	Maximum dry density
MICP	*Microbially-induced calcite precipitation*
NB	Nutrient broth
OD	Optical density
OMC	Optimum moisture content
R^2	Coefficient of determination
SEM	Scanning electron microscopy

C	Carbon
Ca	Calcium
Ca^{2+}	Calcium ions
$CaCl_2 \cdot 2H_2O$	Calcium chloride dihydrate
$CaCO_3$	Calcium carbonate
$CO\ (NH_2)_2$	Urea
CO_2	Carbon dioxide
${CO_3}^{2-}$	Carbonate ions
Fe	Iron
H_2O	Water
K	Potassium
N	Nitrogen
NaCl	Sodium chloride
Mg	Magnesium
NH_3	Ammonia
${NH_4}^{+}$	Ammonium
NH_4Cl	Ammonium chloride
$NaHCO_3$	Sodium bicarbonate
O	Oxygen atom
P	Phosphorus
Si	Silica

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CHAPTER 1

INTRODUCTION

1.1 Background of Study

Microbial-induced calcite precipitation (MICP) is a relatively new and innovative soil improvement technique based on biochemical treatment. Soil improvement, in the context of geotechnical engineering, refers to enhancement of inherent engineering properties of soil (such as increment in shear strength and stiffness, reduction in hydraulic conductivity and compressibility) to accommodate the needs of construction. Existing soil improvement techniques, such as chemical grouting (except sodium silicate) are mostly toxic and hazardous (Karol 2003; DeJong et al., 2010). There are expressed environmental concerns over their applications despite their proven effectiveness in geotechnical engineering (DeJong et al., 2010).

Current construction trend has put great emphasis on sustainable development and construction with minimal pollution. Soil improvement through MICP can provide an alternative to ‘green construction’ as the treatment process exerts minimal disturbances to soil, human health, and environment. It is a process that exists in nature in view of urease-producing microorganism can be found in abundance in natural soil and groundwater (Lloyd and Sheaffe 1973; Swensen and Bakken 1998; Fujita et al., 2000). For the purpose of soil improvement, the MICP process is intensified by increasing the concentration of urease-producing microorganism and cementation reagent in soil matrix. Nevertheless, it should be noted that the MICP process is not perfectly environmental friendly. The process generates by-product of ammonium and its oxidized by-product nitrate, which can be toxic for soil organisms, particularly at high concentrations (van Paassen et al., 2010).

Despite being a relatively young 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 shear stiffness and shear capacity. Treated sand exhibits non-collapsing strain softening shear behavior.

Several researchers attempted to formulate appropriate procedure to distribute and fix urease-producing bacteria homogeneously in soil to promote effective MICP. Harkes et al. (2010) found that two-phase injection procedure could contribute to homogenous distribution of *S. pasteurii* in sand column. The two-phase injection was by first, injection of *S. pasteurii* suspensions and second, injection of a fixation fluid (high salt content). This procedure has successfully retained 100% of urease activity in the sand column, through the retention of *S. pasteurii* in sand column. Besides microbe-induced calcite formation, the urease enzyme can be supplied directly for the MICP reactions. Nemati et al. (2005) investigated the plugging behaviour of porous medium for reduction of soil hydraulic conductivity. They found that both microbe-induced and enzyme-induced calcite formation effectively reduced soil hydraulic conductivity. However, the use of biomass is not recommended because biomass can be readily degraded overtime and hence is not a durable plugging agent.

Martinez et al. (2011) studied the effects of stopped-flow injection and continuous injection on the uniformity of calcite formation in sand column and found that the former offered better uniform cementation in the sand column.

Ivanov and Chu (2008) presented a detailed review on the applications of MICP for soil improvement. At present, promising MICP applications only focus on biocementation and bioclogging. Biocementation improves soil strength by formation of cementation materials through microbial means. Bioclogging reduces hydraulic conductivity of soil by filling of the soil pores through microbial processes.

A measurement technique was developed to investigate the effect of MICP on soil in non-destructive manner. Weil et al. (2012) developed a real-time S-wave and P-wave velocities measurement system to monitor the spatial distribution of MICP in sands. Increased calcite content improves stiffness of the soil and yield a higher S-wave velocity. Al Qabany et al. (2011) found a linear correlation between calcite content and S-wave velocity measurement. This correlation can be used to evaluate the performance of MICP for soil improvement without affecting the engineering properties of soil (DeJong et al., 2006; Martinez et al., 2011).

Most studies of MICP treatments have been performed on a laboratory scale (Whiffin et al., 2007; DeJong et al., 2006; Ivanov and Chu 2008; DeJong et al., 2010; Mitchell and Santamarina 2005). van Paassen (2011) provided an overview of research development in the Netherlands, using scale-up laboratory tests and field-scale experiments. The MICP technique has been applied successfully in field to strengthen the wall of borehole from soil collapsing during drilling process.

The applications of MICP are not restricted to soil improvement. Durability of mortar specimens can be improved by MICP (De Muynck et al., 2008). Resistance against deterioration, characterized by carbonate rate and chloride mitigation, can be decreased by as much as 40 %. Compressive strength of MICP treated mortar, which has been amended with fly-ash, improved by 10 to 19 % (Achal et al., 2011). Durability of bricks that is characterized by resistance against water absorption, can be improved by as much as 45% (Sarda et al., 2009).

This study evaluates the improvement in engineering properties, i.e. increment in unconfined compressive strength and reduction in saturated hydraulic conductivity, of a typical tropical residual soil by MICP. The preference conditions for promoting the effective MICP treatment are investigated.

1.2 Problem Statement

The state of the art research of MICP soil improvement has so far focused primarily on fine sands (Harkes et al., 2010; Ruyt and Zon 2009; Qian et al., 2010), but very little studies on other soil types. Optimum grain size for MICP treatment is between 50 μm and 400 μm (Rebata-Landa 2007). Fine soil where pore throat-size is sufficiently small to limit the free passage of bacteria is not favourable. On the other extreme, coarse soil would require large amounts of calcite for effective improvement. Nevertheless, it is of particular interest to many geotechnical engineers to assess the performance of MICP for soils that containing fine and coarse grains that reflects soil particle distribution in reality. van Paassen (2011) attempted the MICP technique on gravels, and Mortensen et al. (2011) tested on a wide range of soil grain sizes including sand, silty sand, and silt. They concluded that the MICP treatment was equally robust in these soil types.

Most *Bacillus* strains can produce urease enzyme for urea hydrolysis (Hammes et al., 2003). Reported studies have mostly adopted *S. pasteurii* as the urease-producing microorganism (DeJong et al., 2006; Martinez et al., 2011; Harkes et al., 2010; Stocks-Fischer et al., 1999). Studies on alternative bacilli 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 shear strength of sand may not be directly proportional to the calcite content (Whiffin et al., 2007). For instance, the improvement in shear strength of soil was not measurable for calcite content below 3.5 % w/w or 60 kg/m^3. This is 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 non-destructive indicator for calcite precipitation and stiffness improvement in soil specimens. Martinez et al. (2011) and Weil et al. (2012) also used the non-destructive technique to monitor calcite precipitation process in soils. These indirect measurements have shown good correlations with physical and mechanical properties of soil i.e. stiffness, dry density, porosity etc. However, they may not be appropriate indicators for engineering properties of soil, such as shear strength and hydraulic conductivity which are of greater interests to geotechnical engineers. Direct measurement of shear strength using unconfined compression test or direct shear test, and hydraulic conductivity using constant pressure or falling head permeability test are preferred for assessing effectiveness of MICP in improving soil engineering properties for geotechnical applications.

From the foregoing, it can be concluded that the procedures and materials required for performing MICP soil improvement have been well studied (Martinez et al., 2011; Harkes et al., 2010; De Muynck et al., 2010b; Al Qabany et al., 2011). However, the preference conditions to promote MICP for soil improvement, particularly for soil media other than sand, have not been studied thoroughly. This research gap forms the basis for the initiation of the present study.

1.3 Aims and Objectives

The aim of this research is to determine the preference conditions for MICP treatment on residual soil. The effectiveness of MICP treatment is assessed by comparing the engineering properties of the residual soil before and after treatment. The objectives set out to achieve the aim are:

i. To investigate the feasibility of applying MICP soil improvement technique on a typical tropical residual soil.
ii. To investigate the effects of MICP soil improvement technique on shear strength and hydraulic conductivity of the residual soil.
iii. To identify the preference conditions for the MICP treatment.
iv. To examine the relationship between calcite content and enhanced engineering properties in the MICP treated residual soil.

1.4 Scope of Study

This research was conducted using a small scale experimental approach. A steel column was fabricated with an inlet and an outlet at each end. The design was to effect one-dimensional flow of cementation reagent through the soil specimens.

The main soil material investigated in this research was a typical tropical residual soil. The soil was extracted from Kuala Lumpur campus of the Universiti Tunku Abdul Rahman. In addition, typical concreting sand was also adopted for the preliminary testing stage. MICP technique has been proven effective in improving engineering properties of sand material. Findings from preliminary tests would provide a baseline for the proper study of the MICP treatment for the tropical residual soil. The effectiveness of MICP treatment was assessed by evaluating two engineering properties of the residual soil, i.e. unconfined compressive strength and saturated hydraulic conductivity. The distribution of cemented sites in the soil was beyond the scope of this study.

Urease enzyme is required to trigger the precipitation of calcite. Urea is decomposed by urease into carbonate ion, and forms calcite if calcium ion presents. This study acquires the production of urease enzyme through microbial activity. *B. megaterium* was the only urease-producing microorganism adopted throughout this experimental work.

Four treatment variables were considered in the study: (i) reagent flow pressure that regulates the rate of cementation reagent flowing through the soil specimens, (ii) treatment duration of MICP on the soil specimens, (iii) concentration of *B. megaterium* used in the soil mixtures, and (iv) concentration of cementation reagent used for flowing through the soil specimens. These four variables were identified as the main controlling factors for MICP soil treatment. Other treatment conditions, i.e. temperature, pH, bacteria type, soil grain size, injection method etc. were remained invariable.

1.5 Structure of Report

This thesis is divided into six chapters: Introduction (Chapter 1), Literature Review (Chapter 2), Research Methodology (Chapter 3), Preliminary Experimental Results (Chapter 4), Results and Discussion (Chapter 5), Conclusion and Recommendation (Chapter 6). Concluding Remarks are presented at the end of Chapters 2, 4 and 5 to summarize the content of the chapters.

Chapter 1 provides a brief background of the MICP soil improvement technique, problem statement, aim and objectives, scope of study, and the thesis chapter organization.

Chapter 2 contains findings of previous literature on residual soil and MICP. This chapter includes introduction of MICP and biochemical reactions responsible for improving engineering properties of soil. Besides, the applications of MICP on other construction materials such as sand, concrete, and brick are discussed. This chapter also provides detailed reviews on factors affecting the MICP performance for soil improvement. The states of the art in researches of MICP soil improvement technique are reported. Research gaps are identified from the reported studies and they form the basis for the initiation of the present study.

Chapter 3 provides detailed description on how the research project is planned and implemented. This chapter includes experimental design, standard procedure descriptions, and apparatus adopted in the experimental works.

Results and discussions are presented separately in two chapters: Chapter 4 and Chapter 5. In Chapter 4, results from preliminary experimental tests are presented. Sand and tropical residual soil specimens are treated under identical MICP treatment conditions, and their performances are compared. The findings from these preliminary tests are essential to prove the feasibility of applying the MICP technique on tropical residual soil.

Chapter 5 reports the main results of the present study. The treatment parameters that contribute to the highest engineering properties improvements with the lowest reagent / effort input are identified as the preference treatment condition. The findings are compared with previous reported studies, and discussed in relation to hypothesis of the present study.

The final chapter, Chapter 6, concludes the important findings obtained from this experimental study. Limitations of current study and recommendations for future research are also discussed.

CHAPTER 2

LITERATURE REVIEW

2.1 Introduction

This chapter provides an overview on the emergence of MICP soil improvement technique and its advantage over the current techniques. The biochemical reactions associated to MICP are explained. The potential applications of MICP technique on various construction materials are reviewed. The technique was delved through the summarization of factors affecting the effectiveness of MICP in soil improvement. Finally, considerable state of the art literature pertaining to the topic of MICP soil improvement was critically reviewed.

2.2 Emergence of MICP Soil Improvement

Nowadays, construction on problematic soils is inevitable owing to the growing scarcity of land worldwide. Problematic soils are commonly characterized by low strength and high compressibility (Huat 2006; Kazemian et al., 2011; Ho and Chan 2011). In tropical regions like Malaysia, soils are subject to further softening due to infiltration of intense and prolonged downpour. Consequently, development on the problematic soils is highly susceptible to severe geohazards including excessive settlement of embankment or foundation, debris flow, and catastrophic landslide.

Parts of this chapter were published in:

1. **Ng, W.S.**, Lee, M. L., & Hii, S. L., 2012. An overview of the factors affecting microbial-induced calcite precipitation and its potential application in soil improvement. *Proceeding of World Academy of Science Engineering and Technology*, 19-21 February 2012 Kuala Lumpur, Malaysia. Kuala Lumpur: WASET, (62), pp. 723-729.

Studies on soil improvement techniques can be found in abundance. The important features of soil improvement include: improving the shear strength of soil, reducing potential for total and differential settlement, reducing the time during which the settlement takes place, reducing potential for liquefaction in saturated fine sand or hydraulic fills, reducing the hydraulic conductivity of soil, removing or excluding water from soil etc (Kazemian and Huat 2009; Leonards 1962; Krebs and Walker 1971). Conventionally, the norm is to replace low strength soil deposits with engineering fill. Presently, the use of chemical grouting is becoming increasingly popular owing to its economical benefit. Chemical grouting can be achieved with a variety of additives including Portland cement, lime, asphalt, sodium silicate, acrylate, lignin, urethane, and resins. While many of these additives have proven successful (Karol 2003; Xanthakos et al., 1994; Anagnostopoulos and Hadjispyrou 2004; Peethamparan et al., 2009; Basha et al., 2005), the additives often modify the pH of the soils, and may contaminate the soils and groundwater (DeJong et al., 2006; Karol 2003).

In recent years, with increasing awareness of environmental issues, there has been a remarkable shift toward ‘green’ and sustainable technologies. As all chemical grouts except sodium silicate are toxic and hazardous, there are expressed concerns over their use for soil improvement (DeJong et al., 2010). A new soil improvement technique that utilizes a biological process, which is termed technically as *Microbial-Induced Calcite Precipitation (MICP)*, has emerged recently. MICP has been enabled through interdisciplinary researches at the confluence of microbiology, geochemistry, and geotechnical engineering, to find natural treatments for soil improvement (DeJong et al., 2010). MICP is a biological process that occurs in nature. It is intensified by introducing a large population of calcite forming microorganisms and cementation reagent into the soil matrix, whereby a cement compound is generated to improve the engineering properties of the soil. The environmental friendly characteristics of the calcite forming microorganisms will cause very little, if not no impairment to the soil, human health, and environment.

Despite the fact that MICP soil improvement technique is still a relatively young technology, many studies pertaining to the topic have been reported including Baveye et al. (1998), Castainer et al. (1999), Ehrlich (1999), Mitchell and Santamarina (2005), Lian et al. (2006), Ivanov and Chu (2008), Dejong et al. (2010), Okwadha and Li (2010), Harkes et al. (2010) and, Lu et al. (2010). These studies have contributed to a strong basis for further developing this innovative technique as a practical solution for soft ground problems.

2.3 Biological Reaction of MICP

Calcite precipitation can be induced in several MICP processes. Castanier et al. (1999) have conducted an extensive review on potential carbonate precipitation pathways, associated species of microorganism and environments. The main categories of calcite precipitation process include photosynthesis, sulphate reduction, nitrogen cycle and other unspecified pathway.

Photosynthesis could promote carbonate precipitation with the aid of fungi, algae and other geochemical agents (Ehrlich 1998; McConnaughey and Whelan 1997). Calcification is the result of calcareous plants' autotrophic nutrient acquisition physiologies in nutrient-deficient environment. For sulphate reduction, the dissolution of gypsum and removal of suphate by sulphate-reducing bacteria can eliminate the inhibitors for carbonate formation (Wright 1999). These activities increase the resultant pH and offer mechanism that favors the dolomite precipitation. A nitrogen cycle involves ammonification, nitrate reduction or urea hydrolysis. These three mechanisms are all capable of producing calcium carbonate (Stocks-Fischer et al., 1999; Castainer et al., 1999; Fujita et al., 2000).

The potential of urea hydrolysis, aerobic oxidation, denitrification, and sulphate reduction for MICP were studied and compared by van Paassen et al. (2010). They concluded that urease production possesses a greater calcite conversion rate compared to other processes. Urea hydrolysis is the most common MICP process for soil improvement. This is supported by the fact that most of the studies pertaining to the topic of MICP have adopted the urea hydrolysis process for calcite precipitation.

Urease enzyme decomposes urea ($CO(NH_2)_2$) in soil through a chemical reaction known as urea hydrolysis. The enzyme can be either supplied externally into soil (Nemati and Voordouw 2003) or produced in-situ by urease-producing microorganism (Whiffin et al., 2007; Martinez et al., 2011; DeJong et al., 2006). Quantitatively, 1 mole of urea is hydrolyzed to 2 moles of ammonium, in the presence of urease enzyme:

$$CO(NH_2)_2 + 2H_2O \xrightarrow{\text{urease}} 2NH_4^+ + CO_3^{2-} \qquad (2.1)$$

The ammonium (NH_4^+) released from the urea hydrolysis results in a local pH rise that commences the precipitation of calcium carbonate (calcite). Calcite is precipitated through the reaction between carbonate ions (CO_3^{2-}) from the urea hydrolysis and calcium ions (Ca^{2+}) from the supplied calcium chloride:

$$Ca^{2+} + CO_3^{2-} \rightarrow CaCO_3 \qquad (2.2)$$

The calcite ($CaCO_3$) formed is responsible for improving the engineering properties of soil.

The process of calcite precipitation by microbes can be described in sequences by Figures 2.1a – 2.1d (De Muynck et al., 2010a). At Figure 2.1a, calcium ions in the solution are attracted by negative charges to the cell wall of microbes. Addition of urea leads to the release of dissolved inorganic carbon (DIC) and ammonium in the microenvironment of the microbes. At Figure 2.1b, the existence of calcium ions causes local supersaturation and thus heterogeneous calcite precipitation on microbial cell wall. At Figure 2.1c, after certain period of time, the microbes become encapsulated by calcite, resulting in limited or no nutrient transfer and eventually exterminate the microbes. Figure 2.1d shows the imprint of microbial cells that take part in calcite precipitation.

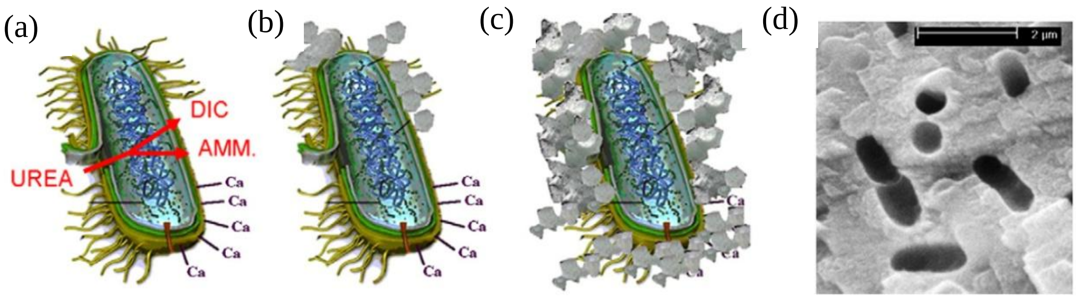


Figure 2.1: Simplified representation of the events occurring during the ureolytic induced carbonate precipitation (a) calcium ion attracted to cell wall; (b) calcite precipitated near cell wall; (c) calcite increased in quantity and encapsulate cell; (d) imprint of cell that take part in calcite precipitation (De Muynck et al., 2010a).

2.4 Applications of MICP in Construction Materials

Potential applications of MICP in construction materials have been reported by numerous researchers. The studied construction materials mainly focused on sand, concrete, mortar and brick. MICP improves properties of sand by enhancing the strength and reducing hydraulic conductivity. Concrete, mortar and brick are improved in strength and durability. The details of these applications of MICP are reported in the following sections.

2.4.1 Sand

Whiffin et al. (2007) evaluated the feasibility of MICP as a sand strengthening technique. A sand column of 5 m in height was treated with bacteria and reagent. The strength of sand was increased by 1.8 to 3.4 times, proportional with the amount of calcite precipitated. However, the study proposed that a minium 3.5 % or 60 kg/m^3 of calcite was required for measurable improvement in compressive strength. The study on soil was conducted by Lu et al. (2010) to determine the effect of calcite precipitation on its compressive strength. The compresive strength of soil was improved by 140 %, relative to original soil. Besides, the result implied that the soil treated with reagent only (without the addition of bacteria suspension) experienced a reduction in compressive strength. The authors explained that this observation was caused by the hygroscopicity of substrates.

The calcite content in MICP treated sand is not uniformly distributed. The calcite tends to decrease with increasing distance from reagent injection point (Whiffin et al., 2007). The calcite content was in the range of 85 to 105 kg/m^3 near the injection point (10 to 120 cm from injection point) and gradually drop to the range of 2 to 30 kg/m^3 near the other end of sand column (260 to 500 cm from injection point).

Besides enhancement in strength, calcite precipitation could clog the pores and reduce the hydraulic conductivity of sand. Nemati and Voordouw (2003) investigated the plugging of porous media (mixture of coarse sand and glass beads) by MICP. The hydraulic conductivity of sand was reduced though the accumulation and plugging of calcite at sand pore spaces. The urease enzyme was supplied into the porous media, instead of produced in-situ by urease-producing microorganism. Remarkable reduction in hydraulic conductivity was observed and the greatest reduction recorded was 98 %.

Nemati and Voordouw (2003) have also found that sand specimen could be treated mutliple times, and each treatment has delivered further diminution in hydraulic conductivity. The decrease in hydraulic conductivity after the first and second injections were 92 % and 72 %, respectively, making the overall diminution to be 98 %. The third injection failed to deliver measurable diminution in hydraulic conductivity. The result implied that increase in urease concentration (from 0.01 to 0.1 g/l) has lead to increase in calcite production. Besides, the increment in reagent concentration up to some extent (0.5 M) increased the amount of calcite precipitated. In addition, the temperature increment from 20 to 30 ℃ altered the rate and amount of calcite precipitation. The effects of these variables on calcite precipitation are further discussed in Section 2.6.

Another study of Nemati et al. (2005) compared the hydraulic conductivity reduction of porous media (mixture of coarse sand and glass beads), using urease enzyme supplied directly into sand or produced in situ by *Proteus Vulgaris* (urease-producing microorganism). The reduction in hydraulic conductivity for specimens treated with biomass only, combination of biomass and reagent, and combination of urease enzyme (direct supply) and reagent were 52 %, 65 %, and 62 %, correspondingly. The experimental results indicated that bacterial and enzymic treatments have both delivered an approximately equivalent plugging effect on sand. The plugging induced by the combination of biomass and reagent was however not reliable as the reduction in hydraulic conductivity was mainly due to the accumulation of biomass, which is not a durable plugging agent.

MICP was adopted as innovative method to increase the seismic properties of sand and its resistance against liquefaction. Montoya et al. (2012) evaluated the potential of calcite precipitation in improving the sand resistance against seismic loading. The resistance towards liquefaction and deformation was improved dramatically, contributed by the binding of soil particles. The bonding between soil particles and calcite precipitated promoted small-strain stiffness and strength of treated sand (Montoya et al., 2012; Mortensen and DeJong 2011). Mortensen and Dejong (2011) reported that the peak normalized shear strength of treated sand was about 1.5 times the original sand.

2.4.2 Concrete

Durability of cementitious materials depends on their resistance against deteroriation. De Muynck et al. (2008) investigated the effect of calcite precipitation on the durability of mortar. The precipitation of calcte on the surface of mortar has reduced its water absorption for 65 % to 90 %. The diminution in water absorption has limited the migration of chloride and gas into mortar, and hence reduced the deterioration caused by carbonation and chloride-induced corrosion. The study conducted by Achal et al. (2011) reported a 50 to 72 % diminution in water absorption of fly ash amended mortar.

The compressive strength of mortar was also increased as the result of MICP treatment. Achal et al. (2011) reported a 10 to 19 % increments in compressive strength of mortar cubes, as the result of crystal subtances formation. The calcite may have deposited on the surface of fly ash amended mortar or within the cement-sand matrix, and plug the pores within mortar (Ramachandran et al., 2001; Ramakrishnan et al., 1998). Siddique et al. (2008) and Vijay et al. (2009) found that the increments of compressive strength for mortar cubes were 5 to 26 %, relatively consistent with findings of Achal et al. (2011).

2.4.3 Brick

Sarda et al. (2009) utilized the precipitation of calcite to improve the durability of brick. Most of the deterioration of brick is caused by the presence of moisture. The penetration of water, especially those carries chlorides, have corrosive effect on brick. The deposition of calcite on brick surface and void spaces restricted its water absorption. The durability of brick was improved as the result of lower water absorption. The treatment conditions considered by Sarda et al. (2009) include a normal water treatment (as a control), and MICP treatments with nutrient broth (NB) and brain heart infusion (BHI) media. The water absorption of the control specimen (treated with water only) recorded was 25 %. The MICP treatment lowered its water absorption to 21.5 % (NB) and 14.8 % (BHI media). The difference in water absorption reduction observed for the brick specimens treated in NB and BHI media was however not discussed by the authors.

2.5 Mechanisms of Soil Improvement through MICP

Microbial geotechnology is an emerging technology, which derived from geotechnical engineering and biology. DeJong et al. (2006) stated that this innovative soil improvement that consists of combination of microorganisms, nutrients, biological processes application that naturally present in soil subsurface could effectively improve the engineering properties of soil. There are diverse kinds of potential application of microorganisms in soil improvement; so far there are only bioclogging and biocementation resulting in more reliable and effective results.

The main purpose of biocementation in soil matrix is to enhance the strength and stiffness of the soil through the microbially-induced products, while bioclogging reduces the soil porosity and hydraulic conductivity. According to Ivanov and Chu (2008), biocementation can be utilized to prevent the soil avalanching, minimize the risk of liquefaction of soil, mitigate the swelling potential of clayey soil, and densify the soil of reclaimed land sites. Bioclogging has the potential to reduce the hydraulic conductivity of dam, forming barrier to confine soil pollution sites, prevent soil erosion and etc.

2.5.1 Biocementation

Biocementation improves shear strength of soil through production of soil particle-binding materials, by introduced bacteria and cementation reagent into the soil. Soil cementation materials are mostly carbonates, silicates, phosphates, sulphides and hydroxides (Ivanov and Chu 2008). Calcium carbonate (calcite) is an attractive element to be studied in biocementation because calcite formation is commonly found in nature. In addition, urease positive bacteria are widespread in the environment, and this made the in situ soil treatment does not likely require the introduction of foreign ureolytic bacteria (Fujita et al., 2000). The native ureolytic bacteria can be multiplied through nutrient injection until their growths reach a desired concentration.

Dejong et al. (2010) explained the mechanism of strength improvement contributed by calcite precipitated. The calcite precipitation results in a decrease in void space (porosity), and subsequently provides perception into a change in overall properties. Beyond that, the distribution of calcite within the void space of soil (mm scale) is critical. Figure 2.2 provides schematics of the two extreme possibilities of how calcite may be dispersed around soil particles (DeJong et al., 2010). “Uniform” distribution indicates the calcite precipitated on the surface of soil particles evenly, at an equal thickness. As a result, the bonding formed by calcite to cohere two particles is relatively small, and consequently negligible improvement to soil properties may be anticipated. “Preferential” distribution refers to a condition in which the calcite only precipitated at particle-particle contacts. This is the preferred spatial distribution as all calcite precipitated contributes directly to the enhancement in soil properties. Unfortunately, bio-geo-chemical processes do not naturally optimize for soil engineering properties. For that reason the “preferential” distribution is impracticable. Auspiciously, the “uniform” distribution is also not viable. Both of the analysis of scanning electron microscope, SEM (Figure 2.3) and X-ray computed tomography images demonstrate that the balance of these two extreme conditions is the “actual” distribution of precipitated calcite (Figure 2.2) (DeJong et al., 2010).

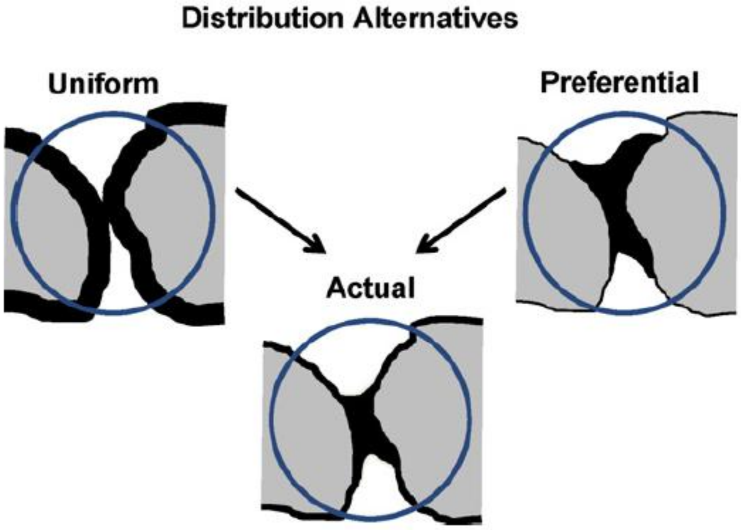


Figure 2.2: Illustration of calcite distribution alternatives within pore space (DeJong et al., 2010)

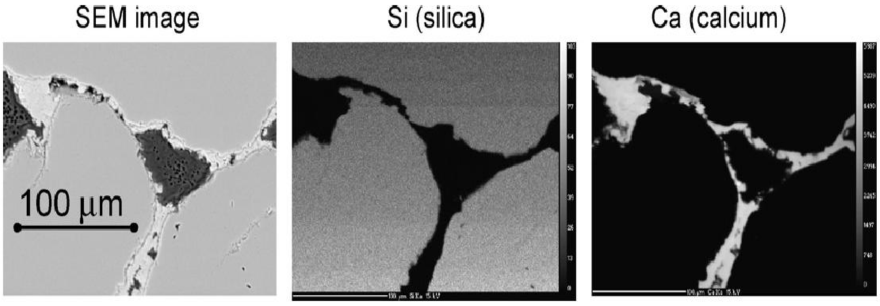


Figure 2.3: SEM elemental mapping of silica sand particles, precipitated calcite, and pore space (n*ote*: lighter gray scale in each image denotes respective element concentration)(DeJong et al., 2010)

Fortunately for the MICP process, there is a considerable fraction of the calcite is in the neighborhood of the particle–particle contacts. The formation of calcite in the soil pore space can be clearly seen in Figure 2.3. The spatial distribution of calcite is determined by biological behavior and filtering processes. Microbes have an inclination to keep away from exposed particle surfaces and instead desire to locate themselves in smaller surface features, such as near particle–

particle contacts. This partiality is due to reduced shear stresses in the area and a greater availability of nutrients at the soil grain contacts. A larger concentration of microbes near the particle-particle contacts promotes greater portion of calcite precipitation in that region (DeJong et al., 2010).

Besides MICP, there are some other potential microbial processes that can lead to biocementation as summarized in Table 2.1 (Ivanov and Chu 2008). These processes include binding of soil particles with sulphides of metals produced by sulphate-reducing bacteria; carbonates of metals produced due to hydrolysis of urea; and production of ferrous solution, ferric salts and hydroxides due to activities of iron-reducing bacteria.

Table 2.1: Possible microbial processes that can lead potentially to biocementation (Ivanov and Chu 2008)

Physiological group of microorganisms	Mechanism of biocementation	Essential conditions for biocementation	Potential geotechnical applications
Sulphate-reducing bacteria	Production of undissolved sulphides of metals	Anaerobic conditions; presence of sulphate and carbon source in soil	Enhance stability for slopes and dams
Ammonifying bacteria	Formation of undissolved carbonates of metals in soil due to increase of pH and release of CO^2	Presence of urea and dissolved metal salt	Mitigate liquefaction potential of sand. Enhance stability for retaining walls, embankments, and dams. Increase bearing capacity of foundations.
Iron-reducing bacteria	Production of ferrous solution and precipitation of undissolved ferrous and ferric salts and hydroxides in soil	Anaerobic conditions changed for aerobic conditions; presence of ferric minerals	Densify soil on reclaimed land sites and prevent soil avalanching. Reduce liquefaction potential of soil

2.5.2 Bioclogging

Bioclogging is a process where the soil void is filled by the product from microbial-induced biochemical process. The clogging of soil restricts water flow through soil, and hence reduces its hydraulic conductivity. Vandevivere and Baveye (1992) and Abdel Aal et al. (2010) found that the hydraulic conductivity of soil reduced significantly through the accumulation of biomass and production of exopolymeric substances. The accumulation can occur at soil pore throat or uniformly on soil particle surface. The reduction in hydraulic conductivity induced by the accumulation of biomass in soil matrix is not permanent.

Different possible microbial processes that may lead to bioclogging are summarized in Table 2.2. These processes include a formation of impermeable layer brought by algal and cyanobacterial biomass; slime in soil induced by aerobic and facultative anaerobic heterotrophic bacteria, oligotrophic microaerophilic bacteria and nitrifying bacteria; production of undissolved sulphides of metals by sulphate-reducing bacteria. In addition, ammonifying bacteria induces formation of undissolved carbonates of metals. However, not all of these processes have been tested in laboratory and field (Ivanov and Chu 2008).

Table 2.2: Microbial processes that can lead potentially to bioclogging (Ivanov and Chu 2008)

Physiological group of microorganisms	**Mechanism of bioclogging**	**Essential conditions for bioclogging**	**Potential geotechnical applications**
Algae and cyanobacteria	Formation of impermeable layer of biomass	Light penetration and presence of nutrients	Reduce of water infiltration into slopes and control seepage
Aerobic and facultative anaerobic heterotrophic slime-producing bacteria	Production of slime in soil	Presence of oxygen and medium with ratio of C:N > 20	Avoid cover for soil erosion control and slope
Oligotrophic microaerophilic bacteria	Production of slime in soil	Low concentration oxygen and medium with low concentration of carbon source	Reduce drain channel erosion and control seepage
Nitrifying bacteria	Production of slime in soil	Presence of ammonium and oxygen in soil	Reduce drain channel
Sulphate-reducing bacteria	Production of undissolved sulphides of metals	Anaerobic conditions; presence of sulphate and carbon source in soil	Form grout curtains to reduce the migration of heavy metals and organic pollutants
Ammonifying bacteria	Formation of undissolved carbonates of metals in soil	Presence of urea and dissolved metal salt	Prevent piping of earth dams and dikes

2.6 Factors Affecting MICP

Calcite precipitation is a relatively straightforward chemical process regulated mainly by four key elements: (i) calcium concentration; (ii) concentration of dissolved inorganic carbon (DIC); (iii) pH; and (iv) availability of nucleation sites (Kile et al., 2000; Castainer et al., 1999). 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; Rivadeneyra et al., 2004; De Muynck et al., 2010b; Maier et al., 2009).

2.6.1 Reagent Concentration

By referring to Equation (2.1) and (2.2), the products from 1 mole of urea and 1 mole of calcium chloride would react to form calcite. A solution containing equimolar of both reactants would provides better conversion to calcite (Nemati et al., 2005). In terms of weight, the stoichiometric ratio of 2.5 for urea and calcium chloride is critical in order to achieve complete production of calcite, considering the molecular weights of urea ($CO\ (NH_2)_2$) and calcium chloride ($CaCl_2 \cdot 2H_2O$) are approximately 60 g/mole and 147 g/mole, respectively.

Urea and calcium chloride at high concentrations (0.5 to 1.0 M) can generate significant amount of calcite. The study of De Muynck et al. (2010b), demostrated the weight gain of limestone specimen due to carbonate precipitation increased with increased concentration of reagent. The weight gain increased from 0.33 g to 0.56 g and 0.66 g when reagent concentration increased from 0.25 M to 0.5 M and 1.0 M, respectively.

High concentrations (0.5 to 1.0 M) of urea and calcium chloride was however possess relatively low efficiency in calcite formation, compare to low concentrations (0.05 to 0.25 M) (Nemati et al., 2005; Okwadha and Li 2010). The efficiency of calcite composition is defined as the ratio of actual calcite precipitated to that of theoretically estimated. At high concentration, the efficiency decreased with increasing reagent concentrations (Nemati and Voordouw 2003; De Muynck et al., 2010b; Ferrer et al., 1988). De Muynck et al. (2010b) found that the efficiency of calcite formation ratio dropped from 0.66 to 0.56 and 0.33 as the concentration of urea and calcium chloride increased from 0.25 M to 0.5 M and 1.0 M, correspondingly.

2.6.2 pH

Calcite precipitation commences when urea is decomposed by urease enzyme. The urease enzyme is produced by microbial metabolic activities and released to environment. As a result, urea hydrolysis normally occurs around the microbe cell. Like all other enzymes, urease enzyme only active at certain range of pH.

With the exception of a small group of acid ureases, microbial ureases generally possess an optimum pH of near neutrality (Mobley et al., 1995). The urease activity of alkalotolerant bacteria, such as S. pasteurii has an optimum pH value of 8 (Stocks-Fischer et al., 1999; Ciurli et al., 1996). At pH values below 5, the 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 values between 8.7 and 9.5 (i.e. 9.5 (Stocks-Fischer et al., 1999); 9.3 (Ferris et al., 2003); 9.1 (Fujita et al., 2004); and 8.7 - 9.5 (Dupraz et al., 2009)). Arunachalam et al. (2010) who performed MICP treatment using B. *sphaericus* reported that the calcite precipitation peaked at pH 8. van Elsas and Penido (1982) found that *B. megaterium* phage was stable between pH 6-8. Only 19% and 59% of the bacteriophage survived at pH 5 and 9, respectively. Khan et al. (2011) reported that *B. megaterium* has an optimum pH of 7-9, and the urease activity peaked at pH 7. Production of ammonia from urea hydrolysis will increase the medium pH during MICP process. Bicarbonate from urea hydrolysis and microbial respiration, on the other hand, acts as a buffer to the pH rise.

2.6.3 Bacteria Cell Concentration

A high bacterial cell concentration supplied to the soil sample would certainly increase the amount of calcite precipitated from MICP process (Okwadha and Li 2010). The rate of urea hydrolysis has a direct relationship with the bacterial cell concentration, provided sufficient cementation reagent is available. A high concentration of bacteria produces more urease per unit volume to commence the urea hydrolysis.

Li et al. (2011) and Stocks-Fischer et al. (1999) both suggested that bacteria cell served as nucleation sites for calcite to precipitate in biochemical reaction. Lian et al. (2006) studied the crystallization by *Bacillus Megaterium*. They identified from

SEM images that nucleation of calcite takes place at bacteria cell walls. The availability of nucleation sites is one of the key factors for calcite precipitation (Knorre and Krumbein 2000). Stocks-Fischer et al. (1999) also demonstrated that calcite precipitation is associated with the concentration of *Bacillus Pasteurii,* one of the urease positive bacteria.

2.6.4 Nutrients

Nutrients are the energy sources for bacteria, and hence it is essential to provide proper and sufficient nutrients for urease-producing bacteria. Nutrients are supplied to bacteria during culture stage and soil treatment stage. Common nutrients for bacteria include CO_2, N, P, K, Mg, Ca, Fe, etc (Mitchell and Santamarina 2005). Lack of organic constituents in soil is a limitation for bacteria growth. The supply of nutrient into soil specimen during soil treatment process is essential. Numerous previous reported studies have included 3 g/l of nutrient broth into the treatment solution to sustain the growth and viability of urease producing bacteria (DeJong et al., 2006; Stocks-Fischer et al., 1999; Al Qabany et al., 2011). The supply of nutrient is to ensure the bacteria can sustain sufficiently long to support calcite precipitation in order to achieve the desired level of improvement.

2.6.5 Types of Bacteria

The bacteria types that are suitable for MICP application should be able to catalyst urea hydrolysis, and they are usually urease positive bacteria. The typical urease positive bacteria are genera *Bacillus*, *Sporosarcina*, *Spoloactobacilus*, *Clostridium* and *Desulfotomaculum* (Kucharski et al., 2008). The aerobic bacteria are preferable as they release CO_2 from cell respiration, and CO_2 production is paralleled by the pH rise due to ammonium production.

Bacillus sp. is a more common type of bacteria used to precipitate calcium carbonate in their micro-environment through catalytic conversion of urea to ammonia and carbon dioxide (Castainer et al., 1999; Hammes et al., 2003). The common types of *Bacilli* used in previous studies were B. sphaericus in repairing or improving the durability of concrete (De Muynck et al., 2008; Van Tittelboom et al., 2010), *B. megaterium* in improvement of concrete strength and durability (Achal et al., 2011; Siddique et al., 2008), and *B. pasteurii* in concrete and soil improvement (Sarda et al., 2009; Whiffin et al., 2007; Vijay et al., 2009). The amount of calcite produced in MICP varied with the types of *Bacillus* strains (Dick et al., 2006).

2.6.6 Geometric Compatibility of Bacteria

Bacteria are the most abundant microbes in soil (Schloss and Handelsman 2004; Janssen 2006). Their sizes are mostly ranging from 0.5 to 3.0 μm (Mitchell and Santamarina 2005). Soil microbes transport across soil through pore throats between soil particles, either by self-propelled movement or by passive diffusion. The geometric compatibility of urease-producing bacteria is critical whenever the transportation of bacteria within the soil is required for soil treatment. Small pore throat size would limit their free passage, depends on the size of microbes and soil composition. For an example, bacteria with size ranging from 0.3 to 2 μm can move freely within sandy soil having particle sizes of 0.05 to 2.0 mm (Maier et al., 2009).

Significant amounts of silt and clay (size < 2 μm) in soil would have inhibitory effect on movement of bacteria. Furthermore, sediment-cell interaction may also result in puncture or tensile failure of the cell membrane (Rebata-Landa and Santamarina 2006). This inhibitory effect obstructs the bacteria distribution in soil. It is thus essential to take into considerations the type of soil, its pore throat size, and size of bacteria when selecting the appropriate type of bacteria for MICP treatment.

Rebata-Landa (2007) found that the optimum range of soil particles sizes for MICP reactions is ranged between 50 to 400 μm. Finer particles sizes are not favourable for bacterial activity and movement, as it hinders migration of bacteria itself and transport of nutrient and reagent. The restriction on nutrient transportation would influence the survivability of bacteria. The constraint on reagent migration would decrease the intensity of MICP reactions due to limited reagent available. Coarser soil would require large amount of calcite for effective soil improvement.

DeJong et al. (2010) discovered that the size of pore throat largely depends on a small portion of soil particles. This small portion can be determined by mechanical sieve analysis and anticipated as 20% of the soil particles size that corresponding to 10% passing. This information has provides an approximate lower bound limit on soil treatment by in-situ mixing which confides on the soil particle size relative to the microbe size. The assessment of typical sizes of soil particles and microbes is demonstrated in Figure 2.4 (DeJong et al., 2010).

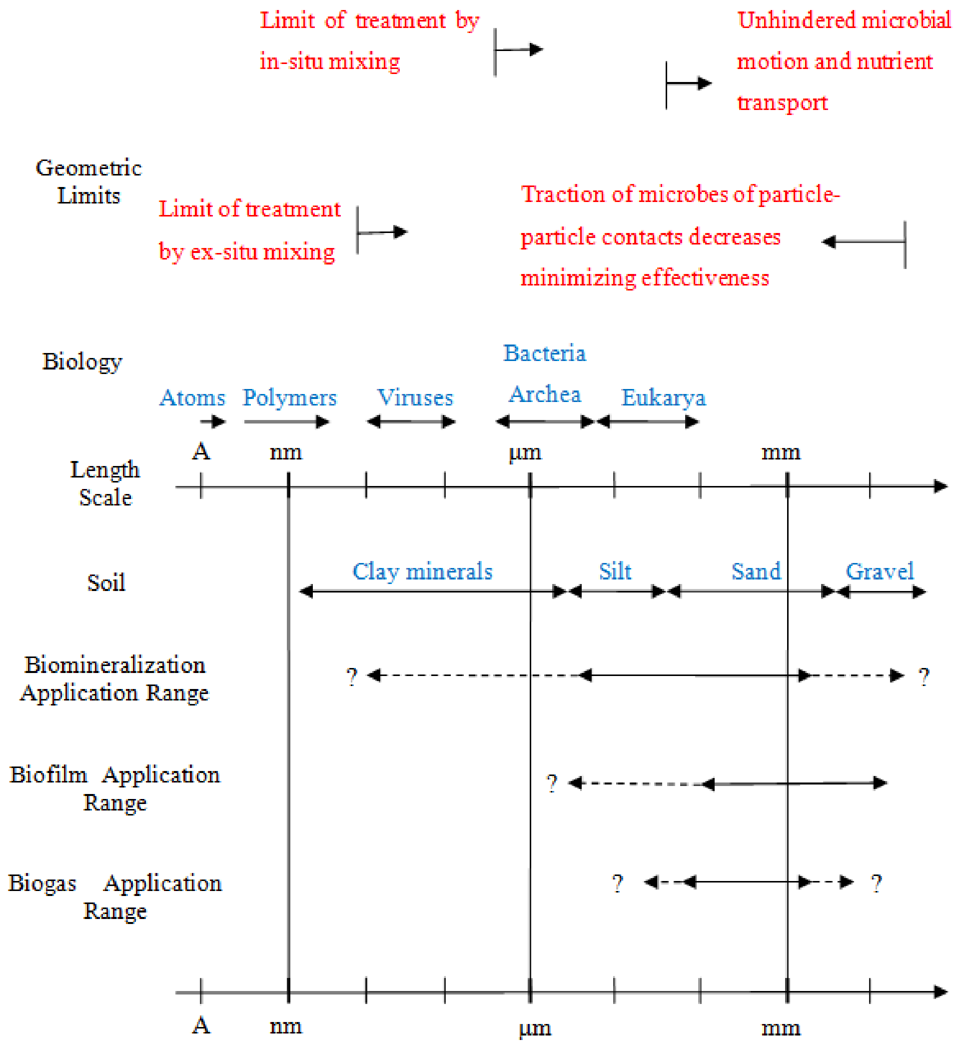


Figure 2.4: Comparison of typical sizes of soil particles and bacteria, geometric limitations, and approximate limits of various treatment methods (DeJong et al., 2010)

2.6.7 Fixation and Distribution of Bacteria in Soil

Ideally, urease positive bacteria should be distributed evenly and fixed in place when they are injected into soil for MICP treatment. Improper method of injection maycause the bacteria to be located only in certain part of soil or be flushed out from the soil. Harkes et al. (2010) studied on the methodologies to dispense bacteria and settle them over a 18 cm long sand bed. They found that injection of undiluted bacteria suspension, followed by one pore volume of high salinity fixation fluid (50 mM of calcium chloride) could successfully retain almost all bacteria suspension in the sand bed.

High salinity solution encourages flocculation, and this promotes the adsorption of bacteria and retention in sand column (Ritvo et al., 2003; Torkzaban et al., 2008). Nevertheless, a low salinity solution (e.g. fresh surface water) has its advantage where homogenous distribution of bacteria is required at large sand body. Low ionic strength and adsorption strength of bacteria in the low salinity solution allow them to transport over great distances (Harkes et al., 2010). Fixation fluids with a high flow rate flush bacteria cell over a longer distance than that of a low flow rate.

2.6.8 Temperature

Temperature has a significant influence on the urease activity, and hence on the rate of MICP. At temperatures below 5^{o}C, the urease activity is negligible (van Paassen 2009). Whiffin (2004)studied the effect of temperature on urease activity in *Sporosarcina pasteurii*. He found that the urease activity increased proportionally with temperatures between 25 ^{o}C and 60^{o}C. The enzyme had an optimum temperature of 70^{o}C, after which the urease activity dropped significantly to almost half of the optimum urease activity at 80^{o}C. Despite the urease activity peaks at 70 ^{o}C, most of the MICP treatments were performed at room temperatures (i.e. 20-30^{o}C). This is because most of the urease producing bacteria used in the existing

MICP treatments (i.e. *S. pasteutii*, *B. megaterium*) are of mesopilic type with the optimum growth temperatures ranging from 30 - 45°C (Todar 2005).

For urease enzyme, Sahrawat (1984) suggested that the optimum temperature for urease activity lies at approximately 60 ℃. Urease activity increased with increasing temperature from 10 ℃ and reached the peak at 60 ℃. The activity was inhibited at 100 ℃ when temperature is raised further. The optimum temperature reported by Sahrawat (1984) is consistent with the findings from Liang et al. (2005) and Chen et al. (1996). This optimum temperature for urease activity, however, is impractical to be applied for soil treatment either on site or in laboratory.

It is recommended to select urease-producing bacteria that live optimum at soil temperature. Soil temperature varies with latitude, altitude, incident solar radiation, moisture content, conduction, type of soil, depth of soil and etc (Selinus 2005; Jacobson 2005; Doty and Turner 2009). Rahim Nik et al. (1986) performed a study on soil temperature in Malaysia at open area and forest. They found that the average soil temperature for open areas (from depth 0 to 30 cm) is approximately 30 ℃. This makes *Bacillus megaterium* suitable for MICP application in Malaysia considering the optimum growth temperature for this bacterium is 30 ℃ (International Society for Environmental Biotechnology and Wise 1997; Bergey and Boone 2009; de Bary 1884). Besides, the temperature of injected cementation solution may also slightly affect the ambient temperature in soil, given that the specific heat of water is higher than soil (Jacobson 2005).

2.6.9 Injection Methods

Studies pertaining to the favorable and proper treatment method of MICP can be found in abundance. Most researches on MICP were performed by injection method which is similar to the grouting of artificial material for soil improvement. Harkes et al. (2010) found that two-phase injection procedure could contribute to homogenous distribution of *B. pasteurii* in sand column. The two-phase injection was by first, injection of *B. pasteurii* suspensions and second, injection of a fixation fluid (high salt content). This procedure has successfully retained 100% of urease activity in the sand column. Martinez et al. (2011) studied the effects of injection methods (stopped-flow injection and continuous injection) on the uniformity of calcite formation in sand column. They found that stopped-flow injection method (injection of 1.5 pore volume of reagent, followed by 2.5 hours of rest period) offered better uniform cementation. On the other hand, continuous injection method promoted abundant calcite precipitation near the injection point, but the calcite content decreased with the distance from the injection point. Similar finding was obtained from the numerical modeling developed by Barkouki et al. (2010). The stopped-flow injection is capable of distributing cementation fluid evenly in sand column before the composition of calcite.

Repeated injection of reagent or number of treatment to the soil would increase the composition of calcite. The repeated injection of reagent is very similar with the stopped-flow injection adopted by Martinez et al. (2011). Studies on the effect of repeated injection on the carbonate precipitation on limestone and diminution in hydraulic conductivity of sand were conducted (Nemati et al., 2005;

De Muynck et al., 2010b). The limestone treated for second and third times experienced additional 36 % and 33 % of weight gains, respectively (De Muynck et al., 2010b). The diminution in hydraulic conductivity for sand possessed the same trend, where first injection contributed to an approximately 65 % of reduction (Nemati et al., 2005). The second and third re-injection of bacteria culture and reagent contributed to another 12 % and insignificant reductions, respectively. The introduction of urease enzyme directly into the sand delivered a greater reduction in hydraulic conductivity, i.e. 28 % and 7 % of diminution for second and third treatments, respectively.

2.7 Concluding Remarks

Studies on MICP soil improvement have so far focused primarily on fine sands (Harkes et al., 2010; Ruyt and Zon 2009; Qian et al., 2010), and very little studies on other soil types. In addition, reported studies have mainly adopted *S. pasteurii* as the urease-producing microorganism (DeJong et al., 2006; Martinez et al., 2011; Harkes et al., 2010; Stocks-Fischer et al., 1999). Studies on alternative species of bacteria and media other than fine sand are still very limited.

The present study focuses on the effectiveness of MICP treatment for improving engineering properties of a natural tropical residual soil (silt). The urease enzyme was produced by *Bacillus megaterium* (ATCC 14581). The treatment conditions studied included concentration of *B. megaterium*, concentration of cementation reagent, flow pressure of cementation reagent, and treatment duration.

The effectiveness of MICP was evaluated by direct measurements of unconfined compressive strength and saturated hydraulic conductivity of the soil specimens.

CHAPTER 3

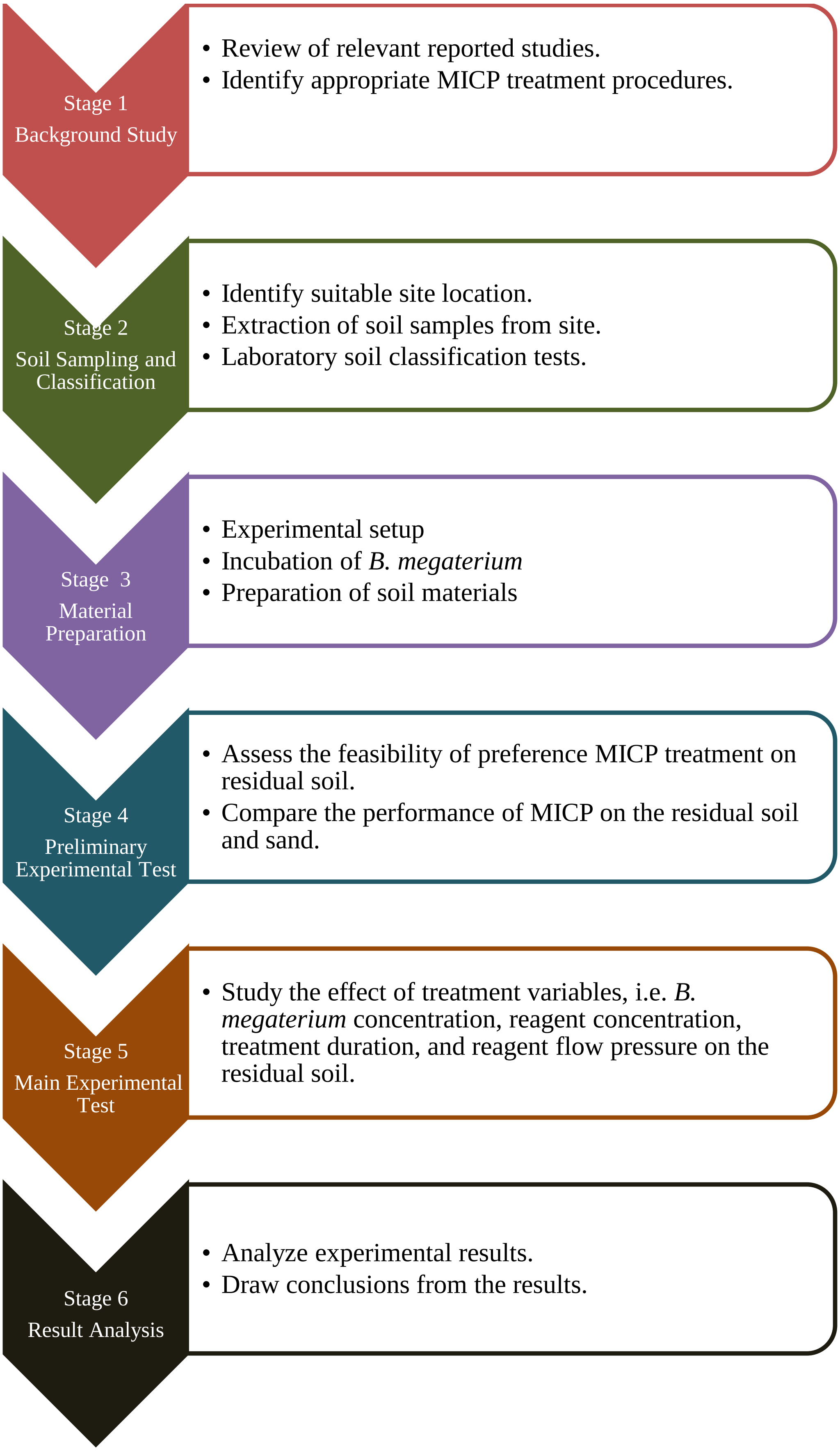
METHODOLOGY

3.1 Introduction

This study aims to determine the preference MICP treatment conditions for residual soil, and to study the effect of MICP treatment on engineering properties of the soil. A systematic methodology was developed in this chapter to achieve the research goal.

3.2 Research Framework

In general, this research can be divided into six major stages: background study, soil sampling and classification, material preparation, preliminary experimental test, main experimental test, and result analysis. Figure 3.1 provides an overview of the research framework.



Stage 1
Background Study

- Review of relevant reported studies.
- Identify appropriate MICP treatment procedures.

Stage 2
Soil Sampling and Classification

- Identify suitable site location.
- Extraction of soil samples from site.
- Laboratory soil classification tests.

Stage 3
Material Preparation

- Experimental setup
- Incubation of *B. megaterium*
- Preparation of soil materials

Stage 4
Preliminary Experimental Test

- Assess the feasibility of preference MICP treatment on residual soil.
- Compare the performance of MICP on the residual soil and sand.

Stage 5
Main Experimental Test

- Study the effect of treatment variables, i.e. *B. megaterium* concentration, reagent concentration, treatment duration, and reagent flow pressure on the residual soil.

Stage 6
Result Analysis

- Analyze experimental results.
- Draw conclusions from the results.

Figure 3.1: Research framework

The study was initiated by exploring the previous works on MICP soil improvement reported by numerous researchers. The collected literature was critically reviewed and research gaps were identified. The effects of different MICP treatment variables on soil engineering properties were the main focus of literature review. Besides, the appropriate treatment method for residual soil was identified from the results of published works.

The second stage of research involved the preparation of main test materials, which were residual soil and sand. The residual soil was extracted from the Universiti Tunku Abdul Rahman (UTAR) Kuala Lumpur campus. Soil classification test: wet sieve, hydrometer, liquid limit, and plastic limit tests were performed. The sand tested in preliminary experimental tests was typical concrete sand.

The experimental apparatus was designed and setup prior to the commencement of experiment works. Relevant published works were referred as the foundation of the experimental setup. Steel moulds were fabricated to contain the soil during the MICP treatment. Stock cultures of *B. megaterium* were prepared and stored for treatment use.

The feasibility of MICP improvement on residual soil was studied in Stage 4. Sand cementation through MICP has been proven successful in previous studies. Performances of MICP in soil were evaluated based on the variation of engineering properties. Therefore, the feasibility of MICP treatment on residual soil was investigated by comparing the performance of MICP-treated residual soil with that of sand.

Investigation of preference MICP treatment conditions on residual soil was performed in Stage 5. Four treatment parameters were studied, i.e. *B. megaterium* concentration, reagent concentration, treatment duration, and reagent flow pressure. The effectiveness of treatment was assessed by comparing the engineering properties of untreated and treated soil. Supportive data, i.e. ammonium concentration, pH, and calcite content were collected for verification purposes.

The results obtained from the Stage 4 and Stage 5 were analyzed in Stage 6. Improvements of residual soil treated with various combinations of treatment parameters were assessed, by weighing up the improvement level with the input of treatment effort and materials.

3.3 Materials

3.3.1 Soil Media

The soil media used for the preliminary tests consisted of tropical residual soil and sand. The tropical residual soil specimen was obtained from a site in Universiti Tunku Abdul Rahman, Kuala Lumpur campus compound, while the sand specimen was of typical concrete sand. Table 3.1 tabulates the values of physical indices of the soil specimens obtained from the standard soil properties tests. Based on the Unified Soil Classification System (USCS), the residual soil was classified as Silt (ML). It has 32% of 50-400 μm particle grains, the ideal size range for MICP (Rebata-Landa (2007)). Typical concrete sand adopted in the preliminary study was classified as well graded sand (SW).

Table 3.1: Soil properties

Properties	**Residual Soil**	**Concrete Sand**
Composition:		
Gravel (%)	0	2
Sand (%)	38	96
Silt (%)	43	1
Clay (%)	19	1
Liquid Limit (%)	40.4	-
Plastic Limit (%)	25.9	-
Plasticity Index	14.5	-
Unified Soil Classification (USCS)	ML (Sandy Silt)	SW (Well graded Sand)
Maximum Dry Density (MDD)	1688.5 kg/m^3	-
Optimum Moisture Content (OMC)	16.6 %	-
Maximum Index Density (ρ_{max})	-	1842 kg/m^3
Minimum Index Density (ρ_{min})	-	1439 kg/m^3
For main experimental test:		
Original friction angle:		
(i) 0.85ρ_{max}	-	39.9°
(ii) 0.90ρ_{max}	-	44.2°
(iii) 0.95ρ_{max}	-	48.8°
Original Undrained Shear Strength, c_u (kPa)	38	-
Original Saturated Hydraulic conductivity, k_{sat} (m/s)	5.4×10^{-8}	-
Original Carbonate Content (%)	0.7	-

3.3.2 *Bacillus megaterium*

The urease-producing microorganism used in the present study was *Bacillus megaterium* (ATCC 14581). *B. megaterium* is a Gram-positive bacterium that can be found in a broad habitat range, but mainly 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). Despite of the fact that *B. megaterium* is one of the largest eubacteria (2 – 5 μm x 1.2 - 1.5 μm) and it has a relatively low urease enzyme activity compared to other bacteria (i.e. *S. pasteurii*) that are commonly used for calcite precipitation (Whiffin 2004; Kaltwasser et al., 1972; Bachmeier et al., 2002), the selection of *B. megaterium* as the urease-producing bacterium in the present study was made based on three reasons: (i) *B. megaterium* can be found in abundance in natural tropical soil (van Elsas and Penido 1982; Lian et al., 2006), (ii) the large and elongated rod-shaped *B. megaterium* may confer advantage of preventing flushing out of the bacterium during field injection of cementation reagent and intense rainfall, and (iii) *B. megaterium* can form resistant endospores that has a high resistance to different extreme environmental conditions. These characteristics of *B. megaterium* provide enormous advantages for promoting field implementation of this soil improvement technique in tropical regions that are commonly characterized by high heat exposure and intense rainfall.

The *B. megaterium* was cultivated at pH 7 under aerobic batch conditions in a sterile culture medium 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 rpm and constant temperature of 37 ℃. The *B. megaterium* was grown to 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 cfu/ml and 1×10^7 cfu/ml) were obtained by dilution with sterile sodium chloride solution (9 g NaCl/l). Viable cell concentrations were determined by direct plate counting.

3.3.2.1 Stock Culture Preparation

First, 150 ml nutrient broth solution was prepared in a number of 250ml conical flasks with concentration of 13 g/L. 30 % glycerol solution was prepared separately in a Scott bottle. Next, the conical flasks which contained the nutrient broth solution were sealed with cotton and aluminium foil, and were sterilized together with the glycerol solution in an autoclave machine for 15 minutes at 121 °C. After the sterilization process in autoclave, the conical flasks and Scott bottle were put aside to cool down to room temperatures before use.

Both conical flasks and Scott bottle were then placed into laminar flow cabinet for addition of freeze-dried *Bacillus megaterium*. The LyfoCults Plus device was removed from foil punch. After that, the applicator was unscrewed gently from vial to vent. Ampoule was slowly squeezed to disperse the hydration fluid into the vial as illustrated in Figure 3.2. The applicator was then re-inserted into the vial, suspension was aspirated and expelled with ampoule device until suspension appeared homogenous. The applicator was used to insert 1 drop of microbes into every conical flask as presented in Figure 3.3. Subsequently, the conical flasks were incubated for 24 hours at 37 °C.

Figure 3.2: Applicator was used to insert hydration fluid and mix suspension

Figure 3.3: One drop of microbes was added into nutrient broth

The conical flasks were then placed into the laminar flow once again to pour 15 ml of suspension of cell into every single sterile centrifuge tube. The centrifuge tubes were centrifuged at 6500 rpm for 10 minutes. The supernatant in centrifuge tubes was discarded while the cell pellet at the bottom was remained.

2 ml of sterile 30 % glycerol was added into the centrifuge tubes. The glycerol was mixed thoroughly with cell pellet by using vortexmixer to obtain a heavy cell suspension. The centrifuge tubes were then sealed with a parafilm. The sealed centrifuge tubes were stored in freezer (-20 °C) for future preparation of cell suspension.

3.3.2.2 Concentration Measurement and Incubation

Bacteria growth is defined as the increase in viable cell number in a population. The dynamics of bacteria growth follows a predictable pattern which is called growth curve. Figure 3.4 shows a typical cell growth curve that consists of four stages, namely lag phase, exponential phase, stationary phase, and death phase (Pörtner 2007).

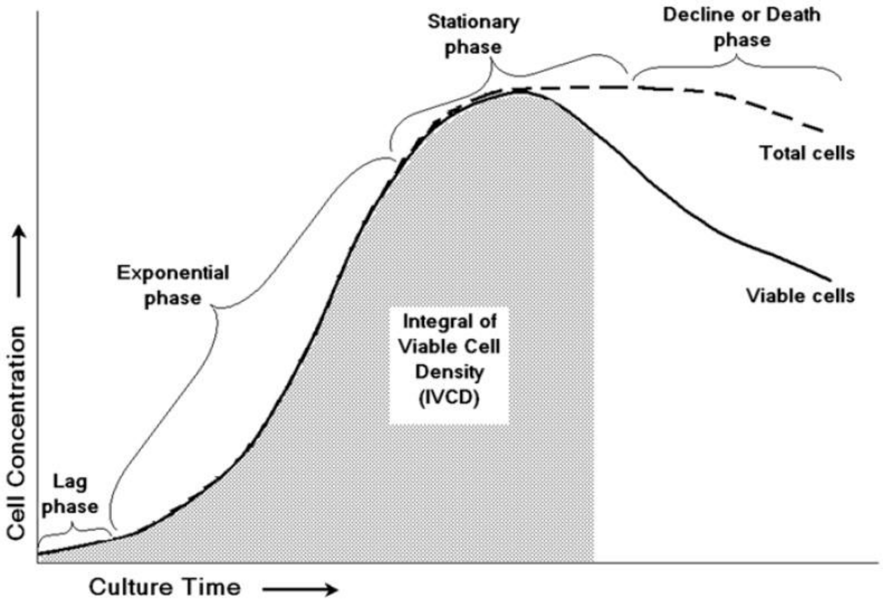


Figure 3.4: Typical cell growth curve

Newly inoculated bacteria might experience no or insignificant growth and it is called lag phase at this stage (Pörtner 2007; Pommerville 2010). This is attributed to the adaption process of bacteria to new environment including pH, temperature, nutrient and etc. The cell then enters an active stage which is known as exponential phase. The cell is growing at the greatest and steadiest growth rate, and the cell-doubling times are typically measured at this phase. The energetic cell growth in the exponential phase will eventually lead to stationary phase. The growth rate of cell declines gradually and achieves flat grow rate curve (no variation in cell quantity), where the cell growth and death rate are in a balance state. This may be caused by the exhaustion of nutrient and inhibit cell rapid growth. Finally, the death rate of cell may exceed the growth rate and this indicates the death phase of cell culture. The viable and total cell concentration reduces with time due to the depletion of nutrient or accumulation of metabolites or other toxic substances.

In the present study, *B. megaterium* was incubated to early stationary phase before it was used for soil treatment. At the early stationary phase, the concentration of *B. megaterium* was relatively constant compared to exponential phase. This is important to ensure that the concentration of *B. megaterium* does not vary greatly when the measurements are taken. In addition, the relatively constant concentration at early stationary phase allowed the *B. megaterium* to be harvested at particular point of their growth. Besides, the accumulation of metabolites and other substances would contribute adverse effect on the activity of *B. megaterium*. For all the reasons above, the practice of harvesting the *B. megaterium* at early stationary phase was justified.

The *B. megaterium* was incubated in laboratory prior to residual soil treatment. The stock culture was first extracted from freezer where the stock culture was stored at temperature of – 20 °C. The *B. megaterium* was spread on an agar plate using a sterile wire loop. This technique is known as streak plate technique(Figure 3.5). The *B. megaterium* was isolated into numerous discrete colonies on the agar plate. The colonies were only visible by naked eye after it grew for certain period, i.e. 24 hours, as illustrated in Figure 3.6.

The colonies or colony forming unit (cfu) were then transferred to the sterile nutrient broth using a sterile wire loop, within the laminar flow cabinet. The *B.*

megaterium was then incubated in a shaking incubator under constant temperature of 37 °C and rotation speed of 200 rpm until early stationary growth phase. The *B. megaterium* was then used in residual soil treatment. The growth curve of *B. megaterium* in this study was illustrated in Appendix A.

Figure 3.5: Stock culture was spread on agar plate using wire loop

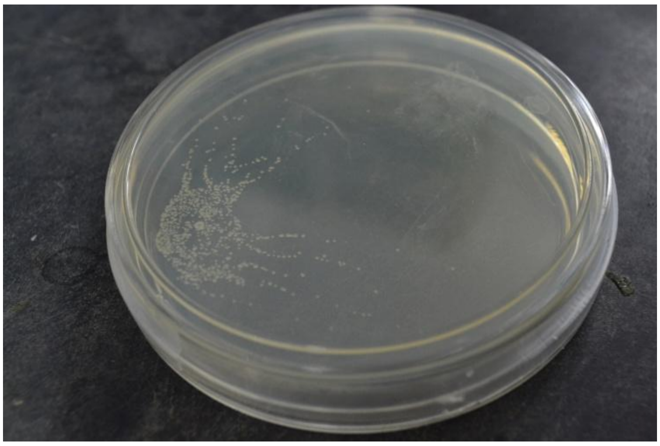


Figure 3.6: Colony forming units grew on agar plate

The concentration measurements of *B. megaterium* were performed using two methods: optical density (OD) and spread plate technique, consistent with typical cell concentration measurement (Stephenson 2010). The time when colonies were transferred from agar plate to nutrient broth was assumed as time zero. The *B. megaterium* then experienced lag phase (as shown in the growth curve above) and exponential phase. Samples of culture were extracted periodically for OD determination using *Jenway 6320D* Visible range *Spectrophotometer,* at 600 nm wavelength. The OD shall be in the range of 0.2 to 1.0 to ensure the measurements are of acceptable accuracy. Dilution was required if the OD of culture exceeded 1.0. For spread plate technique, a sample of culture was serially diluted in sterile saline solution (9 g/l of sodium chloride). The dilution is meant to provide easily countable and well isolated colonies when they are spread on an agar plate, as demonstrated in Figure 3.7. The plates were incubated overnight and the number of colonies was determined. The number of colonies shall be in the range of 30 to 300 colonies, to ensure acceptable accurateness of result (Figure 3.8). The OD of culture at various sampling times was correlated with the cell concentration determined using the spread plate technique, as showed in Appendix B. For subsequent incubation under similar incubation conditions, the more complicated spread plate technique may not be required as the concentration of *B. megaterium* can be extrapolated by measuring the OD only.

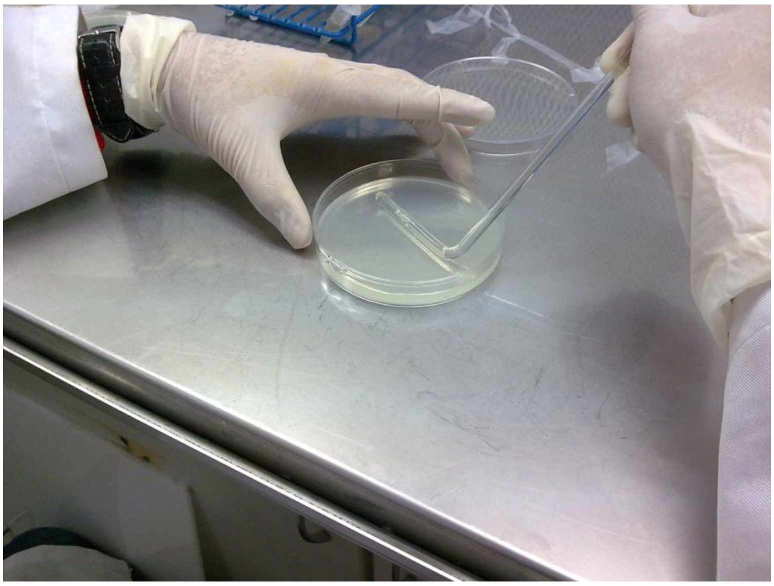


Figure 3.7: Bacteria suspension was spread on agar plate after serial dilution

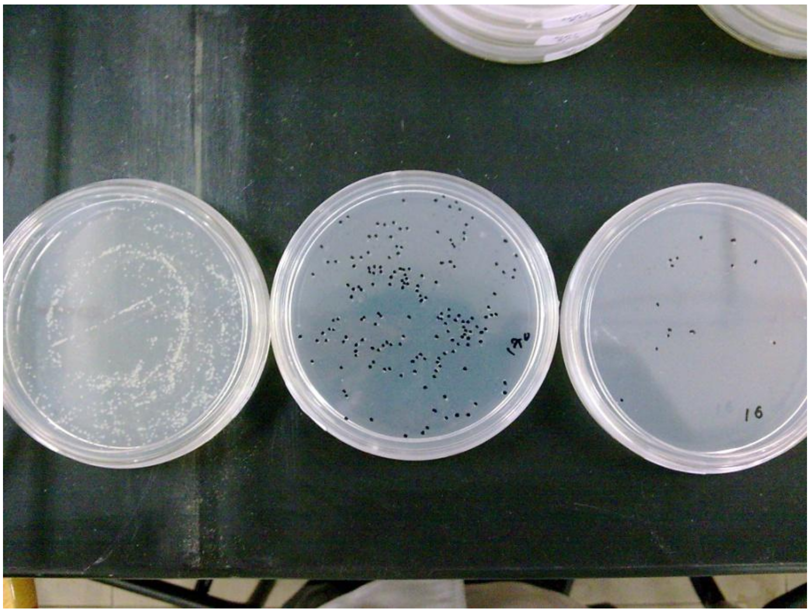


Figure 3.8: Different level of serial dilution contributed different concentration of colony forming units on agar plate

3.3.3 Cementation Reagent

The cementation reagent for the MICP treatment consisted of urea and calcium chloride. The urea and calcium chloride serve as important ingredients for promoting calcite precipitation. The cementation reagent solution also contained 3 g of nutrient broth. The chemicals adopted in this research are in analytical reagent (AR) grade.

3.3.4 Specimen Preparation for MICP Treatment

The soil materials were mixed with the nutrient broth and B. *megaterium* solutions prepared in the Section 3.3.2. The soil mixtures were then compacted to the desired densities in five layers with each layer was about 30 mm thick. The total height of the soil specimen was 150 mm, sandwiched by a 10 mm thick filter layer (gravel) at each end.

During the preliminary tests, the residual soil specimens were compacted at three different densities, i.e. 85%, 90%, and 95% of maximum dry density (MDD) to investigate the effectiveness of the MICP treatment for soils of varying density. For the sand specimen, the minimum (ρ_{min}) and maximum (ρ_{max}) index densities were determined in compliance with the procedures of ASTM D4254 (ASTM 2000b) and ASTM D4253 (ASTM 2000a), respectively. Three densities were compacted within the range of the minimum (ρ_{min} = 1439 kg/m^3) and maximum (ρ_{max} = 1842 kg/m^3) index densities, i.e. 85% of ρ_{max}, 90% of ρ_{max}, and 95% of ρ_{max}. These densities can be achieved by setting up a standard compaction procedure for each density through trial and error. For the main experimental test, the density of residual soil was fixed at 90% of MDD.

Upon MICP treatment, the soil specimen was extruded from the mould for various testing i.e. unconfined compression test, calcite and ammonium content measurements etc (Figure 3.9).

Figure 3.9: Residual soil was extruded from prefabricated mould by pushing it against extruder

3.4 Laboratory Setup

Figure 3.10 shows the schematic diagram of the experimental setup for the MICP treatment. The apparatus consisted of a steel mould of 50 mm in diameter and 170 mm in length (Fig. 3.11), an air compressor, a pressure tank, and an effluent collector.

The steel mould was coated with anti-corrosion paint to prevent potential formations of rust throughout the course of the tests. In addition, the inner tube of the steel mold was coated with grease prior to commencement of each test to provide additional rust protection, and functioned as a lubricant in the sample extrusion process.

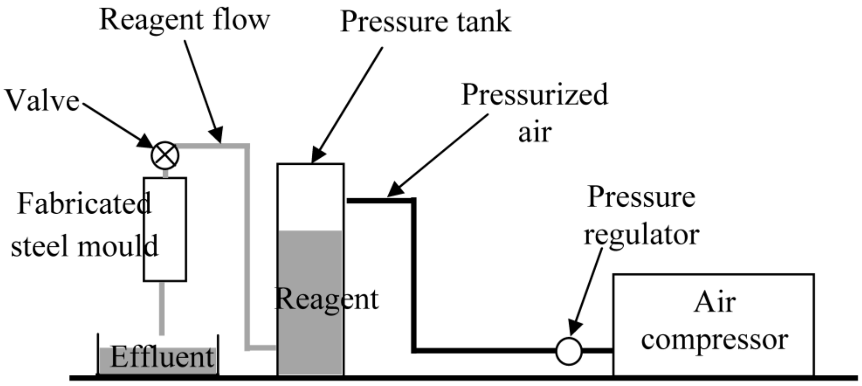


Figure 3.10: Laboratory setup for MICP treatment

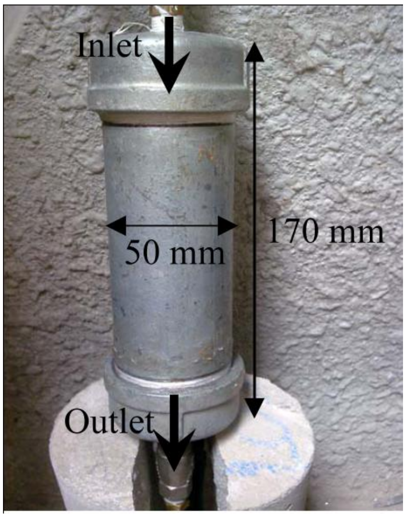


Figure 3.11: Prefrabicated steel mould

To prepare the soil specimens, air-dried residual soil was first mixed with a *B. megaterium* culture (urease-producing microorganism). The *B. megaterium* culture is not inserted into the soil specimen through injection, due to the relatively large size of *B. megaterium* (2 – 5 μm x 1.2 - 1.5 μm) compare to residual soil particles (mostly in the range of 2-20 μm). The small pore throat in residual soil would be the obstacle for *B. megaterium* movement.

Sufficient water was added to attain a moisture content of 16.6% (consistent with the optimum moisture content obtained from compaction test). The soil specimen was then compacted into the prefabricated steel mould to a dry density of 1519 kg/m^3 (90 % of the maximum dry density). The soil specimen was sandwiched by two filter layers (gravel) of 10 mm thick each to avoid turbulent inflow and clogging at the inlet of the specimen.

The specimen mould was held vertically by a clamp attached to a retort stand. The inlet of the mould was connected to a pressure tank where cementation reagent fluid was stored. The reagent fluid was supplied into the specimen mould at a desired flow pressure. This was done by regulating the air pressure of the air compressor. All the treatments were performed at room temperatures (ranging from 22 to 27 ℃). Ammonium concentration and pH were monitored by sampling the effluent from the outlet of the specimen mould at an interval of 12 hours.

During the course of the experiments, effluent samples were collected periodically (every 12 hours) from the outlet of the specimen mould for the measurements of pH. Afterward, the effluents were frozen at -20 ℃ and ammonium concentration measurement was conducted on the effluent samples within 48 hours.

Treated specimen was in water-saturated state and hydraulic conductivity test was conducted. The specimen was then extruded from the mould for shear strength test.

3.5 Soil Classification Tests

3.5.1 Wet Sieve

Wet sieve method covers the quantitative determination of the particle sizes distribution in an essentially cohesionless soil, down to the fine sand size. The wet sieve method used in this research complied with BS1377-2:1990 Section 9.2. Soil specimen was oven dried to a constant weight before the test. The soil specimen was washed until the water passing through the test sieve was virtually clear. The sieves were stacked in following aperture sizes: 75 mm, 63 mm, 50 mm, 37.5 mm, 28 mm, 20 mm, 14 mm, 10 mm, 6.3 mm, 5 mm, 3.35 mm, 2 mm, 1.18 mm, 600 μm, 425 μm, 300 μm, 212 μm, 150 μm, 63 μm, and pan receiver. Soil slurry retained at pan receiver (particle size < 63 μm diameter) was tested using hydrometer test. The main processes of wet sieve are shown in Figure 3.12 and Figure 3.13.

Figure 3.12: Soil was washed by distilled water in test sieve

Figure 3.13: Soil retained on each test sieve was transferred to an evaporating dish

3.5.2 Hydrometer Test

Hydrometer method covers the quantitative determination of the particle size distribution for soil grains smaller than 63 μm. The hydrometer method performed in this research was in compliance with BS1377-2:1990 Section 9.3 (Figure 3.14). The readings of hydrometer and temperature of mixture were taken at exponential intervals of 1 minute, 2 minutes, 4 minutes, etc. until 24th hour.

Figure 3.14: Inserting hydrometer in mixture of water and fine soil

3.5.3 Liquid Limit and Plastic Limit Tests

Liquid limit is an empirically established moisture content at which a soil passes from the liquid state to the plastic state. It provides a means of classifying a soil, especially when the plastic limit is also known. There are two types of liquid limit test: cone penetrometer method and Casagrande method. Cone penetrometer test is fundamentally more satisfactory than Casagrande method. Thus, the cone penetration test was used in this study in compliance with BS1377-2:1990 Section 4.3 (Figure 3.15).

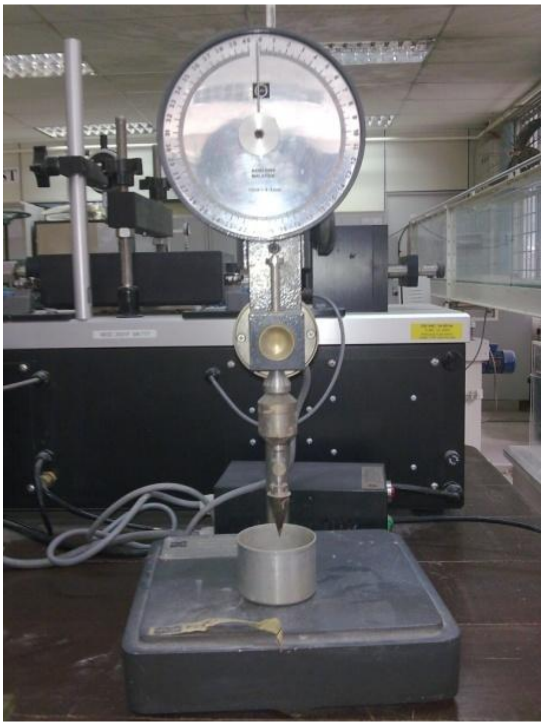


Figure 3.15: Cone penetrometer with cup

Plastic limit of fine-grained soil is the water content where soil ceases to be plastic and changes to semisolid. The soil sample crumbled when it was rolled into 3 mm diameter. The plastic limit test was performed in accordance to BS 1377-2.

3.5.4 Compaction Test

The purpose of compaction test is to determine the relationships between compacted dry density and soil moisture content. The test covers the determination of the dry density of soil passing through 20 mm sieve size when it was compacted in a specific manner over a range of moisture contents. The range of moisture content of soil should include the optimum moisture content where the maximum dry density of soil could be obtained for this degree of saturation. The compaction test was carried out using standard proctor in accordance with BS1377-4:1990 Section 3.3 (Figure 3.16).

Figure 3.16: Soil compaction using standard proctor

3.6 Soil Properties Measurements

3.6.1 Unconfined Compressive Strength

Unconfined compression test is used widely to determine the consistency of cohesive soils. It was used to determine the ultimate compressive strength and undrained shear strength of cohesive soils. The unconfined compression test is convenient, simple and quick compare to other shear strength test. It is ideally suited for measuring the unconsolidated undrained shear strength of saturated clays (Reddy, 2008). Unconfined compression test adopted for residual soil in this study was in compliance with BS1377-7:1990 clause 7.2 (BSI 1990).

A cylindrical vertical specimen of 50 mm in diameter and 100 mm in height was set up between plates. The specimen was subjected to a steadily increasing axial compression until failure. The maximum value of the compressive force per unit area which the specimens could sustain was referred to as the unconfined compressive strength of the soil. In very plastic soils in which the axial stress does not readily reach a maximum value, an axial strain of 20 % was used as the criterion of failure. The images of compression machine and cylindrical specimen are shown in Figure 3.17 and Figure 3.18 respectively.

Figure 3.17: Compression machine

Figure 3.18: Cylindrical soil specimen

3.6.2 Hydraulic Conductivity

The hydraulic conductivity of residual soil specimens was measured by falling head test. Falling head test covers the determination of the coefficient of hydraulic conductivity for saturated fine-grained soils. It is suitable for soils having coefficients of hydraulic conductivity less than 10^{-4} m/s.

In this study, the falling head test commenced by connecting the soil specimens contained in the prefabricated steel mould to standpipe tubes (Figure 3.19). The time of water dropping within a specified distance along the standpipe tube was recorded. These procedures were done in triplicates and the average saturated hydraulic conductivity of soil was calculated. The falling head test was setup in accordance with publication manual produced by Head (1982).

Figure 3.19: Prefabricated moulds were connected with glass standpipe

3.6.3 Direct Shear Test

Direct shear test was conducted to measure the shear strength of sand. A small sand box was restrained and sheared laterally, while subjected to a vertical confining stress. The test was repeated for three vertical confining stresses, i.e. 50 N, 100N and 200 N.

The shearing resistance of sand was measured when one layer of sand was sheared against another layer at its mid-height. Failure occurred when shearing resistance reached maximum. The direct shear test adopted in this study complied with BS1377-7:1990 clause 4 (BSI 1990). The direct shear test machine was shown in Figure 3.20.

Figure 3.20:Direct shear test machine

3.6.4 Constant Head Test

The hydraulic conductivity of soil is a measure of its capacity to allow the flow of water through the pore spaces between solid particles. Constant pressure head of water was applied to a sample of saturated sand specimen. The hydraulic conductivity of sand was determined by measuring the volume of water at the outlet of sand specimen over time.

The coefficient of hydraulic conductivity was expressed as a velocity. The constant head permeability test procedure used for this research is complied with BS1377-5:1990 clause 5.5 (BSI 1990).

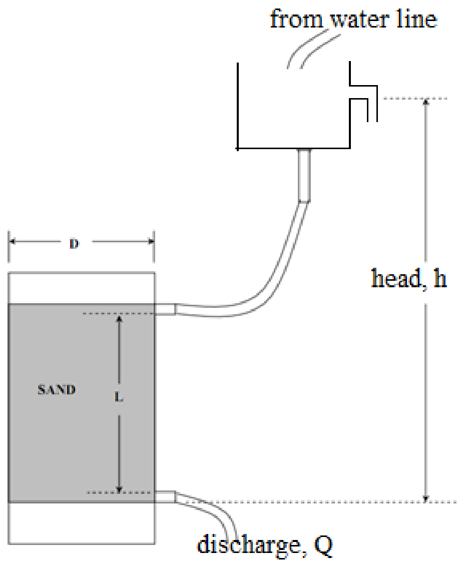


Figure 3.21: Illustration of constant head test setup

3.6.5 Ammonium Concentration Measurement

Ammonium concentration of the effluent was determined by phenate method (Greenberg et al., 1992; APHA et al., 2005). This method is accurate for measuring ammonium over the range of 0.02 – 2 mg NH_4/l. For this reason, effluent samples of higher concentrations than this range were diluted with distilled water. 10ml of the effluent sample was added to a universal bottle and mixed with 1 ml of oxidizing agents, 0.4 ml of sodium nitroprusside and 0.4ml of phenol solution. The oxidizing agent was prepared by mixing 100 ml alkaline citrate solution with 25 ml sodium hypochlorite (5 %). The alkaline citrate solution was prepared by dissolving 200 g trisodium citrate and 10 g sodium hydroxide in 1000 ml deionized water.

The mixture was allowed to react for 60 minutes at room temperatures (22 to 27 ℃), and under a subdued light condition. Blue color was developed in the mixture solution, in which the intensity of the color was proportional with the ammonium concentration (Figure 3.22). The sample was then analyzed using the UV-Vis spectrophotometer (Varian-Cary 100). The resulting peak's absorbance was 640 nm. The measurement was taken within 2 hours upon the completion of the reaction, although the color intensity would normally be stable for a period of 24 hours. The area under base peak was calibrated with several NH_4Cl standards measured under the same conditions. A calibration curve (ammonia concentration vs area under curve) was constructed with NH_4Cl standards, as illustrated in Appendix C.

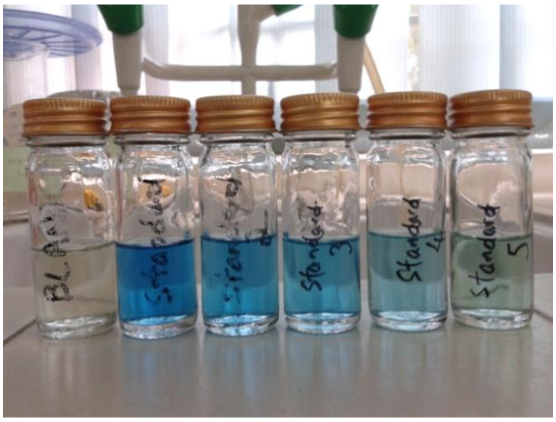


Figure 3.22: Different concentration of ammonium contributed to different intensity of blue colour

3.6.6 pH

Effluents were collected at various sampling times during the MICP treatment for pH measurement. The measurements were performed using Sartorius PB-10 Standard pH meter (Figure 3.23). The pH meter was calibrated with commercial pH standard before the measurement on a collective of effluent samples was performed. The pH sensor was flushed with enormous amount of distilled water before the pH of next samples was determined.

Figure 3.23: pH meter for effluent pH measurement

3.6.7 Carbonate Measurement

Quantitative measurement of the carbonate content in soil was carried out by gravimetric analysis using the acid-treatment weight loss technique. 20g of dry soil sample was prepared for the test. The carbon dioxide deliberated from the reaction between diluted hydrochloric acid (2 molar) and carbonate in soil was indicated by the effervescence.

The residue was collected using a filter paper as shown in Figure 3.24 and oven-dried at temperature of 105 ℃. The measured weight loss of the soil sample was used to estimate the percentage of carbonate content in the soil specimen. Two samples were measured concurrently for each soil specimen. The test was repeated if the percentage difference between the two samples was more than 0.5 %. It was assumed that the increment of carbonate content in the soil specimen after the MICP treatment was purely caused by the formation of calcium carbonate (calcite). The calcite content was presented as a ratio of weight of calcite to weight of soil specimen before the acid treatment in percentage.

Figure 3.24: Soil sample reacted with hydrochloric acid was filtered using filter paper

3.7 Treatment Variables

As mentioned in earlier sections, the experimental works of this study can be essentially divided into two stages, namely preliminary experimental tests, and main experimental tests. The variables considered in each experimental test are described in detail in the following sections.

3.7.1 Preliminary Experimental Tests

The experimental design focused mainly on the effects of soil types (residual soil and sand), soil densities (85%, 90%, and 95% of maximum density), and treatment conditions (untreated, treated with *B. megaterium* only, treated with cementation reagent only, and treated with *B. megaterium* and cementation reagent) on the shear strength and hydraulic conductivity of soils. Both sand and residual soil were compacted with 16.6 % water content. They were in water-saturated state after the MICP treatment. Each experiment was done in triplicates, and the average shear strength and hydraulic conductivity were computed.

Untreated soil specimens served as controls. The treatment with cementation reagent only was used to investigate the existence of naturally inhibited calcite forming microorganisms in the soil specimens. The specimens treated with microorganism only were used to monitor the effect of biomass on the shear strength and hydraulic conductivity of the soils. The details of the experimental design are tabulated in Table 3.2. A total of 24 combinations of experiments were performed in this preliminary test.

Table 3.2: Experimental design for preliminary tests

No.	Experiment Abbreviation	Soil Type	Soil Density	Treatment Method
1.	0.95RU	Residual soil	0.95 MDD	Untreated
2.	0.95RM	Residual soil	0.95 MDD	*B. megaterium* only
3.	0.95RR	Residual soil	0.95 MDD	Reagent only
4.	0.95RT	Residual soil	0.95 MDD	*B. megaterium* & Reagent
5.	0.90RU	Residual soil	0.90 MDD	Untreated
6.	0.90RM	Residual soil	0.90 MDD	*B. megaterium* only
7.	0.90RR	Residual soil	0.90 MDD	Reagent only
8.	0.90RT	Residual soil	0.90 MDD	*B. megaterium* & Reagent
9.	0.85 RU	Residual soil	0.85 MDD	Untreated
10.	0.85RM	Residual soil	0.85 MDD	*B. megaterium* only
11.	0.85RR	Residual soil	0.85 MDD	Reagent only
12.	0.85RT	Residual soil	0.85 MDD	*B. megaterium* & Reagent
13.	0.95SU	Sand	$0.95\rho_{max}$	Untreated
14.	0.95SM	Residual soil	$0.95\rho_{max}$	*B. megaterium* only
15.	0.95SR	Sand	$0.95\rho_{max}$	Reagent only
16.	0.95ST	Sand	$0.95\rho_{max}$	*B. megaterium* & Reagent
17.	0.90SU	Sand	$0.90\rho_{max}$	Untreated
18.	0.90SM	Residual soil	$0.90\rho_{max}$	*B. megaterium* only
19.	0.90SR	Sand	$0.90\rho_{max}$	Reagent only
20.	0.90ST	Sand	$0.90\rho_{max}$	*B. megaterium* & Reagent
21.	0.85SU	Sand	$0.85\rho_{max}$	Untreated
22.	0.85SM	Residual soil	$0.85\rho_{max}$	*B. megaterium* only
23.	0.85SR	Sand	$0.85\rho_{max}$	Reagent only
24.	0.85ST	Sand	$0.85\rho_{max}$	*B. megaterium* & Reagent

3.7.2 Main Experimental Tests

Four treatment variables were considered in the main experimental tests: (i) concentration of *B. megaterium* (*M*), (ii) concentration of cementation reagent (*R*), (iii) treatment duration (*D*), and (iv) flow pressure of cementation reagent (*F*). The values of these variables are tabulated in Table 3.3. These sets of variables form a total of 81 combinations of experiments. Furthermore, 7 additional sets of control experiments were performed to investigate the effect of biomass, reagent alone, and reagent flow pressure on the engineering properties of soil. All the experiments were done in triplicates and only the average readings were reported considering the measurements obtained from the sample replicates are reasonably consistent.

Table 3.3: Treatment variables in MICP treatment on residual soil specimen

B. megaterium Concentration	Reagent Concentration	Treatment Duration	Reagent Flow Pressure
1×10^6 cfu/ml	0.25 M	24 hours	0.2 bar
1×10^7 cfu/ml	0.5 M	48 hours	1.1 bar
1×10^8 cfu/ml	1.0 M	72 hours	2.0 bar

During the experimental test, it was found that certain combinations of treatment variables contributed to insignificant alterations in the soil engineering properties. The 81 combinations of experiment were not performed entirely in the present study. The variables and details of experimental test that have been done in this study are listed in Appendix D. These variables have totalled 58 experimental tests.

The concentrations of *B. megaterium* studied in this research were 1×10^6, 1×10^7, and 1×10^8 cfu/ml. The different concentrations of *B. megaterium* were prepared by dilution using sterile saline solution (sodium chloride with concentration 9 g/l). The amount of sterile saline solution added to the culture was calculated based on the desired *B. megaterium* concentration. The OD of incubated *B. megaterium* was determined before and after the dilution, and the concentration was determined using the calibration curve in Appendix B.

The reagent flow pressures were adjusted using the air pressure regulator, as illustrated in Figure 3.21. The compressed air from the air compressor provided pressure to the reagent contained in confined air tank, and pushed the reagent to flow through residual soil specimen. The flow pressures approximated from previous studies testing on coarse sand medium were in the range of 0.1 to 0.3 bars (Nemati et al., 2005; Nemati and Voordouw 2003; Whiffin et al., 2007; Martinez et al., 2011). However, it is impractical to adopt this range of flow pressure for residual soil which has a relatively low hydraulic conductivity. Three cementation reagent flow pressures were considered in this study, i.e. 0.2 bar, 1.1 bar, and 2.0 bar. These pressures were selected because higher flow pressures were required to provide considerable amount of reagent for MICP reactions. Theoretically, a high flow pressure would result in a high amount of cementation reagent flowing through the specimen and provide more cementation reagent per unit time for urea degradation. In addition, a high flow pressure would also extend the injection / treatment distance in the soil specimen.

CHAPTER 4

PRELIMINARY EXPERIMENTAL RESULTS

4.1 Introduction

This chapter presents preliminary results of this research, which are essential for determining the feasibility of MICP treatment on tropical residual soil prior to further extensive experimental works. Typical concrete sand and residual soil were treated with MICP at various densities (e.g. 85, 90 and 90 % of maximum dry density / maximum relative density). Since the improvement of MICP treatment in sand has been proven successful by numerous previous studies (as reported in the *Chapter 2: Literature Review*), comparison of MICP performances between sand and residual soil provided a good basis to insight the mechanism of MICP treatment in residual soil.

The effectiveness of MICP treatment was examined based on variations of engineering properties before and after the treatment. The indicative engineering properties were shear strength and hydraulic conductivity. The calcite content was determined and related with the variations in soil engineering properties. Scanning electron microscope (SEM) imagery was performed on selected soil specimens to visualize the calcite precipitation in the soil specimens.

Parts of this chapter were published in:

1. **Ng, W. S.**, Lee, M. L., Tan, C. K., and Hii, S. L., 2012. Improvements in Engineering Properties of Soils.. *KSCE Journal of Civil Engineering*, Springerlink (Accepted).

4.2 Shear Strength of Residual Soil

Figure 4.1 compares the stress-strain curves of the untreated residual soil specimens (RU) and those treated with *B. megaterium* and cementation reagent (RT). The unconfined compressive strength (q_u) of the soil is defined as the peak stress or the stress that yields 20% of axial strain, whichever is lower. The shear strengths of the residual soils in this study were characterized by the undrained shear strength parameter (c_u), which was taken as half of the unconfined compressive strength (q_u), i.e. $c_u = ½ q_u$. Figure 4.2 summarizes the shear strength results of the residual soil specimens. The shear strength of MICP-treated residual soils was improved for all densities (0.85RT, 0.90RT, 0.95RT). The shear strength improvement ratio increased with increased density, i.e. 1.41 (41%), 2.59 (159%) and 2.64 (164%) for specimens of 0.85RT, 0.90RT, and 0.95RT, respectively.

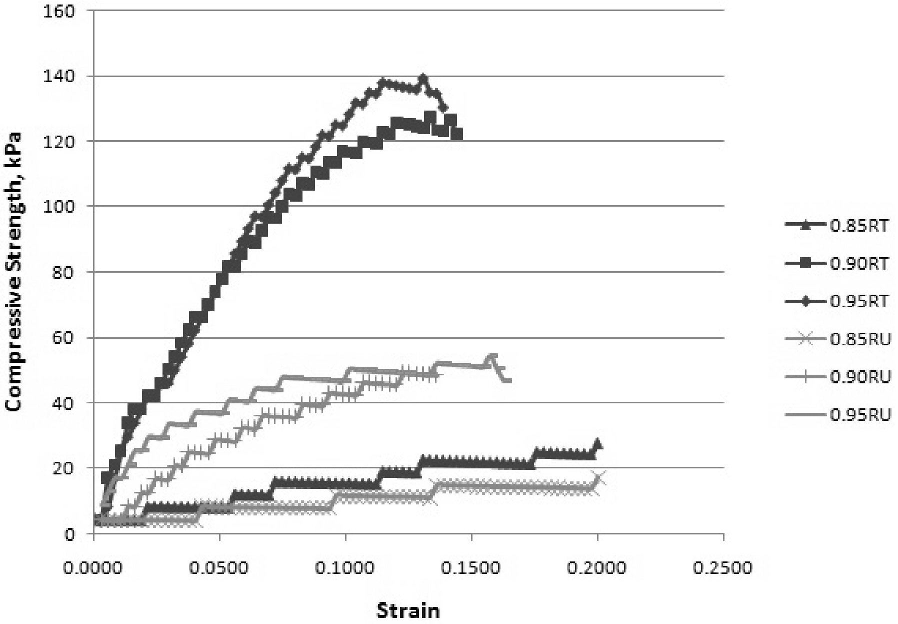


Figure 4.1: Stress-strain curve for residual soil specimens

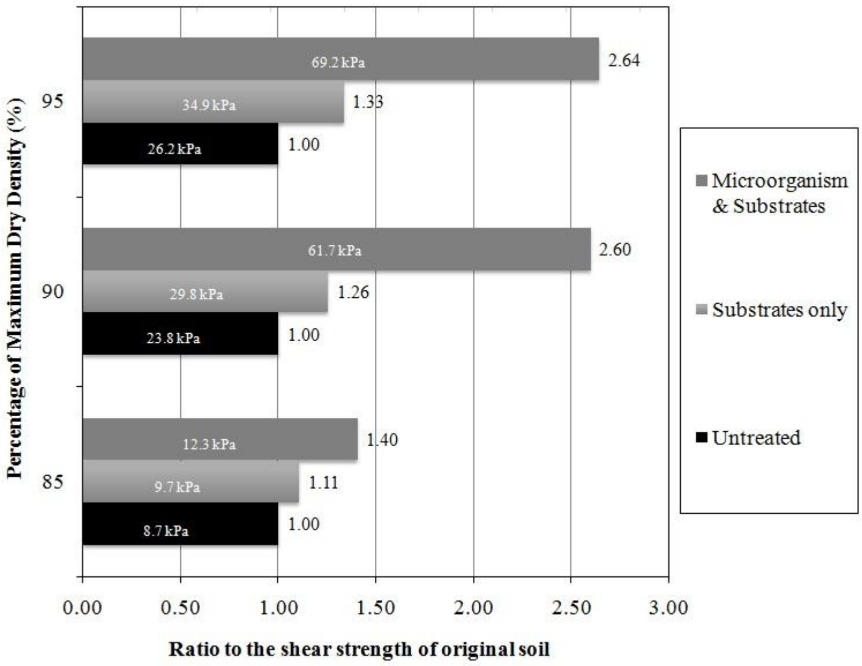


Figure 4.2: Shear strength result for residual soil specimens

The soil specimens treated with cementation reagent only (0.85RR, 0.90RR, and 0.95RR), also exhibited increased shear strength. The undrained shear strength parameter improved by 1.11, 1.25 and 1.33 for the specimens of 0.85RR, 0.90RR, and 0.95RR, respectively. The results implied that MICP was triggered by the microorganisms inhibiting naturally in the soil deposits. The improvement (ranging from 1.11 – 1.33), however, was lower compared to the specimens treated with *B. megaterium* and cementation reagent (ranging from 1.41 – 2.64). This was because the introduction of *B. megaterium* resulted in a higher production of urease enzyme. The enzyme triggered more calcite precipitation and led to greater enhancement in shear strength. The results for the specimens treated with microorganism only, i.e. 0.85RM, 0.90RM and 0.95RM were not shown in Figure 4.2 because no visible improvement in shear strength was observed in these specimens. The results implied that biomass was ineffective in improving the shear strength of the residual soil.

4.3 Hydraulic Conductivity of Residual Soil

Figure 4.3 shows the results of hydraulic conductivity for the residual soil specimens. The saturated hydraulic conductivity (k_{sat}) of untreated residual soil specimens ranged between 1.0 × 10^{-7} m/s and 9.3×10^{-7} m/s, in which the values were directly proportional to the soil density. The saturated hydraulic conductivity of tropical residual soil typically lies in the range of 1×10^{-6} to 1×10^{-8} m/s (Tan et al., 2008).

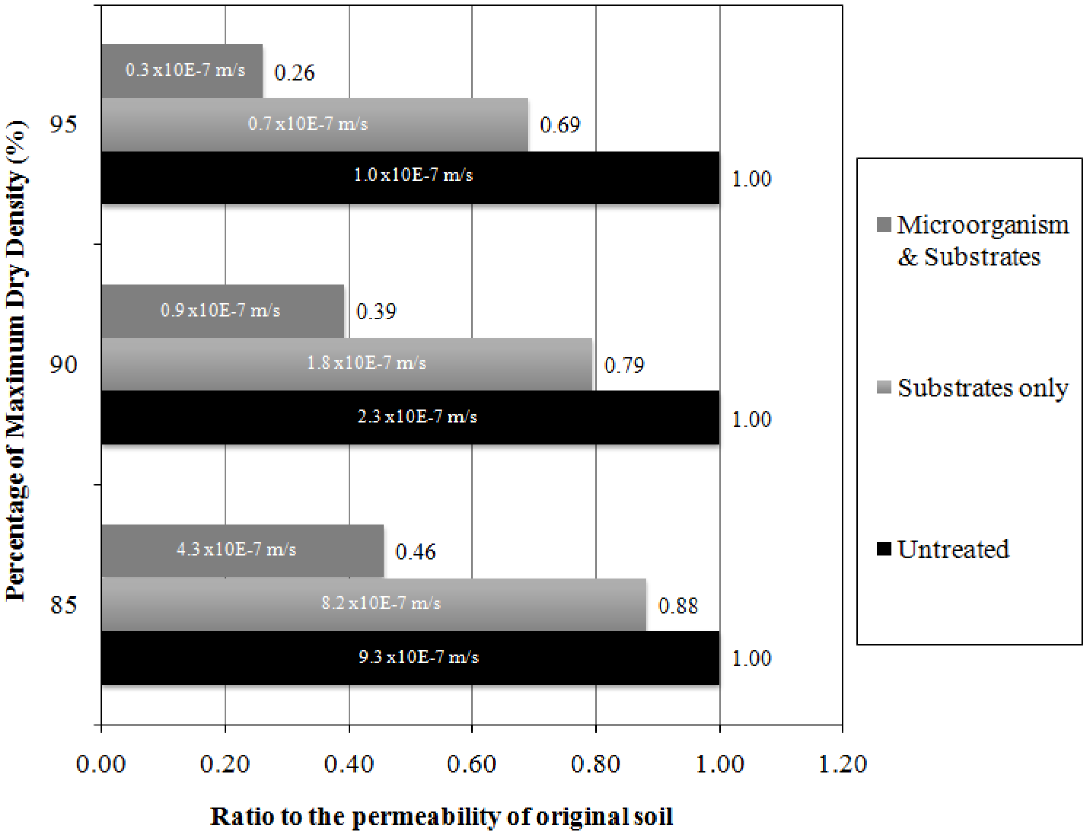


Figure 4.3: Hydraulic conductivity result for residual soil specimens

The saturated hydraulic conductivity of MICP-treated soil was markedly reduced for all densities. The reduction in hydraulic conductivity inflicted by the calcite can be explicitly seen by observing the margin between the saturated hydraulic conductivities of the untreated specimens (0.85RU, 0.90RU and 0.95RU) and those treated with *B. megaterium* and cementation reagent (0.85RT, 0.90RT and 0.95RT). The greatest reduction in hydraulic conductivity occurred in the densest specimen (0.95RT) where the ratio of saturated hydraulic conductivity between treated and untreated specimens was 0.26 (a reduction of 74%). The reduction ratios for 0.90RT and 0.85RT specimens were 0.40 (60%) and 0.45 (55%), respectively.

Similar to the observation in shear strength, the residual soil specimens treated with cementation reagent only (0.85RR, 0.90RR and 0.95RR) exhibited slight alteration in saturated hydraulic conductivity (decreased by not more than 30% of untreated). This observation confirmed earlier finding that a relatively small amount of urease producing microorganism exists naturally in the residual soil. Furthermore, the residual soil specimens treated with microorganism only, without the supply of cementation reagent (0.85RM, 0.90RM, and 0.95RM) experiences no significant diminution in hydraulic conductivity. In the nutshell, diminution in hydraulic conductivity of soil was mainly inflicted by calcite and the effect was proportional to the soil density. The formation of calcite clogged most of the pores and reduced the saturated hydraulic conductivity of the residual soil effectively.

4.4 Shear Strength of Sand

Figure 4.4 shows samples of shear stress versus horizontal displacement results obtained from direct shear tests for the untreated sand specimens (SU), and those treated with *B. megaterium* and cementation reagent (ST). A sample of vertical displacement (dV) versus horizontal displacement (dH) of sand is illustrated in

Figure 4.5. The plot shows that the sand specimens remained as loose sand even it experienced MICP treatment. Most of the tests give almost identical relationship between dV and dH, and thus the results are not discussed explicitly herein.

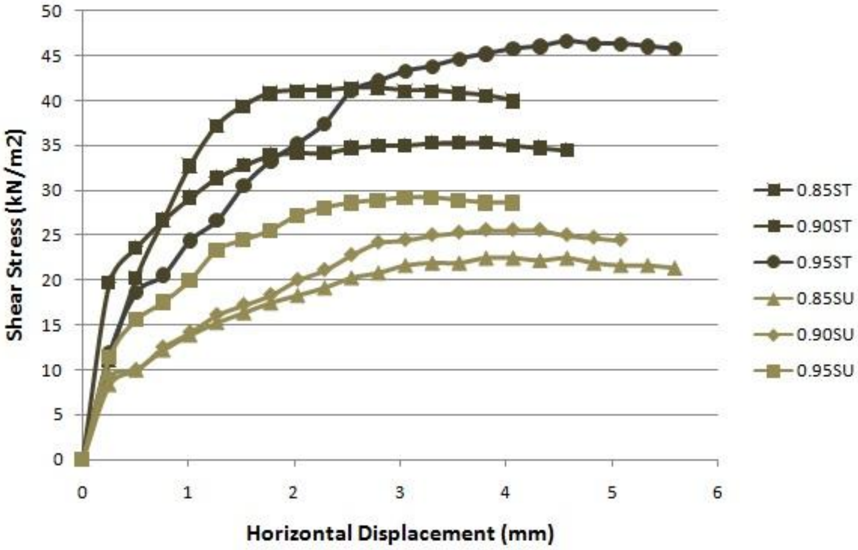


Figure 4.4: Stress-strain curve for sand specimens

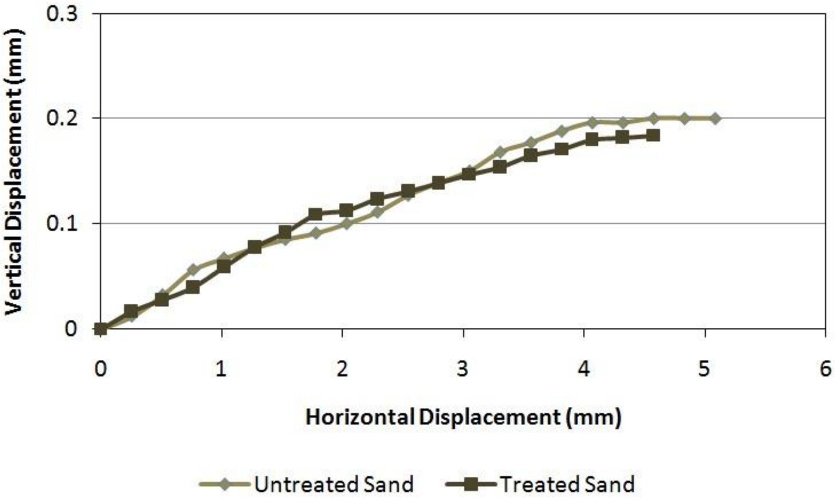


Figure 4.5: Plot of vertical displacement versus horizontal displacement for untreated and MICP-treated sand specimen

Two properties contribute to shear strength is cohesion of particles and internal angle of friction. Sand lacks of cohesion, and hence the shear strength of the dry sand specimens used in this study was characterized by the effective internal friction angle (ϕ') only. Figure 4.6 summarizes the improvement in the effective internal friction angle of the sand specimens.

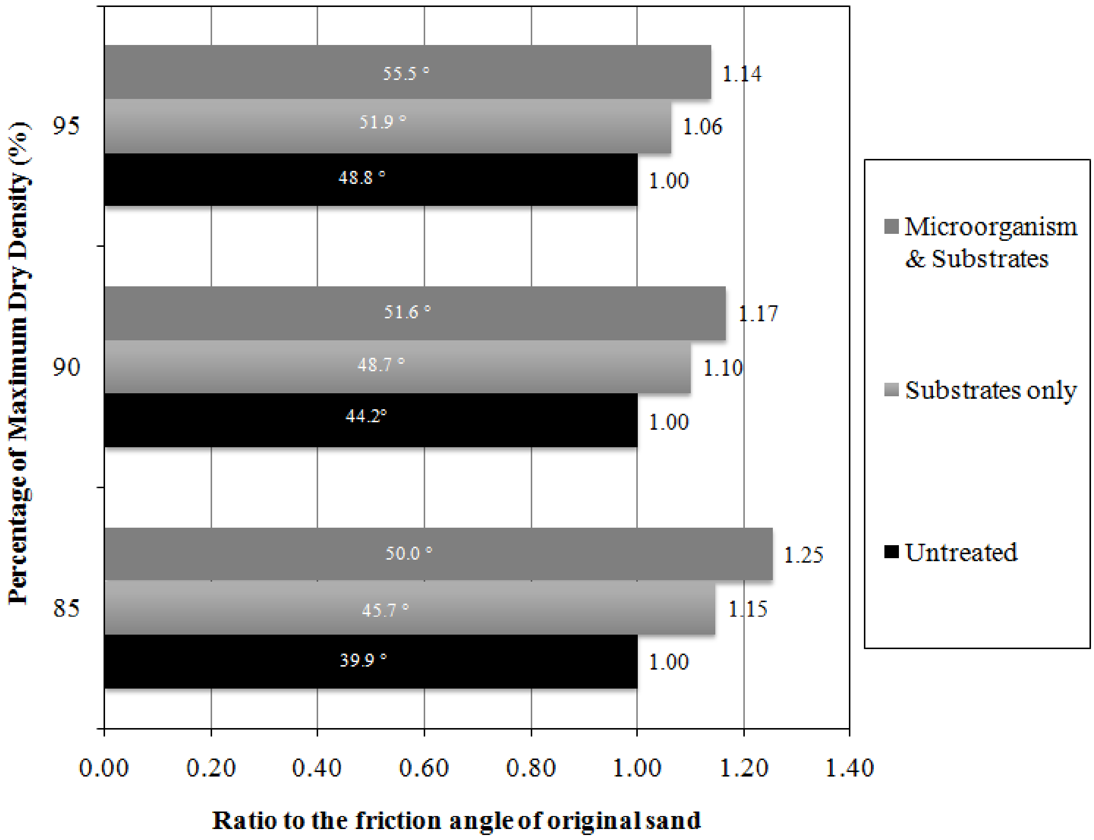


Figure 4.6: Shear strength result for sand specimens

The effective internal friction angles of the untreated sand specimens were between 39.9^o and 48.8^o. The internal friction angles of the MICP-treated sand specimens (0.85ST, 0.90ST and 0.95ST) were generally higher than the untreated specimens (0.85SU, 0.90SU and 0.95SU). The 0.85ST specimen had the greatest improvement ratio (1.25), followed by 0.90ST specimen (1.17) and 0.95ST specimen

(1.14). The improvement ratio decreased with increased density. The trend was opposite to the results observed in the residual soil specimens. Furthermore, the sand specimens (1.14– 1.25) exhibited significantly lower improvement ratios than the residual soil specimens (1.41 – 2.64).

For specimens treated with cementation reagent only (0.85SR, 0.90SR and 0.95SR), the shear strength improvement ratio was markedly lower (1.06 – 1.15) compared to the specimens treated with *B. megaterium* and cementation reagent (1.14 – 1.25). The specimens treated with microorganism only (0.85SM, 0.90SM, 0.95SM) barely had any effect on the shear strength alterations.

4.5 Hydraulic Conductivity of Sand

The saturated hydraulic conductivity (k_{sat}) of the sand specimens is illustrated in Figure 4.7. The saturated hydraulic conductivities of the untreated sand specimens were in the orders of 10^{-4} to 10^{-3} m/s. These values are in close agreement with typical saturated hydraulic conductivity of fine to medium sand specimens (Brassington 1988).

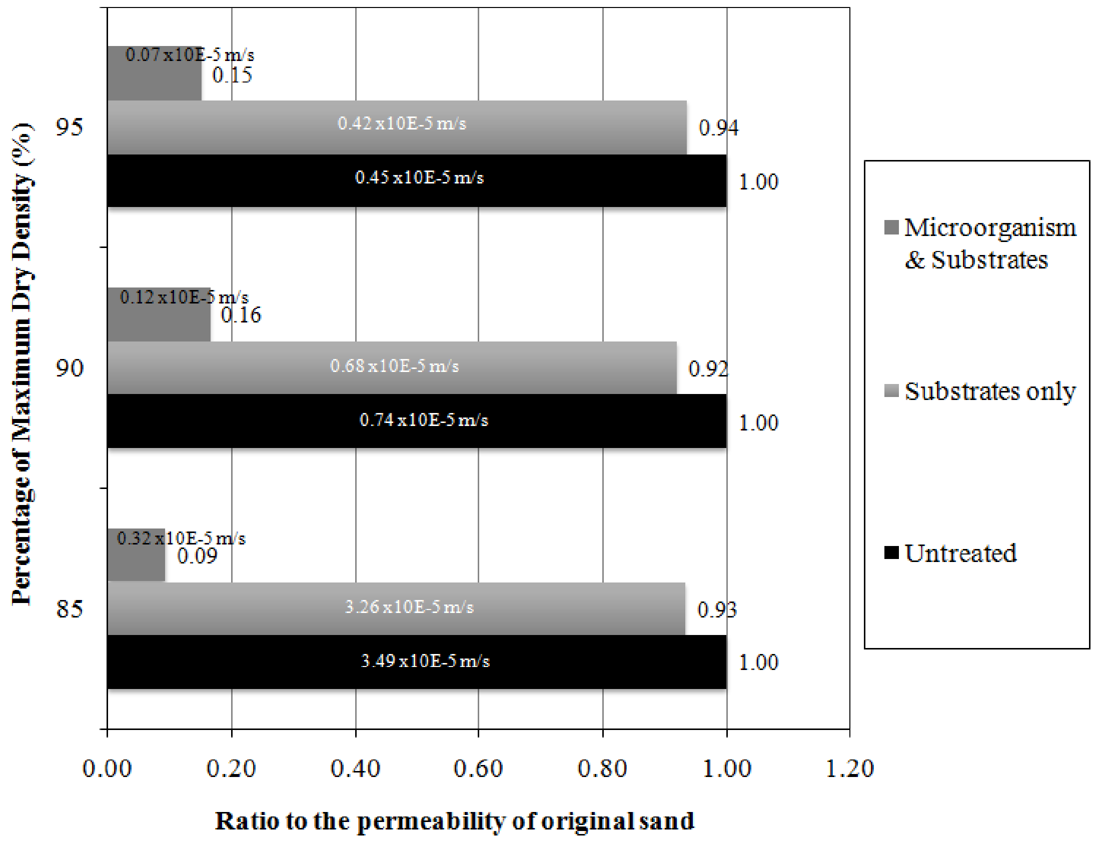


Figure 4.7: Hydraulic conductivity result for sand specimens

Similar to the trend observed in shear strength of sand specimens, the reduction in hydraulic conductivity becomes less effective with the increased density. The greatest reduction in hydraulic conductivity occurred in the 0.85ST specimen in which the hydraulic conductivity decreased by approximately one order of magnitude from 3.5×10^{-5} m/s to 3.2×10^{-6} m/s (a reduction ratio of 0.09). As the density of the specimen increased, the reduction ratios of hydraulic conductivity were marginally lesser as observed in 0.90ST (0.14) and 0.95ST specimens (0.15). For the sand specimens treated with cementation reagent only, the reductions in hydraulic conductivity were negligible. The hydraulic conductivities of the 0.85SR, 0.90SR and 0.95SR were only reduced by an average of 7%. Similarly, the reductions in hydraulic conductivity of sand specimens treated with microorganism only (0.85SM, 0.90SM, and 0.95SM) were also negligible.

4.6 Quantitative Analysis of Calcite Precipitated

The carbonate contents of the untreated residual soil and sand were 0.7 % and 0.3 %, respectively. Figure 4.8 correlates the carbonate content of the MICP-treated residual soil specimens (0.85RT, 0.90RT and 0.95RT) with their shear strength and hydraulic conductivity improvements. The carbonate contents of the 0.85RT, 0.90RT and 0.95RT soil specimens were found to be 1.8 %, 2.6 %, and 2.4 %, respectively. By subtracting their initial content in untreated specimens, the increments of calcite contents after the MICP treatment were 1.1 %, 1.9 %, and 1.7%, respectively.

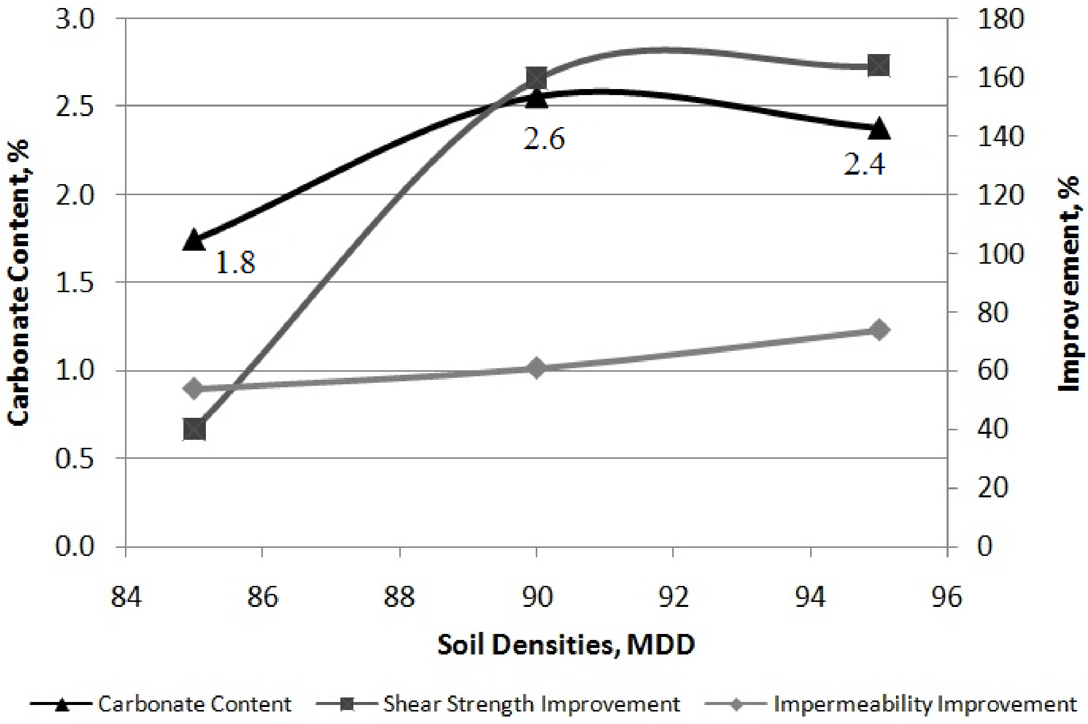


Figure 4.8: Carbonate content and improvement for residual soil

The 0.85RT specimen has the lowest carbonate content, and hence contributed to the lowest improvement in shear strength and reduction in hydraulic conductivity. The highest carbonate content was measured in the specimen of 0.90RT, followed by the specimen of 0.95RT with slightly lower carbonate content. The overall trend of the carbonate content showed reasonably good comparisons with the trends of the shear strength improvement and hydraulic conductivity reduction. Dense soil has a close arrangement of soil particles, and this contributes to more inter-particle contact points per unit volume. The precipitation of calcite at these inter-particles contact points reduces the pore size (reduces hydraulic conductivity) and improves the bonding between soil particles (improves shear strength).

Correlations between carbonate content of the MICP-treated sand specimens (0.85ST, 0.90ST and 0.95ST) and their shear strength and hydraulic conductivity improvements are demonstrated in Figure 4.9. The calcite contents of the 0.85ST and 0.90ST specimens were almost identical to each other, i.e. 6.4 % and 5.9 %, respectively. However, a significant drop of carbonate content (2.9%) was observed in the 0.95ST specimen. Nevertheless, the amounts of calcite in the treated sand specimens (ranging from 2.7 % to 6.1 %) were still generally higher than those of the residual soil specimens (ranging from 1.8 % to 2.6 %). This observation can be explained by the large pore spaces in sand. It can thus accommodate for more reagent and allow for more calcite precipitation. Dense sand (0.95ST) experiences low calcite precipitation after the MICP treatment because it has a relatively smaller pore space.

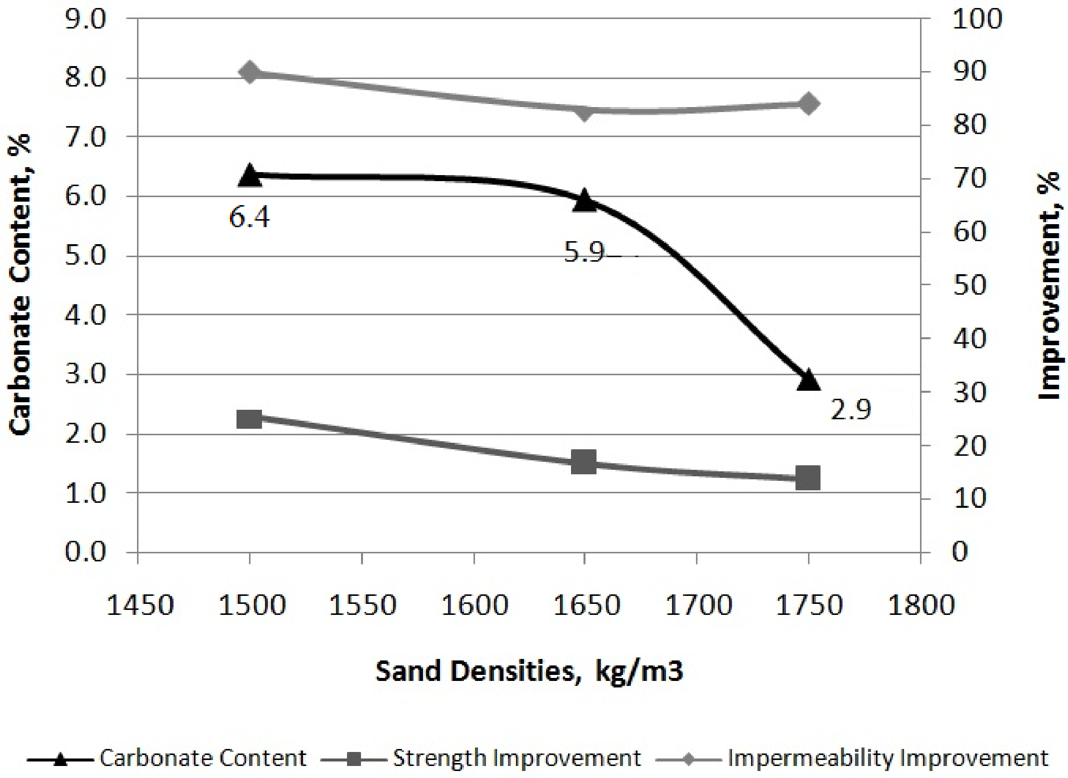


Figure 4.9: Carbonate content and improvement for sand

From the Figure 4.9, it is apparent that the overall trend of the calcite content showed good agreement with the trends of improvement in shear strength and reduction in hydraulic conductivity of the treated sand specimens. It should be noted that despite of significantly higher calcite content precipitated in sand as compared to the residual soil, the improvements in shear strength of sand specimens were still much lower than those of residual soil specimens. The reason could be the calcite was not formed at the inter-particle contact points in view of large pore size in sand. Nevertheless, there was still a great reduction in hydraulic conductivity due to the high calcite contents in treated sand. The precipitated calcite caused clogging at the sand pore spaces and pore throats.

4.7 Scanning Electron Microscope (SEM)

Scanning electron microscope (SEM) imagery was performed on the selected soil specimens to visualize the calcite precipitation in the soil specimens. The SEM images were captured using the Hitachi S-3400N Scanning Electron Microscope. The tests were of particular interest on the formation of calcite crystals upon MICP treatment. Figure 4.10 shows the SEM images of the residual soil specimens treated under three different conditions, i.e. untreated, treated with cementation reagent only, and treated with *B. megaterium* and cementation reagent. Relatively smooth particle surfaces were observed in the untreated specimen (Figure 4.10a). For the treatment with cementation reagent only, some calcite crystals or crystal-shaped substances were observed on the soil particles (Figure 4.10b). Abundance of calcite crystals were observed in the specimens treated with *B. megaterium* and cementation reagent (Figure 4.10c). On closer observations, rod-shaped *B. megaterium* was found in intimate contact with the calcite crystals.

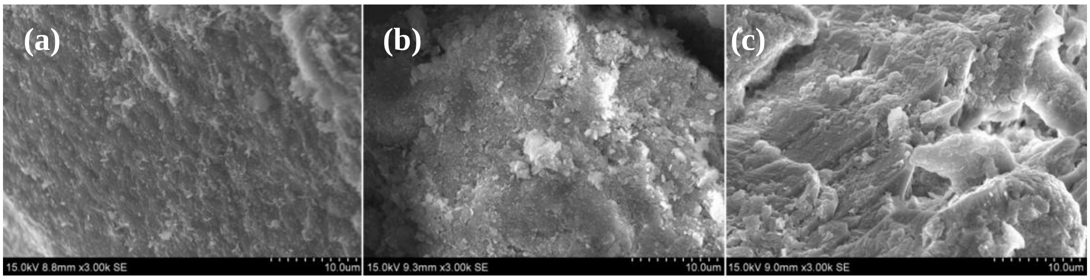


Figure 4.10: SEM images (a) untreated soil; (b) treated with substrates only; (c) treated with microorganisms and substrate.

Similar patterns were observed for the SEM images of sand specimens (Figure 4.11). Comparatively, the quantity of the precipitated calcite crystal for the sand specimen treated with cementation reagent only (Figure 4.11b) was less than that observed in residual soil specimen (Figure 4.10b). This observation confirmed the results from the shear strength and hydraulic conductivity tests that the natural microorganisms only exist for an insignificant amount in the sand specimens, and hence induced slight alterations to their properties.

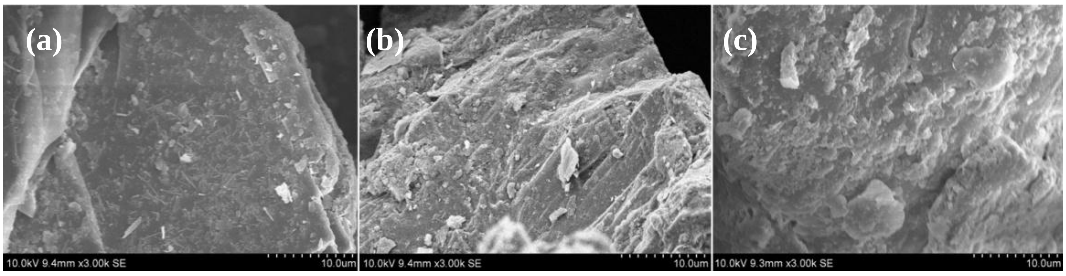


Figure 4.11: SEM images (a) untreated sand; (b) treated with substrates only; (c) treated with microorganisms and substrate.

SEM mapping was performed on an arbitrary spot of the MICP-treated soil to prove the presence of calcium element. Numerous light-coloured spots, as observed in Figure 4.12, indicate the presence of calcium element. Since calcium is one of the main elements in calcite, it can thus confirm the occurrence of calcite precipitation in the soil specimens.

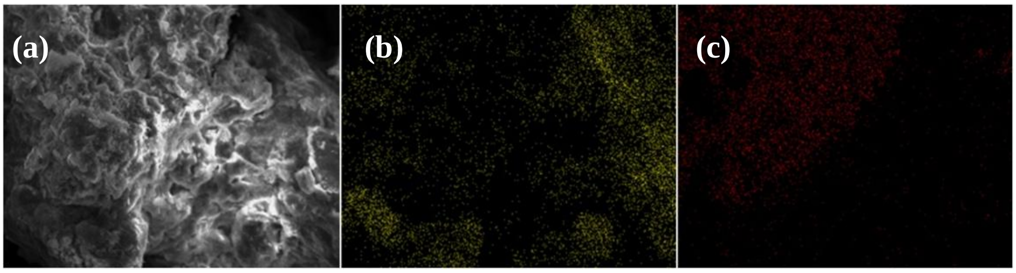


Figure 4.12: SEM mapping (a) soil; (b) silica; (c) calcium

Furthermore, energy dispersion spectroscopy (EDS) was performed on the MICP-treated specimens using EDAX (AMETEK Materials Analysis Division) to analyze the concentrations of calcium (Ca), carbon (C), Oxygen (O), and Silica (Si) elements. It is an analytical technique used for elemental analysis or chemical characterization of samples. Figure 4.13(a) illustrates the EDS performed on a soil particle at different positions. The intensity of Si element was the highest among all elements concerned since it is the major element in soil. As for the Figure 4.13(b), Ca and O recorded the highest intensity. An increased intensity was also encountered for C. As these three elements form the main components of calcite ($CaCO_3$), the EDS was probably performed on a calcite crystal.

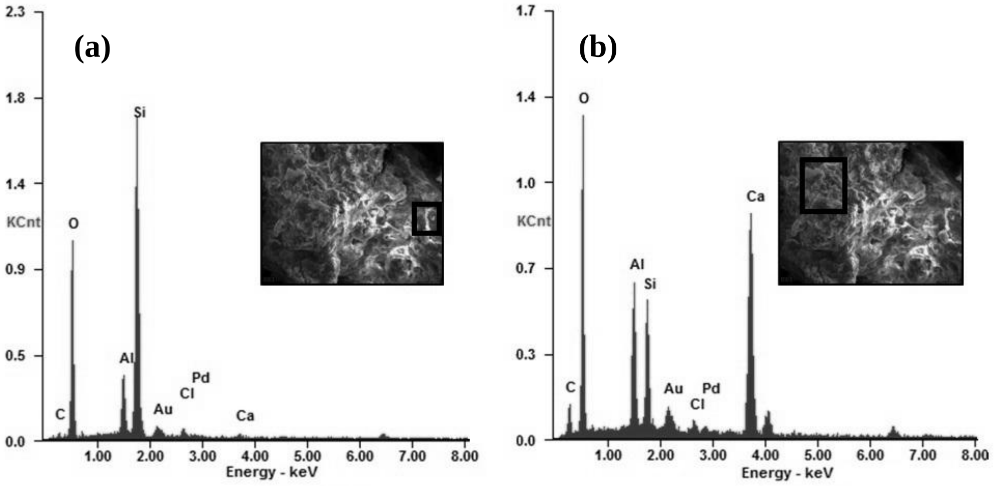


Figure 4.13: SEM images at different positions of treated residual soil specimen

4.8 Discussion

Microbial induced calcite precipitation has been shown to be an effective method to enhance the shear strength and reduce hydraulic conductivity of soil. The soil with enhanced strength can contribute to a greater ground bearing capacity, while reduced hydraulic conductivity can minimize settlement, shrink-swell tendency, seepage, and infiltration of rainfall into soils.

The experimental results indicated that MICP was more effective in improving shear strength for residual soil (1.40 – 2.64 increment ratio) than for sand (1.14 – 1.25 increment ratios). With respect to the reduction in hydraulic conductivity, the sand specimen (0.09 – 0.15 reduction ratio) was found to be more effective than the residual soil (0.26 – 0.46 reduction ratio). Improvement in shear strength and reduction in hydraulic conductivity of residual soil are greater with increased density. However, the sand specimens exhibited a reverse trend. These different observations between residual soil and sand need clarification through an insight into the behaviour of the *B. megaterium* in soil.

According to Achal et al. (2009), the effectiveness of MICP treatment on a soil specimen can be attributed to both the ability of the microorganism to move freely throughout the pore space and on the sufficient particle-particle contacts per unit volume at which cementation occurs. These two attributes, however, are contradicting each other as the soil with large pore space tends to have less particle-particle contacts per unit volume, and vice versa. These conditions require a balance relationship between the microorganism size and the pore structure characteristics, namely the pore throats.

The relatively low improvements in shear strength of sand compared to residual soil can be explained by the insufficient concentrations of particle-particle contacts per unit volume. This is because the sand specimen contains coarser granular particles. The contacts between the coarse particles are lesser compared to the residual soil specimen that consists of wide range of particle sizes (ranging from smaller than 1 μm to 2 mm). The pores between the coarse particles in residual soil are filled with the smaller grains, thus results in greater particle-particle contacts.

The improved shear strength and reduced hydraulic conductivity with increased density of residual soil can also be explained by the particle-particle contacts. As the residual soil compacted to a higher density, the particle-particle contacts increase. This facilitates greater calcite bonding at particle-particle contacts. Pore spaces also decrease with increased compaction. However, the long treatment duration (2 days) and intermittent cementation reagent injection method (flush the cementation reagent through the sample every 6 hours at a moderately high velocity of 1.7×10^{-5} m/s) employed in this study are believed to have minimized the inverse impact of small pore throat on the MICP treatment. The sand specimen has a higher hydraulic conductivity reduction ratio than the residual soil. This can be explained by the greater porosity of sand. Greater porosity means more pore space available for calcite deposition by *B. megaterium*, and hence results in greater reduction in hydraulic conductivity.

As mentioned earlier, the pore throat size can also affect the effectiveness of MICP. The improvement to the shear strength and hydraulic conductivity reduction of the sand specimens decreased with increased density. This is because denser sand contributed to a smaller pore throat size. Consequently, the movement of *B. megaterium* within the sand specimen was restrained, and hence retards the MICP process slightly. Although denser specimen may have greater particle-particle contacts leading to enhanced improvement in the soil properties, however, particle-particle contacts of sand have not been improved by greater compaction due to lack of finer particles that act as filler to the voids between the large particles. The smaller pore throat in the sand specimen plays a more dominant role in controlling the effectiveness of MICP.

The residual soil and sand specimens treated with cementation reagent only exhibited slight increments in the shear strength and reduction in hydraulic conductivity. The results imply that the amount of natural calcite forming microorganisms is insufficient to trigger effective MICP. Comparatively, the residual soil specimens show marginally greater improvements than the sand specimens. This is because the residual soil specimen was taken in-situ from a site which could be rich of natural microorganisms, while the sand specimen was of typical concrete sand which was left exposed under the extreme tropical climate. As the result, the natural inhibited microorganisms in the sand specimens are lesser than the residual soil specimens.

4.9 Concluding Remarks

Twenty four configurations of experimental variables were designed to investigate the effectiveness of MICP in improving the shear strength and reducing hydraulic conductivity of sand and residual soil. The following findings are drawn from this preliminary study:

i) *B. megaterium* was used in MICP treatment to enhance the shear strength and reduce the hydraulic conductivity of both residual soil and sand specimens. The improvement in the engineering soil properties varied with soil densities, soil types, and treatment conditions.

ii) The MICP-treated residual soils exhibited significant increment ratios in shear strength, i.e. 1.41 – 2.64. The rate of improvement increased with increased density. This can be explained by high particle-particle contacts in residual soil particles.

iii) The MICP-treated sands improved in shear strength by ratios of 1.14 – 1.25. The lower improvement compared to residual soils can be attributed to the lesser contacts between sand particles. The rate of improvement decreased with the increased density. The results implied that the particle-particle contacts of sand have not been improved markedly by the higher degree of compaction.

iv) The saturated hydraulic conductivities of the MICP-treated residual soils exhibited reduction ratios of 0.26 – 0.45. The reduction is less significant than the reduction ratios of the sand specimens (0.09 – 0.15). This can be explained by the greater porosity and pore spaces in sand that are available for bioclogging.

v) The amounts of calcite precipitated in the treated residual soil specimens ranged from 1.1 % to 1.9 %. The calcite content increased with the increased soil density. Opposing trend was observed for the sand specimens, whereby the calcite content decreased with the increased sand density. Nevertheless, the amount of calcite in the treated sand specimens (ranging from 2.7 % to 6.1 %) was generally higher than those of the residual soil specimens.

vi) For both residual soil and sand specimens, treatment with cementation reagent only exhibited slight improvement in shear strength and diminution in hydraulic conductivity. The results indicated the presence of natural calcite forming microorganisms which existed in insignificant amount. The results from SEM analysis confirmed this finding.

vii) The effects of microorganism only (biomass) on the shear strength and hydraulic conductivity of both residual soil and sand specimens were negligible.

CHAPTER 5

RESULTS AND DISCUSSIONS

5.1 Introduction

The preliminary experimental results reported in the previous chapter have shown that MICP treatment is feasible for tropical residual soil. In this main experimental test, extensive experimental works were carried out predominantly on the residual soil to determine the preference MICP treatment conditions for the soil. The preliminary results also indicated that the improvement in engineering properties of different soil density exhibited an invariably trend. Therefore, the density of the soil in the main experiment was set as a constant (90 % of MDD). The effects of four variables: cementation reagent flow pressure, treatment duration, concentration of *B. megaterium*, and cementation reagent on MICP performances were investigated. In addition, several control tests were performed to clarify the influences of other factors on the experimental results.

The increment in calcite content upon the MICP treatment was correlated with the improvement in engineering properties of residual soil. Besides, ammonium and pH of the effluent was measured as indirect indicator for the bioactivity. Qualitative elemental analysis on the performance of MICP treatment was also performed on selected soil specimens using Scanning Electron Microscopy (SEM).

Parts of this chapter were under reviewed and published in:

1. **Ng, W. S.**, Lee, M. L., Tan, C. K., and Hii, S. L. Factors affecting improvement in engineering properties of residual soil through microbial induced calcite precipitation. *ASCE Journal of Geotechnical and Geoenvironmental Engineering* (Revised and under re-review).
2. Lee, M.L., **Ng, W.S.**, Tan, C.K., and Hii, S.L. (2012). Bio-mediated soil improvement under various concentrations of cementation reagent. *Applied Mechanics and Materials*, Trans Tech Publications. 204-208, pp. 326-329.

5.2 Control Tests

Effective MICP treatment requires the supply of both *B. megaterium* (urease-producing microorganism) and cementation reagent into soil. Besides calcite formation, other factors may also contribute to alterations in engineering properties of soil. For instances, resting and dead cells may slightly improve shear strength of soil (Chou et al., 2011). For these reasons, 7 control tests were carried out to identify to what extent these factors would affect the engineering properties of soil. These control tests consisted of: (i) an original soil specimen (C1); (ii) a specimen with the inclusion of *B. megaterium* (1×10^8 cfu/ml) only (C2) to study the effect of biomass on the soil engineering properties; (iii) a specimen treated with cementation reagent only (0.5 M) for a duration of 48 hours at a low flow pressure of 0.2 bar (C3); (iv) three specimens treated with cementation reagent only (0.5 M) for durations of 24 hours, 48 hours, and 72 hours, respectively at an intermediate flow pressure of 1.1 bar (C4 - C6); (v) a specimen treated with cementation reagent only (0.5 M) for a duration of 48 hours at a high flow pressure of 2.0 bar (C7). The specimens C3, C5 and C7 were used for comparing the influence of reagent flow pressure on the soil engineering properties, while specimens C4 – C6 were used to study the effect of treatment duration on the soil engineering properties.

Figure 5.1 and 5.2 show the isolated effects of biomass (*B. megaterium* cells) and cementation reagent fluid on the shear strength improvement and hydraulic conductivity reduction, respectively. Alterations (improvement or reduction) in the soil properties of all the control specimens were presented in percentage by making reference to the original control specimen (C1). The shear strengths of all the control specimens were not improved, but reduced by varying percentages. The shear strength reduction in the specimen treated with 2.0 bar reagent pressure (C7) was particularly significant, i.e. 20%. This observation is probably caused by the development of pore-water pressure in soil which will be discussed in a later section. All the control specimens treated with cementation reagent only (C3 - C6) exhibited slight decreases (4 – 10%) in shear strengths. This can be explained by the hygroscopic behavior of reagent as suggested by Lu et al. (2010). The physical and engineering properties of soil (i.e. plasticity index, shear strength) can be improved by the additions of salts such as calcium chloride or sodium chloride. However, a reverse effect may be encountered (reduction in shear strength) when these salts were added in excessive amounts, i.e. more than 4 % (Phanikumar and Sastry 2001; Naeini and Jahanfar 2011).

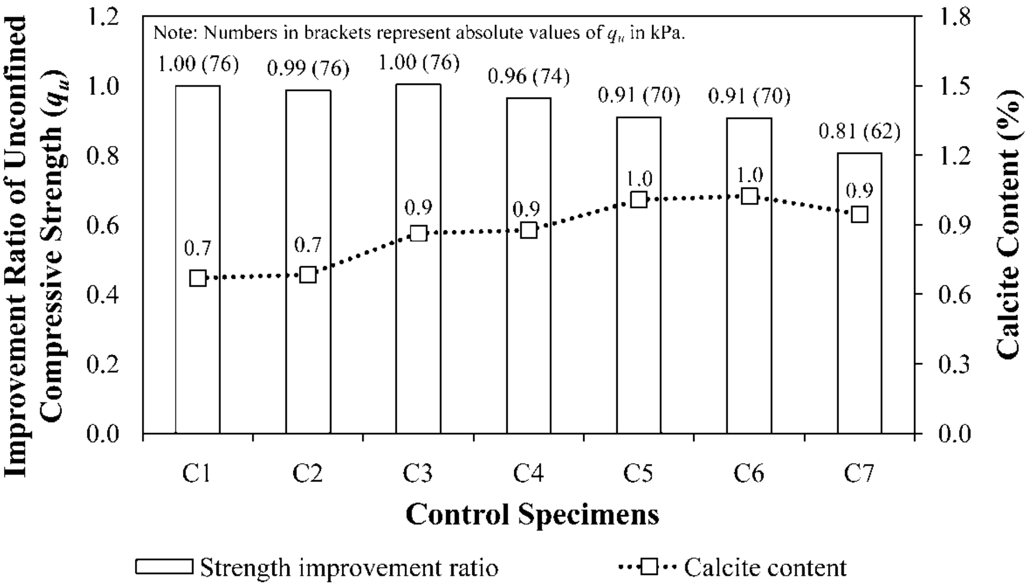


Figure 5.1: Unconfined compressive strength (q_u) and calcite content of control specimens: C1: original soil; C2: with the inclusion of *B. megaterium* (1×10^8 cfu/ml) only; C3: treated with cementation reagent only (0.5 M) for 48 hours at 0.2 bar flow pressure; C4,C5,C6: treated with cementation reagent only (0.5 M) for 24 hours, 48 hours, and 72 hours, respectively at 1.1 bar flow pressure; C7: treated with cementation reagent only (0.5 M) for 48 hours at 2.0 bar flow pressure.

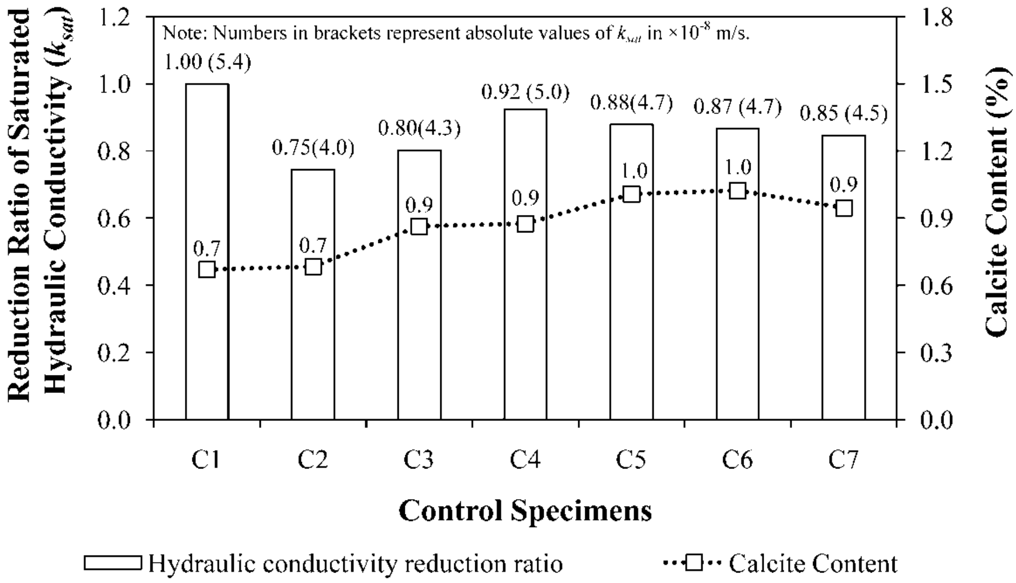


Figure 5.2: Saturated hydraulic conductivity (k_{sat}) and calcite content of control specimens: C1: original soil; C2: with the inclusion of *B. megaterium* (1×10^8 cfu/ml) only; C3: treated with cementation reagent only (0.5 M) for 48 hours at 0.2 bar flow pressure; C4,C5,C6: treated with cementation reagent only (0.5 M) for 24 hours, 48 hours, and 72 hours, respectively at 1.1 bar flow pressure; C7: treated with cementation reagent only (0.5 M) for 48 hours at 2.0 bar flow pressure.

Biomass effect through the inclusion of *B. megaterium* only (C2) reduced hydraulic conductivity significantly by 25 %. This reduction is caused by the plugging of biomass in the soil pores. The control specimen with 0.2 bar of cementation reagent flow pressure (C3) experienced greater reduction in hydraulic conductivity (19 %) than those treated with higher flow pressures, i.e. 1.1 bar (C5) and 2.0 bar (C7) (12 % and 15 %, respectively). This is because the low flow pressure (0.2 bar) encouraged the growth of indigenous bacteria in soil and prevent the flushing out of the biomass from soil.

The calcite content in the original residual soil specimen (C1) was 0.7 %. Inclusion of *B. megaterium* only (C2) did not promote calcite precipitation. Slight increments (0.2 – 0.4%) in calcite content were observed in the control specimens treated with cementation reagent only. This observation could be attributed to two contributing factors: (i) presence of indigenous urease-producing bacteria in the soil, and (ii) oversaturation state of reagent in the soil leading to chemical precipitation of calcium carbonate or other carbonate minerals.

5.3 Effects of Cementation Reagent Flow Pressure

Typical reagent flow pressures used for fine sands were 0.1 - 0.3 bar, as deduced from flow rates reported in previous literature (Nemati et al., 2005; Nemati and Voordouw 2003; Whiffin et al., 2007; Martinez et al., 2011). Martinez et al. (2011) performed MICP treatments on a sand column compacted to 80% of maximum relative density. A vertical pressure of 100 kPa was applied on the top of the sand column to normalize the pressure of reagent injected to the column from bottom to top. In the present study, no vertical pressure was applied on the column as the cementation reagent was supplied to the column from top to bottom. To maintain an equivalent flow rate through the residual soil specimen, higher pressure should be used because the residual soil has a lower hydraulic conductivity than the fine sand. Three cementation reagent flow pressures were considered in this study, i.e. 0.2 bar, 1.1 bar, and 2.0 bar. Theoretically, a high flow pressure would result in a high amount of cementation reagent flowing through the specimen and provide more cementation reagent per unit of time for urea degradation. In addition, a high flow pressure would also extend the injection / treatment distance in the soil specimen.

Figure 5.3 compares the shear strength of the soil specimens treated with flow pressures of 0.2 bar, 1.1 bar, and 2.0 bar. The concentration of *B.Megaterium*, cementation reagent, and treatment duration were kept at 1×10^8 cfu/ml, 0.5 M, and 48 hours, respectively. The shear strength improvements in these MICP treated soils were compared with the control specimen of original soil (C1). The shear strength improvements were significant for the specimens of 0.2 bar (100 %) and 1.1 bar (69 %). The increment in shear strength for the specimen at 0.2 bar was higher than that of 1.1 bar, despite more calcite content produced in the specimen treated at 1.1 bar (2.64%) than at 0.2 bar (2.31%). The results implied that a low flow pressure (i.e. 0.2 bar) encouraged calcite cementation at particles contact points. At a high flow pressure (1.1 bar), more calcite was formed. However, some portions of the calcite may concentrate at certain point of injection which was ineffective in promoting shear strength improvement. At an excessively high flow pressure (i.e. 2.0 bar), shear strength reduced by 13 % despite considerable calcite content (1.36%). A plausible explanation to this observation is that the high flow rate may lead to the buildup of pore-water pressure in soil due to clogging of the soil body and mold outlet, and eventually result in a decrease in the effective stress. The high hydraulic gradient may also result in detachment of soil particles or disturbance of soil structures, and hence causes an adverse impact on the soil strength, as demonstrated in the results of control tests.

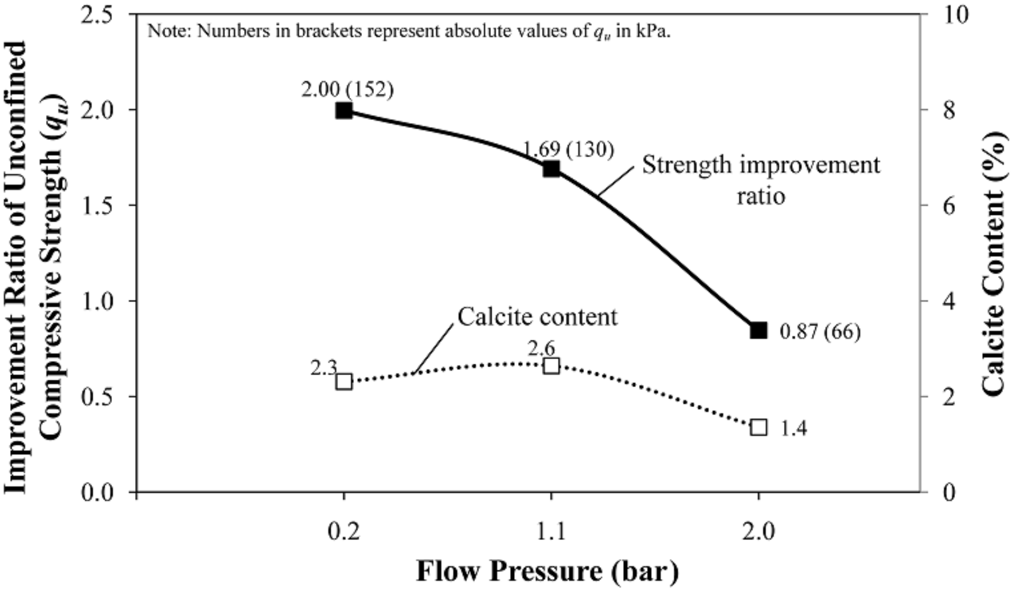


Figure 5.3: Effect of cementation reagent pressure (0.2 bar, 1.1 bar and 2.0 bar) on unconfined compressive strength (q_u) and calcite content of MICP-treated residual soil specimens.

Figure 5.4 shows the hydraulic conductivity of soils treated under various flow pressures. Both the 0.2 bar and 1.1 bar specimens experienced significant reductions in hydraulic conductivity. There were opposing trends observed with respect to the results of hydraulic conductivity and shear strength. For the hydraulic conductivity reduction, the 1.1 bar specimen has a slightly lower hydraulic conductivity than that of 0.2 bar, whereas for the shear strength improvement, the 0.2 bar specimen has significantly outperformed the 1.1 bar specimen. The contrary results implied that the hydraulic conductivity reduction has a different mechanism from the shear strength improvement. The reduction of hydraulic conductivity in soil matrix is mainly attributed to the clogging of calcite in pore spaces or pore throats. The formation of calcite in pore space would be suspended in the pore fluid, and eventually be filtered by the soil pore throat as the fluid flowing through the soil. This filtering phenomenon is controlled by the size ratio of precipitated calcite particle to pore throat (Valdes and Santamarina 2006). The larger the size of precipitated calcite particle relative to pore throat, the more significant the filtering phenomenon. No specific binding of soil particles are required for obstructing the water flow. Therefore, the reduction in soil hydraulic conductivity tended to be proportional with the amount of calcite precipitated in the MICP treatment. By comparing the calcite amount precipitated in the specimens treated with 0.2 bar, 1.1. bar, and 2.0 bar, it was apparent that the 1.1 bar specimen had the highest calcite content, and thus the greatest reduction in hydraulic conductivity was observed. At an excessively high flow pressure (i.e. 2.0 bar), the flow has flushed out the bacteria in soil resulting in a low calcite precipitation, and hence a low reduction in hydraulic conductivity.

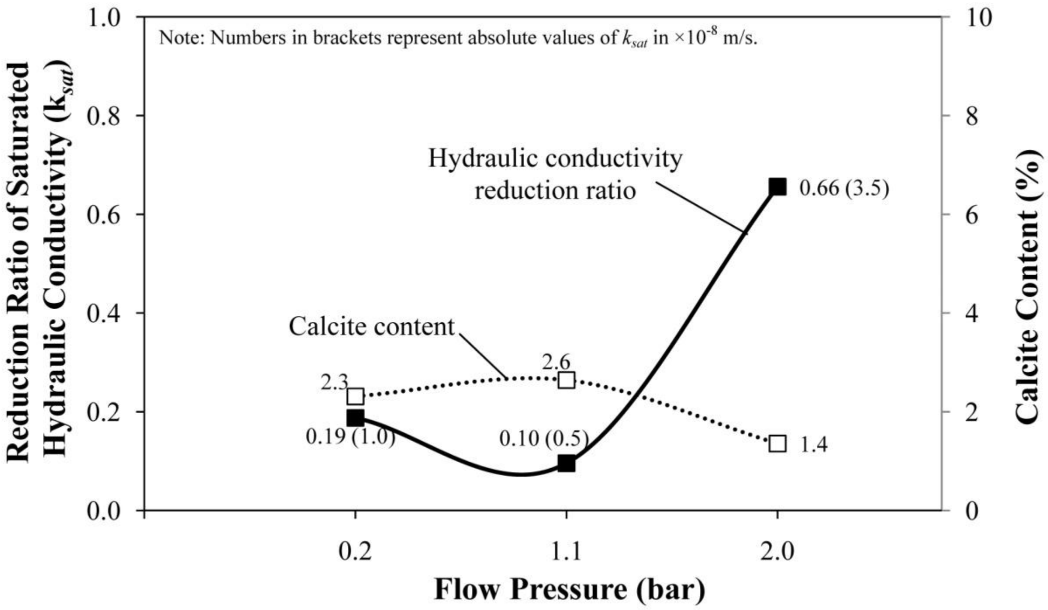


Figure 5.4: Effect of cementation reagent pressure (0.2 bar, 1.1 bar and 2.0 bar) on saturated hydraulic conductivity (k_{sat}) and calcite content of MICP-treated residual soil specimens.

5.4 Effects of Treatment Duration

Figure 5.5 shows the shear strength improvements corresponding to various treatment durations, i.e. 24 hours, 48 hours and 72 hours. To enable indisputable comparisons, the concentration of *B. megaterium* 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 M and 0.5M were presented.

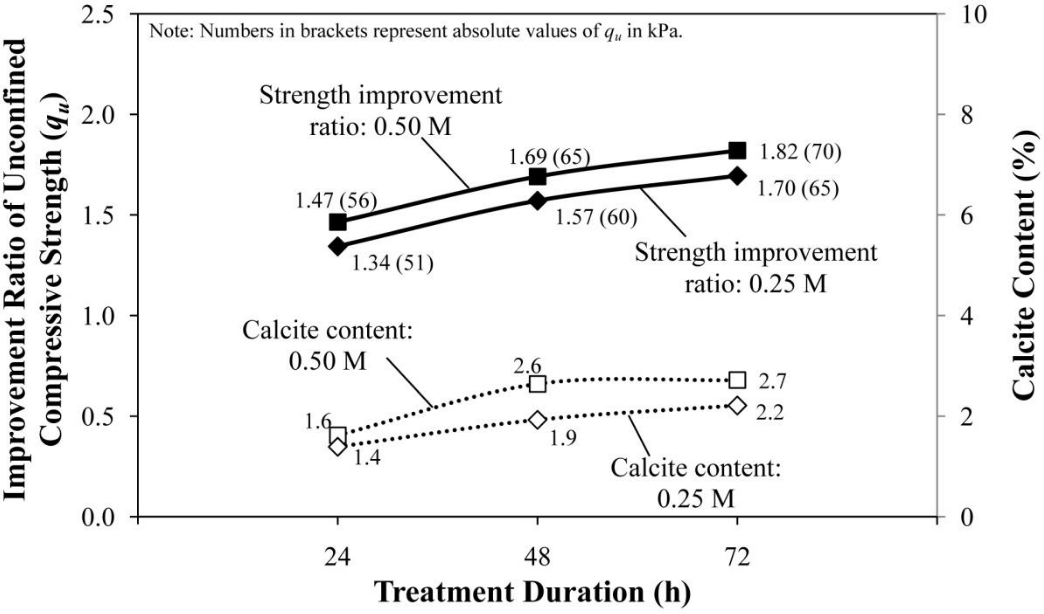


Figure 5.5: Effect of treatment duration (24 hours, 48 hours, and 72 hours) on unconfined compressive strength (q_u) and calcite content of MICP-treated residual soil specimens.

Treatment with cementation reagent of 0.25 M and 0.5 M indicated that longer treatment duration produced greater shear strength improvement. The improvement by 0.25 M and 0.5 M were in the range of 34 - 70 % and 47 – 82 %, respectively. The specimens treated with 0.5 M cementation reagent possessed slightly greater improvements than those treated with 0.25 M cementation reagent for all the three treatment durations concerned. This is because, under the same experimental conditions, the reagent concentration of 0.5 M could provide greater amounts of ingredients (urea and ammonium) per unit of time for promoting MICP process compared those of 0.25 M.

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

Figure 5.6 shows the reduction in hydraulic conductivity for various treatment durations. Hydraulic conductivity reduction showed good comparison with the shear strength results. The reduction rate of hydraulic conductivity decreased with the increased treatment duration. The hydraulic conductivity reductions for the specimens treated with 0.5 M cementation reagent ranged between 78 % and 91 %. These reduction rates were marginally higher than those of 0.25 M specimens (68 – 82%).

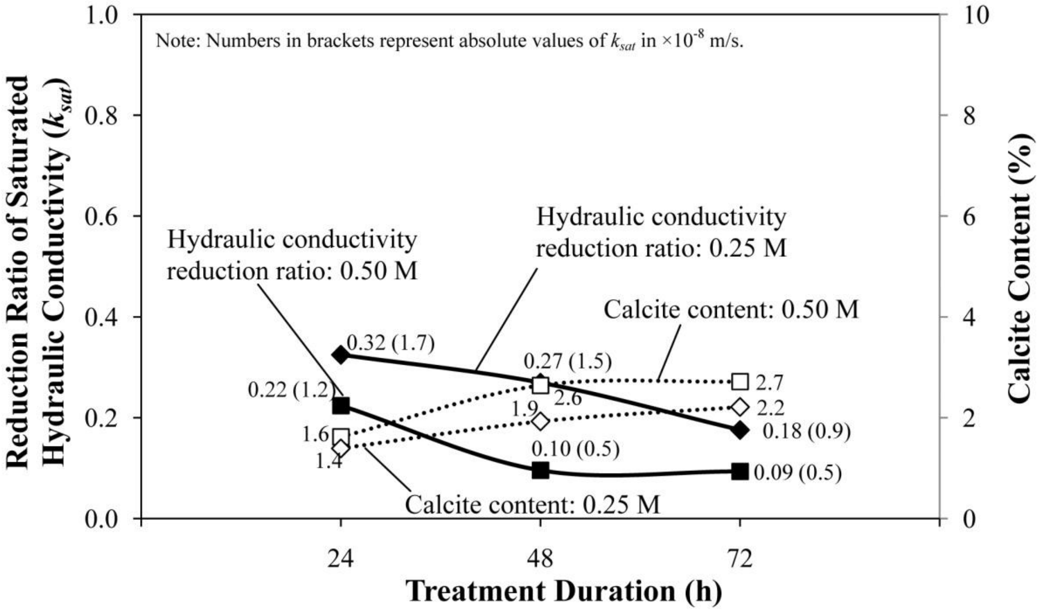


Figure 5.6: Effect of treatment duration (24 hours, 48 hours, and 72 hours) on saturated hydraulic conductivity (k_{sat}) and calcite content of MICP-treated residual soil specimens.

5.5 Effects of Concentration of *B. megaterium* and Cementation Reagent

An increase in the concentration of cementation reagent should be complemented by an increase in the urease enzyme (produced by the *B. megaterium*), and vice versa. The results of shear strength and hydraulic conductivity with treatments of various concentrations of cementation reagent and *B. megaterium* at flow pressure of 1.1 bar and duration of 48 hours are presented in Figure 5.7 and 5.8, respectively. Treatments with 1.0 M of cementation reagent did not show any measureable changes in shear strength and hydraulic conductivity. The measurements of ammonium content and pH have further confirmed that no visible urease activity was detected for these specimens. Kunst and Rapoport (1995) reported that the microbial growth under a salt stress condition has an adverse impact on the production of degradative enzyme. High salinity (i.e. 1.0 M) would strongly retard the growth of *B. megaterium* (Nekolny and Chaloupka 2000). Calcium chloride, which is one of the main components in the cementation reagent, is a salt that may contribute to the salinity of reagent solution. For these reasons, cementation reagent of excessively high concentration (i.e. higher than 1.0 M) was not recommended for the MICP treatment. However, this limiting concentration (1.0 M) may only valid for the *B. megaterium* adopted in the present study, as other species of bacteria may have different adaptability to the changes in salinity.

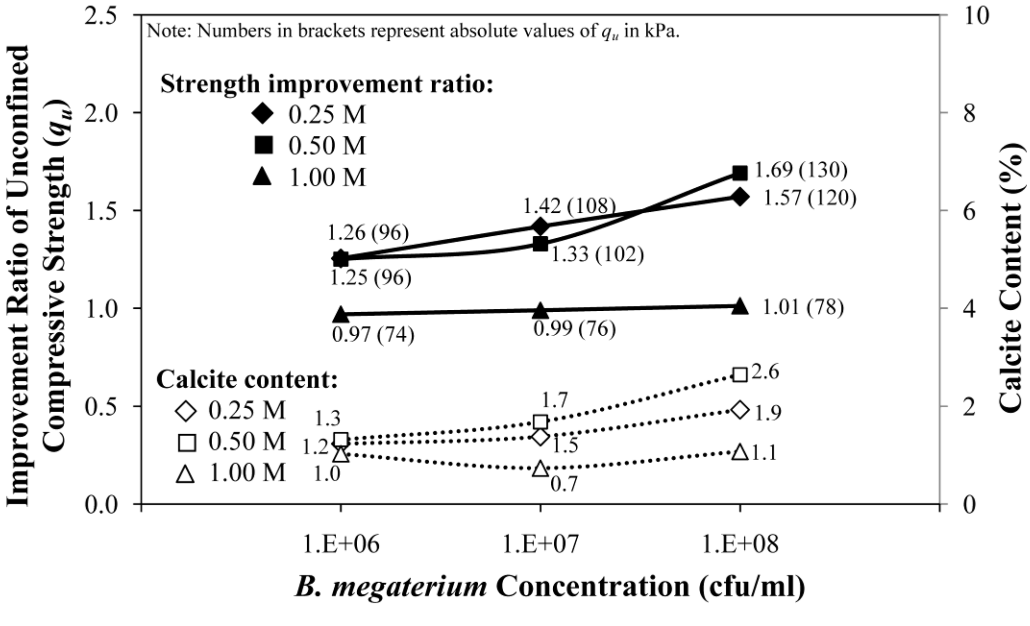


Figure 5.7: Effects of concentrations of *B. megaterium* (1×10^6 cfu/ml, 1×10^7 cfu/ml, and 1×10^8 cfu/ml) and reagent (0.25 M, 0.5 M, and 1 M) on unconfined compressive strength (q_u) and calcite content of MICP-treated residual soil specimens.

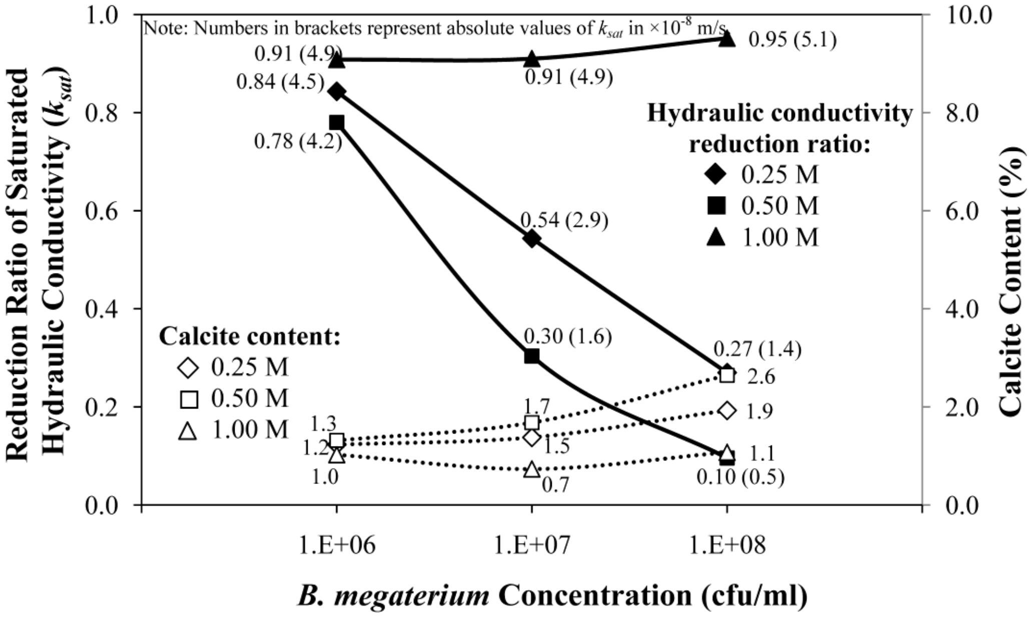


Figure 5.8: Effects of concentrations of *B. megaterium* (1×10^6 cfu/ml, 1×10^7 cfu/ml, and 1×10^8 cfu/ml) and reagent (0.25 M, 0.5 M, and 1 M) on saturated hydraulic conductivity (k_{sat}) and calcite content of MICP-treated residual soil specimens.

For the specimens treated with 0.25 M and 0.5 M of cementation reagent, the highest improvement occurred at *B. megaterium* of 1×10^8 cfu/ml, followed by 1×10^7 cfu/ml and 1×10^6 cfu/ml. The shear strength of the specimens treated with 0.25 M of cementation reagent was improved by 26 - 57 %, while the 0.5 M reagent recorded improvements of 25 - 69 %. The reductions in hydraulic conductivity for the 0.25 M and 0.5 M cementation reagent were 16 – 73%, and 22 – 90 %, respectively.

At low concentrations of *B. megaterium* (i.e. 1×10^6 cfu/ml and 1×10^7 cfu/ml), the increase of cementation reagent from 0.25 M to 0.5 M has not promoted measurable alterations in both soil engineering properties and calcite content. It can be deduced that the concentration of *B. megaterium* was the limiting factor for the

MICP. The cementation reagent (i.e. urea and calcium chloride) supplied was in excess of the urease enzyme produced. As the concentration of *B. megaterium* was increased to 1×10^8 cfu/ml, sufficient urease enzyme has been produced and the increase of cementation reagent concentration from 0.25 M to 0.5 M has caused significant improvements in both soil engineering properties and calcite content. The preference concentration of cementation reagent should lie between 0.5 – 1 M. It is interesting to find that Al Qabany et al. (2012) also obtained a similar preference concentration of cementation reagent, i.e. 0.66 M, despite a different bacterium (*S. pasteurii*) was used in their study.

5.6 Correlations between Calcite Content, Shear Strength, and Hydraulic Conductivity

Figure 5.9 shows the correlations between calcite content, shear strength and hydraulic conductivity. The graph is plotted from all test results. No visible shear strength and hydraulic conductivity improvements were observed for the calcite contents below 1.0 %. The calcite contents between 1.0 - 2.5 % were proportional to the shear strength improvement (R^2= 0.8741) and hydraulic conductivity reduction (R^2= 0.6525). The maximum enhancement in shear strength was achieved at 2.50 % of calcite content, while the hydraulic conductivity still exhibited a steady rate of reduction. This can be attributed to different mechanisms of shear strength improvement and hydraulic conductivity reduction, as explained in the earlier section.

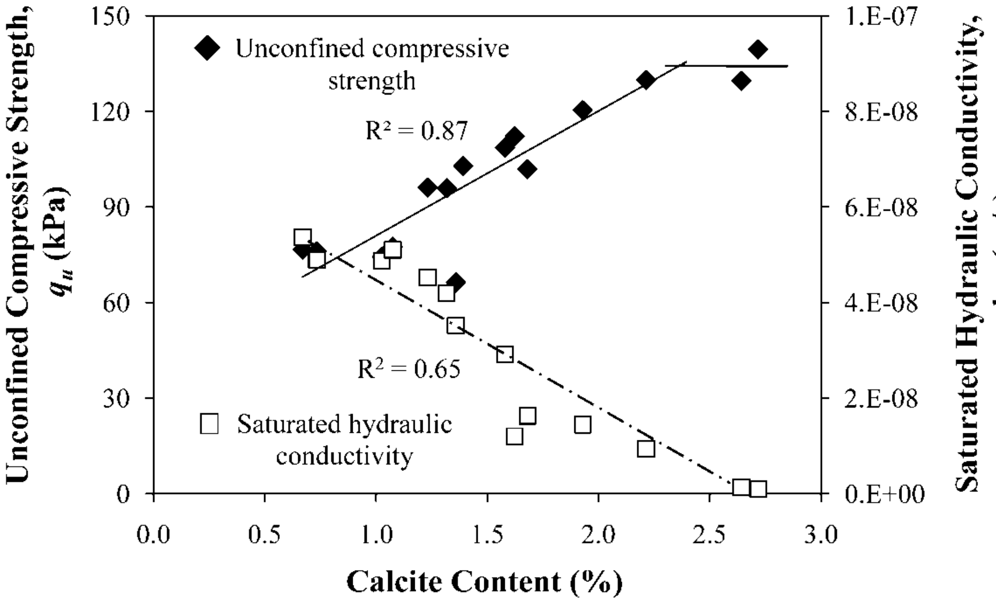


Figure 5.9: Correlations between unconfined compressive strength (q_u), saturated hydraulic conductivity (k_{sat}) and calcite content.

5.7 Ammonium Concentration and pH

Figure 5.10 and 5.11 present the variation in ammonium concentration and pH of effluent over time, respectively for the specimens treated with three different concentrations of cementation reagent, i.e. 0.25 M, 0.5 M, and 1.0 M and a control specimen treated with 0.5 M of cementation reagent (C5). Except the control specimen (C5) that was not supplied with *B. megaterium*, all these specimens have an identical *B. megaterium* concentration (1×10^8 cfu/ml), treatment duration (48 hours), and fluid pressure (1.1 bar). The measurement of ammonium content and pH is used as supportive indicators for the presence of urea hydrolysis activity in soil. Both the specimens treated with 0.25 M and 0.5 M cementation reagent showed dramatic increments in the ammonium content after 10 hours of treatment. The ammonium content of 0.25 M specimen reached a peak value after about 24 hours of treatment. Longer treatment duration has not promoted further urea hydrolysis. This could be attributed to insufficient cementation reagent supplied into the soil. The ammonium content of 0.5 M specimen peaked after about 40 hours of treatment. The peak concentration of ammonium in the effluent of 0.5 M specimen was about 2.5 times higher than that of 0.25 M specimen. Similar trends were observed for pH. The ammonium content and pH of the 1.1 M specimen was almost identical to that of the control specimen (C5), and this is consistent with earlier findings.

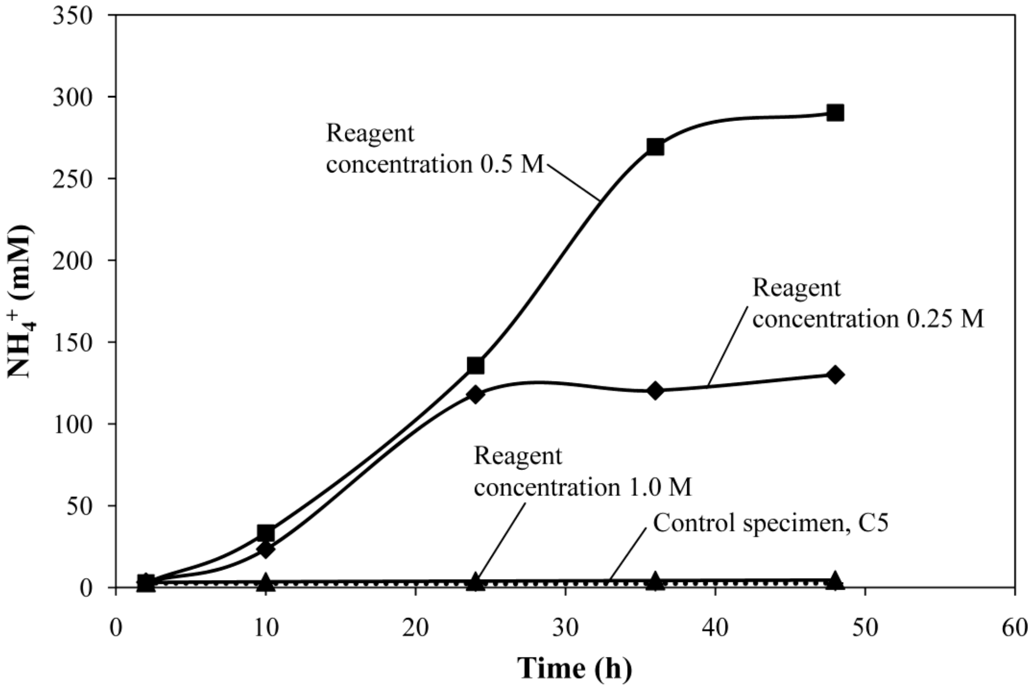


Figure 5.10: Variations of ammonium concentration over time during MICP treatment for an original control specimen (C1), and three MICP-treated specimens with 0.25 M, 0.5 M, and 1.0 M cementation reagent , respectively.

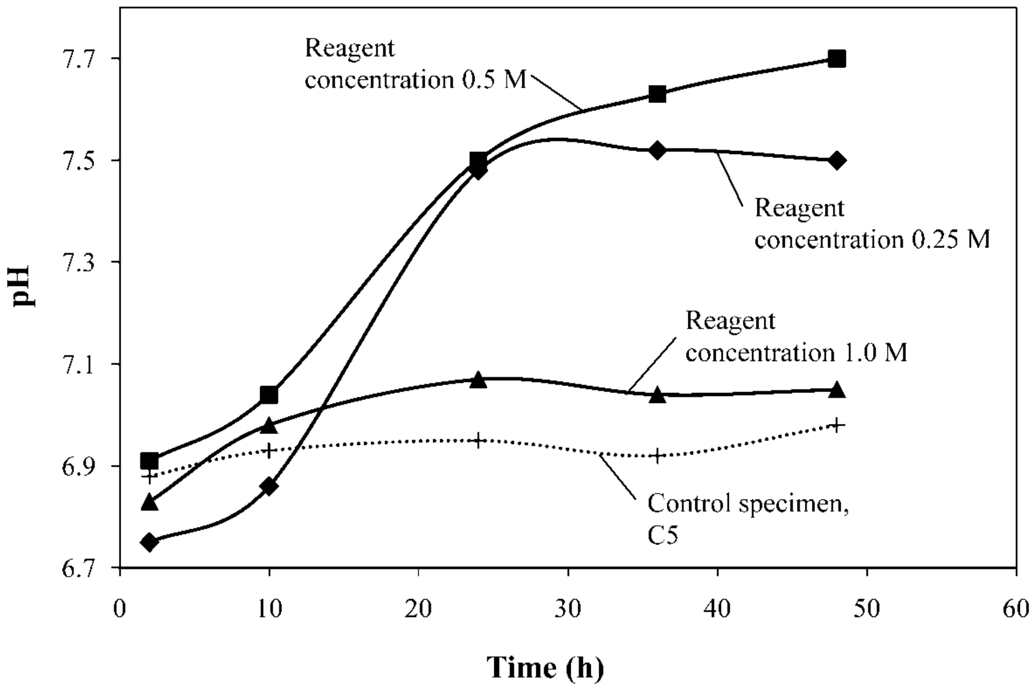


Figure 5.11: Variations of mean pH over time during MICP treatment for an original control specimen (C1), and three MICP-treated specimens with 0.25 M, 0.5 M, and 1.0 M cementation reagent , respectively.

The MICP reaction was commenced by injecting cementation reagent solution into the soil specimens. The initial pH in the soil was slightly acidic (lower than pH 7) attributed to the acidic nature of the residual soil. The production of ammonium ion in the urea hydrolysis increases the pH of the soil environment gradually. The increase in pH further improves the rate of urea hydrolysis as the optimum pH for urease enzyme is in the range of pH 7 - 8 (Stocks-Fischer et al., 1999; Evans et al., 1991). This repetitive cycle continues until the pH is no longer optimum (excessively alkaline) for the urease enzyme or survival of *B. megaterium*. The results of ammonium concentration and pH rise in the effluent showed reasonably good agreements with the earlier results of calcite content and improvements in soil properties.

5.8 Scanning Electron Microscope (SEM)

Scanning Electron Microscopy (SEM) analyses were carried out on selected samples to visualize qualitatively the calcite bonds and their distributions in the soil grains. Figure 5.12, 5.13, and 5.14 show the SEM images for the original control specimen (C1), and specimens treated with 0.25 M and 0.5 M cementation reagent, respectively. The particles of the original control specimen (C1) have a smooth surface (Figure 5.12). Both the 0.25 M and 0.5 M specimens which experienced considerable calcite precipitation showed abundant of calcite crystals forming at the particles contact points and particle surfaces. Comparatively, the distribution of the calcite crystals for the specimen treated with higher concentration of cementation reagent, i.e. 0.5 M (Figure 5.14) was denser than that of 0.25 M specimen (Figure 5.13).

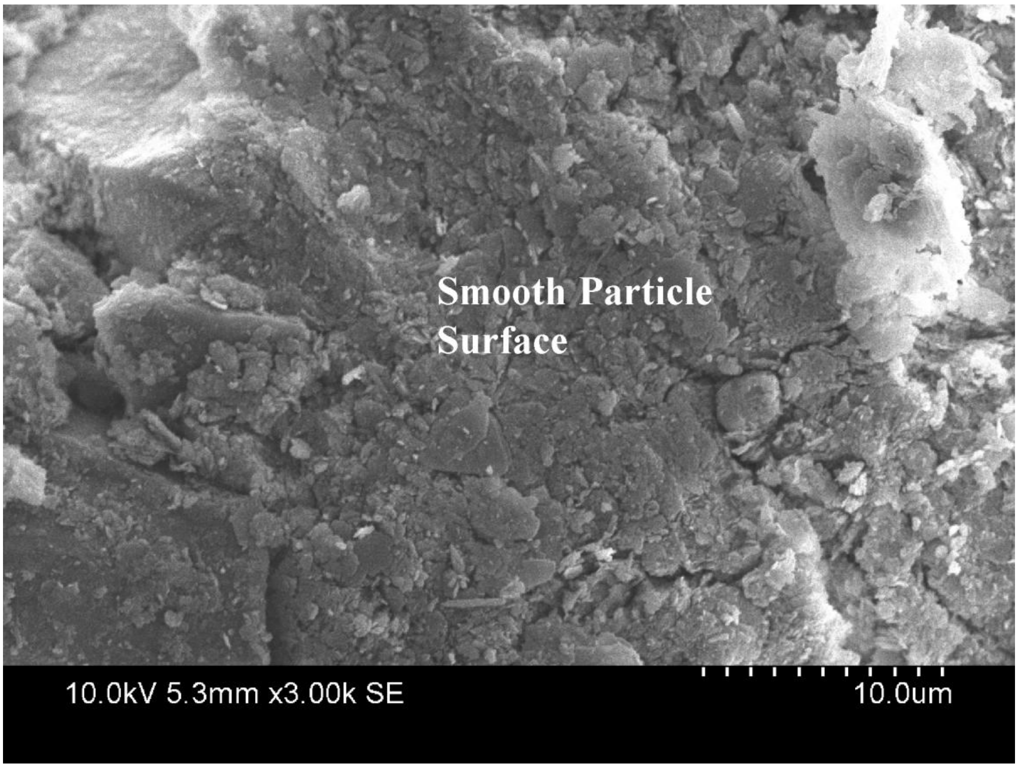


Figure 5.12: SEM image of Control Specimen C1

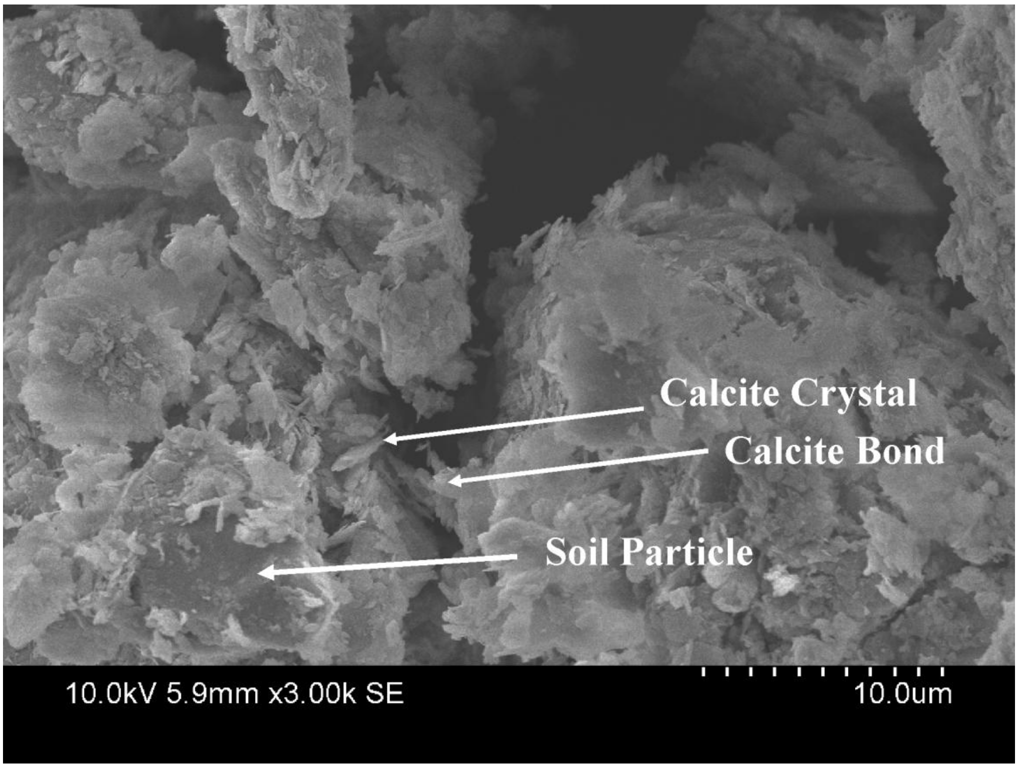


Figure 5.13: SEM image of specimen treated with 0.25 M cementation reagent

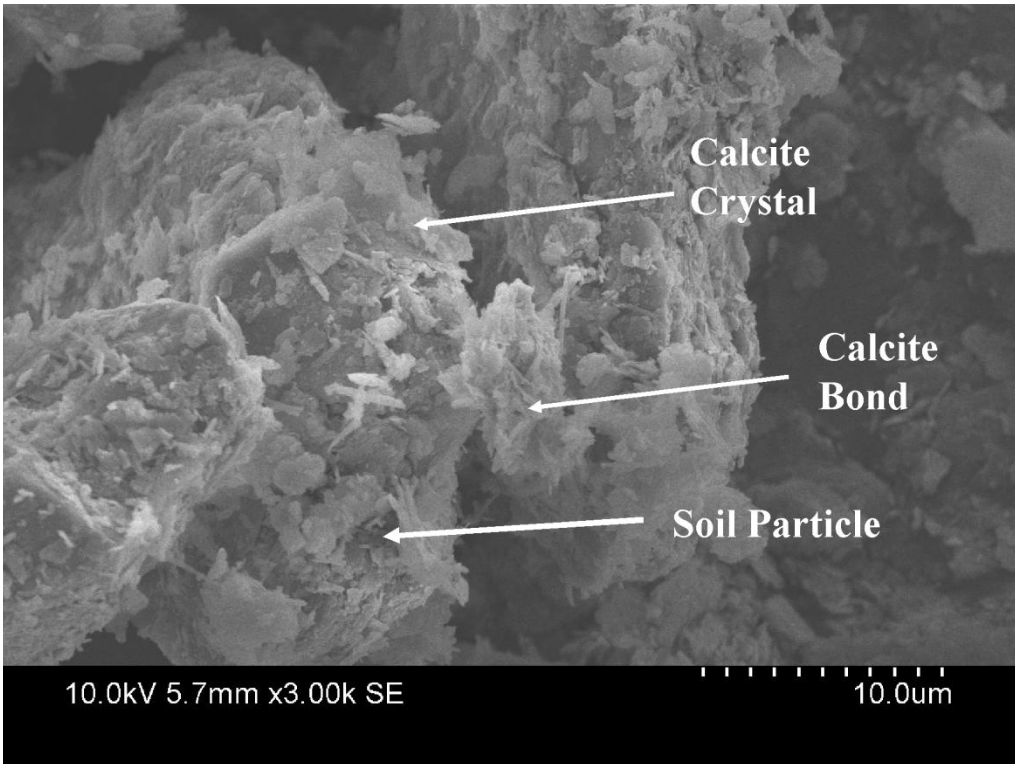


Figure 5.14: SEM image of specimen treated with 0.5 M cementation reagent

5.9 Discussion

From the present experimental study, it can be concluded that the MICP treatment has contributed to considerable improvements in engineering properties of tropical residual soil. The enhanced shear strength and reduced hydraulic conductivity were in the range of 25 – 100 % and 5 – 91 %, respectively depending on the several factors considered in this study, i.e. concentration of *B. megaterium*, concentration of cementation reagent, treatment duration, and flow pressure of cementation reagent. The improvement in shear strength is highly desirable in building construction. For instance, an improvement in shear strength of soil could reduce the construction cost/size of footing. The decrease in hydraulic conductivity of soil, however, may leave both positive and negative impact depending on its application. For example, a decrease in hydraulic conductivity of soil could control the infiltration of water into soil slope, and hence minimize the risk of landslide. However, the reduction in hydraulic conductivity may not favourable for controlling failure induced by earthquake as a low permeability soil is more prone to liquefaction. The successful attempts of the MICP treatment on residual soil could broaden the practical applications of the MICP technique in soil improvement, which has been thus far limited to the sand medium only.

The flow pressure of cementation reagent is an important controlling factor for the MICP treatment. As shown in the present laboratory tests, the high reagent pressure (i.e. 2 bar) has led to development of excess pore-water pressure in soil mold, and eventually reduced the shear strength of soil. For field treatment, it is anticipated that such effect may only be critical at the injection point of cementation reagent. The effect of excess pore-water pressure may not be permanent in field as the pressurized cementation fluids will eventually drain away slowly and lead to dissipation of pore-water pressure. The disturbance of soil structures by the high flow pressure, however, could be permanent for in-situ soils. The soil particles could be detached or loosened by the high pressure flowing fluids. On the other extreme, the reagent flow pressure cannot be excessively low as sufficient pressure is required to offer acceptable injection distance in the soil specimen. Long injection distance would minimize the number of injection well per area of land, and hence reduce the cost of treatment. From the calcite content measurements, the reagent flow pressure of 0.2 bar produced a slightly lower calcite content than that of 1.1 bar. It can be deduced that the low reagent flow pressure (i.e. 0.2 bar) has caused bioclogging near the inlet of the specimen, and eventually retard the flowing through of cementation reagent into the soil specimen. Therefore, a cementation reagent flow pressure in between 0.2 and 2 bar (i.e. 1.1 bar) is recommended for the residual soil treatment.

The treatment duration is defined as the time period over which cementation reagent is supplied into soil specimen. The amount of calcite precipitated increased with the increased treatment duration. However, from an economical point of view, the soil improvement needs to be completed within as short a time period as possible to minimize the cost of treatment. Therefore, it is important to determine the peak rate of calcite precipitation and the calcite content for promoting effective improvements in soil engineering properties. At the initial stage of the MICP treatment, the calcite precipitation rate increased with time. The production of ammonium ion during the urea degradation increased the pH of reagent solution and provided a favorable environment to further promote the urea degradation. In the present study, the calcite precipitation reached a plateau at pH between 7.5 – 7.7 (Fig. 5.11). Numerous studies performed using *S. pasteurii* found that the MICP reached a plateau at pH values between 8.7 and 9.5 (i.e. 9.5 (Stocks-Fischer et al., 1999); 9.3 (Ferris et al., 2003); 9.1 (Fujita et al., 2004); and 8.7 - 9.5 (Dupraz et al., 2009)). Longer treatment duration would further increase the pH and create an excessively alkaline environment, which is unfavorable for bacteria survival and urea degradation. Hammes and Verstraete (2002) and De Muynck et al. (2010b) suggested that long treatment duration in the presence of calcium ions may also result in a local supersaturation and heterogeneous calcite precipitation on the bacteria cell wall. This would eventually lead to cell deaths and impair the efficiency of MICP. From the Fig.

5.9, the effective calcite content for promoting measurable improvements in soil engineering properties is in the range of 1.0 - 2.5 %. From the foregoing results, it is justified to suggest that the preference treatment duration for the residual soil is 48 hours. By adopting 48 hours of treatment duration 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 7.7 and the calcite precipitated was about 2.64 % (marginally exceeded the effective calcite range of 1.0 - 2.50 %).

From the results of shear strength and hydraulic conductivity above, it is apparent that the improvements in the first 24 hours of treatment were greater than the second 24 hours, despite calcite precipitated within the first and second 24 hours were almost identical. The initial bonding formed between the soil particles is crucial in shear strength improvement because the calcite precipitated at the initial stage could effectively form bonding at the soil particles contact points (DeJong et al., 2010). As treatment continues, most of the particle contact points have been occupied and the calcites precipitated thereafter are deemed to be less effective in improving the shear strength.

The concentrations of *B. megaterium* and cementation reagent are interdependent factors in MICP treatment. *B. megaterium* produces the urease enzyme required in urea degradation, and acts as nucleation sites for calcite to precipitate. The amount of calcite precipitated would increase with the increased concentration of *B. megaterium*, provided sufficient cementation reagent is supplied into the soil. The cementation reagent contains urea and calcium chloride which serve as important ingredients for calcite precipitation. The urea and calcium chloride should be provided in equimolar. One mole of carbonate ion that degraded from one mole of urea is react with one mole of calcium ion, as perceived from the Eq. 1 and 2. Excessive amount of either reagent relative to the other one would cause unnecessary waste as the surplus reagent is not utilizable for calcite precipitation.

Despite the fact that lesser calcite content can be precipitated in the residual soil due to smaller pore throat for free passage of bacteria, the improvement in shear strength of residual soil through MICP treatment (25 – 100 %) was comparable to those previously reported studies using fine sand material, i.e. about 25 - 120 % of improvements (Whiffin et al., 2007; Lu et al., 2010). The effective calcite content as identified in this study was 1.0 – 2.50 % w/w or 15 – 37.5 kg/m^3. Whiffin et al. (2007) suggested that the minimum calcite content required for promoting effective improvement in engineering properties of fine sand was 3.5 % or 60 kg/m^3. This discrepancy can be explained by the higher particle-particle contacts per unit volume of residual soil compared to that of fine sand. The residual soil used in the present study consisted of a mixture of coarse and fine grains. The pores between the coarse grains were filled with the smaller grains, thus resulted in greater particle-particle contacts. This has created a favourable environment for the calcite bonds to be formed effectively on these particle-particle contacts, and hence improve the inherent shear strength of soil. With respect to the reduction in hydraulic conductivity, the MICP treatment of residual soil (reduction ranged 0.3 to 0.9 order of magnitude) was comparatively less effective than the fine sand, i.e. about half to one order of magnitude reduction (Nemati et al., 2005; Nemati and Voordouw 2003). Ng et al.

(2012) claimed that the high porosity in sand provided more pore spaces for calcite deposition, and hence results in a greater reduction in hydraulic conductivity than residual soil.

One of the downsides of the MICP soil improvement technique is the generation of ammonium ion as a side product of urea degradation. The ammonium ion plays an essential role in the MICP process to increase the pH of solution and accelerate the rate of urea degradation. However, the toxicity of ammonium ion may cause soil contamination. According to the United States Environmental Protection Agency (1993), ammonia concentration of 0.1 to 10.0 mg/l may cause negative impact on fish health, reproduction or even mortality. Ammonia comes in two forms: ionized form (NH_4^+) and ammonium salt form (NH_3), while the toxicity is mainly contributed by the ammonium salt form. Most ammonium produced in urea hydrolysis would be converted to ammonium salt form if the pH is higher than 9.5. In addition, a fraction of the ammonium may be converted to nitrate (NO_3^-) through bacterial denitification (Hamdan et al., 2011). The maximum allowable concentration of nitrate in soil is 130 ppm, as regulated by the United Nations Environment Programme (1998).

5.10 Concluding Remark

This chapter presents the results of a series of experimental works to investigate the viability of MICP technique for improving the engineering properties of a typical tropical residual soil. Four treatment parameters, i.e. concentration of *B. megaterium*, concentration of cementation reagent, treatment duration, and flow pressure of cementation reagent were considered to determine the preference treatment conditions for the MICP treatment. The following conclusions can be drawn from this study:

i) MICP treatment is capable of improving shear strength and reducing hydraulic conductivity of residual soil. The greatest improvement in shear strength and reduction in hydraulic conductivity were 100 % and 90 %, respectively. The rate and magnitude of improvement were controlled by the treatment parameters considered in this study.

ii) Excessively high cementation reagent flow pressure (i.e. 2 bar) may lead to a build-up of pore-water pressure and disturbance of soil structures, and hence leave an adverse impact on soil improvement. On the other extreme, excessively low flow pressure (i.e. 0.2 bar) may precipitate calcite close to the inlet and prohibit the flow of reagent through the soil specimen. A moderate flow pressure (i.e. 1 bar) is recommended to maintain the adequate injection distance of the cementation reagent while avoiding the potential development of excess pore-water pressure.

iii) The preference 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 hours. The shear strength improvement and hydraulic conductivity reduction obtained from this combination of treatment parameters are 69 % and 90 %, respectively.

iv) A minimum calcite content of 1.0 % (15 kg/m^3) is required for provoking measurable improvements in shear strength and reduction in hydraulic conductivity of residual soil. The shear strength improvement and hydraulic conductivity reduction are linearly proportional with the calcite content between 1.0 and 2.5 %. Beyond 2.5 % of calcite content, the shear strength improvement becomes less effective because most of the particle-particle contact points have been bonded by the calcite. The hydraulic conductivity reduction does not exhibit this limitation.

v) The improvements in soil engineering properties obtained from the control specimens that treated with cementation reagent only are negligible. The inclusion of *B. megaterium* only reduces the soil hydraulic conductivity by about 26 % through biomass, which is deemed to be a temporary effect.

CHAPTER 6

CONCLUSIONS AND RECOMMENDATIONS

6.1 Introduction

Four conclusions are drawn in this study keeping in view the objectives formulated in Chapter 1 of this thesis. The conclusions are clearly presented in the following sections.

6.1.1 Feasibility of applying MICP soil improvement technique on tropical residual soil

The preliminary experimental results have confirmed the effectiveness of MICP technique in improving engineering properties of tropical residual soil specimens. The MICP treatment promoted an increase in shear strength of residual soil (from 20 kPa to 61 - 69 kPa). The hydraulic conductivity of MICP treated soil was reduced from approximately 3.0×10^{-7} m/s to $0.3 – 0.9 \times 10^{-7}$ m/s.

Comparison between residual soil and sand has shown that the improvements in engineering properties of residual soil were considerable. The shear strength increment ratios of residual soil (1.41 to 2.64) were significantly higher than that of sand (1.14 to 1.25). This is because residual soil has higher particle-particle contacts per unit volume of soil than sand. Opposite result was observed for hydraulic conductivity where the sand (reduction ratios of 0.09 to 0.15) has outperformed the residual soil (reduction ratios of 0.26 to 0.45). The relatively high porosity in sand provides more pore spaces for calcite deposition, and hence results in a greater reduction in hydraulic conductivity than residual soil.

6.1.2 Effects of MICP on Shear Strength and Hydraulic Conductivity of Residual Soil

The effect of MICP treatment on shear strength and hydraulic conductivity of residual soil is functions of *B. megaterium* concentration, cementation reagent concentration, flow pressure, and treatment duration. From the present experimental study, it can be concluded that the MICP treatment has contributed to considerable improvements in engineering properties of tropical residual soil. The enhanced shear strength and reduced hydraulic conductivity were in the range of 25 – 100 % and 5 – 91 %, respectively. The rate and magnitude of improvements were controlled by the four treatment parameters considered in this study.

The concentrations of *B. megaterium* and cementation reagent are interdependent factors in MICP treatment. *B. megaterium* produces the urease enzyme required in urea degradation, and acts as nucleation sites for calcite to precipitate. The amount of calcite precipitated would definitely increase with the increased concentration of *B. megaterium,* provided sufficient cementation reagent is supplied into the soil. Excessive amount of either reagent relative to the other one would cause unnecessary waste as the surplus reagent is not utilizable for calcite precipitation.

Excessively high cementation reagent flow pressure (i.e. 2 bar) may cause development of excess pore water pressure in soil and leave an adverse impact on soil improvement. On the other extreme, excessively low flow pressure (i.e. 0.2 bar) may precipitate calcite close to the inlet and prohibit the flow of reagent through the soil specimen.

The amount of calcite precipitated increased with the increased treatment duration. However, from an economical point of view, the soil improvement needs to be completed within as short a time period as possible to minimize the cost of treatment. Therefore, it is important to determine the peak rate of calcite precipitation and the calcite content for promoting effective improvements in soil engineering properties.

6.1.3 Preference Treatment Conditions For MICP Soil Treatment

Based on the variables considered in the main experimental results, it can be concluded that the preference conditions for MICP treatment on residual soil were *B. megaterium* concentration of 1×10^8 cfu/ml, cementation reagent concentration of 0.5 M, flow pressure of 1.1 bar, and treatment duration of 48 hours. These preference conditions resulted in a shear strength improvement of 69% and hydraulic conductivity reduction of 90%. However, the effectiveness of MICP technique should be assessed on a case-specific basis by evaluating the cost effectiveness of the soil improvement method on a specific application.

6.1.4 Effective calcite content for promoting MICP improvement in residual soil

The effective range of calcite content for shear strength improvement and hydraulic conductivity reduction was between 1.0 to 2.5 %, or approximately 15 to 37.5 kg/m^3. The maximum enhancement in shear strength was achieved at 2.50 % of calcite content, while the hydraulic conductivity still exhibited a steady rate of reduction. This can be attributed to different mechanisms of shear strength improvement and hydraulic conductivity reduction, as explained in the earlier section.

6.2 Limitations

This study is bounded by several limitations which may have restricted the application of the outcomes in real life problem. These limitations were identified as a means for potential strategies in future research:

i. The MICP treatment conducted in this study was conducted by directing the flow of cementation reagent from top to bottom of soil column. This method permits only one-dimensional flow of cementation reagent, while fluid moves in three-dimensional flow within the field porous media like soil.

ii. The properties of residual soil used in this study were assumed to be homogeneous. In reality, residual soil is normally characterized by high variability in terms of their physical and engineering properties. This inhomogeneity and variability may significantly affect the performance of MICP treatment.

iii. The residual soil used in the MICP treatment was remoulded samples. The compaction process may alter the properties of original soil, even though remoulded soil was compacted to an identical density to its natural state by a controlled compaction effort.

iv. The side product of urea degradation, i.e. ammonium ion assists the urea degradation itself by increasing the pH of the solution and accelerating the degradation rate during the treatment process. However, ammonium ion could be a threat in the soil environment due to its toxicity. Ammonia comes in two forms: ionized form (NH_4^+) and ammonium salt form (NH_3), while the toxicity is mainly contributed by the ammonium salt form. Most ammonium produced in urea hydrolysis would be converted to ammonium salt form if the pH is higher than 9.5. In addition, a fraction of the ammonium may be converted to nitrate (NO_3^-) through bacterial denitification (Hamdan et al., 2011). The maximum allowable concentration of nitrate in soil is 130 ppm, as regulated by the United Nations Environment Programme (1998). The MICP soil treatment can create enormous concentration of ammonium, depending on the treatment conditions. No specific research to deal with the ammonium produced by the MICP reaction was reported so far.

6.3 Recommendations

Several study areas are recommended for further improving practical applications of the MICP soil improvement technique in residual soils:

i. Study of two or three-dimensional flow of cementation reagent in residual soil. This can be achieved by up-scaling the laboratory model or performing field soil treatment.

ii. Studies to include more soil types or grain sizes. The grain sizes of soil may affect the passage of microorganism and cementation reagent through the soil matrix. Thus, it is worthwhile to further investigate to what extent that these particle grain sizes may affect the performance of MICP treatment.

iii. The by-product of MICP reactions, i.e. ammonia needs to be mitigated or controlled to minimize the risk to environment. Future researches may look into the potential mitigation measures.

iv. Study of distribution of cemented sites in treated soil. These results could be useful for explaining the mechanism of alteration in engineering properties of soil.

v. Study on microstructure of the MICP treated soil. This could explain the effect of pore throat size on the effectiveness of MICP treatment.

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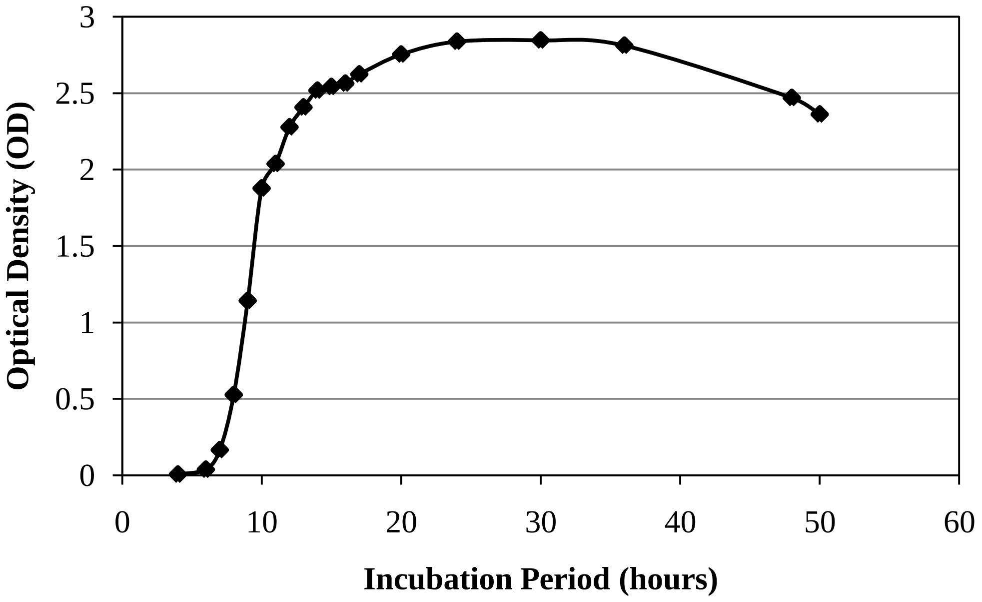
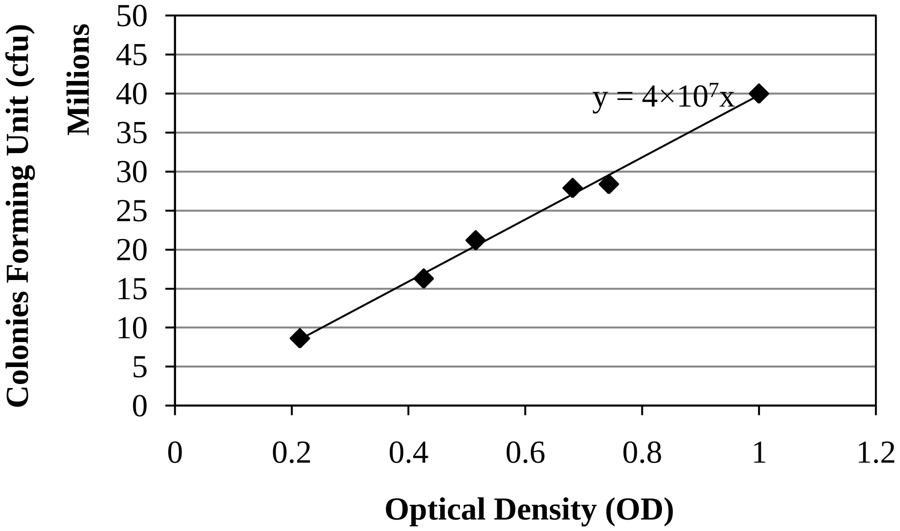
APPENDICES

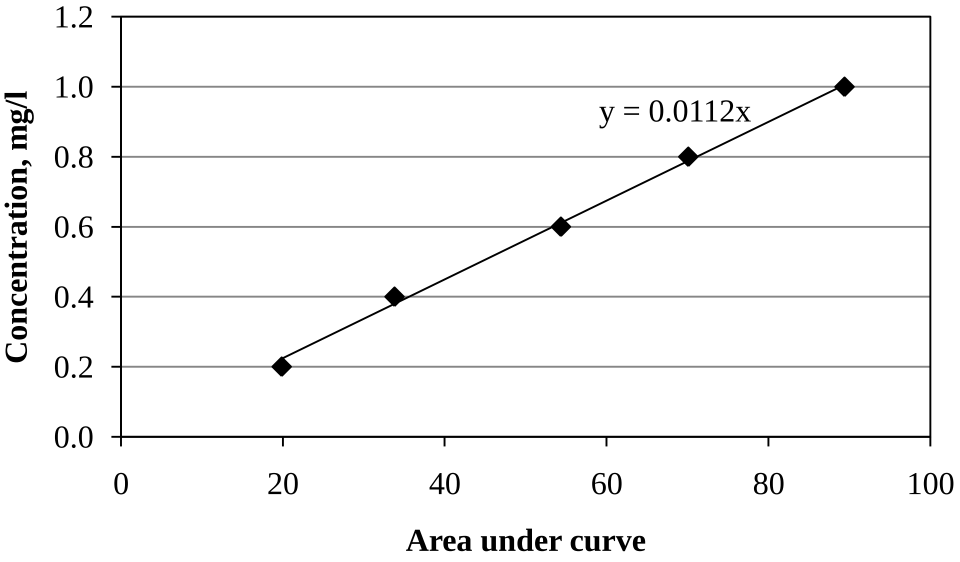
APPENDIX A: *Bacillus megaterium* Growth Curve



APPENDIX B: OD vs cfu Calibration Curve



APPENDIX C: Ammonium Concentration Calibration Curve



APPENDIX D: List of Main Experimental Tests

Test no	*B. Megaterium* Concentration (cfu/ml)	Reagent Concentration (M)	Treatment Duration (h)	Reagent Flow Pressure (bar)
C1	-	-	-	-
C2	1×10^8	-	24	1.1
C3	-	0.5	48	0.2
C4	-	0.5	24	1.1
C5	-	0.5	48	1.1
C6	-	0.5	72	1.1
C7	-	0.5	48	2.0
1	1×10^6	0.25	24	0.2
2	1×10^6	0.25	48	0.2
3	1×10^6	0.25	72	0.2
4	1×10^6	0.5	48	0.2
5	1×10^6	0.5	72	0.2
6	1×10^6	1	48	0.2
7	1×10^6	1	72	0.2
8	1×10^7	0.25	48	0.2
9	1×10^7	0.25	72	0.2
10	1×10^7	0.5	24	0.2
11	1×10^7	0.5	48	0.2
12	1×10^7	0.5	72	0.2
13	1×10^7	1	48	0.2
14	1×10^7	1	72	0.2
15	1×10^8	0.25	48	0.2
16	1×10^8	0.25	72	0.2
17	1×10^8	0.5	24	0.2
18	1×10^8	0.5	48	0.2
19	1×10^8	0.5	72	0.2
20	1×10^8	1	48	0.2
21	1×10^8	1	72	0.2
22	1×10^6	0.25	48	1.1
23	1×10^6	0.25	72	1.1
24	1×10^6	0.5	48	1.1
25	1×10^6	0.5	72	1.1
26	1×10^6	1	72	1.1
27	1×10^7	0.25	24	1.1

28	1×10^{7}	0.25	48	1.1
29	1×10^{7}	0.25	72	1.1
30	1×10^{7}	0.5	24	1.1
31	1×10^{7}	0.5	48	1.1
32	1×10^{7}	0.5	72	1.1
33	1×10^{7}	1	48	1.1
34	1×10^{7}	1	72	1.1
35	1×10^{8}	0.25	24	1.1
36	1×10^{8}	0.25	48	1.1
37	1×10^{8}	0.25	72	1.1
38	1×10^{8}	0.5	24	1.1
39	1×10^{8}	0.5	48	1.1
40	1×10^{8}	0.5	72	1.1
41	1×10^{8}	1	48	1.1
42	1×10^{8}	1	72	1.1
43	1×10^{6}	0.25	48	2.0
44	1×10^{6}	0.25	72	2.0
45	1×10^{6}	0.5	48	2.0
46	1×10^{6}	0.5	72	2.0
47	1×10^{6}	1	72	2.0
48	1×10^{7}	0.25	48	2.0
49	1×10^{7}	0.25	72	2.0
50	1×10^{7}	0.5	48	2.0
51	1×10^{7}	0.5	72	2.0
52	1×10^{7}	1	72	2.0
53	1×10^{8}	0.25	48	2.0
54	1×10^{8}	0.25	72	2.0
55	1×10^{8}	0.5	48	2.0
56	1×10^{8}	0.5	72	2.0
57	1×10^{8}	1	48	2.0
58	1×10^{8}	1	72	2.0

LIST OF PUBLICATION

Journal:

Lee, M.L., Ng, W.S., Tan, C.K., and Hii, S.L., 2012. Bio-mediated soil improvement under various concentrations of cementation reagent. *Applied Mechanics and Materials, Trans Tech Publications*. 204-208, pp. 326-329. [Indexed by Scopus]

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Ng, W.S., Lee, M.L., Tan, C.K., and Hii, S.L. Improvements in engineering properties of soils through microbial-induced calcite precipitation. *KSCE Journal of Civil Engineering*, Springerlink. (Accepted). [Sci. IF 0.377]

Conference paper:

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