CALCITE PRECIPITATION IN BIO-MEDIATED TROPICAL RESIDUAL SOIL

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A project report submitted in partial fulfilment of the requirements for the award of Bachelor of Engineering (Honours) Civil Engineering

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DECLARATION

I hereby declare that this project report is based on my original work except for citations and quotations which have been duly acknowledged. I also declare that it has not been previously and concurrently submitted for any other degree or award at UTAR or other institutions.

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APPROVAL FOR SUBMISSION

I certify that this project report entitled **"CALCITE PRECIPITATION IN BIO-MEDIATED TROPICAL RESIDUAL SOIL"** was prepared by **YEE JASON** has met the required standard for submission in partial fulfilment of the requirements for the award of Bachelor of Degree (Honours) Civil Engineering at Universiti Tunku Abdul Rahman.

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ABSTRACT

Biomediation in soil for improvement is a relatively new approach and environment friendly method in civil engineering. This method induces calcium carbonate (calcite) through the biological process of microbes which known as Microbial Induced Calcite Precipitate (MICP). There are less studies on the evaluation of biomediation in fine-grained soil such as clay in tropical residual soil, so far fine sands were used as the primary material for the research of MICP treatment. This purpose of this project is to study the effect of reagent concentrations on strength properties and calcite content of biomediated tropical residual soil as well as the uniformity of calcite precipitation. 3 sets of soil specimens were prepared by well mixing 1 x 10^8 cfu/ml of urease-forming bacteria, *Sporosarcina Pasteurii* with the tropical residual soil (<2mm) and each treated with 0.25M, 0.50M, and 1.0M of cementation reagent (combination of urea, calcium chloride and nutrient broth), respectively. The pH values of effluent over the treatment duration were measured as the indicator for ureolytic activities. Strength test and calcite test were respectively performed to examine the strength properties and calcite content of bio-mediated tropical residual soil. Untreated soil specimen was used in this study as the benchmark for strength properties and calcite content. The results of this study showed that 0.5M of cementation reagent had contributed the highest strength (66.97kPa), trailed by 1.0M of cementation reagent (64.40kPa). 0.25M of cementation reagent resulted the lowest strength (55.58kPa). Same trend was found for the calcite content in the tropical residual soil. Besides, it has been found that the correlation between calcite content and strength properties of tropical residual soil. The higher the calcite content obtained in the soil, the higher the strength properties that can achieved by the soil. The increment of dry density was examined as well upon the MICP treatment in tropical residual soil. Overall dry density was increased by 2.09% to 5.19% upon the MICP treatment. Furthermore, the variation of pH value was observed along the treatment duration. Overall pH value was increased from 5.5 to 8.0 over the 6 days treatment duration for bio-mediation in tropical residual soil.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF SYMBOLS / ABBREVIATIONS

BS	Brithish Standard
cfu	Colony forming unit
MDD	Maximum dry density
MICP	*Microbial induced calcite precipitate*
OMC	Optimum moisture content
R^2	Coefficient of determination
SEM	Scanning electron microscopy
S. pasteurii	*Sporosarcina pasteurii*
UCS	Unconfined Compression Strength
USCS	Unified Soil Classification System

Ca	Calcium
Ca^{2+}	Calcium ions
$CaCl_2$	Calcite chloride
$CaCO_3$	Calcium carbonate
$CaSO_4 \cdot 2H_2O$	Gypsum
CH_4N_2O	Urea
CO_3^{2-}	Carbonate ions
HCl	Hydrochloric acid
HCO_3^-	Bicarbonate ions
NH_3	Ammonia
NH_4^+	Ammonium
H_2O	Water
Cl^-	Chloride ions
OH^-	Hydroxyl ions
Si	Silica

CHAPTER 1

INTRODUCTION

1.1 General Introduction

Bio-mediation in soil for improvement is a relatively new approach of method in civil engineering. This method technically will produce calcium carbonate (calcite) through the biological process of microbes which known as microbial induced calcite precipitate (MICP). The calcite induced in the particle-particle contacts of soil are used to enhance the geotechnical properties of soil (i.e. increasing the stiffness, shear strength and reducing the compressibility and permeability) by the process of biocementation and bioclogging (Ivanov and Chu, 2008; Ng, 2013; Wani and Mir, 2020;). MICP offers a more sustainable and environment friendly alternative to the conventional type of soil improving methods such as cementing and jet grouting by reducing the harmfulness of chemicals to the environment (DeJong, et al., 2010; Montoya, 2012).

Many researchers have claimed that the shear strength, stiffness and permeability are enhanced after a few chains of biochemical reactions (Feng and Montoya, 2015; Nafisi and Montoya, 2018). Moreover, MICP via denitrification has shown the potential for improving earthquake-induced liquefaction resistance of soils for long term mitigation (Muttaqa, Khairul, and Abubakar, 2018; Kavazanjian, O'Donnell and Hamdan, 2015). Besides, erosion resistance of mine sand can be improved by MICP as well as increased the surficial stability, dust control and accessibility for future re-vegetation (Gomez, et al., 2013). Erosion of the coastal deposits can be prevented by MICP to combine the sand particles together as one body (Montoya, Feng, and Shanahan, 2013).

Numerous contributing factors may lead to different outcome of effectiveness in MICP treatment. This includes the biological activity, spatial distribution of microbes and chemical, concentration of microbes and chemical reagent, time control, etc (DeJong, et al., 2010).

Tropical residual soil describes a soil is being weathered from a rock structure at its original place without any moving. (Ishak, Zolkepli and Affendy, 2017). Salih (2012) stated that residual soil is being destroyed by the mechanical and chemical weathering due to several factors such as climatic element, geographic

origin, parental materials, and age of body. The geotechnical characteristics of residual soil were generally determined by these factors. Formation process of the residual soils is highly dependent on the tropical weather in Malaysia.

There are several researchers have studied the behavior of MICP on sandy soil, but a few in-depth experimental researches have been studied to evaluate the ureolytic activities of bacteria for bio-mediation in tropical residual soil. The research gap is found as the present study.

1.2 Importance of the Study

This research provides the before and improved engineering properties of tropical residual soils. The relevant correlations and facts pertaining to MICP treatment on residual soil are discussed. These data and information gathered can be served as a future reference for local construction personnel.

MICP treatment can potentially be used in tropical residual soil as well to enhance the engineering properties of soil, i.e. increasing the shear strength and reducing the permeability. MICP treatment on residual soil can potentially be implemented on slope to achieve the optimum shear strength in order to prevent landslides without any large cuttings in the construction. MICP treatment can be used to replace other traditional techniques without eliminating any harmful substances as well as be applied in the field on a large scale without the need for heavy machinery.

1.3 Problem Statement

More than 80% coverage of wide land area in Peninsular Malaysia are residual soils (Taha, Hossain and Mofiz, 2000). Intensive rainfall weather leads to serious slope failures or landslides in tropical residual soil every year.

There are less studies on the evaluation of biomediation in fine-grained soil such as clay in tropical residual soil, so far fine sands are the primary material for the research of MICP treatment (Harkes, et al., 2010). Traditional grouting has the risk of eliminating poisonous substances to the environment. Besides, most of the traditional grouting techniques have the issue of low “certainty of execution”. Feasibility of MICP treatment on tropical residual soil to overcome the problems is discussed in the present study.

1.4 Aim and Objectives

The aim of this study is to investigate the calcite precipitation in bio-mediated tropical residual soil.

The objectives set out to achieve the aim are:

1. To investigate the pH changes versus treatment duration for bio-mediation in tropical residual soil.
2. To investigate the calcite content and compressive strength of bio-mediated soil with different cementation reagent concentrations.
3. To investigate the effect of bio-mediation on the dry density of tropical residual soil.
4. To correlate the relationship between shear strength and calcite content in bio-mediation in tropical residual soil.

1.5 Scope and Limitation of Study

This project was conducted using a small scale of experimental approach to study on possible microbial treatment on the residual soil. The scope of study includes the basic cultivating of bacteria, MICP treatment by flowing cementation reagent through soil samples, pH test on effluent to check the ureolytic activities, and calcite test by acid wash to check the performance of MICP.

The treatment variables were considered as the main factors that controlled the effectiveness of MICP treatment.in the study such as the dry density of residual soil and concentration of cementation reagent through the soil specimen.

Other treatment conditions such as temperature, pH, bacteria type, soil grain size, and injection method were considered as the limitation in this study. The distribution of bacteria in the pore spaces of soil was out of the scope of this study. And, the potential processes and application of MICP treatment are further studied.

1.6 Layout of Report

This study is categorized into five chapters include: Introduction (Chapter 1), Literature Review (Chapter 2), Research Methodology and Work Plan (Chapter 3), Results and Discussions (Chapter 4) and Conclusion and Recommendations (Chapter 5).

Chapter 1 shows the general introduction of the MICP treatment and tropical residual soil. Besides, the importance of study, problem statement, aim and objectives, scope and limitation of study, and layout of report are provided in this chapter.

Chapter 2 shows the previous researcher's studies on the MICP treatment and the findings on tropical residual soil in Malaysia. Other than that, the leading factor to the emergence of MICP treatment and its advantages are discussed. Furthermore, the biological process and chemical reaction of MICP treatment are shown. The application of MICP treatment and its leading factors are further elaborated in this chapter.

Chapter 3 demonstrates the detailed description on how the project works by a flowchart. This chapter provides the description of the material used, laboratory setup and several tests. A work plan of the study is shown in this chapter as a Gantt Chart as well.

Chapter 4 explains the experimental results of the study. The optimum concentration of cementation reagent is identified by comparing the ultimate compressive strength and calcite content from the result. The results in this study are further discussed with the findings from previous researchers in this chapter.

Lastly, Chapter 5 concludes the findings of this study and provides the recommendations for future study.

CHAPTER 2

LITERATURE REVIEW

2.1 Introduction

This chapter provides a brief introduction of tropical residual soil in Malaysia and its characteristics that result in slope failure. The factors lead to the emergence of MICP soil improvement method and its advantage over the current techniques are reviewed. A summary of MICP soil improvement method was explained. Biological processes and chemical reactions associated to MICP are discussed. The potential application of MICP was delved through the implementation of bio-cementation and bio-clogging in recent years. Finally, considerable state of the art literature pertaining to the topic of MICP soil improvement was critically reviewed.

2.2 Tropical Residual Soil

Residual soils are created by the process of weathering of parental rocks and without any moving from its origin. (Rahardjo, et al., 2004). Mechanical and chemical weathering are the main factor on destroying of residual soil from its parental rock (Townsend, 1985). The behaviour of in-situ weathered soils is complicated because it is totally dependent on several factors (Salih, 2012). These factors include the climatic element, geographic origin, parental materials, and age of body.

Malaysia is one of the countries within the equatorial zone which have the tropical rainforest climate. Abundant of down-pour with high average temperature is the characteristic of tropical climate. In fact, tropical weather is the main factor influenced in the formation of the residual soils in Malaysia. Intense chemical weathering of parent rocks under the intensive and prolonged rainfall, consistent day length of high temperature and approximate perpendicular of sun angle throughout the year is the main agent which lead to the extensive formation of tropical residual soil in Malaysia.

More than three-quarters of tropical residual soils are widespread on the land of Peninsular Malaysia (Taha, et al., 2000; Rahardjo, et al., 1995). Major type of residual soils in Malaysia are in granite and sedimentary form, whereby covered most of the land area in Malaysia excluded the contribution soft clay in coastal sites (Salih, 2012). These soils may preserve part of the characteristics of their parental

rocks. Figure 2.1 shows the granite residual soils profile under the subsurface. The raw or parental rocks (un-weathered) transform to partially weathered, then further transform into soil which as shown in Figure 2.1.

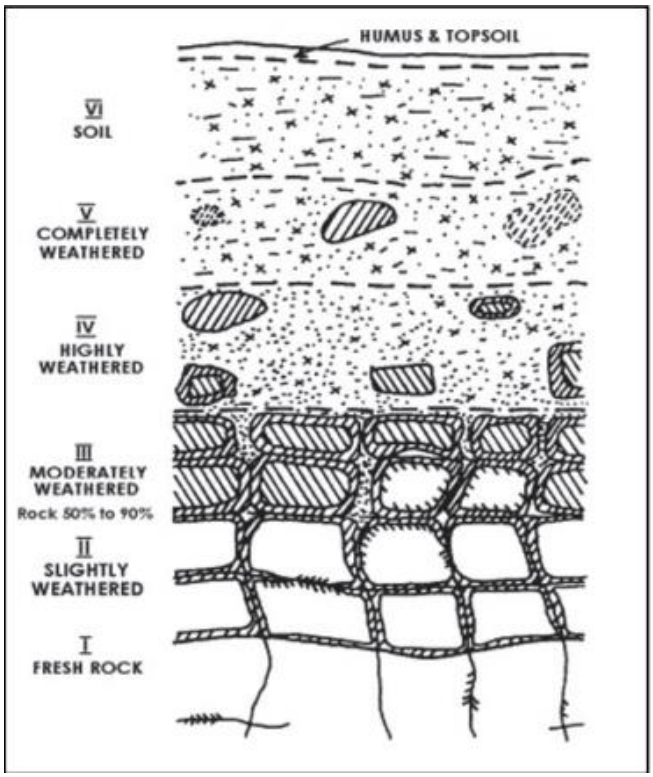

Figure 2.1: Profile of Residual Soil Based on Degree of Weathering (Huat, et al., 2010).

The different of soil types are formed according to its parental rocks. For instance, coarser residual soils like sand are weathered from granite rocks while soils from silt type to gravel resulted from igneous and metamorphic type of rocks. However, sedimentary rocks like shale formed a fine-grained residual soil like clay. In fact, the characteristics of the residual soils may differ from their parental rocks after a long processing time. In other words, the characteristics of residual soils from its original parental rocks may be eliminated over the time.

When ground water table dropped to a certain low elevation, further evapo-transpiration effects will result in the residual soils to be extremely unsaturated. These unsaturated soils have a characteristic of instability in volume under the changes of water content. These soils are generally to be swell when water absorption, shrinkage when reduction in water content, and collapse upon saturation.

These soils are generally categorised into part of the residual soils which may lead to soil avalanching upon high degree of saturation in soil (Huat, et al., 2008). These residual soils usually have the characteristic of low undrained shear strength with high void ratio.

Due to the instable potential risk upon high degree of saturation in tropical residual soil, slope soaked in water after raining has the high possibility lead to landslides (Huat, et al., 2008). Landslide is one of the most frequent natural disaster in Malaysia due to the intense climate and geological condition. Landslides are usually susceptible to periods of heavy rainfall. High moisture content in the soil after raining lead to a significant degradation of suction power in soil especially at shallow area. Loss of suction power results in a significant decrease in shear strength especially for the soils which are merely bonded by the internal suction power from soil. Several function such as rain intensity, rainfall duration, and infiltration rate into the soil have positive correlations with pore water pressure as well (Huat, et al., 2008).

Conditions that unsaturated soil is likely to be collapsed are (Huat, et al., 2008): (i) the soil has an exposed area, unstable soil composition, and unsaturated soil matrix with high void ratio, (ii) in a metastable condition whereby a high external stress is acted on top of the soil, (iii) reduction in suction power upon wetting, and (iv) low bonding strength between particle-particle.

2.3 Emergence of Bio-mediated Soil Improvement Systems

Majority of land areas in Malaysia are covered by the residual soils (Huat, et al., 2007, Taha, et al., 2000). By developing of country, buildings and structures are certainly constructed on these widespread soils. However, Malaysia is experiencing abundance of rainfall in every single month throughout the year. Due to the tropical weather, residual soils become wet and soft under the intense and continuous rainfall. Eventually, construction upon soft ground is highly susceptible to problems such as instability, settlement and failure issues. Therefore, soil improvement is required to enhance the engineering properties of soil for construction with safe and economical design. The main purposes to improve the engineering properties of soil include enhancing the stiffness and shear strength of soil, reducing the long term settlement, reducing the time taken for settlement, reducing the potential risk of liquefaction, removing the existing water from soil and so on (Kazemian and Huat, 2009).

There are plenty of soil improvement methods are used in the construction recently. A popular practice in the construction site to bind the pore spaces is chemical grouting by injecting the artificial materials, such as micro-fine cement, silicates, and other chemicals into the ground (DeJong, et al., 2010; Montoya, 2012). However, all these chemicals may contaminate the environments with their poisonous nature of state except sodium silicate (DeJong, et al., 2006; Karol, 2003). Moreover, low "certainty of execution" is one of the issues that current grouting techniques suffering from.

Nowadays, environmental issues are under an extremely high attention from the public. Other than sodium silicate, all the chemical grouts consist of toxic and hazardous substances may create environmental issues if certain limit has been reached over the use (DeJong, et al., 2010). With the increasing of awareness to environment, government promotes the Sustainable Development Goals (SDG) which shifted the traditional soil improvement methods toward environment friendly and sustainable technologies. Hence, a relatively new soil improvement treatment has emerged recently which is known as Microbial Induced Calcite Precipitation (MICP). MICP refers to few chains of chemical reactions with controlled by biological process from calcite forming microorganisms which pertained naturally. MICP treatment for soil improvement has been introduced to the research field at the joint of biochemistry and civil engineering (DeJong, et al., 2010). Due to the natural existence characteristic of the calcite producing microorganisms, they are highly being introduced in the laboratory by mixing high concentration of calcite producing microorganisms together with preference amount of cementation reagent into the soil. As the result shows byproducts are capable to enhance the geotechnical properties of the soil without eliminating any harmful substances to the environment.

2.4 Overview of MICP soil improvement method

Bio-mediated soil improvement method has been focused by researchers recently due to its environment friendly and sustainable characteristics. However, microbes have already been used in research to improve the soil properties with the nature of biological process by ancient (DeJong, et al., 2010). MICP soil improvement method has been utilized in research and claimed that this method is "a chemical reaction network that is managed and controlled within soil through biological activity and whose byproducts alter the engineering properties of soil" (DeJong, et al., 2010;

Montoya, 2012). Despite the chemical network reaction in MICP treatment has been confirmed that it could be successfully occurred in solution without any occurrence of biological process. These byproducts formed in the chemical network reaction has been identified to have the potential to enhance the properties of soil as well (DeJong, et al., 2010; Ivanov and Chu, 2008; Ng, 2013). But, the formation of the calcite precipitate is carried out before reaching to the specific pore spaces. Eventually, the chemical reaction chain although has been occurred, but the improvement of soil properties has not been achieved.

An overview of MICP soil improvement method is presented schematically in Figure 2.2. The biological mediation plays an important role in the MICP treatment, whereby the capability of controlling and managing the retention time, and spatial distribution of the microbes and chemicals. Under the chemical reaction, the formation of byproducts can be categorized into inorganic precipitation, organic precipitation, and gas generation which considered as biomineralization, biofilm formation, and biogas generation, respectively. Preference conditions such as pH, negative or positive ion charges, and resistivity are the main factor which lead to the successive reactions.

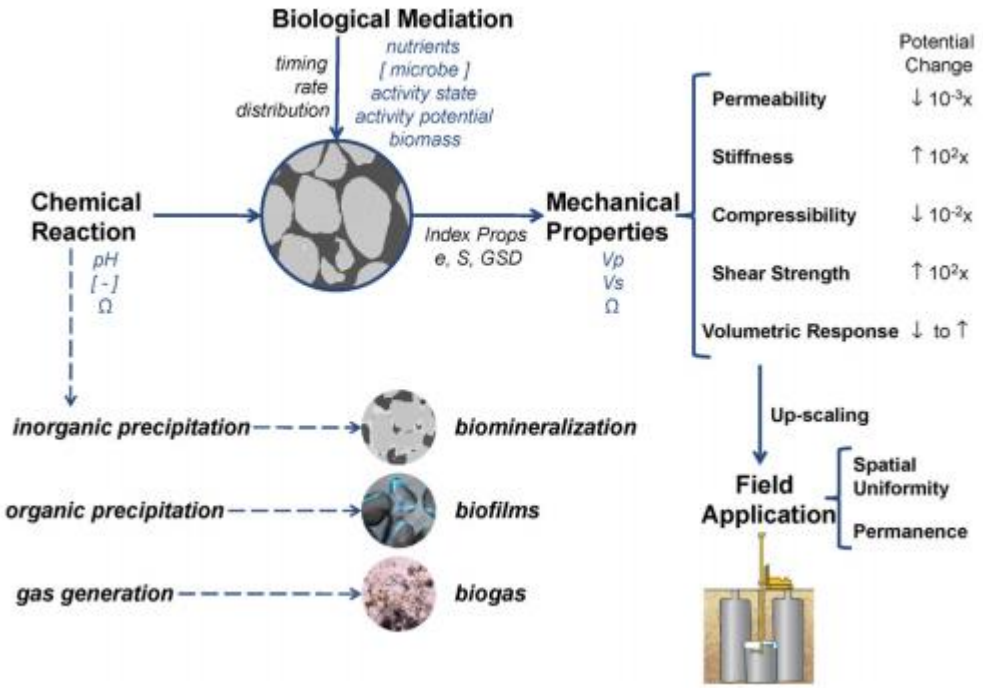

Figure 2.2: Overview of MICP Soil Improvement Method (DeJong, et al., 2010).

Geometric correspondence of the microbes and soil particles is essential to the effectiveness of MICP treatment. It is considerably important since the microbial bacteria must move freely in the soil particles to induce calcite precipitate. Majority of microbes are small, usually with the size of 0.5 to 3 mm. As preferred to be used in MICP treatment, microbes are capable to pass through the pore spaces between the soil particle-particle (Ng, 2013). Comparisons between sizes of microbes and soil particles, and the limitation range on application of MICP treatment, are as shown in Figure 2.3. Generally, sand has the optimum pore size in term of its suitability for all the treatment; whereas treatment of biomineralization may be conducted in the silt; biofilm can be applied in gravel. However, DeJong, et al. (2010) stated that all the treatments are limited to clay minerals due to its relatively small particle size which may block for the delivery of bacterial cells. Therefore, bio-mediated of clay may be carried out by other methods without any passage of microbial bacteria.

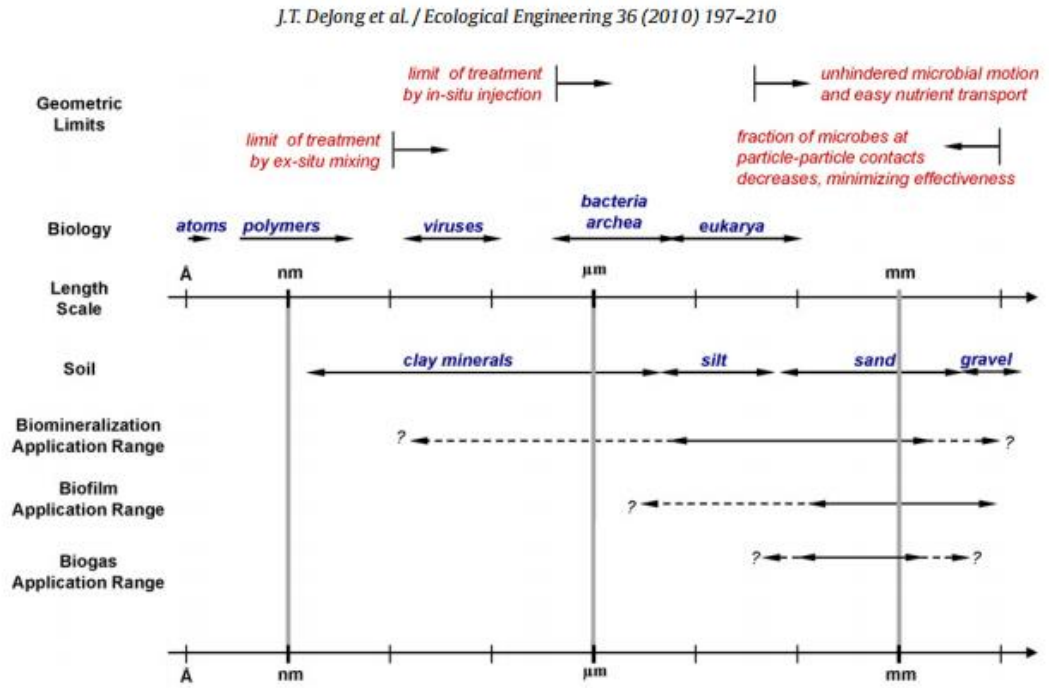

Figure 2.3: Comparison of Sizes of Microbes and Soil Particles, Limitation Range on Application of MICP Treatment (DeJong, et al., 2010).

2.5 Biological processes and chemical reactions of MICP

By promoting the MICP treatment to improve the soil properties is increasing promptly in recent years. Biomineralization processes identified in the literature include production of magnetite, greigite, amorphous silica, and calcite (Kohnhauser,

2007). In fact, calcite precipitate is the important products from MICP treatment that enhance the engineering properties of soil. There are several methods can be used to induce calcite precipitation for MICP treatment. Castanier, et al. (1999) found that different species of microorganism have the capability to produce calcite precipitate in specific conditions. The primary processes of formation of calcite precipitate include photosynthesis, urea hydrolysis, denitrification, iron reduction, sulphate reduction, and other pathways (De Jong, et al., 2010; Ivanov and Chu, 2008). However, the most efficient and applicable method in present day is by using urea hydrolysis method to produce calcite precipitation.

A summary of chemical network reaction involved to produce calcite precipitate is tabulated in Table 2.1. The most common biological process is through photosynthesis to produce calcite precipitate by cyanobacteria and algae. With increasing of pH value, algae utilized dissolved carbon dioxide (CO_2) in water and thus shifting the equilibrium of bicarbonate ions (HCO_3^-) and carbonate ions (CO_3^{2-}) (McConnaughey and Whelan, 1997; Ehrlich, 1998). Calcite precipitate is then formed through the reaction between bicarbonate ions (HCO_3^-) and calcium ions (Ca^{2+}). When the presence of dissolved gypsum ($CaSO_4 \cdot 2H_2O$) in the environment, sulfate reduction bacteria enables to further reduce CH_2O to HCO_{3-} and induce calcite precipitate ($CaCO_3$) with the combination of Ca^{2+}. Calcite precipitate can also be produced by microbial groups of nitrates reducing bacteria through denitrification under the reaction chains which shown in Table 2.1. Under the anaerobic oxidation, calcite precipitate is formed after the methane oxidation is occurred with the aid of methanogens. Ureolysis is the most applicable reaction that occurred in these microbial groups, which results in the production of carbonate ions (HCO_3^-) and ammonium (NH_4^+). Calcite precipitation ($CaCO_3$) is then produced in the presence of calcium ions (Ca^{2+}) (Zhu and Dittrich, 2016). DeJong, et al. (2010) claimed that ureolysis is more efficient based on the existence of urea in the system since urea hydrolysis has relatively low free energy in computed changing at standard conditions as compared to other processes. Thus, ureolysis is more focused in the bio-mediated soil improvement systems.

Table 2.1: Chemical network reaction involved in different metabolism pathways to produce calcite precipitate (Zhu and Dittrich, 2016).

Microbial groups	Metabolism	Reference	Reactions	By-product
Cyanobacteria Algae	Photosynthesis	Baumgartner, et al. (2006)	$2HCO_3^- + Ca^{2+} \rightarrow CH_2O + CaCO_3 + O_2$	O_2 CH_2O
Ureolytic bacteria	Ureolysis	Achal and Mukherjee (2015)	$CO(NH_2)_2 + 2H_2O + Ca^{2+} + Cell \rightarrow 2NH_4^+ + Cell\text{-}CaCO_3$	NH_4^+
Nitrate-reducing bacteria	Denitrification	Erşan, et al. (2015b)	$CH_2COO^- + 2.6H^+ + 1.6NO_3^- \rightarrow 2CO_2 + 0.8N_2 + 2.8H_2O$ $Ca^{2+} + CO_2(aq) + 2OH^- \rightarrow CaCO_3(s) + H_2O$	Complete reaction: $CO_2 + N_2$ Incomplete reaction: $NO + N_2O$
Myxobacteria	Ammonification	González-Muñoz, et al. (2010)	–	NH_3
Sulfate reduction bacteria	Sulfate reduction	Baumgartner, et al. (2006)	$SO_4^{2-} + 2[CH_2O] + OH^- + Ca^{2+} \rightarrow CaCO_3 + CO_2 + 2H_2O + HS^-$	CO_2 HS^-
Methanogens	Methane oxidation	Reeburgh (2007)	Anaerobic oxidation: $CH_4 + SO_4^{2-} + Ca^{2+} \rightarrow CaCO_3 + H_2S + H_2O$ Aerobic oxidation: $CH_4 + 2O_2 \rightarrow CO_2 + 2H_2O$	H_2S

Sporosarcina pasteurii, an urease-forming bacteria with high pH resistance, is introduced in current bio-mediated soil improvement method (Whiffin, 2004). Hydrolysis of urea is technically termed as ureolysis, it is introduced by bacteria through its urease to decompose urea. Urease is a highly active enzyme that can be used to catalyse the hydrolysis of urea into carbon dioxide (CO_2) and ammonia (NH_3). After these chemicals diffuse into the surrounding solution, ammonia is further reacted with water (H_2O) and converted into ammonium (NH_4^+) while carbon dioxide (CO_2) is reacted with hydroxide ion (OH^-) to form bicarbonate ions (HCO_3^-). The net pH is increased due to hydroxyl ions (OH^-) generated from the production of ammonium (NH_4^+) which exceeds the free calcium ions (Ca^{2+}) for calcite precipitation (Figure 2.4). This creates an alkaline environment and supersaturated conditions for carbonate required to react with the presence of calcium ions (Ca^{2+}). The formation of calcite precipitate ($CaCO_3$) is completed at the nucleation site

whereby positive charged calcium cation (Ca^{2+}) are attached on cell surfaces with negative charged.

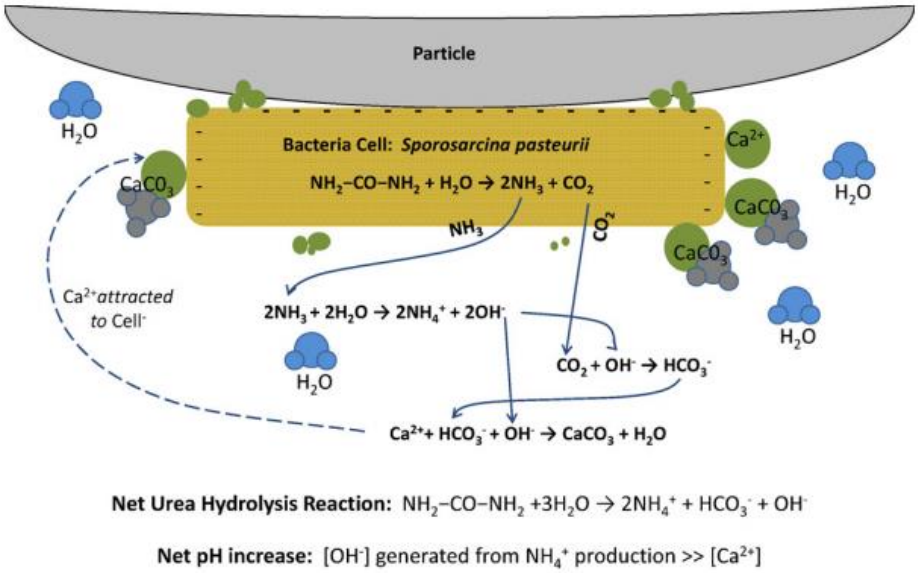

Figure 2.4: Overview of Ureolysis in MICP Treatment (DeJong, et al. 2010).

2.6 Application of MICP treatment

2.6.1 Biocementation

Biocementation has been focused on studies regarding its advantages and applications in recent years. According to Ivanov and Chu (2008), biocemenation has the potential to prevent landslides, lower down the risk of swelling in clayey soil, minimize the risk of liquefaction on sand, and stiffen soil on reclaimed land. Biocementation improves shear strength and stiffness properties of the soil through the introduction of microbial bacteria, nutrients and cementation reagent into the soil matrix by binding the soil particles together. Biocementation can form strong and stable binding materials for soil particles which couldn't be degraded by other microorganisms. These materials are majority from carbonates, silicates, phosphates, sulphides and iron hydroxides (Ivanov and Chu 2008). However, calcium carbonate ($CaCO_3$) is the most attractive element to be referred in biocementation due to the calcite formation is readily found with the aid of ureolytic bacteria. Furthermore, ureolytic bacteria are widespread in the subsurface which they have been active. In the top part of subsurface, there are more than 10^9 cells per gram of soil are existing naturally (DeJong, et al., 2010). The number of ureolytic bacteria can be multiplied by bio-stimulation through nutrient injection or augmented (bio-augmentation)

through additional microbes injection until their growths reach a desired level to propose and maintain a chemical reaction.

De Jong, et al. (2010) claimed that the spatial distribution of calcite precipitate is an essential to the effectiveness of MICP treatment. The calcite precipitations lead to a reduction in void space (porosity) and change in phase relationship properties as a result. Based on Figure 2.5, a schematically diagram shows the two extreme possibilities of distribution alternatives of calcite precipitate (DeJong, et al., 2010). “Uniform” distribution refers to the calcite precipitate is distributed evenly with an equal thickness on the surface of soil particles. In the case of “uniform”, the bonding formed between two particles by calcite is considerably small, thus the improvement to soil properties may be neglected as a result. “Preferential” distribution shows a condition in which the calcite precipitate merely formed between the soil particle-particle. This is the optimum spatial distribution as all calcite precipitates focus on the specific particle-particle contacts to contribute directly to the enhancement in soil properties. Unfortunately, these processes do not easily fulfill for the engineering properties of soil. As a reason of the “preferential” distribution is impractical in the reality. Same as the “uniform” distribution is infeasible due to extremely inefficient from engineering perspective. Analysis of scanning electron microscopy (SEM) is shown in Figure 2.6, clearly revealed that the calcite precipitate coats the open surface of soil particles but higher amount of calcite form at the soil particle-particle. This condition is the balance of these two extreme possibilities of distribution which known as “actual” distribution of precipitated calcite (Figure 2.5). Fortunately, there is a considerable amount of the calcite precipitate which is located at the desired soil particle-particle. Based on the X-ray computed tomography images in Figure 2.6, Silica (Si) represents the soil particle while Calcium (Ca) represents the calcite. The formation of calcite in the pore space of soil can be clearly seen in Figure 2.6. The spatial distribution of calcite is controlled by biological activities as well as managed by filtering processes (DeJong, et al., 2010).

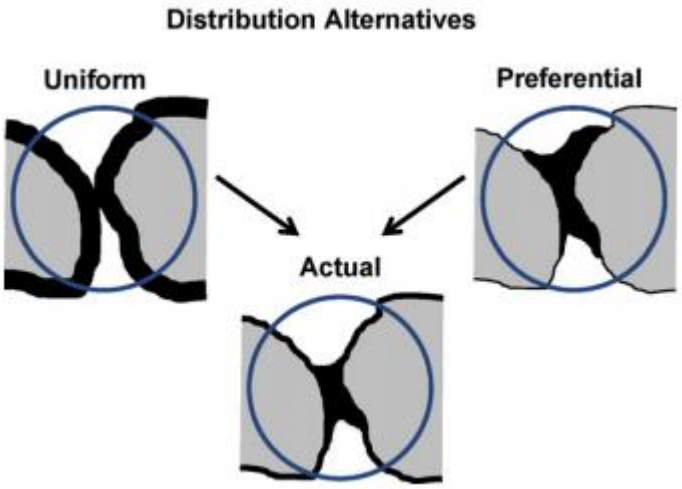

Figure 2.5: Schematic Diagram of Distribution Alternatives of Calcite Precipitate Within Pore Space (DeJong, et al., 2010).

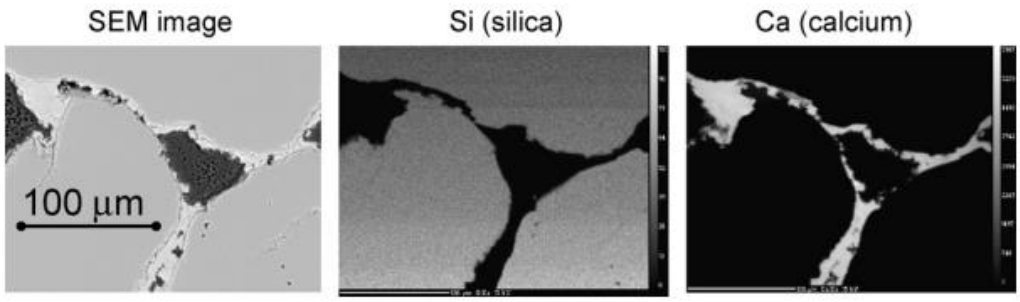

Figure 2.6: Scanning Electron Microscopy (SEM) and X-ray Computed Tomography Images of Calcite Precipitate and Soil Particle (DeJong, et al.,2010).

Other than utilized ureolytic bacteria to induce calcite precipitate in bio-cementation, there are some other potential microbial induced bacteria that may render to bio-cementation as well. These microorganisms are shown in Table 2.2 includes sulphate-reducing bacteria and iron-reducing bacteria. Sulphate-reducing bacteria produces metal sulphides to form a grout barrier to mitigate the pollution of heavy metal. Besides, iron-reducing bacteria can be used to prevent piping of dams by introducing the production of undissolved ferrous and ferric salts and hydroxides in soil.

Table 2.2: Different microbes that can lead to bio-clogging (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	Form grout curtains to reduce the migration of heavy metals and organic pollutants
Ammonifying Bacteria	Formation of undissolved carbonates of metals in soil due to increase of pH and release of CO2	Presence of urea and dissolved metal salt	Prevent piping of earth dams and dikes
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	Prevent piping of earth dams and dikes

2.6.2 Bio-clogging

Bio-clogging is another recent development area in microbial geo-technology. It is mainly used to control the drain channel erosion, prevent piping of earth dams, and form grout curtains to reduce migration of heavy metal, enclose bioremediation zone and etc. With the formation of undissolved precipitates, these precipitates block the water flowing through the pore spaces, eventually lead to reduction in hydraulic conductivity. Besides, calcite precipitates clogged in the void result in reduction in void ratio of soil and a low compressibility as a result. Bio-clogging form a barrier to reduce infiltration rate from the retention ponds to the ground as well as to prevent toxic leakage from landfills, protect the site from soil pollution in consequence (James, et al., 2000; Seki, et al., 2005). Wu, et al. (1997) stated that bio-clogging established a positive relationship between mass of microbes attached at the pore throat and the hydraulic conductivity of soil. In other words, reduction in soil hydraulic conductivity can be achieved by promoting the growth of microbes in soil with injection of nutrition. The overall microbes accumulated at soil pore throat or uniformly on soil particle surface to hinder the passage of water.

Different microbial processes that can lead to bio-clogging are summarized in Table 2.3. These processes can be induced by several microorganisms include algae and cyanobacteria, aerobic and facultative anaerobic slime-producing bacteria, oligotrophic microaerophilic bacteria, nitrifying bacteria, sulphate-reducing bacteria, ammonifying bacteria, and iron-reducing bacteria. A formation of impermeable layer of biomass by algal and cyanobacteria under the presence of light and nutrients may be used to reduce of water infiltration into slopes and control seepage. Production of slime in soil is mainly used to control the soil erosion through aerobic and facultative anaerobic heterotrophic bacteria, oligotrophic microaerophilic bacteria and nitrifying bacteria under their respective conditions. Sulphate-reducing bacteria, ammonifying bacteria and iron-reducing bacteria are not only can be used for bio-cementation, but also bio-clogging. They have the same characteristics of byproducts which is not dissolvable. These characteristics can be focused on the protection of soil from the infiltration of water to the subsurface. However, there are only some of these processes have been tested in laboratory and field (Ivanov and Chu, 2008).

Table 2.3: Different microbes that can lead to bioclogging (Ivanov and Chu, 2008).

Physiological group of microorganisms	Mechanism of bioclogging	Essential conditions for bioclogging	Potential 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 slime-producing bacteria	Formation of slime in soil	Presence of oxygen and medium with ratio of C:N>20	Avoid cover for soil erosion control and slope protection
Oligotrophic microaerophilic bacteria	Formation of slime in soil	Low concentration oxygen and medium with low concentration of carbon source	Reduce drain cannel erosion and control seepage
Nitrifying bacteria	Formation of slime in soil	Presence of ammonium and oxygen in soil	Reduce drain channel erosion
Sulphate-reducing bacteria	Formation 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 due to increase of pH and release of CO2	Presence of urea and dissolved metal salt	Prevent piping of earth dams and dikes
Iron-reducing bacteria	Formation 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	Prevent piping of earth dams and dikes

2.7 Affecting factors on MICP treatment

The formation of calcite precipitates is influenced by several causative factors include concentration of cementation reagent, concentration of bacteria, pH level, and other relative factors.

2.7.1 Concentration of cementation reagent

DeJong, et al. (2010) stated the chemical equation of one molecular of urea reacts with one molecular of calcium chloride equally to one molecular of calcite precipitate. Nemati, et al. (2005) also claimed that same molecular of urea and calcium chloride in a solution could convert into a better outcome of calcite carbonate. With a relatively high concentration of cementation reagent (> 1.0 M), the efficiency of reaction between urea and calcium chloride has significantly dropped. However, the concentration of cementation reagent within a range of 0.5 M to 1.0 M could produce a considerably high amount of calcite precipitate from the reaction. De Muynek, et al. (2010) concluded that the increment of concentration of cementation reagent from 0.25 M to 0.5 M in the solution may lead to 70% of increment of weight in the soil specimen, while increasing from 0.5M to 1.0 M may result in another 18% of weight increment.

2.7.2 Concentration of bacteria

The concentration of bacteria has a positive correlation with the level of ureolytic activities. Production of urease from ureolytic bacteria is one of the important process in the ureolytic activities, and an essential key for the calcite formation. Okwadha and Li (2010) mentioned that a substantially increment of calcite precipitate is found by introducing adequate amount of concentration of bacteria in the soil. The formation of calcite precipitate is found to be complete at the nucleation site on the cell surface (Stocks-Fischer, et al., 1999). Thus, the high concentration of bacteria provides a sufficient nucleation space for the positive calcium ion to attach for the completion of calcite precipitate.

2.7.3 pH value

Urease is an alkaline type of enzyme that produced by ureolytic bacteria. Urease produced by *Sporosarcina pasteurii* usually can be functioned well in alkali within the pH range of 8-9 (Stocks-Fischer, et al., 1999). However, Mobley, et al. (1995)

stated that the functionality of urease will be degenerated in acidic environment. The production of hydroxyl ions from the second ureolysis by the urease generally cause the increment of pH value in the cell surrounding. The increment of pH value would not affect the efficiency of urease, whereas promote the reaction of calcite precipitate.

2.8 Summary

In this chapter, previous studies on basic characteristics of tropical residual soils and problems encountered in Malaysia were presented. In recent years, many studies were focused on MICP soil improvement on fine sands, but only few studies on other soil types such as tropical residual soil. Besides, many types of microorganisms were studied which have the ability of inducing calcite precipitate. Potential application of MICP treatment includes biocementation and bioclogging were discussed by previous researchers. Last but not least, several causative factors on MICP treatment such as concentration of cementation reagent, concentration of bacteria and pH value were evaluated.

CHAPTER 3

METHODOLOGY AND WORK PLAN

3.1 Introduction

This chapter reviews the methodology of the study by presenting a research flow chart, description of material preparation and main tests. The work plan of the study is shown in this chapter as a Gantt Chart as well.

3.2 Research Flow Chart

This research study generally can be categorised into six major stages which are in the sequence of background study, soil retrieving and classification, material preparation, preliminary experimental test, main experimental test, and result analysis. An overview of the research flow chart is shown in Figure 3.1.

Stage 1: Literature review

- Review relevant reported studies.
- Synthesized ideas from literature.
- Identify research gaps.

Stage 2: Problem formulation and hypothesis

- Formulate problem statement.
- Introduce MICP treatment on residual soil

Stage 3: Preparation of material and apparatus

- Experimental setup.
- Cultivation and incubation of *Sporosarcina pasteurii.*
- Preparation of soil materials

Stage 4: Main experimental test

- Investigate the applicability of MICP treatment on residual soil.
- Study the effects of treatment variables include concentration of cementation reagent, and the maximum dry density of residual soil.

Stage 5: Result & Data analysis

- Analyze the data from experiment.
- Discuss the experimental results.

Stage 6: Conclusion & Report writing

- Draw conclusions from the results.
- Write a final report.

Figure 3.1: Research Flow Chart.

Abundance of relevant literatures published by previous researchers were found and studied. The research ideas regarding MICP soil improvement were then systhesized from researchers. Research gaps were identified by comparing the previous research work done by several researchers.

Problem statement was found based on the research gaps. MICP treatment on residual soil was introduced in this research. The appropriate methods and procedures for MICP treatment on residual soil were identified from the published works. Besides, the affecting factors and preference conditions of MICP treatment were determined. The experimental variables were identified to determine the effectiveness of MICP treatment on soil engineering properties.

In the third stage of research involved the preparation of soil materials such as tropical residual soil. Relevant reported journals were studied as the reference of the experimental setup. The apparatus was prepared and setup by following the reference of study before the commencement of experiment. Stock cultures of *S.pasteurii* were prepared and readied for the use of treatment.

In stage 4, the applicability of MICP treatment on residual soil was studied. Preference conditions for MICP treatment on residual soil were investigated as well. Two treatment variables were focused in this experiment which are the concentration of cementation reagent and maximum dry density of residual soil. The remaining affecting factors such as concentration of *S.pasteurii*, concentration of nutrient, retention time and temperature were remained constant.

The results obtained from the experiment were analyzed under Stage 6. The optimum conditions controlling the effectiveness of MICP treatment were investigated by comparing the engineering properties of untreated and treated soil. Some significant data includes pH value, compressive strength and amount of calcite produced were collected for evaluation in this stage.

Last but not the least, the conclusion of the results from MICP treatment was made in this stage. All the relevant information and results were written into a final report.

3.3 Materials

3.3.1 Soil specimen

In this research, the soil specimens used in the experimental tests were made by tropical residual soil. The tropical residual soil was extracted at a collecting site

which is nearby Universiti Tunku Abdul Rahman, Sungai Long campus. The retrieved residual soils were classified based on Unified Soil Classification System (USCS). Total six residual soil specimens were made for this research. All the soil specimen has the dimension of 55 mm diameter and 50 mm in height

Each of the soil specimens from were compacted into 90 % of maximum dry density (MDD). The targeted density was achieved by introducing a standard compaction method as shown in Figure 3.2.

Figure 3.2: Standard Compaction Method

3.3.2 *Sporosarcina pasteurii*

The urease-producing microorganism used in the present study was *Sporosarcina pasteurii* (ATCC 11859). *S. pasteurii* is a gram-positive bacterium that widespread in a broad range of soil which able to survive in highly alkaline environments (pH~10). *S. pasteurii* are introduced in the research field in recent years based on its features whereby producing calcite precipitate through MICP under certain circumstances in natural soils. Tobler, et al. (2014) found that *S. pasteurii* has an ability to induce calcite precipitate although low urease activity is found in the indigenous communities. *S. pasteurii* has a longer lifespan in the soil for more than 30 days due to its spore formation. (Sabermahani, et al.,2019). These beneficial characteristics support *S. pasteurii* to be implemented in soil improvement technique in tropical regions.

3.4 Cultivation of bacteria

3.4.1 Stock Culture Preparation

Sporosarcina pasteurii was cultivated in laboratory prior to residual soil treatment. All the relevant equipment and ingredients such as Petri dishes, conicalflasks, Scott bottle, nutrient broth, agar powder, etc were prepared. Then, all containers were sterilized by Autoclave machine at 121 °C before using.

Agar plate medium was prepared in the first step. 14 g nutrient broth and 5 g agar powder were added into the Scott bottle filled with 500 mL of distilled water as shown in Figure 3.3 and 3.4. Then, the mixture was shaken well in the Scott bottle and put into an autoclave machine at 121 °C for sterilization as shown in Figure 3.5 and 3.6. After the process of sterilization, the Scott bottle were put aside for cooling down until it reached the room temperatures. Subsequently, the mixture was poured into 10 - 12 Petri dishes as shown in Figure 3.7.

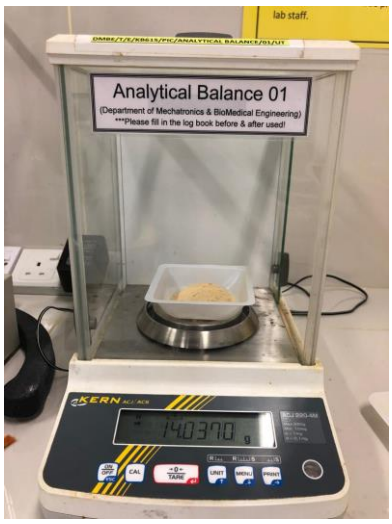

Figure 3.3: 14g of Nutrient Broth.

Figure 3.4: 5g of Agar Powder.

Figure 3.5: Mixture was shaken well.

Figure 3.6: Scott Bottle was put into the Autoclave Machine.

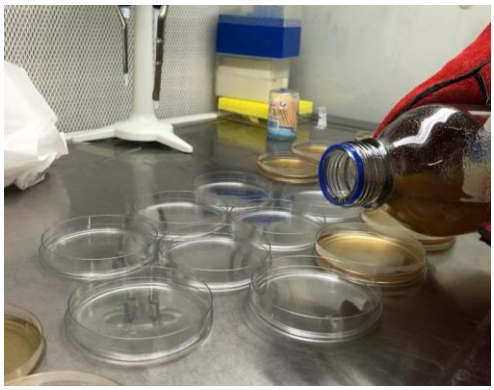

Figure 3.7: Agar Mixture was Poured into Pettri Dishes.

Next, *S. pasteurii* was extracted from the stock culture which stored in freezer. *S. pasteurii* was spread on an agar plate using a sterile wire loop. The spreading method is well known as streak plate method was utilized in this cultivation. Streak plate method can be used to dilute the concentration of *S. pasteurii* gradually with the increase of streak number on the agar plate. Eventually, *S. pasteurii* was isolated into several discrete colonies on the agar plate. *S. pasteurii* in Petri dishes were incubated in a normal incubator under constant temperature of 37 °C for 24 hr. *S. pasteurii* was a newly inoculated bacteria and experienced the lag phase at this current stage. After 24 hours, the colonies of *S. pasteurii* were visible by naked eye, as illustrated in Figure 3.8.

Figure 3.8: Colony Forming Units (cfu) Grew on Agar Plate.

3.4.2 Incubation of Bacteria

Once the *S. pasteurii* has grown into a visible stage, the colonies or colony forming unit (cfu) on agar plate were readied to be transferred to the sterile nutrient broth in a

laminar air flow cabinet. First of all, the sterile nutrient broth were prepared in several conical flasks, then proceeded to the process of sterilization as well in an autoclave machine at 121 °C. Conical flasks were put aside to cool down until it reached the room temperatures before used. All the equipment was sprayed with ethanol for sterilization purpose as well as laminar air flow cabinet was cleaned by UV-ray and air flow as shown in Figure 3.9. After that, the transferred of the colonies or colony forming unit (cfu) on agar plate to the conical flasks were completed within the laminar air flow cabinet. Total ten loops of colony forming unit (cfu) were extracted from the agar plate and transferred into conical flasks as shown in Figure 3.10. During the transferring of colonies, after each loop of colonies was sent into the conical flasks, the sterile wire loop was sterilized by heating under a Bunsen burner.

The *S. pasteurii* was then incubated in a shaking incubator with a constant temperature of 34 °C and rotation speed of 150 rpm for the next 24 hr. Until the early stationary growth phase, the *S. pasteurii* was then readied to be used in the MICP treatment in residual soil.

Figure 3.9: Sterilized the Apparatus under UV-light.

Figure 3.10: Colony Forming Unit (cfu) was collected from Agar Plate by Using Sterile Wire Loop.

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

3.4.3 Bacteria Concentration Checking

Optical Density (OD) test was carried out to determine the concentration of bacteria in the nutrient solution by using spectrophotometer in Figure 3.11. A little portion of nutrient solution was extracted from both conical flask and filled into the plastic cuvette as shown in Figure 3.12. The surface with stripe on the plastic cuvette was wiped before placing into the spectrophotometer. The normal nutrient solution was put into spectrophotometer first as a benchmark then followed by the nutrient solution with bacteria. The spectrophotometer was set with 600 μm of UV-light for the checking. Lastly, the simple reads report for this test was shown in Figure 3.13.

Figure 3.11: Spectrophotometer for OD Test.

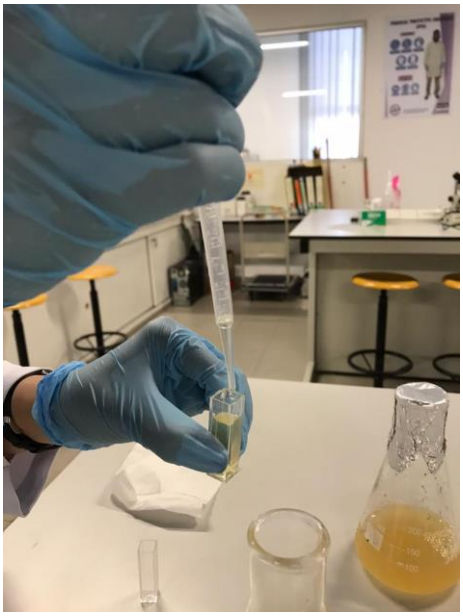

Figure 3.12: Transferring Both Bacteria Solution and Normal Nutrient Solution from Conical Flask into Plastic Cuvette.

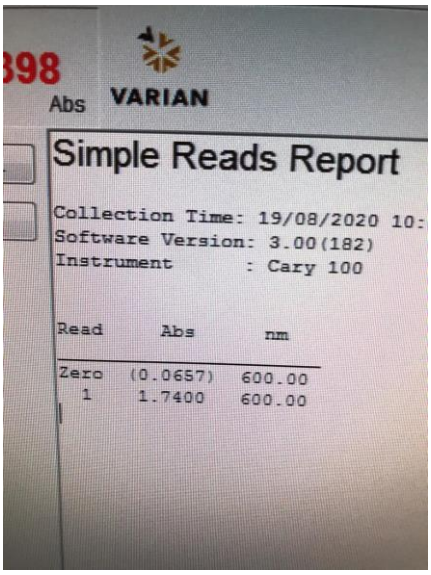

Figure 3.13: Simple Reads Report of OD Test.

3.4.4 Cementation Reagent

The cementation reagent for in this research consisted of urea (CH_4N_2O) and calcium chloride ($CaCl_2$). The urea and calcium chloride were the essential ingredients for MICP treatment to initiate the calcite precipitation. Three different concentration of cementation reagent include 0.25 M, 0.5 M and 1.0 M were added into the nutrient broth with *S. pasteurii* from part 3.3.2 to form a final solution, respectively. White colour precipitates were found in the fluid as a proven of the formation of calcite precipitate. These precipitates were shown as an evidence to prove the successful cultivation of *S. pasteurii* from the previous step in part 3.3.2. The final solution was then added 3 g of nutrient broth as the nutrient supplied for *S. pasteurii* during the next MICP treatment.

3.5 Laboratory Setup

Prior to the commencement of MICP treatment, all the apparatus such as plastic mould, tube, tank, and effluent collector were prepared. Firstly, the soil specimen was compacted into the mould to the desired percentage of maximum dry density (MDD) with optimum moisture content. The soil specimen was then sandwiched by two layers of gravel (10 mm thick) at each end to prevent the occurrence of unstable inflow and protect the inlet of the mould from blockage. Next, the mould was supported vertically by the aid of retort stands. The final solution with *S. pasteurii*was stored into the tank whereas the inlet of the mould was connected to a

tank with the tube. The tank was located at the level above the mould to create a pressure head. The cementation reagent fluid was then forced into the soil specimen by the atmospheric pressure. The whole processes of treatment were conducted under the room temperatures. Effluents from the outlet of the specimen mould were collected at the effluent collector. The pH value of effluents was then monitored at an interval of 24 hours. After the completion of MICP treatment, the soil specimen was extruded from the mould as shown in Figure 3.14 for various testing such as compressive test and acid washing test.

Figure 3.14: Soil Specimen was extruded from mould after MICP Treatment.

3.6 pH measurement

During the experiments, effluent samples were collected periodically in every 12 hours from the effluent collector for the pH measurements. For pH measurement, effluents were collected at various times during the MICP treatment. The checking of pH values was done by using Sartorius PB-10 Standard pH meter as shown in Figure 3.15. The standard of pH meter was calibrated in accordance with the typical pH standard before the measurement was performed. Before and after every checking, the sensor on the pH meter was flushed with large amount of distilled water, rinse and dried with the filter paper to avoid any collection of error data.

Figure 3.15: pH Meter.

3.7 Compressive strength test

The compressive strength test was performed in this study to determine the compressive strength of the treated soil sample. The compression machine is shown in Figure 3.16.

Firstly, the soil sample was placed vertically and tightened between two Then, the soil sample was subjected to an axial compression force with a 1.25mm/min rate until the soil collapsed. The reading on the electric load cell was recorded after every 20mm took place on the dial gauge. The compressive strength of soil sample was obtained from the highest value of reading on the electric load before it collapsed. Figure 3.17 shows the tested soil sample after compressive strength test.

Figure 3.16: Compression Machine.

Figure 3.17: Test Soil Sample After Compressive Strength Test.

3.8 Acid washing test

The amount of the calcite precipitate ($CaCO_3$) content was measured by using gravimetric acid washing technique in the test as shown in Figure 3.18. 2.0M of concentrated hydrochloric acid (HCl) was placed in a 50 ml volumetric flask, then diluted with distilled water to form a diluted acid solution.

Before washing by acid, the air-dried soil specimen was put into an oven to achieve oven dry at temperature of 105 °C for 24 hr. Next, about 20 g of the soil samples were collected from the top and bottom part of the oven-dried soil specimen and labelled respectively. Then, each of the soil samples from top and bottom part were put into funnel together with filter paper on top of conical flask respectively. Consequently, each of the soil samples were washed by the diluted acid solution for several times until no additional effervescence from the soil samples. The effervescence was considered as the carbon dioxide from the reaction between diluted acid solution and calcite precipitate in soil samples. Then, the residues were collected with the filter paper together for the next 24 h of oven dry.

Measurement of the weight of oven-dried soil samples before and after acid washing test was performed. The weight loss between the soil samples was assumed as the mass of calcite which washed out by acid. The calcite content was expressed in percent in terms of the mass of calcite divided by the mass of the untreated soil sample. Noted that the test was repeated if the percentage difference between the two samples was more than 0.5 %.

Figure 3.18: Effervescence Deliberated During Acid Washing Test.

CHAPTER 4

RESULTS AND DISCUSSION

4.1 Introduction

This chapter shows and elaborates the results of this project. The effect of reagent concentration on calcite content and strength properties were demonstrated under this chapter. Besides, the relationship between strength properties and calcite content in bio-mediated tropical residual soil was correlated. The effect of bio-mediation on the dry density of tropical residual soil and the pH changes over treatment duration upon MICP treatment were investigated in this chapter as well. In addition, other possible affecting factors on the experimental results and clarifications were discussed in this chapter.

4.2 Effect of Cementation Reagent Concentration on Properties of Compressive Strength

The comparison between the stress-strain curves from different concentration of bio-mediated samples and untreated sample is shown in Figure 4.1. The overall result shows that the 0.5M cementation reagent had the best outcome in term of strength properties, followed by the 1.0M cementation reagent. The 0.25M of cementation reagent contributed the least amount of strength among the treated soil samples. Untreated soil sample was used as the benchmark for the strength properties of tropical residual soil in this study. Hence, the effect of *S.pasteurii* on the strength properties of soil was proven as well as the concentration of cementation reagent was concluded as a vital factor on the magnitude of strength for tropical residual soil in this study.

Untreated soil specimen without the mixture of the urease bacteria and cementation reagent was used as the benchmark for strength properties in this study. Based on Figure 4.1, 43.15kPa was found as the threshold of strength properties under the untreated soil specimen. The strength of the soil specimen was slightly increased under the treatment from 0.25M cementation reagent. The results from both samples were recorded as 55.58kPa and 27.93kPa. However, the second sample has decided to be eliminated from the strength result due to serious cracking was

found after demoulding. Thus, the overall strength of soil sample from 0.25M cementation reagent was concluded as 55.58kPa.

Based on the experimental results, the strength properties of soil specimen after MICP treatment through 0.5M of cementation reagent had increased significantly which recorded as 89.17kPa. Although another soil sample treated by 0.5M cementation reagent only achieved the strength of 44.76kPa, but the average outcome from both samples was resulted as the highest strength as well which is 66.97kPa. It has been showed that the improvement of strength properties of tropical residual soil while the concentration of cementation reagent increased. However, when the concentration of cementation reagent increased to 1.0M, the reduction of strength properties of tropical residual soil was observed. As shown in Figure 4.1, the compressive strength of the soil samples from 1.0M cementation reagent were recorded as 68.74kPa and 60.05kPa which resulted in an average strength of 64.40kPa.

Unconfined compression strength (UCS) is usually used as the standard to measure the strength properties of soil specimens for bio-mediation. Umar, et al., (2016) claimed that the unconfined compressive strength of the treated samples had relatively improved after the MICP treatment. A L/D ratio between 2 to 3 is generally recommended for the unconfined compression test. However, a L/D ratio around 1.0 was used to obtain a shorter soil sample in this study. In term of UCS, the results from this strength test (L/D=1) is assumed to be 0.7 of UCS (L/D=2). Güneyli and Rüşen (2015) found that the UCS for (L/D=1) was 0.70 of (L/D=2) in Almanpinan Clay, where UCS for (L/D=1) in Handere Clay was 0.72 of (L/D=2). This shows a reduction in UCS for clayed soil while increasing L/D ratio from 1.0 to 2.0. The correlation between UCS for 1.0 L/D ratio in rocks was proved to be 0.8-0.9 for 2.0 L/D ratio by Ergu¨n and Nilsun (2009) as well.

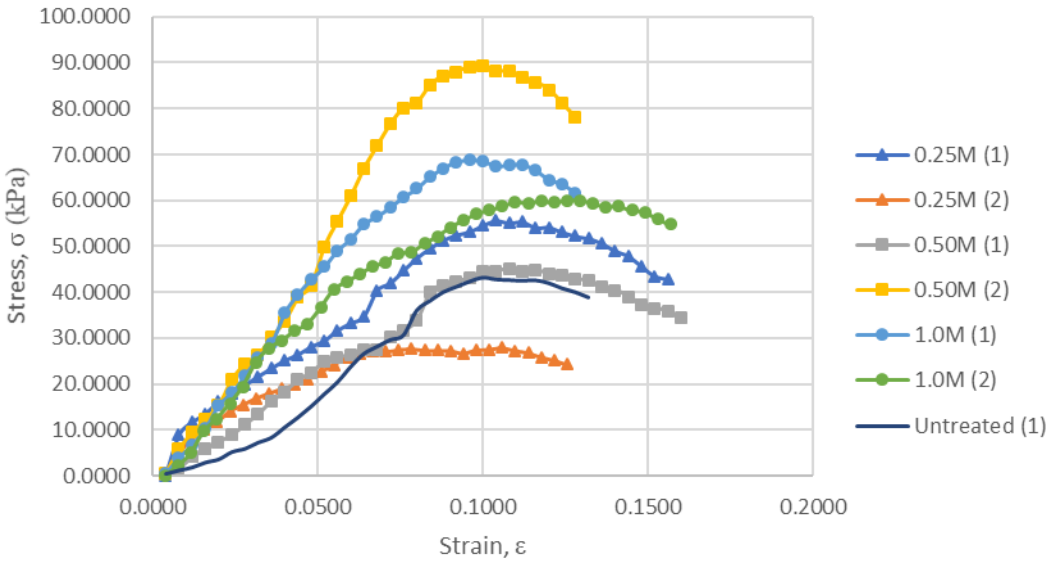

Figure 4.1: Stress-strain Curve of Treated and Untreated Soil Specimens.

Nayanthara, et al., (2019) stated that the 0.5M cementation reagent is the most suitable condition for MICP treatment compared to 1.0M due to higher favourable to strength improvement. This is because 0.5M cementation reagent is the optimal concentration for bio-mediation which could produce the highest amount of calcite content in the soil particles. As the calcite precipitate formed in the soil particles, it filled up the voids between the particle-particle in the soil. These calcite precipitates become a connection which could link all the soil particles together. Eventually, these connections resulted in the improvement of strength of soil after the MICP treatment. Sharma and Ramkrishnan (2016) stated that the formation of calcite precipitates in the soil sample after MICP treatment act as a bond between the soil particles, which enhance the strength properties of soil. According to the experimental result from Whiffin, et al. (2007), it showed the properties of strength, stiffness and load bearing capacity were considerably increased after the MICP treatment.

4.3 Effect of Cementation Reagent Concentration on Calcite Content

Figure 4.2 shows the comparison of the average calcite content between the bio-mediated samples from different concentration as well as untreated sample. Untreated soil sample was used as the benchmark for the calcite content in this study as well. The general results stated the calcite content in the treated soil samples had increased from the concentration of 0.25M, 1.0M and 0.5M accordingly. Hence, the

amount of calcite content in tropical residual soil has related to the concentration of cementation reagent.

There was only 0.05% of calcite content has been found in the untreated soil sample as shown in Figure 4.2. It can be said that the tropical residual soil contains a very little portion of calcite precipitate only in nature. However, calcite content in those soil samples which contained *S.pasteurii* with cementation reagent had dramatically increased after the MICP treatment. The calcite content found in the soil sample which treated by 0.25M cementation reagent is the lowest amongst all the treated samples. 2.28% of average calcite content was resulted from 2.35% and 2.21% of calcite content in the first and second sample after the treatment by 0.25M of cementation reagent. Under 0.5M of cementation reagent, the second sample has been found with the highest amount of calcite content, 5.31% in the soil. With the calcite content of 2.24% in the first soil sample, the average calcite content for soil under 0.5M of cementation reagent was measured as 3.78%. However, the calcite content is starting to drop when the soil samples were treated by 1.0M of cementation reagent. The calcite content in the first and second sample which treated by 1.0M of cementation reagent were recorded as 3.40% and 2.30%, respectively. This resulted the average calcite content of 2.85% in the soil under 1.0M of cementation reagent.

Ng, et al., (2014) stated that the calcite content in the sample of 0.5M cementation reagent is denser than 0.25M cementation reagent. It has been found that 0.5M is the optimal concentration for *S.pasteurii* in bio-mediation (Umar, et al., 2016). Under the optimum condition, *S.pasteurii* grows faster and produces urease efficiently for the ureolytic activity. Whiffin (2004) also concluded that the activity of *S.pasteurii* have been reduced due to the high concentration of calcium ion from the cementation reagent. Microbes such as *S.pasteurii* could not work efficiently under the environment of high concentration. According to Okwadha and Li, (2010) the concentration of cementation reagent above 0.5 M reduces the efficiency of MICP treatment. This is because the urease is essential for the activity of hydrolysis of urea. Apart from microbes, high concentration of cementation reagent also inhibits the activity of urease. The production of urease was affected under salt-stressed conditions, just like the what Kunst and Rapoport (1995) had concluded with enzyme. This is because enzyme such as urease is not active under the high concentration due to the inhibitory effect. The inactive of urease in the soil resulted in the reduction of

the calcite precipitates induced by the ureolytic activity. Muynek, et al. (2010) concluded that the increment of concentration of cementation reagent from 0.25 M to 0.5 M in the solution may lead to 70% of increment of weight in the soil specimen, while increasing from 0.5M to 1.0 M may result in another 18% of weight increment. Hence, 0.5M cementation reagent has the higher amount of calcite content compared to 0.25M and 1.0M.

The calcite content in the top and bottom part from each treated soil samples and untreated sample were shown in Table 4.1. The top and bottom part of the soil samples were tested separately by using gravimetric acid washing technique to study the uniformity of the calcite content over the soil sample as shown in Figure 4.3. It has been observed that top part of the soil sample contained more calcite precipitate compared to the bottom part. The variation of calcite content along the soil sample could be explained by the cementation reagent was available to flow in single direction which is from top to bottom of the soil. At the same time, the compacted soil sample with 90% of maximum dry density (MDD) might slowed down the flow rate of cementation reagent from top to the bottom part. Therefore, one possibility is the ureolytic activity has been commenced in the top part of soil before the cementation reagent flowing into the bottom part. The calcite precipitate may clog the soil particles in the top part after several times of treatment by cementation reagent. This blockage might cause the death of *S.pasteurii* at the bottom part of soil due to the absence of nutrient from cementation reagent. It could also be possible to consider the depletion of cementation reagent when it flows to bottom part since the cementation reagent has been consumed for bio-mediation at the top part of soil. Another possible reason might be the restraint of the activity of *S.pasteurii* while the availability of oxygen has become lower from top to the bottom part of soil. MICP treatment was considerably restrained under the anaerobic condition (Surabhi and Arnepalli, 2019).

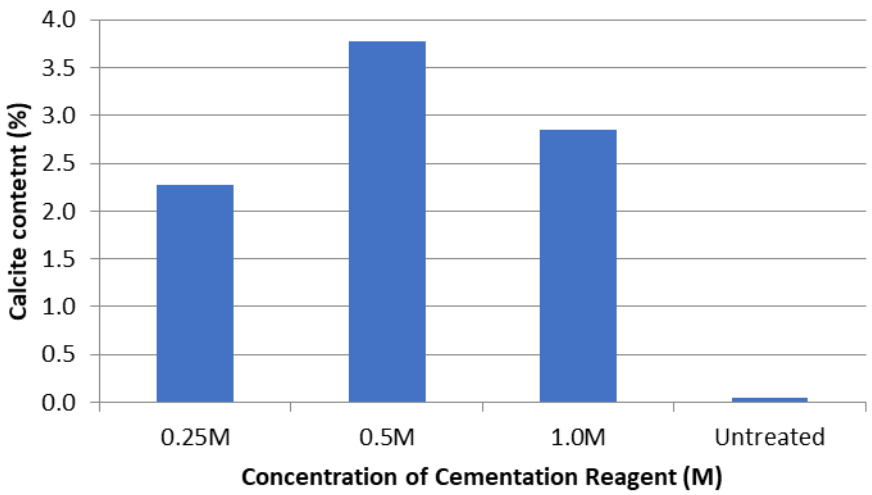

Figure 4.2: Effect of Concentration of Cementation Reagent on Calcite Content

Table 4.1: Effect of Concentration of Cementation Reagent on Average Calcite Content

Concentration of cementation reagent	Specimen	Calcite content (%)	Average calcite content (%)
0.25M	S1, top	3.03	2.35
	S1, bot	1.67	
	S2, top	2.26	2.21
	S2, bot	2.16	
0.5M	S3, top	2.33	2.24
	S3, bot	2.15	
	S4, top	6.88	5.31
	S4, bot	3.74	
1.0M	S5, top	4.01	3.40
	S5, bot	2.79	
	S6, top	2.69	2.30
	S6, bot	1.92	
Untreated	U1, top	0.05	0.05
	U1, bot	0.05	

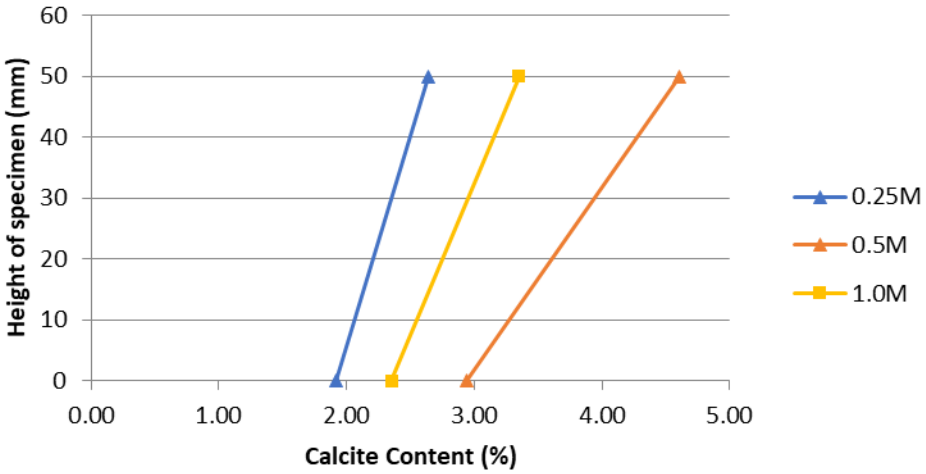

Figure 4.3: Uniformity of Calcite Precipitate in Each Sample.

4.4 Relationship between Properties of Compressive Strength and Calcite Content

Same trend on strength properties was found for the calcite content in the soil sample as shown in Figure 4.4. The overall result demonstrated the improvement of compressive strength and calcite content are both dependent on the concentration of cementation reagent. Figure 4.5 shows the linear relationship between the compressive strength and calcite content.

It has been observed that the property of compressive strength was proportional to the calcite content (R^2=0.9549). This concluded that the higher amount of calcite content is found in the soil particles, the higher the compressive strength it has, and vice versa. As shown in Figure 4.4, the highest amount of calcite content with 3.78% corresponds the highest compressive strength with 66.97kPa under 0.50M of cementation reagent. The trend is found under the 1.0M of cementation reagent as well, by contributing the second highest amount of calcite content with 2.85% and 64.40kPa of strength properties, respectively. Similarly, the lowest amount of calcite content with 2.28% showed only 55.58kPa of compressive strength can be achieved by the soil sample under 0.25M cementation reagent. The untreated soil was used as the benchmark for both calcite content and compressive strength, thus the value for all treated samples were above the benchmark to justify the improvement after the MICP treatment.

DeJong, et al. (2010) showed the improvement of soil strength corresponding to the formation of calcite precipitate in the soil particles after MICP. The presence

of calcite precipitate increases the initial stiffness by contributing a high stress over small strain. During the MICP treatment, calcite precipitate formed within the soil particles at the earlier stage is important as it acts as bond between the particle-particle. This bonding effect shows the improvement in shear strength after the calcite precipitates formed at the exact surface in the soil matrix. The formation of calcite precipitates has been proved to increase the shear strength of the tropical residual soil effectively (Ng, 2013). The mixture of coarse and fine particles in the tropical residual soil provides a greater space in the particle-particle of soil which could enhance the shear strength of soil by calcite bonds favourably. Hence, the calcite precipitates induced by *S.pasteurii* during MICP treatment could enhance the strength properties of soil is proven in this study.

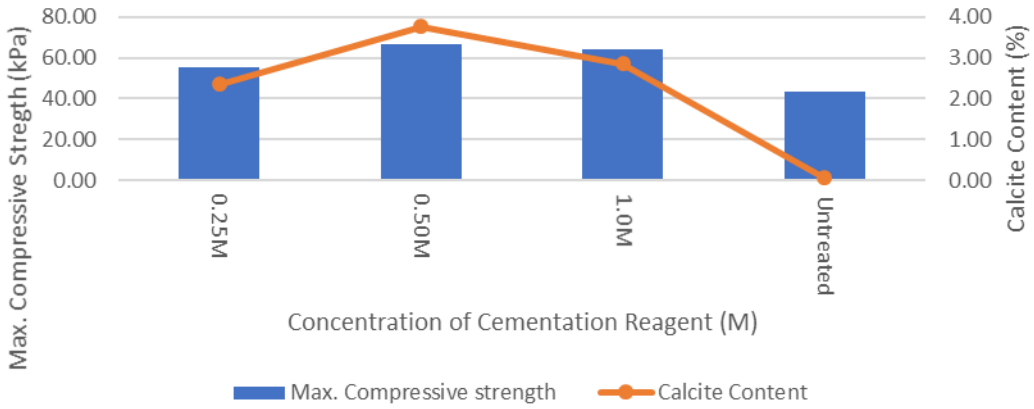

Figure 4.4: Relationship Between Average Compressive Strength and Calcite Content.

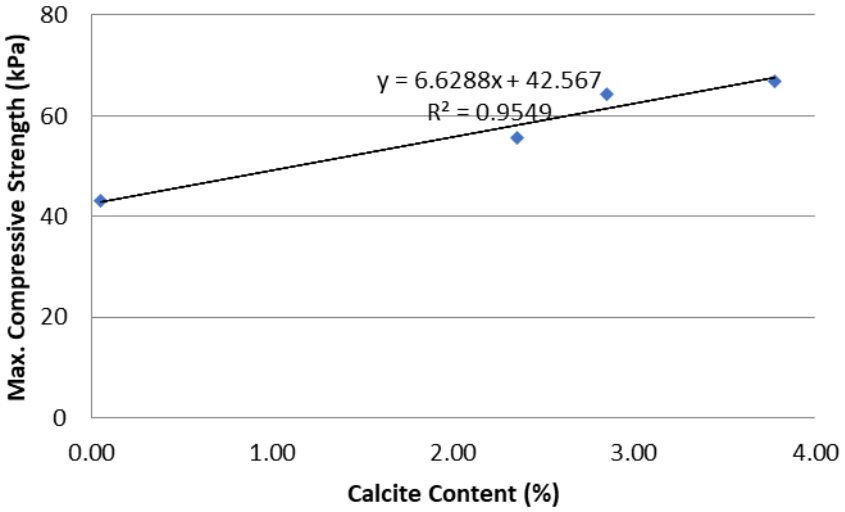

Figure 4.5: Linear Correlation Between Average Compressive Strength and Calcite Content

4.5 Effect of Bio-mediation on Dry Density of Tropical Residual Soil

Figure 4.6 reported the dry density of treated samples varied due to the different concentration of cementation reagent. The overall result showed the higher concentration of cementation reagent for bio-mediation contributing the higher dry density in the soil. Besides, higher dry density of the treated samples was found compared to the untreated samples. This showed the increment of dry density of treated samples was due to the formation of calcite precipitates in the soil. Hence, the concentration of cementation reagent indirectly influenced the result of dry density of tropical residual soil.

Based on Figure 4.6, the highest dry density was recorded as 1674.47 kg/m^3 for the sample treated by 0.5M cementation reagent, followed by 1.0M cementation reagent at 1661.28 kg/m^3 and 0.25M cementation reagent at 1658.75 kg/m^3. The initial dry density of untreated sample was used as the initial dry density for the comparison for each treated sample. As compared to the initial dry density, the final dry density for sample under 0.5M cementation reagent had increased 2.7%, trailed by 1.0M at 1.9% and 0.25M at 1.7%. The measurement of dry density was done by dividing the dried mass of soil sample which contained the calcite precipitates over the volume of soil sample. In fact, the amount of calcite precipitates increases according to the concentration of cementation reagent. This was proved by the Scanning Election Microscopy (SEM) analysis which reported by Okwadha and Li,

(2010). The SEM analysis shows the calcite precipitates formed in the particle-particle of soil whereby reducing the pore space in the soil, and hence resulting in a denser soil with high amount of solid content. According to Ng (2013), the higher the concentration of cementation reagent such as 0.5M induced a denser soil compared to the 0.25M sample. Thus, it has been found that the dry density of soil is directly proportional to the concentration of cementation reagent.

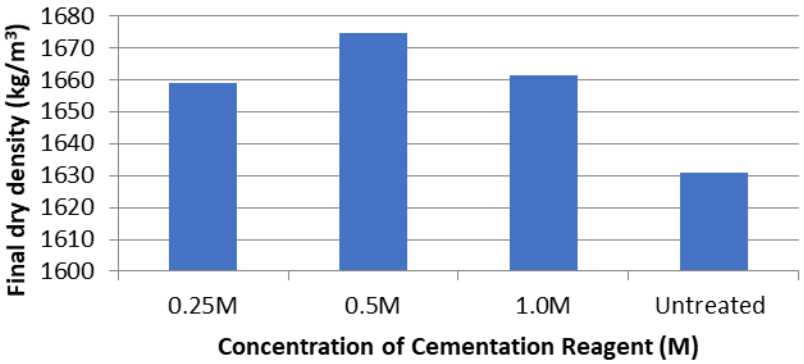

Figure 4.6: Effect of Bio-mediation on the Dry Density.

4.6 pH changes over treatment duration upon MICP treatment.

The variation of pH values of the cementation reagent against the treatment duration is shown in Figure 4.7. The experimental outcome shows the pH values had increased gradually from 5.5 to 8.0 along the MICP treatment. MICP treatment is proved to be occurred in an alkaline environment due to the ureolytic activities.

The cementation reagent composed of urea and calcium chloride is used to flow through the soil sample during the treatment. Figure 4.7 shows the initial pH value of the effluent from each sample was around 5.5 which is slightly acidic. The effluent remained at acidic for the first two days since the cementation reagent was 'warming up' for the ureolytic activity in the further cycle of treatment. The ureolytic activity might take some time to happen after the mixing of *S.pasteurii* and cementation reagent due to the high flow rate during the treatment. The reaction of ureolytic was deemed to begin when the pH value was found to be increased at the effluent. This is because the hydroxide ions with its alkaline nature were released during the urea hydrolysis process. The increment of pH value created an alkaline condition in the soil which is favourable toward the urease activity of *S.pasteurii*. In the last day, the pH value of the effluent from each sample were observed at around

8.0. Under the optimum pH at 8.0, the highest amount of calcite precipitates has been expected to be induced through the ureolytic activity. This can be justified by a high production of urease enzyme was found in the *S.pasteurii* between the pH range 7.5–9.0 (Nayanthara, et al., 2019). Ciurli, et al. also reported that 8.0 is the optimum pH value for the urease activity of *S. pasteurii*. Besides, the total spending duration for each cycle was 2 hours per day and the treatment had carried out successively for 6 days in this study. Ng (2013) stated the duration of treatment which implied the most effective result in term of providing a consistent improvement in shear strength is within 48 hours. Sharma and Ramkrishnan (2016) has also reported that the unconfined compressive strength in had significantly increased after 3 days of treatment duration. Therefore, the minimum treatment duration to achieve a significant increase in strength properties is 2-3 days.

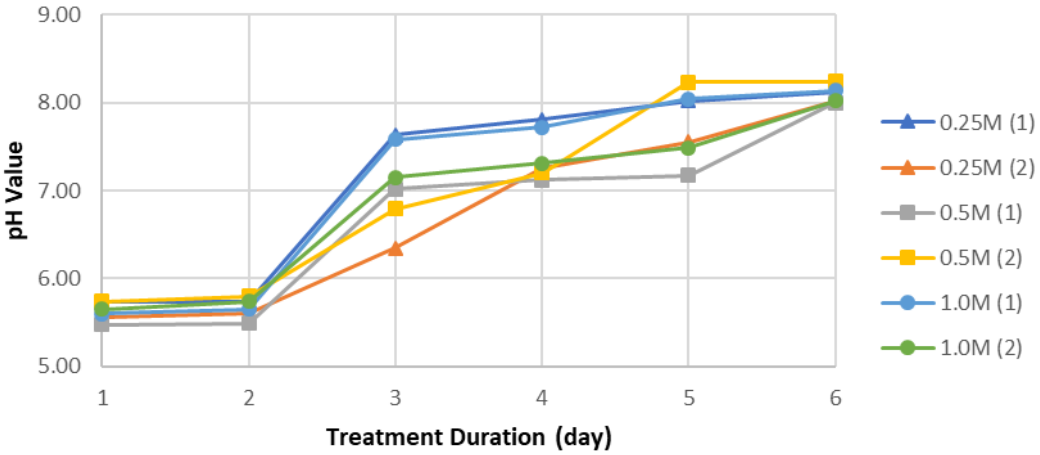

Figure 4.7: pH Changes versus Treatment Duration

4.7 Discussion

Based on the results as stated earlier, *S.pasteurii* with specific concentration of cementation reagent was proved to enhance the strength properties of soil MICP treatment. It can be concluded that the concentration of cementation reagent was an important factor which could influence the compressive strength, calcite content, dry density and pH value in the treatment. Throughout the experiment, 0.50M of cementation reagent was proven to be the most suitable concentration for MICP treatment, followed by 1.0M and 0.25M.

Other than that, the initial density of soil is another controlling factor which might affect the effectiveness of MICP treatment. Achal, et al. (2009) demonstrated

that effectiveness of MICP treatment is affected by the mobility of microorganism throughout the pore space in the soil for bio-cementation. Besides, Ng (2013) showed that the 90% of MDD is the optimum density which could provide a greater pore space for urease-producing bacteria to pass through. Thus, 90% of MDD was used for the soil sample in this study.

Besides, the flow rate of cementation reagent is one of the vital factors which could control the effectiveness of MICP treatment. A high flow rate of cementation reagent introduces into the soil sample might correspond with a high pore-water pressure in the soil sample (Ng, 2013). This could also disturb the soil sample by flushing out the soil particles from the treated soil due to a very high flow rate of flowing reagent. However, with a relatively low flow rate during the treatment process might clog the inlet of the soil sample. Therefore, the cementation reagent in this study which flowed under a pressure of 0.2 bar was viable for the MICP treatment in tropical residual soil.

Lastly, concentration of *S.pasteurii* shows the restraint on the performance of MICP treatment. According to the previous studies by Ng (2013), $1x10^8$ cfu/ml is the preferable concentration for bacteria to provide a better strength improvement in soil. Umar, et al. (2016) also stated that a greater strength improvement due to higher calcite formation could be obtained by introducing a higher bacteria concentration. Hence, $1x10^8$ cfu/ml was adopted as the concentration of bacteria in this study.

4.8 Summary

This chapter shows the experimental results for the improvement of properties of topical residual soil after the MICP treatment. The preferred concentration of cementation reagent was proven as 0.5M for the MICP treatment in term of providing the greatest strength improvement and the highest amount of calcite content, followed by 1.0M and 0.25M. The relationship between the compressive strength and calcite content was examined. The effect of bio-cementation on the dry density of soil and the pH changes against the treatment duration was analysed in this chapter as well. The other controlling factors for the performance of MICP treatment were further discussed.

CHAPTER 5

CONCLUSIONS AND RECOMMENDATIONS

5.1 Conclusions

In this study, Microbial Induced Calcite Precipitation (MICP) treatment had proved the improvement of strength properties in the tropical residual soil. The objective of this research which is to investigate the calcite precipitation in bio-mediated tropical residual soil was done. The effectiveness of MICP treatment was examined through the strength properties of soil and calcite content present in the treated soil. The combination of $1x10^8$ cfu/ml of bacteria concentration with 0.5M of cementation reagent was examined as the preferred condition for bio-mediation in tropical residual soil, trialled by 1.0M of cementation reagent and, lastly followed by 0.25M of cementation reagent which indicated the positive results. This can be proven by the result of this study whereby the 0.5M of cementation reagent had contributed the highest strength (66.97kPa), trailed by 1.0M of cementation reagent (64.40kPa). 0.25M of cementation reagent resulted the lowest strength (55.58kPa). Same trend was found for the calcite content in the tropical residual soil by showing 3.78% of calcite content for the 0.5M of cementation reagent, followed by 2.85% for the 1.0M of cementation reagent and 2.28% for the 0.25M of cementation reagent. This trend shows the relationship of calcite content and compressive strength was examined. The results were enhanced by the increment of dry density after the MICP treatment. An overall dry density under the 0.5M of cementation reagent was increased by 2.7%, trailed by 1.0M at 1.9% and 0.25M at 1.7%. Besides, the viability of MICP treatment was proven by the increment of pH value after the ureolytic activity. Overall pH value was increased from 5.5 to 8.0 over the treatment duration of 6 days for bio-mediation in tropical residual soil. In conclusion, calcite precipitation in MICP treatment had improved the strength properties on the tropical residual soil.

5.2 Recommendations

There are some recommendations on the further improvement for the performance of MICP treatment could be carried out in the next study. Firstly, number of samples for each test could be added on. This could be increased the accuracy of results for each test. Next, the height of the soil sample could be varied to at least 100mm. This could

make sure the L/D ratio be at least 2.0 for the unconfined compression test. The arrangement of calcite precipitate in the pore space could be investigated. These could be useful to explain the way of how the soil being improved by the distribution of calcite precipitate. Besides, different types of soil specimen could be used for further study since the particle size of soil may affect the movement of microorganism throughout the soil. The pore space between the soil particle might affect the formation of calcite precipitate and the effectiveness of MICP treatment as well. Furthermore, the mitigation measures for the by-product such as ammonia could be examined. This is because large amount of ammonia could arise the negative impact to environment.

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