MICROBIAL INDUCED CALCITE PRECIPITATION TREATMENT ON TROPICAL RESIDUAL SOIL

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To my beloved parents, lover and sibling

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ABSTRACT

Biomediated soil improvement, also known as Microbial Induced Calcite Precipitation (MICP), is a promising new branch of microbial geotechnology. Earlier MICP studies only focused on sandy soils, but more recent studies have also investigated the potential and feasibility of MICP on tropical residual soil. The main objective of this study is to examine the feasibility of MICP and the mechanisms involved between two types of bacteria and chemical reagents in improving the strength of tropical residual soil. Essentially, this was about identifying the optimized treatment conditions as well as the effects of some specific MICP parameters and curing on unconfined compressive strength (UCS) development and calcite distribution. Two bacteria from the *Bacillus* family, namely, *Bacillus subtilis* and *Sporosarcina pasteurii*, were used as urease producing bacteria. The tropical residual silt soil with 80% fine soil was compressed to a cylindrical sample measuring 100 mm in height and 50 mm in diameter as well as having a dry density of 1.31 Mg/m^3. Continuous injection method was employed. Series of tests were carried out, with each having different chemical reagents concentrations (0.15, 0.25, 0.35 or 0.45 M), reagent flow pressures (0.1, 0.2, 0.3 or 0.4 bars), and treatment durations (24, 48, 72 or 96 hours). Strength improvement of about 56.70% and 38.14% was immediately discovered after MICP treatment using *Sporosarcina pasteurii* and *Bacillus subtilis*, respectively. Additionally, strength improvement of about 30 to 104.12% were recorded after curing the samples for 3, 7, 14, 20, and 28 days. The optimum curing period was 14 days. The optimum treatment condition of MICP treatment for both bacteria consisted of 0.25 M of reagent concentration, 0.2 bars of reagent flow pressure, and 48 hours of treatment duration. The reagent flow pressure was the only MICP parameter that affected calcite distribution. No clear correlation was observed between calcite precipitation and strength. The two bacteria had distinctive responses to the designated treatment conditions as the behaviour of living microorganisms could differ although they are from the same family. This information were analysed to establish design charts for MICP practitioner in selecting optimal MICP parameters under different circumstances.

ABSTRAK

Pembaikan tanah secara *Biomediated* yang juga diketahui sebagai pemendakan kalsit cetusan mikrob (MICP) merupakan bidang Geoteknologi mikrob yang baru dan berpotensi. Kajian-kajian awal MICP hanya tertumpu kepada tanah berpasir, tetapi beberapa kajian baru MICP telah dijalankan untuk mengkaji potensi dan kebolehlaksanaan MICP ke atas tanah baki tropika. Objektif utama kajian ini adalah untuk mengkaji kebolehlaksanaan MICP dan mekanismanya antara dua jenis bakteria serta reagen kimia untuk meningkatkan kekuatan tanah baki tropika, mengenalpasti keadaan rawatan yang optima, kesan parameter MICP yang tertentu dan pengawetan terhadap perkembangan kekuatan tak terkurung dan taburan kalsit. Dua jenis bakteria dari keluarga *Bacillus* yang sama, *Bacillus subtilis* dan *Sporosarcina pasteurii*, digunakan sebagai bakteria penghasil urease. Kelodak dari tanah baki tropika dengan 80% butiran halus telah dimampatkan kepada sampel berbentuk silinder dengan ketinggian 100 mm, garispusat 50 mm serta berketumpatan kering 1.31 Mg/m^3. Kaedah suntikan berterusan digunakan untuk rawatan ini. Siri ujian MICP telah dilakukan dengan mengubah kepekatan reagen kimia (0.15, 0.25, 0.35, dan 0.45 M), tekanan aliran reagen (0.1, 0.2, 0.3, dan 0.4 bars), dan tempoh rawatan (24, 48, 72, dan 96 jam). Peningkatan kekuatan sebanyak 56.70 % dan 38.14 % telah diperhatikan sejurus selepas rawatan MICP yang menggunakan *Sporosarcina pasteurii* dan *Bacillus subtilis*. Disamping itu, peningkatan kekuatan sebanyak 30 hingga 104.12 % telah direkodkan selepas proses pengawetan selama 3, 7, 14, 20, dan 28 hari. Masa pengawetan optimum adalah 14 hari. Keadaan optimum MICP untuk kedua-dua bacteria adalah 0.25 M kepekatan reagen, 0.2 bar tekanan aliran reagen, dan 48 jam tempoh rawatan. Tekanan aliran reagen adalah satu-satunya parameter MICP yang memberikan kesan terhadap taburan kalsit. Tiada hubungan yang jelas antara mendakan kalsit dan kekuatan. Kedua-dua bakteria memberi tindak balas yang berbeza terhadap rawatan dalam kajian ini kerana mikroorganisma hidup mempunyai kelakukan yang berlainan walaupun dari keluarga yang sama. Maklumat ini telah dianalisa bagi menghasilkan carta rekabentuk untuk pengguna MICP dalam membuat pemilihan parameter MICP yang optima bagi keadaan yang berbeza.

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LIST OF ABBREVIATIONS

ACC	–	Amorphous Calcium Carbonate
AEC	–	Anion Exchange Capacity
ATCC	–	American Type Culture Collection
BIM	–	Biologically Induced Mineralization
BCM	–	Biologically Controlled Mineralization
BSCS	–	British Soil Classification System
CEC	–	Cation Exchange Capacity
DDL	–	Diffuse Double layer
DIC	–	Dissolve Inorganic Carbon
DTA	–	Differential Thermal Analysis
FESEM	–	Field Emission Scanning Electron Microscope
FTIR	–	Fourier Transform Infrared Spectroscopy
LOI	–	Loss of Ignition
LL	–	Liquid Limit
MICP	–	Microbial Induced Calcite Precipitation
MEOR	–	Microbially Enhanced Oil Recovery
OC	–	Organic Content
PI	–	Plasticity Index
PL	–	Plastic Limit
PVC	–	Polyvinyl Chloride
UCS	–	Unconfined Compressive Strength
UCT	–	Unconfined Compressive Test
XRF	–	X-ray Fluorescence
XRD	–	X-ray Powder Diffraction

USCS – Unified Soil Classification System

UV – Ultraviolet

LIST OF SYMBOLS

$C_{\mathbf{c}}$	–	Compression Index
$C_{\mathbf{r}}$	–	Recompression Index
$k_{\mathbf{h}}$	–	Hydraulic Conductivity
$S_{\mathbf{c}}$	–	Total Consolidation Settlement
$\rho_{\mathbf{d\ max}}$	–	Maximum Dried Density
$w_{\mathbf{opt}}$	–	Optimum Moisture Content
$P_{\mathbf{s}}$	–	Pre-consolidation Stress
$G_{\mathbf{s}}$	–	Specific Gravity

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CHAPTER 1

INTRODUCTION

1.1 Background of Study

The rapid and unprecedented population growth rate of the modern world's population are fuelling the demand for faster infrastructure development to meet the ever-growing societal needs. However, its expansion is often limited by geographical boundaries, undesired soil condition, and also the unavailability of competent soil upon which facilities can be constructed. Fortunately, there exists different type of soil stabilization or soil improvement methods that are able to treat and improve the quality and the engineering properties of soil to enable infrastructure construction.

In Malaysia, the major problem specific to tropical residual soil is the high annual rainfall which infiltrates into slopes and causes erosion, landslide and slope failure at hillside area and highway (Huat *et al.*, 2012). The strength and compressibility of tropical residual soil arise from the effect of cementation of secondary deposition, but this is often disrupted by the brittleness of the deposit (Kamarudin, 2004). To overcome this, a more effective and environmental friendly soil improvement such as bio mediated soil stabilization has to be examined to understand its feasibility.

The current massive soil stabilization methods often come with some environmental issues. For example, the production of cement used pervasively for construction and to improve ground condition is believed to be one of the major contributors to carbon release and global warming through the burning of fossil fuels (DeJong *et al.*, 2010). Furthermore, most of the grouting injection fluid used for chemical soil stabilization is toxic and hazardous to the environment and public (Karol, 2003). Therefore, a more sustainable and environmental friendly method is needed to replace and improve the conventional soil improvement techniques. In this regard, the bio mediated soil stabilization method is a promising soil improvement approach since it utilizes biological processes to alter the engineering properties of soil such as strength, stiffness, and permeability.

Bio mediated soil stabilization, also known as Microbial Induced Calcite Precipitation (MICP), is a new and innovative application in the geotechnical engineering field that has attracted the attention and great interest of researchers worldwide. Similar rigour can be seen in research works on microbial induced carbonate precipitation with varying type of soil, injection depth and bacteria as well as methods to improve and practically implemented at site being studied. In fact, the usage of microbial induced precipitation is not new in many industries. It has been applied to treat contaminated soils (Khachatoorian *et al.*, 2003); stabilize metal (Etemadi *et al.*, 2003); recover reservoir oil (Nelson and Launt, 1991); improve the strength of concrete and cement mortar (Ghosh *et al.*, 2005); retrofit and fix cracks in concrete (Van Tittelboom *et al.*, 2010); and others as described in Chapter 2.

DeJong *et al.* (2010) were among the researchers who had successfully started the application of microbial inducted precipitation in geotechnical engineering to alter the permeability and shear strength of a sandy soil. In relation to MICP of tropical residual soil, Soon *et al.* (2014) conducted a series of MICP treatments on a tropical residual soil (sandy silt) using *Bacillus megaterium*. They discovered that the unconfined compressive strength (UCS) improved by 69% and the permeability reduced by 90% compared to the untreated soil.

Although numerous studies have been published, literature on biomediated soil stabilization mostly remains at the research stage due to inconsistencies in results reported. The limitations of this approach are most commonly associated with the soil type, the bacteria compatibility, and the complexity of the reactions. To this date, these limitations are still unanswered and remain one of the biggest challenges to researchers (Ivanov and Chu, 2008).

1.2 Problem Description

Most studies on MICP technique are done on sandy soil rather than fine grained soil. Although a few studies have been published on fine-grained soils and tropical residual soil (Soon *et al.*, 2014; Lee *et al.*, 2013), the results are inconsistent and remained at the laboratory stage due to uncertainties and complexity of tropical residual soil, soil geochemistry, bacteria, and other environmental factors.

On the other hand, *Sporosarcina pasteurii* and *Bacillus subtilis* have been widely used as a urea-hydrolysing bacterium in numerous MICP studies, but its application and feasibility in MICP treatment of typical fine-grained soils has never been examined.

The sizes of soil particle and pore throat are the main attributes in determining the feasibility of the MICP treatment. The migration of the chemical reagents and bacteria into the soil might be hindered by the fine grained soil and subsequently affect the calcite precipitation distribution along the sample length. For this reason, the application of MICP technique for fine grained soil remains largely an unexplored territory.

Not only that, the small clay pore size and its low permeation rate for nutrient and oxygen tend to limit cell growth on the particles' surface. This makes the usage of injection method inevitable despite the inherent problem of uneven calcite distribution. In order to reach a uniform calcite distribution, the MICP parameters such as chemical reagents, treatment duration, and reagents flow pressure have to be optimised, controlled and counterbalanced

The implication from the problems stated above on the bacteria and other environmental factors shows that there is no one recipe or typical formulation applicable to all soil types for MICP treatment. Different soil type requires different combination of MICP parameters due to the variations in mineralogy, pH, and geochemistry of the soil.

1.3 Objectives

The aim of this research is to improve the engineering properties of tropical residual soil through the utilization of the bacteria and chemical reagents. The main objectives in this research are listed as below.

(a) To determine the feasibility and relationship of MICP treatment by using two different bacteria strains, *Sporosarcina pasteurii* (ATCC® 11859™), and *Bacillus subtilis* (ATCC® 55422™) to improve shear strength and examine change in permeability of treated tropical residual soils.

(b) To optimize MICP treatment and examine subsequent changes in strength, calcite distribution and microstructure of the treated tropical residual soil with reference to different MICP parameters, i.e., treatment duration, reagent concentration, and reagent flow pressure.

(c) To establish a design chart for MICP treatment of tropical residual soil and to examine the effects of curing period on MICP treated soil.

1.4 Scope of Study

This bio-mediated soil stabilization research focused on small scale laboratory physical modelling. Tropical reddish residual clayey soil retrieved from Universiti Teknologi Malaysia (UTM) at Skudai, Johor and passing 2 mm sieve was used. The length and diameter of MICP treated cylindrical sample were 100 and 50 mm, respectively.

The MICP treatment system was designed based on continuous injection method in which the chemical regents was injected uninterrupted throughout the treatment duration. This treatment was conducted in an air-conditioned room with temperature controlled at 25.5°C. *Sporosarcina pasteurii* (ATCC® 11859™) and *Bacillus subtilis* (ATCC® 55422™) were the bacteria used. It was hypothesized that both *Sporosarcina pasteurii* and *Bacillus subtilis* are feasible for MICP treatment of tropical residual soil. The formation of calcite tend to increase the shear strength of the soil after treatment. However, it decreases the permeability of the treated soil. Geotechnical engineering properties of the bio-mediated treated soil such as permeability and shear strength were examined extensively with every recorded changes further studied and compared with those of the untreated soil samples.

In order to understand the mechanism and relationship of each of the MICP components, six different treatment conditions were set up, which were:

1. Treatment with chemical reagents only
2. Treatment with nutrient broth only
3. Treatment with inclusion of *Sporosarcina pasteurii* only
4. Treatment with inclusion of *Bacillus subtilis* only
5. MICP treatment using *Sporosarcina pasteurii*
6. MICP treatment using *Bacillus subtilis*

The three most important parameters in this MICP treatment were reagents concentration (0.15, 0.25, 0.35 and 0.45 M), reagent flow concentration (0.1, 0.2, 0.3, and 0.4 bars), and treatment duration (24, 48, 72 and 96 hours). Each parameter was altered to reach optimization and examine their effects on the unconfined compressive strength (UCS), microstructure, and calcite distribution of the treated soil sample. Additionally, the curing period was set at seven, 14, 20, and 28 days to examine the long term performance of *Sporosarcina pasteurii* on the MICP treated soil.

This study has also established the MICP design chart of tropical residual soil for strength improvement using *Sporosarcina pasteurii* and *Bacillus subtilis* with reagent concentration set at 0.15, 0.25, 0.35, and 0.45 M and treatment duration fixed at 24, 36, and 48 hours only.

1.5 Structure of Thesis

This thesis consists of seven chapters: Introduction (Chapter 1); Literature Review (Chapter 2); Methodology (Chapter 3); Variation of MICP treatment conditions (Chapter 4); Effect of chemical reagents flow pressure, concentration, and treatment duration for MICP of *Sporosarcina pasteurii* and *Bacillus subtilis* (Chapter 5); The MICP treatment design charts and effect of curing on MICP treatment (Chapter 6); and Conclusion and Recommendation (Chapter 7). Concluding remarks are provided at the end of each chapter to highlight and summarise the findings and outcomes of each chapter.

Chapter 1 is the introductory chapter on bio-mediated soil stabilization. It also highlights the research background and problems; objectives; scope; and limitation of the present research in relation to bio-mediated soil improvement.

Review of literatures is presented in Chapter 2 with the main focus put on the origin of tropical residual soils and bio-mediated soil stabilization. Reviews on the bacteria, MICP processes, MICP treatment on different type of soil, engineering application, and factors are also included and discussed.

Research methodology, experiment procedures, material preparation, bacteria cultivation and maintenance process and equipment specification exercised in this study are explained and presented in Chapter 3. Physical and geotechnical properties test were performed in accordance to procedures outlined by the British Standard. However, for those testing methods which are not included in any specific standard, general accepted methods established by researchers have been used in the determination of parameters such as calcite determination, CEC, X-ray Fluorescence (XRF) and X-ray Diffraction (XRD) analyses.

The results and outcome of this study are divided and discussed separately in 3 different chapters, i.e., Chapter 4, Chapter 5, and Chapter 6. Chapter 4 presents the physical and geotechnical properties of tropical residual soil. It also presents an in depth discussion on each MICP component, i.e., bacteria, chemical reagents, nutrient broth, and tropical residual soil. Results for both samples treated by two different bacteria, *Sporosarcina pasteurii* and *Bacillus subtilis* were also studied and discussed. The main subjects discussed in this chapter are UCS and permeability of the MICP treated soil.

Chapter 5 mainly focused on the optimization of MICP treatment using *Sporosarcina pasteurii* and *Bacillus subtilis*. Additionally, the effect of MICP parameters such as chemical reagents flow pressure, concentration, and treatment duration for both MICP of *Sporosarcina pasteurii* and *Bacillus subtilis* were examined. The discussion mainly focused on the calcite distribution pattern, effect of calcite precipitation on UCS, effectiveness of calcite precipitation, and some comparisons with other MICP studies.

Chapter 6 compiles the results and outcome in this study for the MICP design chart establishment. It should be noted that the MICP treatment design charts, which are based on *Sporosarcina pasteurii* and *Bacillus subtilis*, only serve as a guideline, information and reference for MICP treatment of tropical residual soil. Nevertheless, the charts are useful for determining the optimum treatment duration for a given reagent concentration and vice versa. In addition, the effect of curing on the MICP treated soil is also examined and discussed in Chapter 6.

Lastly, Chapter 7 concludes the findings and outcomes of this study. Recommendations for future research are also given in this last chapter.

1.6 Significance of Study

This particular research is crucial for the evolution of geotechnical ground improvement technique to provide a more environmental friendly alternative to conventional methods. The proposed MICP technique has the potential to resolve geotechnical problems including healing of crack surface of lime stabilized soil; sealing of tension crack of a slope; and coating for road embankment to prevent water infiltration and for slope protection (where the early strengthening of soil is not a concern). Besides, the exceptional advantage of using MICP treatment with bacteria is also able to heal degraded calcite bonding after deformation; a trait not common in conventional methods.

The research provides a more profound understanding and knowledge for bio-mediated soil stabilization on tropical residual soil. This research has evaluated the feasibility of *Sporosarcina pasteurii* and *Bacillus subtilis* in MICP treatment of tropical residual fine-grained soil, which has not been reported and attempted before the time of this writing. The application of these two bacteria in MICP treatment had been proven favourable and effective in UCS and permeability improvement. The research also provides more understanding on the some important MICP parameters that affect calcite distribution.

Another contribution is in a more in-depth understanding on the effects of calcite distribution on the strength development of MICP treated soils. This research has also evaluated the effect of curing period on UCS. The outcome of this research have been assembled to establish a design charts for the bio-mediated soil stabilization of tropical residual soil.

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