Faculty of Engineering and Science

Investigation of Strength and Consolidation Behaviour of Peat Treated Using Microbial-Induced Calcite Precipitation (MICP)

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DECLARATION

To the best of my knowledge and belief, this thesis contains no material previously published by any other person except where due acknowledgement has been made.

This thesis contains no material which has been accepted for the award of any other degree or diploma in any university.

Ignatius Phang Ren Kai

Date: 22 June 2021

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The following publications were produced during my PhD research:

Journal papers

1) Phang, I.R.K., San Chan, Y., Wong, K.S. and Lau, S.Y., 2018. Isolation and characterization of urease-producing bacteria from tropical peat. *Biocatalysis and Agricultural Biotechnology*, 13, pp.168-175.

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Conference papers

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2) Phang, I.R.K., Wong, K.S., Chan, Y.S., and Lau, S.Y., 2019, April. Effect of surcharge load on Microbial-Induced Calcite Precipitation (MICP) treatment of tropical peat. In *IOP Conference Series: Materials Science and Engineering* (Vol. 495, No. 1, p. 012068). IOP Publishing.

ABSTRACT

Peat is known as an inappropriate material in the geotechnical engineering application, especially for road and building construction. Peat has low bearing capacity and excessive compressibility, resulting in a long-term settlement, which lead to cracking of structures and pavement. Approaches such as surcharging, electro-osmosis treatment, pile foundation, deep mixing and mass stabilisation were applied to mitigate these problems. However, these methods are time-consuming, expensive, and environmentally unfriendly. Bio-cementation technology emerged as an effective approach to overcome these problems. Biocementation of soil via microbial-induced carbonate precipitation (MICP) has shown to be effective through the natural calcite precipitation ($CaCO_3$) process to bind soil particles which increases its strength and stiffness. Most of the studies were based on sand and fine-grained soil with limited studies on MICP treatment on peat. This study intends to investigate the potential of MICP to improve the geotechnical properties of peat, which includes the shear strength and the consolidation behaviour. The study showed successful isolation of MICP suitable bacteria strains P19 (MH639002) and P21 (MH639001) from acidic tropical peat. Those isolated strains are found to be the genus of *Enteractinococcus* and *Staphylococcus*. The isolated strains yield high urea hydrolysis activities, with isolate P19 shown the urease activity up to 815 U/mL. Both isolated strains showed carbonic anhydrase activity as well. The finding of high urease activity and capability of carbonic anhydrase of these isolates suggested its suitability for MICP usage. These isolates precipitated $CaCO_3$ in polymorph of vaterite and calcite. Isolate P21 produced higher $CaCO_3$ precipitation and higher unconfined compressive strength (UCS) than isolate P19 in the bio-cementation of sand. The possibility of MICP with indigenous urease activity of peat was explored and found that calcium carbonate precipitation was possible by utilising indigenous urease activity in peat which altered the pH of its natural acidic condition to facilitate carbonate crystal precipitation. The strength of the peat samples were observed to improve through the precipitation of $CaCO_3$ from 1.92 kPa for the untreated peat to 22.46 kPa for the treated sample, suggesting improvement from MICP. The precipitated $CaCO_3$ was tested with XRD and confirmed to be calcite. Further exploration was done with 25%, 50% and 75% sand filler with the isolated strains. It was observed that strain P21 showed the most significant improvement of strength at 28 days upon curing at 108 CFU/mL in peat with 25% of sand and at 2 mol/kg of cementation reagent dosage. The strength of the test samples increased with increasing cementation reagent dosage up to 2

mol/kg and decreased after 4 mol/kg. UCS for the treated peat with 25%, 50% and 75% sand showed increasing trends with increasing curing duration, with the highest observed at 75% sand with 94.85 kPa. $CaCO_3$ precipitation increased with increasing sand contents along with a more extended curing period. Permeability of the treated test sample of stabilised peat is extremely low compared with untreated samples and observed that it reduced with time. This study also indicates that peat varies with sand content treated with MICP resulted in a lower void ratio due to calcium carbonate precipitation. Increasing sand content increases the Coefficient of consolidation, C_v for both treated and untreated peat. However, the treated peat samples yield lower C_v as compared to the untreated samples at same sand content (0 – 75%). Reduction of hydraulic permeability was found in the treated peat compared to the untreated peat at the same sand content. Compression Index, C_c and Swelling Index, C_s values for the treated peat were lower than the untreated peat at the same sand content. Secondary compression Index, C_α for the treated peat (25% sand) was higher compared to the untreated peat (25% sand) at 100 kPa effective stress onwards, while C_α for the treated peat with 50% sand was higher compared to the untreated peat with 50% at 200 kPa effective stress onwards. This phenomenon leads to higher C_α/C_c for the treated peat with 25, 50% and 75% sand compared with its untreated counterpart showing a decreasing C_α/C_c with increasing sand content. For durability study, MICP stabilised peat showed as high as 66.37% strength loss when submerged in peat slurry compared to distilled water as a control. Extended calcium carbonate precipitation was observed for samples submerged in distilled water (Control), whereas declining trends were observed for stabilised peats submerged in peat slurry. This suggested that although MICP is possible, a large peatland area may present a challenge with the surrounding acid attack towards the treated column. The outcome of this research reveals an alternative peat ground improvement method by MICP treatment with indigenous bacteria isolated belonging to the genus *Enteractinococcus* and *Staphylococcus* from peatland.

Keywords: Microbial Induced Carbonate Precipitation (MICP); Peat; Urease; Geotechnical

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LIST OF NOMENCLATURES

BLAST – Basic local alignment search tool.

FTIR - Fourier-transform infrared spectroscopy

MICP - Microbial induced calcite precipitation

NCBI – National Centre for Biotechnology Information

PCR – Polymerase chain reaction

SEM - Scanning Electron Microscopy

TAE – Tris-acetate-EDTA

TGA - Thermo-Gravimetric Analysis

XRD - X-ray Powder Diffraction

XRF - X-ray fluorescence

NOTATION

C_c - Compression index

C_α - Secondary compression index

C_v - Coefficient of Consolidation (m^2 /yr)

e - Void ratio

$\Delta e / \Delta \log \sigma'_v$ - Change of void ratio against the change of effective vertical stress

C_s - Swelling index

CHAPTER 1: INTRODUCTION

1.1. Background

Peatlands cover approximately three percent of the global land (Immirzi et al., 1992; Page et al., 2011). More than 80 % of total peatlands situated in the northern hemisphere covering large parts of Russia, North America and Europe, while 11 % is tropical peatland covering a land area of 441,025 km^2 situated in the tropics, mainly in Southeast Asia but also in mainland East Asia, the Caribbean and Central America, South America and Africa where regional environmental and topographic conditions favour formation of peat (Andriesse, 1988) (Figure 1.1). Tropical lowland peatlands cover approximately 23Mha in Southeast Asia, with the largest coverage in Southeast Asia's coastal zone, especially in Indonesia and Malaysia. Malaysia has peatlands cover of approximately 2.6 Mha (Mutalib, 1992), with Sarawak state covering the largest extent of peatland over 1.6 Mha, about 70 percent of peatlands in the country (Melling, 2016). Peatlands are important as sources of carbon pool and wildlife species pool (Immirzi et al., 1992; Strack, 2008), timber production, and land use for agriculture, especially for oil palm plantation and pulp trees (Koh et al., 2009).

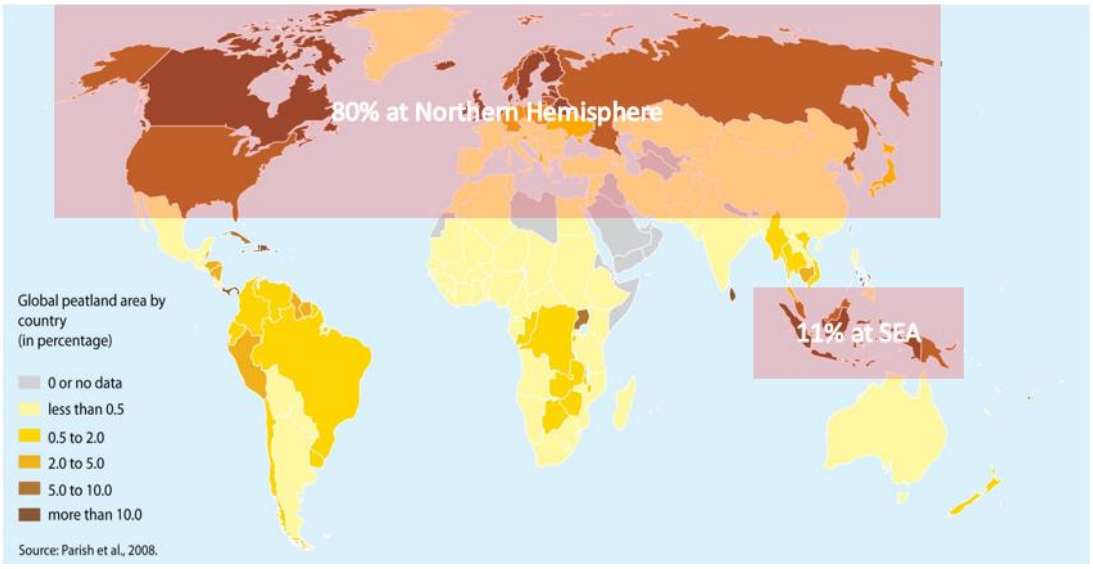


Figure 1.1. Global distribution of peatlands (Parish et al., 2007).

Peat has more than 75% of organic content and consists of finely mixture of partially decomposed plant remaining (Hampton & Edil, 1998). Peat formed in wetlands or

ground that are fully undrained with the waterlogging condition when suitable climates and topographic conditions lead to high accumulation of organics compared to decaying (Dhowian & Edil, 1980; Fuchsman, 2012). Munro (2004) defined that actual peat comprises of decomposed fragments and water with no measurable strength. Therefore, peat with extreme compressibility and low bearing capacity is often considered a geotechnical engineering problem (Sing, Hashim, et al., 2008).

Peat is highly soft and is subject to instability and well-known for its substantial primary and long-term settlement when subjected to loading during construction (Huat, 2004). This rendered difficult accessibility to peat sites. Buildings constructed on peat are usually suspended on piles driven into underlying mineral soil and bedrock; however, the soft peat around such buildings may still settle, resulting in cracks formation in pavement and driveways and broken drains around the building structure. Settling of roads built on peat ground may result in bulging and tilting houses situated near or alongside the roads (Huat, 2004). Due to peat problems, the complication of construction on peatland and the inevitable high costs involved, engineers and developers opt to avoid building on problematic peat ground. Scarcity and unavailability of suitable construction ground, especially in coastal lowland areas, often faced high land development pressure (Zulkifley et al., 2014). Peatland development is unavoidable.

Various methods to tackle the peat problem have been introduced to improve its geotechnical properties, including improving the shear strength and peat deformation. These include the use of cementations materials such as synthetic polymer and cement-based stabiliser into organic soils and peats to improve the mechanical properties. Various approaches were effective but rather costly and harmful to the environment (Ivanov & Chu, 2008).

Recent studies utilize simulating of a natural process to treat unsaturated soils. Among those methods, microbial soil cementation or known as microbial-induced carbonate precipitation (MICP), has shown promising results which follows natural depositing of calcite ($CaCO_3$) on the soil particles to improve stiffness, strength, and reduction of erodibility (DeJong et al., 2006; Li et al., 2016; Lin et al., 2016; Omoregie et al., 2019; Saricicek et al., 2019). The microbial mechanism based on ureolytic non-pathogenic bacteria such as *Sporosarcina pasteurii* to hydrolyse urea and in the presence of

calcium ions in a system inducing the precipitation of calcium carbonate crystals (Achal et al., 2009a, 2009b; Zhao et al., 2014).

The application of the MICP technique has demonstrated its potential in increasing soil stiffness and strength, reductions in foundation settlement and soil permeability. However, limited study was found on the MICP approach towards strengthening the peat. Hence, this study explores the potential of the MICP approach in tropical peat and its effect on shear strength and peat's consolidation behaviour.

1.2.Problem statement

Road and building construction on peat experiences time-dependent settlement, and the excessive settlement may result in structures and pavement distress or cracking. Peat is considered a problematic soil in geotechnical terms with low bearing capacity and extreme compressibility (Huat et al., 2011; Huat et al., 2014; Kazemian, Prasad, Huat, & Barghchi, 2011). Avoidance is inevitable with decreasing land for development, and physical solutions are time-consuming. Chemical additives are less environmentally friendly and may contribute to blockage and contamination of groundwater (Basha et al., 2005; DeJong et al., 2006; Karol, 2003). MICP provides a natural alternative as it simulates natural calcite precipitation in soil. However, to the best knowledge of the authors, a limited MICP study was conducted based on highly organic and acidic peat.

1.3.Research Scope

The study focused on urease-based bio-precipitation of calcite by microbial means or term as microbial-induced carbonate precipitation (MICP) in tropical peat soil. Indigenous isolates of bacteria from the source peat sample were screened and isolated for their urease activity and utilized as the urease source to study calcium carbonate precipitation in peat. Geotechnical tests were then conducted to evaluate MICP activity towards peat in terms of unconfined compression strength (UCS), consolidation behaviour, primary and secondary consolidation, and permeability.

1.4. Research Objectives

The purpose of this study is to explore the potential application of Microbial-induced Calcite Precipitation (MICP) in improving the strength and consolidation behaviour of peat.

This study embarks on the following objectives:

I. To isolate, identify and characterize ureolytic calcium carbonate ($CaCO_3$) precipitating bacteria from peat.

II. To evaluate MICP performance of isolated bacteria towards unconfined compression strength and hydraulic conductivity of peat.

III. To investigate the effect of MICP on the consolidation behaviour of peat.

IV. To determine the durability of MICP stabilised peat.

1.5. Significance of the Study

MICP of tropical peat required further research as there is a lack of understanding of how MICP will affect highly organic and acidic tropical peat. Thus, this study attempts to provide insight into the knowledge gap.

This research will enrich the knowledge of the characteristics of MICP towards tropical peat soil as a very limited study on the use of MICP for peat improvement has been carried out. Although related studies were identified by the author but there is still no study done with tropical peat especially the use of indigenous strains (Canakci et al., 2015a, 2015b; Sato et al., 2016). Gap in knowledge in this study included the suitability and possibility of urease activity to raise the pH of peat suitable for MICP, the use of local indigenous bacteria isolates from peat and sand as filler with MICP were also addressed.

The study involved urease induced MICP process, which used local indigenous strain. The isolation and identification of tropical indigenous urease based MICP bacteria, including screening from peat itself were not done in the previous study. The bacteria used in the previous study was not isolated in the tropical region, and they were not used in tropical peat. This will provide an additional alternative of suitable bacteria for MICP application in tropical peat.

The outcome of this research will reveal the possibility of using indigenous strains for MICP of peat as an alternative solution for peat improvement while contributing to the gap of knowledge in MICP of organic soil. As peat from Sarawak is used in the research, the outcome is expected to provide valuable insight on the future development of suitable MICP based soil improvement for local and tropical usage.

1.6. Outline of the thesis

The chapters included in this study are as followed:

Chapter 1 outlines the background, research significance and objectives of the study.

In Chapter 2, a literature review was done on the geotechnical issue of peat and the possibility of MICP used as peat improvement alternative. A summary of the previous MICP studies that have been performed on inorganic soil was also presented.

In Chapter 3, the methodology of the study, including experimental design, testing procedure and materials were presented. The chapter consisted of the methodology for results discussed in Chapter 4, 5, 6 and 7.

Chapter 4 focused on the isolation and characterisation study of calcite precipitation bacteria from tropical peat. The enzyme activity, including urease (urea hydrolysis) and carbonic anhydrase of the selected isolates and calcium carbonate precipitation performance, were studied. Isolated strains were identified and to be used for MICP treatment in the next chapter.

In Chapter 5, the study was done by using indigenous microbial sources in peat to induce bio-precipitation of calcium carbonate in treated peat samples. This experiment provided evidence that MICP is possible in tropical peat based on the urea hydrolysis pathway and may lead to a significant strength increase. Then the study explored factors of MICP using indigenous isolates from Chapter 4 with parameters including different bacteria concentration, cementation reagent concentration and filler effect of sand towards geotechnical properties of constituted peat sand mixture. Bio-cementation effect of MICP was evaluated through the Unconfined Compression Test, and bio-clogging effect was observed with the permeability test.

In Chapter 6, by using the results from the previous chapter, changes in consolidation behaviour of MICP treated peat and non-treated in term of primary and secondary

consolidation at different levels of sand filler is presented and discussed. The results were obtained from One-dimensional oedometer testing with treated and untreated samples cured at different duration.

Chapter 7, the durability of MICP stabilised peat is presented, and the results suggest the feasibility of MICP of peat in long-term soaking of acidic peat condition.

Finally, Chapter 8 concludes the whole study by reviewing the completeness of the research objectives The chapter also recommend possible directions to improve the present work.

CHAPTER 2: LITERATURE REVIEW

2.1. Issue with geotechnical properties of peat

2.1.1. Compressibility of peat

The compression behaviour of peat varies from other types of soils. Peat has more extensive compression, and its creep settlement is more significant in defining the total settlement than other soil types. Peat undergoes rapid primary consolidation due to its high initial permeability in which naturally a thousand times that of soft clay or silt deposits. Although peat undergoes dramatic reductions in permeability under compression, an extensive secondary or even tertiary compression still follows after primary consolidation (Kazemian & Huat, 2009; Mesri & Ajlouni, 2007). Various reasons affect the compression behaviour of peat including fibre content, water content, void ratio, initial permeability, soil particles arrangement and inter-particle chemical bonding in certain soils (Mesri & Ajlouni, 2007). Compression index, C_c can be defined as the change of the void ratio against the change of effective vertical stress, $\Delta e/\Delta \log \sigma'v$ and can be used for evaluating primary consolidation of soil (Madaschi & Gajo, 2015; Mesri et al., 1997). Peat has high void ratio due to its porous nature of peat particles which contributes to high compression index value (Huat et al., 2011). The larger void ratio results in larger compression index which leads to higher degree of primary consolidation settlement. However, the rate of consolidation will be decreased as the applied stress is increased (Huat et al., 2014; Mesri et al., 1997). The compression index (C_c) of peat ranges between 2 to 15, and the secondary compression happens before the complete dissipation of excess pore water pressure (Gofar & Sutejo, 2007; Leonards & Girault, 1961). Secondary compression occurs as fibrous peat continues to compress at a gradually decreasing rate under constant effective stress. Mesri et al. (1997) reported that the secondary compression could be due to the decomposition of fibres in peat which is assumed to occur at a slower rate after the primary consolidation. The slope at the final part of the graph of void ratio versus logarithmic of time curve, C_α is defined as the rate of secondary compression. Secondary compression index, C_α, which shows the creep behaviour of soil under constant stress is known as varies depending on time and its behaviour which varies depending on applied stress (Mesri & Castro, 1987). The ratio of C_α / C_c are commonly

used to evaluate the behaviour of peat (Dhowian & Edil, 1980) and Mesri et al. (1994) reported C_α / C_c value of peat range between 0.05 - 0.07.

2.1.2. Shear strength of peat

The shear strength of peat is low and it will increase with consolidation. The peat shear strength is affected by various factors, such as its moisture, state of decomposition and inorganic minerals. Peat with increasing moisture content and decomposition will yield lower shear strength, while increasing mineral contents can improve the shear strength (Munro, 2004; Munro & MacCulloch, 2006). The triaxial test can be used to study the shear strength of peat in a laboratory environment under a consolidated-undrained (CU) state by submerging peat in a waterlogged condition. In general, the internal friction angle of peat is higher than inorganic soil. Friction angle of amorphous peat and fibrous peat falls between 27 – 32° (normal pressure of 3−50 kPa), whereas for amorphous granular peat, the adequate internal friction is 50° and for fibrous peat, it ranges between 53- 57° (Edil & Dhowian, 1981; Landva & La Rochelle, 1983).

2.2. Recent technology to tackle the peat problems

Some feasible approaches were developed to tackle peat ground construction, these include avoidance, excavation-replacement (practice in peats that are up to 5 meters in peat depth) and various other ground improvement methods (Edil, 2003; Kazemian, Prasad, Huat, & Barghchi, 2011; Moayedi & Nazir, 2018). Ground improvement method for peat mainly focused in improvement of geotechnical properties of peat, including its shear strength and deformation characteristics such as preloading, pilling, vertical drains, mass stabilization for shallow peat (Celik & Canakci, 2013; Hampton & Edil, 1998; Hebib & Farrell, 2003; Holm & Ahnberg, 1999) and deep in situ mixing that use cementations materials (Islam & Hashim, 2009; Islam & Hashim, 2008; Kazemian & Huat, 2009) such as synthetic polymer and cement or granular additives to improve the mechanical properties of soft soils. Deep soil mixing, which was first developed three decades ago, is known to be the most widespread approaches for stabilising soft soil, including organic and peaty soil (Fang et al., 2001; Saitoh et al., 1985). Generally, various amounts of additives are mixed into organic soils and peats to improve strength significantly.

2.2.1. Additive approach

Chemical cementation or chemical grouting is a widely used technique in geotechnical engineering. The method involved the usage of chemical grouts that is known to induce polymerization and the binding effect that bridge the soil and sediments particles together to achieve cementation-liked properties. Subsequently, the soil strength is improved as the voids between the soil particles are filled. Calcium based binders, including cement products and lime, were commonly used for soil stabilisation and traditionally preferred due to their highly robust nature and ease of obtained (Pourakbar & Huat, 2017; Prusinski & Bhattacharja, 1999). Other chemicals used may include acrylamides, calcium chloride, polyurethanes, sodium silicate, acrylates based (Indraratna et al., 2015; Karol, 2003). The chemical additives method is more economical and required a shorter time (Kazemian, Huat, et al., 2011; Kazemian, Prasad, Huat, & Barghchi, 2011; Kazemian, Prasad, Huat, Bazaz, et al., 2011).

Recently, there is a rise in awareness concerning the use of manufactured binders as there are known to be detrimental to the environment (Pourakbar & Huat, 2017). Cement products like Ordinary Portland Cement (OPC), for example, has contributed more than seven percent anthropogenic carbon dioxide (CO_2) emissions globally due during its manufacturing process (Gartner, 2004; Matthews et al., 2009). A study has reported that the one tonne of CO_2 release from every tonne of manufactured cement has caused serious global warming (Lothenbach et al., 2011). Nitrous oxide (N_2O) generated from cement production process also contributes to depletion of the ozone (Mosca et al., 2014). Apart from CO_2, clinker burning in cement production produces N_2O, SO_2 and dust, leading to acid rain and the greenhouse effect (Hendriks et al., 1998; Mosca et al., 2014). Furthermore, intensive usage of artificial chemical requires substantial production energy, which leads to energy wastage, economically unfeasible and generally environmentally harmful (Ivanov & Chu, 2008). Thus, it makes the large-scale peat treatment less feasible due to the limitation of fast hardening at the point of treatment, causing uneven distribution and viscosity nature. Such treatment methods will significantly reduce the permeability of the strengthened soil and may lead to blocked groundwater flow. In addition, uncontrol use of chemical grout in environmental soil improvement will cause extreme pH alteration on the treated soil area and groundwater contamination with increasing toxicity (Basha et al., 2005; DeJong et al., 2006; Karol, 2003).

2.2.2. Sustainable approach

The environmentally-friendly biological technique known as bio-grouting was then introduced (Ivanov & Chu, 2008). Many biological approaches with the use of microbes have been developed to treat soils naturally (Khatami & O'Kelly, 2012; Kumari & Xiang, 2019; K. S. Wani & B. Mir, 2020). These methods included structural microbial grouting or known as microbial cementation. Microbial bio-cementation (Figure 2.1.) works by forming soil particle-binding material after the introduction of microbes and specific additives into the soil which differs from bio-binding. Bio-binding, on the other hand, involved the formation of the particle-binding cellular chains and usually utilized mechanism from mycelial fungi, actinomycetes, and filamentous phototrophic and heterotrophic bacteria. Meadows et al. (1994) reported the presence of some fungal strains binds the soil grains and leads to higher shear strength in the soil. Generally, fungal hyphae bound soil aggregates leading to the formation of macroaggregates that contribute to soil stabilisation (Degens, 1997; Miller & Jastrow, 2000). However, traditional bio-binding is not suitable for improving the liquefaction resistance of land reclamation sites due to the fact that biological bindings are generally unstable and biodegradable. This shortcoming has led to the development of microbial induced bio-cementation through microbial-induced carbonate precipitation (MICP) by naturally mimicking the depositing of calcium carbonate crystal on the soil particle surface, which thereby increase the material's stiffness, strength and significant reduction of erodibility (DeJong et al., 2010; Rong et al., 2012).

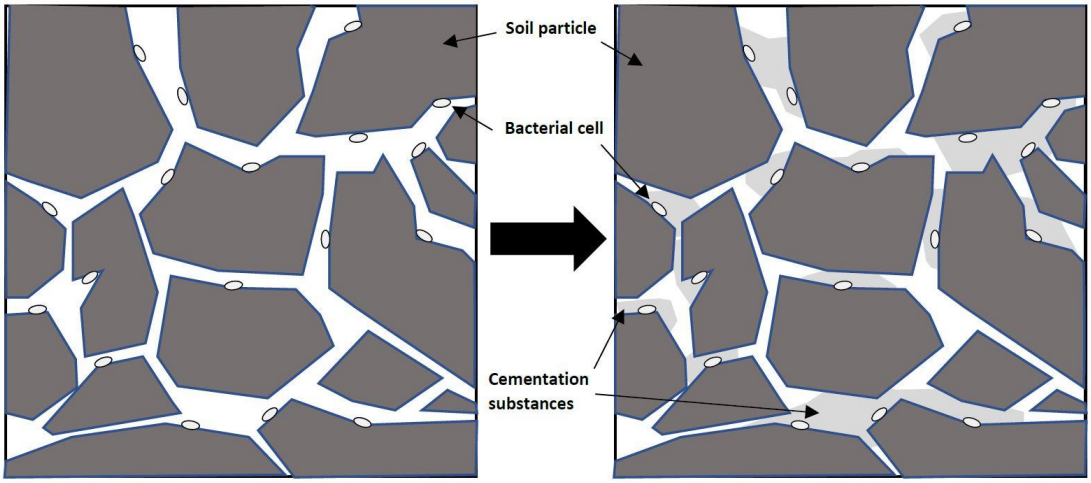


Figure 2.1. The process of bio-cementation. Bacteria are adsorbed onto the surface of loose soil (left); then the cementation substance is formed and bridges the gaps between soil particles (right) (Rong & Qian, 2012).

2.3. Microbial induced calcite precipitation (MICP)

Bosak (2011) defines Microbial Induced Calcite Precipitation (MICP) as calcium carbonate ($CaCO_3$) crystal formation in various polymorphs in the presence of microbial activity. The micro-organisms present can produce metabolites, in this case, carbonates ions (CO_3^{2-}) which, with the availability of calcium ions (Ca^{2+}) in the environment, can induce subsequent deposits of $CaCO_3$ minerals.

The microbial mineral precipitation occurs via three different mechanisms. Firstly, direct precipitation of the mineral occurs through a cellular mechanism where the microbes control the formation and growth of specific minerals via formation of intracellular magnetite crystals by magnetotactic bacteria (Phillips et al., 2013). Secondly, passive mineral precipitation can be induced biologically through the presence of cell surfaces of negative charge or other organic components such as extracellular polymeric substances (EPS) (Benzerara et al., 2011; Decho, 2010; Phillips et al., 2013). Thirdly, mineral precipitation occurs through indirect alteration of the surrounding environment and biological activity that caused increased saturation and precipitation of minerals (De Muynck, De Belie, et al., 2010). Often combinations of those different processes may be present at the same time.

The precipitation of calcium carbonate in the environment can occur via different microorganism group of different mechanisms categories such as photosynthesis,

urealysis, denitrification, ammonification, sulfate reduction, anaerobic sulfide oxidation and methane oxidation (Anbu et al., 2016). The present study utilized urealysis pathway mechanism for MICP. The MICP method was previously applied in other soil type and has reported promising results in soil consolidation (De Muynck, Verbeken, et al., 2010; DeJong et al., 2010). Urea hydrolysis is the most desired $CaCO_3$ precipitation method adopted by researchers as the process is straightforward and easily controlled, facilitating up to 90% chemical conversion efficiency of $CaCO_3$ precipitation amount in a short amount of time (Al-Thawadi, 2011; Dhami et al., 2013).

2.3.1. Ureolysis induced calcite precipitation

In MICP, ureolytic bacteria such as *S. pasteurii* hydrolyses urea in the presence of calcium ions leading to the precipitation of calcite crystals on soil particles. The presence of hydroxide ions increases the pH of water induces the precipitation of calcite. The calcites bridge and bind the soil particles together and increase the strength and stiffness of sand. Generally, a previous study suggested that higher mineral salts content would contribute to higher shear strength (Munro, 2004).

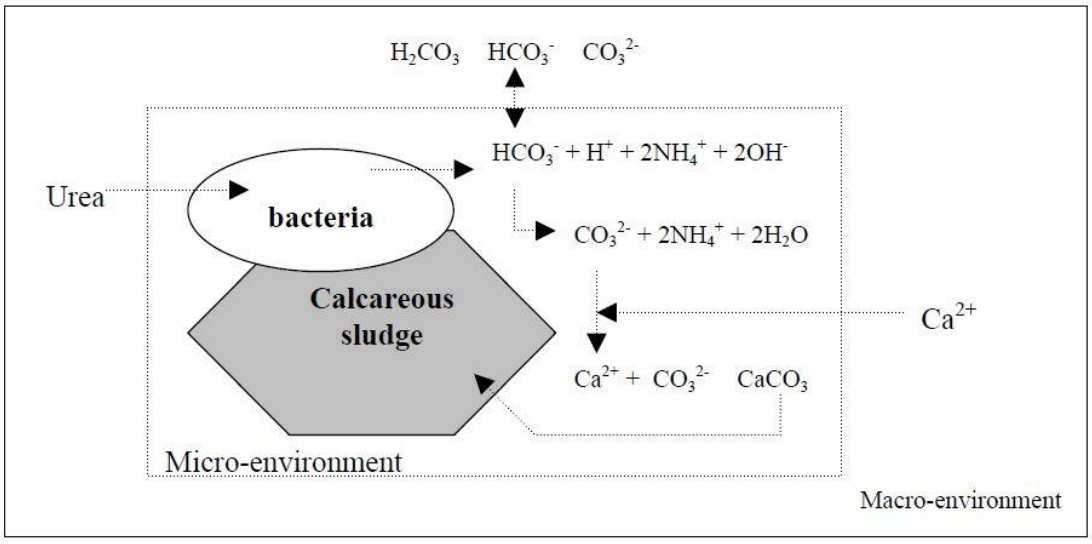


Figure 2.2. Schematic presentation of hypothesized chain-of-events for ureolytic based microbial $CaCO_3$ precipitation resulting in a localization of urease bacteria as a surface of crystal nucleation sites.

Figure 2.2. showed the process of ureolytic carbonate precipitation in a macro environment. Ureolytic bacteria first hydrolyse urea to ammonia and carbamic acid as shown in Eq. (2.1). The carbamic acid is hydrolysed spontaneously to ammonia and carbonic acid shown in Eq. (2.2).

$$CO(NH_2)_2 + H_2O \rightarrow NH_3 + NH_2COOH \quad (2.1)$$

$$NH_2COOH + H_2O \rightarrow NH_3 + H_2CO_3 \quad (2.2)$$

The ammonia and carbonic acid equilibrate in water to form bicarbonate, ammonium, and hydroxide ions shown in Eq. (3-4). Hydroxide ions produced will increase the surrounding pH and shift the bicarbonate equilibrium equation to the right resulting in carbonate ions production (Fujita et al., 2008).

$$H_2CO_3 \leftrightarrows H^+ + HCO_3^- \quad (2.3)$$

$$2NH_3 + 2H_2O \leftrightarrows 2NH_4^+ + 2OH^- \quad (2.4)$$

$$HCO_3^- + H^+ + 2OH^- \leftrightarrows CO_3^{2-} + 2H_2O \quad (2.5)$$

The continuous generation of NH_4^+ will create an alkali condition where calcium carbonate is formed with the bacteria serving as nucleation side and facilitating $CaCO_3$ precipitation at the bacterial cell surface as shown by Eq. (6-7) (Mitchell & Ferris, 2006).

$$Ca^{2+} + \text{Bacterial cell} \rightarrow \text{Cell-}Ca^{2+} \quad (2.6)$$

$$\text{Cell-}Ca^{2+} + CO_2^{-3} \rightarrow \text{Cell-}CaCO_3 \quad (2.7)$$

Urease activity, commonly found in bacteria and diverse in its strain, has diverse levels of urease activity. Bacillus group is a common type of bacteria used to produce urease and calcite precipitation. *Sporosarcina pasteurii (*previously known as *Bacillus pasteurii)*, a non-pathogen gram-positive aerobic soil bacterium with endospore formation, was found to yield high urease activity in an optimum pH at 9.0, which has been extensively used for MICP study (Al Qabany & Soga, 2013; Al Qabany et al., 2011; Cuthbert et al., 2012; Gorospe et al., 2013). Other species and strains that has been studied for urease induced calcium carbonate precipitation included *A. aerogenes*,

B. megaterium, B. subtilis, B. thuringiensis, D. halophila, H. eurihalina, H. pylori, K. flava, L. sphaericus, M. parvum, M. xanthus, P. mirabilis, P. denitrificans, Spoloactobacillus sp., and S. ginsengisoli (Anbu et al., 2016).

In general, different bacteria have different capability of urease activity and calcium carbonate precipitation in different condition. Aside from wild type, a mutant strain of urease bacteria such as *S. pasteurii* MTCC 1761 was also studied and found with a higher level of urease activity and calcite formation in comparison to wild strain (Achal et al., 2009b).

2.4. Application of MICP in geotechnical Engineering

The use of biological technologies in geotechnical engineering has been increasing recently. The application of the MICP technique has shown potential in various studies, including improvement in the strength of sand (Rong et al., 2012); improvement of foundation settlement (DeJong et al., 2010); soil hydraulic conductivity (Dennis & Turner, 1998); liquefaction mitigation (Montoya et al., 2012); self-healing concrete, strength improvement and cracks filling (Seifan & Berenjian, 2018; Seifan et al., 2016). Although many literatures have discussed the use of MICP but this field of study is relatively new and required more years of exciting study ahead to fully optimize its potential through laboratory experiments to full-scale *in-situ* implementation and development of reliable monitoring of performance in real-life situations as well as commercialization of the product to meet society needs (Parmar & Singh, 2014).

For the use in soil improvement, most MICP study focuses on bio-cementation in the sand (Al Qabany & Soga, 2013; Al Qabany et al., 2011; Harkes et al., 2010; van Paassen et al., 2010; Whiffin et al., 2007). Whiffin et al. (2007) reported sand stabilization with *S. pasteurii* inoculation with a calcium chloride solution has demonstrated a significant reduction in porosity and improved its strength in packed columns condition. van Paassen et al. (2010) scaled up the MICP study of sand towards 100 $m^{3,}$ and promising results were obtained in terms of ground improvement. Burbank et al. (2011) then proceed with a field test to study how MICP improved the strength of liquefiable soils. The shore of Snake River, USA were exposed to MICP treatments, and results showed approximately 1% by weight of $CaCO_3$ of soil cementation in the near-surface with more than 1.8% calcite formation below 90 cm of the soil (Burbank et al., 2011). Sharma and Ramkrishnan (2016) described that calcite precipitates

formed were bound intimately with soil composite and thus enhanced the bonding between the soil particles. The bond formation between soil particles leads to an increase in cohesion of soil which is one of the parameters for shear strength of soil leading to higher shear strength.

Ng et al. (2012) applied *Bacillus megaterium* to treat residual soil and found that the shear strength ratio of treated to untreated soils was increased at values ranging from 1.40 to 2.64. Recently, the application is extended to residual soil consisted of about 40% of sand particles and 60% of fine-grained particle (Lee et al., 2013; Soon et al., 2014). To date, most of the application of MICP is limited to coarse-grained soil, mainly sand. Though Mitchell and Santamarina (2005) stated that organic soil is a good candidate soil for bio-modification; however, to the best of the author's knowledge, there is limited application of MICP to peat.

2.5. Related study of MICP on organic soil

Table 2-1 Summary of related study on MICP for organic soil stabilisation

Soil type	Urease source	Treatment	Testing	Source
Organic soil - Sakarya, Turkey (60% LOI, pH 6)	*S. pasteurii*	Dry loose peat placed in container. Flushed with cementation reagent at 20 ml/min.	Direct shear	Canakci et al. (2015a)
Organic soil - Sakarya, Turkey (60% LOI, pH 6.5)	*S. pasteurii* NCIMB 8221	Soil dried at 80°C and packed at dry density of 0.6 and 0.69 g/cm^3. Flushed with cementation reagent at 20 ml/min.	Direct shear tests; One-dimensional consolidation tests	Canakci et al. (2015b)
Peaty soil – Hokkaido, Japan (56.65% LOI, pH 4.1)	Indigenous, urease (from *Canavalia gladiata*)	Peaty soil adjusts pH with sodium bicarbonate. Premix method and cured without compaction at 20°C.	Unconfined compression test	Sato et al. (2016)

A preliminary study was done by Canakci et al. (2015a) on MICP to stabilise organic soil obtained from Turkey using *S. pasteurii*. The soil was packed loosely (0.69 gm/cm^3) and pre-treated with *S. pasteurii*. Treatment was done by gravitational flushing of urea and $CaCl_2$ at 20 mL/min every 6 hours interval up to 5 days at 28 ◦C in a temperature-controlled room. Then, the samples were cured at room temperature (25 ◦C) for five days before a direct shear test was performed. The study showed $CaCO_3$ precipitation up to 16% by sample weight and increased shear stress between treated and untreated under normal stress (15 kPa).

In another study carried out by Canakci et al. (2015b), organic soil of the same region was used with 60% organic content, 15% silt and clay, and 25% sand. The soil was dried at 80°C to sterilise it before packed at 0.6 - 0.69 g/cm^3 and followed with pre-treatment by flushing (gravitational at 20 mL/min) with 500 mL bacteria media consisting of *S. pasteurii* NCIMB 8221 (1.5 x 10^8 – 12 x 10^8 CFU/ml), nutrient broth (3g/L), urea (20 g/L), NH_4Cl (10 g/L), $NaHCO_3$ (2.12 g/L) and $CaCl_2$ (18.5 g /100 ml). After the initial treatment, 12 hours were allowed for the sample to sit before flushing with $CaCl_2$ and urea for every 6 hours interval up to 4 days at 28 °C. The sample was then cured for another five days at 25 °C before subjecting to direct shear and one-dimensional consolidation test. Reduction of compression index (C_c) and primary consolidation based on the coefficient of consolidation (C_v) were shown by the treated sample compared with the untreated ones.

The screening was done by Sato et al. (2016) and found that indigenous urease activity was present in various peaty soil in Hokkaido, Japan, including those from Iwanai (93.81 % organic content; pH 4.3), Ebetsubuto (56.65 % organic content; pH 4.1) and Tomikawa (39 % organic content; pH 2.5). The effort was made to solidify peat soil from Ebetsubuto at 20°C for 1 and 4 months by pre-mixing various mixing proportions consisting of $CO(NH_2)_2$, $CaCl_2$, $NaHCO_3$ and urease from sward beans (*Canavalia gladiate*). It was found that unconfined compressive strength at one month after sample treatment was 25 kN/m^2 following an increased to 53 kN/m^2 after four months, which was sufficient for transport by dump trucks (50 kN/m^2) (Sato et al., 2016). It is noted that the treatment was done without compaction or preload.

Table 2.1 summarises the previous related study of MICP on stabilisation of organic soil. Although MICP technology were applied on organic soil treatment, there is still a wide gap of knowledge needed to be filled. The following section will discuss on the challenges that may occur for MICP on peat.

2.6. Challenges of MICP on peat

Mujah et al. (2017) reviewed that most studies on MICP were conducted on inorganic soil, including sand and fine-grained soil like residual soil. The previous study of MICP involved limited use of peat, and challenge may arise in terms of factors that may affect the $CaCO_3$ precipitation when utilizing MICP on peat. Peat is known to be

in acidic bog condition in a natural environment. This may raise certain challenges like pH, which affect the performance of MICP directly.

MICP can be applied to fibrous peat, which consists of quite large particles and large void by nature. Bacteria have a variety of shape, including oval, rod-like, or spiral with cell diameter ranging from 0.5 to 3 µm, and bacterial spores, stress-resistant resting stages of some species, may be as small as 0.2 µm (Madigan & Martinko, 2005). Motile microorganisms have the capability of moving freely in the pore spaces of coarse-grained materials, either by self-propelled movement or by passive diffusion, which means the presence of smaller pore throats of fine-grained soils prohibit their entry and free passage. Therefore, bacteria are not expected to enter through pore throats smaller than approximately 0.4 µm, and a previous study found that bacteria activity is hindered in kaolinite with particles less than 2 µm (Mitchell & Santamarina, 2005; Rebata-Landa, 2007). Hence, for amorphous peat, most of the particles are less than 2 µm (Huat et al., 2011); this may have rendered MICP treatment expected to be ineffective in amorphous peat. However, there is no study to prove the hypotheses yet. Landva and Pheeney (1980) found that stem diameters of 20 to 500 µm and leaf with a thickness of 10 to 15 µm, length and width of 100 to 1200 µm are common for fibrous peat. Thus, MICP may show to have potential in fibrous peat.

2.6.1. Bacteria strain suitability

Sporosarcina pasteurii (previously *Bacillus pausterii*) were commonly used for bio-cementation in the sand and residual soils due to their high urease activity in a short time frame that favours the production of high carbonate precipitates (Dhami et al., 2013; Wei et al., 2015). However, such aerobic strain was mostly used in an alkaline environment and was found to be inhibited in anoxic condition (Martin et al., 2012). Urease itself is one type of hydrolases, and generally, hydrolases are not affected by the presence or absence of oxygen to function (Freeman et al., 2001). From a recent study, by using purified enzyme, urease activity was shown to be independent of the oxidization-reduction environment (Jiang et al., 2016). This finding encourages the development of ureolytic based MICP at anoxic peat condition. However, the major concern would be the ability of bacteria to acclimate and grow at anoxic condition while producing urease enzyme. The use of *Bacillus megaterium* (ATCC 14581) was found to performed better than *S. pasteurii* for MICP uses in anoxic condition and

suggested the strain used in subseafloor (Jiang et al., 2016). As peatlands are usually anoxic with waterlogged in undrained condition with an acidic pH environment (Ehrenfeld, 1989), isolation and identification of alternative strain from indigenous sources is essential for utilizing MICP approach with tropical peat.

2.6.2. Acidic nature and organic acid of peat

An alkaline environment is important for the MICP to occur. High pH condition favours the formation of CO_3^{2-} from HCO^{3-} (Knoll, 2003). Tropical lowland peats are generally acidic between pH 3.2 to 3.8, but they may vary from pH 3.3 to 7.3 (Huat et al., 2011; Kazemian, Prasad, Huat, & Barghchi, 2011). Peat with extremely low pH (<3.2) is generally influenced by acid sulphate properties (Maltby et al., 1996), but the pH of peat water will be higher (pH 4.0 to 4.5) due to the dilution effect. The pH of the surrounding environment can affect calcite formation since the urease enzyme will only be active at pH values specific for urea hydrolysis. Stocks-Fischer et al. (1999) and Gorospe et al. (2013) reported that MICP were optimum at a pH level of 8 while higher pH may decrease enzyme activity yields. They also discovered that urease activity for MICP at pH 6 is relatively slow and increases linearly with pH until a peak at pH 8 before decreasing linearly with pH. The carbonate will tend to dissolve in lower pH rather than precipitate out as solid (Lowenthal & Marais, 1976). Basically, calcite precipitation forms under alkaline conditions between pH 8 to 9.5 (Ferris et al., 2004; Stocks-Fischer et al., 1999). However, Mobley et al. (1995) found that the possibility of optimum urease activity in neutral pH. Typical aerobic bacteria released CO_2 via cell respiration, which is paralleled by an increase in pH due to ammonia production (Ng et al., 2012). The urease activity may further increase the pH until it is favourable for MICP. Thus, the challenge is in the starting initial pH condition, where the bacteria start to acclimate and reproduce. Bang et al. (2001) employed polyurethane-encapsulated bacterial cells to protect them from high cement pH. However, no effort was discussed on employing bacteria for MICP effort at lower pH.

Peat has been known to have a high amount of humic substances, including humic acid and fulvic acid, that contribute to its high organic content and low pH environment (Urban et al., 1989; Wiłkomirski & Malawska, 2004). Organic acid has been known to affect calcium-based stabilisation effort due to the reactivity of humic and fulvic acid towards cations (Van Dijk, 1971). Humic and fulvic acid were found to form a

complex with cations (Ong & Bisque, 1968; Shi et al., 2007). This will reduce the availability of calcium ions needed for peat stabilisation. The previous study has shown that organic matter retard cement-based (calcium cation are required for reaction) stabilisation effort and required more additives to be added to enhance stabilisation performance (Chen & Wang, 2006). Another study also found that humic acid would disrupt stabilisation that uses silica and alumina in soil (Jawad et al. 2014). This leads to the decline in the strength of lime stabilised soil as Ca^{2+} ions react with humic acid and lead to ineffectiveness of calcium crystallisation and pozzolanic reaction in soil (Jawad et al., 2014). Furthermore, humic was found to inhibit calcite nucleation (Lin et al., 2005). However, Morse et al. (2007) has suggested that the presence of natural organic matter in nucleation inhibition of calcite may be speculation without solid data. Natural environment condition, however, is complex and may behave differently. This can be a challenge for MICP treatment when used in peat as Ca^{2+} are essential for forming calcium carbonate crystal that leads to the bio-cementation effort.

2.6.3. Durability of MICP induced peat

Another concern about the feasibility of MICP in peat is the durability of calcite presence in lower pH of natural peatland when expose to surrounding acidic environment. The previous study done mainly was based on the non-acidic environmental condition. Stabnikov et al. (2011) utilized halotolerant alkaliphilic Bacillus sp. VS1 to seal a layered sand pond producing a nearly impermeable crust (> 1 mm). Subsurface MICP barriers were durable enough to solve the saltwater intrusion issue during groundwater extraction (Rusu et al., 2011). In contrast to the coastal environment and freshwater pond, there are challenges for applying MICP in acidic peat. Due to the acidic environment of peat ground, the calcite in the treated peat column may react with humic acid from the surrounding peat to form calcium humate. However, calcium humate is partially soluble (Kříženecká et al., 2014) and may accumulate surrounding the treated peat. This might prevent a further acid attack on the calcite and enhance the durability of MICP treated peat. MICP studies were mostly focus in sand and inorganic soil. MICP of tropical peat required further research as there is a lack of understanding of how MICP might react in a highly organic and acidic environment of tropical peat. Hence, this study will provide a valuable insight into the knowledge gap of MICP process in peat.

CHAPTER 3: RESEARCH METHODOLOGY

Materials and methodologies adopted in the experiments are introduced in this chapter. The research flow is shown in Figure 3.1.

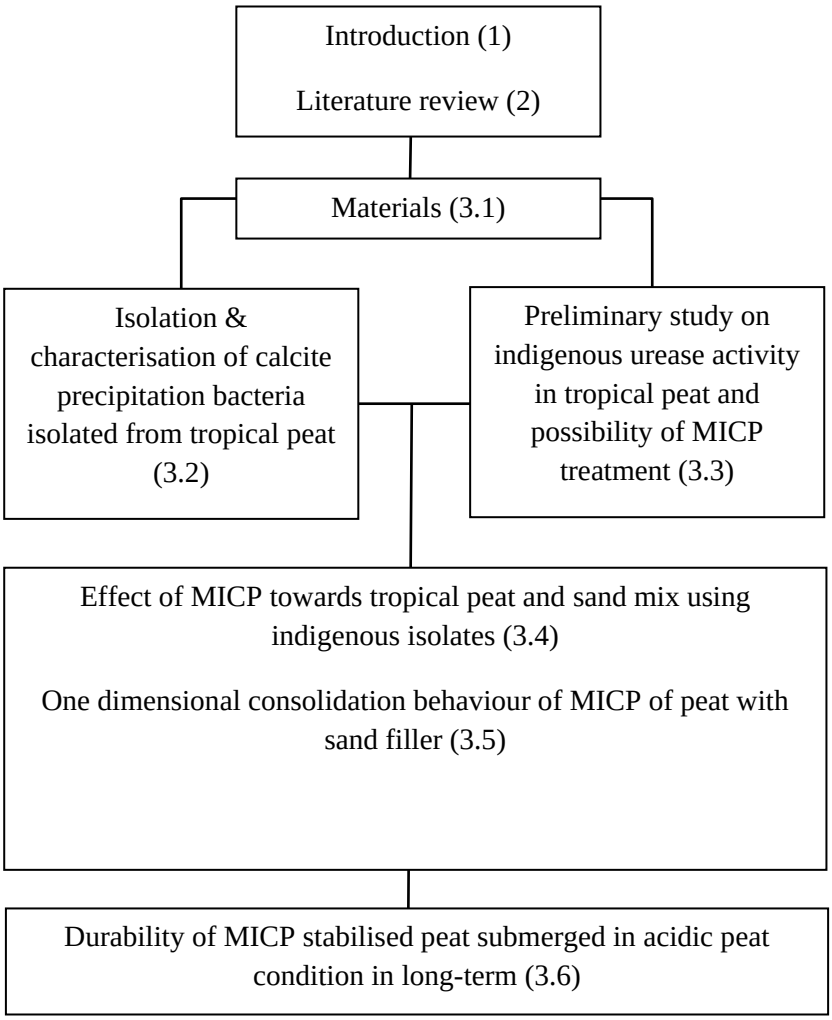


Figure 3.1. An overview of the flow of study.

3.1. Materials

3.1.1. Chemicals and reagents

All analytical grade chemicals used were purchased either from Sigma (USA), Fischer Scientific or Merck (Darmstadt, Germany). Nutrient agar was purchased from Sigma (USA), while Urea Agar Base (Christensen) was obtained from Himedia (India). Calcium chloride ($CaCl_2$) and urea used in the microbiological study were from Sigma (USA). $CaCl_2$ and urea of technical grades were also used for application study on peat. Chemical composition analysis was done for technical grade chemicals.

3.1.2. Peat sampling, characterization, and preparation

Peat sample was obtained from Curtin Malaysia (4°30'43.1"N 114°00'45.7"E) in Miri, Sarawak, shown in Figure. 3.2. Tropical peat with high organic content (94-96%) and low pH (pH 3.9-4.9) were preferable and collected to differentiate this study from the previous study done by Sato et al. (2016) and Canakci et al. (2015a). Peat was classified and characterized using ASTM D4427 method (ASTM, 2018). Summary of the properties of collected peat, including the moisture content, the fibre content, the organic content, the ash content, the pH, the type of peat and the specific gravity, are listed in Table 3.1. The peat characterizations were done if required in between or after any treatment throughout the study period. Before performing any test, roots, and coarser fibre (Figure. 3.4.) from peat were first removed, and the wet peat was passed through a 2 mm sieve (Figure. 3.5.). The wet peat slurry was homogenized with a kitchen mixer to ensure uniform moisture distribution throughout the soil and stored for future use (Figure. 3.6.).

For bacteria isolation, peat was collected aseptically in a sterilized polyethene zipper bag. The sample collected was from 1 m depth of peat where anoxic occurs (Yavitt et al., 1990), and pH was recorded to be between 3.8 - 4.9 *in-situ* indicating the peat environment is acidic. The sample stored in a zipper bag was transferred back to the laboratory with an icebox and preserved at 4°C before subjected to further analysis (Refer to section 3.2.1). Peat used for MICP application study were dug (Figure. 3.3) and collected in bulk. The sample was stored in a container with peat water covering its surface to ensure saturation as of in the natural environment for the condition in the laboratory at room temperature (26°C ± 1°C).

Figure 3.2. Peat sampling was done locally in Curtin University Malaysia, Sarawak

Figure 3.3. Peat sampling for MICP application.

Figure 3.4. Coarse roots and other vegetative matters were removed from peat.

Figure 3.5. Wet sieving of peat. Peat passing 2 mm sieve were used.

Figure 3.6. Homogenized peat after manual removal of coarse material and sieving.

3.1.3. Sand

River sand was obtained locally, and its compositions was identified with XRF (Table 3.2). River sand was pre-washed before drying and sieving. Figure 3.7 showed the particle size distribution curve of river sand. According to ASTMD 2487-11, sand that passed through 2 mm sieve and retained on a 0.425 mm sieve is classified as medium size sand, while sand that passes through 0.425mm is classified as fine sand (ASTM, 2011a). For this study, fine sand (< 0.425mm) was used.

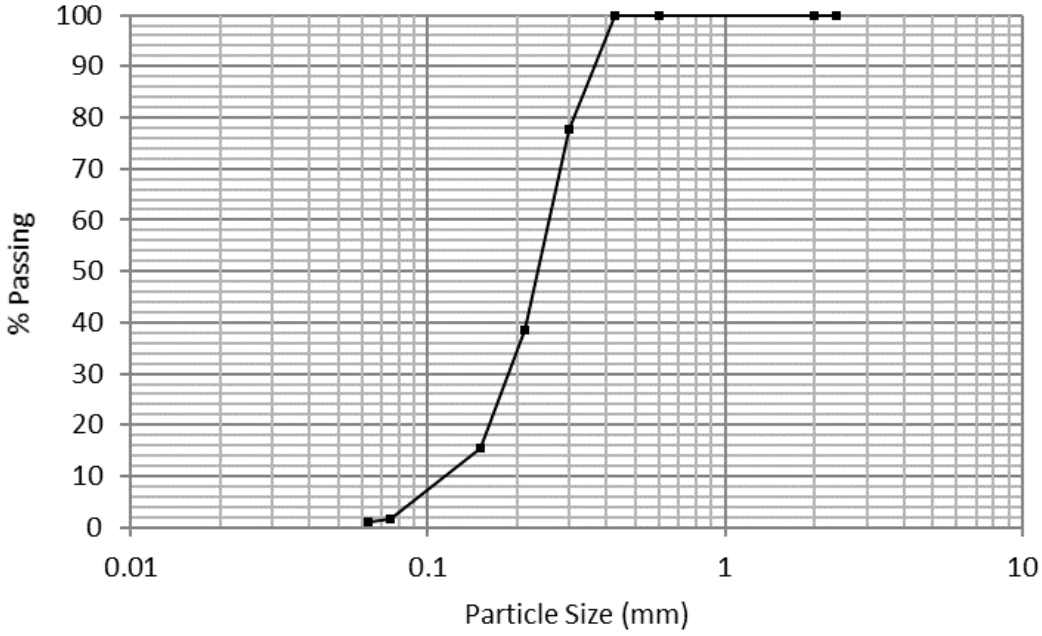


Figure 3.7. Particle size distribution curve of the river sand.

Table 3-1. Basic properties of natural peat used in the study.

Basic soil property	
Natural moisture content (%)	670 – 800
Fibre content (%)	50 - 60
Organic content (%)	94 - 96
Ash content (%)	4
pH	3.9 - 4.9
Von Post Designation	H3 - H5
Specific gravity	1.11 - 1.23

Table 3-2. Chemical composition of materials for the stabilised peat based on XRF analysis.

	Sand (%)	Calcium chloride (%)	Urea (%)
SiO_2	89.28	-	-
CaO	1.21	47.44	0.15
K_2O	0.96	-	0.02
Fe_2O_3	1.33	-	-
P_2O_5	1.48	0.39	0.35
MgO	0.25	-	-
Al_2O_3	4.26	-	0.01
SO_3	-	-	0.07
Cl	0.88	52.14	0.11
TiO_2	0.28	-	-
SrO	0.0083	0.02	-
CuO	0.0071	0.007	0.01
Rb_2O	0.0033	-	-
ZnO	0.0049	-	-
ZrO_2	0.05	-	-
V_2O_5	-	-	0.01

3.2. Isolation and characterisation calcite precipitation bacteria isolated from tropical peat

3.2.1. Isolation and characterisation

Tropical peat samples were aseptically collected at a metre depth from peatland near Curtin University Malaysia, Miri Sarawak and stored at 4°C as described in Section 3.1.2. Isolation was done by placing 1g of peat in 50 ml of Tryptic Soy Broth (TSB) (Merck, USA) supplemented with sterile urea (2%) (Sigma, USA) and incubated in an orbital shaker for 48 hours at 28 °C and 120 rpm. Potential isolates were serial diluted and purified by repetitive streaking and sub-culturing on Tryptic Soy agar (TSA) (Merck, USA). TSB, as well as TSA, were selected as it is widely used as isolation media and were used for culturing of bacteria for bio-cementation study (Pakbaz et al., 2018; Stabnikov, 2016; K. M. N. S. Wani & B. A. Mir, 2020). The preparation of the media was done following the manufacturer's instruction. The media comes buffered with a final pH 7.3 +/- 0.2 at 25°C. Selected purified strain were then streak on Urea Agar (Christensen's medium) (Himedia, India) containing peptone (1 g/L), dextrose (1 g/L), NaCl (5 g/L), KH_2PO_4 (2 g/l), phenol red (0.012 g/l) and agar (15 g/L) supplement with 2% sterile urea (Sigma, U5378). Strains were selected for their ability

to turn Urea Agar from pale yellow to pink within 12 hours, suggesting high urease activity. The strains were maintained on TSA slants at 4°C.

Cell morphology of the selected strains was examined by using light microscopy (model DM3000; Leica). For further characterisation, gram staining and the KOH lysis test was also carried out (Gregersen, 1978; Reddy et al., 2007). The spore formation of the selected strain was determined by endospore staining (Reddy et al., 2007). Cell motility determination was conducted by the development of turbidity using a semisolid medium (Leifson, 1960). Catalase activity was determined based on the evolution of bubbles with 3% H_2O_2 (v/v) solution (Reddy et al., 2007). The catalase enzyme serves to neutralize the bactericidal effects of hydrogen peroxide by expedites the breakdown of hydrogen peroxide (H_2O_2) into water and oxygen ($2\ H_2O_{2\,(aq)} + \text{Catalase}_{(aq)} \rightarrow 2\ H_2O_{(aq)} + O_{2\,(g)}$) (MacFaddin, 2000). The test serves to differentiate aerotolerant bacteria and obligate anaerobic bacteria. Growth at different temperature (20 – 50°C, an interval of 5°C) and growth tolerance of various salt (NaCl and KCl) concentrations of 0 - 12% (w/v) of 1% interval at 30°C were also done using Tryptic soy agar (TSA). High salt tolerance isolates are not crucial for the study but may have advantages of survival and adaption when exposed to high osmotic stress present with cementation reagents with high salts and chloride ions. Growth at various buffered pH (4.0 -10.0) were determined using Tryptic soy broth (TSB) at 30°C.

3.2.2. Molecular analysis

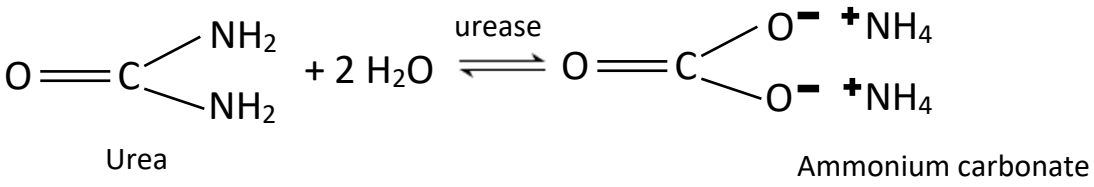
The selected isolated strains were grown overnight, and cells suspension were harvested by microfiltration (0.2 µm; Millipore), and the cell supernatant was washed in Phosphate-buffered saline (PBS). Genomic DNA was extracted from the isolated strains using MYgen^{TM} Genomic DNA Prep Kit (GeneXpress, MY). For polymerase chain reaction (PCR), the following universal primers, 27F (5'-AGAGTTTGATCCTGGCTCAG-3') and 1492R (5'-GGTTACCTTGTTACGACTT-3') were used. The PCR program was started with an initial denaturation at 95 °C for 3 mins followed by 30 cycles of 20 s at 98 °C, 15 s at 64 °C, 50 s at 72 °C, and final extension at 72 °C for 50 s. The PCR products were then purified using MYgenTM Gel & PCR Purification System (GeneXpress, MY) and sequencing were done at Centre For Chemical Biology, USM (Malaysia).

16S rRNA gene sequence was compared with database from GenBank using BLAST (Basic Local Alignment Search Tool) available online at National Center for Biotechnology Information (NCBI) (https://blast.ncbi.nlm.nih.gov/Blast.cgi) (Altschul et al., 1997; Zhang et al., 2000). A phylogenetic tree was constructed by the neighbour-joining method and maximum parsimony algorithms using MEGA X software (version 10.0.4) evaluated by bootstrap analysis (1000 replications) for confidence limits of the tree topology. Evolutionary distances matrices were computed using the Maximum Composite Likelihood method and shown in units of the number of base substitutions per site (Felsenstein, 1985; Kumar et al., 2016; Saitou & Nei, 1987; Tamura et al., 2004). The 16S rRNA gene sequences were then deposited in GenBank (NCBI) with their respective accession numbers.

3.2.3. Enzyme assay

The purpose of this section is to observe the effect of different routes of enzyme activity that leads to $CaCO_3$ precipitation, including production of urease and carbonic anhydrase enzymes for both isolated strains. The enzyme production and bacterial growth were observed throughout the incubation period for an interval of 24 hours up to 5 days, where the strain was incubated at 37°C in an orbital shaker (120 rpm). The bacterial medium used for the experiment consist of Tryptic soy broth (Merck, USA) supplemented with 5 μM nickel chloride along with 2 % urea.

Urease (UA or urea amidohydrolase, EC 3.5.1.5) catalyses the hydrolysis of urea by converting 1 mole of urea to 2 moles of ammonia and 1 mole of CO_2:



The hydrolysis of urea to ammonia is stoichiometric, hence the activity of urease was identified through ammonia formation by using modified Nessler method (APHA, 1992; Nakano et al., 1984). The bacteria pellet was washed 3 times with PBS and re-suspended into 100 mM Tris/HCl buffer at pH 8 containing 300 mM of urea and incubated at 30°C for 10 min. Then, the sample was centrifuged to obtain the supernatant. 1 mL of supernatant was added into 100 μL of Nessler reagent (Fischer Scientific) in the cuvette and allowed to react for a minute before taking the reading

with a spectrophotometer (Lambda 25 UV/Vis Double Beam, Perkin Elmer) at 425 nm. Nessler reagents react with ammonia under an alkaline reaction to produce a yellow-coloured species. Hence, one unit of urease activity is defined as the amount of enzyme that would hydrolyze 1 μmol urea per minute (corresponding to 2 μmol of ammonia) per min under the assay conditions. The absorbance readings were calibrated by using different concentration of NH_4Cl standards measured with the same conditions as above. Samples were diluted in the required range if necessary.

The carbonic anhydrase (CA, EC 4.2.1.1) activity was tested for both selected strains by using UV-Vis spectrometer (Lambda 25 UV/Vis Double Beam, Perkin Elmer) at 348nmwherep-nitrophenyl acetate (Acros) hydrolysed to form 4-nitrophenolate ion in the presence of the enzyme at 25°C (p-nitrophenyl acetate + H_2O → p-nitrophenol + acetate) (Capasso et al., 2012; Dhami et al., 2016b; Dinçer et al., 2016; Smith & Ferry, 1999). Between the interval of 24, 48, 72, 96 and 120 hours, the cultures were collected and centrifuged at 4000 rpm for 5 minutes. 200 μL of culture supernatant was added to a mixture containing1.8 ml of 100mM phosphate buffer at pH 7 and 1 ml of 3 mM p-nitrophenyl acetate solution. The increase in absorbance at 348 nm was recorded for 5 minutes. One unit of carbonic anhydrase activity is defined as the amount of enzyme required to form 1 μmole of p-nitrophenol per minute.

Optical density (OD) was known to be linearly proportional to the cell concentration in a solution based on Beer-Lambert Law (Parks, 2009). Hence, cell growth was done by measuring Optical density (OD_{600}) as an indication of bacterial growth in the culture medium using a spectrophotometer at a wavelength of 600 nm (Harkes et al., 2010). Generally, one millilitre of culture broth was harvested at the selected interval and immediately transferred to a glass cuvette, and its OD was measured as mentioned above. According to the Beer-Lambert law, the concentrated samples were diluted as needed. The final concentration of the sample was obtained by multiplying the dilution coefficient with the OD value.

3.2.3. Calcium carbonate ($CaCO_3$) precipitation study

An *in-vitro* study was done to evaluate the precipitation capability of both strains in different precipitation medium. The precipitation medium was selected based on a previous study, as shown in Table 3.3. Broth A and B were media based on urea hydrolysis by mean of urease activity for calcite precipitation, while Broth C was used

to study the possibility of precipitation with urea addition mainly through carbonic anhydrase activity. Broth B was used to study the possibility of $CaCO_3$ production in high calcium chloride content as the previous study has also shown an inhibitory effect of $CaCO_3$ production with $CaCl_2$ more than 40 g/L (Nemati et al., 2005). Broth C or commonly as B4 medium, was used in the study of $CaCO_3$ precipitation from carbonic anhydrase mechanism (Silva-Castro et al., 2013; Uad et al., 2014). Bacterial suspension at the exponential phase of growth was used with 100 mL of sterile precipitation media and the flask were incubated in orbital shaker at 28 °C and 120 rpm. The precipitated carbonates were collected by filtration (Whatman No. 1 filter paper), washed with distilled water, and dried at 50°C for 48 h. The filters were weighed before and after the collection of crystals to estimate the amounts of carbonate crystals precipitated by the different strain at different medium. The supernatant was subsequently discarded, and the resulting precipitate was washed with distilled water, dried in an oven at 50 °C for one hour and then weighed. (Achal & Pan, 2011; Rangamaran & Shanmugam, 2018; Zamarreño et al., 2009)

To identify the presence of carbonate crystal phase in the precipitate, the precipitates retained on the membrane filter was analysed through the X-ray diffraction (XRD). X-ray diffraction (XRD) measurements were taken by a SEIFERT diffractometer (XRD 3003 PTS, Germany) using monochromatized Cu K radiation to follow the $CaCO_3$ precipitation pattern. X-ray Diffractometer (XRD) was done to identify crystal phase of precipitated $CaCO_3$. Samples were analysed using XRD, and crystalline mineral phases search done using Crystallography Open Database (COD) (Rev. 198327) (http://www.crystallography.net/cod/) (Gražulis et al., 2011).

3.2.4. Bio-cementation study

In order to test the bio-cementation process on sandy soil under laboratory conditions, a polyvinyl chloride tube with an internal diameter of 2.6 cm was positioned vertically and packed with fine river sand (grain size characteristics: d_{10} = 0.13 mm (10% of the grains have a diameter of this size or lower); d_{50} = 0.24 mm; d_{90} = 0.37 mm) up to a height of 7cm lightly packed with a dry density of 1.51 g cm^{-3}. The sand column was soaked with bacteria suspension at the exponential phase of growth for 4 hours. Then, sand columns were fed continuously by gravity with 100 mL of Media B (Table. 3.3) at room temperature to mimic the natural environmental conditions. This was done by

collecting the effluent every 12 hours, and the effluent was reused to feed the column. The effort was continued up to 14 days by replacing with fresh media every three days.

The bio-cementation process on the sand column was evaluated based on strength gain (initial sand cohesion is assumed to be zero) determined by the unconfined compression test as done by a previous study (Moosazadeh et al., 2018). This test was performed on an unconfined compression machine following ASTM D2166 specifications with an axial strain at a rate of 2% min^{-1} (ASTM, 2016a).

Table 3-3. Selected precipitation medium used for the study.

Media	Composition	Reference
Broth A (YE–Ur–$CaCl_2$)	Yeast extract (20g/L), urea (20g/L), $CaCl_2$ (50g/L)	De Muynck, Verbeken, et al. (2010)
Broth B	Yeast extract (20g/L), Urea (60.06g/L), $CaCl_2$ (110.98g/L)	-
Broth C (B4 medium)	Yeast extract 1g/L, $Ca(C_2H_3O_2)_2$ (5g/L), Glucose (1g/L)	Baskar et al. (2006); Zamarreño et al. (2009)

3.3. Preliminary study on indigenous urease activity in tropical peat and possibility of MICP treatment

3.3.1. Sample preparation

Peat samples with coarse fibres roots were removed, and wet peat slurry passed through 2 mm sieve were used for the testing as described in Section 3.1.4. Peat slurry was then collected and stored in a single container to ensure homogeneity in its moisture distribution. The moisture content of the peat slurry was about 400%. 500g of the peat slurry were mixed separately with 100 mL of three different combinations of cementation mixtures containing urea (Sigma-Aldrich) and $CaCl_2$ (Merck, Germany), namely: with urea only (60.06g), with $CaCl_2$ only (110.98g), and another containing both urea (60.06g) and $CaCl_2$ (110.98g). The reagents were pre-sterilized to ensure free from any microbial agents. Peat without any admixture was mixed with 100 mL of distilled water which acting as a control. The peat slurry was homogenized for 5 minutes with a kitchen mixer to ensure uniform moisture distribution. The curing method was adopted from EuroSoilStab (2001). The peat mixture was poured into a PVC tube with a size of 53 mm internal diameter and 260 mm height with porous stone covering both openings. The tubes were placed vertically submerged in water with a

surcharge load of 9 kPa, simulating 1m of sand layer. The samples were cured at room temperature for the period of 3, 7, 14 and 28 days before subjecting to testing.

3.3.2. Unconfined Compression Tests

The effectiveness of bio-cementation to peat strength was evaluated by measuring Unconfined compression strength (UCS) according to the procedure described in ASTM D2166 (ASTM, 2016b). The samples were extruded from the cylindrical pipe after curing and trimmed carefully with minimum disturbances to form samples with a diameter-to-height ratio of 1:2 (53 mm x 106 mm). The samples were then tested using Universal Testing Machine (Lloyd Instruments) with a loading rate of 2.0 mm/min. After the testing, each sample was cut into three portions (top, middle and bottom), and 1g of each portion was subjected to ammonia determination. Then, the rest of the portions are combined and dried for calcium carbonates determination. For the sample with the highest unconfined compressive strength, a portion of peat fabric was cut and preserved for SEM imaging and XRD analysis.

3.3.3. Calcium Carbonate ($CaCO_3$) precipitation and ammonia determination

The percentage of $CaCO_3$ precipitation of the samples was determined by acid washing technique (Keykha et al., 2017; Mortensen et al., 2011). Samples were initially rinsed with distilled water to remove excess $CaCl_2$ and urea on peat particles. Samples were dried in the oven with their mass measured before and after rinsing with HCl (5M). Generally, the samples were rinsed multiple times on filter paper to allow HCl (Merck, Germany) to dissolve the carbonate salt while passing through the filter. The difference between the measured mass before and after rinsing was taken as the mass of $CaCO_3$ and the results were expressed at percentage of precipitated $CaCO_3$ over the dry mass of peat. pH of wet peat after curing was also measured based on ASTM D4972. Ammonia concentration was determined using the Nessler method (Cheng et al., 2014; Greenberg et al., 1992). All tests were done in triplicates, and results were shown in mean value.

3.3.4. Scanning Electron Microscopy (SEM) and X-ray diffraction analysis

For the sample with the highest unconfine compressive strength, a portion of peat fabric was cut and preserved for SEM imaging and XRD analysis. The fabric of the

peat surface with calcium carbonate precipitation was observed by scanning electron microscopy (SEM). The collected dry samples were mounted directly into the SEM stubs and sputter-coated with a gold/palladium mixture. X-ray Diffractometer (XRD) was done to identify the crystal phase of precipitated $CaCO_3$ shown in SEM analysis. Samples were analysed using XRD, and crystalline mineral phases search done using Crystallography Open Database (COD) (Rev. 198327) as described by (Gražulis et al., 2011). SEM (ZEISS EVO) was performed at an external laboratory at the University College of Technology Sarawak (UCTS), while XRF (Malvern Panalytical) was performed at Central Laboratory, Universiti Malaysia Pahang.

3.4. Effect of Microbial-induced Calcite Precipitation towards tropical peat and sand mix using indigenous isolates

MICP based on urea hydrolysis pathway was used to induce bio-cementation reaction in this study. Previous study makes use of exogenous bacteria as urease source. Ureolytic non-pathogenic bacteria such as *S. pasteurii* and *B. megaterium* were commonly introduced to the target soil to induce urea hydrolysis to facilitate bio-cementation mechanism (Achal et al., 2009b; Ng et al., 2012). Indigenous sources of ureolytic bacteria isolated from tropical peat (isolated from the same area of peatland used for the current study), bacteria strain P19 (GenBank MH639002) which was identified as *Enteractinococcus sp.* and bacteria strain P21 (Genbank MH639001) identified as *Staphylococcus sp.* were obtained from Chapter 4. Literature has also shown the presence of ureolytic microbial sources in tropical peat (Blonska, 2010; Phang et al., 2018). Hence, MICP by indigenous microbial sources in peat itself were also observed in this study.

3.4.1. Bacterial culture preparation

Seed culture was prepared by transferring a small amount of the bacterial culture into 100 ml of Tryptic Soy Broth (TSB) (Merck, USA) supplemented with sterile urea (2%) (Sigma, USA) and incubated in an orbital shaker up to 48 hours at 28 °C and 120 rpm. The cells culture was harvested by centrifugation for 10 min at 5000g at 4°C. The harvested cells were then washed twice in sodium phosphate buffer 0.1 M of pH 7 to remove metabolic waste products from bacterial growth that may cause interference with the experimental study. Bacterial cells were resuspended with saline solution at

the required concentration of 10^5, 10^6, 10^7 and 10^8 CFU/mL. The indigenous ureolytic source was also observed in this study without bacteria addition.

3.4.2. Reconstituted peat samples preparation

Reconstituted peat samples were used for the experiment. Sand was used as filler for a previous study related to calcium-based stabilisation effort (Nikookar et al., 2012; Rahgozar & Saberian, 2016; Saberian & Rahgozar, 2016; Sing, Hashim, et al., 2008; Venuja et al., 2017; Wong et al., 2013). Previous study has shown the used of sand as a filler up to 50 % dry sand to wet weight of peat (Sing, Hshim, et al., 2008; Zain et al., 2019). For this study, peat slurry was mixed with 25%, 50% and 75% of sand to weight of wet peat slurry. For example, to prepare 50% sand peat mixture, 50 kg of sand (dry weight) was added to 100 kg of slurry peat (wet weight). Cementation reagent was prepared by adding equal molars of urea and calcium chloride. XRF analysis of urea and calcium chloride are shown in Table 3.2. Study for MICP done on inorganic soil has cementation reagent between 0.1 M to 1 M (Al Qabany & Soga, 2013; Maleki et al., 2016). In circumstances that the samples used in the study are highly organic peat, the dosage was increased up to 4 mol/kg to evaluate its performance. Dosage applications were done in range of 0.1 – 4 mol/kg towards wet weight of peat slurry (Table 3.4.). To produce stabilised peat admixture, peat slurry was mixed with dried sand followed by mixing different dosage of cementation reagents and 100 mL of bacteria culture followed by homogenising for 5 minutes with kitchen mixer to ensure that uniform distribution. The peat mixture was then carefully poured into the mould with the best effort without introducing too much air bubbles. Table 3.5. summarised the mix design for testing.

The moulds used for the unconfined compression test were PVC tubes with 50 mm internal diameter and 250 mm long. For falling head tests, the moulds were cylindrical PVC tubes of 63 mm internal diameter and 300 mm tall. MICP treatment for soil was commonly performed through flushing or injection technique and surface percolation method (Mujah et al., 2017). Those techniques ensure continuous feeding of oxygen and flow of cementation reagent with or without bacteria agent. Peat has low permeability or hydraulic conductivity, where most peat area are in swampy, waterlogged and anoxic condition (Chason & Siegel, 1986; Landva & Pheeney, 1980; Quinton et al., 2008). Hence, for this study, wet curing for soft soil stabilisation

simulating saturated field condition was performed (EuroSoilStab, 2001; Hebib & Farrell, 2003). The tubes containing mixed samples were placed vertically submerged in water with a surcharge load of 9 kPa as discussed in Section 3.3.1. The samples were cured at room temperature for the required period before subjecting to testing.

Table 3-4. Dosage concentration of cementation reagent consisting of calcium chloride and urea to wet weight of peat slurry.

mol/kg	$CaCl_2$ (%)	Urea (%)	Total (%)
0.1	1.11	0.60	1.71
0.2	2.22	1.20	3.42
1	11.10	6.01	17.10
2	22.20	12.01	34.21
4	44.39	24.02	68.42

Table 3-5. Experimental design for peat stabilisation study for geotechnical testing

Set test	Test	Sand (%)	Bacteria	Cementation reagent dosage (mol/kg)	Curing duration (days)
1	UCS	25	P19, P21, Indigenous	2	28
2	UCS	25	Selected from Test 1, Indigenous	0.1, 0.2, 1, 2, 4	28
3	UCS	25, 50, 75	Selected from test 2	Selected from test 2	3, 7, 14, 28
4	Permeability	25, 50, 75	Selected from test 2	Selected from test 2	3, 7, 28

3.4.3. Geotechnical tests

Unconfined compression test and falling head permeability tests were performed to quantify the mechanical properties of the test samples. MICP study previously shown

that MICP occurring in soil may resulted bio-cementation of soil that leads to improve of unconfined compressive strength of soil and reduction in permeability. The effectiveness of bio-cementation to peat strength was evaluated by measuring unconfined compressive strength (UCS) according to the procedure described in ASTM D2166 (ASTM, 2016a). After curing, the samples were extruded with soil lab extruder and trimmed carefully with minimum disturbances with standard soil lab trimmer to form samples with a diameter-to-height ratio of 1:2 (53 mm x 106 mm). The samples were then tested using Universal Testing Machine (Lloyd Instruments) with a loading rate of 2.0 mm/min. The unconfined compressive strength recorded as the peak stress of the soil stress-strain curve or is identified as peak stress correspond to vertical strain reaches 20% as described by a previous study (Sing, Hashim, et al., 2008). All tests were done in triplicates, and results were shown in mean value. Selected samples were dried and proceed to quantify for calcium carbonate.

Permeability changes due to MICP were evaluated through hydraulic conductivity measurements of peat with conventional falling head apparatus (ELE International, UK) according to ASTM 4511 method (ASTM, 2011b). The time taken for a measured quantity of water to flow through the sample was recorded, and the coefficient of permeability, k (m/s) was calculated based on the formula (ASTM, 2011b) below:

$$k = \frac{L}{A\,(\Delta\, H)}\; x\; Q/t$$

where:

k = hydraulic conductivity, m/s,
Q/t = rate of water outflow, m3/s,
A = cross-sectional are of sample, m2,
L = length of sample, m, and
Δ H = value of constant head, m, required to maintain a
sustained flow rate, Q/t.

3.4.4. Calcium Carbonate ($CaCO_3$) precipitation

The amount of $CaCO_3$ precipitation in the samples was quantified to evaluate the MICP performance for peat-sand mixtures. The amount of precipitated $CaCO_3$ was quantified based on the method described in Section 3.3.3.

3.4.5. Scanning Electron Microscopy with Energy Dispersive Spectroscopy (SEM-EDS) and X-ray diffraction analysis

Selected samples of the treated and untreated sample were dried and crushed before X-ray Diffractometer (XRD) analysis to study the crystal phase precipitation that occurred in the samples. XRD (Brunker) analysis was performed at the external laboratory at Universiti Malaysia Pahang (UMP). XRD analysis was commonly used to study polymorph crystal phase of calcium carbonate from microbial induced including calcite, vaterite and aragonite from microbial induced precipitation (Ganendra et al., 2014; Rodriguez-Navarro et al., 2003). Then, the selected representative was analysed using Scanning Electron Microscopy with Energy Dispersive Spectroscopy (SEM-EDS) to study the micro-surface of MICP treated peat-sand mix samples. The samples were pre-coated with thin layer of gold/platinum mix coating to provide SEM with conduction path while protecting the fragile peat samples by dissipate heat build-up and minimize beam damage. Low acceleration voltage of 10kV – 20kV was used as higher voltage will damage the samples and unable to obtain a clear image resolution. SEM analysis coupled with X-ray spectroscopy were commonly used to visualise bio-cementation or bio-precipitation on inorganic soil due to MICP (Achal et al., 2012; Burbank et al., 2012; DeJong et al., 2006; Kim & Youn, 2016; Mahawish et al., 2018).

3.5. One-dimensional consolidation behaviour of Microbial Induced Calcite Precipitation (MICP) of peat with sand filler

3.5.1. Samples preparation

Peat and sand used were prepared as described in Section 3.1.2. and Section 3.1.4. respectively. Selected isolates and bacterial culture for MICP preparation were described in 3.4.1. Reconstituted peat samples with different percentage of sand were used for the experiment. Collected peat slurry was then mixed and stored in a single

container to homogenise it. For this study, peat slurry was mixed with 25%, 50% and 75% of sand (dry weight) to weight of wet peat slurry. Cementation reagent was prepared by adding equal molars of urea and calcium chloride at 2 mol/kg of wet pet slurry. XRF chemical analysis of urea and calcium chloride are shown in Table 3.2. To produce each stabilised peat, peat-sand mixture was mixed with cementation reagents and 100 mL (10^8 CFU/mL) of bacteria culture for treated samples (PTS0, PTS25, PTS50 and PTS75) followed by homogenising for 5 minutes with kitchen mixer to ensure that uniform distribution. Sample preparation was illustrated in Figure 3.8. Untreated samples were also prepared without cementation reagent to compared with treated samples. Table 3.6. summarised the mix design for testing.

Figure 3.8. Sample preparation flow of peat samples treated with MICP (bacteria and cementation reagent.

Table 3-6. Experimental design for treated and untreated peat samples varies with different sand content.

Sample Abbreviation	Sand to peat slurry (%)	Dry density (kg/m^3)	MICP Treatment
PS0	0	0.23	Untreated samples
PS25	25	0.52	
PS50	50	0.79	
PS75	75	0.90	
PTS0	0	0.27	Treated samples
PTS25	25	0.73	
PTS50	50	1.00	
PTS57	75	1.35	

The curing method was performed according to Section 3.4.2. A similar curing method was performed by Bobet et al. (2011) to study the consolidation study of cemented organic soil. In general, PVC tubes of 160 mm diameter containing mixed samples were placed vertically submerged in water with surcharge loading of 9kPa with an ending covered with geotextile and porous stone. The samples were cured at room temperature up to 28 days before subjecting to testing.

3.5.2. One-dimensional odometer testing

Incremental loading Oedometer tests were performed to determine the consolidation behaviour of the samples according to ASTM D2435/D2435M (ASTM). The loading conditions were at 50, 100, 200 and 400 kPa and the following unloading at 200, 100 and 50kPa. Each loading was performed for 24 hours, and loading-unloading cycles for each sample take around 8 days.

Specific gravity tests were performed according to ASTM D854-14 (ASTM). The average value of three pycnometers of specific gravity was used in the Oedometer results analysis for each sample. Void ratio, e and height of solid, Hs were calculated for e vs log σ' graph for each sample according to ASTM D2435/D2435M to obtain Compression Index, C_c and Swelling Index, C_s (ASTM, 2011c). Compression index, C_c can be defined as the change of the void ratio against the change of effective vertical stress, $\Delta e/\Delta \log \sigma'_v$ and can be used for evaluating primary consolidation of soil (Madaschi & Gajo, 2015; Mesri et al., 1997). Coefficient of Primary Consolidation, C_v were obtained from square root time method based on $C_v = 0.848*H_d^2/t_{90}$ (ASTM, 2011c). Secondary Compression Index, Cα for each loading condition for each sample was calculated from void ratio vs log time graphs (Mesri & Castro, 1987). C_α/C_c were obtained with C_α and C_c at each applied stress for each sample according to previous method (Mesri & Castro, 1987).

3.6. Durability of MICP stabilised peat submerged in acidic peat condition at long-term

3.6.1. Preparation

For this study, peat slurry was mixed with 25% of sand to weight of wet peat slurry based on the study conducted in Chapter 5. Cementation reagent was prepared by adding equal molars of urea and calcium chloride at 2 mol/kg of wet pet slurry. To

produce each stabilised peat, peat-sand mixture was mixed with the cementation reagents and 100 mL of bacteria culture at 10^8 CFU/mL for followed by homogenising for 5 minutes with kitchen mixer to ensure that uniform distribution.

The curing method was performed according to Section 3.4.2. In general, PVC tubes of 160 mm diameter containing mixed samples were placed vertically submerged in water with surcharge loading of 9kPa with an ending covered with geotextile and porous stone. The samples were cured at room temperature (26°C± 1°C) for up to 28 days.

3.6.2. Durability study

The durability study was evaluated by submerging the stabilised peat into peat slurry and distilled water up to 90 days. Peat slurry used was to simulate actual acidic peatland conditions, and distilled water was used as control. The stabilised peat was carefully extruded and was trimmed to about 53 mm diameter x 106 mm height which then placed in a PVC tubing with holes that facilitated diffusion and wrap with geotextile (Figure 3.9.). Minimum disturbance was practised in order not to damage the stabilised peat samples. The samples were then prepared in bulk and place vertically submerged into a cylinder tank containing peat slurry and left for up to 90 days at room temperature (27 °C ± 1°C) (Figure 3.10.). Control sample was placed and submerged into distilled water instead. The samples were removed (Figure 7.5.) from the submerged condition at a various time interval of 1, 30, 60 and 90 days and subjected to soil strength testing, pH, and calcium carbonate residue estimation.

Soil strength was evaluated through Unconfined Compressive Strength (UCS) testing based on the standard procedure described in ASTM D2166 (ASTM, 2016a). The samples were tested using Universal Testing Machine (Lloyd Instruments) with a loading rate of 2.0 mm/min. The unconfined compressive strength recorded as the peak stress of the soil stress-strain curve or is identified as peak stress correspond to vertical strain reaches 20% as described by a previous study (Sing, Hashim, et al., 2008).

The amount of $CaCO_3$ precipitation in the samples was determined by acid washing method, which measured the dry weight of the soil before and after exposure to acid (Keykha et al., 2017; Mortensen et al., 2011). Samples were dried in the oven with their mass measured before and after rinsing with HCl (5M). Generally, the samples

were rinsed multiple times on filter paper to allow HCl (Merck, Germany) to dissolve the carbonate salt while passing through the filter. The difference between the measured mass before and after rinsing was taken as the mass of $CaCO_3$ and the results were expressed at weight of precipitated $CaCO_3$ over the dry mass of samples. pH of the sample was also measured based on ASTM D4972. All tests were done in triplicates, and results were shown in mean +/- standard deviations.

Figure 3.9. Stabilised peat sample trimmed and placed in a PVC tube with holes and wrap with geotextile before submerged to peat slurry and distilled water.

Figure 3.10. Peat samples were placed vertically into a tank containing peat slurry and submerged up to 90 days.

CHAPTER 4: ISOLATION AND CHARACTERISATION OF CALCITE PRECIPITATION BACTERIA ISOLATED FROM TROPICAL PEAT

4.1. Introduction

Biomineralization or mineral precipitation induced by the organism is commonly found in the environment (Dupraz et al., 2009; Marvasi et al., 2010). The bacterium that induces calcium carbonate in the form of calcite with the availability of calcium ions is commonly known as calcite precipitation bacteria or calcite forming bacteria (CFB) (Kim et al., 2014; Park et al., 2012). CFB can induce calcite formation by utilizing various microbial metabolic pathways, namely carbonic anhydrase (CA), sulphate reduction, ammonification, denitrification, and urea hydrolysis (Hammes et al., 2003). Among the mechanism, urea enzymatic hydrolysis or urease has advantages over other pathways as it is easily controlled and shows great potential to produce large amounts of carbonate in a short time (De Muynck, De Belie, et al., 2010). Urease, common in many microorganisms catalyses the urea intracellular hydrolysis to one mole of ammonia and one mole of carbamate, which spontaneously forms an additional mole of ammonia and carbonic acid (Hammes et al., 2003; Siddique & Chahal, 2011). The latter is equilibrated in water, thus forming bicarbonate, two moles of ammonium and two moles of hydroxide ions. This causes a pH increase that changes the bicarbonate balance leading to the formation of carbonate ions, which in the presence of soluble calcium ions are precipitated as $CaCO_3$ (Al-Thawadi, 2011; Hammes & Verstraete, 2002; Siddique & Chahal, 2011). The previous study has suggested synergistic between urease and carbonic anhydrase in carbonate mineralization (Dhami et al., 2016b; Dhami et al., 2014). Carbonic anhydrase plays an essential role in the interconversion of CO_2 and HCO_3^- while urease increase the system pH during $CaCO_3$ precipitation (Dhami et al., 2016a).

The chapter aims to isolate, identify, and characterise $CaCO_3$ precipitating bacteria from peat. The chapter will focus on the study urease and carbonic anhydrase activity, characterising of calcite precipitation and bio-cementation potential of two isolates from tropical peat. 16S rRNA sequencing and phylogenetic study were done for the identification of selected strains. The current study would provide an insight into the

urease and carbonic anhydrase activity of isolates from peat, leading to $CaCO_3$ crystal precipitation.

4.2. Isolation and Identification of isolates

Two strains, namely strain P19 and P21, were selected for their ability to turn urea agar from pale yellow to pink within 12 hours, suggesting high urease activity (Figure 4.1e & Figure 4.1f). The colony of isolate P19 and P21 shown grown on TSA (Figure 4.1b & Figure 4.1c). Cells of isolate P19 and P21 were observed to be coccoid under light microscope and both strains were Gram-positive, catalase-positive, non-spore formation and non-motile (Table. 4.1.). The isolate P19 was able to grow between pH of 4 – 10 with noticeable slow growth at pH 4 and pH 5 (colony were obvious only after a week) while isolate P21 was able to grow between pH 4 – 10. Both isolates P19 and P21 were found to grow between 20 – 40°C and were able to tolerate salt concentrations of 0.5 - 12 and 0 - 12 % for NaCl and KCl (w/v) respectively.

Due to the limitations of morphological and characterization study identification above, the ribosomal RNA (16S rRNA) was also used for identification in this study. Sequence analysis of 16S rRNA, genes encoding small-subunit ribosomal RNA are commonly used for the phylogenetic classification and reconstructing of prokaryotic phylogenies (Case et al., 2007; Janda & Abbott, 2007). The study was able to generate a nearly complete sequence length of 1364 bp and 1368 bp of the 16S rRNA gene for isolated strain P19 and strain P21, respectively. The basic local alignment search tool (BLAST) nucleotide sequence analysis for P19 revealed that the strain showed a high degree of similarity (99.19%) with *Enteractinococcus viverrae* YIM 101632 (NR148333) followed by *E. coprophilus* YIM 100590 (NR132287) with 97.27 % similarity and third closest of *E. lamae* YIM 101617 (NR148334) 96.24% similarity. For the strain P21, it was found with highest similarity of 99.93% with *Staphylococcus warneri* AW25 (NR025922) followed with 99.49% for *S. pasteuri* ATCC 51129 (NR114435) and 98.75% with *S. epidermidis* NBRC 100911 (NR113957). The sequence of the 16S rRNA gene of the isolated strain P19 and P21 from this study have been deposited in GenBank under accession number MH639002 and MH639001, respectively.

Phylogenetic analysis (Figure 4.2.) based on 16S rRNA gene sequences revealed that the two strains formed a clade with their respective species in the same genus, which

indicated that the strain P19 and P21 belong to the genus *Enteractinococcus* and *Staphylococcus*, respectively. The genus *Enteractinococcus* represent a novel genus separated from genus *Yaniella* within the family *Micrococcaceae* (Cao et al., 2012). Previously, urease-producing strain VS8 with similarity to *Enteractinococcus* sp. YIM 101632 (98 % similarity) and *Yaniella sp.* YIM 100590 (96 % similarity) was isolated for calcium carbonate precipitation in bio-clogging of sand (Ivanov & Stabnikov, 2017).

Biosafety for the use of the microbial agent in bio-cementation technology has been reviewed and should be taken into consideration as, ultimately, the microbial agent will be used outside of laboratory condition in the field (Ivanov et al., 2019). For strain P19, to the best of the authors' knowledge, there is still no classification biological agent risk for the genus *Enteractinococcus*. From the phylogenetic tree, the nearest *Yaniella* genus was known as Risk group 1 (including *Yaniella flava* and *Y. halotolerans*). For strain P21, high similarity *S. warneri* was known to belong to Risk group 1 while *S. pasteurii and S. epidermidis* were listed as Risk group 2 according to The Technical Rules for Biological Agents (TRBA).

Table 4-1. Basic characteristic of isolated strains.

Characteristic	**Isolated strains**	
	P19	P21
Morphology	Coccoid*; Yellowish circular opaque**	Coccoid*; White circular opaque**
Motility	-	-
Catalase	+	+
Spore formation	-	-
pH	4.0 - 10.0	4.0 - 10.0
Temperature (°C)	20 - 40	20 - 40
NaCl % (w/v)	0.5 - 12	0.5 - 12
KCl % (w/v)	0 - 12	0 - 12

+ indicates positive and - indicates a negative test
* Cell shape observed under light microscope
** Colony morphology grown on TSA

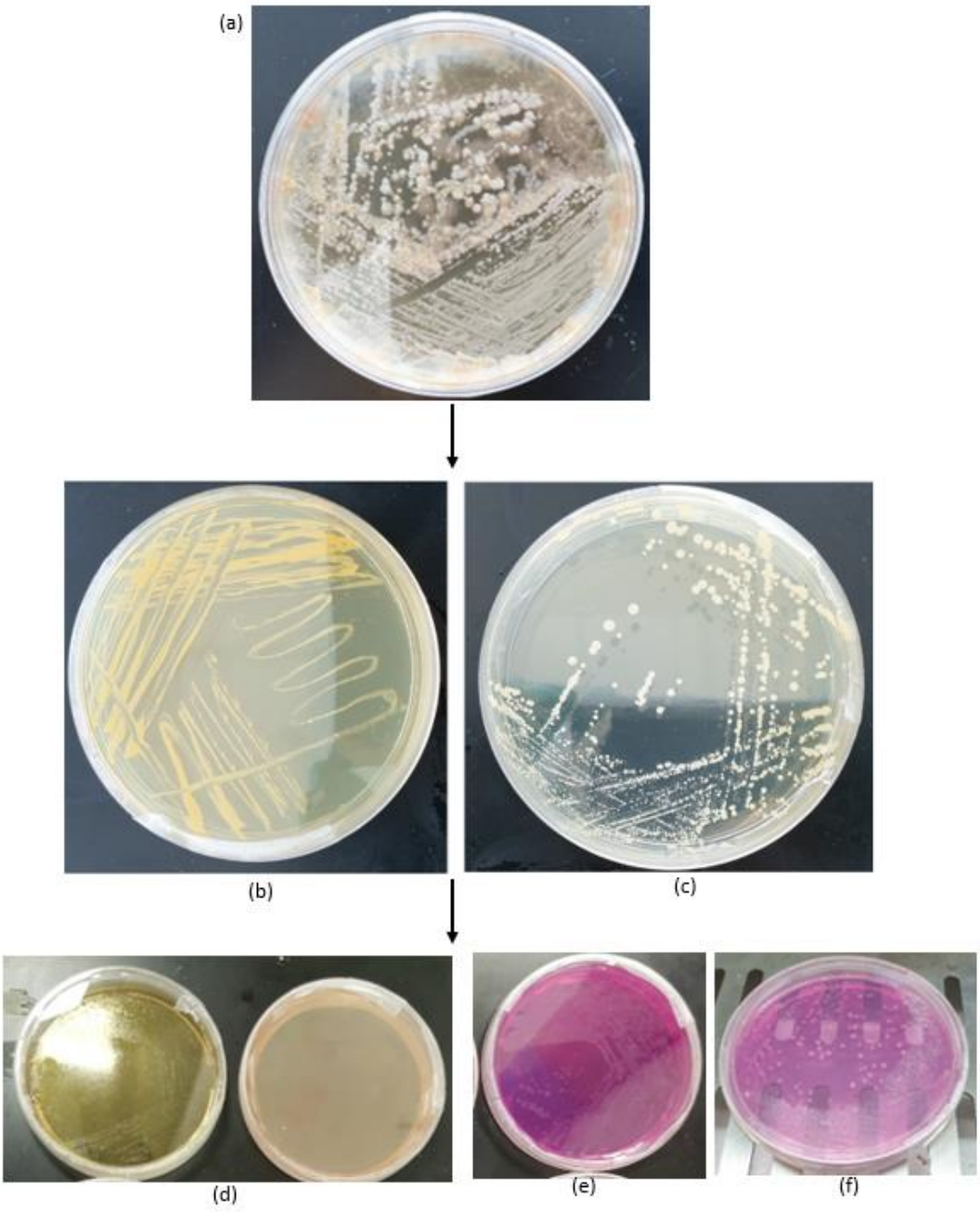


Figure 4.1 . (a) Mixed bacteria isolate possible to grow on TSA; (b) Purified isolate P19 grown on TSA; (c) Purified isolate P21 grown on TSA; (d) Urea agar showing urease negative (yellow formation) (left) & blank (pale orange) (right); Urea agar showing positive urease activity (purple) of isolate P19 (e) & isolate P21 (f).

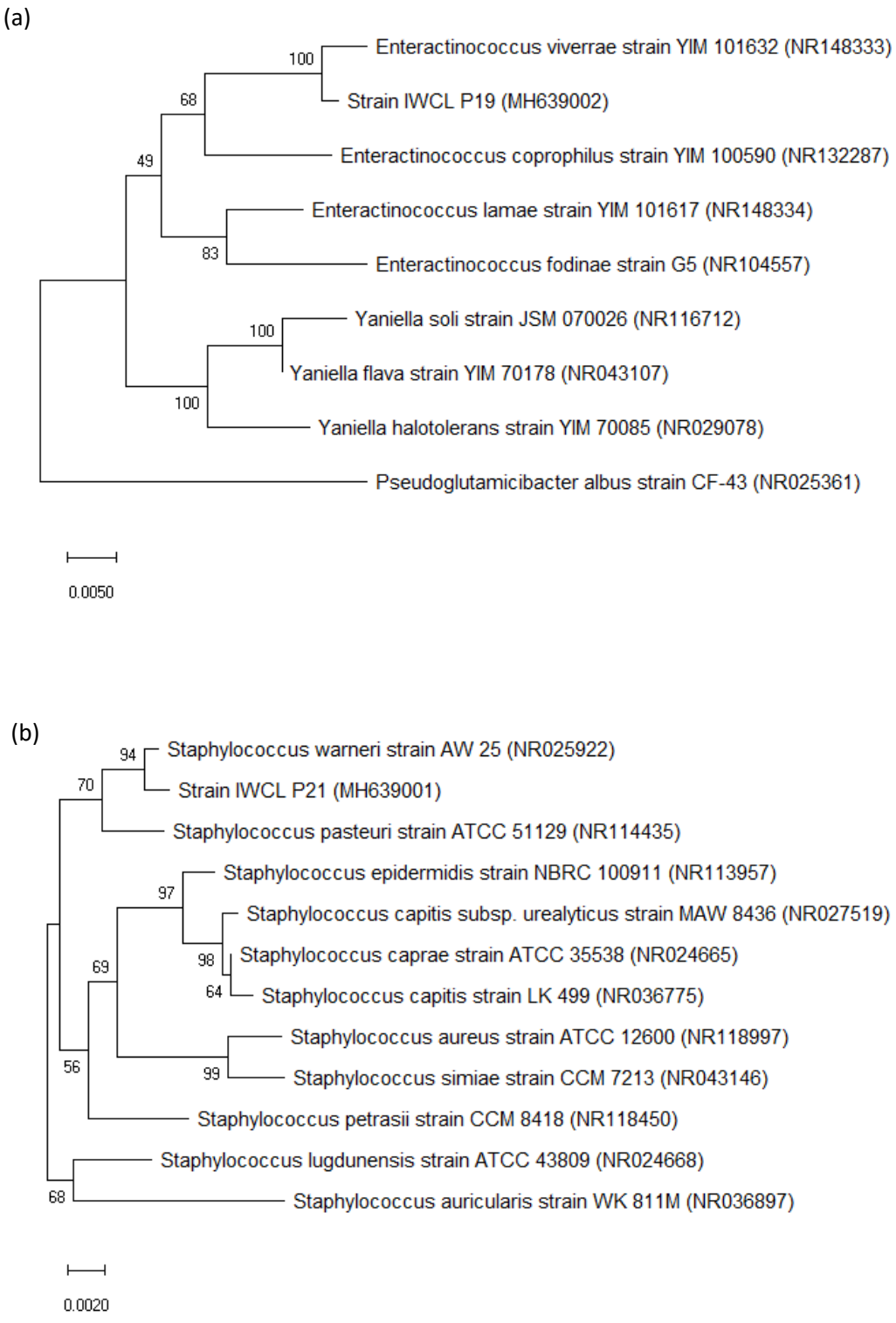


Figure 4.2 Phylogenetic tree based on the 16S rRNA gene sequences of the representative species with GenBank accession numbers showing the phylogenetic positions of strain IWCL P19 (a) and IWCL P21 (b) with their related taxa based on 16S rRNA gene sequence analysis constructed by using the neighbour-joining method. Numbers at nodes indicate bootstrap values based on neighbour-joining analysis of 1,000 resampled datasets. The evolutionary distances were computed using the Maximum Composite Likelihood method with scale bar indicates 0.020 substitutions per nucleotide position.

4.3. Urease and carbonic anhydrase activity

The course of cell growth to concentration by measuring OD_{600}, urease activity, and carbonic anhydrase activity of the selected isolates was studied up to 120 hours of incubation period and observed at an interval of 24, 48, 72, 96 and 120 hours. Figure 4.3. showed bacterial growth in OD_{600}, urease activity and carbonic anhydrase activity of isolated strain P19 and P21 for five days incubated in Tryptic Soy Broth (TSB) supplemented with urea. Strain P19 showed maximum urease activity at 96 h with an average mean of 815 U/mL, while strain P21 showed maximum urease activity at 120 h with 633.3 U/mL. Both showed an increasing trend of enzyme production with bacterial growth. Different bacteria strain may react differently with their growth and urease activity. Such an example can be seen from the urease activity of *P. vulgaris* which was found to be proportional to cell biomass, while increasing growth for *S. pasteurii* were not found to induce any increment of urease production (Whiffin, 2004).

A decline in the enzyme production at day 4 for P19 and day 3 for P21 were observed with a decline in growth. This might be due to various factors such as depletion of available nutrients causing a decline in biomass, accumulation of products such as high concentration of metabolite ions NH^{4+}, $CO_3^{2-}$and OH^- and production of other proteases, extracellular metabolites and proteins that suppressing urease activity (Achal et al., 2009a; Berg & Tymoczko, 2002; Dhami et al., 2016b). A second peak was observed for isolate P21 after loss in growth, suggesting diauxic growth, which occurs in multi-nutrient environments where microbes undergo adaptation for population growth (John et al., 1974; Zaharia et al., 2013). Such trade-off was common in a biological system due to the dynamic environment along with metabolic fluxes and gene regulation (Chu & Barnes, 2016; Deutscher, 2008; Kotte et al., 2014).

Carbonic anhydrase was found to have a synergistic effect with urease in the bio-precipitation of calcium carbonate by regulation of bicarbonates to carbonates (Dhami et al., 2014). Although the initial objective of the study was to identify urease activity potential for MICP, carbonic anhydrase was also screened. Carbonic anhydrase activity of the isolates was evaluated with the presence of urea through incubation up to 120 hours with the same media for urease activity. Both isolates produced extracellular carbonic anhydrase; strain P21 has the highest carbonic anhydrase activity of 2.5 U/mL at 120 h compared to strain P19, with the highest production at

24 h with 1.1 U/mL. Higher carbonic anhydrase activity was observed for both strains at the early stage of 24 h and a decline at 48 h. The increased in carbonic anhydrase activity during the initial growth phase for both strains could be attributed to the phenomenon where CO_2 is required to meet the biosynthetic demand as suggested by the previous study, which is essential to overcome the lag phase according to Charles and Roberts (1968) as cited by Achal and Pan (2011). A decreasing trend was also observed for both strains in the incubation period. A decrease in carbonic anhydrase activity could be due to accumulation of CO_2 and bicarbonate result from urease activity during bacterial growth may occur (Achal & Pan, 2011). Dhami et al. (2014) found that the Carbonic anhydrase (CA) activity of *Bacillus megaterium* was 115 U/mL and CA was lower at 8.2 U/ml with the presence of inhibitors. Dhami et al. (2016b) has shown the introduction of carbon source in the form of $NaHCO_3$ or direct CO_2 injection were able to produce high carbonic anhydrase activity (>90 U/mL) of *Bacillus megaterium and B. pumilis.* Hence, carbonic anhydrase activities were influenced by environmental condition with the presence of inhibitors or regulatory effect by bacteria itself with introduced carbon.

Carbonic anhydrase played a role in the interconversion of carbon dioxide and bicarbonate interconversion for metabolic and physiological processes (Bury-Moné et al., 2008). Fluctuation trends of carbonic anhydrase activity for this study were observed, which may arise due to the metabolic process of the bacteria in response to CO_2 transport and pH homeostasis. Han et al. (2020) shown the fluctuated trends of carbonic anhydrase activities of *S. warneri* with decreasing bicarbonate ions and increasing carbonate ions in the system. Thus, further study is indeed required to understand the complexity of carbonic anhydrase expression in the system with urease activity.

Carbonic anhydrase activity on its own can facilitate $CaCO_3$ precipitation (Li et al., 2011). However, this does not affect the possibility of the urease bacteria for MICP as urease activity is still the primary driving mechanism of $CaCO_3$ precipitation. Carbonic anhydrase regulates CO_2/HCO_3^- metabolism of the cell and the moderation of nickel, which is essential for the catalytic reaction of urease (Achal & Pan, 2011; Dhami et al., 2013, 2014; Park & Hausinger, 1995). Hence, both the urease and carbonic anhydrase played an essential role in calcite precipitation induced by bacteria.

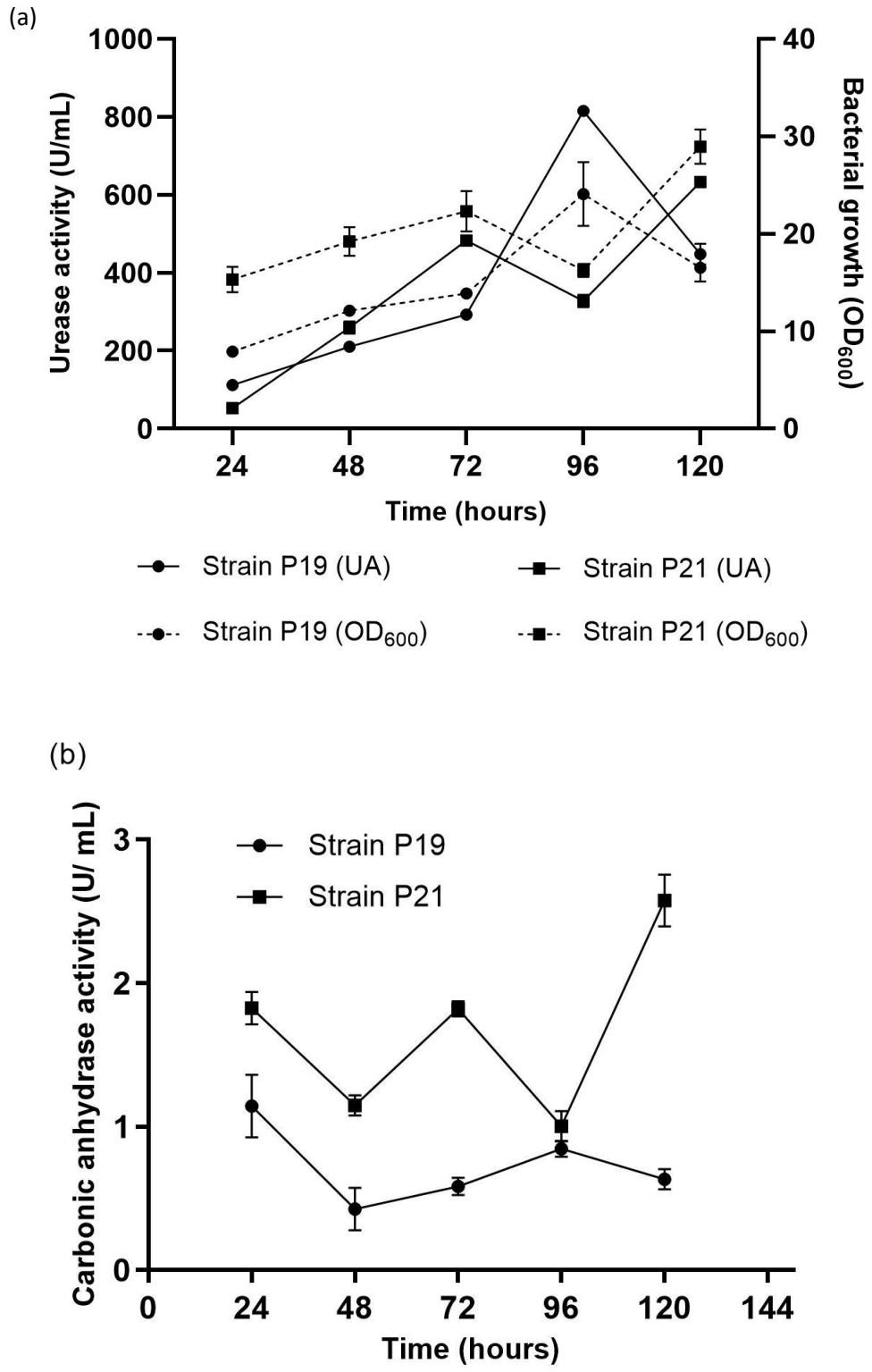


Figure 4.3. Bacterial growth (OD_{600}) with its urease activity and carbonic anhydrase activity of isolated strain P19 and P21 for 5 days incubate in TSB supplemented with urea. Results shown in mean values with standard deviations (error bars) (n = 3).

4.4. Precipitation of calcium carbonate ($CaCO_3$)

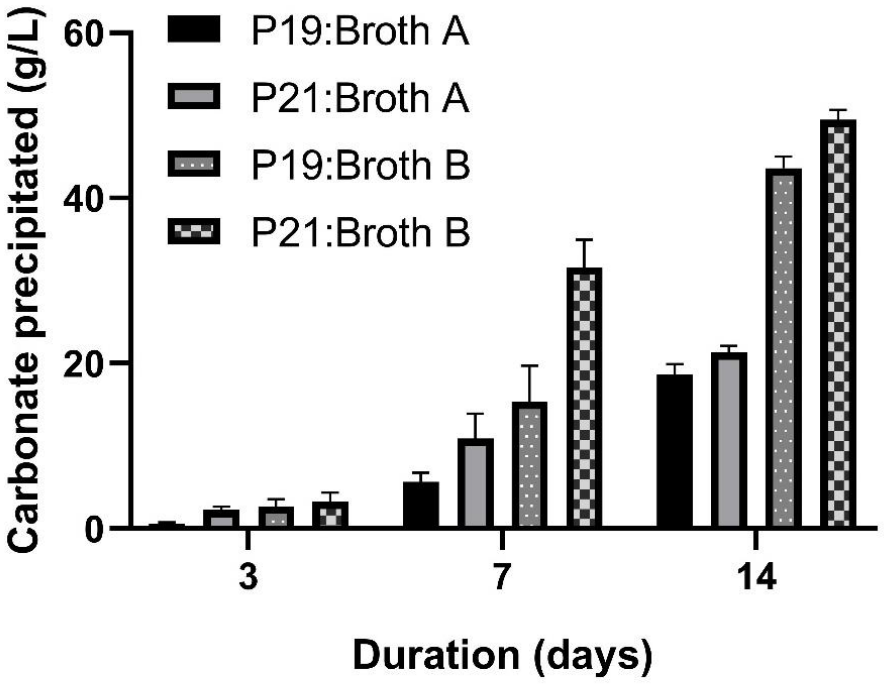
Figure 4.4 presents calcium carbonate precipitation for three different precipitation media (Table 3.3) on day 3, 7 and 14. No carbonate crystals was precipitated in the controls. As shown in Figure 4.4, strain P21 gave the highest productivity of calcium carbonate ($CaCO_3$) in all media as compared to strain P19. The highest production of $CaCO_3$ was observed in Broth B at day 14 with an average value of 38.47 mg/mL for strain P21 and 38.15 mg/mL for strain P19 which is slightly lower than strain P21. Precipitation trends showed increasing precipitation with 3 days incubation at lowest, which may be due to lower urease activity at early stages (Figure 4.3) and increasing with incubation period with highest at day 14. Higher precipitation for broth A and broth B was observed for strain P21 compared to strain P19 in the 7-day incubation period. This may be due to higher early bacterial growth of P21 compared to P19, as seen in Section 4.3. Active carbonate precipitation occurs with bacteria cells during urea hydrolysis, which involved the immobilisation of cations like Ca^{2+} on cell material and followed by bicarbonate ions (Schultze-Lam et al., 1996; Warren et al., 2001). Hence, increasing cells may favour $CaCO_3$ precipitation. Similar precipitation trends were observed between Broth A and B with increasing duration as both media contained the same chemical composition but with broth B containing a higher equal molar of calcium chloride and urea with up to 1 M. The intention was to study the possibility of the isolated strain precipitating $CaCO_3$ at a higher reagent concentration. Ivanov et al. (2019) suggested that urease-producing bacteria in bio-clogging of soil must be active in high salt environments with a concentration of calcium chloride at least 100 g/L producing urease enzyme for $CaCO_3$ production. A previous study has reported the inhibitory effect of $CaCO_3$ production for a higher concentration of calcium chloride (> 40g/L) (Nemati et al., 2005). It can be concluded that both strains shown possible for the use of MICP as $CaCO_3$ were successfully precipitated in Broth A and B with $CaCl_2$ more than 40 g/L.

For broth C, strain P19 was able to produce 1.7 mg/mL while P21 produced 2.09 mg/L of calcium carbonate, which was less than broth A and B due to the lesser molar concentration of available Ca^{2+}. The $CaCO_3$ precipitation using broth A and B is initiated by urea hydrolysis. Urea was hydrolysed to ammonia and carbonic acid, which equilibrated in water to form bicarbonate, ammonium, and hydroxide ions. The hydroxide ions increase the surrounding pH and shift the bicarbonate equilibrium

equation to the right resulting in carbonate ions production (Fujita et al., 2008). Along with the pH alteration, bacterial surfaces contribute to carbonate precipitation by attracting positively charged metal ions with its negatively charged surface acting inducing heterogeneous nucleation (Bäuerlein, 2003). Broth C which is known as B4 broth, is commonly used for organo-mineralisation potential for bacteria (Marvasi et al., 2012). B4 medium has been used to culture microorganisms involved in active $CaCO_3$ precipitation from different environmental sources (Baskar et al., 2006; Cacchio et al., 2004; Giralt et al., 2001; Silva-Castro et al., 2013). B4 medium was also used in the study of $CaCO_3$ precipitation related to the presence of carbonic anhydrase (Silva-Castro et al., 2013; Uad et al., 2014). Overall, both isolates produced a significantly maximum amount of calcium carbonate at the end of the 14th day.

The calcite precipitated by both isolates in broth A at day 14 was dried and characterized using XRD analyses. The calcium carbonate deposits for strain P19 and strain P21 were presented as a mixture of calcite and vaterite crystals, as confirmed by XRD analyses with calcite as the main polymorph (Figure.4.4). Similar spectra confirming the presence of both calcite and vaterite were also observed by a previous study (Zamarreño et al., 2009). Calcite, vaterite and aragonite are three crystalline polymorphs of calcium carbonate commonly found in natural environments. Zamarreño et al. (2009) reported *Pseudomonas* D2 and F2 were responsible for the precipitation of calcite and vaterite primarily while *Acinetobacter* B14 induced more precipitation of vaterite than calcite. Similar phenomena were observed for *Sporosarcina pasteurii* KCTC 3558, *Myxococcus xanthus* and *Bacillus sphaericus* in their ability to produce both polymorphs of calcite and vaterite (De Muynck et al., 2008; Gorospe et al., 2013; Rodriguez-Navarro et al., 2003). Understanding the polymorph produced is critical for bio-cementation work as the strength and durability of carbonate crystal can be affected by the type and structure of $CaCO_3$ polymorphs (Rodriguez-Navarro et al., 2012). Bacteria that can precipitate both vaterite and calcite were used in bio-consolidation work of stone and concrete suggesting that strain P19 and strain P21 is possible for bio-cementation study (De Muynck et al., 2008; Rodriguez-Navarro et al., 2003).

(a)



(b)

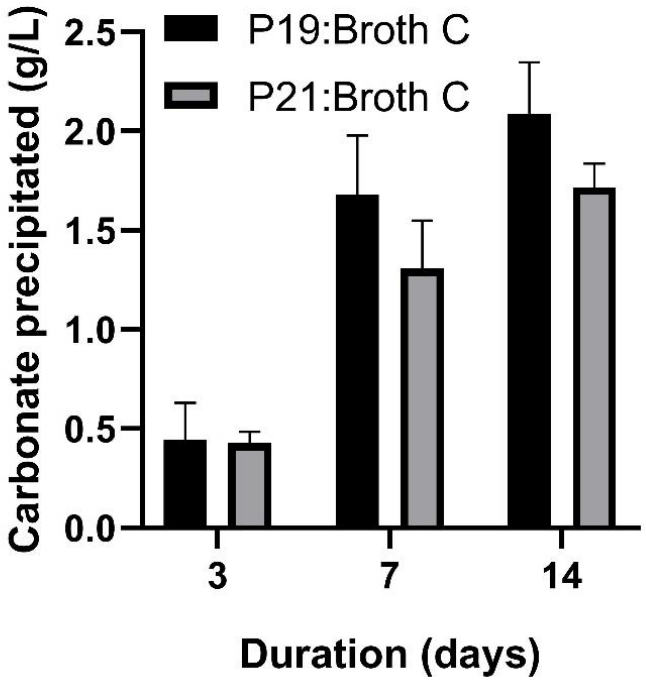


Figure 4.4. In-vitro study of calcium carbonate precipitation for day 3, 7 and 14 in (a) Broth A and B. (b) Broth C. Results in mean values with standard deviations (error bars) (n = 3).

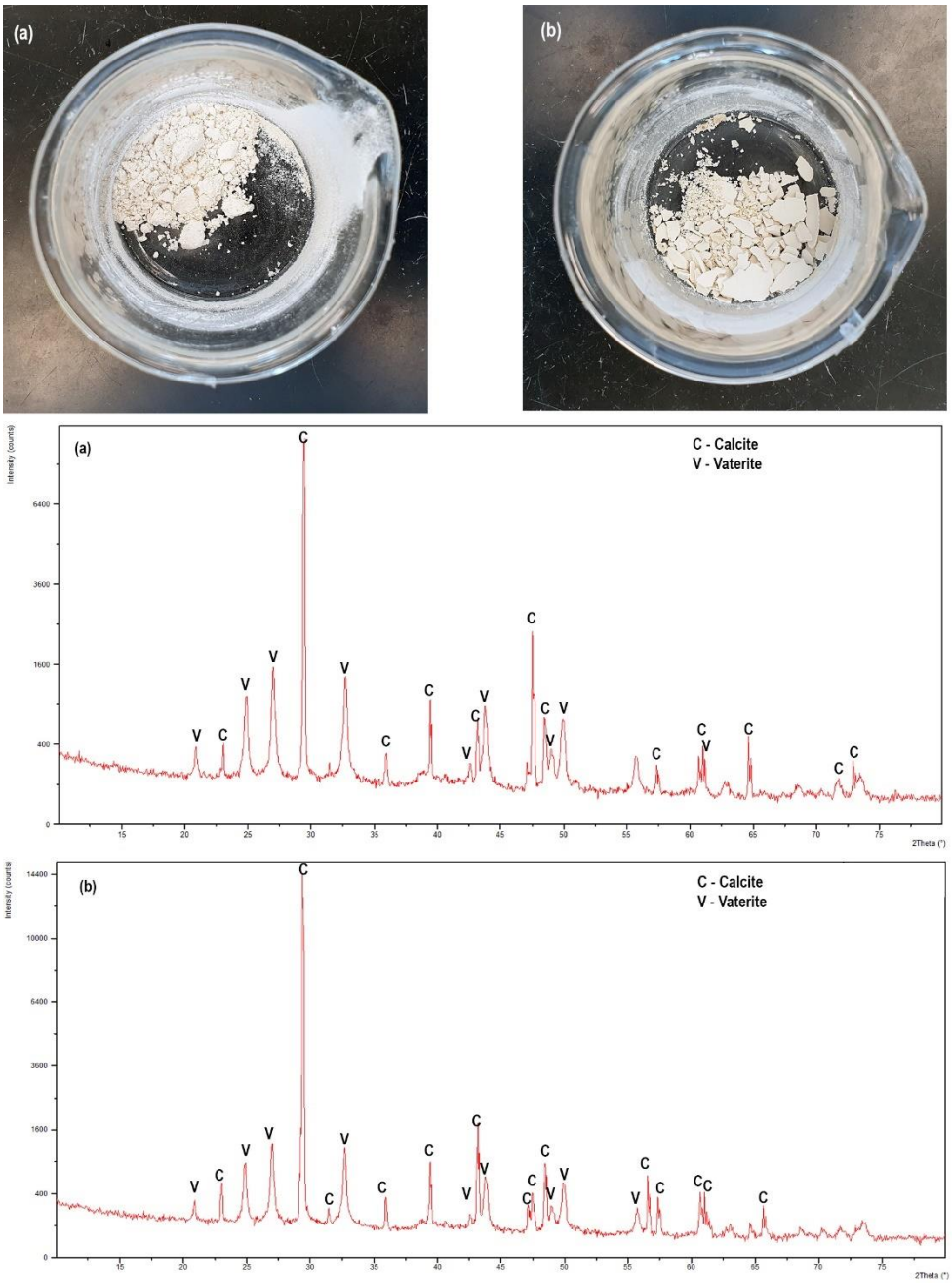
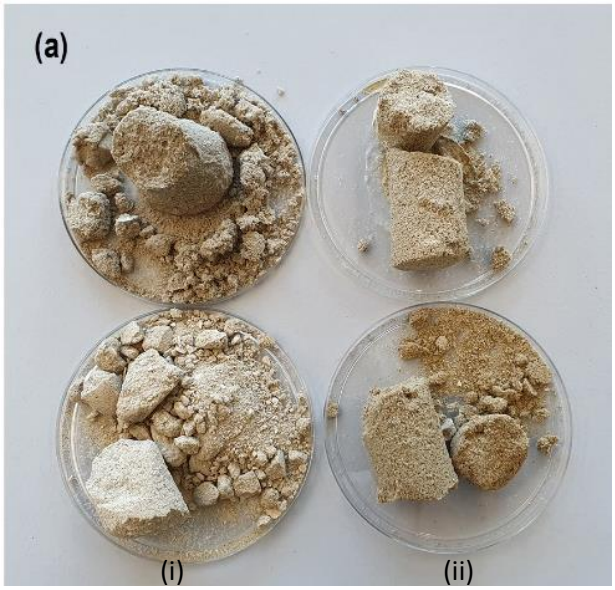


Figure 4.5. XRD analysis of calcium carbonate precipitated. (a) Dried $CaCO_3$ precipitated by strain P19 (with Media A). (b) Dried $CaCO_3$ precipitated by strain P21 (with Media A).

4.5. Bio-cementation study with sand column

The previous section shown the ability of the bacteria to precipitate calcium carbonate in an aqueous solution. Strain P19 was identified as *Enteractinococcus viverrae* and Strain P21 as *Staphylococcus Warneri* in Section 4.2. For the genus *Staphylococcus*, *S. Warneri*, *S. saprophyticus* and *S. aureus* were studied and found to precipitate

calcium carbonate crystal (Ghezelbash & Haddadi, 2018; Han et al., 2020; Wei et al., 2020). Stabnikov (2016) produced bio-cement with the use of *Enteractinococcus sp.* as a bioagent. There is a lack of study of both isolated strains in term of bio-cementation potential. The bio-cementation potential of the isolates was evaluated through the precipitation of calcium carbonate on the sand surface to bridge and hold sand particles together and its unconfined compressive strength. Figure 4.6. showed the results of the treated sand column with bacteria strain P19 and strain P21. Treated sand column for both strains of day three and day seven could not stand on its own weight although precipitation occurs, and sand was partially bio-cemented (Figure 4.6a). The strength yield for control (Untreated) and the treated sand columns for strain P19 and P21 were shown in Figure 4.7. Untreated sand (control sample) was not cemented and unable to stand. The average compressive strength of the treated sand column of day 14 with strain P21 was higher than strain P19 with 131.95 kPa and 112.26 kPa, respectively. This may be due to higher calcium precipitation on sand particles surface, leading to more bio-cementation than isolate P21 and P19. Hence, both isolated strains were found capable of bio-cementation and can be used for MICP purpose of soil.



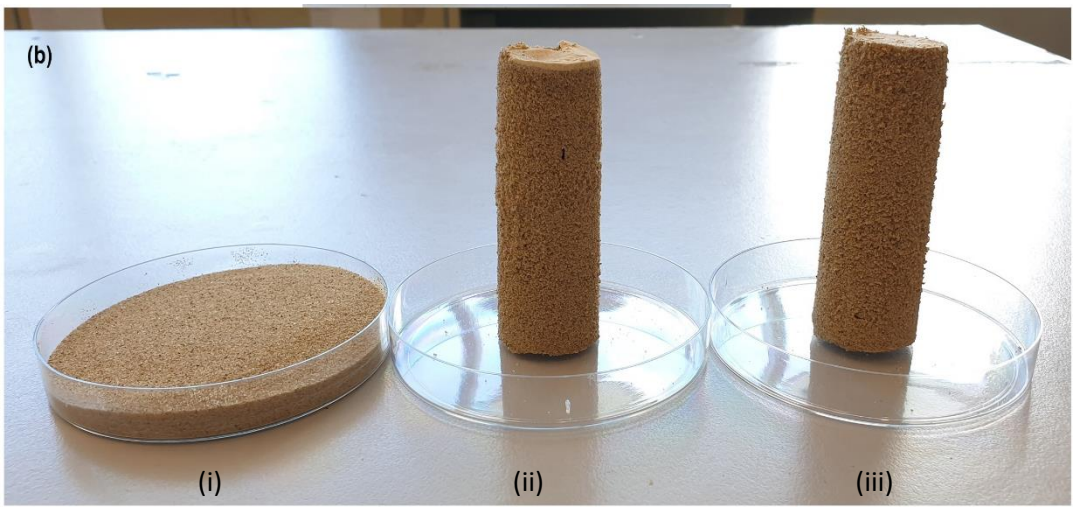


Figure 4.6. Bio-cementation of sand. (a) Representative treated samples for day (i) 3 and (ii) 7. (b). Dried sample at day 14 – (i) control, (ii) treated sand column (P19), (iii) treated sand column (P21).

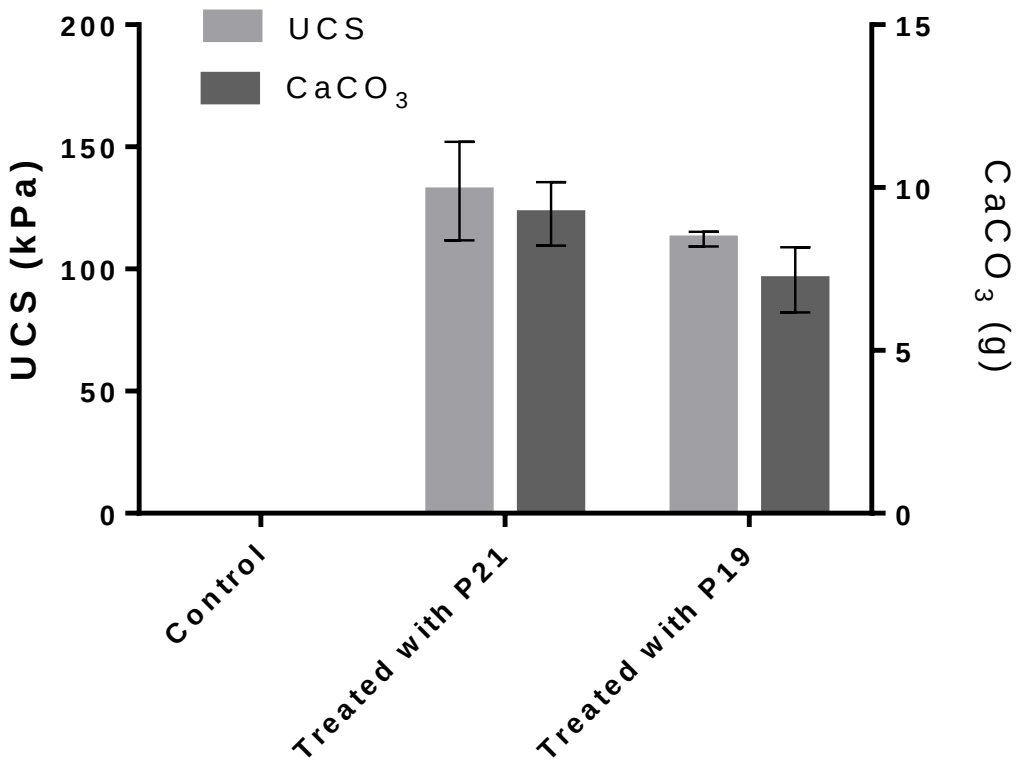


Figure 4.7. Bio-cementation of sand column. Results of Unconfined Compressive Strength (UCS) and calcium carbonate ($CaCO_3$) precipitation in mean values with standard deviations (error bars) (n = 2).

4.6. Chapter summary

The objective I was achieved in Chapter 4 with successful isolation and characterisation of ureolytic $CaCO_3$ precipitating bacteria from tropical peat. The study showed that the isolated urease producing cocci, P19 and P21 from tropical peat belong to the genus *Enteractinococcus* and *Staphylococcus*, respectively. Both strains were capable of urease and carbonic anhydrase activity, in which these enzymes were known to expedite calcium carbonate formation. Isolate P19 showed maximum urease activity at 96 h with an average mean of 815 U/mL, while strain P21 showed maximum urease activity at 120 h with 633.3 U/mL. Both enzyme activity of urease and carbonic anhydrase were present for biomineralization when urea was present. These strains were found to precipitate $CaCO_3$ in mixed vaterite and calcite polymorph. These suggested that the isolates are suitable for MICP purposes as calcite is the most stable polymorph used in the bio-cementation study. Isolate P21 was found to have higher productivity of $CaCO_3$ and with higher unconfined compressive strength in bio-cementation of study of sand as compared with isolate P19. Hence, the isolates were suitable for Microbial-induced calcite precipitation (MICP) treatment and will be used for MICP treatment of peat in the next chapter.

CHAPTER 5: EFFECT OF MICROBIAL-INDUCED CALCITE PRECIPITATION (MICP) TOWARDS UNCONFINED COMPRESSION STRENGTH AND HYDRAULIC CONDUCTIVITY OF PEAT

5.1. Introduction

Microbial bio-cementation or commonly known as Microbial-induced Carbonate Precipitation (MICP), has shown promising results which follow natural precipitation of various polymorphs of calcium carbonate ($CaCO_3$) on the soil particles which improve stiffness, strength, and reduction of soil erosion (DeJong et al., 2006). Originally, exogenous ureolytic non-pathogenic bacteria such as *S. pasteurii* and *B. megaterium* were used to induce hydrolysis of urea and with added soluble calcium (Ca^{2+}) ions in the system that facilitated the precipitation of calcite (Achal et al., 2009b; Ng et al., 2012). Recently, the use of indigenous bacteria for MICP was studied on both laboratory scale and *in-situ* (Burbank et al., 2012; Burbank et al., 2011). The possibility of inducing calcite precipitation with treatment depth up to 12m for granular soil using its native ureolytic microbial community was also explored (Gomez et al., 2018). MICP for soil improvement were intensively studied in inorganic soil, especially sand (Al Qabany & Soga, 2013; Al Qabany et al., 2011; Harkes et al., 2010; Whiffin et al., 2007; Zhao et al., 2014). Recently, the application was extended to residual soil consisted of about 40% of sand particles and 60% of fine-grained particle (Lee et al., 2013; Ng et al., 2012) and marine clay (Ivanov et al., 2015; Kannan et al., 2020). Sandy organic silt was studied, showing strength improvement with the injection of *S. pasteurii* for initiation of MICP. Canakci et al. (2015b) extended the study to organic soil up to 60% organic content and with MICP treatment through injection method of *S. pasteurii* and bio-cementation reagent showing an increase in strength. A preliminary study of peat solidifying was done using natural ureolytic bacteria in peat at Hokkaido, Japan and treated without compaction showing increasing of strength after a month and four months (Sato et al., 2016). However, there is still a lack of understanding of the possibility of MICP towards highly organic and naturally acidic material like peat.

This chapter aims to evaluate the performance of isolated bacteria towards unconfined compression strength and hydraulic conductivity of peat. The chapter is generally divided into two sections. Section 5.2 focus on MICP of peat through microbial urease activity from peat itself (indigenous sources) supplement with sole urea and soluble calcium without introducing external bacteria strain and nutrient sources. As from Chapter 4, we know that MICP potential bacteria were present in tropical peat. Hence, the effort was made to evaluate the indigenous urease activity in tropical peat and the possibility of Microbial-induced Calcite Precipitation (MICP) treatment. Unconfined compressive strength (UCS) and $CaCO_3$ precipitation of MICP treated tropical peat were studied to evaluate its stabilisation performance. pH, ammonia concentration (NH_4^+) and micro-fabric of peat by SEM were also studied. Section 5.3 focus on the stabilisation effort of MICP by indigenous isolates from Chapter 4. The study evaluates the effect of MICP towards stabilisation of peat and peat-sand mixture in term of strength gain and permeability changes under different bacteria concentration, cementation reagent dosage and peat-sand mixture (sand as a filler).

5.2. Study on indigenous urease activity in tropical peat and possibility of Microbial-induced Calcite Precipitation (MICP) treatment

5.2.1. Effect of MICP on Unconfined compressive strength (UCS) of peat

The effect of MICP for stabilisation of peat was evaluated with strength improvement of peat in this section. As shown in Figure 5.1(a), the strength of the MICP treated peat sample (Peat-$CaCl_2$-Urea) was overall higher compared to the untreated peat sample (peat only). Bulging failure was visually observed for all samples. It was observed that the highest strength was obtained for Peat-$CaCl_2$-Urea of 28 days curing periods with strength increased from 1.92 kPa of untreated peat towards 22.46 kPa. It is interesting to note that the UCS was almost the same for Peat-$CaCl_2$ and Peat-Urea. The strength increased with $CaCO_3$ content as seen in sample cured from 3 days to 28 days. Carbonate precipitation was observed only with the peat supplemented with both urea and $CaCl_2$ (Figure. 5.1(b)). This is obvious since urea acts to provide carbonates and increase pH to favour the precipitation, while $CaCl_2$ act as a Ca^{2+} ions source for the precipitation to occur (DeJong et al., 2006). $CaCO_3$ precipitation was observed to be increased in time. The strength was the lowest on Day 3, as shown in Figure 5.1(b), which may result due to the chemical and ecological complexity of peat that leads to

retardation of further $CaCO_3$ precipitation (Borga et al., 1994; Landva & Pheeney, 1980). The previous study has suggested that high organic content in peat, especially those comprised of humic substances or peat colloids, may interfere with the availability of the dissolved cation such as calcium ions (Rate, 1990). These phenomena were is observed in Chen and Wang (2006) whereas organic matter react with calcium retarding cement formation as organic acid in peat tends to decompose crystal such as calcium aluminate hydrate. As urea hydrolysis progresses, ammonium availability may lead to the sorption of humic substances displacing Ca^{2+} from peat particles (Slavek et al., 1982; Tipping, 2002). This may lead to increasing $CaCO_3$ formation between peats fabrics. Increasing the formation of $CaCO_3$ by biological means in the soil will eventually lead to strength improvement (Whiffin et al., 2007).

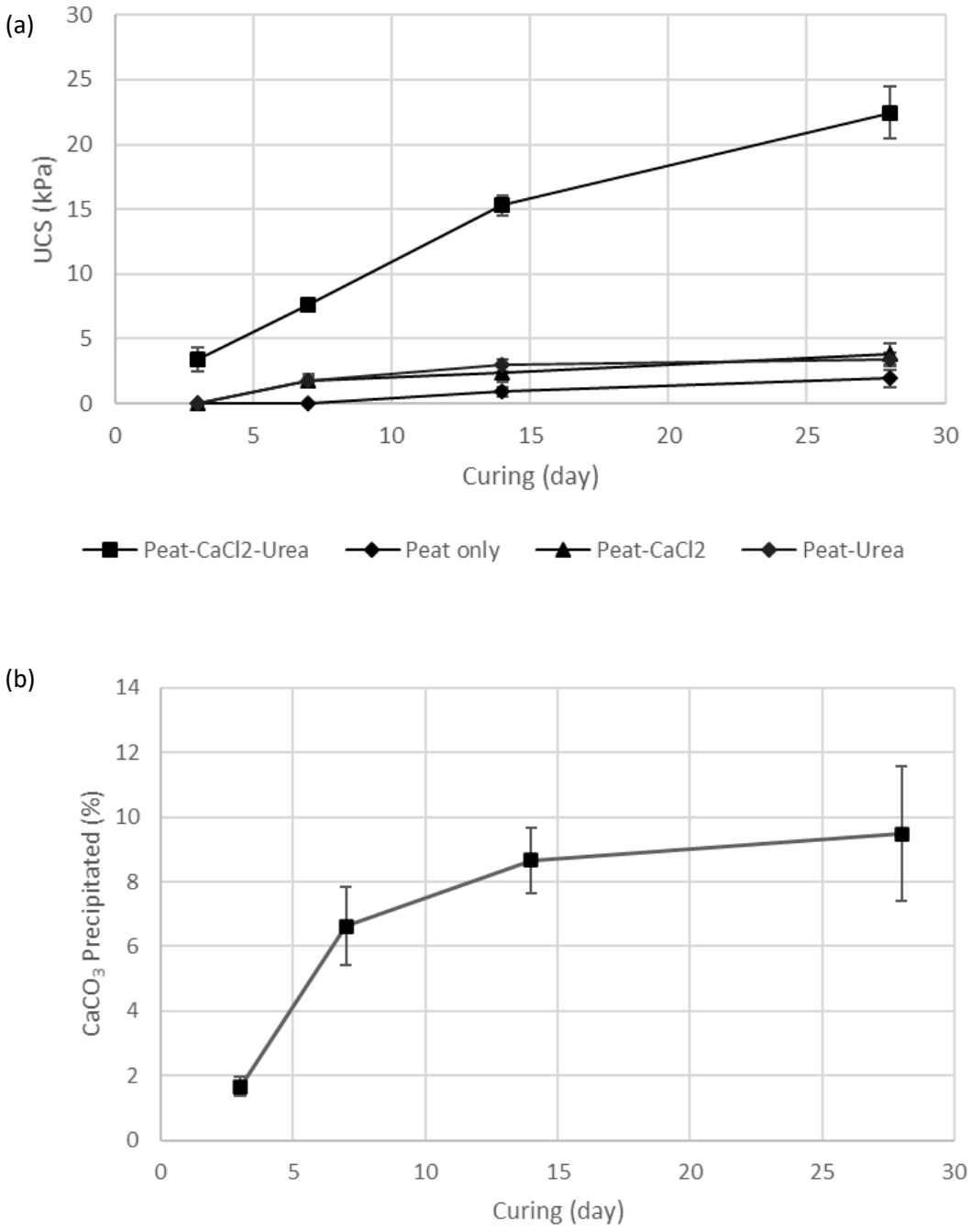
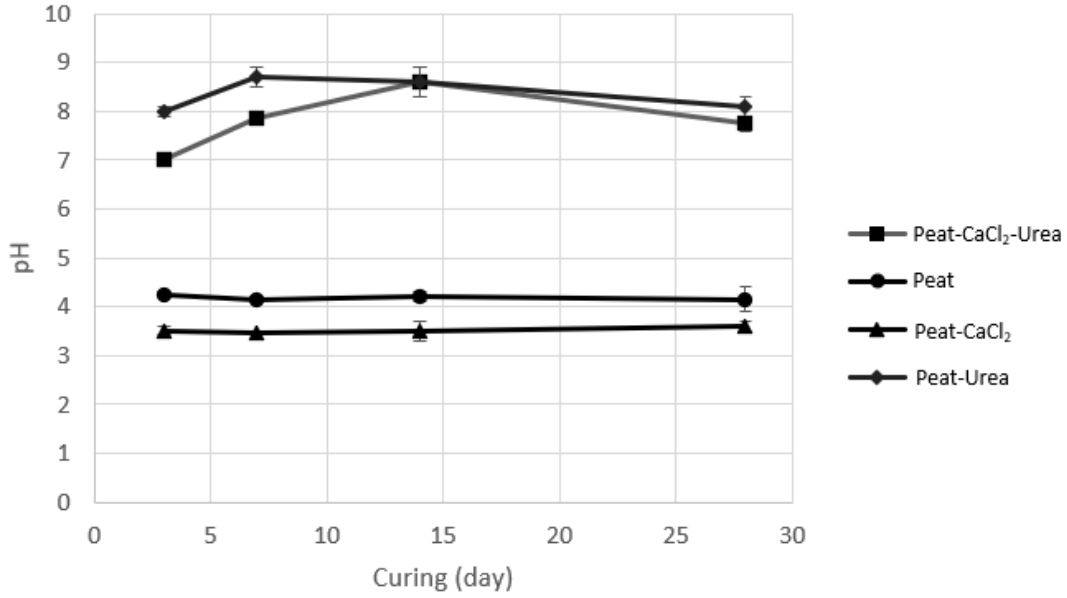


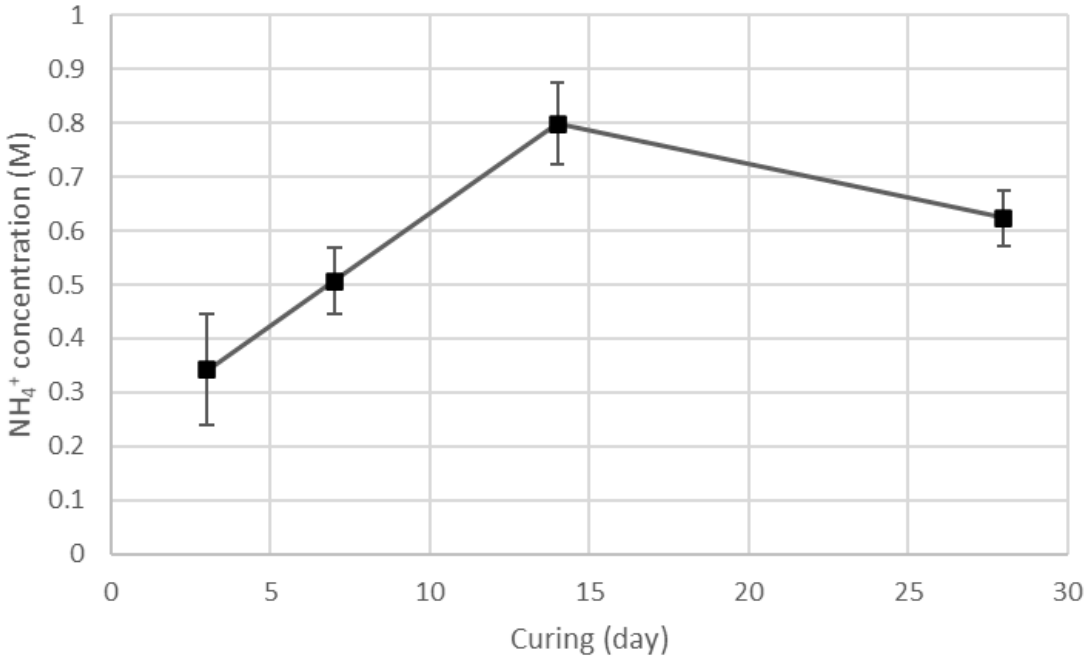
Figure 5.1. MICP of peat samples cured under submerged condition from 3 to 28 days: (a) UCS (kPa); (b) $CaCO_3$ precipitated by sample Peat-$CaCl_2$-Urea. Error bars represent mean ± standard deviations of triplicates.

5.2.2. Urease activity in peat

The increase in pH of peat throughout curing periods indicated urea hydrolysis (Figure. 5.2a). Increasing pH in the system facilitates $CaCO_3$ precipitation in peat leading to strength improvement. However, the formation of carbonate tends to lower the pH. Urease activity increases surrounding pH, but continuous carbonates precipitation will lead to a lower pH (DeJong et al., 2006; Stocks-Fischer et al., 1999). In addition, the biogeochemical process by the microbial community, such as nitrification and anaerobic ammonium oxidation, tends to also lower surrounding pH (Prosser, 2006; Soetaert et al., 2007). Ammonia oxidation is frequently observed in acidic soil, including peat (Stopnišek et al., 2010; Zhang et al., 2012). This can be seen with decreasing trends with ammonium ions after 14 days of curing, which suggested an ammonia removing mechanism in tropical peat (Figure 5.2b). The removal of ammonia and ammonium ions in the system indicates environmental friendliness of MICP on peat as the accumulation of ammonia considered toxic. However, it is not definite as different peat may contain different chemical composition and microbial community that may affect the ammonia removal (de Jong et al., 2020; Girkin et al., 2020; Preston et al., 2012).



(a)

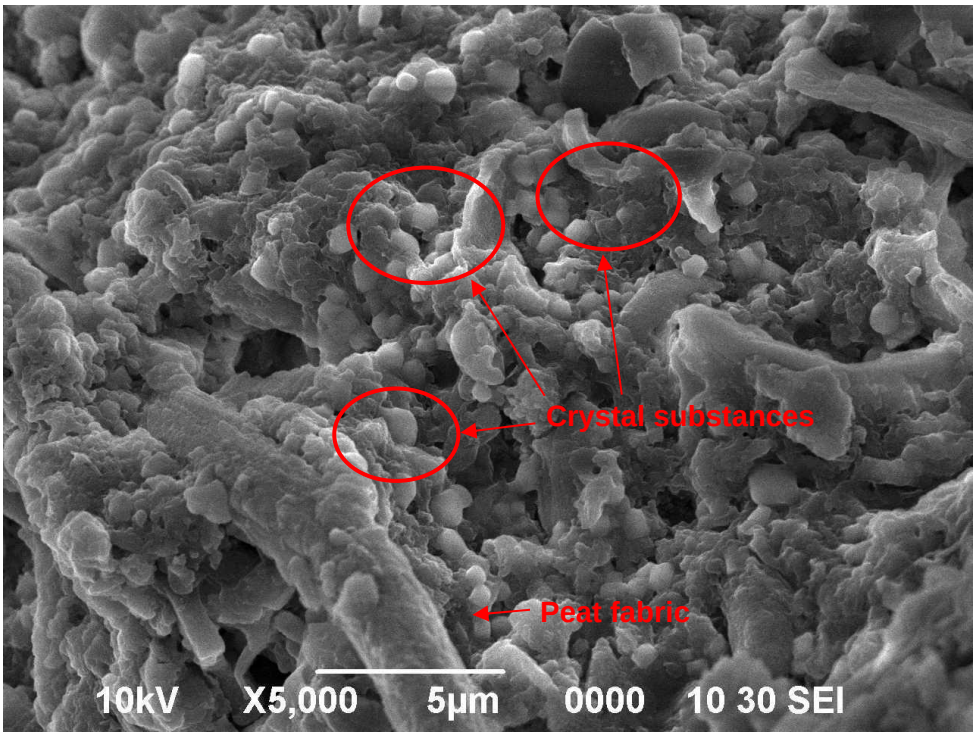


(b)

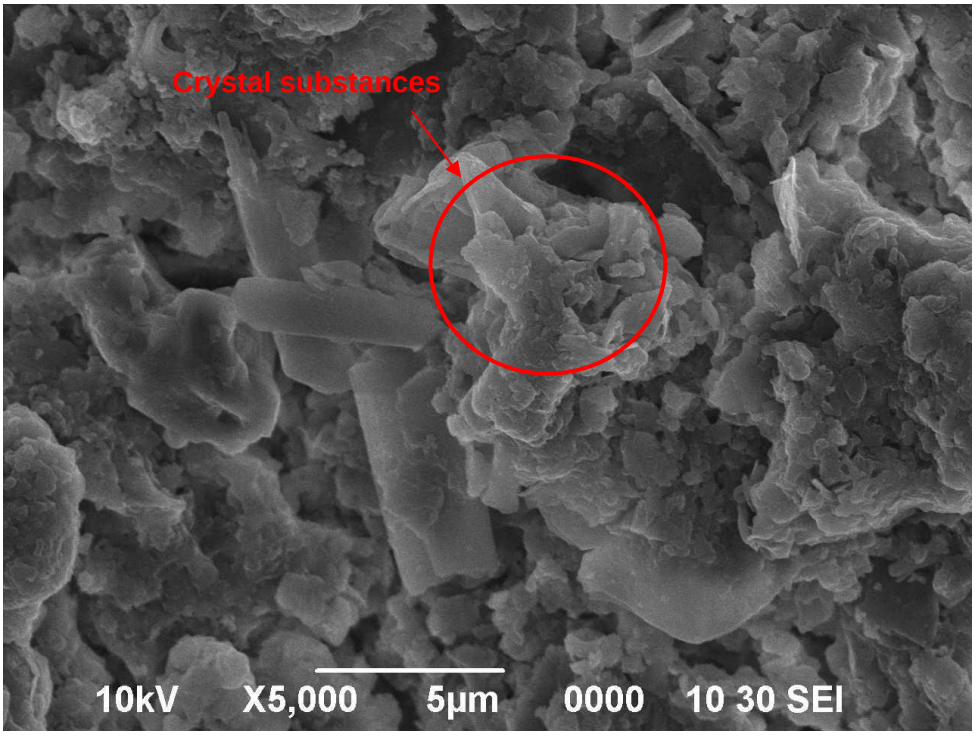
Figure 5.2. MICP of peat samples cured under submerged condition from 3 to 28 days: (a) NH_4^+ measured in MICP treated sample (treated with $CaCl_2$ and urea); (b) pH changes of the samples along curing periods. Error bars represent mean ± standard deviations of triplicates.

5.2.3. Scanning Electron Microscopy (SEM) and X-ray diffraction analysis

SEM analysis is usually used to visualise bio-cementation or bio-precipitation on inorganic soil (Burbank et al., 2012; DeJong et al., 2006). Due to dark coloured natural peat fabric, carbonate crystal precipitation on the surface is hardly noticeable with naked eyes. For this study, SEM imagery was performed on the selected treated sample to visualise the precipitation of calcite crystal on organic fabric. Crystal liked substances were seen precipitated on peat fabric for treated peat sample (Figure. 5.3a). Intense crystal precipitation was observed covering the void and surface of peat fabric, indicating the possibility of bio-cementation occurrence on the organic material surface of peat which may explain the increasing strength of stabilised peat samples (Figure. 5.3b). Furthermore, the crystal phase of the sample was identified by XRD analysis (Figure. 5.3c). Several calcite peaks were found and confirmed that the crystals precipitated as calcites, consistent with that observed in SEM. Calcite was found to precipitate, forming a bridge between soil particles as observed under SEM leading to strength improvement (Lin et al., 2016; Porter et al., 2018). Calcite identification is essential as its polymorph formation is the main reason for strength improvement for MICP effort (Park et al., 2010; Sharma & Ramkrishnan, 2016).

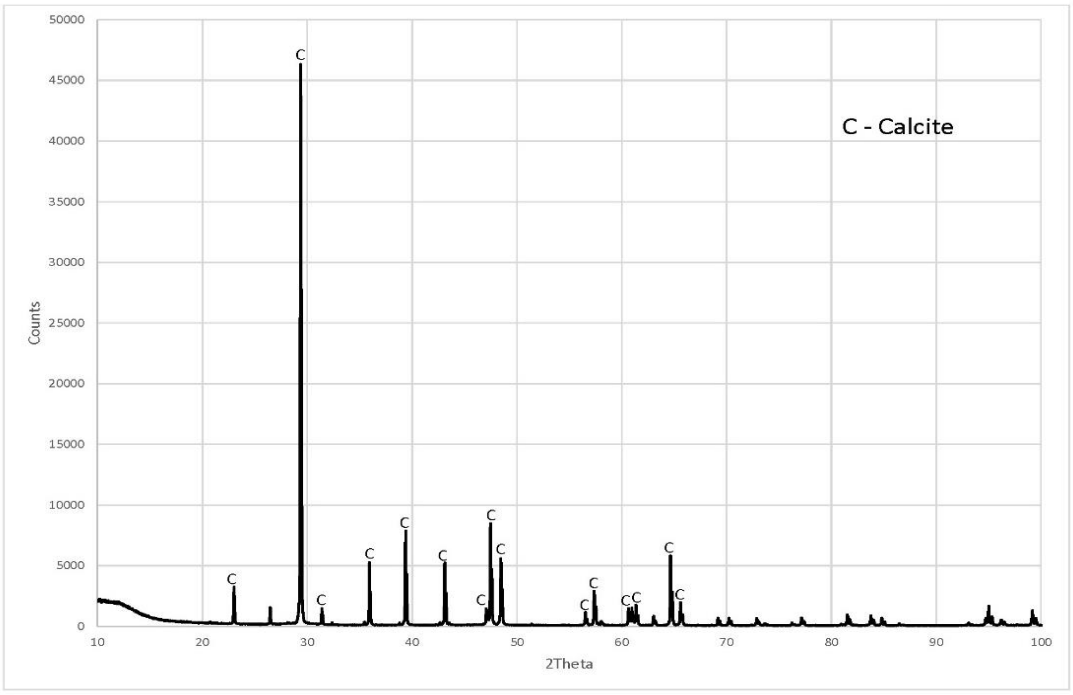


(a)



(b)

Figure 5.3. Scanning electron microscopy images of samples and XRD analysis: (a) Carbonate crystal precipitation along peat fabric; (b) Intense crystal precipitation covering peat surface; (c) XRD analysis of powder crystal precipitated obtain from sample surface. (Continued next page)



(c)

Figure 5.4. Scanning electron microscopy images of samples and XRD analysis: (a) Carbonate crystal precipitation along peat fabric; (b) Intense crystal precipitation covering peat surface; (c) XRD analysis of powder crystal precipitated obtain from sample surface.

5.2.4. MICP for peat stabilisation

It was observed that the addition of urea to peat had induced urea hydrolysis, which led to increasing pH suitable for MICP of peat and with Ca^{2+} ions provided, $CaCO_3$ was formed as shown in Figure 5.1 and Figure 5.4. The $CaCO_3$ formed bridged peat particles and filled up its void spaces, which led to cementation that improved UCS. The effort for isolation and characterisation of urease producing bacteria in Chapter 4 has shown the possibility of using isolated indigenous strain for MICP of peat. In contrast with above effort of using directly indigenous urease sources, previous studies make use of isolated indigenous strains and culture to a certain bacterial concentration before applying MICP towards the local soil (Burbank et al., 2012; Mohammadizadeh et al., 2020). For this study, the microbial urease sources were from tropical acidic peat and different soil might provide different urease bacteria strain. For example, Burbank et al. (2012) had successfully isolated urease bacteria of the genus *Lycinibacillus, Sporosarcina, Arthrobacter, Brevibacterium* and suggested the possibility of using indigenous isolates for urease based MICP of the local soil. Mohammadizadeh et al. (2020) isolated indigenous bacteria of *Acinetobacter calcoaceticus* Nima and shown its use for mechanical properties improvement of Sirjan's native soil (sand with silt) through MICP. However, some studies made use of indigenous urease activity without isolation and enrichment of indigenous strains for soil improvement. Burbank et al. (2011) suggested that the use of enrichment media containing $CaCl_2$ and urea with carbon source to stipulate urease activity leading to precipitation of calcite on locally collected soil (Snake River, USA). Amini Kiasari et al. (2018) biostimulated soil from Karun River, Iran, with enrichment of carbon source, urea, $CaCl_2$, and resulted in improvement of shear strength. Sato et al. (2016) had tested peaty soil from Hokkaido, Japan, showing positive urease activity and solidification while improving UCS by adjusting pH with sodium bicarbonate and mix proportion of urease from sward beans (*Canavalia gladiata)* along with urea and $CaCl_2$. Hence, in this section, the study was done to understand MICP using indigenous urease of tropical peat as it was clear that urease bacteria were present as discussed in the previous chapter (Chapter 4). To differentiate from previous study, apart from the use of tropical peat, carbon source was not added, and pH adjustment was not done. For this study, UCS of the sample was raised from 1.92 kPa of untreated peat towards 22.46 kPa after 28 days of curing.

In comparison, the UCS of treated samples increased from 25 kPa at the 1st month to 53 kPa after 4 months (Sato et al., 2016).

Mujah et al. (2017) reviewed that most of the MICP were performed through the flushing or injection technique and surface percolation method. Those techniques ensure continuous feeding of oxygen and flow of cementation reagent with or without bacteria agent. Peat has low permeability or hydraulic conductivity, where most peat areas are in swampy, waterlogged and anoxic condition (Chason & Siegel, 1986; Landva & Pheeney, 1980). This hinders the possibility of flushing cementation reagent through peat. Hence, the study explores such condition with the direct mixing of cementation reagent containing urea and $CaCl_2$ without adding nutrients to peat samples. The urease activity occurred while submerged for curing in water tank to mimic natural peat conditions and the deep mixing method for peat stabilisation. The deep mixing method for peat stabilisation usually involves the use of binders, including lime and cement (Islam & Hashim, 2008). MICP treatment on peat provides an alternative to conventional binders and as an eco-friendly binder to be used along with the deep mixing method. The laboratory-scale study suggested the possibility of calcite precipitation in acidic peat by utilising indigenous microbial communities and resulted in an improvement in the strength of peat.

5.3. Evaluation of isolated bacteria towards unconfined compression strength (UCS) and hydraulic conductivity of peat.

5.3.1. Unconfined compressive behaviour

To investigate the influence of MICP on stabilized peat, experimental results of unconfined compression tests on the test samples are focused on the effects of bacteria types and concentration, cementation reagent dosage, different amount of sand and curing time. The results of such effects on the UCS of the test samples are discussed.

5.3.1.1. Effect of bacteria type and concentration

Figure 5.4 shows the experimental results of the effect of different type of bacteria, including indigenous bacteria presence in peat and concentration (CFU/mL) of the added ureolytic bacteria strains P19 and P21 isolated (From Chapter 4) from tropical peat on the unconfined compressive strength (UCS) of the stabilized peat. Each test sample was prepared with 25% sand and cementation reagent dosage of 2 mol/kg cured

at room temperature submerged in water for 28 days. Literature suggested urease activity of pure ureolytic bacteria culture proposed for ground improvement should be in a range between 4 to 50 mM urea/min (Al-Thawadi, 2011; Burbank et al., 2012; Whiffin et al., 2007). Bacteria strain P19 and P21 were considered as the indigenous strain as both were previously isolated from the same tropical peat (Chapter 4) and selected for use in this study. Both strains had shown high urease activity (>400 U/mL) in aqueous solution (Figure 4.3) and found to precipitate calcite (Figure 4.5). Bacteria strain P19 and P21 were added at different concentration along with cementation reagent. In contrast, for the study of non-isolated indigenous bacteria, only cementation reagent was added to the peat sand samples before curing. It was shown that with 25% sand as filler, the resulting MICP effort with indigenous bacteria does not provide enough strength gain and rather low compared with bacteria addition. At 10^5 and 10^6 CFU/mL, samples treated with bacteria strain P19 showed 28.36 kPa and 30.91 kPa, whereas for bacteria strain P21, UCS were 30.16 kPa and 30.91 kPa. The UCS for both bacteria strain at 10^5 and 10^6 CFU/mL were low compared to indigenous bacteria with 28.11 kPa. UCS increment was more obvious at 10^7 CFU/mL for both bacteria strains suggesting the threshold of bacteria concentration needed for MICP treatment for the current peat sand mix. The highest UCS was observed with bacteria strain P21 at a concentration of 10^8 CFU/mL at 82.05 kPa and bacteria strain P19 at 70.36 kPa. Hence, the results suggested that bacteria strain P21 has better strength improvement with MICP than bacteria strain P19 in 25% sand mixed with peat. Bacteria addition (> 10^6 CFU/mL) showed higher UCS gain than the use of solely indigenous bacteria. Sharma and Ramkrishnan (2016) reported that highest increment was observed at 1×10^6 cfu/ml at 3.72 kg/cm^3 compared to 1 x 10^5 CFU/mL at 3 kg/com^3 for fined grained soil. However, higher CUF/mL may not result in higher strength, a study showed that the compressive strength of bacterial concrete found to increase in between 10^3 to 10^5 cfu/ml and followed by a decrement after 10^7 cfu/ml (Andalib et al., 2016). The amount of ureolytic bacteria introduced may affect urea hydrolysis rate, influencing calcium carbonate precipitation and ultimately towards the performance of MICP. Apart from urease activity governed by ureolytic bacteria, the bacteria cells also contributed to nucleation sides for stable calcium carbonate formation, leading to bio-cementation (Ferris et al., 1996; Phillips et al., 2013). Hence, it is important to study the required optimize amount for MICP of target materials.

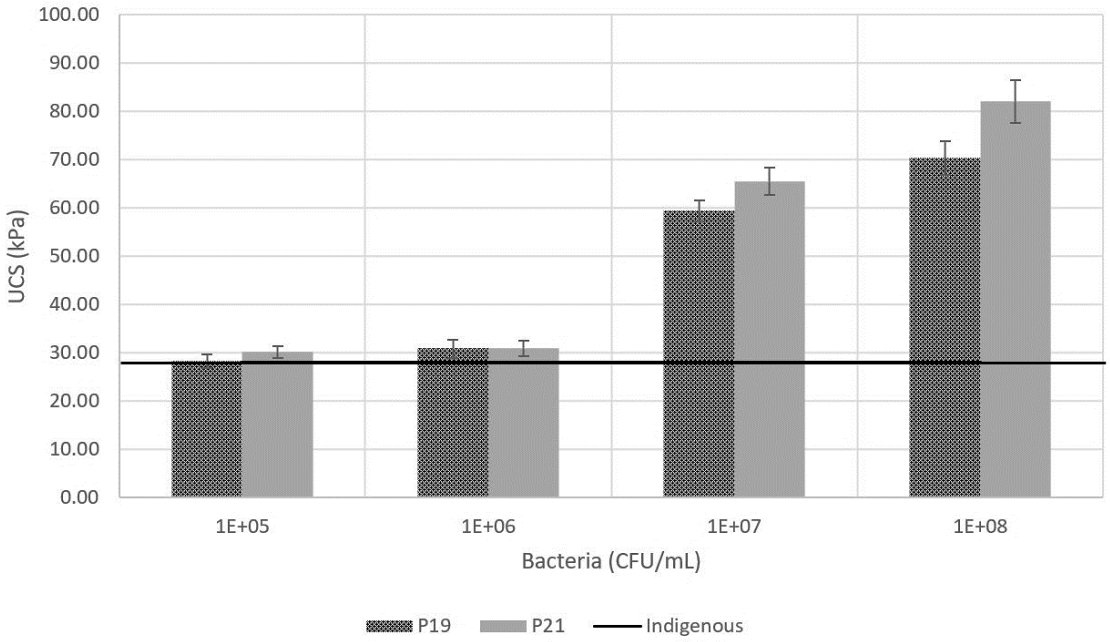


Figure 5.5. Effect of bacteria type and concentration towards unconfined compressive strength for peat mixed with 25% sand.

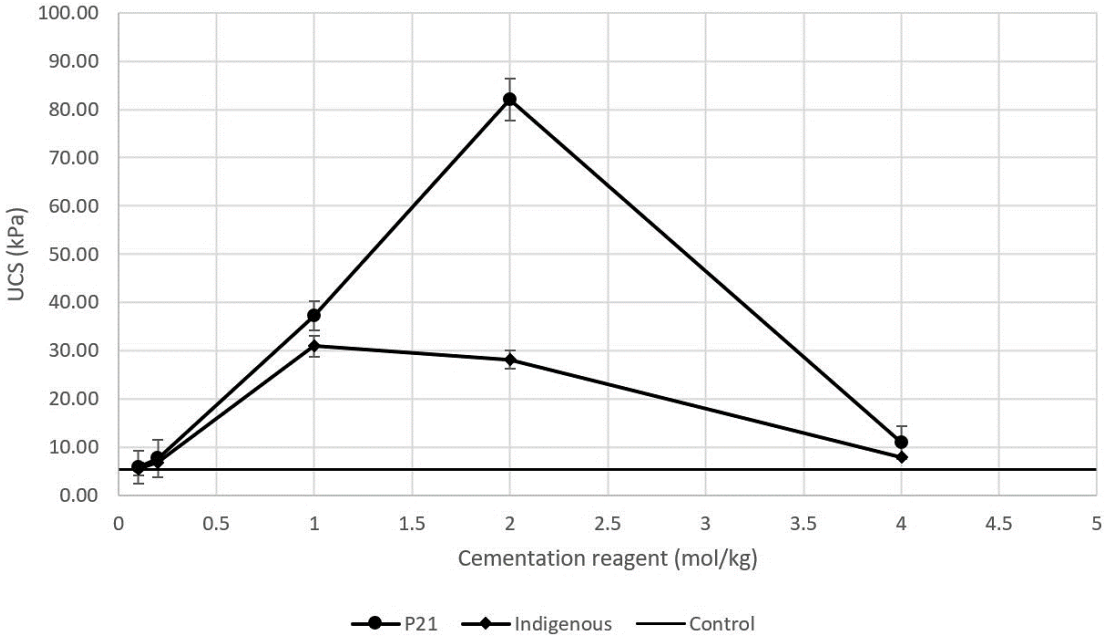


Figure 5.6. Effect of cementation reagent dosage towards unconfined compressive strength (UCS).

5.3.1.2. Effect of cementation dosage

Based on the literature, typical binder dosage for peat stabilization ranged from 50 – 400kg/m^3 (w/v of wet weight peat) (EuroSoilStab, 2001; Wong et al., 2013). For this part of the study, the total cementation components containing urea and calcium chloride were added to peat slurry in the range of 0.1 – 4 mol/kg towards the wet weight of peat slurry to identify its effect on MICP with 25% sand as filler after 28 days curing. Figure 5.5 shows the experimental results of the effect of concentration dosage with added bacteria strain P21 (10^8 CFU/mL) and without bacteria addition (indigenous) on the unconfined compressive strength (UCS) of the stabilised peat after 28 days of curing. Overall, treated samples showed higher strength compared to the control (peat with 25% sand) without treatment with 5.3 kPa. Based on the Figure 5.6, the highest increment was observed at 2 mol/kg for the sample treated with bacteria strain P21 while 1 mol/kg for indigenous bacteria. The UCS observed for the sample treated with bacteria strain P21 and solely with indigenous bacteria at 0.1, and 0.2 mol/kg were rather low with slight improvement as compared to the control (peat with 25% sand only). Peat has a significant high cation exchange capacity (CEC) due to the presence of humic substances, including humic acid and fulvic acid (Chen & Wang, 2006; Kazemian, Huat, et al., 2011). Literature suggests that humic substances may react with calcium ions (Ca^{2+}) and inhibit or retard calcium-based stabilisation of peat which may explain lower strength gain at 0.1 and 0.2 mol/kg (Chen & Wang, 2006; Huat et al., 2014; Jawad et al., 2014). Sample treated with bacteria strain P21 showed increasing strength gain with increasing dosage of 0.1, 0.2, 1.0 and 2 mol/kg up to 82.05 kPa while a drop-in strength at 4 mol/kg to 10.97 kPa. The trend for indigenous bacteria showed strength increment at 0.1, 0.2, and 1 mol/kg up to 30.91 kPa and continued to reduce in strength at 2 and 4 mol/kg down to 7.93 kPa (Figure. 5.5). Such phenomena suggested that 4 mol/kg might present an excessive amount of cementation components which had a detrimental effect on strength gain. The difference between dosage amount before a drop in strength as seen between samples treated with bacteria strain P21 (Figure 5.5), and indigenous bacteria addition showed that with added bacteria, higher cementation reagent dosage up to 2 mol/kg could be added without compromising strength gain. The cementation reagent contains calcium chloride, which is highly soluble and may contribute to ions exchange in the soil (Bache, 1974; Moayedi et al., 2013). Ions exchange of soil may contribute to the hardening of soil

without any cementation reaction (Gray, 1970; Moayedi et al., 2013). Calcium chloride may improve the strength of peat to a certain extent. However, an excessive concentration of $CaCl_2$ may cause strength reduction due to distortion of charge balance, resulting in re-stabilization of the peat colloidal fraction and deflocculating of the larger particles. (Kazemian, Prasad, Huat, Bazaz, et al., 2011). These phenomena may result in strength loss when an amount exceed 2 mol/kg of cementation was added for when bacteria strain P21 at 108 CFU/mL was used.

5.3.1.3. Effect of sand filler and curing duration

The influence of sand % with MICP on stabilisation peat soil was studied, and the UCS results of stabilised peat for 3, 7, 14, and 28 days of curing time are provided in Figure 5.7. Bacteria strain P21 at 10^8 CFU/mL with cementation dosage of 2 mol/kg was used for treated samples for this part of the study. The unconfined compressive strength of MICP treated test samples increased while increasing the duration of curing in water and the sand percentage. When 75% of sand was applied, the UCS of test samples increased progressively from 5.15 to 94.85 kPa at the respective curing time in water from 3 to 28 days. Highest UCS was observed at 75%, followed by 50% and 25% sand at 94.85, 87.56 and 82.38 kPa, respectively. The increment for 25, 50 and 75% sand compared between treated and non-treated are 14.55%, 12.31% and 8.23%, respectively. From Section 5.2.1, it was observed that the highest strength gain for peat with sole indigenous urease source (without the addition of strain P21) at 28days was 22.46 kPa. The strength was higher as compared with peat mixed with sand without MICP treatment. However, those treated with added P21 strain and sand were found to yield higher strength than MICP treated peat using indigenous urease. It was evident from the findings that the magnitude of strength gains of treated samples compared to untreated samples of different amount of sand % and duration of curing in the water suggested the bio-cementation effect of MICP. The bio-cementation process improved the unconfined compressive strength (UCS) of the test samples. Treatment of sands via MICP has resulted in increases of UCS greater than three orders of magnitude and up to four orders of magnitude (Al Qabany & Soga, 2013; Al Qabany et al., 2011; Terzis & Laloui, 2018; van Paassen et al., 2010). Calcium carbonate precipitation in the treated samples was quantified and presented in Figure 5.8. The trends showed increasing $CaCO_3$ content with increasing curing durations. The highest amount was recorded for treated peat with 75% sand at 0.13 g/g $CaCO_3$ precipitated. From the

results, the increasing amount of sand was shown to increase precipitated $CaCO_3$, which may be due to decreasing peat content with the addition of sand. Humic substances in peat or peat colloids may interfere with the availability of the dissolved cation, such as calcium ions (Rate, 1990). As urea hydrolysis progress, the availability of ammonium may lead to sorption of humic substances displacing Ca^{2+} from peat particles and increase the conversion towards $CaCO_3$ (Slavek et al., 1982; Tipping, 2002). These may suggest high late strength of the treated at 28 days along with higher $CaCO_3$ precipitation.

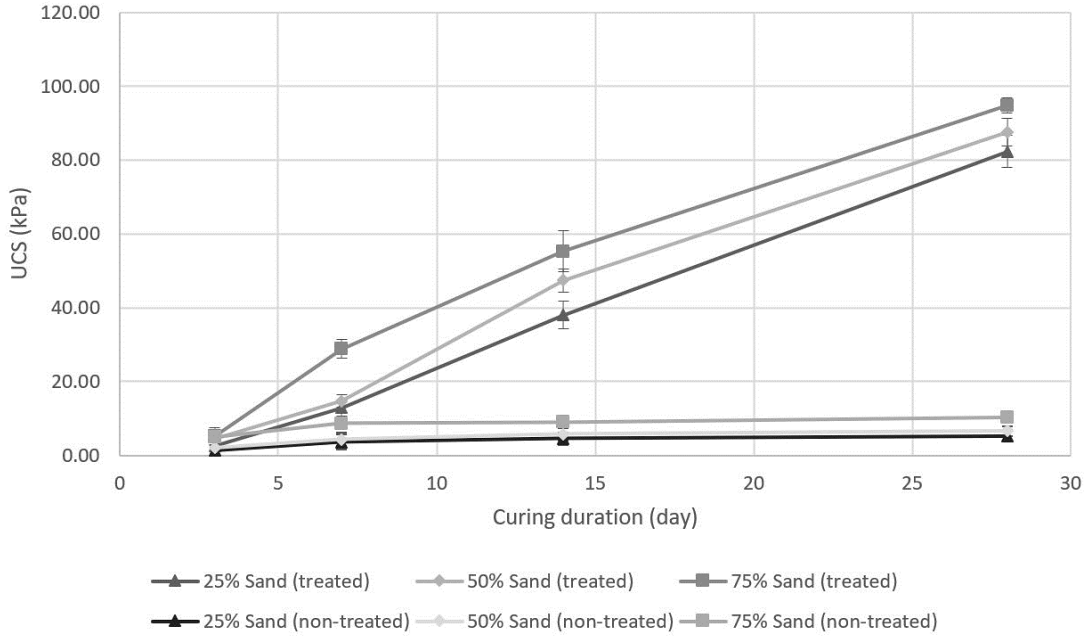


Figure 5.7.Effect of amount of sand as fillers and curing duration.

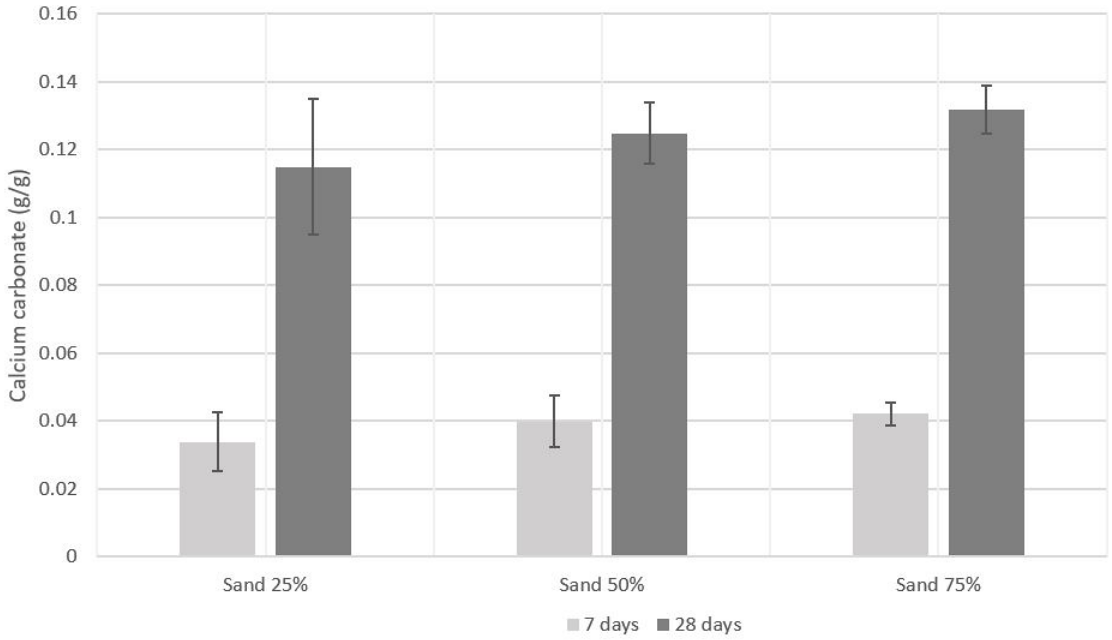


Figure 5.8. Calcium carbonate precipitation of treated peat with 25%, 50% and 75% sand after 7 and 28 days of curing period.

5.3.2. Rate of permeability

The previous study of MICP has shown a bio-clogging effect that reduced permeability of the treated samples with precipitation of calcium carbonate at soil pore space (Chu et al., 2014). Permeability of the treated and untreated peat sand mixture was assessed through saturated hydraulic conductivity (K_{sat}) of samples cured at a duration of 3,7 and 28 days (Table 5.1). Figures 5.8 shows that overall, the hydraulic conductivity of treated samples was reduced compared to control with increasing curing periods. Mesri et al. (1997) has summarised that hydraulic permeability of peat from earlier studies (Berry & Vickers, 1975; Dhowian & Edil, 1980; Lefebvre et al., 1984). The hydraulic permeability ranges between 10^{-8} – 10^{-5} m/s. For this study, the lowest value was observed for treated peat with 25% sand with 3.21 x 10^{-7} m/s as compared to control with 1.28 x 10^{-6} m/s. Generally, the trends for MICP treated and nontreated are as followed: sand 25% (treated) < sand 50% (treated) < sand 25% (control) < sand 50% (control) < sand 75% (treated) < sand 75% (control). Permeability reductions for 25%, 50% and 75% sand were in a range of 51.31 -74.93%, 48.01 – 70.47% and 28.66 – 34.78% respectively (Figure 5.9). MICP treated sand columns were reported to achieve as much as 90%–100% reduction in permeability from initial values (Bang et al., 2001; Gollapudi et al., 1995; Tobler et al., 2011). van Paassen (2009) reported biotreated soils with an approximate 60% reduction in the initial permeability with 100 kg/m^3 $CaCO_3$ precipitation, while Ivanov et al. (2010) had observed permeability reduction at a range of 50 – 99% for MICP treated soil. The result for treated 25% sand showed approximately 10 times reduction in permeability as compare with other additives. Rahman et al. (2016) reported that hydraulic permeability of peat was reduced from 1.73 x 10^{-5} m/s to 1.87 x 10^{-6} m/s with 20% Ordinary Portland Cement (OPC) added curing for 20 days. Rahgozar and Saberian (2016) reported that permeability of peat was reduced from 6.7 × 10^{-5} m/s to 6.4 × 10^{-6} m/s for addition of sand (400 kg/m^3) and 20% tyre chips.

Table 5-1 Hydraulic conductivity, K_{sat} (m/s) of control and treated peat samples curing for 3, 7 and 28 days.

Sand (%)	Hydraulic conductivity (m/s)					
	3 days		7 days		28 days	
	Control	Treated	Control	Treated	Control	Treated
25	1.91E-06	9.32E-07	1.31E-06	5.12E-07	1.28E-06	3.22E-07
50	2.49E-06	1.29E-06	2.21E-06	8.29E-07	2.19E-06	6.47E-07
75	5.59E-06	3.99E-06	5.04E-06	3.69E-06	5.04E-06	3.29E-06

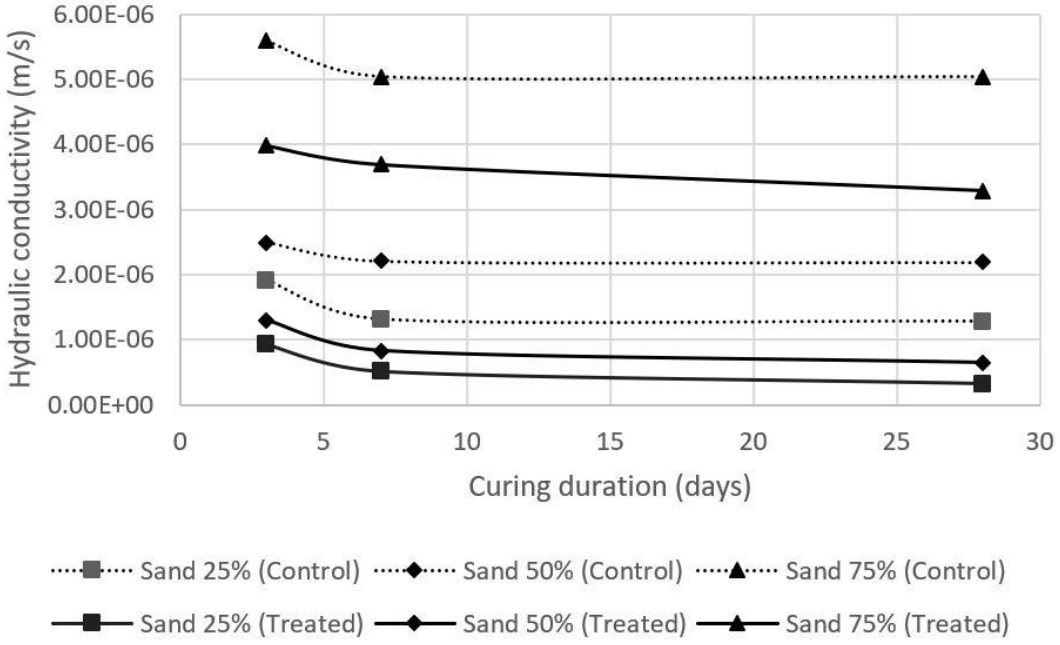


Figure 5.9. Effect of MICP towards hydraulic conductivity of peat at sand mixture of 25%, 50% and 75% up to 28 days curing period.

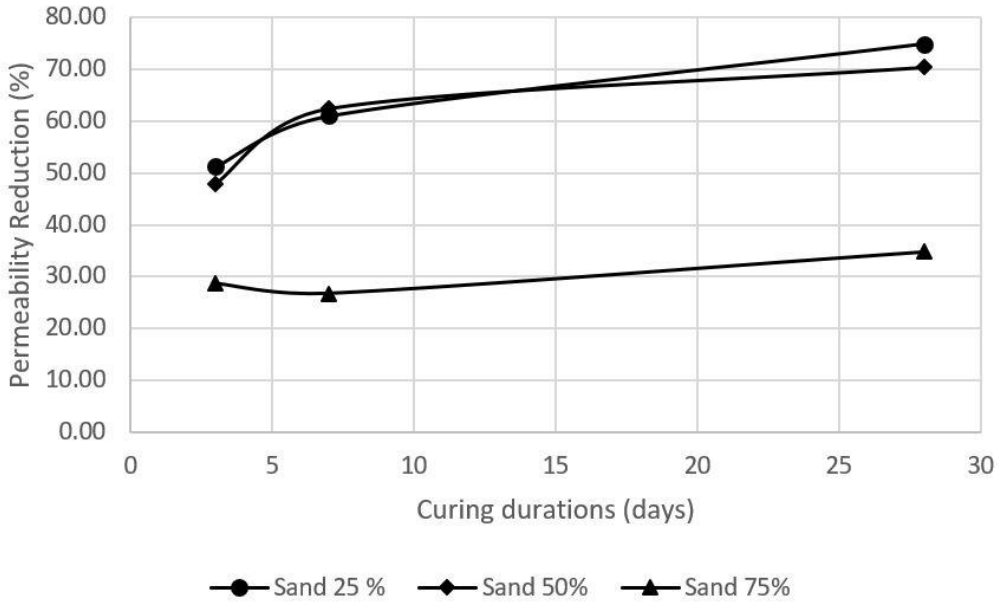


Figure 5.10. Reduction in permeability due to MICP towards peat at sand mixture of 25%, 50% and 75% up to 28 days curing period.

5.3.3. X-ray diffraction analysis and Scanning Electron Microscopy coupled Energy Dispersive Spectroscopy (SEM-EDS) analysis

X-ray diffraction analysis was performed on selected samples to study the polymorph of calcium carbonate formed in the stabilised peat samples. Figure 5.10 shows XRD analysis of crystal phase in the samples for the representative samples of MICP treated and untreated peat with sand crystal. Calcium carbonate in calcite polymorph was seen presence along with sand as quartz compared to untreated samples that were observed with only quartz crystal. SEM analysis coupled with X-ray spectroscopy is commonly used to visualise bio-cementation or bio-precipitation on inorganic soil due to MICP (Achal et al., 2012; Burbank et al., 2012; DeJong et al., 2006; Kim & Youn, 2016; Mahawish et al., 2018). Mineralization due to biological effort may lead to different polymorph of $CaCO_3$ such as calcite, aragonite, vaterite, monohydrocalcite ($CaCO_3 \cdot H_2O$), hexahydrocalcite or ikaite ($CaCO_3 \cdot 6H_2O$) and less favourable amorphous calcium carbonate (Anbu et al., 2016). Calcite and vaterite are the common precipitation, with calcite deemed as the primary and thermodynamically stable product of $CaCO_3$ in many MICPs (Anbu et al., 2016; Ganendra et al., 2014; Spanos & Koutsoukos, 1998; Stocks-Fischer et al., 1999). Figure 5.11 shows the microsurface

of the MICP treated representative peat sample. EDS spectra (a) and (b) show the presence of sand particle along with peat surface, while (c) shows the presence of calcium along with silica suggesting calcium carbonate precipitation on sand and peat fabric. These suggested that bio-cementation bridging sand and peat fabric which results in strength improvement as shown in Section 5.3.1. This, in turn, led to bio-clogging, which might explain the reduction of permeability as discussed in Section 5.3.2.

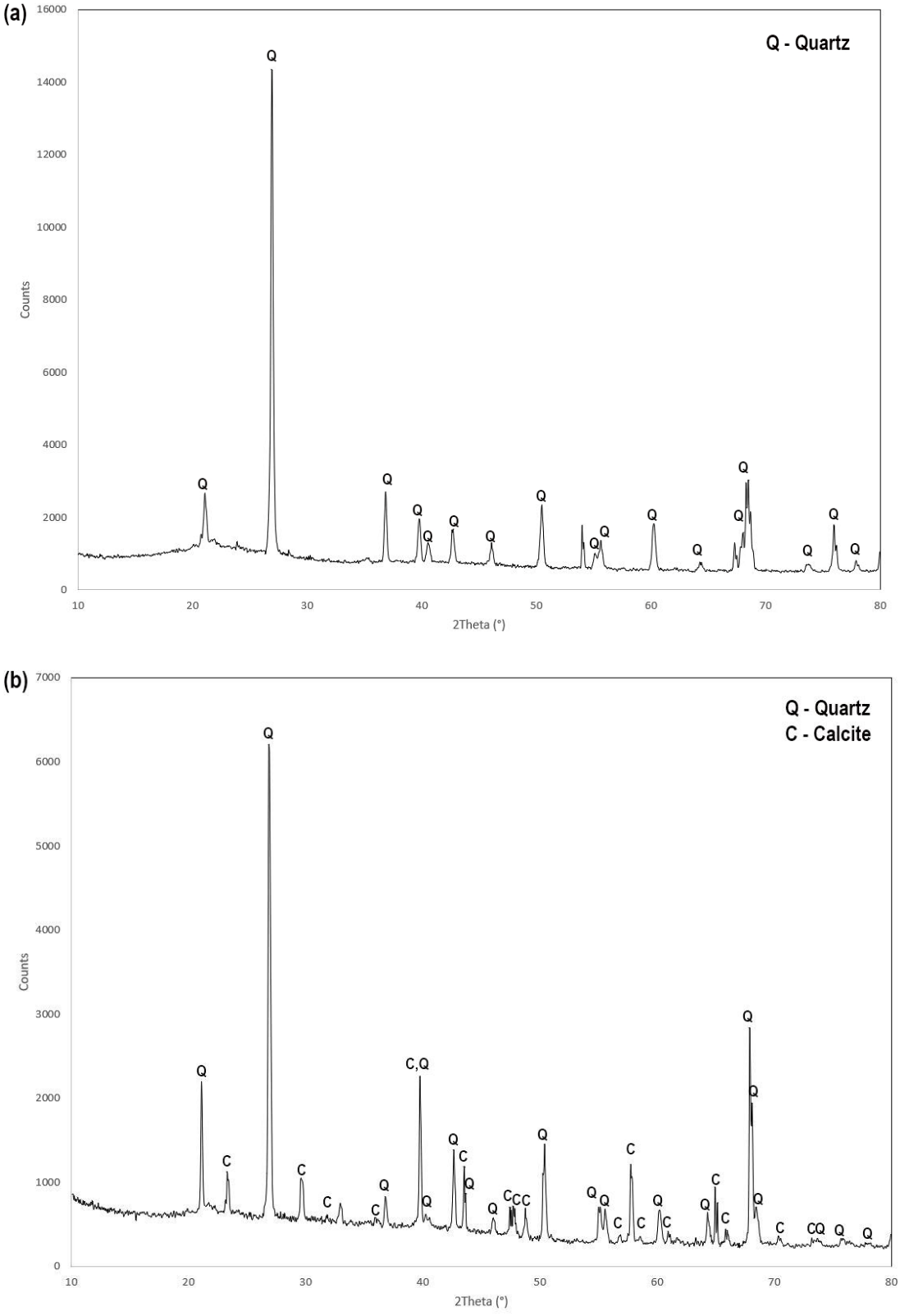


Figure 5.11. X-ray diffraction analysis of (a) Control peat samples (25% sand; 28 days curing) and (b) MICP treated peat (25% sand; 28 days curing)

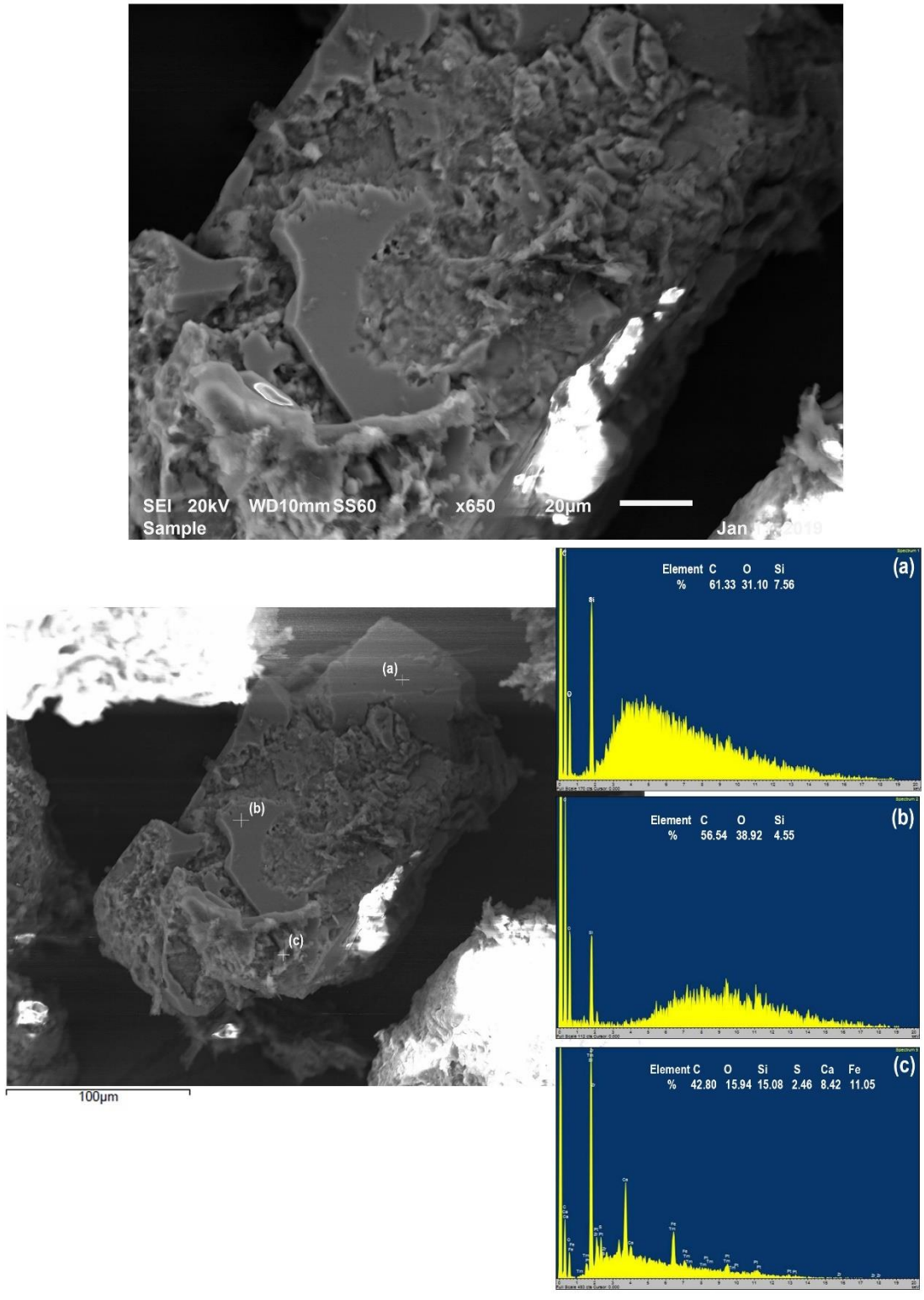


Figure 5.12 SEM and EDS analysis of MICP treated peat (20% sand; 28 days curing).

5.4. Chapter summary

Objective II was achieved in the current chapter. Following are the findings for this Chapter:

1. It was observed that calcium carbonate precipitation was possible by utilising indigenous urease activity in peat. Although the natural acidic condition of peat did not favour carbonate crystal precipitation, it was possible with the presence of urea hydrolysis of indigenous peat bacteria altering the environmental pH for calcite precipitation.

2. The precipitation of $CaCO_3$ was seen with strength improvement of peat samples suggesting its uses for peat stabilisation. $CaCO_3$ precipitated were tested by XRD and shown to be in calcite polymorph.

3. Ammonia accumulation has always been an issue for MICP application. For peat, it was observed that ammonia concentration was reduced at day 28 of curing, suggesting the potential of MICP in the stabilisation of peat as an eco-friendly stabilisation method.

4. For MICP of peat with 25%, 50% and 75% sand, after 28 days of submerged curing, within the range of test, an optimal unconfined compressive strength 82.05 kPa was observed with bacteria strain P21 at a concentration of 10^8 CFU/mL for peat with 25% sand and 2 mol/kg cementation reagent dosage. The UCS of the test samples increased with increasing cementation reagent dosage to an extent where strength reduction will occur.

5. UCS for treated peat with 25%, 50% and 75% showed increasing trends with increasing curing duration. Highest UCS was observed at 75% sand, followed by 50% and 25% sand at 94.85, 87.56 and 82.38 kPa, respectively. $CaCO_3$ precipitation was seen to be increased with increasing sand content and curing duration.

6. The permeability of the treated test sample of stabilised peat in overall was lower as compared with untreated samples, and values reduce against curing time.

7. XRD and SEM-EDS showed that the presence of calcite in MICP treated samples bridging peat and sand particles improving UCS and reducing permeability due to clogging.

CHAPTER 6: CONSOLIDATION BEHAVIOUR OF MICP TREATED PEAT

6.1. Introduction

This chapter aims to investigate the effect of MICP on the consolidation behaviour of peat. Section 6.2 focused on the consolidation behaviour of MICP treated peat. Reagent concentration, bacteria concentration and range of sand content were selected based on the study done in Chapter 5. Consolidation behaviour in term of primary consolidation and secondary compression (creep) was evaluated.

6.2. One-dimensional consolidation behaviour

6.2.1. Effect towards void ratio, e and coefficient of primary consolidation, C_v

Figure 6.1 shows the overall trends that there is a decrement of void ratio compared between treated and untreated peat samples. The compression curves reduced linearly with incremental effective vertical stress for treated and untreated samples. The void ratio for the peat samples decreased with increasing sand content and further decreased with MICP treatment. Peat samples without sand were observed to have the highest void ratio. Natural peat has a high void ratio due to high water content filling the void space of peat particles with a value up to 15 (Hanrahan, 1954). A slight decrement of void ratio was observed for treated peat compared with untreated peat. The initial void ratio for treated sample was observed to be lesser than the untreated sample at the same sand content. MICP treatment for this study was based on urea hydrolysis based on Equation 6.1 and 6.2 (Cheng et al., 2013):

$$CO(NH_2)_{2\,(aq)} + H_2O_{\,(l)} \rightarrow CO_3^{2-}{}_{(aq)} + 2NH_4^{+}{}_{(aq)} \qquad (6.1)$$

$$Ca^{2+}{}_{(aq)} + CO_3^{2-}{}_{(aq)} \rightarrow CaCO_{3\,(s)} \qquad (6.2)$$

Reduction of initial void ratio when comparing with treated and untreated samples at the same sand content may be due to the MICP process where water is consumed for urea hydrolysis, and the precipitation of calcium carbonate crystal filled the voids and reducing water content leads to reducing void space under the same preloading.

Coefficient of Consolidation, C_v was used to evaluate how quickly the consolidation process is completed. Generally, the higher the C_v, the faster is the primary consolidation process C_v increases with increasing permeability and stiffness of soil (Ameratunga et al., 2016). Figure 6.2 shows both treated and untreated peat samples with different sand content had decreasing trends of C_v with increasing effective stress. Previous study has also observed similar reducing trends of C_v of stabilised peat with increasing pressure (Hassan et al., 2013; Makinda et al., 2018). Treated and untreated peat without sand has lower C_v as compared to samples with sands under the same consolidation stress level. Such phenomena were also seen in a previous study where C_v of peat without sand filler were lower compared with sand at a range between 10 – 50% under the same vertical pressure (Hassan et al., 2013). This arose due to higher organic content, which decreases C_v in organic soil (Adejumo, 2012). The results suggested that increasing sand content increased C_v for each treated and untreated peat sample under the same consolidation pressure. This was due to a reduction of organic content with increasing granular materials that increased C_v (Hassan et al., 2013). Treated peat with 50% sand (PTS50) and 75% sand (PTS75) were shown to have lower C_v between effective stress of 50 – 400kPa as compared with their untreated counterpart. As discussed earlier, C_v was affected by permeability, lower C_v for PTS50 and PTS75 as compared to PS50 and PS75 may be due to precipitation of carbonate crystal that lowers the sample's permeability. This might also suggest that why PTS25 had a different trend compared to PTS50 and PTS75, where C_v was observed to be higher compared to PS25 as $CaCO_3$ precipitation was lesser, as suggested in Chapter five.

The general trend among all the peat samples decreased the value of the coefficient of volume compressibility, m_v, with subsequent increase of effective pressures, as shown in Figure 6.3. The trend suggested that increasing sand content decrease m_v and m_v of treated were found to be lower as compared with untreated samples at the same sand content subject at the same stress. This further suggested that the compressibility of treated samples was decreased when calcite formation filling up void space of the treated samples that promoted cementation of organic particles. Such trends were also observed from a previous bio-cementation study involving organic soil with higher m_v values for the untreated sample than for the treated samples (Canakci et al., 2015b).

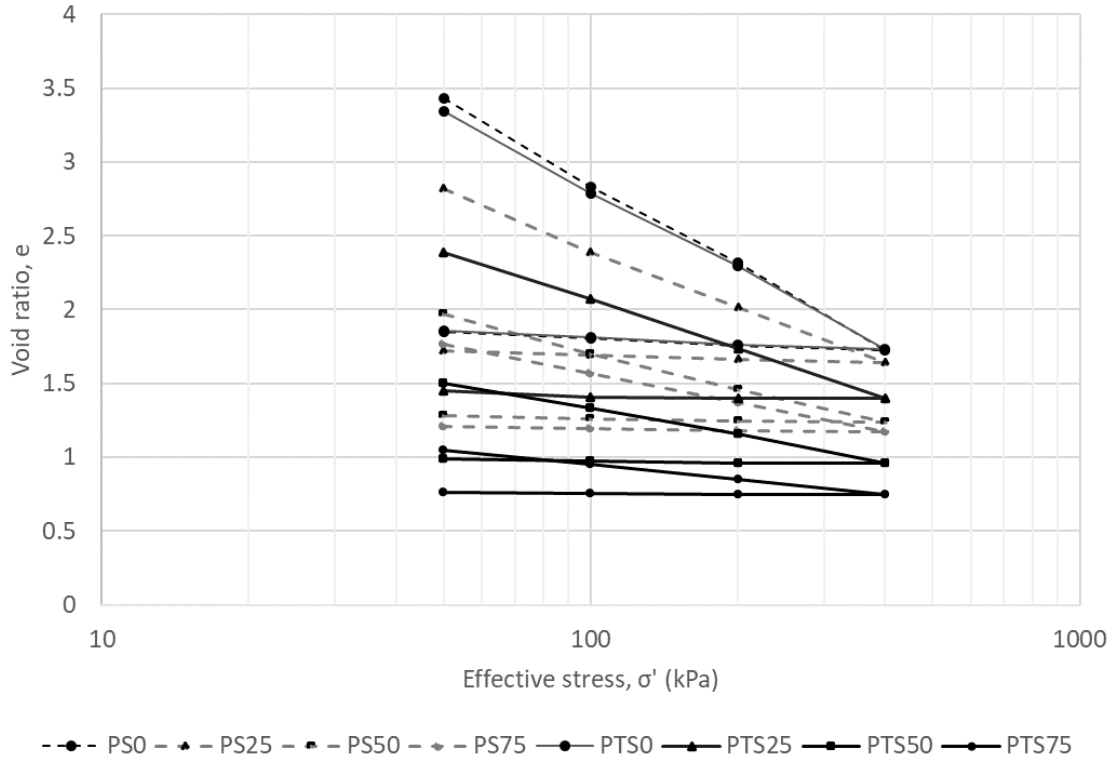


Figure 6.1. Void ratio against log effective stress curve for treated samples with 0% (PTS0), 25% (PTS25), 75% (PTS75) sand and untreated samples of 0% (PS0), 25% (PS25) and 75% (PS75) sand content.

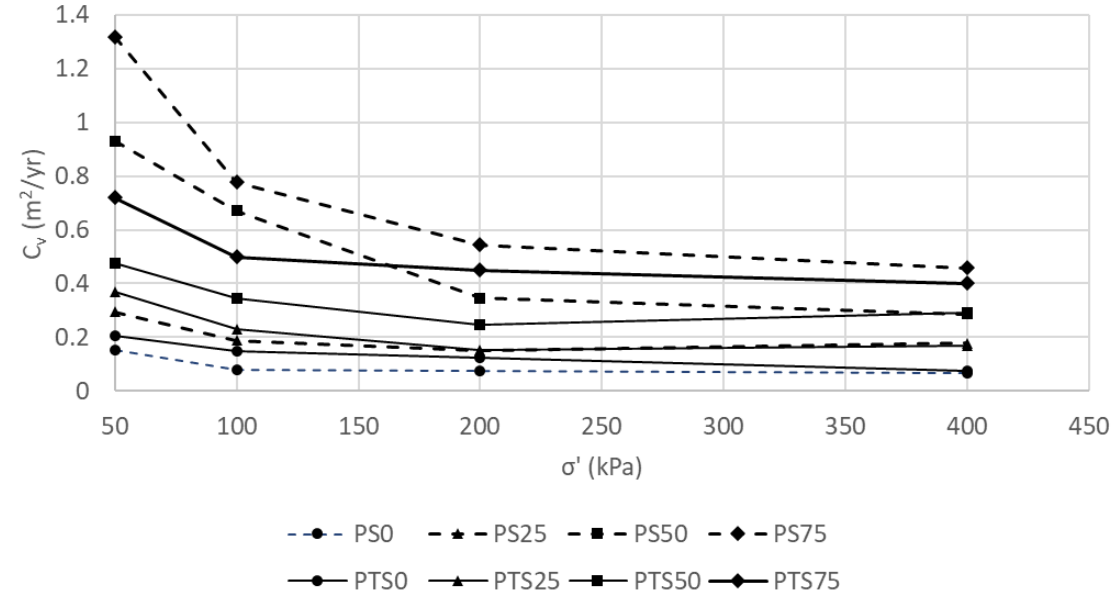


Figure 6.2. Coefficients of consolidation, C_v against effective stress, σ' curve for treated peat samples with 0% (PTS0), 25% (PTS25), 75% (PTS75) sand and untreated samples of 0% (PS0), 25% (PS25) and 75% (PS75) sand content.

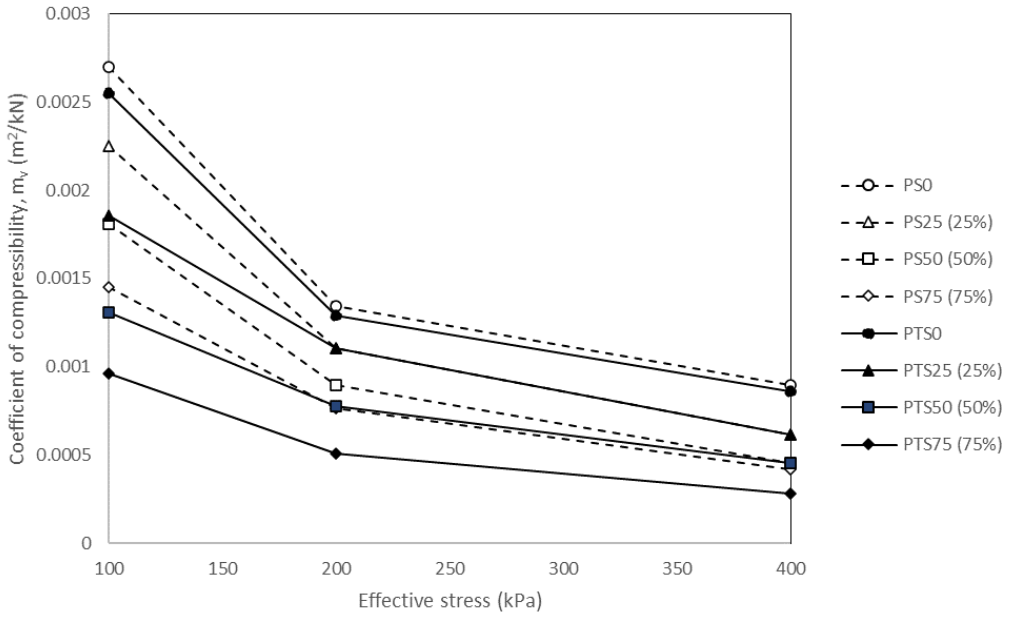


Figure 6.3 Coefficient of compressibility against vertical effective stress of treated peat samples with 0% (PTS0), 25% (PTS25), 75% (PTS75) sand and untreated samples of 0% (PS0), 25% (PS25) and 75% (PS75) sand.

6.2.2. Effect towards hydraulic permeability, k

Hydraulic permeability, k (m/s) can be estimated indirectly with an oedometer test based on C_v and coefficient of volume change, m_v at each vertical stress. A calculation can be done through k (m/s) = $C_v * m_v * \gamma_w$ where γ_w is the unit weight of water. Permeability of treated and untreated peat mixed with 0% to 75% of sand at each vertical stress is shown in Figure 6.4. Hydraulic conductivity was observed to decrease with increment loading. Generally, treated samples showed lower hydraulic conductivity as compared to untreated. Such a trend was observed in a related chemical cementation study where the permeability of treated peat was further reduced from 9.72 x 10^{-13} m/s (Wong et al., 2013). The reduction of permeability was indicated a clogging effect by the MICP process. The formation of calcite in soil void led to clogging, in turn reduced permeability (Whiffin et al., 2007). Canakci et al. (2015b) found a similar trend of permeability reduction due to MICP for organic soil. The trends suggested that higher permeability was observed with a higher C_v value. This may suggest the reason for higher C_v of untreated samples compared treated samples at the same stress level shown in Figure 6.2 as suggested by Canakci et al. (2015b).

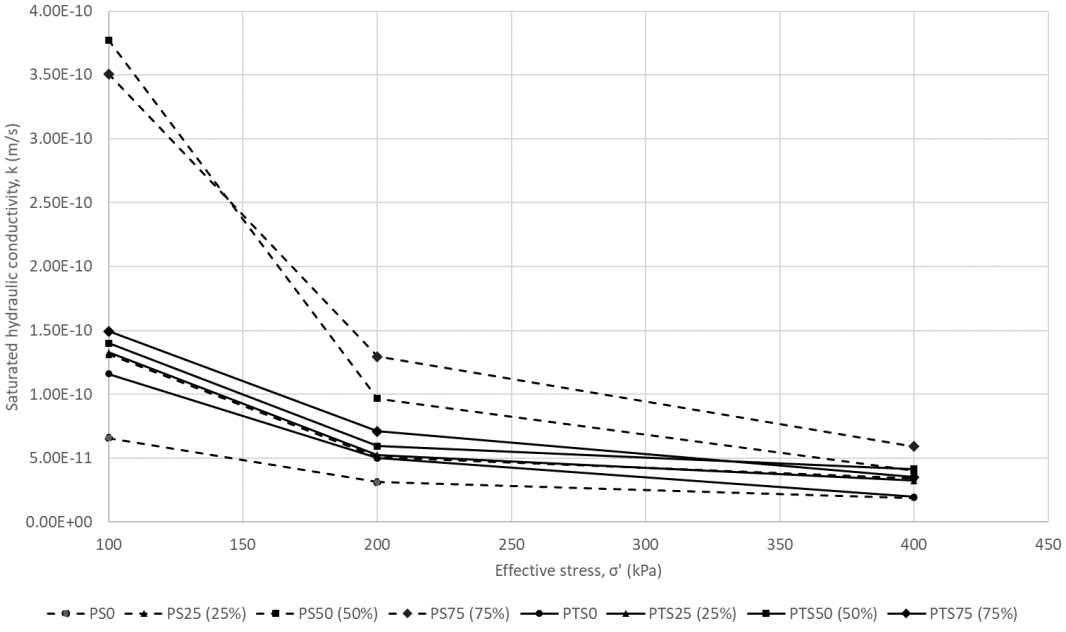


Figure 6.4. Saturated hydraulic conductivity, k against effective stress, σ' curve for treated & untreated peat samples with different sand content.

6.2.3. Effect towards Compression index, C_c and Swelling Index, C_s

Peat has a high void ratio due to its porous nature of peat particles (Huat et al., 2011). Peat, with its high *in-situ* void ratios, generally will display high compression index values due to the high in situ void ratios (Mesri et al., 1997). The larger the void ratio of the peat is, the larger the compression index and the higher will be the primary consolidation settlement of peat. However, the rate of consolidation will be decreased as the applied stress is increased (Huat et al., 2014). Figure 6.5 shows the compression index, C_c for MICP treated and untreated peat sample with different sand content. C_c for both treated and untreated samples shown decreasing trends with increasing sand content. A previous study suggested a similar reducing trend of C_c for peat with increasing sand percentage (Celik & Canakci, 2014; Hassan et al., 2013). Treated sample at the same sand content was shown to be lower as compared with the untreated sample at the same sand content. This is observed as there is a reduction of initial void ratio from treated compared to untreated, which leads to a minor change in void ratio, Δe at the same applied effective stress, σ'_v. Such phenomena suggested calcite precipitation occupying void space and reducing water content in samples. Mesri et al. (1997) suggested decreasing C_c with decreasing water content for peat and clay. Apart from the reduction of water content by filling of the void with calcite precipitation, the reduction of C_c might also be due to cementation effect between peat particles. Previous studies has showed C_c reduction was achieved with increasing cementation grouting (Kazemian & Moayedi, 2014; Youventharan & Duraisamy, 2007). The reduction of C_c of treated samples compared to untreated samples as indicated that MICP led to improvement of compressibility of peat. This could also be supported by a study done by Canakci et al. (2015b) where the formation of calcite during MICP reduced C_c of organic soil, which led to improvement of compressibility.

Stabilized peat is susceptible to swelling when exposed to water (Deboucha & Hashim, 2009). The swelling index, C_s represent the rebound region that occurs when the effective vertical stress is reduced. This index acts as a parameter for the expansion of soil after the applied load on top is reduced. The values of C_s for various test samples are shown in Figure 6.6 above. Generally, decreasing C_s was observed for both treated and untreated with increasing sand content. Treated samples were shown to have lower C_s compared to untreated samples at the same sand content. Cementation in soil was

shown to decrease C_s by flocculation of soil fabric and with cementation of soil particles and filling of cementation products replacing void space (Abbey et al., 2019).

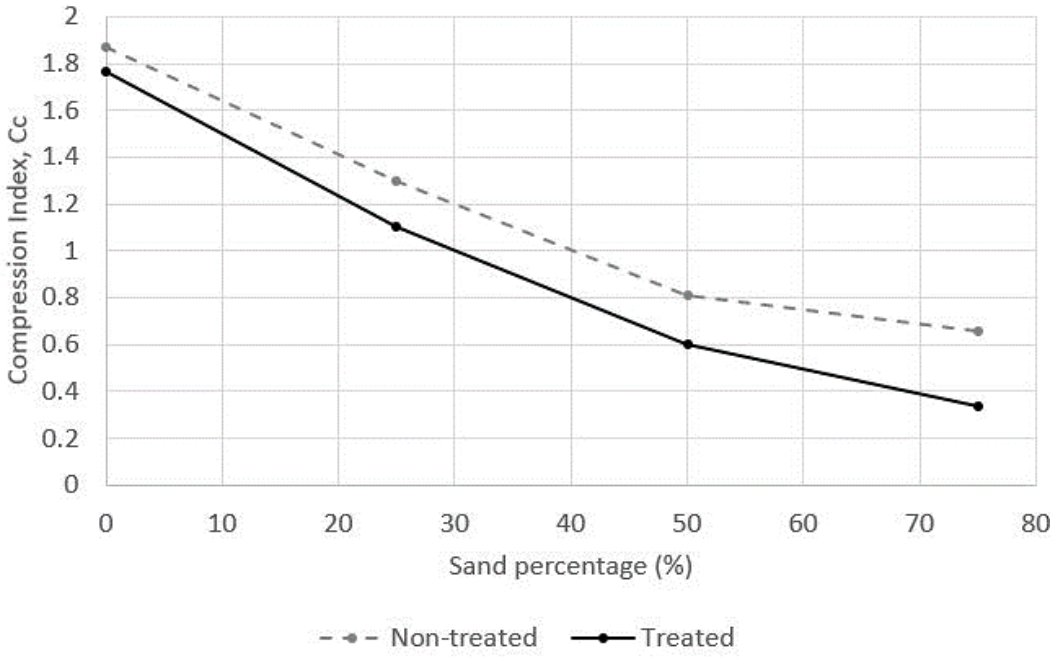


Figure 6.5. Compression Index, C_c for treated and untreated peat samples varies with sand content (%).

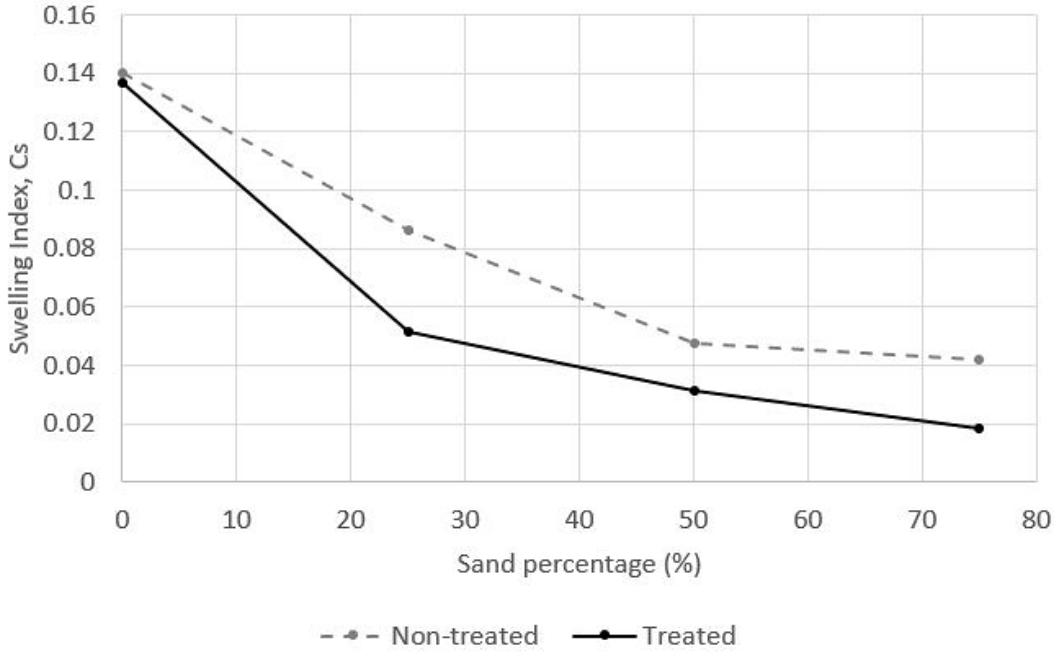


Figure 6.6. Swelling Index, C_s for treated and untreated peat samples varies with sand content (%).

6.2.4. Effect towards Secondary compression index, C_α and C_α/C_c concept

The secondary compression index, C_α, which shows the creep behaviour of soil under constant stress, is known to vary depending on time and its behaviour which varies on applied stress (Mesri & Castro, 1987). C_α can be calculated from e vs log time graph according to with $C_\alpha = \Delta e / \Delta \log t$. Generally, it was observed that C_α decrease with increasing sand content for both treated and untreated. However, C_α for treated peat with 25% sand was higher compared to untreated peat of 25% sand at 100 kPa effective stress onwards, while C_α for treated peat with 50% sand was higher compared to untreated peat with 50% at 200 kPa effective stress onwards (Figure 6.7). Secondary consolidation behaviour was further evaluated with C_α/C_c concept.

$C\alpha/Cc$ concept is used to evaluate the secondary consolidation behaviour of MICP treated and untreated peat with different percentage of sand. $C\alpha/Cc$ can be used to evaluate compressibility of soil, usually, between different soil type with the higher the value of $C\alpha/Cc$, the soil is compressible (Mesri et al., 1997). As discussed previously, $C\alpha$ of the samples at each effective stress were obtained from e vs Log time graphs and Cc of the samples were obtained from slope of void ratio at the end of primary consolidation vs Log σ' graph (Mesri & Castro, 1987). The trends of C_α/C_c for the samples are shown in Figure 6.8. C_α/C_c for treated peat with 0, 25, 50 and 75% sand were 0.058, 0.082, 0.079 and 0.057 respectively while untreated peat with 0, 25, 50 and 75% were 0.057, 0.059, 0.058 and 0.053, respectively. C_α/C_c varies with soil type, for inorganic clays, C_α/C_c were reported to be equal to 0.04 ± 0.01, while for peats, data as high as 0.10 were reported but typically were found in the range of 0.05 – 0.07 (Mesri et al., 1997; Santagata et al., 2008). It was observed for peat with 0 and 75% the C_α/C_c for treated and untreated showed smaller difference while at 25 and 50% the C_α/C_c showed larger difference. From there, we can observe decreasing C_α/C_c from 25% to 75% sand content. This might be due to higher C_α for treated peat of 25 and 50% sand. C_α were affected by the biodegradation of peat fabrics (Mesri et al., 1997). Degradation was typically slowed or retarded at acidic condition of peat (Hobbs, 1986; Wardwell et al., 1983). pH rise and nutrients alteration such as nitrogen input provided a favourable biodegradation environment in peat (O'Kelly & Pichan, 2013). MICP treatments may raise pH by urea hydrolysis, and urea input may increase ammonium

nitrogen which act as nutrients for microbial population that may induce biodegradation which leads to higher C_α.

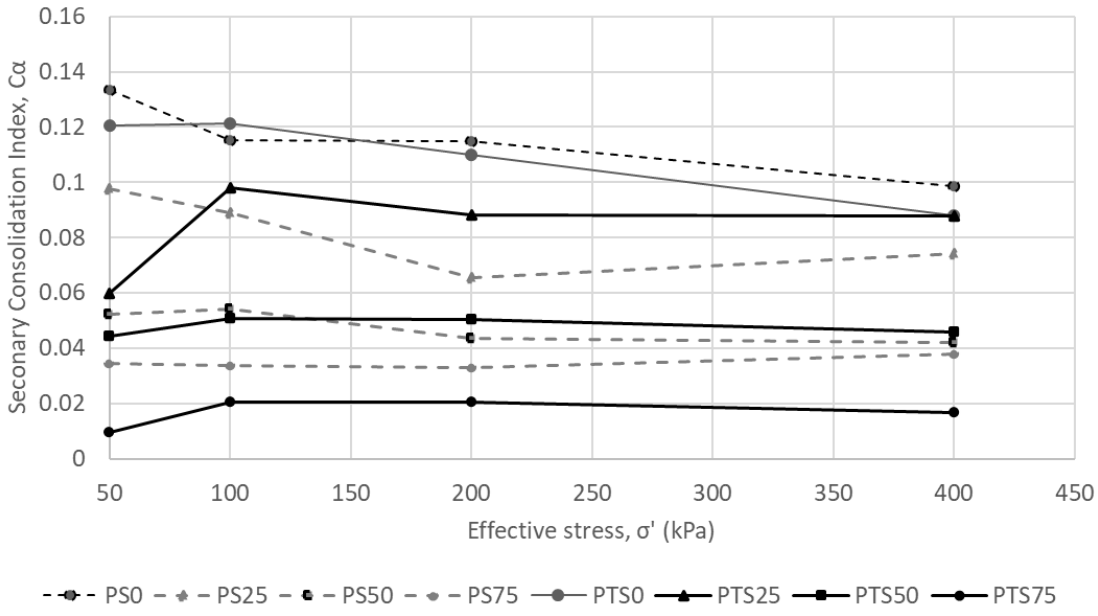


Figure 6.7. Secondary Compression Index, C_α against Effective Stress, σ' for treated and untreated peat samples varies with sand content (%)

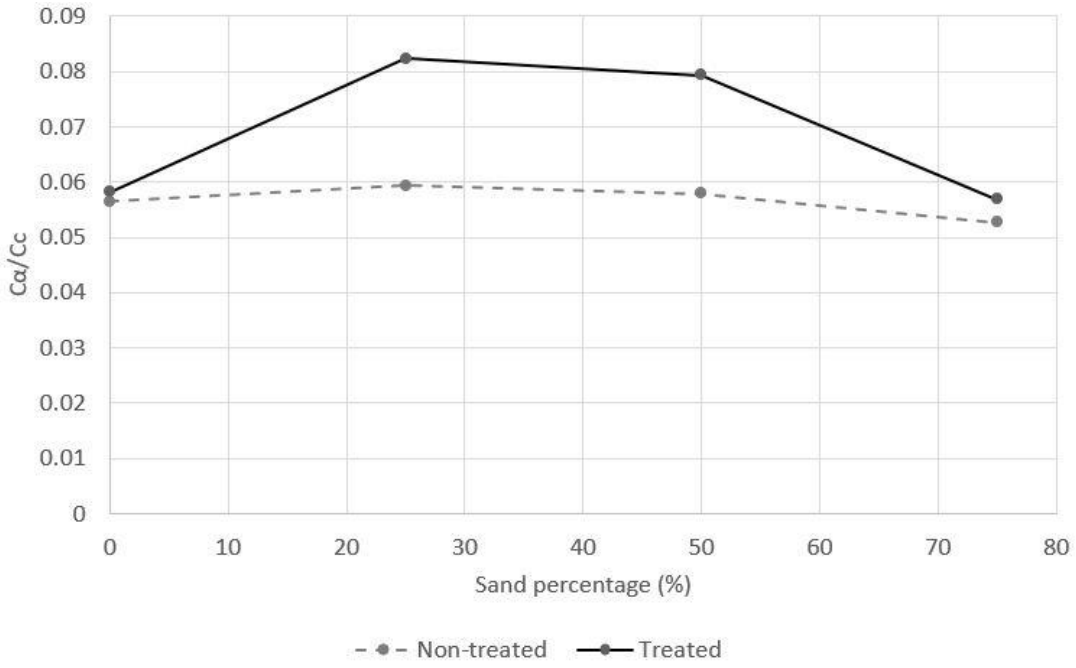


Figure 6.8. C_α/C_c for varies for treated and untreated peat samples varies with sand content (%)

6.3. Chapter summary

Objective III was achieved with the following findings summarised from this chapter:

1. Peat varies with sand content treated with MICP will results in a lower void ratio due to calcium carbonate precipitation.

2. Increasing sand content increased Coefficient of consolidation, C_v for both treated and untreated peat. However, lower C_v was observed for treated peat as compared with untreated at the same sand content. Reduction of hydraulic permeability was observed for treated as compared to untreated at same sand content.

3. Compression Index, C_c and Swelling Index, C_s for treated peat were lower compared to untreated peat at the same sand content.

4. Secondary compression Index, C_α for treated peat with 25% sand was higher compared to untreated peat of 25% sand at 100 kPa effective stress onwards while C_α for treated peat with 50% sand was higher compared to untreated peat with 50% at 200 kPa effective stress onwards which may be due to biodegradation of peat.

CHAPTER 7: DURABILITY OF MICP STABILISED PEAT SUBMERGED IN ACIDIC PEAT CONDITION

7.1. Introduction

The chapter aims to discuss the durability of the MICP treatment of peat. The previous study mostly focussed on MICP in a non-acidic and inorganic environment (Choi et al., 2020). Possibility involved altering of acidic pH to more alkaline condition to facilitate the process in an organic environment were proven (Sato et al., 2016). However, peatland has an acidic pH with a tropical peatland pH range below pH 4.5 (Osaki & Tsuji, 2016). Hence, from the previous chapter, we know that the MICP process was able to initiate in acidic peat, but the possibility of its $CaCO_3$ degradation by surrounding acid attack was not known. The study aims to evaluate the acid attack by immersing stabilised peat column in acidic peat slurry and distilled water at a given duration of up to 90 days. Unconfined compression test, calcium carbonate content and pH of the treated submerged peat column were measured to evaluate for the treatment durability. The current laboratory study would provide an insight on how MICP stabilised peat column will react *in-situ* at acidic waterlogged peatland.

7.2. Strength of MICP stabilised peat with exposure to acidic peat slurry and distilled water

The effect of acidic peat environment towards MICP stabilised peat was evaluated through unconfined compressive strength (UCS) at a different time interval of 1, 30, 60 and 90 days submerged in peat slurry and those submerged in distilled water were used for the control experiment. Figure 7.1. shows UCS of the samples submerged in peat and distilled water, respectively. In general, sample submerged in distilled water showed higher strength as compared to those submerged in peat slurry. Samples submerged in peat slurry showed an extensive decrease in strength as compared to samples submerged in distilled water. It was noted that in Chapter 5, the identical samples' setup that has not been immersed in solution without preload after 28 days curing was able to yield UCS of 82.38 kPa, and from this extended study, after immerging in both solutions, UCS was found to decrease. This may be due to the swelling of peat samples without confinement which was observed in Section 6.2. It

was observed that the UCS for the samples submerged in peat slurry were 36.34 kPa, 28.87 kPa and 23.94 kPa at 30, 60 and 90 days, respectively, whereas 72.19 kPa, 67.99 kPa and 67.19 kPa were obtained for MICP stabilised peat submerged in distilled water at the respective duration.

In general, lower strength was observed for samples submerged in peat water as compared to control. However, a decreasing trend was observed for control with the submerged period (peat submerged with distilled water) due to the swelling nature of peat when immersed in water without any preload. Peat, with its organic materials, is prone to swell when exposed to water and affect the durability of stabilised peat (Deboucha & Hashim, 2009; Rezanezhad et al., 2016).

Lower strength observed for stabilised peat immersed in acidic peat slurry compared to immersion in distilled water. Figure 7.2. showed the change in the strength of the peat samples submerged in peat slurry over peat samples submerged in distilled water (Control) to evaluate strength loss of MICP stabilised peat by exposure to acidic peat environment at a specific duration. The strength loss was 49.67% at 30 days, 57.54% at 60 days and up to 64.37% at 90 days. The decomposition of $CaCO_3$ in the samples may cause strength loss due to acidity, further discussed in Section 7.3.

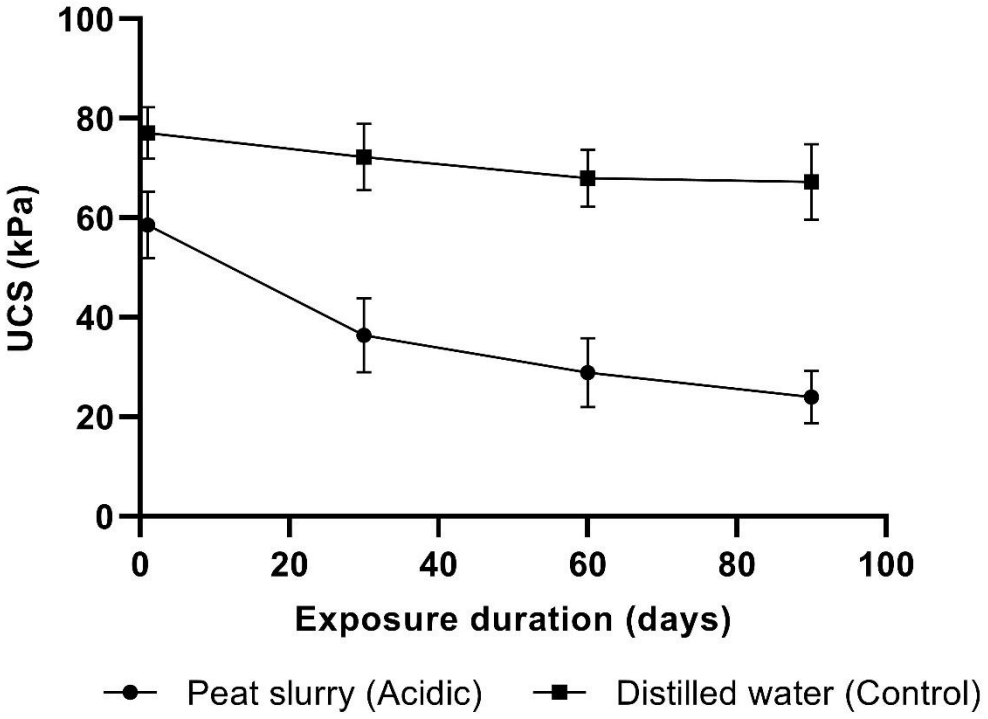


Figure 7.1. Unconfined compressive strength (UCS) of MICP stabilised peat samples submerged in acidic peat slurry and distilled water up to 90 days.

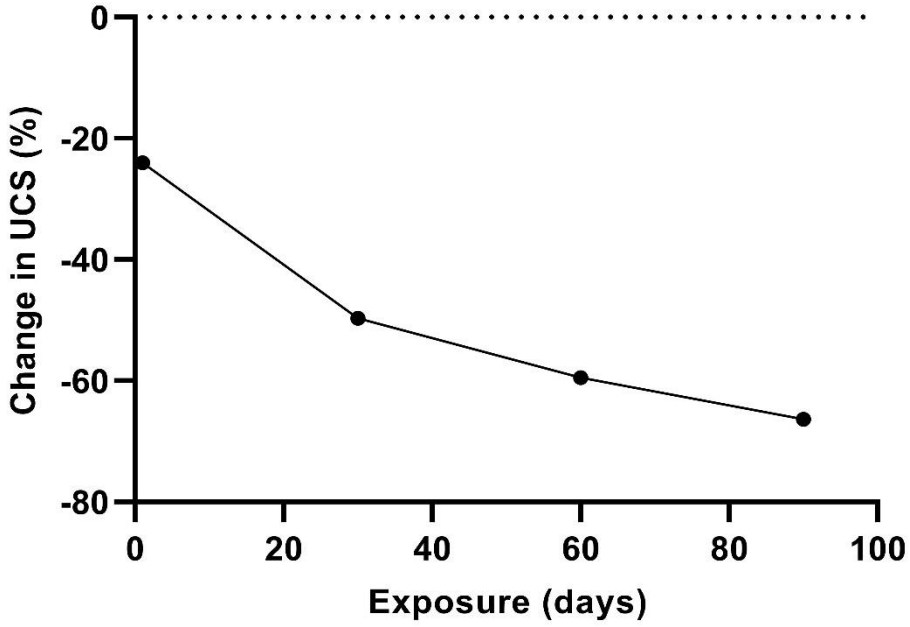


Figure 7.2. Change in UCS between MICP stabilised peat samples submerged in acidic peat slurry with distilled water up to 90 days.

7.3. Calcium carbonate ($CaCO_3$) in stabilised peat and pH changes with exposure to acidic peat slurry and distilled water

The effect of the acidic nature of peat ground towards MICP stabilised peat samples was evaluated through calcium carbonate content and pH changes of the submerged samples in peat slurry and distilled water. Figure 7.3. shows the average pH values of samples submerged in peat slurry and distilled water recorded at submerged time of 1, 30, 60 and 90 days. The overall pH showed that the samples submerged in peat slurry were lower compared to distilled water. It is interesting to note that samples submerged in distilled water showed a slight decreasing pH trend from an average of pH 8.4 at 1-day submerged time to pH 7.8 at 30 days and remain constant at pH 7.5 at day 60 and day 90. Such phenomena may suggest leaching of hydroxide ions from the treated column to surrounding water, rendering decreasing trend of pH until an equilibrium is reached. For MICP stabilised peat samples, fluctuation trends were observed over the 90 days in a range of pH 6.7 – pH 7.3. Figure 7.4. shows an average value of calcium carbonate content estimated at respective samples submerged in peat slurry and distilled water up to 90 days. Increasing carbonate precipitation trends were observed for peat samples submerged in distilled water, whereas decreasing trend was observed for MICP stabilised peat samples submerged in peat slurry. The increments of calcium carbonate content for peat samples submerged in distilled water were more obvious between day 1 to day 30 with recorded average calcium carbonate of 118.13 mg/g, to 133.67 mg/g and remained almost the same at day 60 and 90 at an average of 143.78 mg/g and 144.67 mg/g. Obvious white crystal precipitation was observed at the area exposed directly to water, including the top and bottom of the samples for samples submerged in distilled water (Figure 7.6), while none were observed for peat immersed in peat slurry (Figure 7.5). The calcium carbonate content for peat samples submerged in peat slurry decreased from 96.94 mg/g at day 1 to 72.52 mg/g at day 90.

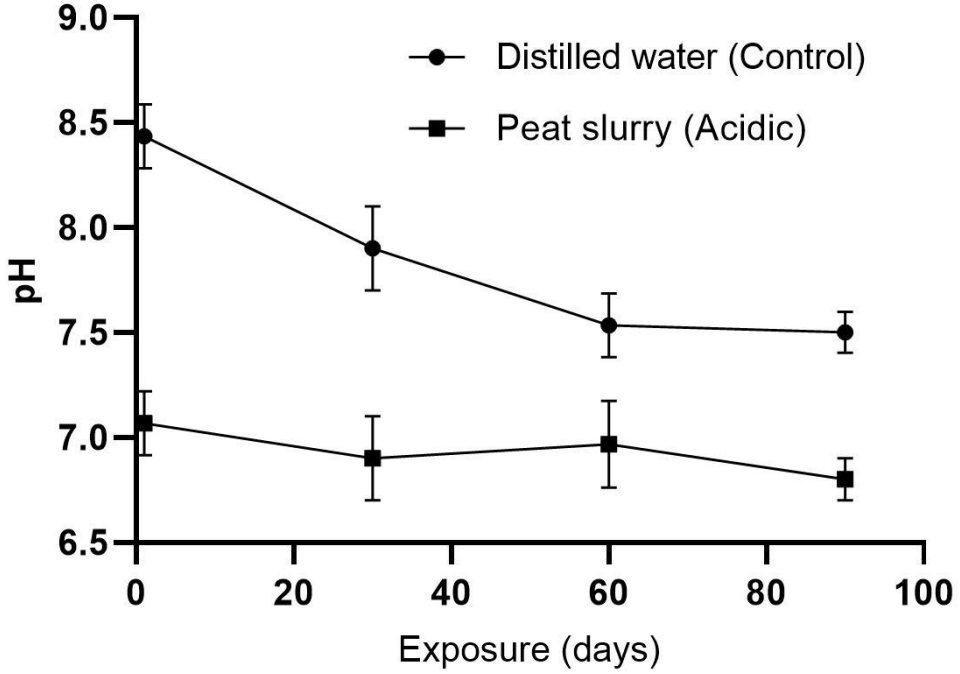


Figure 7.3. pH changes of MICP stabilised peat samples submerged in acidic peat slurry and distilled water up to 90 days.

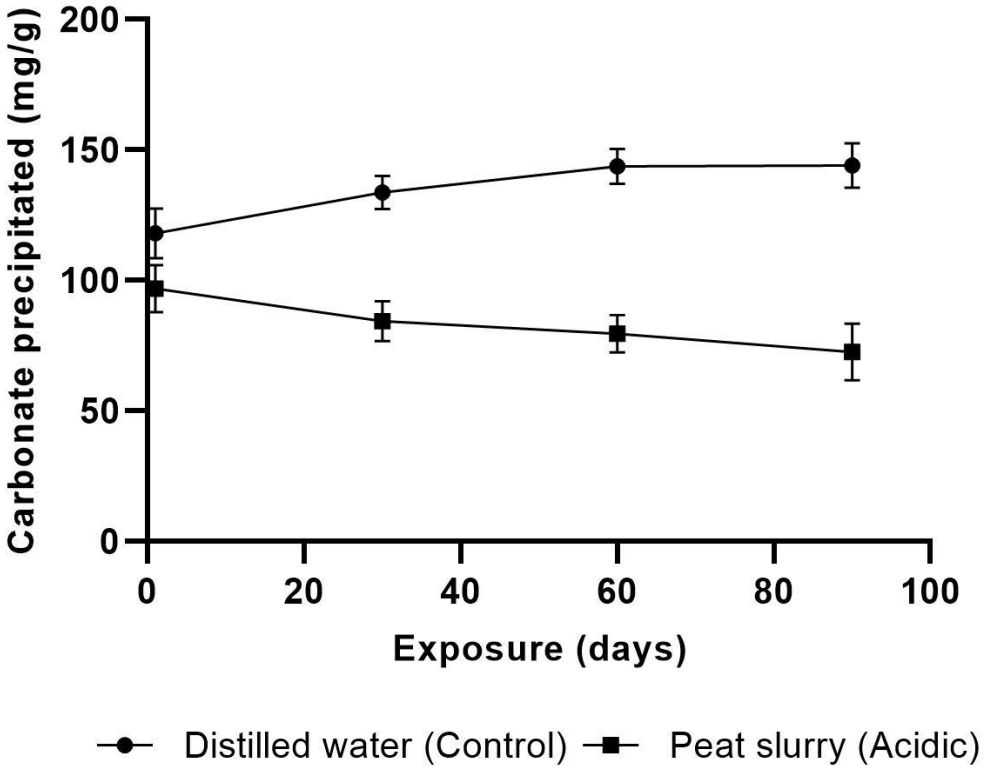


Figure 7.4. Estimation of calcium carbonate ($CaCO_3$) content in MICP stabilised peat samples submerged in acidic peat slurry and distilled water up to 90 days

Figure 7.5. Removal of peat samples from submerged condition prior to testing.

Figure 7.6. Visible observation of calcium carbonate crystal precipitation on surface of the peat samples

7.4. Discussion

The UCS of MICP treatment of peat without submerging to peat slurry showed more than 50 kPa, which was possible for minor ground improvement for dump truck transport. Sato et al. (2016) suggested that peat strength stabilisation to about 50 kPa was required for dump truck transport usage. However, MICP treated samples submerged to peat may pose a challenge as deteriorating UCS were observed.

Application of MICP in acidic condition of peat presented a challenge in contrast with MICP application in sand or other inorganic soil. Chapter 5 suggested among the polymorph of calcium carbonate, calcite was precipitated in treated peat. Calcite, among the rest of the $CaCO_3$ polymorph of vaterite and aragonite, is known among the most stable and the less soluble $CaCO_3$ (Amjad, 2013). Due to the low pH (acidic) environment of peat ground, the calcite may be dissolved with the presence of organic acid. The study evaluated the durability of MICP stabilised peat samples exposing to an environment high in organic acid in peatland. An organic acid in peat, including humic and fulvic acid, was found to dissolved calcium carbonate (Fiskus & Manning, 1998; Klepetsanis et al., 2002). This phenomenon may affect the durability of the MICP stabilised peat column. It was observed that treated samples submerged in peat slurry were found to be low in UCS as compared with those submerged in distilled water. This is observed from the above results showing loss of strength and calcium carbonate content with time when submerged into peat slurry. Peat contained organic acid, including humic and fulvic acid, which is a weak acid. Hence, the dissolution is not instantaneous. The dissolution of $CaCO_3$ in acidic condition may produce calcium cations (Ca^{2+}) which will also form complex with organic acid presence in peat (Tipping, 2002). Residual Ca^{2+} may react with humic acid and fulvic from the surrounding peat to form calcium humate is partially soluble (Kříženecká et al., 2014). Previously, cations were shown to have a stabilisation effect on peat at a certain concentration range (Moayedi et al., 2013; Moayedi et al., 2014). Although $CaCO_3$ dissolution was observed along with decreasing strength, cation stabilisation may occur, contributing to minimum residual strength apart from the contribution of strength from residual $CaCO_3$ in the samples. The decreasing UCS trend was observed for samples submerged in distilled water with a slight increase in $CaCO_3$ content. Swelling may be one of the factors causing decreasing UCS. Hydration by organic matter in peat hindered soil particle-to-particle interactions, thus leading to decreasing

UCS. However, it should be taken into consideration that vertical deformation and irregular formation of $CaCO_3$ without binding peat particles together lead to a lack of strength contribution when swelling due to hydration may also occur. Hence, further study is required for the understanding occurrence of such phenomena on a macroscopic scale.

pH drops for samples submerged in distilled water may be due to extended precipitation of $CaCO_3$ from residual urea and calcium chloride even after the stabilisation effort. The increase in $CaCO_3$ content may suggest the presence of residual urea and calcium chloride as urea acts to provide carbonates, and while $CaCl_2$ act as a Ca^{2+} ions source for the precipitation to occur (DeJong et al., 2006). A previous study suggested that continuous carbonates precipitation will lead to a lower pH (DeJong et al., 2006; Stocks-Fischer et al., 1999). Decreasing pH trend may also be due to the acidification of peat. Peat is biologically active, and its degradation process tends to lower its pH until a certain extend (Martini et al., 2007). Treatment of MICP raised the pH of treated peat which induce biodegradation of peat materials. The previous study has suggested that increasing pH above neutral will promote extracellular enzyme in peat, namely phenol oxidase, to accelerate the degradation process in peat (Kang et al., 2018). This may suggest the trend where the continuous formation of $CaCO_3$ occurred when treated peat was submerged in distilled water with a decreasing trend of strength. Fluctuation of pH observed for peat samples submerged in peat slurry were due to dissolved of $CaCO_3$ in the stabilised samples which buffer the pH. Calcium carbonates were commonly found to increase pH of soil when exposed to acidic condition and used in agriculture (Acosta-Martinez & Tabatabai, 2000; Bertrand et al., 2007; McCauley et al., 2009).

7.5. Chapter summary

Objective IV was completed in this chapter. This study investigates the durability of MICP stabilised peat column submerged to peat slurry, simulating actual low pH and high organic acid condition in peatland for up to 90 days. From this study, it can be summarised that:

1. MICP stabilised peat showed as high as 66.37% strength loss when submerged in peat slurry compared to distilled water as a control.
2. Extended calcium carbonate precipitation was observed for samples submerged in distilled water. However, it was not enough to promote strength gain, whereas higher $CaCO_3$ dissolution was observed for stabilised peat submerged in peat slurry.

CHAPTER 8: CONCLUSIONS AND RECOMMENDATIONS

This chapter concludes the research findings for the whole study and recommendations for future research.

8.1. Key findings and contributions of the study

The focus of this study was to explore the potential of Microbial-induced Calcite Precipitation (MICP) for use in tropical peat. This study also aimed to investigate the use of indigenous bacteria for the improvement of tropical peat geotechnical properties through Microbial-induced Calcite Precipitation (MICP). Based on the results of this study, several conclusions can be drawn as follows.

The objective I was achieved in Chapter 4 with successful isolation and characterisation of ureolytic $CaCO_3$ precipitating bacteria from tropical peat. The study showed that urease activity was present in tropical peat with successful isolated urease-producing strains P19 and P21 from tropical peat belonging to the genus *Enteractinococcus* and *Staphylococcus*. The isolated indigenous strains were successfully used on local peat, and this reduced the need to use non-native species or introduce invasive species to the natural environment. Both strains were found capable of high urea hydrolysis activity, with isolate P19 showing maximum urease activity at 96 hours incubation with 815 U/mL, while strain P21 showed maximum urease activity at 120 hours incubation with 633.3 U/mL. Both strains were also found to have carbonic anhydrase activity, known to have a synergistic effect with urease for bio-mineralisation with the presence of urea. These strains were found to precipitate $CaCO_3$ in mixed polymorph of vaterite and calcite. This suggested the suitability of the isolated strains for the application in bio-cementation as most study relies on calcite, the most stable polymorph among calcium carbonate precipitation. When comparing both isolates, isolate P21 was found to have higher productivity of $CaCO_3$ and higher unconfined compressive strength in bio-cementation of study of sand as compared with isolate P19. Nevertheless, both isolates were deemed to be suitable for Microbial-induced calcite precipitation (MICP) treatment.

Objective II was achieved, as reported in Chapter 5. Following the conclusion from Chapter 4, since urease bacteria were found in tropical peat, an initiative was made to

study the possibility of MICP with solely indigenous urease activity of peat without the introduction of indigenous strains grown to a particular concentration (Chapter 5.2). It was found that calcium carbonate precipitation was possible by utilising indigenous urease activity by increasing the pH of the environment as its natural acidic condition of peat in which low pH does not favour carbonate crystal. The precipitation of $CaCO_3$ was seen with improved strength of the peat samples, suggesting the possibility of MICP treatment of peat. The precipitated $CaCO_3$ were tested by XRD and found to be calcite which is favoured over other calcium carbonate polymorph for bio-cementation. Ammonia accumulation has always been an issue for MICP application. For peat, it was observed that ammonia concentration was reduced at day 28 of curing, suggesting the potential of MICP in the stabilisation of peat as an eco-friendly stabilisation method.

Further exploration was done with isolated strain with the addition of sand as filler in the range of 25%, 50% and 75% for the effect of MICP in terms of UCS and permeability (Chapter 5.3). By comparing between the two strains and direct use of indigenous urease source (no introduction of isolates), strain P21 showed the most significant improvement of UCS at 28 days curing with bacteria concentration of 10^8 CFU/mL for peat at 25% sand and 2 mol/kg cementation reagent dosage. The unconfined compressive strength (UCS) of the test samples increased with increasing cementation reagent dosage, to which strength reduction was observed. UCS for the treated peat with 25%, 50% and 75% showed increasing trends with increasing curing duration. Highest UCS was observed at 75% sand at 94.85 kPa. Precipitation of $CaCO_3$ increased with increasing sand content and with increasing curing duration. The permeability of the treated test sample is extremely low compared with the untreated samples, and the permeability reduces with time. XRD and SEM-EDS were used to confirm the presence of calcite, and the micrograph showed the bridging of peat and sand particles in the MICP process leading to improved strength and reduced permeability due to clogging.

In Chapter 6, objective III of the study was completed with the effort to extend the study of the effect of MICP on consolidation behaviour of peat treated with MICP along with different sand content of 0, 25, 50 and 75% (Chapter 6.2). This study concluded that the MICP of peat with indigenous strain and sand as the filler would result in a lower void ratio due to calcium carbonate precipitation. Increasing sand

content showed an increase Coefficient of consolidation, C_v for both treated and untreated peat. However, lower C_v was observed for the treated peat as compared to the untreated at the same sand content. Reduction of hydraulic permeability was observed in the treated as compared to the untreated at same sand content further supported that bio-cementation could lead to clogging of peat which lower the Compression Index, C_v, and the Swelling Index, C_s for the treated peat were lower compared to the untreated peat at the same sand content. Secondary compression Index, C_α for the treated peat with 25% sand was higher compared to the untreated peat of 25% sand at 100 kPa effective stress onwards. While C_α for the treated peat with 50% sand was higher compared to the untreated peat with 50% at 200 kPa effective stress onwards, which may be due to biodegradation of peat. This leads to a higher difference of C_α/C_c for the treated peat with 25 and 50% sand than its untreated counterpart. Hence, MICP treatment was found to improve the compressibility of peat with the sand mixture.

Chapter 7 reports the results achieved for objective IV. An initiative was done to study the durability of MICP stabilised peat column submerged to peat slurry simulating actual low pH and high organic acid condition in peatland (Chapter 7). This study found that MICP stabilised peat showed 66.37% strength loss up to 90 days when submerged in peat slurry compared to distilled water as a control. Extended calcium carbonate precipitation was observed for samples submerged in distilled water (Control experiment), whereas reducing trends were observed for stabilised peat submerged in peat slurry. This suggested that MICP is possible in peat; however, a large area of peatland may present a challenge with the surrounding acid attack towards the treated column. Hence, further studies are recommended and listed in the next section.

8.2. Future study recommendation

This study provides some understanding of the Microbial Induced Calcite Precipitation (MICP) process for the use on tropical peat. The potential of urease sources from peat and possible MICP effect towards geotechnical were also established. However, the scope that was not covered or potential research areas for future studies on MICP of peat are suggested below.

- Study of consolidated undrained shear strength of MICP treated peat. There is still a lack of knowledge about how MICP treated peat may behave under undrained consolidation condition and how it will affect its undrained shear strength when tested with a triaxial system.

- The study of MICP towards biodegradation rate of peat and its effect towards long term creep. Introduction of nutrients and alteration of pH due to MICP may alter the rate of biodegradation of peat. Its effect on consolidation behaviour and shear strength are not fully understood.

- The effect of gas bubble evolution due to MICP treatment on peat. As urease induced MICP tend to release gas. There is a lack of understanding of how gas bubble may affect the geotechnical properties of peat under undrained condition.

- Study to prevent strength loss of MICP treated peat due to acidic peat infiltration from the surrounding. This study has suggested strength loss after MICP treatment when submerged. Hence a future study could be done to solve the issue.

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