USE OF MICROBIAL TECHNOLOGY TO IMPROVE STRENGTH PROPERTIES AND PERMEABILITY OF SOIL

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USE OF MICROBIAL TECHNOLOGY TO IMPROVE STRENGTH PROPERTIES AND
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ABSTRACT
A recently developed technology known as Microbially Induced Calcite Precipitation (MICP) has been an important breakthrough in the field of geotechnical engineering. It is a viable ground improvement technique that incorporates the use of bacteria to facilitate calcite precipitation in order to bind the soil particles and improve its strength. It is a trans-disciplinary research project since it integrates the fields of microbiology (urease producing microorganisms), geochemistry (precipitation of calcite through urea hydrolysis) and civil engineering applications (ground improvement techniques). *Sporosarcina pasteurii* has been proven to have high urease productivity and it has been used extensively in research works over the decade. Many have agreed that it possesses a high resistance towards chemical and physical agents that enables its use in the field.

The main objective of this research is to be able to (i) develop for the first time, a reliable lab procedure and injection mechanism in order to produce consistent and repeatable lab results and (ii) demonstrate the improvement in unconfined compressive strength in soil and permeability through a laboratory-scale bio-cementation process to justify its practical field application in local conditions.

The study begins with a preliminary laboratory test with a specimen size of 50mm cubes with gravity flow injections. Factors considered for this research study were the percentage of urea used in the growth media with respect to its urease activity, the concentration of cementation solutions (i.e. urea and calcium chloride), the preparation and treatment technique as well as curing duration (i.e. 7 and 28 days). The results were interpreted in terms of the effectiveness of the MICP productivity under local conditions. Throughout the preliminary and main laboratory tests, urease agar base tests and bacterial culture plates showed positive results that indicated that no contaminations occurred. Main laboratory tests were continued thereafter with an improved pressurised injection system with a specimen size of 150 mm cubes; the size of a typical concrete cube for a UCS test. Precipitation of calcite on the surface on both of the samples was further tested and verified with a Scanning Electron Microscope (SEM) and an imaging Energy Dispersive X-Ray Spectroscopy (EDX). The test reported in this research study includes the unconfined compressive strength (UCS) of the sand samples that shows improvement based on 0.75M of an equimolar concentration of cementation solutions as well as a reduction in its permeability. Further, the calcite content value was comparable to other types of ground improvement in soil geomechanical properties.
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DECLARATION

I declare that this thesis contains no material that has been accepted for the award of any other degree or diploma and to the best of my knowledge contains no material previously published or written by another person except where due reference is made in the text of this thesis.

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CHAPTER 1  INTRODUCTION

1.1 Background of Study

In recent years, land development and construction industries in Malaysia have seen intensive activities. Due to rapid development and as a result of the scarcity of good ground, marginal grounds have been earmarked for development. Engineers nowadays are faced with problems related to poor ground conditions. Construction in these areas needs proper ground improvement works to avoid problems such as sand liquefaction on large settlements during or after construction. Soil liquefaction happens when a saturated or partially saturated soil significantly loses strength and stiffness due to increasing or a sudden change of applied stress, causing it to behave like liquid (Perlea 2000).

Due to the relatively low shear strength of marginal ground, suitable ground improvement technology is seen as a viable option that can be used to treat these sites. Soil strength improvement technologies that are normally used in Malaysia are mostly via a chemical grouting process which involves injection or mixing soil with additives such as Portland cement, sodium silicate, lime, fly ash etc. (Sina et al. 2010). As an example, the construction of the SMART Tunnel used several methods of grouting that included fissure grouting and cavity grouting in rock, and also jet grouting and compaction grouting in the soil layers (Yew & Raju 2007).

The emergence of new ground improvement techniques has been a topic of interests among engineers. One that has been gaining popularity in the past decade particularly in the construction and engineering industries is known as construction biotechnology, which the major route under construction biotechnology is either the microbial production of construction materials or the applications of microorganisms in the process of constructions (Ivanov et al. 2015).

The route map of what construction biotechnology can be developed into within these two major directions can be seen in Figure 1.1. The term biotechnology which many are familiar with, refers to the scientific knowledge concerned with developing any high-tech applications which incorporate the use of microorganisms and/or biological systems to produce or modify products or processes for a specific use (What is biotechnology? 2016). It is normally subdivided into agriculture, cell culture, DNA and many others due to its numerous applications to our everyday lives.
One of the biotechnology routes for a construction process is known as Microbially Induced Calcite Precipitation (MICP) or in short, known by most researchers as biocementation. It is relatively new to the field of geotechnical engineering and evolves from time to time, but its potential has been shown to be remarkable. It is a sustainable, environmentally friendly and a cost effective ground improvement technique. It is also known as bio-grouting when naturally harvested microbes are used to trigger precipitating calcite to bind the soil particles through the process of biocementation and bioclogging. This new technique incorporates a microbiological process to facilitate the improvement of soil properties without compromising the surrounding environment. Also, it can be realised through trans-disciplinary research combining the understanding of microbiology processes, geochemistry and also civil engineering (DeJong et al. 2010).
1.2 Problem Statement

In the early stage of construction, engineers have always been confronted with dealing with poor ground conditions, especially those of loose sand, peat soil or soft clay. Improving grounds that are in poor condition results in high construction costs for its foundation, hence marginal plots of land are earmarked each time as it requires less cost. However, due to rapid urbanisation and development in almost every country, good plots of land for construction are scarce nowadays. Engineers are faced with challenges to come up with a ground improvement technology that is viable and less harmful to the surrounding environment. Soils in tropical regions like Malaysia have problems as there are many areas with these types of soils, which are weak and experience further softening due to intense and prolonged downpours (Ng et al. 2013).

The use of cement grouting, one of the conventional techniques for ground improvement, is known to be harmful to the environment (DeJong et al. 2006; DeJong et al. 2010; Karatas 2008). In particular, the use of Ordinary Portland Cement (OPC) has a very high carbon footprint on the environment. Apart from that, production of these materials is rather costly especially if it involves chemical additives for significant improvements in the soil properties. This further clarifies that this particular technique is not favourable since it is not environmentally sustainable over the long term. This shows that most of the techniques require substantial energy for material production and/or installation. Therefore, the process takes a longer time to execute as it also involves relatively high production costs. In general all chemical grouting except sodium silicate is hazardous (DeJong et al. 2010). Hence, research on feasible and environmentally friendly ground improvement techniques is being done rigorously for their practicality and potential in being adopted in the near future.

1.3 Research Objectives

- To conduct respective scientific analyses under local conditions (i.e. the percentage of urea with respect to its urease activity, concentration of cementation solutions, method of mixing and casting and curing temperature).
- To develop for the first time, a reliable lab procedure and injection mechanism in order to produce consistent and repeatable lab results.
- To demonstrate the improvement in unconfined compressive strength and permeability of the soil through laboratory-scale biocementation at a smaller scale.
of 50 mm cubes.
- To assess a detailed methodology for performing the laboratory-scale biocementation with improvised pressurised injection system with an acrylic collapsible mould of 150 mm cubes.

1.4 Significance of Research
This research aims to develop a reliable lab procedure and injection mechanism in order to produce consistent and repeatable lab results and to determine its effectiveness in terms of its unconfined compressive strength and permeability through a laboratory scale biocementation process and finally to justify its practicality in local typical field applications. Besides, a comparison between existing methodologies in other literature shall be evaluated with an improvised methodology in this study in terms of its mixing method, experiment setup and also the treatment cycles. To find out its effectiveness in MICP, respective scientific analyses (i.e. concentration of cementation solution, the percentage of urea used in preparing bacterial culture with regards to its urease activity and curing temperature) were performed in this research study. After finding the optimised conditions, it will then be utilised for a further stage of the laboratory scale of biocementation process (see Chapter 5). By utilising the pressurised injection system, the improved methodology will be expected to help in future upscaling experimental works that shall be implemented in the near future.

1.5 Thesis Documentation Flow
The documentation of this thesis will be divided into six chapters.

**Chapter 2** will review the literature on the existing ground improvement technology, specifically the emergence of this new viable and feasible technology of microbial-induced calcite precipitation in the construction industry especially the geotechnical aspects of it. Besides, this chapter will also discuss the established methods of preparing the specimens as well as the treatment technique. A few parameters involved such as (i) the concentration of the cementation solution, (ii) the treatment technique as well as (iii) the urease enzymatic activity were amongst the most important factors to be considered in MICP, hence their explanation in this chapter.

**Chapter 3** provides information on the specimen preparation method, the mixing and treatment technique for the trial experiments done in this research study. There were two stages of experiments that will be further discussed in detail later in this chapter while
maintaining some of the key parameters for consistency in the results. The first stage is preparing the samples in a 50mm standard cube size and the second stage involved the use of a collapsible mould with the dimension of 150mm cubed. This conforms to the standard sizes of the concrete cube for compressive strength testing. All the methods of preparation and testing were done in accordance with their respective standards as outlined in British Standards as well as other corresponding standards.

Chapter 4 shall discuss the effect of the key parameters used in this study, which is the concentration of the cementation solution, curing temperature and percentage of urea used (with respect to urease activity). The effectiveness of MICP is shown in terms of its effects on unconfined compressive strength and permeability. It is then further verified with images from a Scanning Electron Microscope (SEM) and also from an elemental analysis to ensure that the existing elements conform to the precipitation of calcium carbonate within the samples.

Chapter 5 will conclude the results of the analysis done in the previous chapter and choose the best optimised condition for the scaled-up laboratory experiments with those of 150mm cubed. Interpreted results, in terms of unconfined compressive strength and estimation of calcite content, will be used to evaluate its effectiveness. Image verification, obtained from SEM and also analysis of elements, are conducted as well to ensure that the mineral elements formed within the samples are affirmative.

Chapter 6 will give a general conclusion to the overall experimental tests with a further explanation and justification on the achieved objectives throughout this research study. Limitations of the technology of MICP and the improvised procedure used in this research will also be explained in detail while at the same time providing recommendations that would be needed for forthcoming experimental works should it be realised in the near future.
CHAPTER 2 LITERATURE REVIEW

2.1 Introduction

This chapter provides a detailed overview of the potential of the microbial-induced calcite precipitation technique for the works of ground improvement technology. Not only it has gained the interest of geotechnical engineers and biotechnologists alike, but it is also feasible for the production and manufacturing of construction materials. Besides, the feasibility was also reviewed hereinafter as well as some of the factors involved that were taken into consideration which consisted of (i) the concentration of cementation solutions, (ii) methods of mixing as well as (iii) the treatment technique as published in the literature.

2.1 Ground Improvement Technique

There are many techniques of ground improvement involved in the construction industries and each of them is unique in their own sense. Ground improvement, also known as soil modification, is generally used to improve any poor condition of land area that will be developed for construction such as basic infrastructure, housing development, skyscrapers and much more. Such construction would need a good foundation for the structure to stand up strong for many years to come. Therefore, investigation of underlying soils should be conducted prior to any construction to determine its geomechanical properties to determine the types of ground improvement that would be needed for the construction and modification or improvement required to the soil properties to that of soft rock such as clay shale or slightly cemented sandstone (Soil Modification 2016). Due to the different geomechanical properties of soil, this requires a suitable type of ground modification that must be adapted on site. Many factors affect the choice of ground improvement method to be implemented such as (i) the type of soil; (ii) area, depth and location of treatment required; (iii) environmental concerns and also (iv) economics. Therefore, when engineers face challenges that involve geotechnical problems, these factors are prioritised to decide on the type of solutions that can be best implemented on the site. The type of soil is the most vital factor that determines the type of ground improvement method as some of them have specific requirements that are only applicable to specific soil types and/or grain size as seen in Figure 2.1.
2.1.1 Conventional Ground Improvement Techniques

There are many types of ground improvement techniques being used worldwide and they are classified as (i) mechanical modification; (ii) hydraulic modification; (iii) physical and chemical modification and (iv) modification by inclusion and confinement. Each of them serves different purposes and it is as summarised in Table 2.1. These types of ground modifications are commonly used nowadays. Future undertakings to discover a novel green technology that is cost effective, environmentally friendly and most importantly feasible for implementation on site are increasing in demand.

Table 2.1: Summary of different types of ground improvement (Nicholson 2015a)

<table>
<thead>
<tr>
<th>Types of Ground Modification</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mechanical</td>
<td>Manipulation of earth materials most commonly refers to controlled densification (i.e. engineered fills and in situ).</td>
</tr>
<tr>
<td>Hydraulic</td>
<td>Alteration of flow, seepage, and drainage characteristics in the ground. (i.e. lowering of water table, increasing or decreasing permeability of soils, reducing compressibility and etc).</td>
</tr>
</tbody>
</table>
# Physical and Chemical Stabilisation of Soils

“Stabilisation” of soils caused by a variety of physiochemical changes in the structure and/or chemical makeup of the soil materials or ground.

<table>
<thead>
<tr>
<th>Inclusions, confinement and reinforcement</th>
<th>Includes use of structural members or other manufactured materials integrated with the ground. Reinforcing soil by vegetating the ground surface could also fall into this category.</th>
</tr>
</thead>
</table>

2.1.2 Microbial Induced Calcite Precipitation (MICP)

**Development of MICP**

The perks of MICP in the geotechnical industries have gained the attention of many researchers in exploring its potential for ground improvement technology in the near future. Studies began more than a decade ago and some have already patented their unique technology with regards to MICP. There are different terminologies in MICP that involves the use of microorganisms to induce the precipitation of calcite that helps to bind the soil particles together and improve the engineering properties of soil. The engineering properties of soil include its shear strength, unconfined compressive strength, stiffness and permeability (Cacchio et al. 2003; Chou et al. 2011; Chu et al. 2012; DeJong et al. 2010; van Paassen et al. 2010; Whiffin et al. 2007). The formation of calcite precipitation helps strengthen the bond between the soils, which increases the shear strength as well as reducing its permeability (De Muynck et al. 2008). Examples are as shown in Figure 2.2.

![Micrographs of untreated sand and (b) calcium-based biogrouting](https://example.com/micrographs.png)

**Figure 2.2:** Micrographs of untreated sand and (b) calcium-based biogrouting, (Ivanov et al. 2015).

Over the years, many researchers have successfully gained positive results in which it has been proven that the strength and permeability of soils can be improved through MICP (Rebata-Landa 2007; van Paassen 2009; Whiffin 2004). Bioclogging or bio-
mineralization is a process that aims to reduce the permeability of soil through microbial activity or products. While biocementation (biofilms) is a process where the formation of soil particle-binding material takes place with the aid of microbes or additives. Figure 2.3 shows the overview of the bio-mediated soil improvement system that depicts the potential of improving the soil properties with regards to its mechanical properties such as permeability, stiffness, compressibility, shear strength and volumetric response.

![Figure 2.3: Overview of bio-mediated soil improvement system ([-]=chemical concentration, ^=resistivity, \( V_p =\text{compression wave velocity}, V_s =\text{shear wave velocity}\), DeJong et al. (2010).](image)

MICP, mostly done at an experimental stage over the past few years, was initially implemented on a laboratory scale and mainly focused on fine sands. It can be seen from Figure 2.4 that within five years of laboratory experiments, it has been scaled up from 0.01 m to 1.0 m laboratory column samples (one-dimensional) and from 1.0 m\(^3\) up to the size of 43 m\(^3\).

![Figure 2.4: Biogrout, the bio-mediated ground improvement method based on MICP was scaled up in 5 years from laboratory column to a 100 m\(^3\) experiment (van Paassen 2011).](image)
Currently, the largest experiment so far was done by van Paassen (2009) at the Delft University of Technology, the Netherlands. The prototype was 100 m$^3$ and about 43 m$^3$ was successfully cemented biologically within 12 days (Figure 2.5). According to van Paassen (2011), the results were not entirely satisfactory as some of the key parameters for an in-situ application still need to be further improved. This remains a great challenge in the MICP technology.

A novel field test using a bio-grout technique was performed on coarser materials by van Paassen (2011). It was implemented to reduce the problem of borehole instability in gravel through horizontal directional drilling (HDD) during the installation of pipelines (Figure 2.6). For a large-scale in-situ application to be done, there are many factors to consider beforehand which requires a thorough investigation before the bacteria injection methods can be perfected and applied successfully.
2.2 Microorganisms in Developing Bio-Cementation

There are many microorganisms that are helpful to the MICP technique and there are also other biochemical processes that lead to successful bio-cementation. One that is extensively used in most literature is the calcium and urea-dependent biocementation as stated by Ivanov and Chu (2008). This is further explained later in the section as it is adapted for the experimental works in this research.

However, there are other processes of MICP which are able to induce the precipitation of calcite such as the production of carbonates by heterotrophic bacteria during aerobic or anoxic oxidation of organics, calcium and magnesium-based biocementation, calcium-phosphate biocementation, calcium bicarbonate biocementation as well as iron-based bioclogging and biocementation (Ivanov et al. 2015). These processes are unique by their respective chemical reactions. Each of them has their own benefits and drawbacks. They are summarised in Table 2.2.

2.2.1 Urease Producing Microorganisms

Bacteria are tiny living microorganisms whose habitat is at any suitable location where they will grow in millions of cells. In the field of biotechnology, their applications are mostly for medical, agricultural and also pharmaceutical purposes. In the new era, science and engineering have introduced construction microbial technology which consisted of microbially-induced construction processes and microbial production of construction materials (Ivanov et al. 2015). There are a few other applications rooted in biotechnology and thereafter combined with geotechnical aspects from engineering.

Bacteria have their own natural purposes before they are utilised in different applications. Specifically, in the case of biocements and biogrouts, the bacteria required for this process would be those of which are able to produce a urease enzyme and will help in triggering the precipitation of calcite. The formation of calcite on the surface of the particles will eventually strengthen the bonds between them. Hence, there will be a significant improvement in the soil properties in terms of its shear strength, stiffness, permeability as well as its compressibility (Al-Thawadi 2008; Chou et al. 2011; DeJong et al. 2010; Mitchell & Santamarina 2005; Nicholson 2015b; Stocks-Fischer et al. 1999; Whiffin et al. 2007).
<table>
<thead>
<tr>
<th>Types of Biocementation</th>
<th>Description</th>
<th>Outcome</th>
<th>Drawbacks</th>
</tr>
</thead>
<tbody>
<tr>
<td>(a) Calcium- and Urea-Dependent</td>
<td>Formation of calcium-carbonate minerals such as calcite, vaterite, or aragonite on the surface of soil particles due to:</td>
<td>Use of Gram-positive (S. pasteurii) bacteria in major biocementation process due to its properties:</td>
<td>1) By-product of urea hydrolysis is ammonium and ammonia that are toxic substances for workers, harmful for aquatic environment and atmosphere, and increases the risk of corrosion because of high pH.</td>
</tr>
<tr>
<td></td>
<td>1) Adhesion of cells of urease producing bacteria (UPB) on the surface of the particles.</td>
<td>1) High urease activity and ability to grow at pH above 8.5 and at higher concentration of CaCl₂.</td>
<td>2) The brittleness of calcite crystals bonding the soil particles.</td>
</tr>
<tr>
<td></td>
<td>2) Creating a microgradient of concentration of carbonate and pH in the site of cell attachment due to hydrolysis of urea by urease of UPB</td>
<td>2) Non-pathogenic bacteria which are also non-toxic to human and animals alike.</td>
<td>3) The cost of calcium reagent and urea are higher than the cost of conventional cement.</td>
</tr>
<tr>
<td>(b) Production of Carbonates by Heterotrophic Bacteria</td>
<td>Production of carbonates by heterotrophic bacteria during aerobic or anoxic oxidation of organics was used for biocementation of the porous stones.</td>
<td>Bioreduction nitrate of organics is useful for the combination of biocementation with nitrogen gas in situ during partial desaturation of sandy soil, an effective method for the mitigation of earthquake-caused soil liquefaction.</td>
<td>The success of MICP via denitrification is very much dependent on the interaction among the microbes present in the subsurface, temperature, soil characteristics and composition, pH, and specific soil chemistry.</td>
</tr>
<tr>
<td>(c) Calcium- and Magnesium-Based</td>
<td>Can be produced through dissolution of dolomite, which is common raw material for the production of cement, in hydrochloric acid.</td>
<td>Able to produce the UCS of the biocemented sand column 12.4MPa at the content of precipitated Ca and Mg 6% (w/w).</td>
<td>Even though the strength was high, but the hydraulic permeability of the biocemented sand was also high, recorded at 7 E 10⁻⁴ m/s.</td>
</tr>
<tr>
<td>(d) Calcium-Phosphate</td>
<td>Calcium phosphate precipitation from calcium phytate solution, using phytase activity of microorganisms.</td>
<td>Able to produce a mixture of the crystal forms such as monetite, whitlockite and hydroxyapatite.</td>
<td>The drawback to this type of biocementation is that the calcium phytate is of low solubility, hence big volumes of solution must be pumped through soil.</td>
</tr>
<tr>
<td>(e) Calcium Bicarbonate</td>
<td>Using the removal of CO₂ from solution of calcium bicarbonate as it releases low amount of ammonia and are able to be performed without increase of pH just like the conventional MICP.</td>
<td>Solubility of calcium bicarbonate is relatively high in order to perform a practical and feasible biocementation. It is a model of the naturally occurring dissolution-precipitation of calcium carbonate.</td>
<td>However, this biocementation process has to be conducted at high concentration of calcium bicarbonate and with significantly higher rate than in nature. In addition, the bicarbonate is still unstable so the solution must be produced and stored at elevated partial pressure of CO₂.</td>
</tr>
<tr>
<td>(f) Iron-Based</td>
<td>The use of iron hydroxide as a binding substance accompanied with acidification of solution. Precipitation of iron/manganese minerals by iron-oxidizing bacteria is another promising technology for modifying engineering soil properties and mitigating geologic hazards.</td>
<td>The advantages of this method as the clogging compound are the soil treated by iron minerals is more ductile and able to resist low pH conditions.</td>
<td>Contrary to its benefits, the soil treated with this method is not as strong as those treated using calcium-based biocement.</td>
</tr>
</tbody>
</table>

Table 2.2: Different types of biocementation process with their benefits and drawbacks as reported by the respective authors: (a) Ivanov et al. (2015); Pacheco-Torgal and Jalali (2011), (b) Chu et al. (2009); Hamdan et al. (2011); Rebata-Landa and Santamarina (2012), (c) Ivanov et al. (2015), (d) Ivanov et al. (2015); Roeselers and Van Loosdrecht (2010), (e) Ivanov et al. (2015), (f) Guo et al. (2010); Ivanov et al. (2009); Weaver et al. (2011).
The most common bacteria capable of degrading urea and produce a urease enzyme is in the family of *Bacillus sp.* A few types of known commercial bacteria are used by researchers for their ability to produce a urease enzyme, such as *Bacillus megaterium*; largely found in natural tropical soil (Ng et al. 2014). Some have utilised *B. megaterium* in other applications of MICP such as improving concrete strength as well as its durability (Achal et al. 2011) such as Siddique et al. (2008) who used *Bacillus megaterium* and showed improvement in compressive strength in cement mortars.

Besides *Bacillus sp.* being the common type of bacteria used in bio-cementation, there are other bacteria which possess similar traits but may differ in terms of their enzymatic activity which is one of the important factors to be considered when choosing the best bacteria for MICP. Rebata-Landa (2007) has proven that by using *Pseudomonas fluorescens* he was able to produce urease enzyme and promote the precipitation of calcite. (*P. fluorescens* is a mesophilic, non-spore-forming species that exists naturally in sediment.) On another note, Cheng and Cord-Ruwisch (2012) used *Bacillus sphaericus* (MCP-11) in order to develop a process for bacteria immobilisation which was found to be effective in unsaturated conditions by using the surface percolation method.

![Figure 2.7: Comparison between S. pasteurii with regards to its specific urease activity against previously report urease activity by other types of microorganism as summarised by Whiffin (2004).](image)

However, the one that has been classified as the most suitable bacteria for biocementation and biogrouting is known as *Sporosarcina pasteurii*. Apart from its ability in producing the most urease enzyme at one time and also its characteristics, *S. pasteurii* was also chosen as the best bacteria to be used in biocementation and biogrouting due to its particular traits.
which are gram-positive bacteria and non-pathogenic properties to humans and animals (Al Qabany et al. 2011). Formerly known as *Bacillus Pasteurii* (Yoon et al. 2001), it has been used extensively by researchers; especially the strain DSMZ 33 (or ATCC 11859) over the past decade. This is mainly due to its high urease activity (Figure 2.7), its pervasiveness in nature and also its high resistance and ability to survive in harsh environments (Al Qabany et al. 2011; DeJong et al. 2006; Ivanov & Chu 2008; van Paassen 2009; Whiffin 2004).

### 2.2.2 Geochemical Process

The precipitation of calcium carbonate (calcite) can be achieved through a variety of pathways. One in particular that has been used comprehensively is the hydrolysis of urea. It is the most energy efficient among others and besides, urease activity can be found in abundance among a wide range of microorganisms and plants (Bachmeier et al. 2002; DeJong et al. 2010). The chemical reactions that occur when bacteria reacts with urea to precipitate calcium carbonate are indicated in the equations below.

\[
\text{CO (NH}_2\text{)}_2 + 2\text{H}_2\text{O} \rightarrow 2\text{NH}_4^+ + \text{CO}_3^{2-} \quad (\text{Hydrolysis of urea}) \quad (\text{Eq. 1})
\]

\[
2\text{NH}_3 + 2\text{H}_2\text{O} \underset{\text{Urease}}{\rightarrow} 2\text{NH}_4^+ + 2\text{OH}^- \quad (\text{pH increase}) \quad (\text{Eq. 2})
\]

\[
\text{H}_2\text{CO}_3 + 2\text{OH}^- \leftrightarrow \text{HCO}_3^- + \text{H}_2\text{O} + \text{OH}^- \quad (\text{Eq. 3})
\]

\[
\text{HCO}_3^- + \text{H}_2\text{O} + \text{OH}^- \leftrightarrow \text{CO}_3^{2-} + 2\text{H}_2\text{O} \quad (\text{Eq. 4})
\]

\[
\text{Ca}^{2+} + \text{CO}_3^{2-} \rightarrow \text{CaCO}_3 \quad (\text{Precipitation of Calcite}) \quad (\text{Eq. 5})
\]

It begins when the ammonium ions (NH\(_4^+\)) are released through the hydrolysis of urea (Eq. 1) and because of the net production of OH\(^-\) ions (Eq. 2), it further increases the pH at the surrounding bacterial cell. Thereafter, carbonic acid is then converted to bicarbonate ions (HCO\(_3^-\)) (Eq. 3) then forming carbonate ions (CO\(_3^{2-}\)) (Eq. 4) afterwards. Hence, the precipitation of CaCO\(_3\) is formed through the mixture of a carbonate ion (CO\(_3^{2-}\)) from urea hydrolysis and the calcium ion (Ca\(^{2+}\)) supplied from calcium chloride (Ivanov & Chu 2008).
The bacterial cell acts as a nucleation site where the precipitation of calcite is at the surrounding of the cell and gradually binds the soil particles together (Figure 2.8). As stated by Ferris et al. (1997), *S. pasteurii* is an alkalophilic soil bacterium which possesses a substantially high active urease enzyme, and consumes urea within the microbe hence disintegrating it into ammonia (NH$_3$) and carbon dioxide (CO$_2$). The negatively charged bacterial cell is easily attracted to the soil particle surface due to a higher concentration of nutrients along the surfaces and also the physiochemical properties of both the bacterial cell and soil particle (Garrett et al. 2008; Hall-Stoodley et al. 2004; Oliveira et al. 2003).

2.3 Bio-Cementation Characterisation

2.3.1 Growth Medium

The creation of bio-soil involves many processes that need to be taken care of before, during and after the treatment. There are quality control checks that can be used to monitor most of the key parameters and lead to a successful treatment of MICP. The aspects involved includes the microbiology part which is to ensure the bacteria are able to produce the urease enzyme and are grown at an optimised condition throughout all series of experiments.

---

**Figure 2.8:** Overview of calcite precipitation through hydrolysis of urea (DeJong et al. 2010).
Bacteria are grown in number of ways whether in liquid form (usually identified as bacterial cultures) or grown on agar plates where they will present themselves in colonies. In general, bacteria are grown on agar plates typically with a streaking technique (Figure 2.9) for the purpose of isolation in order to obtain a single colony on these agar plates. These agar plates are kept at 4°C for at least 48 hours before being aseptically transferred by transferring a single colony to a liquid culture prior to bio-cementation (Chou et al. 2011; Whiffin 2004). Many types of medium exist which can be used to grow urease-producing microorganisms. As reviewed in literature, many of the researchers using these common growth mediums for *S. pasteurii* are shown in Table 2.3.

Table 2.3: Growth medium as reported by respective authors.

<table>
<thead>
<tr>
<th>Authors</th>
<th>Growth Medium</th>
<th>Ingredient</th>
</tr>
</thead>
<tbody>
<tr>
<td>Al Qabany et al. (2011); Stocks-Fischer et al. (1999); Chou et al. (2011)</td>
<td>NH₄-YE Medium</td>
<td>20g of yeast extract</td>
</tr>
<tr>
<td></td>
<td>(Ammonium-Yeast Extract)</td>
<td>10g of ammonium sulphate, (NH₄)₂SO₄</td>
</tr>
<tr>
<td>Reference</td>
<td>Medium</td>
<td>Composition</td>
</tr>
<tr>
<td>---------------------------</td>
<td>---------------------------------------------</td>
<td>--------------------------------------</td>
</tr>
<tr>
<td>Stabnikov et al. (2011);</td>
<td>TSB Medium</td>
<td>30g of tryptic soy broth medium</td>
</tr>
<tr>
<td>Chu et al. (2014);</td>
<td>(Tryptic Soy Broth)</td>
<td>20g urea</td>
</tr>
<tr>
<td>Sarmast et al. (2014)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Whiffin et al. (2007);</td>
<td>YE Medium</td>
<td>20g of yeast extract</td>
</tr>
<tr>
<td>Harkes et al. (2010)</td>
<td>(Yeast extract medium)</td>
<td>10g of ammonium chloride, ( \text{NH}_4\text{Cl} )</td>
</tr>
<tr>
<td>Mountassir et al. (2014)</td>
<td>BHI-Medium</td>
<td>37g of brain heart infusion medium</td>
</tr>
<tr>
<td></td>
<td>(Brain Heart Infusion Medium)</td>
<td></td>
</tr>
</tbody>
</table>

Bacteria have their own life cycle and are categorised in different stages namely the lag phase, exponential growth phase, stationary phase and the death phase (Figure 2.10). Bacteria cells grow at their fastest rate possible during the exponential growth phase when under optimised conditions (Case 2011). As the bacteria are grown in suspension, the broth of the culture turns turbid and its turbidity can be measured by using a spectrophotometer usually at a 600 nm wavelength for at least 8 to 10 hours with an interval of up to every 2 hours (Matlock et al. 2015).

![Figure 2.10: Phases in a bacterial culture (Widdel 2010)](image)

2.3.2 *Urease Enzymatic Activity*

The ability to produce an enzyme is further clarified through measurements of electrical conductivity (Whiffin 2004) or other available monitoring methods to determine its enzymatic activity. During the process itself, a few factors which are considered are usually the pH of the effluents and the ammonium concentration of the effluents (Harkes et al. 2010). This is another quality control method to ensure that the samples are progressing well throughout the treatment cycles. If analysis shows some irregularities, justification on the causes can be further explained.
Each urease-producing bacterium has its own rate of enzymatic activity and it is divided into two types in terms of its response towards ammonium. One type is when it is repressed by ammonium while another type does not. According to Mulrooney et al. (2001), those which are repressed are not favourable for bio-cementation due to the production of glutamine inside their cell which further inhibits the hydrolysis of urea. Therefore, those which are not repressed by ammonium are better due to the high concentrations of urea that are hydrolysed during bio-cementation (Whiffin 2004).

It is vital to ensure that the enzymatic activities remain stable over the course of a few days as the bio-cementation process may take upwards from few days to a week depending on the treatment cycles and injection performed on the samples. On the other hand, the successful precipitation of calcite is very much dependent on the urease enzyme, hence it is best to have its activity measured and best maintained at an average rate for a better bio-cementation process. Whiffin (2004) also mentioned that the enzymatic activity of *S. pasteurii* is notably stable for at least 25 days at a controlled temperature of 4°C for storage. Whilst it fluctuates over time, the values only differ in small margins which are essential as the enzyme remains unaffected during the storage duration (Figure 2.11).

![Figure 2.11: Stability of *S. pasteurii* urease stored in a closed vessel at 4°C (Whiffin 2004).](image)

A study done by Harkes et al. (2010) with regards to the fixation and distribution of bacterial activity to induce carbonate precipitation have found that the sand column shows a higher urease activity of up to 3.33 mM urea.min⁻¹ (200 mM urea.hour⁻¹) when treated with a concentrated bacterial suspension and then with fixation and cementation fluid.
thereafter. The hydrolysis of a non-ionic substrate urea to ionic products is due to the reaction of urease, hence producing a proportional increase in conductivity under standard conditions as stated by Whiffin et al. (2007). It should be noted that the conductivity change was recorded over 5 minutes and then it was calculated by taking into account the dilution factor, then correlated with a hydrolysis activity of 11 mM urea.min⁻¹ in the measured range of activities of about 1 mS.min⁻¹ (Cheng & Cord-Ruwisch 2012; Harkes et al. 2010; van Paassen 2009; Whiffin 2004).

While in some other literature, a different method known as an assay of urease activity was used for measuring the enzyme activity. In the works of Stocks-Fischer et al. (1999), they adapted the spectrophotometric method by Natarajan (1995) whereby the ammonia was produced upon the hydrolysis of urea, the absorbance of the samples were recorded at 626 nm. Then, ammonium chloride (NH₄Cl) was used as standard and the amount of enzyme hydrolysing 1µm urea per minute was defined as one unit of urease.

![Figure 2.12: Effect of pH on urease activity from the cell-free extract of B. pasteurii](Stocks-Fischer et al. 1999)

According to Stocks-Fischer et al. (1999) it can be seen that the enzymatic activity for S. pasteurii increased at a steadily fast rate which peaked around pH 8.0 (Figure 2.12). However, at a higher pH the activity decreased gradually and a significant amount of urease activity remained stable at pH 9.0. Besides that, the authors also mentioned that the Michaelis-Menten kinetic parameters were predominantly important in estimating the
enzymatic activities. $K_m$ represents the substrate concentration at which the initial reaction is half maximal (Stocks-Fischer et al. 1999) and the ranges of urease activity obtained were moderate ($K_m$ of 26.2-41.6 mM hydrolysed urea.min$^{-1}$). This generates an ample amount of ammonia to maintain a high pH in its surroundings should the supply of an adequate amount of urea.

On the other hand, Stabnikov et al. (2013) reported that isolated strains obtained urease activity in the range of 6.2 to 8.8 mM hydrolysed urea.min$^{-1}$ which correlates back to the range of urease activity from the strains of $S.$ pasteurii (Table 2.4). The author also mentioned that to have successful bio-cementation, it is unfavourable to possess high urease activity from the bacterial culture as a lower rate of urea hydrolysis promotes stronger aggregates of calcite (Qian et al. 2009).

Most likely there are other ways of measuring the enzymatic activities of these bacteria, but those which were explained above were commonly used in most literature. One of the methods adopted in this research was for the purpose of quality control to ensure that the enzymatic activity remained active and stable prior to the process of bio-cementation.

Table 2.4: Urease activity of urease-producing microorganisms summarised by Stabnikov et al. (2013).

<table>
<thead>
<tr>
<th>Urease Activity (urea hydrolysed.min$^{-1}$)</th>
<th>Bacterial Strain(s)</th>
<th>Authors</th>
</tr>
</thead>
<tbody>
<tr>
<td>5 – 20 mM</td>
<td>$S.$ pasteurii DSMZ 33</td>
<td>Harkes et al. (2010)</td>
</tr>
<tr>
<td>2.2 – 13.3 mM</td>
<td>$S.$ pasteurii ATCC 11859</td>
<td>Whiffin (2004)</td>
</tr>
<tr>
<td>More than 3.3 mM</td>
<td>Bacillus strains isolated from Australian soil and sludge</td>
<td>Al-Thawadi and Cord-Ruwisch (2012)</td>
</tr>
</tbody>
</table>
2.3.3 Urease Agar Base

Quantitatively, as explained earlier on, the electrical conductivity method is used to measure the enzymatic activity of urease produced by *S. pasteurii*. These results are much more credible though there are test methods which allow visualisation of its effectiveness in the hydrolysis of urea (Christensen 1946). Any slight or delayed reactions of urease activity can be seen with this medium. It is known as the urease agar base test which is where an agar medium is prepared in a small tube; bacterial culture will be streaked on the surface of the agar and then the colour changes of the agar medium shall determine the ability of the bacteria to degrade urea.

Christensen’s Urea Agar Base medium has been used among scholars and scientists alike for the detection of urease-producing microorganisms. Other than the urea agar medium, there is another type of medium which also allows for the detection of urease activity but they are for different purposes (Figure 2.13). In the field of microbiology, Christensen’s medium is mainly used to distinguish between urease-positive *Proteae* from other *Enterobacteriaceae*, while Stuart’s urea broth is simply to differentiate the *Proteus* species (Brink 2013).

2.3.4 Calcite Content Estimation

Some other monitoring methods which are performed after the treatments are, for example, the estimation of organic contents within the sample which can be done by the EDTA titration method (Stocks-Fischer et al. 1999). This method is used extensively by researchers in general, to determine the content of calcium that is available within various solid materials (*Determination of Calcium Ion Concentration* 2011). In the works of
Stabnikov et al. (2011), the treated sand showed that almost 95% of the supplied calcium was precipitated, especially the crust on the surface of the sand. The amount of calcium content within the sand by depth presented in the author’s study showed that only 17% – 22% was filled with calcite out of the total volume of the sand voids underneath the crust. Furthermore, about 0.6 g of Ca/cm² or 6kg/m² of the calcium was measured on top of the sand, for the formation of the crust.

![Crust on the surface of the treated sand](image)

**Figure 2.14: Crust on the surface of the treated sand (Stabnikov et al. 2011)**

Other than the EDTA titration method, estimating the content of calcite can also be performed through the acid wash (Burbank et al. 2011). Bio-treated sand is washed with HCl acid of at least 2M and its dry weight before and after the acid wash are measured accordingly. Therefore, the difference in weight would give the estimation of calcite content in a percentage (% w/w) within the sample. It was reported that at a lower concentration of calcite measuring below 60 kg/m³ or 3.5% w/w had no significant effect on the strength of the sand (Whiffin et al. 2007). These methods are implemented to quantify the amount of calcium or calcite content in samples and to evaluate whether it affects the strength performance of the sample (Chou et al. 2011; Harkes et al. 2010; van Paassen et al. 2010).

### 2.3.5 Scanning Electron Microscopy (SEM) Analysis

Other than quantification of these precipitates, the samples are viewed under a scanning electron microscope (SEM) for qualitative images which are significant in order to view the precipitates at high magnification and may as well show the bonding of the soil
particles (Al-Thawadi & Cord-Ruwisch 2012; Cheng & Cord-Ruwisch 2012; Martinez et al. 2013). Some SEM’s are equipped with energy-dispersive X-ray spectroscopy (EDX) which allows elemental analysis to be performed at the same time.

Figure 2.15: SEM image at 50x magnification of untreated silica sand (Li 2013)

Figure 2.15 shows the image of untreated silica sand and it is obvious that the surface is clear of any crystallisation and no other mineral exists within the sample. Those of treated sand by means of MICP have shown crystallisation of calcite and they come in different shapes either rhombohedral or spherical or both of them together and this can be observed in Figure 2.16.

Figure 2.16: (Left) Crystals of CaCO₃ on the surface of treated sand (Stabnikov et al. 2011); (Right) Particle contact point for 0.25 M treatment (80 kg/m³) by (Al Qabany et al. 2011).
2.4 Key Parameters to Mechanisms of Bio-cementation

When the MICP technique was introduced over a decade ago, it started from a standard one-dimensional column size with a ratio of 2:1 and it has evolved from 0.01 m up until 1 m length samples. Only after having scrutinised the key parameters of this MICP technique, the sizes of the samples began with a 2-dimensional radial and then to a 3-dimensional as shown in Figure 2.4, Section 2.1.2. Further, the process of bio-cementation also requires certain setups due to the variation of treatment techniques done by many researchers. These processes are vital for the determination of methods that are best implemented in-situ, hence all soil characterization and geomechanical properties need to be tested in order to fully understand the process of bio-cementation.

2.4.1 Type of Soil

In general, ground improvement techniques are meant for treating poor condition land area, especially sandy soil and clayey soil which is prone to liquefaction causing a tremendous amount of damage when it occurs (Yasuda et al. 1996). The MICP technique comes as one of the recent green technologies used in order to kerb this challenge as it is best implemented for fine granular soil particles. Hence, grain size and pore size are equally significant in the bio-cementation process although, at very fine soil particles, it is difficult for bacterial activity to occur (Rebata-Landa 2007). It is also reported that the particle sizes ranges from 0.05 mm to 0.40 mm are the most optimum range for a successful bio-cementation. For larger sizes it is possible as well, although it would take a longer time and larger amounts of nutrient for a thick cementation layer to occur and strengthen the bonds between the particles.

![Figure 2.17: CaCO₃ content and models as a function of the grain size as reported by Rebata-Landa (2007).](image)
Reduction in hydraulic conductivity in the bio-cementation process is highly dependent on pore sizes and Rebata-Landa and Santamarina (2006) reported that when the sizes are smaller than ~1µm, geometrical and geomechanical conditions inhibit microbial life. Eventually, it also further reduces the formation of biofilms on the surface of the particles, which leads to bio-clogging of soils. On another note, Rebata-Landa and Santamarina (2006) also reported that the accumulation of biofilm is limited by pore flow velocity and ensuing shear forces due to the large pores and soil particles.

On average, the sizes of particle used in most literature were of poorly graded sand with an average of median particle \(d_{50} = 0.165\) mm. Besides that, most of them were silica sand (at least 90% quartz) which is suitable to be used for a bio-cementation process while some have used standard laboratory sand which passes through a 2 mm sieve (Chou et al. 2011; Chu et al. 2014; DeJong et al. 2006; Harkes et al. 2010; Montoya et al. 2012; Stabnikov et al. 2013; Tsukamoto et al. 2012; van Paassen et al. 2010).

Figure 2.18: Compilation of published hydraulic conductivity data summarised by Rebata-Landa (2007).
2.4.2 Specimens Setup

As reported back in the 90s by Stocks-Fischer et al. (1999), a 60 mL plastic syringe column was used for simple bio-cementation tests. They were packed with sand slurries containing either dead or live cells and about 100 g of sand that had been sterilised prior to placing it in the column. The same column was prepared as well but with no bacteria as a control. Al Qabany et al. (2011) adopted the same setup however with a different dimension. A 100 mL plastic syringe column was used instead of the 60 mL and it was connected to tubes for the purpose of drainage of the nutrients at the bottom of the column (Figure 2.19).

![Diagram of experimental setup by Al Qabany et al. (2011).](image)

The bio-cementation test setup by Tsukamoto et al. (2013) was a half-split mould made of PVC. It was 15 cm in height while its diameter was 5 cm. The internal surface of the mould was applied with grease to avoid the appearance of unwanted water paths along the wall. In contrast to the previous method, the method of placing sand in this setup was by air-drop method. This was also due to the objective of this report which was to investigate the influence of relative density when using this MICP technique. After the sand was filled in, it was then fitted with a collar on top and saturated with distilled water that was supplied from the bottom.
Similarly, Harkes et al. (2010) also used a setup which was somewhat alike to Figure 2.19 but differed in terms of the filter material. Instead of a normal metal gauze, about a 1 cm of filter gravel was used instead, along with a layer of scouring pad (Scotch-Brite) on top of it (Figure 2.20). The packing of the sand was conducted in a saturated condition (under water) to prevent unnecessary air voids within the specimen. Finally, it was topped with another layer of the same height of filter gravel and another layer of scouring pad. The column was positioned vertically and it was connected to a peristaltic pump to regulate the flow rate where the injection began from top to bottom.

![Figure 2.20: Schematic diagram (left) and photo (right) of the mould (Tsukamoto et al. 2013).](image)

Whiffin et al. (2007) had a specimen size of 5 m long of PVC tube with an internal diameter of 66 mm that was positioned vertically and was packed with sand at a dry density of 1.65 g/cm³ (porosity at 37.8%). The packing of the sand was done similar to Harkes et al. (2010) which is to prevent any inclusion of air voids. The following experiments performed in this literature was conducted at an ambient temperature of 18°C ± 2°C and the flow rate was kept constant at 0.35 L per hour. It was found that a significant increase in strength was established higher at the top of the column compared to the bottom due to the supply of cementation reactants against the bacterial activity that existed within the column. The hydraulic conductivity of the samples was also significantly reduced by an order of 1 magnitude, from $2 \times 10^{-5}$ m/s to $9 \times 10^{-6}$ m/s after treatment.
The highest confined compressive strength recorded was 570 kPa that was located at approximately 1 m from the injection point where the highest amount of calcite was also measured. Whiffen et al. (2007) also reported that an apparent minimum calcite content of 60 kg/m³ was required for measurable strength improvement.

While the compressive strength was measured, the residual strength was also determined after failure. According to Whiffen et al. (2007), the values were comparable with those which were not treated for biocementation and this shows that despite the calcite content present in the samples, the improvement of strength was completely lost the minute the bonds were broken.
If this technique were to be implemented in-situ, a one-dimensional process would not be sufficient as there are limited conditions in comparison to a 3D process. van Paassen (2009) investigated the potential of bio-cementation process for field applications by performing experiments in 3D under controlled environments (i.e. conditions and injection techniques similar to those proposed in practice). In Figure 2.23, the experiment setup was a multi-box container with a dimension of 0.9 x 1.0 x 1.0 m together with drainage filters on the sides that were covered with a geotextile to prevent loss of sand throughout the injection process. The unconfined compressive strengths reported for these samples varied from 0 up until 9 MPa, which practically correlated to the amount of calcite content of roughly up to 250 kg/m³ or 17% of dry weight.

2.4.3 Mixing and Casting Methods

There are different types of mixing methods reported in literature and their methods have been investigated for a comparative study to evaluate how it affects the strength of the
sample as well as its permeability. Chou et al. (2011) conducted MICP via a direct shear box which was aseptically placed in completely stirred tank reactors along with about 6L of live cell suspension. It appeared that the specimen that was prepared was submerged inside those reactors and the injection of nutrient began after the samples were left for about 24 h for bacterial attachment.

On the other hand, the conventional method which many have applied over the course of the ongoing research with the MICP technique have been by injecting bacterial suspension either through gravitational flow or by flowing it in with the aid of peristaltic pump where lower flow rate could be controlled periodically. Chu et al. (2014) had their bacterial suspension injected from the bottom of the sand to the top and then incubated for 2 h. Thereafter, the solution or suspension was drained off by gravity.

Another mixing method of bacterial suspension/culture with sand was by using a dropper followed by a fixation solution and then cementation fluids as prepared by Sarmast et al. (2014). It appeared to be quite uncommon to see the mixing of the sand together with the bacterial culture in the beginning of the whole process and this is due to the instant calcification that occurred which would have easily clogged the injection points hence affecting the whole process altogether.

However, there are few which adapted this method. Of those that did, their outcome was not to evaluate the improvement in the geomechanical properties of the soil but rather to investigate the geochemical process that occurred within the sample itself and this was shown by Stocks-Fischer et al. (1999). The bacterial cells which they have grown until it reached a late exponential phase were suspended in a medium that contained urea and calcium chloride and then was mixed with 100 g of sterile sand. This pre-mixed slurry of sand was then packed into a plastic syringe before being further fed with urea and a calcium chloride medium continuously by gravity.

A patent was filed by Dosier (2011) which was for making a construction material by using an enzyme producing bacteria. They prototyped a different procedure in manufacturing these materials through the technique of MICP and one of them was by casting the sand sample into the mould in layers of their respective thickness in Figure 2.25.
Figure 2.25: Thickness of each layer in mm for the preparation of construction material as described by Dosier (2011).

In general, the steps for this procedure begins with preparing a lot of bacteria in a broth solution prior to it being fed into the sample and the loose aggregate material is placed into the formwork. Afterwards, an aqueous solution of urea and calcium chloride is fed into the aggregate material and only then the urease enzyme in a broth solution is poured onto the sample. These steps are then repeated until the desired height had been reached. Once the material hardened, the formwork was removed.

2.4.4 Treatment Techniques

The sequence for the injection of the cementation solution for this bio-cementation process may as well be known as a treatment technique that varies among many kinds processes reported in literature. The different techniques give various outcomes depending on the parameters that many have set to achieve each of their aims. The most prominent technique in the beginning of this MICP technology was through the gravitational method whereby the cementation solution is flushed from their injection points either from top to bottom or from the bottom to the top of the samples. Then they are usually left for 12 hours before the next round of injection is commenced. As explained by Yasuhara et al. (2011), the solutions consisting of urea and calcium chloride were injected into the samples for approximately half an hour and the same amount was injected another 4 or 8 times at an interval of 2 hours. After the final injection, the samples were then cured for 24 hours under a stagnant condition.
Furthermore, some provided these cementation solutions in a sterilised condition prior to injecting them into the sample with the exception of urea as it is sensitive to high temperatures. As such, it was sterilised by means of sterile injection passing through 0.2 µm filter (Mountassir et al. 2014). The injection strategy reported in the author’s literature consisted of injecting the bacterial suspension over the course of 30 minutes, followed by an injection of cementation fluid also over the course of 30 minutes with these steps being repeated five times. It was explained that during the initial observation of the experiments, with every injection cycle, more calcite precipitates were formed and located on the fracture surfaces of the samples.

2.4.5 Precipitation of Calcite at Different Temperature

The precipitation of calcite, in general, consists of a variety of processes and for this MICP technique in particular, it involves the natural geochemical process that occurs with the aid of enzyme produced by a urease producing microorganism, then through the hydrolysis of urea and supplied calcium ions, the elements of calcium carbonate are formed (Chu et al. 2012; van Paassen 2009; Whiffin 2004). Vaterite and aragonite are also a form of calcite, but they are less stable at ambient temperatures when compared to the calcite composition itself which is more durable and has more strength. Also agreed by Al-Thawadi (2008), the crystal structure of the calcite varied in strength which was caused by its formation during the geochemical process. The crystals are either spherical and rhombohedral, whereby the rhombohedral shaped-crystal are the result of a fully-formed calcite which has the most stable strength when compared to those of the spherical-shaped. The occurrence of calcite is induced in abundance at a normal temperature as it is the most thermodynamically stable under ambient conditions (Weiss et al. 2014).

![Figure 2.26: SEM images of calcium carbonate crystals precipitated at (a) 30°C; (b) 50°C and (c) 80°C.](image-url)
Moreover, the study conducted by Weiss et al. (2014) showed that temperature will have an effect on the precipitation of calcite as observed in Figure 2.26. It was found that aragonite was the most abundant polymorph under an elevated temperature of 50°C and appeared to be the only phase that occurred at a temperature of 80°C. Likewise, it was further proven that at a high temperature, the rate of calcium carbonate precipitation was enhanced and the amount of \( \text{CaCO}_3 \) precipitate increased significantly and the formation of aragonite was more apparent (Mejri et al. 2014). In the works of Thriveni et al. (2013), it was mentioned that the polymorph of aragonite at a higher temperature was much more stable and possessed a higher strength in comparison to calcite. Further, calcite at a higher temperature was less stable which further led to a lower strength of the crystals. In terms of strength, Li (2013) reported that the bio-cemented samples which were oven-dried at a temperature of more than 100°C obtained a higher strength as shown in Figure 2.27.

![Figure 2.27: UC strength of soil samples at different oven-dried temperatures (Li 2013).](image-url)
2.5 Other Applications of Biocementation

The scope of utilising the technology of MICP has expanded over the years. This technology began as a viable technique for ground improvement, particularly to prevent soil liquefaction as it improves the geomechanical properties of the soil. It has now become a trend in the construction industry where its application is being utilised for many purposes. It is also feasible in sustainable materials for construction such as bio-bricks (Bernardi et al. 2014). Therefore, production of sustainable materials can be achieved with this emerging green technology. Most materials used in conventional construction materials are clay for bricks and normal Portland cement, fine aggregates for concrete pavers or concrete blocks. The production of these materials requires a lot of energy and releases significantly high carbon emissions.

Production of bio-bricks by Bernardi et al. (2014) was successfully developed which produced compressive strengths that exceed 2.0 MPa. The treatment method involved in producing these bio-bricks consisted of introducing the bacterial culture through a percolation method which ensured bacteria attachment at particle contacts within the samples. The treatment of cementation solutions varied from 1 to 5 times per day and they were treated for 7 days, 14 days and 28 days respectively. After the treatment was completed, the moulds were disassembled and the bricks were dried in an oven at a temperature of 77°C overnight. The recorded strength of the bio-bricks ranged from 1.0 to 2.2 MPa which have demonstrated the feasibility of sand-based bricks being produced.

The remediation of cracks in concrete was inspired by an established microbial enhanced oil recovery method which has profited the oil industry by efficiently exploiting all the mineral resources from a particular digging site (Ramachandran et al. 2001). Also reported by the authors, the microbially induced technique in remediation of cracks in concrete was effective but no improvement of strength in a cement mortar mix was observed. In some other literature, this technique was also beneficial for the protection of ornamental stones by means of a microbial deposited carbonate layer (De Muynck et al. 2010) which incorporates the use of calcinogenic bacteria on stone surfaces and this concept was materialized under the collaboration of the University of Nantes, the Laboratory for the research of historic monuments (LRMH) and the company of Calcite Bioconcept (Le Métayer-Levrel et al. 1999).
2.6 Concluding Remarks

Studies conducted over the past decade have been dedicated to determining and evaluating the parameters that affect the geomechanical properties of the soil. Most of the procedures discussed and conducted in previous research works varied in terms of their specimen size, injection procedures, type of bacteria, type of soil and also the concentration of the cementation solutions. However, the feasibility of the various techniques still needs to be investigated in depth should it be applied for field application which was also suggested by van Paassen et al. (2010).

Most of the specimen sizes used in previous works were one-dimensional, typically in columns. However, conditions favourable for 1D specimens applied on a 3D scale produce different results. Hence, this research focuses on a smaller scale of 50 mm and 150 mm cubes to study the feasibility of the method of injection as well as to evaluate its effectiveness in providing homogeneity within the sand matrix. Different types of injection methods were used, gravitational injection and by using peristaltic pumps, a few of which were used extensively in previous works (Chu et al. 2014; Harkes et al. 2010; Mortensen & DeJong 2011; Tsukamoto et al. 2012; van Paassen et al. 2010; Whiffin et al. 2007; Yasuhara et al. 2011).

This research aims to develop a detail methodology to achieve practicability and repeatability for implementation on a large scale MICP in the near future. Improvements in terms of unconfined compressive strength, hydraulic conductivity and calcite content for the methods used in this research were investigated. For the purposes of large-scale applications, the improvised technique through a pressurised injection system for the 150 mm cubes was also studied and evaluated for its capabilities and limitations.
CHAPTER 3 RESEARCH MATERIALS AND METHODOLOGY

3.1 Introduction
This research study aims to investigate the effectiveness of the strain *Sporosarcina pasteurii* in terms of strength, permeability and its calcite content with a cube size of 50 mm and thereafter with a controlled pressurised system with a cube size of 150 mm. A systematic methodology was developed in this chapter in order to achieve the objective of this research study.

3.2 Research Framework
This research requires a few sets of procedures that involve fieldwork, laboratory tests, experimental standards and chemical analyses in order to be able to achieve the objectives of this research. As for the laboratory tests, the procedures will be done in accordance with the relevant standards. Below is the flow chart that shows how the research was implemented.

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<table>
<thead>
<tr>
<th>Stage 1</th>
<th>Review of Past Reported Studies</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stage 2</td>
<td>Material Preparation</td>
</tr>
<tr>
<td>Stage 3</td>
<td>Trial Setup</td>
</tr>
<tr>
<td>Stage 4</td>
<td>Result Analyses</td>
</tr>
</tbody>
</table>
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3.3 Materials Preparation

3.3.1 Sand Preparation

The sand specimens used in this present research study were all typical uniform sand. The particle sizes ranged from fine sand (0.075 mm) to fine gravel (4.75 mm). It was classified as a poorly graded medium sand according to British Standards, BS5930. The specimens had a coefficient of uniformity, \( c_u = 1.6 \) and a coefficient of gradation, \( c_c = 0.907 \) (\( D_{10} = 0.220 \) mm, \( D_{30} = 0.265 \) mm, \( D_{60} = 0.352 \) mm).

Table 3.1: Grain size characteristics based on plot in accordance with BS 5930

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Percentage (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>FINE Sand</td>
<td>6.72</td>
</tr>
<tr>
<td>MEDIUM Sand</td>
<td>87.95</td>
</tr>
<tr>
<td>COARSE Sand</td>
<td>3.96</td>
</tr>
<tr>
<td>FINE Gravel</td>
<td>1.37</td>
</tr>
</tbody>
</table>

As shown in Table 3.1, 87.95% contributed to the medium size of sand ranging from 0.212 mm to 0.600 mm while 6.72% was of fine sand. This conforms to the works of Rebata-Landa (2007), whereby the optimum particle sizes to promote a significant amount of calcite precipitation for biocementation are between 0.05 mm to 0.40 mm. The specific gravity of the sand used in this study is 2.67 g/cm³ with a minimum and maximum dry density at 1.27 g/cm³ and 1.67 g/cm³ respectively.
3.3.2 Bacteria Strain

The microorganism used for this research study was *Sporosarcina Pasteurii* (DSMZ33), a urease producing, gram-positive and non-pathogenic microorganism. This type strain was purchased from the Leibniz Institute DSMZ-German Collection of Microorganisms and Cell Cultures (Braunschweig, GERMANY).

3.3.3 Growth Medium

*S. pasteurii* was cultivated under aerobic conditions in a medium containing 30 g/l of Tryptic Soy Broth (Merck) and 20 g or 40 g of urea (HmBG) per litre of deionized water with a pH of 7.5. All the ingredients are laboratory grade chemicals sterilised at 121°C for 25 min. However, the urea was filtered using sterilised cellulose acetate syringe filters with a pore size of 0.22 µm as it degrades under high temperature.

The bacterium was incubated in a water bath shaker at 30°C for about 16h to 24h under shaking condition at 150 rotations per minute (rpm). The bacteria were incubated and grown until it reached approximately OD$_{600}$ (Optical Density at 600nm wavelength) of 0.8 – 1.2 in order to yield about $10^7$ cells/ml according to Al Qabany et al. (2011).

3.3.4 Stock Culture Preparation

The initial cultures of *S. pasteurii* were inoculated in cryovial tubes containing 50% glycerol for working stocks and kept in a -80°C freezer for preservation as done by Whiffin (2004). Prior to harvesting the bacteria in a large amount, the bacteria were streaked on a tryptic soy agar with 2% and 4% urea plates for revival and after incubation of 24 h, one single colony was inoculated into 10 ml of broth culture which was kept at 4°C for at least 48h before use. Preparation of the bacteria in an amount of 1 L was done in geometrical order for an appropriate and sufficient amount of nutrient resources. 10 ml of the initial culture was transferred aseptically to 100 ml and grown in a 30°C water bath shaken at 150 rpm for approximately 16 h – 24 h. Then, the same procedure was repeated but the bacteria were transferred from a 100 ml to a 1000 ml growth media solution, aseptically.

3.3.5 Cementation Solution Treatment

The amount used for the cementation solution treatment consisted mainly of urea and calcium chloride throughout the samples is as follows: (i) 0.5 M and (ii) 0.75 M equimolar concentrations of the two main chemical reagents. Besides these two main reagents, 10 g/L of ammonium chloride as well as 3 g/L of tryptic soy broth were provided for pH
balance and nutrients for the microorganism respectively. All the chemicals used in this research study were of analytical grade (AR). The treatment for samples in this study varied according to its corresponding parameters. Hence, it is explained further in Section 3.7.

3.4 Experimental Set-Up

3.4.1 Mixing Method

*Conventional Method*

Prior to placing the sand into the mould, dry sand was first pre-mixed with a cementation solution at their respective concentration. After obtaining a paste-like mix of sand, it was then mixed with an aqueous solution of bacteria which was grown accordingly as explained in 3.3.4. It was again mixed thoroughly for about 5 minutes before being placed into the mould to ensure homogeneity. The sand mix was then compacted directly into three (3) layers inside the cube mould, where each layer was approximately about 16 – 17 mm thick.

*Lamination Method*

As explained in Section 2.4.3, this method was inspired by Dosier (2011) in order to obtain homogeneity of the cementation solution throughout the samples. Dimensions of the small cube were only 50 mm x 50 mm x 50 mm (H x W x L), and each of the layers was kept at 25 mm. The first layer of sand mixed with bacteria solution was compacted inside the mould filling half of the thickness of the mould. Similar to the conventional method, samples were also left for at least 6 hours to allow for bacteria attachment. Then, the sample was injected with the cementation solutions 4 times with an interval of 12 hours each until visible signs of hardness were observed. After the last injection of the first layer, the second layer was applied and compacted on top of the existing layer. This layer was then injected another 4 more times on the top. The samples were rotated 180° afterwards with 4 more injections which were done from the bottom side.
3.4.2 Set-Up of 50mm Cubes

The moulds were fabricated (Figure 3.2) and each mould was a set of three pieces of 50 mm (Width) x 50 mm (Length) x 60 mm (Height). The additional 10 mm for the height of the steel mould was for the allowance of the filter material (Scotch Brite pad) at both top and bottom that contributed about 5 mm respectively. The steel frame for the cubes was of high standard stainless steel to avoid any major rusting or corrosion of any sort from the chemicals used in this study. The stainless steel frames were screwed tightly together to hold the shape of 50 mm cubes. Then, they were tightened with screws to the bottom plate. The plate was made from smooth polythene sheets that are of rigid high-density polyethylene (HDPE).
Two layers of Scotch Brite pad were placed at the top and bottom of the mould respectively and layered with a plastic mesh for filtering purposes. This was to avoid any sand particles being washed away during the treatments. The moulds were also sealed with silicone sealants to prevent any leakage during injections. There were five drain holes on the bottom of the plates for drainage of effluents while another five holes on the top plates were for gravity flow injection to the samples. The holes on top were fitted with 4 mm silicone tubes that came with a pressure regulator to control the flow during the cementation solution treatment. As it is a gravity flow injection with a manual pressure regulator, the flow rate was able to be maintained in a range of 200 – 300 mL/hr.

3.4.3 Set-Up of 150mm Collapsible Cube Mould

Acrylic collapsible cube moulds were fabricated (Figure 3.4) with each of the moulds having the size of 150 mm x 150 mm x 150 mm similar to standard dimensions of a concrete cube used for the compressive strength test in accordance with BS 1881: Part 108:1983. The moulds were assembled with screws and silicone sealant with five injections and drain holes on top and bottom respectively which were fixed with ball valves to control the flow and also to enable saturations of samples during treatment.

Figure 3.4: Acrylic collapsible mould (left) with pressurised injection system (right).

The holes on top of the moulds were fitted with silicone tubes with a diameter of 6mm connected to 15 L pressure vessels that were supported by an air compressor for a constant flow of cementation solutions passing through the sand. Non-woven geotextiles were placed on each side within the mould to reduce the loss of sand particles during the treatments for the first few experimental set-ups. It was then switched to a normal plastic
mesh with the smallest available apertures to avoid flushing of sand particles during the flushing of the cementation solutions. This is explained hereinafter in Section 5.2.3. Prior to placing the sand into the moulds, the bacteria were mixed with sand using a heavy-duty mechanical mixer to obtain a homogeneous paste of bacteria-sand mix (Figure 3.5). The bacteria-sand mix was compacted directly into the cube mould (150 mm x 150 mm x 150 mm) in 3 layers with each layer about 50 mm thick.

Figure 3.5: Mixing of aqueous bacteria solution and dry sand using a heavy duty mechanical mixer.
3.5 Quality Check Methods

3.5.1 Agar Plates

Agar plates are Petri dishes that contain a growth medium that in general means agar is added with the nutrients then poured onto the plates. It is used to culture microorganisms and with their respective condition the microorganisms will grow in colonies. The growth medium used in this study consisted of Tryptic Soy Agar (Merck, Germany) with 2% urea (HmBG). The medium used was laboratory grade which was sterilised at 121°C for 25 min using a Hirayama autoclaving machine. As explained in 3.3.4, prior to preparing the initial cultures of the bacteria, they were streaked on the plates and placed in an incubator at a temperature of 30°C for at least 24 hours for three days. This test method was done at every stage of culturing the bacteria from small to large amounts to ensure that only one type of bacteria was grown on the plates. Those plates that were contaminated were discarded to prevent contamination to other plates.

![Figure 3.6: Sporosarcina pasteurii grown on Tryptic Soy Agar Plates](Image)

3.5.2 Urease Agar Base Test

The urease agar base test is another qualitative method that was conducted for the detection of bacterial urease activity. The preparation of the media was in accordance with the directions provided in the media detail sheet provided together with the product. It is known as Christensen’s medium by OXOID and the content is as shown in Table 3.2 and the pH was kept at 6.8 ± 0.2. This medium was used to determine whether the bacteria were urease-positive or urease-negative. In the case of this research, it was to ensure the growth of *S. pasteurii* used throughout all the experiments for the purpose of showing that they were able to produce a urease enzyme at a steady and rapid rate while at the same time to evaluate if any other bacteria other than *S. pasteurii* were present.
Table 3.2: Typical ingredient for Christensen's urea agar base medium

<table>
<thead>
<tr>
<th>Typical Formula</th>
<th>gramme/Litre</th>
</tr>
</thead>
<tbody>
<tr>
<td>Peptone</td>
<td>1.0</td>
</tr>
<tr>
<td>Glucose</td>
<td>1.0</td>
</tr>
<tr>
<td>Sodium chloride</td>
<td>5.0</td>
</tr>
<tr>
<td>Disodium phosphate</td>
<td>1.2</td>
</tr>
<tr>
<td>Potassium dihydrogen phosphate</td>
<td>0.8</td>
</tr>
<tr>
<td>Phenol red</td>
<td>0.012</td>
</tr>
<tr>
<td>Agar</td>
<td>15.0</td>
</tr>
</tbody>
</table>

This urease agar base test was conducted prior to all experiments to ensure that the effectiveness of biocementation was not affected. The indication of urease-positive bacteria was observed via the change of its colour that was from orange-coloured to pink-coloured agar. As for urease-negative bacteria, it changes to yellow-coloured gel or remains the same. At each stage of growing the bacteria from 10 mL to a larger amount, this test method was conducted to ensure that the growth culture had no contamination. Should that happen, wherein the colour of the agar turned to yellow instead of pink, that particular culture was to be discarded to prevent further contamination. New bacterial culture was cultured from the original bacterial glycerol stock for subsequent experimental trials.

Figure 3.7: Urease agar base test shows that the agar turned pink (S. pasteurii) which indicates urease-positive while the orange-coloured agar is a control specimen with no bacteria streaked on it.
3.5.3 *Urease Enzymatic Activity*

The measurement of urease activity was done in accordance with established methods (Whiffin 2004; Whiffin et al. 2007) and it was measured each time prior to mixing the sand with the bacteria solution. There were two different parameters involved in the growth of the bacteria, one was grown with 20 g/L (2%) of urea while the other was grown with 40 g/L (4%). This was to evaluate whether the difference in the urea percentage for the growth of bacteria affected urease activity and concurrently affected the end result which is the unconfined compressive strength of the samples. Prior to the measurement of the conductivity, 1.11 M urea was prepared; 9 ml of this medium was added with 1 ml of bacterial suspension, then the relative conductivity change was measured and recorded over 5 minutes at 20°C ± 2.

The urease activity was then calculated taking the dilution into account. The urease activity was measured using a conductivity meter with a range of 0 – 199 milliSiemens (mS) per cm. Standard solutions of 1.11 M of urea were prepared prior to the conductivity measurement of the bacterial solution. The different percentage of urea used to grow the bacteria was translated in terms of mM urea hydrolyzed per minute. The value of the conductivity recorded was multiplied by the dilution factor and the constant, 11.11 mS.min⁻¹ which was derived by Whiffin (2004). In the measured range of activities, the relationship between urea hydrolysed (mM) and the conductivity change was established in which 1 mS.cm⁻¹.min⁻¹ conductivity change was proportional to a hydrolysis activity of 11.11 mM urea.min⁻¹ (Cheng & Cord-Ruwisch 2012; van Paassen 2009; Whiffin 2004).
3.6 Laboratory Test Methods

3.6.1 Particle Size Distribution
The particle size distribution (PSD) test was performed on typical laboratory sand that was used in this research. The properties of the sand used were as explained in 3.3.1. This test was to determine the mass percentage of individual particle size ranges found in the soil. It was conducted in accordance to Eurocode 7 corresponding to British Standards (BS) 5930:1999. Sizes of sieve used by a sieving machine ranged from 75µm to 6.3mm. Generally, the test was done in about 15 to 20 minutes and a graph of PSD was plotted in order to obtain respective fractions of each type; silt, sand and gravel.

![Vibrator machine used for sieving (Left); Sieve sets in accordance with BS 5930:1999 (Right)]

3.6.2 Compaction Standards
The purpose of compaction in this study was to have a higher soil density and to also remove the air voids within the saturated sample in order to promote the bonding of the soil particles through the crystallisation of calcite whilst improving the geomechanical aspects of the soil. The mixture of soil and bacteria were compacted under a saturated condition into 3 layers to a height of approximately 16 – 17 mm respectively reaching about 35% of the sand maximum density. Due to the nature of the study, the soil sample in this study was saturated with a bacteria solution as it was justified by having the bacteria premixed with the soil to further help in the attachment of the bacteria to the surface of the soil particles. The bacteria could then act as a nucleation site which enabled the calcite to crystallise and create a bonding structure amongst the soil particles.
3.6.3 Measurement Methods

Estimation of Calcite Content

The estimation of the calcite content was done with reference to Burbank et al. (2011) and Bernardi et al. (2014). Nonetheless, it was also in accordance with BS EN 1997-2:2007, Section 5.6.3. The amount of calcite precipitated throughout the samples was estimated by washing it with acid either hydrochloric acid (HCl) or acetic acid (vinegar). In addition, this method also served as an index to classify the calcite content in soil samples to specify the degree of cementation. Samples for testing were collected from the surface and also from the core of the samples for both the 50 mm and 150 mm cubes.

![Process of an acid wash of samples with HCl and filtered using filter paper.](image)

The samples were dried for 24 hours at 105°C and were weighed accordingly. Then, they were washed with 2 Molar (M) of HCl to dissolve the calcite and thereafter rinsed with deionized water (Figure 3.9) to wash off the remaining acids on the filter paper. The samples were dried again with the same condition and weighed again after drying. The difference in weight after the HCl acid wash was estimated to be the weight of the calcite precipitated within the samples. A control (untreated) sand sample which was not treated nor mixed with bacteria was also washed with HCl to determine the average loss of fine particles or any existing calcite in the sand sample. This value was estimated at about 0.01% and the value was adjusted accordingly.

Permeability Test

The permeability of the typical laboratory sand used in this study was measured by a constant head permeability test. This test method was conducted in accordance with a standard laboratory set-up of the constant head and the measurements were recorded.
accordingly. This test was conducted immediately after the treatment of cementation solution was completed and they were saturated with water prior to the test. Results were recorded with regards to the key parameters and they were calculated in metres per second (m/s). The test was also conducted for untreated sand for comparative purposes.

Unconfined Compressive Strength Test

The unconfined compression tests were carried out on specimens of soil which possessed low permeability to maintain undrained conditions during the test. The tests were done with reference to EN 1997-2:2007 (E), Section 5.8.4. Tests were performed on an NL Scientific Compression Machine (Figure 3.10) with a maximum load of 300 kN. The compression machine was fully automated and parameters inside the software were set accordingly. The maximum load which was applied on the samples was recorded and thereafter measured against the surface area of the samples to obtain its compressive strength in kiloPascal (kPa). After the samples were tested, the results were recorded accordingly and the samples were kept in a separate container for the purpose of the acid wash as explained in Section 3.6.3 and also for Scanning Electron Microscopy Analysis in Section 3.6.4.

Pocket Penetrometer Test

The pocket penetrometer tests were conducted mainly to evaluate consistency and approximate unconfined compressive strength of soils according to ASTM standards (New Test Method for Pocket Penetrometer Test 2010). There is no current standard available for this method. However, this method can be used by field and/or laboratory personnel to describe the characteristics of soil instantly on the spot. The direct reading

Figure 3.10: Compressive strength test machine (Left); Testing of 50mm cube sample (Right)
scale on the penetrometer was in tons/ft² or kg/cm² (i.e. 1 kg/cm² = 0.0980665 MPa). However, the readings obtained from the penetrometer is not recommended to replace laboratory results due to the fact that it could give deceptive results as it only involves a small penetration area. The readings were taken at the lower part of the red ring and the maximum reading of the penetrometer of 700 psi (~approximately 4.82633 MPa). This method was used for the main experimental tests to evaluate the surface strength and the values were recorded accordingly.

Figure 3.11: Pocket penetrometer to check visual classification of soil (Soil penetrometer: pocket style product manual 2016).

3.6.4 Scanning Electron Microscopy Analysis

Of those samples which had undergone the UCS test as explained earlier on, some of the fractured samples were kept accordingly before they were analysed. The analyses of the fractured samples were performed on a Scanning Electron Microscope (SEM), Hitachi S-3400N. The analyses were done at the University of Malaysia Sabah (UMS) in Sabah with the assistance of the lab officer in-charge. Prior to SEM analysis, the samples were
coated with gold in a Quorum Sputter Coater. There were 13 samples selected in total for analysis and every three to four samples were done at a time. Only a small amount of fractured samples was required and they were placed on specimen stubs using “carbon tabs” (Figure 3.13). It was then placed in the Quorum Sputter Coater for about 15 to 20 minutes for gold coating.

![Figure 3.13: Fractured samples were placed on aluminium stubs before mounted in SEM](image)

Three fractured samples were placed on aluminium stubs and mounted on the SEM to begin the analysis. The techniques used in this analysis were with reference to Stocks-Fischer et al. (1999), in which they used secondary electron imaging (SEI) for microscopic examination whilst for electron micrographs, back-scattered electron imaging (BSEI) were engaged. Five images were captured which were of different magnification and at an accelerating voltage ranging from 5 to 15 kV (i.e. 5 µm, 10 µm, 50 µm, 200 µm and 300 µm). The images at 200 µm and 300 µm are mainly for viewing the bonding structure or the particle contact point, whereas, for 5 µm, 10 µm and 50 µm, the purpose was to view the calcite crystals on the surface of the soil particle. A control sample that was untreated with no bacteria and no cementation solution was also viewed on the SEM to benchmark against the treated samples. In addition, an elemental analysis at a magnification of up to 5µm was done as explained hereinafter.
3.6.5 *Energy Dispersive X-Ray Spectroscopy*

Energy dispersive X-ray (EDS) elemental analyses of the pieces of fractured samples were conducted apart from acquiring qualitative results from the SEM examination. The signal processor unit by BRUKER was attached with the SEM and conducted once the images were obtained from the SEM. The purpose being that this elemental analysis works in a way that it disseminates a high-energy beam of X-rays and focused at the samples that are being studied. The qualitative elemental information can be obtained by the intensity of backscattered electrons that is then correlated to the atomic number of the element within the volume of the sample (Mountassir et al. 2014; Stocks-Fischer et al. 1999).

![Signal processor unit (BRUKER) for EDX analysis which is attached together with the SEM equipment.](image)

Besides that, the analysis of characteristic X-rays (EDX analysis) will be able to give more quantitative information of the elements emitted from the sample. Subsequently, after obtaining images from SEM, the same image of the size up to 5µm was chosen for elemental analysis. The service provided allowed three points to be taken for elemental analysis. These EDX analyses of the samples were carried out using Bruker Quantax and Esprit Software. The analysis for each point took exactly one minute before the spectrum was obtained to show the mass percentage (wt %) and atomic percentage (at %) of each element that presents at that particular point. The results obtained from EDX analysis is further discussed in Chapter 4 and Chapter 5.
3.7 Treatment Techniques

3.7.1 Initial Experiments of 50mm Cubes

There was a total of 32 initial experimental samples done altogether that involved different key parameters to evaluate the effectiveness of the bio-cementation process. The tests mainly focused on the concentration of cementation solutions (i.e. 0.5 M and 0.75 M), the method of casting the samples (Section 3.4.1) and also its percentage of urea in the bacterial culture preparation (i.e. 2% and 4%). The samples were kept in an incubator room wherein the temperature was maintained at 35°C, taking into consideration the tropical climate in Borneo which was taken into account if this bio-cementation process is to be implemented in-situ.

The curing of samples at 105°C was to discover whether temperature would have any effect on the precipitation of calcite as well as the compressive strength obtained. Besides, not only would it determine its effect with a high temperature, but most likely it may be adapted to the construction materials industry which may be feasible. For example, the production of red clay bricks requires a high temperature for it to have a high strength of more than 20 MPa (Bernardi et al. 2014) and this procedure can be imitated for producing sustainable construction materials through the MICP technique.

Table 3.3: Initial experimental test design for 0.5M equimolar concentrations of cementation solutions

<table>
<thead>
<tr>
<th>No.</th>
<th>Experimental Codename</th>
<th>Mixing Method</th>
<th>Curing Temperature (°C)</th>
<th>Bacterial Broth (% Urea)</th>
<th>Curing Duration (days)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.52A</td>
<td>Lamination</td>
<td>35</td>
<td>2</td>
<td>7</td>
</tr>
<tr>
<td>2</td>
<td>0.52A</td>
<td>Lamination</td>
<td>35</td>
<td>2</td>
<td>28</td>
</tr>
<tr>
<td>3</td>
<td>0.54A</td>
<td>Lamination</td>
<td>35</td>
<td>4</td>
<td>7</td>
</tr>
<tr>
<td>4</td>
<td>0.54A</td>
<td>Lamination</td>
<td>35</td>
<td>4</td>
<td>28</td>
</tr>
<tr>
<td>5</td>
<td>0.52B</td>
<td>Lamination</td>
<td>105</td>
<td>2</td>
<td>7</td>
</tr>
<tr>
<td>6</td>
<td>0.52B</td>
<td>Lamination</td>
<td>105</td>
<td>2</td>
<td>28</td>
</tr>
<tr>
<td>7</td>
<td>0.54B</td>
<td>Lamination</td>
<td>105</td>
<td>4</td>
<td>7</td>
</tr>
<tr>
<td>8</td>
<td>0.54B</td>
<td>Lamination</td>
<td>105</td>
<td>4</td>
<td>28</td>
</tr>
<tr>
<td>9</td>
<td>0.52C</td>
<td>Conventional</td>
<td>35</td>
<td>2</td>
<td>7</td>
</tr>
<tr>
<td>10</td>
<td>0.52C</td>
<td>Conventional</td>
<td>35</td>
<td>2</td>
<td>28</td>
</tr>
</tbody>
</table>
The initial experiments were categorised into two main categories that were differentiated by their concentration of cementation solution and further divided by their method of mixing. Table 3.3 and Table 3.4 shows the variables which were designed for all the initial experimental tests and hereinafter all the samples shall be addressed in accordance with their identifying code name as shown in Table 3.3 and Table 3.4.

Table 3.4: Initial experimental test design for 0.75M equimolar concentrations of cementation solutions

<table>
<thead>
<tr>
<th>No.</th>
<th>Samples Code Name</th>
<th>Mixing Method</th>
<th>Curing Temperature (°C)</th>
<th>Bacterial Broth (% Urea)</th>
<th>Curing Duration (days)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.752E</td>
<td>Lamination</td>
<td>35</td>
<td>2</td>
<td>7</td>
</tr>
<tr>
<td>2</td>
<td></td>
<td>Lamination</td>
<td>35</td>
<td>2</td>
<td>28</td>
</tr>
<tr>
<td>3</td>
<td>0.754E</td>
<td>Lamination</td>
<td>35</td>
<td>4</td>
<td>7</td>
</tr>
<tr>
<td>4</td>
<td></td>
<td>Lamination</td>
<td>35</td>
<td>4</td>
<td>28</td>
</tr>
<tr>
<td>5</td>
<td>0.752F</td>
<td>Lamination</td>
<td>105</td>
<td>2</td>
<td>7</td>
</tr>
<tr>
<td>6</td>
<td></td>
<td>Lamination</td>
<td>105</td>
<td>2</td>
<td>28</td>
</tr>
<tr>
<td>7</td>
<td>0.754F</td>
<td>Lamination</td>
<td>105</td>
<td>4</td>
<td>7</td>
</tr>
<tr>
<td>8</td>
<td></td>
<td>Lamination</td>
<td>105</td>
<td>4</td>
<td>28</td>
</tr>
<tr>
<td>9</td>
<td>0.752G</td>
<td>Conventional</td>
<td>35</td>
<td>2</td>
<td>7</td>
</tr>
<tr>
<td>10</td>
<td></td>
<td>Conventional</td>
<td>35</td>
<td>2</td>
<td>28</td>
</tr>
<tr>
<td>11</td>
<td>0.754G</td>
<td>Conventional</td>
<td>35</td>
<td>4</td>
<td>7</td>
</tr>
<tr>
<td>12</td>
<td></td>
<td>Conventional</td>
<td>35</td>
<td>4</td>
<td>28</td>
</tr>
<tr>
<td>13</td>
<td>0.752H</td>
<td>Conventional</td>
<td>105</td>
<td>2</td>
<td>7</td>
</tr>
<tr>
<td>14</td>
<td></td>
<td>Conventional</td>
<td>105</td>
<td>2</td>
<td>28</td>
</tr>
<tr>
<td>15</td>
<td>0.754H</td>
<td>Conventional</td>
<td>105</td>
<td>4</td>
<td>7</td>
</tr>
<tr>
<td>16</td>
<td></td>
<td>Conventional</td>
<td>105</td>
<td>4</td>
<td>28</td>
</tr>
</tbody>
</table>
3.7.2 Treatment Techniques for Initial Experimental Samples

The treatment techniques adapted for the initial samples was in accordance with some of the procedures as reported in past studies (DeJong et al. 2006; Ebigbo et al. 2012) where samples were left for at least 4 – 6 hours to allow the attachment of the bacteria to the sand particles. It was then injected with the cementation solution four (4) times with an interval of 12 hours between each injection for the conventional method. As for the lamination method, the injection frequency was explained in Section 3.4.1. However, the concentrations of the cementation solutions used in these initial samples were 0.5 M and 0.75 M equimolar concentrations of urea and calcium chloride. Alongside the cementation solutions, was 3 g/l of Tryptic Soy Broth to allow extra nutrients for the bacteria once mixed with the sand. The injection was conducted with a gravitational flow that was maintained at 200 – 300 ml per hour. Each of the samples was injected with a cementation solution of about 1 pore volume (40% porosity) at an interval of 12 hours for two days. For the lamination method, the injection was also done at an interval of 12 hours for 4 days in total for two layers of sand compacted inside the mould.

3.7.3 Main Experiments of 150 mm Collapsible Cube Mould

The samples of 150 mm cubes commenced after all the findings from previous initial experimental tests were analysed and the conditions which were deemed best was used for further experimentation works to improve the procedure of bio-cementation along with a pressurised injection system. A total of 4 independent tests were carried out with key parameters which were kept constant after the results from the initial experiment tests were finalised.

Samples were cured with a controlled temperature of 35°C and one sample was cured at 105°C to further investigate the effect of a high temperature against the strength and/or the calcite content of the samples. The tests conducted were merely a repetition of the same conditions to improve the procedure of bio-cementation with the use of a pressurised injection system. The results obtained from these main experimental tests are reported in the latter section (Section 5.2). Table 3.5 shows the details of each sample which have been designed and prepared for this research study.

The treatment techniques for these samples were performed similar to the initial samples except for the casting method as well as the number of injections. The samples were left for at least 4 – 6 hours to allow the attachment of the bacteria to the sand particles. It was then injected with the cementation solution 6 times with an interval of 12 hours between
each injection. The flow rate was maintained at $400 \text{ – } 500 \text{ ml per hour}$ corresponding to the pressure exuded by the compressor ($0.05 \text{ – } 0.06 \text{ bar}$) and sustained inside the vessel for flushing of cementation solutions.

<table>
<thead>
<tr>
<th>No.</th>
<th>Sample</th>
<th>Mixing Method</th>
<th>Curing Temperature ($°C$)</th>
<th>Curing Duration (days)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>T1</td>
<td>Normal Mix</td>
<td>35</td>
<td>28</td>
</tr>
<tr>
<td>2</td>
<td>T2</td>
<td>Normal Mix</td>
<td>35</td>
<td>28</td>
</tr>
<tr>
<td>3</td>
<td>T3</td>
<td>Pre-Mix</td>
<td>35</td>
<td>28</td>
</tr>
<tr>
<td>4</td>
<td>T4</td>
<td>Pre-Mix</td>
<td>105</td>
<td>28</td>
</tr>
</tbody>
</table>

3.7.4 Treatment Techniques for Main Experimental Samples

The pressure was monitored every few hours during the injection as it would decrease over time. All four samples were injected with about 1 pore volume (40% porosity) of around 1.4 L of 0.75 M concentration of cementation solutions at an interval of 12 hours. The samples were rotated after the first three injections from the top and then another three injections from the bottom to ensure that the circulation of cementation solutions saturated the whole sample evenly to achieve homogeneity within the samples. The reason for this 180° rotational injection is discussed further in the report (Section 6.1).
4.1 Introduction

The results from the experimental trials performed in this research are presented in this chapter. The preliminary trials setup of 50 mm cubes were prepared to determine which conditions were best applied prior to the setup of the 150 mm cubes with an improved pressurised system which was assumed to have comparatively better results in terms of strength and also its hydraulic conductivity. Studies also included the effect of different percentages of urea in preparing bacterial culture in regards to its urease activity, different concentration of cementation solutions as well as methods of casting under controlled curing temperatures of 35°C and 105°C at 7 and 28 days.

4.2 Bacterial Growth Curve

The preparation of bacterial culture throughout the whole experimental series, both initial and main experimental tests, was able to be maintained at OD$_{600}$ in the range of 0.9 – 1.2; in line with the works of Al Qabany et al. (2011) having OD$_{600}$ around 0.8 – 1.0 corresponding to $10^7$ cells/ml. While Harkes et al. (2010) reached up to 2.88 OD which was at the late exponential phase but was then diluted by a factor of 10 with a saline solution to obtain lower initial urease activity.
4.3 Results of Initial Experimental Tests

4.3.1 Measurement of Conductivity and Urease Activity

Prior to introducing the bacterial culture in the 50mm cubes samples, it was tested for the enzymatic activity to ensure that the value corresponded to what had been reported in past literature. The bacterial cultures were provided with two different percentages of urea that was 2% (20 g/L) and 4% (40 g/L) to investigate whether the difference in its enzymatic activity would have shown any effect towards the outcome of biocementation process. In Figure 4.2, the conductivity increased greatly the instant it was introduced to the urea and the graph shows that the enzymatic activity increased up to 60%. Each of them recorded 13.95 and 36.78 mM urea hydrolysed.min⁻¹ respectively. The conductivity recorded in Figure 4.2 was averaged from each experimental test and these values are referred hereafter as 2% (13.95 mM) and 4% (36.78 mM) of urea.
Table 4.1: Value of conductivity change and urea hydrolyzed per minute of the bacterial cultures grown with 2\% and 4\% of urea.

<table>
<thead>
<tr>
<th>Percentage of Urea (%)</th>
<th>Conductivity Change (mS.cm(^{-1}).min(^{-1}))</th>
<th>Conductivity (mS.cm(^{-1}).min(^{-1})) x dilution factor (df)</th>
<th>mM urea hydrolysed.min(^{-1})</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>0.1256</td>
<td>1.256</td>
<td>13.954</td>
</tr>
<tr>
<td>4</td>
<td>0.3311</td>
<td>3.311</td>
<td>36.785</td>
</tr>
</tbody>
</table>

The calculation of the enzymatic activity in terms of urea hydrolyzed per minute was performed in accordance with Harkes et al. (2010) and Whiffin et al. (2007). This was also explained in Section 2.3.2 whereby the measured range of activities of about 1 mS.min\(^{-1}\) was correlated with a hydrolysis activity of 11 mM urea.min\(^{-1}\) by taking into account the dilution factor which in this case, was 10 hence its final values as shown in Table 4.1. The results show that the enzymatic activity was within the range of what has been reported in past literature wherein the strain of S. pasteurii, particularly DSMZ 33 is between 5 – 20 mM urea hydrolysed.min\(^{-1}\) (Harkes et al. 2010), except for those of 4\% urea which had a higher activity, which might be due to the ingredient of their growth medium (Table 2.3), compared to the one that was used in this research study (Section 3.3.3). This also explains that different amounts of urea used to prepare the bacterial culture affects the urease activity and presumably affects the precipitated calcite and compressive strengths of the samples. This is further explained with findings as presented in Section 4.3.3 and Section 4.3.4.

4.3.2 Hydraulic Conductivity

The saturated hydraulic conductivity (\(k_{sat}\)) were all tested prior to extracting the samples from the mould to prevent any disturbance to the structure of the sample which may have affected the results altogether. As a control, the untreated samples were set up for a constant head permeability test as explained in Section 3.6.3 which was recorded at 4.84 x 10\(^{-4}\) m/s wherein it was also in accordance with the empirical equation designed by Hazen (1892) with his equation as shown below (Eq. 6) in which \(c\) represents the Hazen’s empirical coefficient with a value between 0.0 to 1.5 depending on grain size. Salarashayeri and Siosemarde (2012) used a value between 1.0 to 1.5. \(D_{10}\) is the diameter of the 10\(^{th}\) percentile grain size of the material.

\[
K = c \ (D_{10})^2 \quad \text{(Eq. 6)}
\]
Figure 4.3: Hydraulic conductivity of preliminary samples for 0.5M of cementation solutions for 2% and 4% urea.

The permeability test on all samples was conducted and repeated three times and the values were averaged accordingly. As shown in Figure 4.3, the results demonstrated a reduction of hydraulic conductivity with regards to different mixing methods which was conducted for this study. The reduction was significantly high for the conventional method which was up to 1 order of magnitude (decreasing rate of up to 90%) for both 0.5 M and 0.75 M of cementation solutions. It ranged between $5.60 \times 10^{-5}$ m/s for the conventional method while it came in the range of $0.70 \times 10^{-4}$ m/s for the lamination method.

The lowest recorded hydraulic conductivity reported in this study for 0.75 M was $5.69 \times 10^{-5}$ m/s at 4% urea, which was achieved by using the conventional method (Figure 4.4).

The difference demonstrated in comparison to the untreated samples showed a significant reduction in the hydraulic conductivity wherein, the precipitation of the calcite within the samples were successful, which further inhibited the flow rate. No significant difference in the reduction of hydraulic conductivity between these two methods with regards to its urease activity was indicated. Mixing and casting of the samples by the conventional method proved to be more effective.
Corresponding to the results of urease activity from the previous section (4.3.1), a higher urease activity at 4% urea demonstrated a lower permeability in comparison to those at 2% regardless of its mixing method. To understand this in depth, the amount of calcite precipitated within these samples was investigated to further evaluate its correlation where highly moderate urease activity promoted a well-precipitated calcite which led to clogging of the pores (Yasuhara et al. 2011) and thereafter, a reduction in hydraulic conductivity.
4.3.3 Unconfined Compressive Strength Tests

Figure 4.5: Some of the samples which were treated with 0.75M cementation solutions and 4% of urea after extraction from the mould.

The unconfined compressive strength results were measured for all samples and are illustrated in Figure 4.9 for both lamination and conventional methods. The samples were cured over the course of 7 and 28 days to investigate the effect of the curing duration against its compressive strength. In Figure 4.9 (a), it was observed that all samples for the lamination method at 35°C recorded an average 200 kPa which was counterintuitive in the beginning. The increased rate in strength at 28 days was only about 3 – 4 % except for 0.52A. With 2% urea, the strength of the sample at 7 days was only 84 kPa but increased up to 70% in strength, measured at 244 kPa at 28 days. The rest remained unaffected or with a very minimal change occurring over the course of the curing duration with the lamination method.

On the other hand, with the conventional method, the strength increased over time though again, very minimal changes were observed except for the sample 0.754G which saw an increase over twice the 7-day strength, from 224 kPa to 564 kPa (Figure 4.9 (b)). Also through the findings, the effect of urease activity against the compressive strength was negligible throughout the initial experimental samples for those cured at 35°C except sample 0.754G which demonstrated the opposite to the others.

Figure 4.6 shows the samples at 28 days of 0.75 M at different urease activity of 2% (13.95 mM) and 4% (36.78 mM) before the UCS test and their failure patterns. Qualitatively, the colour of the sand also acted as an indicator with regards to the precipitation of calcite throughout the whole sample. Figure 4.6 (a) showed the darker colour of the sand in comparison to Figure 4.6 (c) proving that the sample was well precipitated with calcite (white precipitates) for the conventional method.
In Figure 4.7 qualitative observation of the colour between the samples deduced that a higher urease activity might have precipitated more calcite; Figure 4.7 (a) and Figure 4.7 (c). This is further explained in Section 4.4 with reference to Figure 4.19 (a). The calcite precipitation is seen in between the layers; a thin layer of white precipitate bonding the two layers together for those prepared from the lamination method.

Moreover, it is possible that the handling process would have affected the strength of the samples in one-way or another. This is due to the fact that lower strength samples were much more difficult to handle especially during extraction or during testing and this was further supported by Whiffin et al. (2007). While samples cured at 35°C showed an average strength of 200 kPa, those which were cured at 105°C demonstrated a higher compressive strength from 684 kPa to 732 kPa as can be seen in Figure 4.9 (d) especially those prepared by the conventional method. In contrast to samples done with the lamination method, results exhibited a lower strength improvement except for sample 0.752F, which was treated with 0.75 M of cementation solutions at a lower urease activity (2% urea), reading at 484kPa and 572 kPa for 7 and 28 days respectively as observed in Figure 4.9 (c).
With regards to those samples that were treated with 0.5 M of cementation solutions, the overall findings have shown that a very slight improvement occurred, irrespective of the method of casting as well as the urease activity at 2% or 4%. With the exception of sample 0.52D and 0.54D (Figure 4.9(d)), cast by the conventional method, improvement from 220 kPa to an average of 686 kPa was seen. Results have shown that the curing of the samples at a high temperature influenced the strength over time, particularly those treated with higher concentrations of cementation solutions. Li (2013) also reported similar results wherein the unconfined compressive strength of bio-cemented samples increased when cured at an oven-dried temperature over 100°C.
Figure 4.9: The unconfined compressive strength of all initial experimental samples with different mixing method (i.e. lamination and conventional) as well as their curing temperature (i.e. 35°C and 105°C).
4.3.4 Calcite Content

The precipitated calcite throughout the samples can be estimated through an acid wash and the percentage of dry weight before and after the acid wash was calculated and the difference obtained would be the estimated calcite precipitate within the collected samples. This test was performed on all samples by collecting about 5 – 10 g each from the outer surface and core of the samples, then washed with hydrochloric acid (HCl) at a concentration of 2 M.

Figure 4.10 shows the results of the average calcite content (% w/w) of samples which were cured at 35°C, while Figure 4.11 shows those cured at a higher temperature of 105°C with regards to their method of casting (i.e. lamination and conventional). It was apparent that the percentage of calcite content over the course of 28 days was somewhat irregular which countered what was preliminarily assumed wherein the calcite content would increase if cured longer. However, some of the results showed otherwise, hence it shall be evaluated on reasons that led to these results (Section 4.4).

Figure 4.10 (a) illustrates that those of a lower urease activity (2%) had an increase of calcite content of from 4.04% to 5.26% for sample 0.52A, while sample 0.752E increased from 4.38% to 6.15% and both of them recorded a strength ranging from 80 kPa to 220 kPa. However, the calcite content of sample 0.54A and 0.754E decreased at a rate of 20% to 40% from the original content which had a relatively big difference.
Figure 4.10: Calcite content for samples cured at 35°C for lamination method (a) and conventional method (b) over 28 days.

This may be due to the collected sand samples for estimation of calcite content through an acid wash which was quite localised wherein the results only represent the location of collected samples, hence affecting the overall results. Also, another reason that may have led to the decrease in this result was due to the method itself. Samples cast in lamination showed a thin layer of precipitated calcite in between those layers. The portion of the sample that was under those layers may not have had a sufficient calcite bonding and once the samples were crushed, the distribution of calcite of the collected samples was random (mix of high and low calcite content) leading to the lower value of calcite content after being cured for 28 days.
The affected samples with regards to the reasons explained earlier occurred with 0.52A, and 0.752E only and it is apparent that only those that used the lamination method were greatly affected as the decrease rate was quite large ranging from 15% to 40%. It was observed as well that the strength recorded for these three samples showed an average of 214 kPa. It was also deduced that due to the random distribution of calcite bonds within the samples, the overall strength and the average calcite content was affected.

In addition, it can be explained in detail with reference to the images obtained from the SEM as well as the elemental analysis by EDX later in the next section (4.3.5 and 4.3.6). Besides that, there is another sample from those from the conventional method; sample 0.754G which may have been affected similar to 0.52A and 0.752E, but the decrease rate was very marginal showing only 0.02%. Other samples that used the conventional method showed an improvement rate from 20% up to 65%.

For samples cured at 105°C as shown in Figure 4.11, results demonstrated that all samples except for 0.754F had their average calcite content increased over the course of 28 days of curing. Sample 0.752F resulted in a high improvement rate where the average calcite content increased from 2.79% to 5.81% in line with their compressive strength that increased from 484 kPa to 572 kPa under higher temperature. In the case of the conventional method, the rest of the samples were improved too, except for sample 0.754H, that decreased at a rate of 0.1%, which was minimal. The increasing rate of the other samples was from 0.1% to 26%, lesser than those of the conventional method which were cured at 35°C.

To sum it up, the relationship between the calcite content and strength were found out to be non-relatable (further discussion in Section 4.4) and the results demonstrated that the content of calcite precipitation was not entirely indicative with regards to the strength of the samples. However, it was deduced that it is the strength of the calcite crystals themselves affected the overall strength of the whole sand matrix. This observation is further supported by Al-Thawadi (2008).
In general, throughout the overall samples from the initial experimental tests, it was established that the effect of urease activity against the average content of calcite was hardly consistent due to the random distribution of calcite bonding that existed within the samples. However, this is not to be mistaken to counter the statement that a higher urease activity promoted better calcite precipitation but rather, due to the heterogeneity of calcite distributed throughout the samples that led to a relatively low strength. Although, reports do establish that at a certain percentage of calcite (dry weight % w/w) an unconfined compressive strength averaged at 200 kPa.

Figure 4.11: Calcite content for samples cured at 105°C for lamination method (left) and conventional method (right) over 28 days.
4.3.5 SEM Images

The visualisation of the calcite formation within the samples was viewed under a Scanning Electron Microscope (SEM) and this test was conducted on selected samples. An untreated sample was viewed to act as a control sample for comparison purposes. The viewing of these samples was at 200 µm showing the particle contact and also the bonding that existed between particles. Four samples of those cured at 35°C with a 0.75 M equimolar concentration were viewed under a SEM with regards to their method of casting as well as the percentage of urea with reference to the urease activity.

Figure 4.12: (a) Untreated sample as control; Lamination method (b) Sample 0.752E; (c) sample 0.754E at 300µm

Figure 4.12 above shows the images of samples which were cast with the lamination method and it was viewed at a larger scale of 200 µm where the particle contact was visible though it was not exactly in full contact probably due the bonding which was seen to be quite minor. There were voids (darker areas) observed among these particles showing less calcite crystal clogging the pores. This may have caused the lower strength as shown in Figure 4.9 (a). It can also be seen that at higher urease activity (4%) at Figure 4.12 (c) there is a better result in terms of the precipitation of calcite in comparison to that of lower urease activity (2%) as seen in Figure 4.12 (b). However, this lamination method shows that a more random distribution of calcite crystals may be caused by the insufficient bonding in-between and under those layers. This said, when compared to the untreated sand, crystallisation was observed to be non-existent on the surface of the particles.

Figure 4.13: (a) Untreated sample as control; Conventional method (b) Sample 0.752G; (c) Sample 0.754G 754E at 300µm
In the case of those done by the conventional method, results show the contrary to that of the lamination method. Voids were observed in Figure 4.13 (b) but the crystallisation was distributed better in terms of its arrangement on the surface of the particle. As shown by Sample 0.754G in Figure 4.13 (c), it demonstrated that calcite precipitations congested the pores and particle contact was evident. This led to the creation of stronger bonds, hence the correlation to a higher strength as portrayed in Figure 4.9 (b).

4.3.6 EDX Analysis
To further analyse the crystallisation of calcite within the sample, an analysis of the element concentration was conducted for these selected samples by Bruker Quantax, another piece of equipment that was fitted alongside a SEM for EDX analysis. The analysis was performed immediately after being viewed under the SEM using the Esprit software, which took one minute to investigate the concentrations of the elements that existed in the sample. The main elements that were to be focused on in these analyses were those of Silica (Si) and Oxygen (O₂), which represent sand (Quartz, SiO) and CaCO₃, the most important component of calcite.

From Figure 4.15 until Figure 4.18, it can be seen that the calcite crystals are those that are white-coloured precipitates and the darker surface areas show little or none of the calcite on the surface of the particle. All four samples showed a high value of the element Calcium (Ca), and the main component CaCO₃. They were measured with a mass percentage (% wt) of 17.42% (0.752E), 16.56% (0.754E), 16.71% (0.752G) and finally 15.73% (0.754G). Other elements such as carbon (C) and oxygen (O) which are also part of the calcite component also showed a high intensity as observed in Figure 4.15 until Figure 4.18 except for Si. In comparison to the untreated sample, the element Ca was 0%, in other words it was non-existent (Figure 4.14). A lesser amount of those SiO₂ elements within these samples demonstrated that the surfaces of the sand particles were mostly covered with the precipitated calcite, hence the higher concentration of CaCO₃ elements.

![Figure 4.14: EDX analysis of Untreated Sample demonstrating element Calcium (Ca) was not present.](image)
Figure 4.15: SEM images at 5µm and EDX analysis at a different point for Sample 0.752E.

Figure 4.16: SEM images at 5µm and EDX analysis at a different point for Sample 0.754E.

Figure 4.17: SEM images at 5µm and EDX analysis at a different point for Sample 0.752G.

Figure 4.18: SEM images at 5µm and EDX analysis at a different point for Sample 0.754G.
4.4 Discussion

The correlation between the strength and the estimated calcite contents were not entirely indicative throughout the whole initial experimental samples as explained in the earlier section (4.3). In Figure 4.19, it can be observed that there was no significant difference in their calcite content (% w/w) for strength shown by samples with the lamination method regardless of its concentration of cementation solution and urease activity. Though the average calcite content of the samples does show an increase from 7 days to 28 days, the rate of increase was very minimal.
Figure 4.20: Comparison between lamination (a) and conventional (b) method in terms their average calcite content against the unconfined compressive strengths at 105°C.

Figure 4.20 shows the relationship between average calcite contents against the compressive strength for samples cured at 105°C. Similar to those which were cured at 35°C, no clear correlation was established because even at a comparatively lower calcite content, a relatively higher strength could be achieved. However, a higher temperature does affect the increase in compressive strength which was also established by Li (2013) who suggested that higher temperatures may prompt a mineral forming reaction within the sample hence a higher strength was achieved. Although in practice, curing soil samples at a high temperature is very unlikely due to the limitation of the technique.
On the other hand, samples of the lamination method may have experienced an insufficient bonding between layers as the casting of the samples were done in separate layers. Formation of calcite crystals may not have transformed fully to those of rhombohedral crystal (ones that have higher crystal strength) and at higher concentrations of cementation solutions, they were less uniform although having larger crystal sizes (Al Qabany et al. 2011). Hence, it has been assumed that this would have affected the overall strength of the samples.

At 0.5 M of an equimolar concentration of cementation solutions, the estimated calcite contents were measured at an average of 3% and above. While at higher concentration of cementation solutions, it was reported to have a less uniform distribution of larger calcite crystal size which measured up to 70 kg/m³ (approximately 4.01% w/w). This also conformed to the results obtained in this study where at least 4% was recorded for the equimolar concentration of 0.75 M. Nonetheless, it does show that at an average compressive strength of about 200 kPa when the calcite content varied from 4% to 8%.

Likewise, for the conventional method, it also showed that at the same average compressive strength of 200 kPa, the average calcite content was similar to the other method though at a relatively lower range of 3% to 6%. The highest recorded strength for samples cured at 35°C was those of the conventional method, measuring at 4% urea and with 0.75 M cementation solutions which read at 564 kPa of unconfined compressive strength. Despite the high strength, the calcite content was only about 4% compared to the lamination method sample (with the same condition).

Regardless of the irregular findings of the calcite content among all of the samples, the values were still conforming to the range reported by Whiffin et al. (2007). It was stated that lower concentrations of calcium carbonate (below 60 kg/m³ or 3.5% w/w) will not have any significant effect on strength or stiffness properties in comparison to the untreated sand. In reference to this value as a benchmark, Al Qabany et al. (2011) stated that at 0.5 M equimolar concentrations of cementation solutions resulted in a more random distribution of calcite crystals with its value of up to 67 kg/m³ of calcite (approximately 3.9 % w/w) which fit into the values obtained in this research.

On another note, the findings of different concentrations of cementation solution and urease activity had little effect on the calcite content which may be partly due to other characteristics during the process of bio-cementation itself. For example, the formation
of calcite crystals precipitated was highly dependent on the geochemical process that occurred within the samples. Different types of calcite crystal shapes can be observed as explained in Section 2.3.5, categorised as either spherical or rhombohedral crystals. It was also observed in the SEM analysis conducted in this study (Section 4.3.6), a mix of rhombohedral and spherical shaped crystals were found in abundance for some samples while some others showed the distribution of the crystals quite randomly.

Nonetheless, Al-Thawadi (2008) stated that spherical shaped calcite crystal gives a relatively lower strength as it is the first stage of calcite before it formed into rhombohedral crystals either from or within the spherical crystals and thereafter led to an increasing in the size of rhombohedral crystals arrangement. Consequently, Al-Thawadi (2008) also explained that the strength of the formation is dependent on the size and type of the calcite crystals. Due to the changes over the course of the reaction, qualitative factors may have also attributed to the formation of the calcite crystal shapes which at the same time would have affected the strength of the crystals itself (Whiffin et al. 2007).

Furthermore, this also correlates back to the hydraulic conductivity as reported in Section 4.3.2. Samples of 0.75 M cementation solutions at 4% urea recorded the lowest value, which read at $5.69 \times 10^{-5}$ m/s. It is comprehended that at a higher concentration of the cementation solutions it would have demonstrated well-precipitated calcite crystals clogging the pores within the samples and reducing the permeability by 1 order of magnitude than those of untreated sand ($4.84 \times 10^{-4}$ m/s).

From the findings of these initial experimental works, it can be deduced there were inconsistencies in the results in terms of the calcite content and unconfined compressive strengths with regards to the curing duration of 7 and 28 days. It demonstrated that even at 7 days, the calcite content was at a high of up to 8.59% for 0.754E and it decreased to 5.22% at 28 days. One of the reasons that could have led to this result was due to the nature of the sample whereby, each of the samples was prepared independently although all the conditions were kept exactly the same. Also, the limitation to this was that the reaction that occurred within the sample was unknown and this shall be investigated even further for more accurate results and for a better understanding of the reactions. Although the volume of bacteria culture and cementation solution was injected at the same rate and amount, what happened inside was still uncertain which should be addressed in future research.
After having scrutinised each key parameter which were tested in this initial experimental set of tests and after evaluation the justifications made on each sample, it was finalised that the sample of those cast by the conventional method was chosen to be best applied for further experimental tests by using 150 mm cubes along with an improved pressurised injection system. From the findings, preparation of the bacterial culture to produce higher urease activity was also deemed to be suitable for a larger scale application. It was also decided that the 150 mm cubes should be cured for at most 28 days. Also, 0.75 M equimolar concentrations of cementation solutions (i.e. urea and calcium chloride) was found to be the most suitable as it showed at a normal drying temperature of 35°C, a strength of at least 500 kPa could be achieved. In the case of surface strength treatment such as preventing erosion from a riverbank (sand dunes) or improving subgrades when constructing the road, 500 kPa would be sufficient for these types of applications.
CHAPTER 5 MAIN EXPERIMENTAL RESULTS AND DISCUSSION

5.1 Introduction

Findings from initial experimental samples carried out previously have concluded the specific conditions that are deemed fit for further experimental works involving a larger size cube of 150 mm. All the tests carried out hereafter were all conducted independently and were repeated a few times to ensure some of the parameters of the process were kept constant throughout whilst at the same time improving the procedure of bio-cementation along with the pressurised injection system. The growth of bacterial culture was also kept constant during the course of the bio-cementation process as explained in Section 4.2. The OD$_{600}$ was in the range of 0.8 – 1.2 corresponding to the works of Al Qabany et al. (2011).

Injections conducted with the pressurised system were able to maintain flow rate at a range of 400 – 500 ml per hour corresponding to a 0.05 – 0.06 bar exuded by the compressor. Lower than the value mentioned earlier on would result in no cementation solution flushing through the samples as the flow rates would be observed to be non-existent. Higher than the value mentioned would affect the bacterial positioning within the sand particles, which might be washed away after the first injection. Hence, the value of a 0.05 – 0.06 bar was finalised after a few runs of flushing water into the saturated sand in the collapsible mould without any bacterial culture. Hence, the value was kept constant throughout all the independent tests carried out in this research. However, it was found out that as it reached the last injection, the flow rate was much slower as it took about 4 to 5 hours of injection to complete (250 – 300 ml per hour). This shows that the clogging of the pores occurred within the samples and may have inhibited the rate of the conductivity during the final injection.

The cementation solution injected into the samples was kept at a 0.75 M equimolar concentration of urea and calcium chloride. This value was obtained after having conducted the initial tests previously to identify the key parameters that were deemed to be suitable for the main experimental tests. The method of casting used the conventional method; the bacterial-sand mix was compacted directly into the mould conforming to the
standard compaction method as stated in Section 3.6.2. With the exception of samples T3 and T4 (Table 3.5), instead of mixing only bacterial culture into the sand (Section 3.4.3), the culture was pre-mixed with the solutions of urea and calcium chloride (0.75 M) with a ratio of 1:3 against the amount of the bacterial culture before it was compacted into the mould with reference to the standard compaction method.

5.2 Results of Main Experimental Tests

5.2.1 Measurement of Conductivity and Urease Activity

![Figure 5.1: The measurement of conductivity at an average for 1.4 L of bacterial culture prior to mixing with sand and then compacted into a 150 mm cube.](image)

Similar to the calculation explained in Section 4.3.1, the value of mM urea hydrolyzed per minute obtained for this main experimental test is as shown in Table 5.1. The value was averaged among all four batches of bacterial culture prepared which was found to be at least 20 to 25 mM of urea hydrolysed.min⁻¹. The increased rate of the electrical conductivity was instant the moment urea was introduced into the bacterial culture. After the first minute, the conductivity remained stable and the slope would give a conductivity change that was multiplied by the dilution factor of 10, and thereafter translated into the unit of mM urea hydrolysed.m⁻¹.
Table 5.1: Value of conductivity change and urea hydrolyzed per minute of the bacterial cultures grown in 1.4L

<table>
<thead>
<tr>
<th>Conductivity (mS.cm⁻¹.min⁻¹)</th>
<th>Conductivity (mS.cm⁻¹.min⁻¹) x dilution factor (df)</th>
<th>mM urea hydrolysed.min⁻¹</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.2167</td>
<td>2.167</td>
<td>24.075</td>
</tr>
</tbody>
</table>

5.2.2 pH of Effluents

Apart from the measurement of urease activity for the samples, the effluents from every injection performed on the samples were collected and measured to ensure that the activity of the bacteria within the sample was affirmative. The introduction of urea into the bacterial culture will increase the pH due to the release of ammonia, in which the process in precipitation of calcite begins at pH in between 8.3 and 9.0 where urease activity remains high (Stocks-Fischer et al. 1999). Therefore, the pH value of 9.0 – 10.0 was observed in the beginning of the injection series.

After few injections, it can be seen that from Figure 5.2, the pH decreased until about a pH of 6.0 – 7.0 assuming a lesser activity within the samples. Although by observing only the pH of the effluents has its limitations for evaluating activity, generally it is understood that S. pasteurii are not active at pH below 8.0 – 9.0 while at the same time reducing the urease activity to trigger the precipitation, hence the declining of pH was apparent.
5.2.3 Unconfined Compressive Strength Tests

The unconfined compressive strength tests were performed on the universal compression machine. All of the 150 mm cube samples were tested at 28 days of curing duration. The experimental tests were done periodically along with further improvements after each test. An independent test, sample T1 recorded the lowest strength, at only 52 kPa although the top surface of the sample demonstrated a notable hardness as shown in Figure 5.4 (a). However, after placing it under increasing load under a compression machine, the highest load it could sustain was only 1.16 kN. In this test, non-woven geotextiles were used on all sides of the sample inside the cube mould to prevent loss of sand particles during the injection of cementation solutions. However, it appears that the geotextiles may have unknowingly trapped the solution as it was wedged with the samples themselves and the reaction only occurred at the surface of the sample instead of the core of the samples. The surface of the sample was tested with a penetrometer and the strength was about 4.83 MPa (the maximum value of the penetrometer). It is also evident that the sand particles in the core of the samples showed similar traits (qualitatively) to those of untreated sand, loose and not completely bio-cemented. Precipitation of calcite probably occurred but it is assumed that it was insufficient in that it did not create strong bonds amongst the sand particles. It was also reasoned that once the surface was already hardened, it was difficult for the rest of the cementation solution to be further injected throughout the sample.

Figure 5.3: UCS results from all the independent test carried out for 150mm cubes.
Figure 5.4: (a) Top surface of sample T1; (b) Crushing of sample T1 under UCS machine; (c) Crushed sample T1.

After observation and analysis were performed for sample T1, sample T2 was then prepared having tweaked some of the possible reasons which could have affected the results. In sample T1, non-woven geotextiles were used but for sample T2, a normal plastic mesh with the smallest aperture available was used. However, it was doubtful that the fine sand particle would not get washed out, hence the plastic mesh was triple-layered to minimise the risk of losing fine sand particles during injection. T2 recorded a strength of only 70 kPa with the highest load sustained at 1.58 kN. In Figure 5.5, it can be observed that the outer surface of the samples was harder in comparison to the core of the samples. Once crushed, the sample was moderately brittle in the core although some fractured (outer surface) samples retained some shape.

Figure 5.5: Sample T2 prior to crushing under UCS machine and its failure mechanism.

Referring back to Table 3.5, T1 and T2 adapted the method of normal mixing wherein the bacterial culture was mixed with sand to obtain a homogeneous paste-like mix prior to placing it into the mould. However, for sample T3 and T4, this method was slightly improvised in which the bacterial culture was pre-mixed with the cementation solutions (i.e. urea and calcium chloride) then was mixed with the sand there after. This pre-mix was presumed to be affirmative due to the instant calcification that occurred and it was
believed the attachment towards the sand particles would be better. It was then left at 4 – 6 hours (stagnant condition) prior to the first injection and then onwards, the treatment technique remained the same throughout the whole process. With this improvised technique, the strength of sample T3 was able to achieve about 200 kPa with a sustained load at 4.45 kN being the highest. As demonstrated by sample T2 at failure as shown in Figure 5.5 (b) and (c), it was not able to maintain the shape as the surface crushed into smaller pieces in comparison to sample T3 as observed in Figure 5.6 (b) and (c).

Sample T1 until T3, all cured at 35°C, were able to achieve a compressive strength of up to 200 kPa with further improvements on the procedure after each test along with the utilisation of the pressurised injection system. However, the homogeneity of the whole sample was still a drawback in this research study as the core was observed to have few traits of being bio-cemented. This shall be further explained in Section 5.3.

![Figure 5.6: Sample T3 prior to crushing under UCS machine and its failure mechanism.](image)

Another independent test was conducted to investigate whether curing at a higher temperature would affect the structure of the sample as well as its strength, sample T4 was initiated. The method pre-mixed was applied to this sample and the treatment technique remained unchanged. The compressive strength measured at an impressive 688 kPa with a maximum load sustained at 15.49 kN (Figure 5.7) after it cured at 105°C. At failure, the sample showed a moderate amount of untreated sand within the sample and the rest of the fractured samples were in big pieces holding its shape. This explains that a higher temperature affects the structure and the strength of the sample, similar to what was investigated when performed on the initial experimental samples (Figure 4.9). However, the feasibility of adapting a high temperature in-situ is not recommended, hence it is suggested that this procedure may only be applicable in another industry.
5.2.4 Distribution of Calcite

Estimation of calcite content precipitated throughout the samples was investigated and conducted for each sample by an acid wash similar to the tests conducted for the initial experimental samples (Section 4.3.4). Small fractured samples were collected from the surface and the core of the sample to evaluate homogeneity and thus evaluating its effect on the strength and also the structure. It can be seen from Sample T1 in Figure 5.8, the percentage of calcite (% w/w) on the surface recorded the highest among the rest, 10.82% against its core measured at 3.09%, also the lowest among all.
This explains the structure after it was crushed using the compression machine, where the outer part of the sample showed a harder surface compared to its core (Figure 5.4). While at sample T2, with the improved procedure of the bio-cementation along with the pressurised system, development of calcite in the core of the sample increased up to 3.75% while the surface recorded 8.34%. However, the percentage difference in between the surface and the core was less than that of T1 which was 4.59% for T2, while the latter was 7.73%.

Figure 5.9: Fractured samples after UCS test; (a) Top surface; (b) Outer surface from the side; (c) Part of the core of the sample.

Figure 5.9 qualitatively shows the fractured samples after a UCS test; (b) shows more calcite (white) precipitates in comparison to the core (c) wherein the distribution of the precipitates was random. Furthermore, sample T3 shows a lesser percentage difference in between its core, which was only at 3.26%, while the surface and core measured at 7.43% and 4.17% respectively. On the contrary, sample T4 which was cured at higher temperature shows slightly better results against T3, where the surface and the core was recorded at 8.93% and 5.78% each, with a difference of only 3.16%. This shows that, even at a higher temperature, the improvement of calcite content was demonstrated. In terms of strength, there is a large difference in the improvement rate and this may be due to the structure of the calcite component that was formed within the samples and further details are explained hereinafter.

The homogeneity in terms of calcite content of the whole sample can be observed through estimation of calcite content in the core and at the surface of the sample. The surface showed a higher calcite content than the core, this led to a relatively lower strength of the calcite bonding precipitated within the sample. It can be seen that the trend of calcite content on the surface decreased while the core increased giving a lower percentage difference in between, and this demonstrated reasonably more homogeneity within the samples as observed from samples T1 – T4 irrespective of the curing temperature.
5.2.5 SEM Images

Further evaluation of the calcite formation within the samples was conducted through the use of a Scanning Electron Microscope (SEM). Selected samples, both of the surfaces and the cores of the samples T3 and T4, were viewed under Hitachi-3400N. An untreated sample was also viewed to act as a control for comparison purposes. The viewing of these samples was at 300µm showing the particle contact and also the bonding’s that existed amongst the particles.

![Figure 5.10: Images of SEM on the (a) untreated sample, (b) surface of sample T3 and (c) core of sample T3 which were cured at 35°C.](image)

It can be observed in Figure 5.10, that the calcite bonds formed at the surface of the sample were more in comparison to the core. Figure 5.10 (c) showed there were still voids existing within the sample, which explains the value of the calcite content as shown in the previous section. There were less calcite bonding and very few particle contacts within the core of the sample while the untreated sample showed none of the precipitation of calcite on the surface of the particles and also the particle contact was non-existent. Similar to sample T4 which was cured at 105°C (Figure 5.11), it showed more particle contacts and calcite bonding at the surface rather than the core itself which was much less, hence conforming to the results obtained in Section 5.2.4.

![Figure 5.11: Images of SEM on the (a) untreated sample, (b) surface of sample T4 and (c) core of sample T4 which were cured at 105°C.](image)
5.2.6 EDX Analysis

An SEM observation was conducted for the purpose of the visualisation of the calcite precipitation and particle contact that existed within the samples. However, to analyse whether elements of calcite actually formed within the sample, further tests were performed through energy dispersive X-ray analysis as explained in Section 3.6.5, to find the concentrations of calcium (Ca), carbon (C), oxygen (O) and Silica (Si) elements. This analytical technique was conducted to analyse the element as well as characterisation of a chemical within samples. Being the major component in sand, it was observed that Si and O recorded the highest concentration that makes up SiO$_2$ (quartz).

Besides that, the component Ca, which forms CaCO$_3$, was about 5.18 (% wt) while C was recorded at 8.71 (%wt) as illustrated in Figure 5.12. For the surface of sample T3 (Figure 5.13) a comparatively higher concentration of Ca was shown and a slightly lower value of C that is 6.95% and 6.17% (% wt) respectively. The element O was at its peak, recorded at 22.45% taking into account that the main element of quartz and also CaCO$_3$, hence its high intensity. While at the core of the samples as shown in Figure 5.14, the analysis of elements showed the concentration of Si at 12.44%, while the highest intensity was by the element C at 26.14%. Other than that, each of the elements Ca and O were measured at 8.49% and 18.51% respectively.

Figure 5.14 shows the SEM images and EDX analysis of those at the surface of sample T4 and each of the elements resulted in a higher concentration compared to sample T3. This explains that it was in line with the results of calcite distribution in Section 5.2.4 wherein the calcite content for T4 was more than those of T3 at both surface and core of the sample. The elements recorded at surface of sample T4 (Figure 5.15) were 7.84% (C), 47.98% (O), 18.15% (Si) and 13.01% (Ca). Even though there were other visible elements existing when the samples were analysed, it may be due to the coating used when viewed in a SEM.
Figure 5.12: SEM images at 5µm and EDX analysis at a different point for Sample T3 – Core.

Figure 5.13: SEM images at 5µm and EDX analysis at a different point for Sample T3 – Surface.

Figure 5.14: SEM images at 5µm and EDX analysis at a different point for Sample T4 – Core.

Figure 5.15: SEM images and EDX analysis at a different point for Sample T4 – Surface.
5.3 Discussion

In general, the improvement of compressive strength obtained by the samples for the 150 mm cubes demonstrated positive results although the maximum that it could sustain was only at 200 kPa of those cured at 35°C even though the condition which was deemed to be the most appropriate key parameter chosen during the initial experimental tests. Only when it was cured at 105°C did the strength increase to 688kPa. However, it is not feasible to have cured the soil at a high temperature in field application.

It was assumed that the reaction of the geochemical process during the first few injections might have clogged the pores on the top part hence the rotation was introduced to ensure the saturation of the cementation within the sample would be uniform. However, even after three separate experimental tests, the homogeneity of the whole sample was not achieved as the hardness was observed only on the surface but not so much in the core of the samples. This is further supported by Harkes et al. (2010) where it was discovered that the moment injection starts, bacterial cells attach at a rapid rate and subsequently accumulate close to the injection point, hence hindering the flow rate of the subsequent injection treatment throughout the sample.

One of the reasons to this lies in the pressurised injection system itself, which limits the contact of the injection points towards the sample in particular. Referring to the set-up of the mould in Section 3.4.3, even though there were five injection points at top and bottom, the moment the cementation solutions were injected, the instant they are in contact with the surface of the sand, the process of calcification occurs. This eventually inhibits the flow of the cementation solution into the core of the sand, hence affecting the strength and the estimated calcite content of the samples.

Generally, it is well known that S. pasteurii possesses traits wherein it is highly tolerant of harsh surrounding conditions and is able to survive in alkalophilic environments (Al Qabany et al. 2011; Stabnikov et al. 2013). However, due to its aerobic nature, its growth and hydrolysis of urea are sensitive to the concentration of O₂ in the soil, a reduction pattern of calcite formation and an increase in porosity with depth was not an unexpected factor (Achal et al. 2009). Therefore, this explains one of the reasons that the formation of calcite within the sample was affected as well as the relatively low strengths obtained in this experimental study.
Figure 5.16: Relationship between the average calcite content (% w/w) obtained on the surface and core of the samples against the compressive strength for each independent tests conducted in this study.

Figure 5.16 shows the relationship between the average calcite content (% w/w) obtained on the surface and the core of the samples against the compressive strength for each experimental test including one that was cured at 105°C. The trend shows that as the strength increased, the difference between calcite content on the surface and in the core gradually decreased although at a minimal rate. As explained in Section 5.2, after each test, the methodology was improved in terms of the experimental set-up and the treatment method. It does show an improvement in strength, which increased from 52 kPa up until 200 kPa, about 2.85 times more than the initial strength.

Also in terms of the calcite content within these samples, as the strength goes higher, the calcite content has also increased progressively at both the surface and core. The percentage difference between these two locations decreased at a higher strength, indicating that homogeneity within the samples could be achieved although it was only a minor change. Results from a SEM also showed that there were more voids observed in the core of the sample compared to those from the surface, hence this was also reasoned to be one of the effects that led to a relatively lower compressive strength. Furthermore, supported by the elemental analysis results from EDX it showed less calcite was formed, which recorded the lowest percentage at 5.18 (% wt) in the core while the highest is 13.08% (% wt) located at the surface.
CHAPTER 6 GENERAL CONCLUSION AND RECOMMENDATION

6.1 General Conclusion and Discussion

The main objective throughout this research study was to develop a detailed methodology after having optimised the key parameters at a smaller scale of laboratory works prior to the upscaling of the bio-cementation process. A general conclusion of overall experimental works can be explained in view of the objectives that have been set in the earlier stage of this research study.

The growth of the bacterial culture with different percentages of urea showed improvement in terms of the urease enzymatic activity. Although, results did not show much difference in between 2% (13.95 mM) and 4% (36.78 mM) of urea with regards to its strength and calcite content, but had pretty much affected the hydraulic conductivity of the samples. In the main experimental tests, it was expected that the urease activity would fall within the range of what had been obtained during the initial experimental tests, which was moderately high measuring about 20 mM urea hydrolysed.min⁻¹. A moderate value of urease activity is one of the key parameters to a successful bio-cementation process as the reaction would not be instant and cause immediate clogging near the injection points (Harkes et al. 2010).

The concentration of cementation solutions has rather a big impact on bio-cementation and has been reported in previous works. Therefore, the evaluation of the appropriate concentration to be opted for in this research was conducted to ensure the repeatability and its feasibility in the surrounding environment. As suggested by many, equimolar concentrations of cementation solutions were best utilised in the process of bio-cementation (Al Qabany et al. 2011; Martinez et al. 2013; Mountassir et al. 2014). Although a higher concentration of cementation solution would inhibit the geochemical process in the beginning of the reaction, Al-Thawadi (2008) suggested that this inhibitory effect is beneficial should the technique be implemented on the site. This is because when those of a higher concentration of cementation solutions are introduced, it will lower the urease activity at the injection point hence allowing the calcium and urea to move much further in depth, at the same time forming calcite precipitation and strength along the packed porous material.
In the initial experiments, the curing duration of 7 and 28 days was evaluated to see if it had affected the strength or the calcite content. Truly, inconsistencies were discovered from all the findings showing even at 7 days, a higher calcite content was measured while samples which were cured for 28 days showed a lower calcite content. Besides, the collected samples after the UCS test was random and it was assumed that the calcite content was too random due to its heterogeneity. At 7 days, the collected sample was probably from a localised point that had more calcite content in comparison to those that were chosen for 28 days and they were not of the same samples. As explained briefly in Section 4.4, the preparation of the samples was conducted independently for each sample even though all the conditions required were all kept at the exact same volume of bacteria and cementation solutions. This has become a slight drawback to the initial experimental works and this shall lead to a more detailed and further investigation should this research to be continued in the near future.

Another key parameter that was investigated in this study was the effect of temperature on the sample in terms of strength and calcite content. As stated by Weiss et al. (2014), calcite produced at a normal temperature is thermodynamically stable at an ambient temperature. There are different types of calcium carbonate crystals, with calcite being the most stable and one that has more strength, while vaterite and aragonite are those which are less stable at normal temperature, relatively lower strength and they are produced typically in abundance and much more stable at a higher temperature (Mejri et al. 2014; Thriveni et al. 2013). Further explained by Al-Thawadi (2008), the amount of calcite content that exists within the sand matrix does not correlate to the strength directly but rather it is highly dependent on the strength of the calcite crystals. Even with a high amount of calcite content, it is possible to have samples with lower strength and vice versa, which justifies further why the structure of the calcite crystal is important in view of the strength of the whole sample.

The mixing method that was applied throughout the main experimental tests was through the pre-mix of the bacterial culture with the cementation solution and it affected the strength obtained as explained in Section 5.2.3. Whilst minor changes were observed, this can still be improved in time. Further, with this method it is evident that the instant calcification that occurred will help in the attachment of this precipitation, which will create bonding between those particles.
Last but not least, the performance of the pressurised injection system was also evaluated during the course of the main experimental works conducted in this research. It was found out that the one-directional flow was not able to sustain the reaction that happened within the sample due to the reaction that occurred near the injection point, which inhibited the flow of the subsequent injection. Hence, it was improved through a rotational injection method wherein the first three injections were flushed from the top while the other three injections were flushed from the bottom after the mould was rotated 180°.

However, the results from the core of the sample were still unsatisfactory due to the injection points being in contact only on the surface instead of having it penetrate to the core of the samples to give a more uniform distribution of the cementation solutions throughout the whole sample. Therefore, it resulted in the strength that is relatively low at only about 200 kPa at most. Despite the results obtained from the initial experimental works that have reached approximately about 600 kPa, this shows that depth has also played an important role in successful bio-cementation process.

Nonetheless, 200 kPa of strength is still sufficient if it were to be implemented on site but on a rather smaller scale of application that involves surface strength treatment or prevention of erosion along the riverbanks by producing man-made sand dunes. Likewise, the potential of this methodology to be adopted in future endeavours shall be looked upon closely with further works of investigation and evaluation of key parameters for it to be practical to be used on site.
6.2 Limitations to the Technology

Due to some limitations that have been raised in this research, the methodology of using the pressurised injection system still needs to be investigated in depth. Besides that, the treatment/injection method applied throughout the whole research work may have adapted only a one-dimensional flow pattern in a 3D scale and conditions may behave differently. Therefore, research on other key parameters with respect to a three-dimensional flow should be further evaluated before the technique can be realised. Besides, one of the major drawbacks of this technology would be the production of ammonia, which is not so much of a ‘green technology’ throughout the process of bio-cementation as it has detrimental effects to the surrounding environment including humans. However, this would be one of the key factors that should be addressed sooner rather than later so that it will not be an issue in the future.

6.3 Recommendations for Future Research Works

From the findings obtained from this research study, there are some portions of the experimental works that are lacking and below would be some recommendations which can be taken into account should there be any future endeavours ensuing from this research study.

i) Being in one of the most diverse parts of this region, it is possible that there would be many urease-producing microorganisms that can be found on the island of Borneo considering the tropical climate, which is in favour of the bio-cementation process as well as the nature of the microorganism itself.

ii) An improved pressurised injection system should be further evaluated with more key parameters, which should be considered for further experimental works such as the introduction of the push-pull injection system which might improve the homogeneity of the whole sample.

iii) A cheaper nutrient is best when the technique needs to be enlarged or to be done in-situ; a cost-effective nutrient for the bacteria would be beneficial.

iv) A study using industrial grade materials would be advantageous for the bio-cementation process to be implemented on site, because if the industrial grade chemical is able to perform almost as good as the analytical grade, it is a plus for a cost effective ground improvement method.
CHAPTER 7 REFERENCES


Al-Thawadi, S 2008, *High strength in-situ biocementation of soil by calcite precipitating locally isolated ureolytic bacteria*, PhD Dissertations thesis, School of Biological Sciences and Biotechnology, Murdoch University, Western Australia.


Burbank, MB, Weaver, TJ, Green, TL, Williams, BC & Crawford, RL 2011, 'Precipitation of Calcite by Indigenous Microorganisms to Strengthen Liquefiable Soils', *Geomicrobiology Journal*, vol. 28, no. 4, 2011/05/01, pp. 301-312.


Christensen, WB 1946, 'Urea Decomposition as a Means of Differentiating Proteus and Paracolon Cultures from Each Other and from Salmonella and Shigella Types', *Journal of Bacteriology*, vol. 52, no. 4, pp. 461-466.

Chu, J, Ivanov, V, He, J & Stabnikov, V 2012, 'Use of microbial technology in geotechnical engineering,' *Second International Conference on Geotechnique, Construction Materials and Environment*,


95


Hazen, A 1892, in Annual ReportMassachusetts State Board of Health, Massachusetts, USA, pp. 539-556.


Rebata-Landa, V & Santamarina, JC 2006, 'Mechanical limits to microbial activity in deep sediments', *Geochemistry, Geophysics, Geosystems*, vol. 7, no. 11, pp. n/a-n/a.


Tsukamoto, M, Inagaki, Y, Sasaki, T & Oda, K 2013, 'Influence of relative density on microbial carbonate precipitation and mechanical properties of sand,'


APPENDIX A

SOIL CLASSIFICATION TEST REPORT
TEST REPORT

ISSUED BY: KUCHING OFFICE

DATE: 14/1/2016

REPORT NO.: GT/16/K/L1779

PAGE NO.: 1 OF 3

1 Customer : Phua Ye Li
(Swinburne University of Technology Sarawak Campus)
Jalan Simpang Tiga,
93350 Kuching, Sarawak.
Tel: 082-416353 Fax: 082-423594

2 Project : TESTING OF SAND SAMPLE

3 Date of Testing : 7/1/2016 to 14/1/2016

4 Sample Description : Sand sample

5 Test Requested & Method of Testing
   : 1) Maximum & Minimum Density of Sands as per BS 1377 : Part 2 : 1990 :
     4.2 & 4.4
   2) Particle Density (Specific Gravity) as per MS 1056 : Part 2 : 2005 : 9.3
       (Small Pyknometer Method)

6 Category of Testing : Laboratory Testing

7 Results : Please refer to following pages

8 Remarks :
   a) This above test is based solely on sample submitted by customer.
   b) No copy of this report is valid without original blue stamp.
   c) (*) Not SAMM Accredited.

Approved Signatory:

[Signature]
Civil Engineer
B. Eng. (Hons) Civil

The accuracy of test measurement are probability at 95% confidence level.

Copyright of this report is owned by the issuing laboratory and may not be reproduced other than in full except with the prior written
Head of the issuing laboratory.
MAXIMUM & MINIMUM DENSITY OF Sands

LAB REF.: GT/16/K/L1779  REPORT NO.: GT/16/K/L1779
CUSTOMER: Phua Ye Li  PAGE NO.: 2 OF 3
PROJECT: TESTING OF SAND SAMPLE
SAMPLE DESCRIPTION: Sand sample
DATE RECEIVED: 6/1/2016
DATE TESTED: 7-14/1/2016
TESTED BY: Halid & Bahsir
VERIFIED BY: Lucy

TEST METHOD: BS 1377: Part 2: 1990: 4.2 & 4.4

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<td>Volume of mould, V (cm³)</td>
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<td>Maximum dry density, ( \rho_{dmax} = \frac{m}{V} ) (Mg/m³)</td>
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<tr>
<td><strong>4.4 Minimum Density of Sands</strong></td>
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<td>Volume of sand in 1L glass cylinder, V (cm³)</td>
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<td>Minimum dry density, ( \rho_{dmin} = \frac{1000}{V} ) (Mg/m³)</td>
<td>1.27</td>
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NOTE: 

Approved Signature:

Clare Frimoe
Civil Engineer
B. Eng. (Hons) Civil

GT-RP-L078/0/15
**PARTICLE DENSITY (SPECIFIC GRAVITY)**

LAB REF.: GT/16/K/L1779  
CUSTOMER: Phua Ye Li  
PROJECT: TESTING OF SAND SAMPLE  
SAMPLE DESCRIPTION: Sand sample  
DATE RECEIVED: 6/1/2016  
DATE TESTED: 13-14/1/2016  
TESTED BY: Kelvin  
VERIFIED BY: Lucy

Test Environmental Condition:-  
Temperature: 25.1°C (Min.) 28.5°C (Max.)  
Relative Humidity (RH): 47% (Min.) 63% (Max.)

**TEST METHOD:** MS 1056 : Part 2 : 2005 : 9.3 (Small Pyknometer Method)

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Remarks:

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Clare Yvonne  
Civil Engineer  
B. Eng. (Hons) Civil

GT-RP-L003/0/12
APPENDIX B

TEST REPORT FOR MAIN EXPERIMENTAL RESULTS
LOAD (kN) VS TIME (s) GRAPH

Sample T1

Sample T2
Sample T3

Sample T4
APPENDIX C
SEM/EDX RESULTS
## SAMPLE 0.752E

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**Bruker**

**UMS** (Universiti Malaysia Sarawak)
LIST OF PUBLICATIONS

Conference Papers

