STRENGTH AND STIFFNESS
IMPROVEMENT OF BIO-CEMENTED
SYDNEY SAND

By

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ABSTRACT

This thesis explores the performance of small scale cemented soil columns produced using soil mixing with cement resulting from bacterially mediated reactions that precipitate calcium carbonate, a process often referred to as bio-cementation. Bio-cementation has received considerable research attention over the last decade as it has the potential to complement existing ground improvement techniques and mitigate environmental concerns with currently used materials. Previous research has concentrated on pumping and injection techniques because of concerns that bacteria will be unable to survive the stresses associated with industrial mixing processes, however it has been difficult to create uniform bio-cemented soil masses. In this thesis the ureolytic bacterium, *Bacillus Megaterium*, not previously reported in bio-cementation studies, has been investigated to determine its viability and efficiency as a microbe for mediating the calcite precipitation. It has been found that the highest hydrolysis rate is recorded when calcium concentrations are double the urea concentrations, and that significant amounts of calcite can be precipitated in a single mixing process. Unconfined compressive strength (UCS) tests and a series of triaxial tests have been conducted to quantify the effects of the bio-cementation on the mechanical response. Bender elements mounted in the triaxial cell have also been used to monitor the shear wave velocity during curing and shearing. The results of mechanical tests on the bio-cemented sand have been compared with tests on gypsum cemented and uncemented specimens. It has been found that bio-cementation by mixing produces homogeneous specimens with similar strengths and stiffnesses to the commonly used flushing or injection technique. To assess the performance of in-situ mixed, 38 mm diameter, bio-cemented sand columns a small scale in-situ mixing technique was used to create the model columns. Foundation tests have been performed at 1-g in a cylindrical tank with diameter of 600 mm. A significant improvement was observed in the response of foundations when placed on bio-cemented columns, and this was similar to the improvement from more conventional gypsum cements. These tests confirmed the feasibility of using an in-situ mixing technique with bio-cementation and provided valuable insight into the factors that must be considered in developing field applications.
This thesis also has demonstrated repair strategies and techniques to encourage healing and self-healing should damage occur in foundations. Results from tests performed to investigate the ability of biocement to repair cemented soil columns are reported.
The need for continual research in biocementation is essential, especially when there is an abundant scope of areas that still need detail investigation. For example, the effectiveness of biocementation as a viable ground improvement technique and the potential environmental impacts are some of them. Even though the technical feasibility of biocementation for a wide range of potential applications has not been fully discovered, there are some on-going research at both the laboratory scale and the field scale. However, the heterogeneity and unpredictability of the engineering parameters, along with the costs of substrates, the cultivation of specific bacteria, and the removal of the by-products have so far limited this process and its competitiveness in comparison to more traditional alternatives.

Previous researchers involved in this area have highlighted some of the key problems with biocementation. For example, the injection strategy developed to homogeneously precipitate calcite, caused what is known as the injection point plugging. Therefore, extending calcite precipitation homogenously may be advantageous to reduce permeability and increase shear strength, while avoiding clogging in the vicinity of the injection point. However, for field applications, it is important for us to address all the limitations highlighted by the previous researchers beforehand. If injected, organisms must be able to penetrate to the targeted pore spaces of target formations, and microbial transport can be restricted by permeability, pore throat size, and other complex factors including the cell cohesion or electrostatic interactions. Should the injection techniques solve the above-mentioned problems; there are still several other challenges that may exist. For example, temperature and the pH of soil could be two factors that will affect the performance of bio-cementation techniques on site. Thus, this thesis focuses on a promising effective bio-cementation technique; the mixing technique that is currently being investigated as an alternative to the injection technique.

This research will only address a limited scope, primarily most important issues highlighted by previous researchers - homogenizing calcite precipitation. The focus here has been to provide a comprehensive study of the problems under discussion, including issues that have so far been afforded little attention, such as alternative to common ureolytic bacteria and the potential of a
self-healing mechanism using biocementation. The current research work demonstrates that the production of biocement by urease producing bacteria (UPB) using an in-situ mixing technique could enhance the strength, strain and the stiffness of sand soil in comparison to gypsum cemented sand, thus providing a more sustainable alternative that could replace industrial binders.
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CHAPTER 1

INTRODUCTION

1.1 Motivation

The production of conventional cement, an essential construction material, results in the release of significant amounts of CO₂, a greenhouse gas (GHG). More specifically, the production of one ton of Portland cement produces about one ton of CO₂ and other GHGs. According to the Intergovernmental Panel on Climate Change (IPCC) report, cement production currently contributes approximately 5 % to global CO₂ emission (IPCC, 2013). If greenhouse gas levels continue to increase, climate models predict that the average temperature at the Earth’s surface could increase from 3.2 to 7.2 °F by the end of this century (USEPA, 2009). Scientists at NASA are certain that human activities are the main reason for the changing composition of the
atmosphere and that increasing the concentration of greenhouse gases will change the planet’s climate. It is therefore clear that the construction industry could play an important role in improving the environment by following “green construction” practices. The latest report by IPCC in 2013 suggested that every possible way to reduce GHG emissions that is associated with construction technologies should be identified and evaluated soon. Timely development and implementation of “green construction” practices will not only reduce CO₂ emissions but also the consumption of global energy. According to Fragaszy et al. (2011), solutions to this challenge will require a multidisciplinary research approach across chemical and biological sciences, as well as engineering. Geotechnical engineering expertise is critical to solving many energy and sustainability-related problems. Hence, geotechnical engineers and academics have the opportunity and responsibility to contribute to the solution of these critical, worldwide problems.

1.2 Background of Study

It has been noted in many regions around the world that the mechanical properties of soils are often/occasionally insufficient for the desired land use, especially when roads and railways undergo settlement and require continuous maintenance. Apart from that, overpopulation in urban areas and an increasing shortage of ideal (suitable) building sites contributes to an increased need for the development of ground improvement techniques within the geotechnical engineering community (DeJong et al. 2010). A wide range of ground improvement techniques are available and the method used will depend on the specific needs of a given project at a given site. The techniques can be classified into one of the following general categories: increasing the shear strength of the soil, to guard against catastrophic failure (e.g. liquefaction); reducing the compressibility of the soil, to prevent excessive ground movements; or reducing the permeability of the soil, to reduce the rate of water seepage (commonly for earth dams or environmental applications).

Earthquakes can cause liquefaction in loose sediments and consequently damage structures founded in or above them. Therefore it is desirable for projects located in earthquake-prone areas to use ground improvement techniques that reduce the hazards associated with an earthquake,
particularly those associated with liquefaction, which can cause catastrophic ground failures (Sadek and Saleh, 2007). Liquefaction is a geotechnical phenomenon that primarily occurs in saturated non-cohesive soils, such as sands and silts that are subjected to cyclic loading during an earthquake. The occurrence of liquefaction is characterized by an increase in pore water pressure, which is caused by the cyclic undrained loading, and leads to a decrease in the effective confining pressure. This, in turn, causes a significant loss of shear strength in the soil (Worthen, 2009). Figure 1.1 shows the potential effects of liquefaction according to Knudsen et al. (2000). This research will explore the potential of microbial induced calcite precipitation (MICP) as a ground improvement technique to solve liquefaction and other geotechnical engineering problems. Recently, research was conducted to mitigate sand liquefaction using a bio-sealing technique. This technique uses biogas which is produced by a type of ureolytic bacteria. The bacterium was cultivated in-situ to activate the denitrification process, which produces gas and reduces the degree of saturation and hence mitigates the liquefaction risk. This was claimed to be more economical than conventional mitigation strategies (Li, 2014).

![Diagram of potential effects of liquefaction](image)

Figure 1.1: Potential effects of liquefaction (Knudsen et al. 2000)

Microbiologically induced calcium carbonate precipitation (MICP) is a bio-geochemical process that induces calcium carbonate precipitation within the soil matrix (Mortensen et al. 2011). Biomineralization in the form of calcium carbonate precipitation can be traced back to the Precambrian period (Ercole et al. 2007). Calcium carbonate can be precipitated in three polymorphic forms, which in the order of their usual stabilities are calcite, aragonite and vaterite.
The mechanism of calcite precipitation and the contributing factors along with importance of the three forms of calcite are explained in details in section 2.4 of Chapter 2. The main groups of microorganisms that can induce the carbonate precipitation are photosynthetic microorganisms such as cyanobacteria and microalgae; sulfate-reducing bacteria; and some species of microorganisms involved in nitrogen cycle (Ariyanti et al. 2011). Several mechanisms have been identified by which bacteria can induce the calcium carbonate precipitation, including urea hydrolysis, denitrification, sulphate production, and iron reduction. Details on these mechanisms can be found in Chapter 2. Because the penetration of microbial cells into soil is limited when the soil pore size is less than 0.5 to 2 µm, biomineralisation is only suggested for limited soil types with sufficiently high hydraulic conductivity to allow the access of bacteria and nutrients. Although technologies used for chemical grouting/cementation could be adapted for microbial grout, the penetration of the grout also depends on the size of microorganisms used, and this can be an obstacle to the penetration of filamentous cells (bacteria) into soil. Some research papers (Whiffin et al. 2007; Dejong et al. 2006; Ivanov and Chu 2008) have shown that the injection of bacteria can result in clogging near the injection points due to the rapid production of calcite. Other papers (Harkes et al. 2010; van Paassen et al. 2010) have shown that there can be an uneven distribution of calcite throughout the cementation material, even when clogging does not occur. Whiffin et al. (2007) introduced a bacterial placement method to overcome the latter mentioned problems in 5 m long sand column experiments, while others have injected bacteria and the nutrients sequentially as an alternative solution (Van Paassen et al. 2008). To date there have been no full scale successful applications of biogrouting for ground improvement although there have been some pilot (small scale) studies (Van Paassen et al. 2009; Montoya et al. 2013) that have shown the potential of biocementation to successfully improve the ground.

Apart from compatibility issues between the sizes of microbes and pores, researchers have also questioned whether it is more effective to use indigenous (native) or exogenous soil microbes. Natural environments, such as soil, contain numerous microbial species that exist in a complex ecological framework. The injection of exogenous bacteria might disrupt the ecological equilibrium that exists prior to injection (Whiffin et al. 2007), which could result in a competitive disadvantage for the injected bacteria, compared to microbes already adapted to the local environment, causing a rapid decline in their numbers (Van Veen et al. 1997). On the other
hand, to activate the locally available microbes requires advanced techniques in diagnostic microbiology and robust knowledge of bacterial morphology in order to supply the right concentration of nutrients and prepare an environment that is conducive in terms of pH and temperature. Furthermore, the size of the bacterial population varies by location and depth, making the process even more difficult to control when it comes to field applications. That being said, it is still worth trying to utilize the indigenous microbes because if they can be used it is likely to be more cost effective and environmentally friendly.

According to Ivanov and Chu (2008), disadvantage of soil bio-cementation in comparison to chemical grouting is that the microbial process is usually slower. The entire bio-grouting process typically takes anywhere between one to two days to complete. Another disadvantage is that the microbial process is more complex and involves many factors, including temperature, pH, concentration of nutrients, and the concentrations of donor and acceptor electrons influence the overall effectiveness of this process. The design criteria for this technique needs to not only consider the soil conditions, but also microbiological, ecological, and engineering aspects of the process. Due to the complexity of this technique, it is yet to be proven successful in a large-scale application (Ivanov and Chu, 2008).

Figure 1.2 shows an overview of the factors influencing the geometric compatibility between the microbes (either indigenous or exogenous species) and the soil into which they are injected. The relatively small size of bacteria, typically between 0.5 and 3 µm (Madigan and Martinko, 2003) is advantageous and, as a result of their size, should be able to travel through many soil types. Unfortunately, the primary restriction on microbial transport is the size of pore throats within the soil matrix through which the microbes must pass as they move from one pore space to another (Mitchell and Santamarina, 2005). The sizes of the pore throats are dependent on the smaller fraction of particles in the soil and have been estimated to be about 20 % of the soil particle size, which corresponds approximately to the size that 10 % passes in a mechanical sieve analysis (Holtz and Kovacs, 1981). This provides an approximate lower bound limit, dependent on the particle size relative to the microbe size, for which treatment by in-situ injection is feasible. Ex-situ mixing of microbes and nutrients with soil can widen the range of soils amenable to treatment to include pure clays as indicated in Figure 1.2.
Most of the previous research conducted on liquefaction mitigation has emphasized the feasibility of precipitating calcite that glues soil particles together rather than considering how best the development of strength in the porous media can provide the required ground improvement. For example, Kucharski et al. (2012) have conducted extensive research to produce high strength cementation in a permeable material using a process known as calcite in-situ precipitation system (CIPS) and have recorded compressive strengths up to 5 MPa. This technique involves injecting chemical solutions that cause the precipitation of calcite within the pore fluid between the sand grains. Alternatively, DeJong et al. (2006) have suggested calcite precipitation mediated by bacteria offers a more sustainable alternative and avoids the need for toxic chemicals that are often injected into soils to improve the ground. The development of strength and stiffness of bio-mediated ground improvement has been assessed by measuring the seismic shear wave velocity using bender elements (DeJong et al. 2013; Al Qabany and Soga 2013) in laboratory and small scale model tests. However, there is a substantial lack of published research related to microbial cementation’s strength contribution in loose, porous material.

According to Mitchell (1995), Kramer and Mitchell (2006) and Douglas et al. (2012) the existing technologies for stabilizing soil in order to mitigate liquefaction are limited to undeveloped sites. While there exist researchers recommending the potential use of bio-induced soil modification to

Figure 1.2: Typical size of soil particles and bacteria with approximate limits of various treatment methods (extended from Mitchell and Santamarina, 2005)
mitigate seismic-induced liquefaction (DeJong et al. 2013), other researchers have expressed doubts about its efficacy. For example, Ivanov and Chu (2008) believe bio cementation is not suitable for large-scale operations, such as enhancing the liquefaction resistance of land. The question of how bio cementation can most effectively be used in ground improvement and the feasibility of this technique for use in preventing liquefaction due to earthquakes has provided the motivation for this thesis.

MICP has been used and investigated for other engineering applications including the repair of concrete structures. According to Ramachandran et al (2001), the concept of using conventional cement in remedial work, including foundation repair is orthodox. Generally, without immediate and proper treatments, cracks in concrete structures tend to expand and eventually require costly repair. Currently, there are a large number of synthetic filler agents that are extensively used in concrete crack repair. Because cracks in concrete structures expand over time, these fillers are applied repeatedly as needed. The use of bacteria to prevent the deterioration of concrete structures is a relatively new approach based on observations in nature that microbial activity constantly deposits minerals (Gollapudi et al. 1995). Since then research continues into details required for practical application. For example Joseph et al. (2010) introduced a self-healing technique that activates the healing agents in concrete via embedded vascular system. Prior to this a group of researchers in the Netherlands have used bacteria and a healing agent that reacts with water to form calcite to autonomously fix cracks in concrete (Jonkers and Schlangen, 2007; Jonkers et al. 2010). With this development concrete structures can not only be healed, but also designed with a self-healing ability and this should reduce maintenance costs in the future (Harbottle et al. 2014; Al Tabbaa and Harbottle, 2015). The development of bio-cements for concrete repair could provide the basis for an alternative and high quality self-healing foundation that is cost effective and environmentally safe. Apart from a centrifuge model study by Montoya (2012) looking at repairing the ground post-earthquake, there appears to have been no previous studies directed towards assessing the potential for the repair and self-healing of foundation elements.
1.3 Scope of Research

The primary objective of this thesis is to investigate the viability of biocementation to remediate loose granular soils that have the potential for liquefaction. As discussed above bio-cementation is a relatively new, cross-disciplinary method of ground improvement that is the focus of considerable current research. Bioclogging, biosealing, and biocementation are some of the names given to applications of biocementation for controlling the porosity, hydraulic conductivity, and shear strength, respectively. Biocementation refers to a process in which particulate materials are generated in the pores of granular materials through microbial activities. As well as filling the pores, the precipitated material can bind the soil particles together so that the shear strength of the soil increases. Due to the complexity of the method, the application of biocementation requires knowledge of microbiology, ecology, geochemistry, and geotechnical engineering to understand the process and assess its viability for effective ground improvement.

Deep soil mixing (DSM), also known as wet soil mixing, is a ground improvement technique that improves the characteristics of weak soil by mechanically mixing them with cementitious binder. It is one of the ground improvement techniques used in practice to mitigate liquefaction hazard. According to Keller Holdings, besides vibro-compaction and vibro-replacement, deep soil mixing method is proven to be effective in reducing liquefaction and is applicable in all soil types (Porbaha et al. 1999). The effectiveness of deep soil mixing method in respect to mitigating or reducing liquefaction was confirmed during the earthquake in Kobe, Japan in 1995 (Topolnicki, 2004).

The research conducted and presented in this thesis has been planned from a geotechnical engineering perspective to explore the ability to create in-situ mixed cemented columns to reinforce loose sand. Since cemented soil columns are an acceptable solution for ground improvement of liquefiable soil but the possibility of biocement has not previously been investigated. A significant effort has also made to understand the biogeochemical process involved to facilitate the practical treatment approach. The project involves three phases of study as set out in Figure 1.3. A fundamental scientific approach was taken to first understand the factors that affect the biochemical process in order to increase the ureolytic rate (Phase 1). The potential of the chosen ureolytic bacteria was assessed by gauging the maximum urea hydrolysis
rate achieved. In order to assess the biocementation in sand, validation of the improvement on engineering properties, such as the strength and the stiffness, is essential. The improvement to the soil properties and the response to loading by varying the amounts of bacteria and nutrients were evaluated using standard drained triaxial compression tests with bender elements being used to monitor the small strain stiffness (Phase 2). Finally, the ability to create a bio-cemented column using the in-situ mixing technique was demonstrated using 1-g physical model tests (Phase 3).

Figure 1.3: Outline of scope of research

1.4 Aims and Specific Objectives

As discussed above liquefaction resulting from earthquakes has resulted in significant failures to man-made structures and occurs predominantly in loose granular soils that are below the ground water table. It has been suggested (Delft Research Institute) that bio-cementation can be used to reduce soil susceptibility to earthquake-induced liquefaction (Van Paassen, 2009). It has an advantage over existing ground improvement techniques (stone columns, vibro-floatation, and
dynamic compaction) by being applicable to already developed sites. Biocementation is also an attractive technique because it has relatively low costs and minimal adverse environmental consequences.

This study is concerned with in-situ mixing, an approach that has received relatively little study. Although bio-clogging is widely used in the oil industry, the applicability of biocementation for large-scale ground improvement has not been proven, leading some to suggest that it is not feasible (Ivanov et al. 2008). Recent research (DeJong et al. 2006) has been focused on the selection of bacteria and conditions to achieve different degrees of bio-cementing in small-scale column experiments. These generally rely on a flow of fluids (in-situ) containing the necessary bacteria and nutrients to produce the cementing effect.

Specific objectives of the study have included:

1. An evaluation of the potential of the chosen ureolytic bacteria and nutrients, and determination of the conditions that produce maximum urease activity and of the mixture that produces the optimum biocementing effect.
2. Determination of the strength and stiffness of bio-cemented Sydney sand using UCS and triaxial tests. An investigation of the effectiveness of the biocementation and a comparison with other cementing agents.
3. To investigate the performance of in-situ mixed biocemented soil columns in small-scale 1-g physical model tests. The development of techniques to prepare and measure the performance of the biocemented sand columns.
4. To investigate the potential of biocement to produce healing and self-healing effect when applied to the repair of cemented column foundations.

### 1.5 Thesis Structure

This thesis consists of seven further chapters:

Chapter 2 provides a review of the work contributing to biocementation from many aspects. Generally, this chapter can be divided into three main parts. The first part of the chapter primarily deals with the different techniques used to apply biocementation, the different
mechanisms and pathways involved in the biocementation process, and the advantages of the process. A fundamental understanding of microbiological and geochemical principles in biocementation is essential to produce the desired engineering properties in soil. This is followed by some discussion of the various applications of biocementation and its potential for future extended application in the engineering field. The third part focuses on the various attempts and approaches used by previous researchers to increase the efficiency of biocementation. The drawbacks and the challenges of each technique are discussed in detail. Finally, a brief summary is included.

Chapter 3 covers the methodology used in designing and commissioning experiments. This chapter is divided into three parts. Each part represents the work carried out to achieve the various research objectives outlined above. The first part deals with the procedures involved in producing calibration charts, setting up batch analysis, and measuring kinetics parameters for urea hydrolysis. The sample preparation and test procedures of standard drained triaxial and unconfined compressive strength (UCS) testing are described in detail in the second part. This includes the use of bender elements to measure shear wave velocity. This chapter ends with detailed description of the apparatus and procedures used in the small-scale model footing tests, and include details of the methods used to create the bio-cemented columns.

Chapter 4 starts with the methods used to quantify bacterial mass, concentration of ammonium, and urea hydrolyzed. The major part of this chapter discusses the results of the batch analyses and the Michaelis Menten model parameters for urea hydrolysis in biocementation. The main objective of this chapter is to highlight the performance of the selected ureolytic bacteria (B. megaterium) in comparison to other commonly used bacterium. The performance of this bacterium is gauged by three indicators; growth rate, urease activity, and urea hydrolysis (ureolysis) rate.

Chapter 5 aims to understand the fundamental geomechanical behavior of the bio-cemented Sydney sand under unconfined compressive strength (UCS) and triaxial tests. Comparison tests are performed on uncemented sand samples and samples cemented with gypsum. Tests were conducted on samples with different amounts of nutrients and gypsum powder. The chapter also compares results from the proposed approach (in-situ technique) with results from other researchers. It also presents the results from bender element tests carried out on uncemented
Sydney sand, gypsum cemented Sydney sand and bio-cemented Sydney sand for a range of effective confining pressure and degrees of cementation. The results from biocemented samples are compared with other artificially cemented soils and are reviewed based on their strength and stiffness improvement.

Chapter 6 discusses the results of a series of 1-g model footing tests. The first part of this chapter is dedicated to the model test results, as the model test offers great advantages in simulating complex systems under control conditions, while simultaneously providing insight into the fundamental mechanism of operating biocementation at a larger scale. The second part of this chapter explores and discusses the results from tests conducted on repaired cemented columns.

Chapter 7 describes an overview of the findings from the results and discussion section, along with the conclusion of the study, followed by development strategies and suggestions for the future research.
CHAPTER 2

LITERATURE REVIEW

2.1 Introduction

Biocementation is a biological process that produces cement or a binding material. Recent research into biocementation has been based on the mechanism of Microbially Induced Carbonate Precipitation (MICP), which is one of a variety of biological processes that can produce cementing agents. The biocementation process can be applied in many fields, including petroleum and construction, as well as environmental protection and erosion control. In the construction field, biocementation has potential applications in soil strengthening and stabilizing, wall and building coating methods, and sand stabilization in earthquake areas. Thus, biocementation can be considered as a ground improvement technology. The bio-cementation technique has also been suggested for strengthening the coastline from progressive erosion, and for stabilizing slopes by creating a cemented zone within weak and vulnerable regions to reduce
the impact of landslides. In the following sections of this chapter the various ground improvement techniques are briefly discussed, and the previous biocementation research reviewed.

Ground improvement is used for numerous environmental and civil engineering applications such as; stabilizing or reinforcing soil to facilitate underground construction stability; improving stability for retaining embankments, walls, and dams; increasing the bearing capacity of non-piled or piled foundations; minimizing the soil liquefaction potential; strengthening tailings dams in order prevent slope and erosion failures; binding of dust particles on exposed surfaces to minimize dust levels; treating pavement surfaces; constructing permeable reactive barriers in environmental and mining engineering, increasing the resistance to degradation of petroleum-boreholes during drilling; stabilizing pollutants in soil through binding; increasing the resistance to erosion of offshore pipelines and sediments beneath offshore foundations; creating water filters and borehole filters; and controlling erosion along rivers and coastal areas. MICP could replace the traditional cements which are used in many of these ground improvement applications.

2.2 Ground Improvement

Kamon and Bergado (1991) classified commonly used ground improvement methods into four categories, as shown in Figure 2.1. While compaction and dewatering only involve work on soil, chemical admixtures and reinforcement techniques require the use of additional materials as inputs into the process. These soil improvement techniques utilize either mechanical energy or man-made materials. A common approach is to inject cementing agents, such as ordinary Portland cement, fly ash, gypsum, epoxy, acrylmide, phenoplasts, silicates, and polyurethane into the pore space to bind soil particles together (Karol, 2003). This is achieved by using a variety of chemical, cement, jetting, and compaction grouting techniques. Except for sodium silicate, all commonly used chemical grouts including cement are toxic and hazardous. The associated toxicity and potential environmental risks of many cementing agents have encouraged the development of alternative soil improvement methods that are more environmentally friendly and more sustainable.
Furthermore, traditional ground improvement methods have several limitations. High pressures are often required to inject the grouts due to their high viscosity or fast hardening time, whereas, other techniques, such as soil freezing only provide a temporary solution during construction. Most of these methods are expensive, and require heavy machinery, in addition to disturbing urban infrastructure and requiring chemicals that have a significant environmental impact. Finally, most of the traditional methods significantly reduce the permeability of the strengthened soil, which hinders groundwater flow and leads to multiple injection points being needed to produce large scale ground improvement. Consequently, these conventional methods are not suitable for treating large volumes of soil and there is also the issue of the CO₂ generation associated with their production as discussed in Chapter 1.

Therefore, there is ample reason for the development of new alternative soil improvement methods. Over the last 20 years, the potential of microbially induced calcite precipitation (MICP), or simply biocementation has been widely recognized in petroleum, geological, and civil engineering (DeJong et al. 2006; Ivanov and Chu 2008). Recently, a number of studies have also been undertaken to investigate the improvement to the mechanical properties of sandy soils using microbially induced biochemical reactions (biocementation) in the subsurface (Whiffin et al. 2007), which according to Van Paassen (2009) simulates the natural geochemical processes that transform loose sand into sandstone.

Deep soil mixing (DSM) is an in-situ soil treatment technique in which the original soil or fill is mixed with cementitious materials (cementing agents), typically referred to as binders. There are
two types of DSM, wet mixing and dry mixing. Wet mixing involves injecting binders in slurry form (wet) to blend with the soil. Dry mixing uses binders in the form of powder (dry) to react with water which is already present in the soil. The use of DSM for liquefaction mitigation is recorded and has been proven to perform well in Japan. Liquefaction mitigation technique which is mentioned in detail in Chapter 1 is reported to be economical. According to Li (2004), in the case of large projects and the engineering properties of the treated soil can be designed to achieve UCS strengths of up to about 4 MPa. Details of the numbers and strengths of the cemented soil columns required for effective liquefaction mitigation using DSM are presented by Matsuo et al. (1996) and Siddharthan and Suthahar (2005).

The properties of DSM-treated columns are influenced by the type and proportion of the cementing agent and the soil properties. The mixing technique and mixing energy also have a strong influence on the strength and stiffness of the DSM columns. The greater the ability to break-down the soil mass into smaller aggregates and the more uniform the mix that can be achieved (either by higher mixing energy and/or better mixing tools), then the greater the resulting strength and stiffness of the DSM are likely to be. This is one of the reasons why the dry mix method is able to achieve high strength in Scandinavian quick clay, which liquefies when disturbed.

Table 2.1 presents a brief comparison of different DSM techniques that are currently available in the Australia and New Zealand region (Bruce, 2000). It can be seen from Table 2.1 that a large range of DSM column strengths can be achieved depending on the method of installation and the proportion of cementing agent adopted. Established design methods (SGI, 1986; Standard, 2005) for DSM are currently limited to relatively compressible and low to moderate strength columns due to concerns regarding brittle failure, and tensile or flexural failure of relatively rigid columns. To resist liquefaction more rigid and higher strength columns are desirable provided the type, size, and spacing of the DSM columns are chosen such that lateral ground movements are kept within limits compatible with the stiffness and strength of the columns used. For example, quadruple augers that form “square” columns with sides of up to about 1.35 m, or Cutter Soil Mixing (CSM) that forms 2.4 m x 0.55 m rectangular columns, have greater lateral resistance and flexural capacity than single columns, which are generally limited to about 0.8 m
diameter. Detailed drained soil-structure analyses have been applied successfully in recent projects in New Zealand (Finlan et al. 2004) and Queensland (Wagstaff, 2006) using high strength wet mix techniques to mitigate liquefaction. All the information in Table 2.1 is considered as important and relevant to this study. It serves as a guide line to choose the right mixing procedure opt for chapter 6. Also relevant was the strength data given can be used to compare with results from footing model test.

Table 2.1 – Brief comparison of various DSM techniques (adapted from Bruce, 2000)

<table>
<thead>
<tr>
<th>1. Dry Soil Mixing with Single Auger/Mixer</th>
<th>2. Wet Soil Mixing with Multiple Augers</th>
<th>3. Wet Soil Mixing using Cutter Soil Mixing (CSM)</th>
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<tr>
<td><strong>Advantages</strong></td>
<td><strong>Advantages</strong></td>
<td><strong>Advantages</strong></td>
</tr>
<tr>
<td>- Relatively small rig implies easy access onto soft ground.</td>
<td>- A more uniform mix is possible due to the action of multiple augers.</td>
<td>- A uniform mix can be achieved.</td>
</tr>
<tr>
<td>- The size of the column varies typically from 0.6 m to 0.8 m diameter.</td>
<td>- The size of column varies with auger arrangement.</td>
<td>- The large treated column size means that fewer columns are required.</td>
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<tr>
<td><strong>Strength</strong></td>
<td><strong>Strength</strong></td>
<td><strong>Strength</strong></td>
</tr>
<tr>
<td>-75 kPa to 250 kPa shear strength, but typically limited to design strength of 100 kPa to 150 kPa.</td>
<td>- UCS of 1500 kPa (shear strength of 750 kPa approximately).</td>
<td>- With high cement content in the slurry mix, UCS in excess of 1 to 4 MPa has been achieved in marine clays.</td>
</tr>
<tr>
<td>- Effective strength parameters of ( c' = 300 ) to 500 kPa and ( \phi' = 25^\circ ) to ( 35^\circ ).</td>
<td>- Appropriate to allow for potential brittle behavior under lateral loading/bending.</td>
<td>- Potential brittle behavior may also need to be considered although the size of the column is more resistant to lateral loading/bending.</td>
</tr>
</tbody>
</table>
2.3 Biocementation

The term biocement was first coined by Cord Ruwisch to describe calcium carbonate (calcite) formation by soil-based bacteria (Whiffin 2004; Al-Thawadi 2008). Biocementation is more formally known as Microbially Induced Calcium Carbonate (Calcite) Precipitation (MICP). Unlike the chemical Calcite In-situ Precipitation System (CIPS), MICP uses the science of the precipitation of minerals by living organisms, which in this case are bacteria. On the other hand, CIPS involves injecting (proprietary) chemical solutions that cause the precipitation of calcite minerals (Ismail et al. 2002), which classifies it as one of the chemical grouting ground improvement methods.

Al_Thawadi (2008) defined biocementation as sand consolidation technology in which the carbonate released from the microbial hydrolysis of urea, in the presence of excess calcium ions and from calcite precipitate. Biocementation improves the shear strength of soil through the production of soil particle-binding materials, as the result of introducing bacteria and cementation reagents into the soil. The soil cementation materials are mostly carbonates, silicates, phosphates, and hydroxides (Ivanov and Chu, 2008). Calcium carbonate (calcite) is an attractive element to study in relation to biocementation because calcite formation is commonly found in nature. In addition, ureolytic bacteria (produces urease enzyme which catalyzes the hydrolysis of urea into ammonium and carbonate) are widely spread throughout the environment (indigenous bacteria), which makes the introduction of foreign bacteria (exogenous bacteria) less important in some cases (Fujita et al. 2000).

2.3.1 Soil Biota

In the soil matrix the solid phase of soils, comprised of sand and finer materials, are usually characterized by an equivalent particle diameter. The range of particle sizes in sand is from 2 mm to 0.06 mm. Finer materials can be split into two particle size fractions, the silt fractions less than 60 µm and the clay fraction less than 2µm. The proportion of each fraction of the fine materials defines the soil texture, which determines the volumes available for the two other soil phases, gaseous (soil-air) and aqueous (soil-water or soil solution). Sandy soils have much higher pore sizes, which allows faster water percolation and evaporation, and can result in rapid shifts
of soil moisture and soil aeration (Buscot and Varma, 2005). Size relationships also play an important role in biological interactions in soil, because the habitat is normally inhabited by microbes of a particular size (Brussaard et al. 1997). According to Krumbein et al. 1977, the process of inducing carbonate precipitation only works when nucleation sites are available, and in MICP the bacteria are believed to provide the nucleation sites. Hence, the ability of bacteria to penetrate the soil pores is considered to be critical in the MICP process.

Mobilization or transport of bacteria is limited in fine grained soil, hence bacterial activity is similarly limited. Given that bacteria have a typical size of 0.5 to 2 µm, they cannot be transported through silty or clay soils as shown in Figure 2.2 (Mitchell and Santamarina, 2005). Although microorganisms are free to move by advection (with the help of water) in the pore space between large soil grains and aggregations, narrow pore throats formed by small soil grains prevent their entry. Bacteria are not expected to enter through pore throats smaller than 0.4 µm, while fungi and protozoa require pore throat sizes greater than 6 µm for entry. This suggests that microbes in fine grained soil would have to have been trapped during deposition and burial. These bacteria could be activated if suitable nutrients are available, but these too have to be transported by advection or diffusion through the soil pores. As these processes are very slow in fine grained soils MICP does not appear to be able to produce effective cementation at the time scale required for engineering projects.

Figure 2.2. Microorganism and pore throat size relationship (Mitchell and Santamarina, 2005)
However, the pore sizes in even clayey soils are sufficient for bacteria to exist, and thus in-situ and ex-situ mixing of microbes and nutrients with soil may extend the range of soils that are amenable to treatment into pure clays. The maximum pore size desired for effective treatment depends primarily on the fraction of microbes acting at particle to particle contacts.

In addition to considering the geometric compatibility before the desired chemical reaction is triggered and byproducts are produced, it is also necessary to consider how an aggregation of byproduct (inorganic precipitant), potentially including the facilitating microbes, may be able to migrate through treated soil. This requires estimation of the aggregate size, as well as a reduction in pore throat size due to an accumulation of precipitation around the pore throat and growth and degradation of microbial communities. Based on these geometric limitations and findings from published research, approximate application limits have been proposed (vertical solid line in Figure 2.3) and a broader range is possible. As as evident, biomineralization appears to be applicable to the broadest range of soil at various ambient (applied external) stress conditions. Basically Figure 2.3 gives an idea on boundary limits in terms of pore and grain size that governs the microbial activities at respective depth during biocementation process.

Figure 2.3: Overview of compatibility regimes considering particle size and ambient stress conditions (after Rebata Landa and Santamarina 2006)
According to Landa (2007), pore and grain sizes play a fundamental role in soil bio-cementation and the nature of the soil is an important factor influencing microbial growth. For example, it has been reported in several studies that bacterial activity cannot take place in very fine soils. Generally, it has been reported that the most active microbial reaction occurs in sand (Dejong et al 2013), and that the relationship between microbe size and void ratio is an important factor for microbial growth. Kim et al. (2014) reported that the amount of calcite precipitated in sand specimens was double that in silt specimens. However this value may differ from one research to another depending on the grain size used. It was inferred that bigger voids between soil particles contributed to the greater amount of calcite precipitated. Based on Velpuri et al. (2016), for an effective biocementation the grain should be neither too fine nor too coarse. Inagaki et al. (2011) reported that the amount of calcite precipitated was nearly equal in Toyoura sand and Edosaki sand, which had similar D$_{50}$ values, but different grain size distributions. More research is required to find out the optimal void ratio that maximizes the microbial cementation. Inagaki et al. (2011) also showed that more calcite deposition occurred for the poorly graded soil. More details on the type of sand used in this study can be found in Chapter 3.

### 2.3.2 Roles of Microorganisms

Bacteria are the most abundant microorganisms found in soil. There are between $10^8$ and $10^{10}$ bacteria per gram of dry soil at the ground surface, with the bacteria population concentration generally decreasing with depth, as shown in Figure 2.4 (Ehrlich, 2002). Whitman et al. (1998) similarly observed that more than $10^9$ cells per gram of soil exist in the top metre of soil and the population concentration generally decreases with depth. At a depth 30 m, the lower limit of most soil improvement engineering applications, microbe concentrations of about $10^6$ cells per gram of soil can be found (Ehrlich, 2002). The types of microbes that can be utilized for biomineralisation are numerous. The relative percentages of various types of bacteria that are commonly found in soil at the depth of 30 cm are provided in Table 2.2. These bacteria catalyze the formations of minerals such as Pseudomonas, which produces naphthalene (Bosch et al. 1999) and Bacillus which produces calcite (Okwadha and Li, 2010). The influence of microorganisms on mineral formation has been recognized for a wide variety of minerals,
including carbonates, oxides, phosphates, sulphates and silicates (Fortin et al. 1997). The chemical transformation of metals and ions in soil are mediated by soil microorganisms, such as the precipitation of iron hydroxide by iron-reducing bacteria (Ivanov and Chu, 2008), and the precipitation of calcium carbonate by the microbial enzymatic hydrolysis of urea (DeJong et al., 2006).

Figure 2.4: Concentration of microorganisms at various subsurface depths (Ehrlich, 1996)

There is significant bacterial activity in the unsaturated organic surface layer of a soil deposit where plant roots are found; this upper layer has been extensively studied and the relation between soil fertility and the presence of microorganisms is well documented. However, details of bacterial activity in the saturated region beneath the organic layer are less investigated. Fierer et al. (2003) observed that bacterial activity decreases by 1 to 2 orders of magnitude by 2 m of depth. Similarly, Cowell (1985) observed a decrease of several orders of magnitude between surface soils and unsaturated soils at a depth of 70 m. These observations contrast with the more moderate reductions in bacterial count at greater depths in saturated sediments.
Table 2.2: The relative percentage of various types of bacteria that are commonly found in soil (Alexander, 1977)

<table>
<thead>
<tr>
<th>Genus</th>
<th>Percentage (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Arthrobacter</td>
<td>5 – 60</td>
</tr>
<tr>
<td>Bacillus</td>
<td>7 – 67</td>
</tr>
<tr>
<td>Pseudomonas</td>
<td>3 – 15</td>
</tr>
<tr>
<td>Agrobacterium</td>
<td>&gt;20</td>
</tr>
<tr>
<td>Alcaligenes</td>
<td>2 – 12</td>
</tr>
<tr>
<td>Flavobacterium</td>
<td>2 – 10</td>
</tr>
<tr>
<td>Others</td>
<td>&lt;5</td>
</tr>
</tbody>
</table>

The ability of microbes to work collectively to degrade any naturally occurring compound (and most man-made ones) has attracted considerable interest in recent decades. Industrial waste, containing chemical substances, high usage of coals and petroleum, and military and public use of nuclear energy have all led to an accumulation of environmental pollution. All regions of the biosphere, including surface water and ground water, soil, and the atmosphere, have been contaminated by toxic chemicals for the sake of the physical development of human civilization. Concern over the growing concentrations of these contaminants has led to the development of bioremediation, in which microbes and other living creatures are used to assist in restoring clean water and soil, and the cleanup of hazardous waste. Recently, biological processes have been used in the geotechnical engineering to maintain a sustainable balance in planetary scale ecosystems (Schaechter et al. 2006) by increasing the storage of CO₂ in the ground. The MICP process does this by increasing the amount of dissolved CO₂ (as carbonate or bicarbonate) in the subsurface water and by the precipitation of dissolved CO₂ in carbonate minerals (Mitchell et al.)
Sequestration of CO$_2$ by different bacterial isolates in soil has been recently shown by many (Ramanan et al. 2009; Wanjari et al. 2011; Yadav et al. 2011). These are some of the many specific functions of soil microorganism in solving environmental and engineering issue.

In addition to their role in the aggregation and development of soil structure via their contribution to humification, soil microbes also act directly to change soil strength. Bacteria and fungi exude colloidal polysaccharides that can bind soil particles. Soil fungi, for example, produce glomalin, which has been shown to represent a high proportion of soil organic matter promoting aggregate formation. However, the mechanical properties of soils with significant organic matter are often insufficient to increase strength.

There has been considerable interest over the last decade in using the cementation produced by bio-mineralisation to improve the soil response. Most of this research has focused on using ureolytic bacteria which when provided with suitable urea and calcium sources can precipitate calcite which binds soil particles (Stocks-Fisher et al. 1999; Bahcemeier et al. 2002; Dick et al. 2006; Whiffin et al. 2007; Achal et al. 2009; Van Paassen et al. 2009; Van Paassen et al. 2010).

The types of microbes or bacteria used for biocementation in the past and their performances are summarized in Table 2.3 (Whiffin, 2004). In the biocementation process, urea is hydrolyzed and each urea molecule is assimilated with two molecules of ammonium. The bacteria produce an enzyme, urease, which is required for this reaction, and also the bacteria must be resistant to high concentrations of ammonium. Bacteria producing urease at high rate is known as bacteria with high urease activity. Urease producing bacteria (UPB) are categorized into two groups, based on the urease response to ammonium. The urease activities of some bacteria are repressed by the presence of high concentrations of ammonium. Examples of ureolytic bacteria which have high tolerance to high ammonium concentrations such as *Alcaligenes eutrophas*, *Klebsiella aerogenes*, *Pseudomonas areuginosa*, and *Bacillus megaterium* but these ammonium concentrations vary in terms of urease activity.
Table 2.3: Characteristics of some ureolytic bacteria used in biocementation

<table>
<thead>
<tr>
<th>Microorganism</th>
<th>High urease activity</th>
<th>Not repressed by NH$_4^+$</th>
<th>Non-pathogenic or genetically modified (GM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sporosarcina pasteurii</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
</tr>
<tr>
<td>Proteus vulgaris</td>
<td>Unknown</td>
<td>✓</td>
<td>Moderately</td>
</tr>
<tr>
<td>Proteus mirabilis</td>
<td>Unknown</td>
<td>✓</td>
<td>X</td>
</tr>
<tr>
<td>Helicobacter pylori</td>
<td>✓</td>
<td>✓</td>
<td>X</td>
</tr>
<tr>
<td>Ureplasmas Moclicutes</td>
<td>✓</td>
<td>✓</td>
<td>X</td>
</tr>
</tbody>
</table>

2.4 Mechanisms of Biocementation

Cemented soils can be classified into two categories depending on the nature of the cementation process. Naturally cemented soils, which can contain carbonate, iron oxide, alumina, or organic matter, that together with other minerals may precipitate at the particle contacts and act as a cementing agent (Mitchell, 1993), and artificially cemented soils in which cementing agents are added to the soil to induce the cementation process during experimental studies or field application. Various soil cementation techniques are used to improve the strength and deformation behavior of soil. Lime and Portland cement are used as cementing agents in many field applications, because of their high performance and availability (Consoli et al. 2000). Cement and lime treated soil have been frequently used for highway, railway, and airport construction to increase the bearing capacity of soft soil subgrades. Calcite or biocement produced by biocementation mechanisms in this study will be treated as one of the binder such as cement and lime used for ground improvement.

2.4.1 Natural Biocementation

Biocementation can be a natural phenomenon that occurs over a long period of time; a classic, recognizable example of this is the formation of stalactites and stalagmites. It involves both the chemical and biological cementation process that occurs naturally. However, for industrial
applications this process has to be accelerated by adding suitable compounds. There are two processes that can lead to in-situ calcite cementation in natural environments. Calcite can be formed when water saturated with calcium ions in marine environments evaporates, a phenomenon that is commonly observed in subtropical areas. The second type happens due to a chemical reaction at the surface of soil grains close to the water-seabed boundary. Numerous factors affect the cementation process such as the chemical and physical environment conditions, soil permeability, soil texture, composition, and the stability of the sediments (Fritzges, 2005). Emerging research evidence suggests that this mechanism can be applied for cementing granular soils, which involves the precipitation of calcium carbonate, by increasing the total carbonate content, the pH, or both in the pore water of the soil through metabolic activity (DeJong et al. 2006; Whiffin 2004; Van Paassen 2009). Thus, calcium carbonate can be precipitated out of a solution either abiotically or biologically. The solubility equilibrium for calcium carbonate is presented by Equation (2.1):

$$\text{CaCO}_3 (s) \leftrightarrow \text{Ca}^{2+} + \text{CO}_3^{2-} \quad (2.1)$$

In calcite precipitation the overall equilibrium reaction is seen in Equation (2.2).

$$\text{Ca}^{2+} + \text{CO}_3^{2-} \leftrightarrow \text{CaCO}_3 \downarrow \quad (2.2)$$

Microbiologically induced calcite precipitation occurs naturally according to the reactions below. (Ramakrishnan et al. 2001)

$$\text{Ca}^{2+} + \text{HCO}_3^- + \text{OH}^- \rightarrow \text{CaCO}_3 \downarrow + \text{H}_2\text{O} \quad (2.3)$$

$$\text{Ca}^{2+} + 2\text{HCO}_3^- + \text{OH}^- \leftrightarrow \text{CaCO}_3 \downarrow + \text{CO}_2 + \text{H}_2\text{O} \quad (2.4)$$

Equation (2.3) and (2.4) are triggered simultaneously by the pH changes into alkalinity induced by bacterial metabolic activity. The high pH environment around 8 to 9 is provided by the decomposition of urea (CH$_4$N$_2$O) according to the reaction in Equation (2.5). One of the by-products of this process is CO$_2$ which will naturally sequestrate in soil with no side effects.

$$\text{CH}_4\text{N}_2\text{O} + 3\text{H}_2\text{O} \rightarrow 2\text{NH}_4^+ + 2\text{OH}^- + \text{CO}_2 \quad (2.5)$$
In addition to calcite precipitation from the above mechanism, calcium ions borrowed from nutrient calcium chloride will deposit on the surface of microorganisms with a net cell surface charge that is negative. Commonly carbonate precipitates develop on the external surface of bacterial cells which are attached to sand particles (Pentecost and Bauld, 1988). After the successive deposition of carbonate, bacteria would be embedded in the growing carbonate crystals (Rivadeneyra et al. 1998; Castanier et al. 1999). The equations for the precipitation of calcite at the cell surface as the nucleation site are as follows:

\[ \text{Ca}^{2+} + \text{Cell} \rightarrow \text{Cell} - \text{Ca}^{2+} \]  \hspace{2cm} (2.6)

\[ \text{Cell} - \text{Ca}^{2+} + \text{CO}_3^{2-} \rightarrow \text{Cell-CaCO}_3 \downarrow \]  \hspace{2cm} (2.7)

Calcite and aragonite are the most common among the several different polymorphs of calcium carbonate found in nature as part of sedimentary rocks, shells and corals. Vaterite can also be encountered, especially in gallstones (Bills and Lewis 1975), repair tissues of shells of gastropods (Carlson and Faulkner 1983) and in sediments precipitated by some microorganisms (Rivadeneyra et al. 1991). Among these calcium carbonate polymorphs, calcite is thermodynamically the most stable, and transformation to calcite from less stable (fluctuation in temperature and pressure) forms of calcium carbonate which are aragonite and vaterite occur through dissolution and re-precipitation naturally. The physical properties of the most relevant common polymorphs of calcium carbonate are presented in Table 2.4. This research has limited calcite as bio-cement and assumed no transformations takes place during the course of experiment by controlling the temperature and atmospheric pressure as explained in Chapter 3.

Table 2.4: Polymorphs of calcium carbonate

<table>
<thead>
<tr>
<th></th>
<th>Density (g/cm³)</th>
<th>Thermodynamic Solubility at 25°C, −logKsp</th>
<th>Hardness (Mohs Scale)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Calcite</td>
<td>2.71</td>
<td>8.33-8.58</td>
<td>3</td>
</tr>
<tr>
<td>Aragonite</td>
<td>2.93</td>
<td>8.18-8.42</td>
<td>3.5-4</td>
</tr>
<tr>
<td>Vaterite</td>
<td>2.54</td>
<td>7.78</td>
<td>3</td>
</tr>
</tbody>
</table>
2.4.2 Artificial Biocementation

Generally bio-mineralization is the process by which living organisms produce minerals. Bio-
mineralization process in nature occurs at a very slow rate over geological times (Stockes Fisher
et al. 1999) and can result in the formation of limestone, sandstone, siltstone and mudstone. Bio-
cementation, as being investigated for ground improvement, is a process occurring at a much
shorter timescale. In bio-cementation, the microbially induced precipitated calcium carbonate
(CaCO$_3$) acts as the cementing agent. There are four parameters that govern bio-cementation
which are the calcium concentration, the carbonate concentration, the pH of the environment and
the presence of nucleation sites (Hammes and Verstraete 2002).

Precipitation of calcite through urea hydrolysis by ureolytic bacteria is the most straightforward
and the most easily controlled mechanism of MICP with potential to produce high amounts of
calcite in short periods of time provided large amount of bacteria and nutrients are supplied
(Velpuri et al. 2016). It can make use of non-pathogenic microorganisms such as Sporasarcina
pasteurii (formerly known as Bacillus pasteurii), an alkaliphilic bacterium found in soil, sewage
and urinal incrustations (Sneath 1986 and Whiffin 2004). This aerobic, spore forming, rod
shaped bacterium is one of the most robust ureolytic bacteria. It is the most commonly used
urease source due to: i) high urease production capacity; ii) ability to produce urease of
ammonium; iii) high stability (robust); iv) consistent production (reliable), and v) no further
down-stream processing prior to use in bio-cementation (Whiffin 2004). Thus, Sporasarcina
pasteurii are used in industrial applications such as in the bioremediation of cracks (Ramakrishna
et al. 2005), the strengthening (durability) of concrete (Ramachandran et al. 2001) and
biogrouting (van Paassen 2009).

Calcite precipitation may be achieved by many different processes (DeJong et al. 2010)
including urea hydrolysis, denitrification (Karatas et al. 2008; Van Paassen et al. 2010), sulphate
reduction (Warthmann et al. 2000), inducing dolomite precipitation and iron reduction (Roden et
al. 2002), inducing ankerite and other mixed mineral precipitation. Further details are provided in
Chapter 4. Enzymatic hydrolysis of urea by microbes opted in this research is the most energy-
efficient (DeJong et al. 2010) because it only involves natural resources and helps in CO$_2$
sequestration, and urease activity (refer to definition in section 4.3 of Chapter 4) is found in a
wide range of micro-organisms and plants (Bachmeier et al. 2002). Thus most of the research on bio-cementation that involves MICP has used urea hydrolysis. Highly ureolytic bacteria obtained from American Type Tissue Culture (ATCC) are cultivated in the laboratory and introduced in the soil using injection technique, where urea and calcium chloride solutions or any type of calcium source are supplied. Selection of the process ultimately implemented in the field should consider the differences in efficiency rates, contaminant byproducts and energy consumption between these processes. Bacillus pasteurii and a more recent strain, Sporosarcina pasteurii a bacterium which is highly active in producing urease enzyme, have been used widely in laboratory studies (Ferris 1996). Up to this point, ureolytic bacteria of the genus Bacillus (a group of bacteria from different species under the same subfamily which is Bacillus) were used as agents in different studies with various applications (Ramachandran et al. 2001; De Muynck et al. 2008). The bacteria catalyze the hydrolysis of urea and produce ammonium and calcium carbonate crystals (calcite) provided a conducive environment was present at the time of the reaction takes place which is similar to natural bio-cementation. These crystals form cohesive ‘bridges’ between sand grains, increase strength and stiffness of sand with significant decrease in permeability.

### 2.5 Applications of Bio-cementation

Several possible applications of MICP are being investigated, which include bioremediation of cracks in concrete and stone, bio-deposition on cementitious material, improvement in brick durability, and bio-cementation for ground improvement. Some of these applications are detailed below.

#### 2.5.1 Bioremediation of Cracks

The injection of suitable bacteria and nutrients into cracks in concrete and other building materials can lead to the cracks being filled up with precipitate. This enhances the strength and durability of the structures because the crack sealing results in a decrease in water permeability and reduces the penetration of water (Ramachandran et al. 2001; Bang et al. 2001; Ramakrishna et al. 2001; De Muynck et al. 2008). This method has also been used by Tiano et al. (1999) to conserve natural stone monuments. Figure 2.5 shows how cracks in concrete allow the ingress of
water and chemicals which can reach to the reinforcement and accelerate corrosion. Filling up the cracks retards the corrosion and can be achieved with conventional cements and biogrouts. One of the reasons for the interest in bacteria is that they offer the potential for self-healing. It has been tested that if bacteria are incorporated into concrete they can be activated by cementation fluid ingress with the resulting precipitation products which will work as sealing material and preventing further cracks.

Figure 2.5: Schematic diagram of conventional concrete (A-C) versus bacteria based self-healing (D-F) concrete (image source: Jonkers 2007)

2.5.2 Biodeposition on Cementitious Materials

MICP is a good sealant as it forms a new layer on the surface of existing concrete. Cementitious materials (stone, concrete, mortar) are prone to ingress of water and other deleterious substances that lead to their deterioration (De Muynck et al. 2008). Therefore, surface treatments play an important role in the protection of such materials. The deposition of a layer of CaCO₃ on the surface of the material will decrease the permeability thus improving the durability as a result of calcite filling up pores and void of aggregate in concrete. For all types of surface treatments, the depth of penetration depends on a variety of parameters; in addition to climatologic conditions, it is influenced by viscosity, surface tension, rate of deposition, application procedure, and rate of solvent evaporation (De Muynck et al. 2013). The applications and the functions of the calcite may vary but the mechanisms involved still remains the same.
2.5.3 Microbially Enhanced Oil Recovery (MEOR)

In principle, microbially enhanced oil recovery (MEOR) method is similar to microbially induced calcite precipitation (MICP). Calcites precipitated from MEOR reactions have been used to plug highly permeable rock in oil reservoirs (Leonard, 1986; Bryant, 1991; Nemati et al. 2005). Because of preferential flow through these highly permeable regions normally, only between 8 to 30 % of the total oil present in an oil reservoir is recovered using conventional oil production methods (Leonard et al. 1986). In the microbially enhanced oil recovery method (MEOR), plugging of the pores occurs due to bacterial multiplication which hydrolyses urea and produces precipitation (MacLeod et al. 1988). Research was focused on the plugging of highly permeable zones as this offers a feasible alternative to block the rock pores and improve residual oil recovery (Nemati et al. 2005).

2.5.4 Biogrouting

Bio-mediated ground improvement has been investigated in the Netherlands as a means of maintaining and repairing dikes to prevent floods in low lying areas. Whiffin et al. (2007) has shown that biogrouting is able to stabilize soil and other particulate matter. This method is useful for tunneling, earthquake repair and instant pavements (Whiffin et al. 2007; DeJong et al. 2006; Van Paassen 2009). During the biogrouting application, loose sand or gravel is injected with a mixture of bacteria and nutrients which stimulates the biochemical reactions leading to the precipitation of calcium chloride. The mechanism of calcite precipitation in biogrouting is similar to biocement and bioconcrete if urea hydrolysis pathway chosen. The term biogrouting is used here depending on the purpose of the application and the techniques involved in the process of making it.

2.5.5 Improved Durability of Brick

Most of the deterioration of brick structures takes place because of the presence of moisture. Sarda et al. (2009) were able to increase brick strength by biologically depositing calcite (bio-calcite) on the surface and in the voids of the bricks using biochemical approach. Bricks were procured from a construction site and immersed in an inoculated medium using MICP process.
After 4 weeks, bricks were removed and dried at room temperature before testing for water absorption capacity, which was found to have been reduced by up to 45%.

2.5.6 Bio-manufactured Bricks

Dosier (2014) investigated the bio-manufacturing of bricks using the MICP process as an alternative to conventional clay-fired bricks. Figure 2.6 shows the process of making bricks without using heat by using non-pathogenic bacteria which are mixed with the sand before putting it in moulds. If bio-manufactured bricks could replace conventional bricks, it would save nearly 800 million tons of CO₂ annually (Dosier, 2014) because it would remove the need for the large amount of energy currently used in brick manufacture. However, there are no comments seen so far from any publications on the uniformity of calcite in bricks. Thus a thorough investigation should be carried out in order to find out the feasibility of bio-manufactured bricks when used in the construction industry.

Figure 2.6: Process of making bio-manufactured brick (image source: Dosier, 2014)

2.5.7 Bio-concrete

Al Thawadi (2008) has been able to use MICP as a cementing agent to bind sand particles together which was then proposed for concrete structure. With multiple bacterial applications sandstone has been replicated at a much shorter timescale than in nature. This approach is deemed not cost effective as the current research work proposed using single bacterial application. Successful experiments have been conducted where soft sand turns into a substance as hard as marble with unconfined compressive strengths of up to 30 MPa (Al Thawadi 2008). This is now being commercialized to produce precast products.
Almost all of the research applications mentioned above have been conducted by microbiological, geological, and environmental engineers; and involvement of geotechnical engineers in these research efforts has been limited. Participation of geotechnical engineers is necessary for development of ground improvement technology based on microbial mechanisms.

2.6 Experimental Procedures for Biocementation

In early research works mixing bacteria with soil was found with no promising results for future application. Hence most research into bio-cementation has pumped the bacteria and nutrients (urea and calcium source) into the soil, an approach similar to that used in cement grouting for soil improvement. The most important factor in achieving an even deposition of precipitated calcite throughout the sand mass is the uniform distribution of the bacterial cells. In order to induce MICP in the soil subsurface, bacteria and nutrients need to be injected and transported to the location where strengthening is required. Mixing bacteria and nutrients prior to injection can result in the immediate flocculation of bacteria and calcite growth. This would cause rapid clogging of injection points and surrounding pore space for fine sands. In order to prevent clogging and encourage a more homogenous distribution of calcite, different MICP injection strategies have been investigated in sand and some of them are discussed here. Only the most recent treatment techniques in MICP were included here as the previously used treatment methods by many researchers deemed unsuitable and completely ignored from discussion.

Whiffin (2004) proposed a two-stage process for achieving bio-cementation in sand column experiments. The first being the growth stage and the second being the cementation stage. The two stages were intended to prevent the premature precipitation of the CaCO₃ and clogging of the front part of the test column, which occurred when the bacteria and cementation solution were premixed. For each stage solutions are injected independently of each other and may be supplied in either batch form or continuous flow.
**Bacterial growth stage:**

The bacteria are cultivated in a growth medium, containing a protein source (such as yeast or beef extract), calcium chloride, $\text{Ni}^+$ ions (used by bacteria to synthesize urease), until the urease activity is at its highest (assessed by optical density). The bacteria are harvested and stored at 4 °C and suspended in the growth medium, until required (Van Paassen 2009).

**Cementation stage:**

The bio-cementation stage involves four phases starting with flushing the matrix material with distilled water, in order to remove any impurities. Then the bacterial solution is injected through the porous media for approximately 24 hours. After this the cementation solution made up of calcium ions (calcium chloride/nitrate) and urea is injected and pumping continued until all the urea is hydrolyzed. This process could be repeated as many times as necessary. Finally the matrix material is rinsed to remove the waste ammonium chloride. The batch injection process for bio-cementation as suggested by Whiffin et al. (2007) is shown in Table 2.5. Column dimensions used in this research were 38 mm internal diameter by 170 mm PVC pipe.

Table 2.5: Summary of batch phase treatment method (Whiffin et al. 2007)

<table>
<thead>
<tr>
<th>Phase</th>
<th>Description</th>
<th>Duration (hr)</th>
<th>Flow rate (L/hr)</th>
<th>Details</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rinse</td>
<td>Water flush</td>
<td>30.7</td>
<td>0.35</td>
<td>Tap water</td>
</tr>
<tr>
<td>Placement</td>
<td>Bacterial injection</td>
<td>18.1</td>
<td>0.35</td>
<td>$\text{OD}_{600}: 1.583$</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>$\text{Act: } 0.23 \text{ mS min}^{-1}$</td>
</tr>
<tr>
<td></td>
<td>CaCl$_2$</td>
<td>17.1</td>
<td>0.35</td>
<td>0.05 M CaCl$_2$</td>
</tr>
<tr>
<td>Cementation</td>
<td>Reaction (cementation) fluid injection</td>
<td>24.9</td>
<td>0.35</td>
<td>1.1 M urea and CaCl$_2$</td>
</tr>
<tr>
<td></td>
<td>No flow reaction</td>
<td>102</td>
<td>0.00</td>
<td></td>
</tr>
<tr>
<td>Rinse</td>
<td>Water flush</td>
<td>23.7</td>
<td>0.35</td>
<td>Tap water</td>
</tr>
</tbody>
</table>

Note: $\text{OD} – \text{ Optical Density}$

$\text{Act} – \text{ Actual turbidity reading in conductivity meter}$
Later studies have modified the procedure to avoid clogging due to crystal accumulation at the injection point and to obtain a more homogenous distribution of CaCO₃. For example, Van Paassen (2009) injected the bacterial solution until it was detected in the outflow, and then immobilized the bacteria within a fixation solution, with a high CaCl₂ concentration. After this a solution comprising urea and calcium chloride in equal molar concentrations was injected to initiate the bio-cementation process. This reagent solution was injected for 8 hours each day until the desired amount of ammonium is converted or CaCO₃ formed. The procedure is summarized in Table 2.6. The column dimensions used in this research were 66 mm internal diameter by 180 mm long.

Table 2.6: Summary of continuous flow treatment (Van Paassen, 2009)

<table>
<thead>
<tr>
<th>Phase</th>
<th>Treatment</th>
<th>Rate (L/hr)</th>
<th>Volume (L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rinse</td>
<td>Tap water is flushed through the entire volume</td>
<td>-</td>
<td>18</td>
</tr>
<tr>
<td>Placement</td>
<td>Bacterial suspension is injected first into the sand core</td>
<td>50</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td>Followed by 0.05 M CaCl₂ injection, a fixation fluid</td>
<td>50</td>
<td>40</td>
</tr>
<tr>
<td>Cementation</td>
<td>Injection of reagent solution containing 0.5M urea and CaCl₂</td>
<td>40</td>
<td>4000</td>
</tr>
<tr>
<td>Rinse</td>
<td>Tap water is flushed through the entire volume</td>
<td>-</td>
<td>14</td>
</tr>
</tbody>
</table>
A more effective MICP treatment is achieved using retention periods between the injection phases that allow more bacteria to be fixed into the pore void spaces (Tobler et al. 2012). Using this approach also excessive crystal accumulation close to the injection points can be prevented. Retention periods or better known as the holding time before the start of next injection also facilitate greater numbers of reactions between bacteria cells and the cementation solution (Al Qabany et al. 2011; Rong et al. 2012). Shahraki et al. (2014) claim to have achieved the most effective cementation in column tests using staged injection coupled with retention periods and downward injection. No clogging observed at the injection point and homogenous calcite precipitation occurred.

Batch and continuous flow methods have been developed to overcome the clogging at the injection points in laboratory scale studies, however the effectiveness of these methods in the field is still to be demonstrated. Laboratory experiments using MICP have had difficulty in producing a homogenous distribution of the cementation within the soil matrix. Martinez et al. (2011) performed an investigation to identify a suitable injection method to obtain uniform calcite distribution in sand columns and recommended a stopped-flow injection technique as continuous injection promoted abundant calcite precipitation near the injection port, and the calcite content decreased with distance from the injection point. Unlike the continuous injection technique, stopped flow injection which is similar to the staged injection technique is normally coupled with retention period. Similar predictions were obtained from numerical modeling (Barkouki et al. 2011). The stopped flow method is capable of distributing the cementation fluid evenly throughout the sand column, but this does not guarantee a uniform deposition of calcite as other factors like in-situ soil pH and temperature does play an important role. Further efforts are required to develop methods that can induce a uniform distribution of microorganisms and at the same time calcite within the soil.
2.7 Effects of Artificial Cementation on Engineering Behavior of Sand

The effects of cementation on the shearing behavior of granular soils have been studied by many researchers using artificially cemented soils (Sitar et al., 1980; Clough et al. 1981; Rad, 1983; Abdulla and Kiousis, 1997; Huang and Airey 1998; Fernandez and Santamarina, 2001; Asghari et al. 2003; Haeri et al., 2005; Kasama et al., 2006; Sharma et al. 2011). These experiments have been performed using Portland cement, gypsum cement, sodium silicates and calcite. Tests on artificially cemented granular soils indicate shear strength increases primarily due to an increase in cohesion among the particles, with only a slight increase in peak and residual internal friction angles for the cemented soil (Sitar et al. 1980; Rad, 1983). Rad (1983) performed a series of tests on sands with varying degrees of relative density and observed increases in unconfined compressive strength with as little as one percent Portland cement by weight. Sitar et al. (1980) reported that cementation with two percent Portland cement by weight induced cohesion on the order of 45 kPa in one sand.

Basically general patterns of any cemented materials including biocemented sand can be idealized. The general patterns of behaviour associated with adding cement are similar for the different cement materials, with increases in strength and stiffness occurring as a result of increases in the amount of cementing material, although the amount of cementing material required to produce a certain cementing effect can vary widely. Cementing effects are referred to the changes in properties of sand behaviour in terms of strength and stiffness. It has been noted that the effectiveness of the cement depends on the density of cemented soil, with the effect of the cement more significant at lower densities mixed manually (Huang and Airey 1998, Consoli et al. 2009). Tests conducted by Clough et al. 1981, suggest that cementation can also increase the initial tangent modulus (i.e, increase the small strain stiffness) of a soil by up to an order of magnitude at low confining pressures, although the effect is much smaller at higher confining pressures. Based on the results of experiments with gypsum cemented gravelly sand, Haeri et al. (2005) reported that the friction angle of sand increases slightly due to cementation but the increase in cohesion is more noticeable and increases as the cement content increases. Fernandez and Santamarina (2001) reported that small strain stiffness of sands can increase by an order of
magnitude or more due to cementation with two percent by weight of Portland cement. The results published by Fernandez and Santamarina (2001) indicate an increase in shear wave velocity (which is proportional to the square root of the small strain stiffness) of fine sub-angular sand from 230 m/s to 620 m/s at 100 kPa confining pressure when mixed with two percent Portland cement by weight and cured before loading. The study also reveals that cemented soils exhibit very limited changes in shear wave velocity (and thus in small strain stiffness) due to stress change until decementation (breaking of cementation bonds) begins (Fernandez and Santamarina, 2001).

Figure 2.6 shows the variations of peak deviator stress (\(\sigma'_1 - \sigma'_3\)), shear wave velocity, and volumetric strain with axial strain for a series of drained triaxial compression tests on weakly cemented sand (cemented using 2.5 % OPC) (Sharma et al. 2011). In all cases, the peak deviator stress, density and shear wave velocity increased consistently with increasing confining stress, density and cement content. According to Sharma et al. 2011, the failure mechanism was relatively brittle (i.e., specimens exhibited dilatant behavior) for all the samples tested except for the 1.8 g/cm\(^3\) samples at low levels of cement content (0 % and 1 %), which exhibited contractive behavior as in Figure 2.7 (a). The volumetric strain response exhibited initial contraction followed by significant dilation, and the amount of initial contraction tended to increase with cement content for a given density and confining stress. In general the observed behavior of these samples during drained shear is consistent with other published results on cemented soil (Clough et al., 1981; Huang and Airey, 1998, Malandraki and Toll, 1996). Figures 2.7: (a) and (b) also show that the shear wave velocity increased during shear to a peak value and then decreased gradually with strain.
Figure 2.7: Typical results of drained triaxial test for weakly cemented sand for (a) $\gamma_b=1.8 \text{ g/cm}^3$, $\sigma'_3=100 \text{ kPa}$ (b) $\gamma_b=2.1 \text{ g/cm}^3$, $\sigma'_3 = 300 \text{ kPa}$ using Portland cement (excerpt from Sharma et al 2011).

However, all these results are for abiotic cementation. Due to a difference in mineral content, structure, particle size and organic content of the cement, soils improved by microbially induced cementation may display different shearing and volume change responses than soils improved with abiotic cementation.
2.8 Effects of Biocementation on Engineering Behavior of Sand

Consistent with other cements it has been found that the UCS strength obtained depends on the dry sand density, with densely packed sand requiring less bio-cementation than looser sand to achieve the same strength (Van Paassen et al. 2009). It has also been suggested that the point to point contact of CaCO₃ crystals, which bridge between two adjacent grains, has an important influence on the UCS strength (Al Thawadi, 2008). The maximum unconfined compressive strengths (UCS) achieved in specimens obtained from small scale experiments have been up to 30 MPa (Al Thawadi, 2008) and from large scale (100 m³) experiments up to 12 MPa (Van Paassen et al., 2009). The UCS strength mildly increases with the strength of the individual soil particles and decreases with particle size, particle pre-coating with CaCO₃ and roundness of particle to identify the maximum cementing effects (Al Thawadi, 2008). Additionally, reactions that take place very quickly precipitate soft and powder like crystals, while natural limestone, which forms slowly, creates a very hard precipitate (Whiffin, 2004). According to Al Qabany and Soga (2013), for the same amount of calcite precipitated, larger UCS strengths were obtained when the solution concentration was low for example 0.1 M in comparison to 1 M. These results were obtained across a range of different initial relative density (refer Figure 2.8). On the other hand, Cheng et al. (2013) reported that higher UCS strength can be obtained at similar CaCO₃ content when treatment is performed under low degrees of saturation (refer Figure 2.9). For example strength achieved by 80 % saturated sample can be similar to fully saturated sample.

![Figure 2.8: UCS data plotted against calcite precipitation for different concentration of solution (excerpt from Al Qabany and Soga, 2013)](image-url)
Homogenous development of strength is influenced by the distribution of bacteria or urease activity as the bacteria are absorbed, strained and detached during the flow and transportation through the grains (Van Paassen et al. 2009). These scenarios could happen to bacteria travelling in soil depending on the flow rate of cementation fluid injected. Ideally bacteria should be distributed homogenously in soil and attached to soil grains. Some of the factors affecting this are: (i) fluid properties such as viscosity and density of the different solutions, (ii) cell wall characteristics such as hydrophobicity, charge and appendages and (iii) solid properties such as grain size distribution, surface texture and mineralogy (Al Thawadi, 2008).

Drained triaxial tests were performed by Mortensen et al. (2011) on bio-cemented samples using urease producing bacteria (Bacillus pasteurii) to compare the drained strength to those of baseline untreated specimens, and the results are shown in Figure 2.10. The treated specimens are all cemented to a moderate cementation level with a shear wave velocity of approximately 450 m/s (produced by about 0.6 % calcite by mass). For comparison, the baseline untreated specimen had an initial shear wave velocity of about 180 m/s. The behavior of the drained compression triaxial tests for the bio-cemented and the untreated loose sand specimen are compared in Figure 2.10 to demonstrate the improvement of the soil behavior. The bio-cemented
specimen exhibited an increase of approximately 1.3 times the peak strength of the loose untreated specimens and was initially much stiffer than uncemented sand. After reaching the peak shear strength, the drained bio-cemented specimen begins to slowly soften as the cementation degrades with continued shearing. The shear strength at large strains are approximately the same for both the bio-cemented and the uncemented specimens. The shear wave velocity data indicate that the cementation in the bio-cemented specimen continues to degrade throughout shearing and that the shear wave velocity also approaches that of the untreated specimen at large strains. The volumetric behavior changes with the addition of precipitated calcite; treated samples exhibits dilation while the untreated sample undergoes contraction. The drained stress-strain behavior and shear wave velocity data both indicate that cemented soil begins to transition towards that of the untreated soil at large strains (>10 % strain).

Figure 2.10: Drained compression test of bio-cemented and uncemented specimen (excerpt from Mortensen et al. 2011)
Similar trends were observed in research conducted by Tsukamoto et al. (2013), DeJong et al. (2013) and others. Figure 2.11 shows the deviator stress- axial strain curves and volumetric strain-axial strain curves. Toyoura sand of various relative densities was saturated with distilled water and injected with 800 ml of nutrients (urea and CaCl₂) and bacteria (Bacillus pastuerii). The amount of calcite precipitated was 51.8 to 63.7 kg/m³. Figure 2.11 shows five test cases including four (P1 to P4) in which the frequency of nutrient injection was varied (from 2 to 12 times) and case N in which Toyoura sand was just saturated by distilled water without the injection of nutrients. It can be seen that increases in calcite precipitation leads to an increase in maximum deviator stress. In specimens with about the same calcite content, the maximum deviator stress was the highest when the relative density was high. Generally the patterns observed in bio-cemented samples are consistent with other artificially cemented sands.

Figure 2.11: Relationship between axial strain, principle stress difference and volumetric strain ($\sigma_c' = 50$ kPa) (excerpt from Tsukamoto et al. 2013)
2.9 Biocementation in Liquefaction Mitigation

Liquefaction is a phenomenon marked by a rapid and dramatic loss of soil strength, which can occur in loose, saturated sand deposits subjected to earthquake motions. Certain types of sand deposits, hydraulic fills, and mine tailings are particularly susceptible to liquefaction. Liquefaction susceptibility depends primarily on the geology, composition, density and stress state of a formation (Seed and Idriss 1983, Kramer and Mitchell 2006). Soil deposits formed in depositional environments that produce uniformly graded, loose deposits are highly susceptible to liquefaction. Compositional factors that influence liquefaction susceptibility include particle size, shape and gradation. Poorly graded sands with rounded particles are more susceptible to liquefaction than well graded sands with angular particles. The liquefaction susceptibility also depends on the initial stress state and density of the soil, with loose deposits that are close to the ground surface with a shallow groundwater table being highly prone to liquefaction.

Loose, saturated sand deposits can liquefy when subjected to earthquake forces due to the tendency of loose sand deposits to decrease in volume when sheared. If the sand is saturated, volume change will not occur until the water drains out from the soil. In the case of earthquake loading, the shearing is so rapid that drainage is almost impossible and therefore the stresses are transferred to the pore water, causing the effective stress and the strength of the sand to decrease. The onset of liquefaction is usually sudden and dramatic and can result in large deformations and settlements, floating of buried structures, or loss of foundation support. A detailed discussion is presented in Seed and Idriss (1983) and Kramer and Mitchell (2006).

According to Figure 2.12 below, uniform sands, such as Sydney beach sand, fall into the most liquefiable soil category. In loose and uniformly graded sands, infiltration can lead to excess pore pressure and induce liquefaction with possible catastrophic consequences. Field experience shows that static liquefaction becomes an issue for sand layers with relative density less than 55%.
Figure 2.12: Limits in the gradation curve separating liquefiable and non-liquefiable soil (after Tsuchida 1970)

It has been suggested that it would be beneficial and sufficient if the unconfined strength of the sand could be increased to 1.5 MPa to prevent liquefaction, but also that only a small increase up to 150 kPa in strength is necessary to prevent sand from flowing (Shibata and Taparaska, 1988). MICP may be effective in mitigating seismic induced liquefaction which generally occurs in relatively loose granular soil deposits that are below the ground water table and thus saturated. According to Mortensen (2012), the MICP treatment process offers a ground improvement method that is capable of reducing liquefaction susceptibility and damage due to seismic loading. The ground treatment provides an increased stiffness arising from the cementation and reduces the cyclic degradation and so minimizes changes to the seismic performance of structures built on, or in the ground. A centrifuge test was conducted (Mortensen, 2012) on loose sand that was cemented by MICP and subjected to seismic loading (refer Figure 2.13). The behavior of the MICP treated soil was significantly different from the untreated loose sand as the level of biocementation increased. For example, the seismic induced settlement was reduced to approximately 100 % when the 3 % calcite by mass was precipitated during the centrifuge test.
One of the difficulties of the injection of nutrients and bacteria to improve the ground through biocementation is that cementation does not occur homogeneously, and this may cause some problems. An alternative strategy to address this problem is to use deep soil mixing (DSM). This technique has been widely adopted in many projects in Japan. DSM has proved to be effective in improving grounds for many projects. Generally, a grid of stabilized soil-cement columns is constructed at sites which are sensitive to strong ground motion. The grid of soil-cement columns acts as a confined shear box which provides additional shear strength for the soil to withstand ground motion. For example Tanaka et al. 1991, have used this technique to improve the engineering properties of liquefiable ground. The most common configuration used in DSM to mitigate liquefaction is the lattice pattern, as shown in Figure 2.14.

Figure 2.13: Centrifuge design used to test MICP treated sand with seismic loading (excerpt from Mortensen 2012)
Most of the current understanding of the seismic behavior of DSM improved ground and the effect of dynamic earth pressures are the result of physical tests such as centrifuge models. Matsuo et al. 1996 and Siddharthan and Suthahar (2005) have given details of how to use deep soil mixing technique to mitigate liquefaction damages. Currently DSM uses conventional cements to form the columns. There do not appear to be any studies in which researchers have used biocement to form the cemented columns by DSM, despite the potential environmental benefits of this approach. Even though there are some concerns about the viability of bacteria during the column forming process (Zamarreno et al. 2009), it is worth to find out the limitations and the challenges of DSM using biocement for future applications.

2.10 Shear Wave Velocity in Biocementation

The shear wave velocity is a property of soil that can be used to indirectly identify the level of cementation and more directly the stiffness. Geophysical methods, such as imaging the shear wave velocity profile of the subsurface, are widely used in liquefaction assessment and to identify the general characteristics of a soil during in-situ testing. Typical loose sands may have a
shear wave velocity between 100-150 m/s depending on the stress level. According to the National Earthquake Hazard Reduction Program (NEHRP) guidelines (see Table 2.7), a liquefiable soil is any soil falling under a shear velocity of 500 m/s. The goal of ground improvement is to raise that shear wave velocity above 500 m/s and to maintain the velocity in the range of 500-1000 m/s throughout any earthquake event.

Table 2.7: Site classification based on shear wave velocity (NEHRP, 2003)

<table>
<thead>
<tr>
<th>Site class</th>
<th>Soil Profile</th>
<th>Shear wave velocity, $V_s$ (m/s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>Hard rock</td>
<td>$V_s &gt; 1524$</td>
</tr>
<tr>
<td>B</td>
<td>Rock</td>
<td>$762 &lt; V_s \leq 1524$</td>
</tr>
<tr>
<td>C</td>
<td>Very dense soil and soft rock</td>
<td>$366 &lt; V_s \leq 762$</td>
</tr>
<tr>
<td>D</td>
<td>Stiff soil</td>
<td>$183 &lt; V_s &lt; 366$</td>
</tr>
<tr>
<td>E</td>
<td>Soft soil</td>
<td>$V_s &lt; 183$</td>
</tr>
<tr>
<td>F</td>
<td>Problematic soil</td>
<td>Site spec. eval.</td>
</tr>
</tbody>
</table>

As discussed above, and shown in Figure 2.10, biocementation can result in significant increases in the shear wave velocity. It should also be noted that shear wave velocity is sensitive to changes in effective stress and changes in soil density. Nevertheless, shear wave velocity is sensitive to the changes in stiffness that accompany cementation and it has been used in many studies for monitoring the bio-cementation process that binds the sand particles. A trend of how shear wave velocity changes during injection of bacteria and nutrients into a sand column is shown in Figure 2.15 (Rong et al. 2011, DeJong et al. 2010). Figure 2.15 was constructed using experimental data obtained from triaxial test with bender elements. This shows the results of the
sequential injection of bacteria and nutrients, which as discussed above are needed to prevent clogging at the column entry. The shear wave velocity is indicated to be constant during injections of nutrients and to rise when calcite precipitation occurs, a process that takes some time.

![Shear Wave Velocity Graph](image)

Figure 2.15: Generalized trend of bio-cementation process using shear wave velocity (after DeJong, 2010)

The stiffness increase of soil specimens due to bio-cementation treatment can be effectively captured by bender elements which allow non-destructive measurement of shear wave velocity and its variation during curing and shearing. For example, DeJong et al. (2006) conducted triaxial tests on bio-treated samples using bender elements mounted in the end platens. The typical variation of shear wave velocity as monitored using bender elements has been shown in Figure 2.15. This and other similar results (DeJong et al. 2006; Mortensen et al. 2011; Martinez et al. 2013) show clearly how shear wave velocity can be used efficiently to monitor and capture the pre cementation and post cementation stiffness response of bio-cemented sand. The shear wave velocity clearly indicates the initiation of cementation bond breakage at peak capacity and
continued degradation with shearing. Future field deployment of bio-cementation will require real-time monitoring to improve the degree of certainty of execution. Geophysical measurements and shear wave velocity measurement in particular, will be useful to determine whether microbially induced calcite precipitation is occurring within the treated soil.

Shear wave velocity and its correlation with the amount of calcite precipitated are shown in Figure 2.16 below. All specimens show a similar increase in the rate of shear wave propagation with time, although it can be seen that specimens with a smaller chemical influx (reaction fluid) have a more gradual increase in stiffness than otherwise, as would be expected because of the lesser amount of reagents (calcium chloride). However, both exhibit stepwise increases of shear wave velocity associated with the sequential injection process. Al Qabany et al. (2011) have reported that there are large variations in the pattern of increase of shear wave velocity with calcite precipitation. However, this may be attributed to non-uniform cementation as dissected bio-cemented samples shows that calcite precipitation was not evenly distributed throughout the samples (Al Qabany et al. 2011, Inagaki et al. 2011). Nevertheless, a reasonable empirical correlation was found between shear wave velocity and the amount of calcite precipitated as in Equation 2.8 (Al Qabany et al. 2011). This empirical equation from bender elements test also shows that shear wave velocity can be a good indicator for the amount of calcite produced and can thus serve as a cementation index.

\[ V_s = 9.7(CaCO_3) + 147 \]  

(2.8)

Where:

\( V_s \) = Shear wave velocity (m/s)

\( CaCO_3 \) = Calcium Carbonate concentration (kg/m\(^3\))
Figure 2.16: Correlation between shear wave velocity and the amount of calcium chloride concentration (after DeJong, 2010)

2.11 Model Foundation Test

Brian and DeJong (2009) have conducted a 1 g physical model test of a scaled shallow footing and the results are shown in Figure 2.17. Specimens were prepared in a rectangular container and subjected to vertical load. Bio-cementation treatment was conducted as outlined by DeJong et al. (2006). A peristaltic pump was used to inject 15 mL cementation solution from the ports on either side of the footing. A cementation solution comprises of Sporosarcina pasteurii (an ureolytic bacteria also known as bacillus pasteurii was grown using ammonium-yeast extract medium) and nutrients (urea and calcium chloride) was injected, followed by even intervals of injections consisting of nutrients and calcium. Results from the bio-cemented specimens indicated a five fold reduction in settlement of the model footing under the same load as the
untreated specimen. Dissection of the bio-cemented sample indicated non-uniform treatment across the target treatment zone. Concentration of calcite precipitation was observed surrounding the injection ports evident of clogging.

Figure 2.17: (a) Shear wave velocity contours across treated area and (b) load-settlement response from model footing test of bio-cemented and uncemented sand (excerpt from Martinez and DeJong, 2009)
Several researchers have reported investigation on bearing capacity of cement improved columns using model test (Probaha, 1998; Probaha et al. 1999; Terashi, 2002; Farouk and Shashien, 2013; Dijkstra et al, 2013; Al Tabbaa and Harbottle, 2015). Researches have been focused on the effect of type of cement, area replacement ratio and cement content in improving the bearing capacity as well as reducing settlement of footing. The bearing capacity and the other improved parameters of the cement improved columns may be affected by changes in diameter, of the column, cement content, area replacement ratio and even the testing method. According to Kitazume and Terashi (2013), the UCS strength of cemented columns may depend on various factors such as the type soil; cement content, curing method and mixing time. It was also highlighted that the surrounding soil could affect the performance of cemented column due the changes of degree of saturation. Farouk and Shashien (2013) concluded that the settlement of the model foundation reduces with larger area replacement ratio and more fines.

2.12 Biocement Potential to Repair Foundations

In recent years, there has been increasing interest in self-healing concrete materials that can repair cracks. These have the potential to significantly increase concrete durability by preventing corrosive agents from accessing the reinforcement and improving their water tightness, consequently reducing the need for inspection and maintenance (Al Tabbaa and Harbottle, 2015). Research into engineering self-healing in concrete was first initiated to reduce the amount of cement needed in concrete mixes (Gerilla et al. 2007; Mora, 2007) as part of global efforts to reduce greenhouse gas generation. This is a significant initiative as cement production is currently responsible for about 5 % of global CO₂ emissions (IPCC, 2013). The inspiration for the study of self-healing concrete is the ability of living organisms to rapidly detect and repair damage. In concrete, degradation usually begins with micro cracks that lead to corrosion and eventual structural failure. The main form of damage is cracking, so self-healing concretes must have the ability to repair small cracks and fissures autonomously. The feasibility of self-healing in concrete has been discussed in several studies (Neville, 2002; Reinhardt and Jooss, 2003, Li and Yang, 2007; Edvardsen, 1999).
Previous research has demonstrated that concrete repair can be produced by three different processes: natural, chemical, and biological. This thesis is primarily concerned with biological processes as these are considered to have self-healing potential and have been shown to be capable of repairing concrete materials. For example, Ghosh and Urban (2009) reported that cement based materials created by biological action exhibited better durability and crack repairing performance than normal concrete. The healing potential of these biological processes are directly related to the amount of calcium carbonate that can be precipitated. The mechanism of foundation involves precipitating calcite, which then bonds to the sand particles. To produce calcium carbonate, a calcium source must be provided, which is commonly provided by adding calcium chloride (CaCl₂), although a wide range of calcium compounds have been used.

The application of bacteria to assist with repair in the construction industry is not a new idea. Biocements have been used to enhance the durability of building structures and the conservation of cultural heritage because of their self-healing potential. Previously, the potential of bacteria to clean concrete surfaces (De Graef et al. 2005), to improve the strength of cement-sand mortars (Dick et al. 2006; Ghosh and Urban, 2009), to repair degraded limestone and ornamental stone surfaces (Rodrigues and Hooton, 2003) and to repair cracks on the surfaces of concrete structures (Bang et al. 2001; Ramachandran et al. 2001) have been investigated. However, less attention has been given to the possibility of repairing sub-structures and foundations.

2.13 Summary

This review has indicated that urease activity is the main factor governing bio-cementation when using ureolytic bacteria. It directly controls the rate of urea hydrolysis and as such the calcium carbonate concentration. It also indirectly controls the pH of the environment depending on the concentration of urea, thus influencing the type of crystals precipitated. Sporarcarcina pasteruii (Bacillus pasteurii) which has been used in most MICP studies is one bacterium that has high urease activity and is not pathogenic to the environment. However, it is not the only option available and there is a need for further research to explore other microbes. The data on the chemical conditions to optimize bio-cementation have received only limited study, but it has
been shown that the concentration of urea has no direct effect on the urease activity, and that the optimum pH for urea hydrolysis by the urease enzyme is 8.5. The major waste product in biocementation using the urea hydrolysis pathway is ammonium salt. The success of biocementation using this pathway depends on the effective approach used in the field to reduce the impact of ammonium salt to the environment.

Shear wave velocity has been used to estimate the magnitude of calcite cementation that has occurred via bio-cementation in laboratory tests due to the precipitated calcite directly influencing the stiffness between particle contacts. Bio-cementation can increase the shear wave velocity of sand from about 200 m/s up to greater than 1500 m/s. Correlations have been developed for a particular sand but these need to be further developed to estimate the evolution in total density, void ratio and shear modulus during bio-cementation. Apart from that, bio-cementation will require a real time monitoring technique to improve the certainty of field scale execution. Shear wave velocity in particular will be useful to determine the degree of cementation occurring within the treated soil.

Widely investigated batch and continuous injecting methods have their limitations as, even in the latest research conducted, the strength improvement is not uniform throughout the soil columns investigated. This is largely influenced by the supply of cementation reactants versus the bacterial activity in the soil column. Therefore, there is a need to explore alternative methods of introducing bio-cement into soil before this approach can be effectively utilized for ground improvement. As the in-situ mixing method allows more homogeneous distribution of soil and bacteria prior to casting/molding and potentially permits application to finer grained soils this method deserves further investigation.

2.14 Research Plan

It has been demonstrated that there is a need to further enhance the biocementation technique initiated by Van Paassen (2009), to better understand the biological process of cementation and how to control it in soil. From the literature research it has been established that soil mixing offers a potential technique that would allow more uniform cementation and allow finer grained
soils to be bio-cemented. Previously this approach has not been followed because of concerns about the inability of bacteria to maintain their integrity during soil mixing. The aims of this thesis are to explore whether alternative bacteria which can resist cell breakage can be used, and to perform model tests to evaluate bio-cemented column creation and responses. The novelty of this research approach is to incorporate the Deep Soil Mixing (DSM) technique with a biogeotechnical engineering application.

To experimentally investigate the potential of an in-situ mixing technique in bio-cementing sand soil, the following three phases listed in Figure 2.18 have been carried out. The details of the equipment used in each phase are in Chapter 3 and the results in Chapters 4 to 6. The first phase in this study focuses on identifying an alternative ureolytic bacterium other than the commonly used *B. pasteurii* which is not adaptive to mixing conditions. This is because the common bacteria used in biocementation process are usually liable to cellysis (puncture) during mixing thus a bacterium with thick wall (membrane) is favorable. Tests in this phase are conducted only to measure the urea hydrolysis rate and not calcite precipitation rate.

The tests in the second phase are designed to investigate how to produce biocement using a mixing technique at laboratory scale. Because of previous concentration on injection methods there is little data of the strength and stiffness from biocemented samples created by mixing techniques. Attempts are also made to correlate actual amount of calcite precipitated through mixing technique with the strength and stiffness achieved.

The final phase is to produce bio-cemented columns to replicate deep soil mixing. In phase 3 of this study, footing model tests have been designed to measure the performance of bio-cemented columns in reducing settlement by mixing technique. Apart from that, for the first time the application of bio-cement to repair foundation has been studied. The potential of biological activity to heal and promote self-healing mechanisms has been investigated through the repair and testing of previously failed columns.
Figure 2.18: List of research works carried out in this study

- **Phase 1**
  - Select suitable bacteria and prepare Bacillus culture solution to establish calibration chart for biomass density.
  - Determine the bacterial growth rate using spectrophotometric technique and urea hydrolysis rate in batch analysis.
  - Determine the urea hydrolysis kinetics parameters using Monod’s Model. (Further details provided in Chapter 4)

- **Phase 2**
  - Perform UCS tests on bio-cemented samples and compared with gypsum cemented samples.
  - Amount of CaCO₃ precipitated calculated.
  - Cementation process was monitored using bender elements and shear wave velocity recorded.
  - Results were compared with gypsum cemented samples.
  - Run standard triaxial test with the biocemented soil sample and shear wave velocity captured throughout the shearing phase.
  - Amount of CaCO₃ precipitated calculated.

- **Phase 3**
  - Designing auger and setting up 1-g footing model test.
  - Bio-cemented and gypsum columns were formed using auger in dry, partially and fully saturated sand.
  - Vertical load and foundation settlement were recorded for each test and amount of CaCO₃ precipitated calculated.
  - Failed cemented columns repaired using self-healing technique and retested.
3.1 Introduction

This chapter describes the materials used and the procedures that were followed during the three phases of experimentation. The tests in these phases include bacteria growth tests, urea hydrolysis tests, unconfined compressive tests, standard triaxial compression tests with bender elements, and 1-g model footing tests. Where relevant, the tests outlined in this chapter were conducted in accordance with international standards, such as the Australian Standards (AS), British Standards (BS), and the American Society for Testing and Materials (ASTM).

To experimentally investigate the potential of in-situ mixing to provide a uniform and substantial calcite precipitate using ureolytic bacteria, the following three phases of study were carried out:
(1) Batch analyses to determine the most suitable amounts of bacteria and nutrients that produce the maximum urease activity for optimum biocementation.

(2) Unconfined compressive strength (UCS) and triaxial tests with bender elements to measure the strength and stiffness improvement of bio-cemented sand and artificially (Gypsum) cemented sand.

(3) Small scale model footing tests (1-g) on ground improved with bio-cemented columns to demonstrate the applicability of the in-situ mixing technique using MICP.

The aim of conducting batch analyses in the first phase of this research is to gauge the performance of the selected ureolytic bacteria. Factors such as urea concentration, biomass concentration and calcium content are expected to affect the urease activity of the bacteria during the hydrolyzation of urea. The second phase involves determining the relationship between the amount of calcite precipitated in biocemented samples and the strength achieved in UCS and triaxial tests. Also in this second phase, the potential of shear wave velocity to monitor the cementation process and the level of cementation achieved is assessed. Finally, the feasibility of preparing bio-cemented columns was explored in small scale model tests. In addition, tests were performed to investigate the possibility of healing of the model columns, which tended to break into two or more pieces during loading. The program of testing is shown in Figure 3.1. This was carefully designed to investigate the targeted objectives and to resolve uncertainties and limitations from previous research.
Figure 3.1: Test program
3.2 Materials

3.2.1 Bacteria

* Bacillus Megaterium * was chosen as the urease producing bacteria (UPB) in this study, as its application has been proven to be effective in improving the strength of soil and durability of concrete. *B. Megaterium* is a rod-shaped, Gram positive, endo-spore forming, aerotolerant species of bacteria used as soil inoculants in agriculture and horticulture. It shows potential as a bio-control for disease caused by fungi or bacteria in wheat plantations (Kildea et al. 2008). Gram positive bacteria are types which are bounded by a single unit of lipid membrane and in general the thickness of the membrane is 20 to 80 nm. One of the reasons for choosing a Gram positive bacterium for this study is because it has a thicker wall that may protect against bursting and puncturing under high overburden site conditions, and also it will resist cell lysis, which is where cells are broken down/destroyed by some external force or puncture during mixing. The bacterium is arranged in the streptobacillus form and is one of the largest eubacteria found in soil, measuring up to four micrometers in length (refer Figure 3.2). It belongs to a group of bacteria that are often found in chains, where the cells are joined together by polysaccharides on the cell walls. *B. Megaterium* is able to survive in some extreme conditions, such as desert environments due to the spores it forms. Sometimes, this particular bacterium can be found on common surfaces that are frequently touched. *B. Megaterium* produces penicillin amidase which is used for making penicillin. It also produces enzymes for modifying corticosteroids, as well as several amino acid dehydrogenases. An isolated bacterial culture of *B. Megatarium* ATCC 14581 was obtained from the American Type Culture Collection (ATCC) in KWIK STIK™. For this study KWIK STIK, which contains an individual microorganism strain, was supplied by BioNovus and produced by Microbiologics®. This bacterium was cultured in the geoenvironmental laboratory of the School of Civil Engineering, at the University of Sydney. Culturing occurred under aerobic batch conditions in a liquid medium, as described further below. Most importantly, this bacterium falls under the category of non-pathogenic bacteria, which pose no harm to humans.
3.2.2 Gypsum

Gypsum has been used in this study to produce artificially cemented samples for comparison with the characteristics of bio-cemented samples. Gypsum has been used in this study because it cures relatively quickly compared to ordinary Portland cement and other conventional binders, and it is also not subject to long term changes in strength (Van Driessche et al. (2012). The selected Gypsum powder was supplied by Boral Investo Co., Australia. The initial setting time specified by the manufacturer is approximately 55 minutes and this is related to the hydration process.

3.2.3 Urea

Urea powder was purchased from Sigma Aldrich. The details of this material can be found at the supplier’s website.

3.2.4 Calcium Chloride

Calcium Chloride was purchased from Sigma Aldrich. The details of this material can be found at the supplier’s website.
3.3 Preparing the Bacterial Culture

3.3.1. Preparing Agar Plates

In this study, a special agar plate utilizing a modified Christensen urea medium was prepared to monitor bacterial growth (Seeliger, 1956). It is recommended to use this medium as the detection of urease producing bacteria (UPB) is more rapid than other medium. On the Christensen medium, ammonia production causes a rise in pH, leading to a color change from yellow to pink, thus serving as an indicator. The medium for the agar plate was prepared as described by Seeliger, (1956) and had the composition shown in Table 3.1. The ingredients were dissolved with boiling water and the pH was adjusted to 6.8 before being cooled to room temperature. The optimum growth of this type of bacteria was recorded when the initial pH value of culture medium was 6.8. Airborne contaminants can easily invade an open Petri dish, thus, sterile Petri dishes were kept sealed until the growth medium was ready to be poured. When the Petri dish was filled with a thin layer of molten agar medium, they were closed with a lid immediately. After cooling down, the Petri disks were stacked upside down in the refrigerator at 2-8 °C to prevent condensation from dripping on the agar surface. The agar plates were removed from the refrigerator and allowed to come to room temperature before being inoculated with bacteria. All the chemicals used in this research were of analytical grade.

Table 3.1: Typical Growth Medium

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>(g/L⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Urea</td>
<td>20</td>
</tr>
<tr>
<td>Ammonium Sulfate</td>
<td>10</td>
</tr>
<tr>
<td>Yeast Extract</td>
<td>20</td>
</tr>
<tr>
<td>Sodium Chloride</td>
<td>5</td>
</tr>
<tr>
<td>Agar</td>
<td>20</td>
</tr>
<tr>
<td>Phenol red</td>
<td>0.012</td>
</tr>
<tr>
<td>Glucose Monohydrate</td>
<td>10</td>
</tr>
</tbody>
</table>
3.3.2. Inoculation of Bacteria

To inoculate bacteria in order to achieve the targeted minimum amount of bacterial content of $9 \times 10^9$ CFU/mL (biomass density, OD = 0.45) in samples, two techniques were used to culture bacteria: the standard agar plate (solid medium) and a nutrient broth (liquid medium). The following section provides details of both techniques.

3.3.2.1 Growing Bacteria in Solid Medium

Petri dishes with a growth medium (refer to Table 3.1) in solid form were prepared in advance according to the procedure described in section 3.4.1 and placed in the incubator at 30 °C for 24 hours in order to dry. The KWIK STIK™ pouch was opened at the notch as shown in Figure 3.3(a) to remove the KWIK STIK™, which was refrigerated at 2-8 °C before opening. This was followed by pinching the fluid ampule located at the cap, just below the fluid meniscus to release the hydrating liquid, Figure 3.3(b). The KWIK STIK™ was then held vertically and tapped on a hard surface to facilitate the flow of hydrating liquid through the shaft to the bottom where a bacteria pellet was located. The pellet was then crushed and mixed with the hydrating liquid by pinching at the bottom of the stick as shown in Figure 3.3(c). Immediately after that, the saturated swab in the hydrated suspension was removed from the holder, Figure 3.3(d). Then the swab was gently rolled over the agar plate as part of the inoculation process Figure 3.3(e). The inoculated agar plate was placed in the incubator at 30 °C for 24 hours, along with a blank agar plate to monitor for contamination Figure 3.3(f).

After each experiment, the KWIK STIK™ was discarded using proper biohazard disposal procedures. This technique was adopted to calibrate and establish a standard growth curve for B. megaterium. All the steps mentioned here were carried out under sterile conditions. For example, petri dishes and other utensils were autoclaved prior to use. Apart from that the lid of the petri dishes were sealed with sticky tape to stop any microorganisms from the air getting in and contaminating the culture.
Figure 3.3: Steps in inoculating bacteria using solid medium
3.3.2.2 Growing Bacteria in Liquid Medium

The growth medium (refer to Table 3.2) in liquid form was prepared in advance and placed in the incubator at 30 °C for 24 hours using a 50 ml beaker, as shown in Figure 3.4(a). There are two ways of introducing bacteria in a liquid medium, either, placing the swab directly into the nutrient broth, or by transferal after inoculation from a solid culture media. The first method was adapted in this study, as shown in Figure 3.4(b), because there is a high chance for contaminants to be transferred into the broth from contaminated culture plates using the second method. After adding the bacteria gentle agitation was used to distribute the organisms throughout the liquid before incubating it. The inoculated bacterial liquid was placed in the incubator at 30 °C for 24 hours. After each experiment, the KWIK STIK™ was discarded using proper biohazard disposal procedures.

Table 3.2: Typical Liquid medium or broth

<table>
<thead>
<tr>
<th>Ingredients (g/L⁻¹)</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Nutrient broth</td>
<td>3</td>
</tr>
<tr>
<td>Urea</td>
<td>20</td>
</tr>
<tr>
<td>NH₄Cl</td>
<td>10</td>
</tr>
<tr>
<td>NaHCO₃</td>
<td>2.12</td>
</tr>
<tr>
<td>CaCl₂</td>
<td>2.8</td>
</tr>
</tbody>
</table>

Nutrient broth is a liquid medium without agar which contains tryptone, yeast powder and other ingredients that support the growth of bacteria.
3.4 Gram Staining Procedure

The gram stain is a technique commonly used to identify unknown bacteria and to control contamination during experiments. This technique provides a means to separate bacteria into two groups, Gram positives and Gram negatives, based upon the different abilities of bacteria in these groups. Gram positive bacteria retain the purple “stain” color after exposure to alcohol, whereas gram negative bacteria lose the purple stain when the alcohol is applied. In order to make the decolorized gram negative cells visible, Safranin was applied to make the gram negative cells pink. In this study, this procedure was used to check if the *Bacillus Megaterium* (which is known to be gram positive bacteria) had been contaminated, by checking for the presence of any gram negative bacteria. The technique for preparing a gram stained smear is as follows:

1. A thin layer of smear from bacterial suspension was prepared on a clean slide and allowed to air dry. The bacterium used in this step was obtained directly from the bacteria culturing liquid medium.
2. The slide with bacteria was passed through a flame three or four times to fix the bacteria on the slide. This ensures that the bacteria will not be washed off during the staining procedure.
3. The smear was flooded with crystal violet solution and was let stand for 30 seconds. Then, it was rinsed with distilled water.
(4) The smear was flooded with Gram’s iodine solution and let stand for 1 minute.

(5) The slide was held at an angle and one drop of 95 % Ethyl Alcohol was added to the smear to decolorize it.

(6) Next, the smear was covered with Safranin for 45 seconds before being rinsed with distilled water. Then, the slide was blotted with bibulous paper and let dry at room temperature.

(7) Finally, the slide was examined under the microscope using 400 x objectives.

Table 3.3 below shows an example of the Gram staining results and the appearance of the *Bacillus megaterium* at each step carried out during batch analysis.

<table>
<thead>
<tr>
<th>Reagent</th>
<th>Time (second)</th>
<th>Appearance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Crystal Violet</td>
<td>30</td>
<td>Purple</td>
</tr>
<tr>
<td>Gram’s Iodine</td>
<td>60</td>
<td>Purple</td>
</tr>
<tr>
<td>95% Alcohol</td>
<td>10</td>
<td>Purple</td>
</tr>
<tr>
<td>Safranin</td>
<td>45</td>
<td>Purple</td>
</tr>
</tbody>
</table>

Based on the results above, the microorganism, *B. megaterium* was purple in appearance, indicating that the bacteria on the slide were gram positive. Any presence of gram negative bacteria cells, which would have contaminated the culture and medium, would have been seen in pink color. This check indicated that satisfactory methods had been followed in growing *B. megaterium* and no evidence of significant contamination was observed.
3.5 Counting Bacteria

Determining the biomass concentration is an important step in ensuring bio-cementation occurs as intended. It is also one of the most delicate, sensitive, and time consuming protocols to be carried out. Care is required because results from all the methods discussed hereafter, whether they are manual or automated, can be unreliable if the broth contains other insoluble particulate matter, which is often the case in a practical application. For example, the optical density measurement has only limited usefulness if the bio-cementation broth is not clear. In addition, these methods cannot distinguish the viable cells from the dead ones. On the other hand, the standard plate count can detect viable cells among other particulate matter but the method requires elaborate preparations, it takes 24-48 hours for the cells to be incubated and counted and the cost of Petri dishes and media can also be prohibitive. Consequently, the direct plate count is unable to provide the feedback control required of a bio-cementation process. It is mainly used industrially to countercheck other measurements, especially the optical density. In this research, the biomass density of a given sample has been measured with the following two methods.

3.5.1 Plate Counting Method

After an incubation period of 24-48 hours, petri dishes were directly inspected with the aid of magnification under uniform and controlled artificial illumination with a hand tally. Where impossible to count at once, plates were stored again for no longer than 24 hours. Serial dilutions were used and the number of colonies on each plate counted. The recorded results are in Table 3.4. Higher magnification was used where necessary to distinguish colonies from foreign matter and the edge of Petri dishes were carefully examined for colonies. Colonies should be well distributed and the area covered should not exceed 25 % of the plate. If there was no plate with 30 to 300 colonies, the plate having nearest to 300 colonies was used. Generally plates with less than 30 colonies were recorded for the lowest dilution. Finally, counts from multiple plates of serial dilution were totaled up as the estimated number of bacteria in the stock (original) solution.
Table 3.4: Bacterial counting results from plate counting and spectrophotometer methods

<table>
<thead>
<tr>
<th>Test Tube</th>
<th>Dilution Factor</th>
<th>Standard Plate Count</th>
<th>Microscopy Count</th>
<th>Absorbance (OD 600nm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td># of Colony</td>
<td>CFU/ml</td>
<td># of Colony</td>
</tr>
<tr>
<td>1</td>
<td>10</td>
<td>&gt;300</td>
<td>TMTC</td>
<td>&gt;300</td>
</tr>
<tr>
<td>2</td>
<td>$10^2$</td>
<td>279</td>
<td>$2.8 \times 10^4$</td>
<td>&gt;300</td>
</tr>
<tr>
<td>3</td>
<td>$10^3$</td>
<td>193</td>
<td>$1.9 \times 10^5$</td>
<td>281</td>
</tr>
<tr>
<td>4</td>
<td>$10^4$</td>
<td>107</td>
<td>$1.1 \times 10^6$</td>
<td>245</td>
</tr>
<tr>
<td>5</td>
<td>$10^5$</td>
<td>88</td>
<td>$8.8 \times 10^6$</td>
<td>231</td>
</tr>
<tr>
<td>6</td>
<td>$10^6$</td>
<td>54</td>
<td>$5.4 \times 10^7$</td>
<td>195</td>
</tr>
<tr>
<td>7</td>
<td>$10^7$</td>
<td>25</td>
<td>$2.5 \times 10^8$</td>
<td>178</td>
</tr>
<tr>
<td>8</td>
<td>$10^8$</td>
<td>10</td>
<td>$1.0 \times 10^9$</td>
<td>164</td>
</tr>
</tbody>
</table>

Note: TMTC-too many to count
The number of bacteria per mL solution was calculated by considering the size of the microscopic fields and the volume of the transferred bacteria solution (10 µL) using equation (3.1) as follows:

\[
BB = (BF \times 10^4 \times DF)/AF
\]  

(3.1)

Where:

- BF = average number of bacteria per field
- \(10^4\) = conversion factor
- DF = dilution factor
- AF = area of microscopic field (mm\(^2\))
- BB = number of bacteria per ml solution

### 3.5.2 Spectrophotometer Technique

This is a rapid and inexpensive method to monitor bacterial growth. Optical density was measured by a visible spectrophotometer VS721G (see Figure 3.5), to measure the concentration or turbidity of bacteria in suspension. As visible light passes through a cell suspension the light is scattered. A greater scatter indicates that more bacteria or other material is present. The amount of light scatter can be measured in a spectrophotometer. Typically, when working with a particular type of bacteria cells, it is possible to determine the optical density at a particular wavelength that correlates with the different phases of bacterial growth. Generally, bacteria cells that are in their mid-log phase of growth are always preferred. The absorbance or optical density (OD) of the bacteria in suspension was read at a wavelength of 600 nm (OD\(_{600\text{nm}}\)) and was used to determine the approximate concentration in the cementation media. Following the plate counting method in this study, the bacterial density in each serial dilution was determined using the spectrophotometer; the corresponding results are shown in Table 3.4.
3.6 Biomass Density Calibration Chart

All dilution tubes and pipette tips were prepared by autoclaving for 15 minutes at 121 °C and 21 psi. A 0.45% urea solution was prepared for dilution of the samples (serial dilution). To quantify the cell numbers, a serial dilution of the original or stock culture was prepared with dilution factor of 10. Subsequently, 1 mL of the diluted sample was aseptically pipetted into three Petri dishes and spread on the agar surface with a sterile glass spreader. Finally, the number of cfu/mL in the agar plates was counted after 2 days of incubation at 30 °C. The viable count (cfu/mL) was plotted against $A_{600}$ readings to establish a calibration chart that has a positive correlation $R^2=0.95$, and is given by Equation (3.2). This relationship was used to convert absorbance readings to viable cell counts during batch experiments for the kinetic study. A Christensen urea growth medium was prepared with the same liquid medium used to prepare cell colonies from stock cultures, but without the agar powder and pH was adjusted to 6.8-7. All cells were grown at 30 °C in this study.

$$Bacteria\ cells\ \left(\frac{CFU}{mL}\right) = 20 \times 10^9 \times A_{600}$$ \hspace{1cm} (3.2)

A detailed discussion on the established calibration chart and the relevant conversion factors are included in Chapter 4, Section 4.4.
3.7 Conductivity Meter Technique

A conductivity meter Model CDM210 from Radiometer Analytical was used in this study (refer to Figure 3.6). It was used to measure the electrical conductivity in liquid media, which is a common technique used to monitor the amount of nutrients in water. In this study, this technique was used to estimate the concentration of urea and ammonium over time during the batch analyses. The conductivity probe gives a reading in the range from 0.01 µS/cm to 400 mS/cm, and this has been calibrated to estimate the urea concentration. Ammonium ions were measured directly using an ammonium selective electrode. We have used a Radiometer electrode (reference no ISE25NH4) with concentration and pH range of $3 \times 10^{-6}$ – $10^0$ M and 3 – 8, respectively.

![Conductivity meter (Model CDM210 from Radiometer Analytical)](image)

Figure 3.6: Conductivity meter (Model CDM210 from Radiometer Analytical)

3.7.1 Ammonium Concentration Calibration Chart

The concentration of $\text{NH}_4^+$ was directly measured using selective electrode of Radiometer Analytical® (reference no. ISE25NH4). The calibration test was conducted using ammonium chloride standards which were diluted with deionized water to the range of 0-1 M. A linear correlation ($R^2 = 0.993$) was established between molar concentration of $\text{NH}_4^+$ and the conductivity of solution (X) in mS/cm, as shown in Figure 3.7. The relationship between
conductivity change and ammonium concentration is shown in Equation (3.3). Changes of pH were monitored using phenol red in growth analysis, and during the batch experiments using a digital pH meter with ±0.1 pH resolution.

\[ NH_4^+ (M) = 0.1871 \times \text{Conductivity} - 1.5014 \]  

(3.3)

![Figure 3.7: Calibration chart of conductivity change versus ammonium concentration](image)

### 3.7.2 Urea Hydrolysis Rate Conversion Chart

The urea hydrolysis rate was determined by measuring the relative change in conductivity when urea was exposed to purified urease enzyme. During the calibration process, conductivity was recorded under standardized conditions with a range of urea concentrations from 0.5 M to 8 M at 30 °C and an initial pH of 7. The calibration is based on the rise of conductivity that results from the ammonium production during urea hydrolysis.

The rate of conductivity increase (mS/min) was converted to a urea hydrolysis rate (mM urea hydrolysed/min) using Equation (3.4) by relating the conductivity measurements of test samples against standards containing purified urease (supplied by Sigma Aldrich U7752). The calibration chart is strictly a function of temperature and the starting pH of the reaction. Figure 3.8 was established for *B. megaterium* under standardized conditions of 30 °C and urease activity was accounted for by standardization of all activities to pH 7. Although Figure
3.8 shows a non-linear response, the calibration assumes linearity. This has been assumed because the measurements are sensitive to temperature, and small temperature changes are believed to explain the apparent non-linearity.

\[
Urea \text{ hydrolyzed (mM/min)} = \text{Conductivity (mS/cm)} \times 66.5
\]  \hspace{1cm} (3.4)

---

![Figure 3.8: Calibration chart of conductivity change versus hydrolyzed urea concentration](image)

3.8 Procedure for Batch Analyses and Measurements

Kinetic data for \textit{B. megaterium} have been obtained by carrying out batch experiments. A liquid medium rich in urea and glucose as the energy and carbon sources, respectively, was poured into a 50 mL beaker in an aerobic environment. Bacterial cultures were then introduced to the system. Samples were collected every half an hour to measure the bacterial concentration using the spectrophotometer. Ammonium and urea concentrations were obtained using the conductivity meter technique.

3.8.1 Measuring Biomass Concentration

Variations in the biomass concentration in batch experiments were estimated from the absorbance using the calibration chart, as described in Section 3.7. The measurement of absorbance is a rapid and inexpensive method of monitoring bacterial growth. In order to use
this chart, samples of 1 mL were taken from the 50 mL beaker and aseptically pipetted into the cuvette. This was followed by placing the cuvette in spectrophotometer and taking an absorbance reading, as explained in Section 3.6.2. Finally, the number of cfu/mL in the cuvette was estimated using the relationship established between viable count (cfu/mL) and absorbance readings as shown in Equation (3.2). This equation was used to convert absorbance readings to viable cell counts.

3.8.2 Measuring Ammonium Concentration

The concentration of ammonium was monitored periodically using a selective electrode probe of Radiometer Analytical® (reference no. ISE25NH4). The conductivity measurement was then converted to concentration using the calibration chart from Figure 3.7.

3.8.3 Measuring Concentration of Hydrolyzed Urea

Hydrolyzed urea was evaluated incrementally, assuming that every mole of urea hydrolyzed produced 2 moles of ammonium based on the chemical reaction explained in Section 2.3. The conductivity measurements were then converted using the calibration chart shown in Figure 3.8.

3.8.4 Measuring Urease Activity

Urease activity was measured using a conductivity meter assay during the lag and exponential growth phases, as described in Section 3.8. Specific urease activity was defined as the amount of urease activity per unit of biomass and was calculated as shown in Equation (3.5).

\[
Specific\ Urease\ Activity (mM\ urea/min/A_{600}) = \frac{Urease\ Activity\ (mM\ urea/min)}{Bacterial\ Density(A_{600})} \quad (3.5)
\]

Cell absorbance was read at a wavelength of 600 nm and the bacterial density (BD) in the solution was determined using the calibration described in Section 3.7. Care was taken not to
shake the samples, as shaking the culture caused cell clumping which produced inaccurate cell counts. Changes of pH were monitored using a digital pH meter with ±0.1 pH resolution throughout the batch experiments.

3.9 UCS and Triaxial Testing Equipment

The sample preparation procedure for both UCS and conventional triaxial testing is described in this section.

3.9.1 Soil

Sydney sand is comprised of uniformly-graded quartz minerals with sub-angular particles and was supplied by Euro Abrasives. No fines were discovered during the sieve analysis test. The particle size distribution of Sydney sand used in this research is shown in Figure 3.9, and the index properties, determined following Australian standard methods, can be found in Table 3.5.

Figure 3.9 Grain size distribution curve
### Table 3.5 Index Properties of Sydney sand

<table>
<thead>
<tr>
<th>Property</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Effective grain size, $D_{30}$ (mm)</td>
<td>0.31</td>
</tr>
<tr>
<td>Mean grain size, $D_{50}$ (mm)</td>
<td>0.39</td>
</tr>
<tr>
<td>Coefficient of Uniformity, $C_u$</td>
<td>2.35</td>
</tr>
<tr>
<td>Coefficient of Curvature, $C_c$</td>
<td>1.45</td>
</tr>
<tr>
<td>Specific gravity, $G_s$</td>
<td>2.65</td>
</tr>
<tr>
<td>Maximum void ratio, $e_{max}$</td>
<td>0.77</td>
</tr>
<tr>
<td>Minimum void ratio, $e_{min}$</td>
<td>0.57</td>
</tr>
</tbody>
</table>

### 3.9.2. Preparation of UCS Samples

#### 3.9.2.1 Mould Fabrication

A split mould was used to prepare cemented Sydney sand using Gypsum and biocement. The mould was fabricated to form cylindrical sample of 55 mm diameter and approximately 110 mm in height. It has been deliberately designed so that the sample can also accommodate bender elements, however in the tests presented in this thesis no bender elements were used in the UCS tests. It consists of four parts, as shown in Figure 3.10, the base, collar, the cylindrical split mould and the top cap. At the middle of the top cap there is a protruding plate (1 mm x 4 mm x 8 mm) to make a slot for the bender elements to fit in. The collar was used to hold the split moulds in place and vertically upright.
3.9.2.2 Mixing Procedure

The gypsum cemented samples have been prepared by mixing gypsum, sand, and water with amounts of gypsum in the range of 5 % to 20 % of the dry weight of the sand. Based on the general practice of deep soil mixing activity on site, this binder range is considered being the most cost effective approach and with minimum socio environmental impact. The dry Sydney sand and gypsum were mixed together in a mixing bowl until no clumps could be seen. Distilled water was then added to the mixture so that the water weight was around 10 % of the total weight of sand and cement used. The mixture was stirred thoroughly for about a minute before it was placed into the mould. In order to prepare repeatable samples with uniform dry density the sand cement mixtures was placed in 5 layers and each was lightly tamped after placing it into the mould.

The preparation of the bio-cemented samples was more involved. The first step in producing the bio-cemented samples was to culture the bacteria. This was done by placing a KWIK STIK™ directly into the liquid medium, as explained in Section 3.4.2.2. It was then placed in an incubator at a temperature of 30 °C for 24 hours. Before mixing with the sand, 1 mL of the bacterial liquid was pipetted into a cuvette so that its absorbance could be checked using the
spectrophotometer. An absorbance value of at least 0.45 was required from the spectrophotometer, indicating a bacterial concentration of $9 \times 10^9$ CFU/mL before it could be used. The highly enriched bacterial solution of 99 mL was then mixed with the pre-mixed dry ingredients (sand, urea powder and calcium chloride powder) before adding some extra water to make up the weight of water in the mixture to about 10 % of the weight of the dry ingredients. The mass of nutrients (urea and Calcium chloride) were in the range of 0.25 % to 20 % of the dry weight of the sand. The sand-cement mixture was placed into the split mould as shown in Figure 3.10.

3.9.2.3 Mould Preparation and Sample Extraction

Before placing the soil in the mould, the internal surfaces of the split mould were lubricated with silicon grease to minimize the wall and soil adhesion during sample extraction as shown Figure 3.11 (a). With the help of an o-ring stretcher, o-rings were used to seal the mould so that no mixture or water could escape during tamping as shown in Figure 3.11 (b). The split mould was then located on its base using the collar as shown in Figure 3.11 (c). The soil-cement mixture was carefully placed into the mould using a scoop and was tamped with a rod as shown in Figure 3.11 (d). The mould was then filled with 5 layers of soil-cement mixture and each layer was lightly tamped 10 times to produce samples with consistent and uniform density. After the final layer, the top surface was leveled using a straight edge as shown in Figure 3.11 (e). The split mould was removed after 24 hours and the samples were left for an additional 6 days to cure as shown in Figure 3.11 (f). The height and mass of the specimen were then measured to compute the dry unit weight before placing it in loading frame.
Figure 3.11: UCS test sample preparation using the split mould technique
3.9.2.4 Testing Program

The sample preparation technique produced cylindrical samples every time with flat, smooth ends a diameter of 55 mm, and a height of approximately 110 mm. After extraction and curing, the specimens were placed directly into a compression machine. A Wykeham Farrance Tritech 50 kN compression test machine (Figure 3.12) was used to perform all the unconfined compressive strength (UCS) tests. The motorized compression machine controlled the axial deformation, which was measured by a displacement transducer (LVDT) mounted on the base platen. A top platen was placed on the samples, and this was connected to the reaction frame through a steel ball seat, an extension rod and a load cell. The axial load was applied using a rate of axial displacement of 1.14 mm/min until failure. The tests were controlled, logged and automated using the in-house developed software, TRIMS.

![Figure 3.12: UCS loading machine (Wykeham Farrance Tritech 50 kN)](image)

3.9.3 Preparation of Triaxial Samples

Triaxial tests have been performed on uncedmented, gypsum cemented and bio-cemented samples. The different procedures used in preparing these samples are described in this section. In all cases the samples were formed directly onto the triaxial base pedestal and the
computer controlling the tests and logging the data was running from the time when the top platen was placed.

3.9.3.1 Uncemented Sample

Preparing uniform samples for triaxial tests has always been a delicate and challenging process, especially when it comes to uncemented samples. Uncemented samples were prepared by pre-weighing the specific amount of sand to obtain the desired relative density and the sand was poured through a funnel at a fixed height to maintain the density each time. Figure 3.13 shows photographs of the various steps of preparation for uncemented sand samples. An O-ring was placed on the pedestal base shown in Figure 3.13 (a) and an annular porous disc was placed on top of it. This was followed by the placing of a filter paper with a slot created for the bender element, as shown in Figure 3.13 (b). The split mould was then attached together using O-rings, one at the bottom, two at the top and then the rubber membrane was stretched and folded over carefully as shown in Figure 3.13 (c). The split mould was then mounted on the pedestal, resting on the O-ring already placed on the pedestal. Vacuum was applied to hold the membrane tightly to the mould during the sand pouring process as shown in Figure 3.13 (d). A funnel was clamped at a fixed height using a magnetic retort stand to control the relative density of the sample prior to pouring of sand, as shown in Figure 3.13 (e). Once the sand reached the anticipated level, the surface was leveled prior to the placement of filter paper and the top porous stone. After placing the top cap at the right position, the folded membrane was then stretched over the top cap carefully and the O-rings were slid from the mould over the top cap and onto the bottom pedestal. A small vacuum was then applied to the top drain to maintain the integrity of the sample while the split mould was then removed slowly from the sample as shown in Figure 3.13 (f) and the dimensions of the samples recorded. After the triaxial cell was assembled and a small confining stress of 10 kPa applied the suction was released and the sample was ready to be tested.
Figure 3.13: Steps in preparing uncemented sand samples for a triaxial test
3.9.3.2 Gypsum Cemented Samples

The procedure to prepare a gypsum cemented sample was similar to that outlined for preparing un cemented samples, except for the mixing of gypsum with sand and water. A known amount of clean and dry sand was placed into a mixing bowl. This was followed by adding gypsum to achieve a predetermined percentage of the dry weight of sand used. Water weighing 10% of the total weight of sand and cement was then added and the mixture was stirred thoroughly for about a minute before being placed into the mould. To prepare uniform and repeatable samples the material was placed in five layers each of which was lightly tamped. Once the mixture reached the anticipated level, the surface was leveled prior to the placement of filter paper and the top porous stone. As soon as the top cap was placed on the sample the data-logging program was started to record the bender element waveforms during the curing process. The procedure for acquiring and interpreting the bender element data is described in more detail below. After 12 hours when curing was complete, the folded membrane was then stretched over the top cap carefully and the O-rings were slid over the end platens. The split mould was then removed slowly from the sample. The height and the diameter of the sample were measured to estimate the volume. After curing, specimens were tested to failure in standard isotropically consolidated drained and undrained triaxial tests, using a range of confining pressures.

Figure 3.14: Procedure of (a) mixing and (b) molding cemented samples for triaxial test
3.9.3.3 Bio-cemented Sample

Most of the procedures outlined here are similar to the procedures necessary to prepare gypsum cemented samples, except, for the mixing of the nutrients and bacterial liquid with the sand. A known amount of clean and dry sand was placed into a mixing bowl. This was followed by adding the required amounts of urea powder and calcium chloride powder, based on a percentage of the dry weight of sand. Prior to this step, the bacterial liquid had to be prepared to reach the optimum biomass before mixing. The procedure to prepare the bacterial liquid has been described in detail in section 3.4.2.2. The mass of nutrients (urea and calcium chloride) were in the range of 5 % to 20 % of the dry weight of the sand. Additional water was added to bring the added moisture up to about 10 % of the weight of the dry ingredients. Preliminary tests indicated that for moisture contents higher than this the additional water was not held by the sand. Hence to obtained uniform and repeatable specimens the moist material was lightly tamped into the mould at this moisture content. It was also important to have sufficient water to ensure dissolution of the urea and calcium chloride powders. Examination of the amounts of calcite precipitation post-test indicated these were consistent with all the nutrients reacting, indicating that 10 % water content sufficient to ensure the full dissolution. The mixture was then stirred thoroughly for about a minute before being placed into the mould. The sand-cement mixture was placed into the split mould as shown in Figure 3.10. In order to prepare uniform and repeatable samples five layers of the mixture were lightly tamped after being placed into the mould and the rest of the steps were similar to the procedure used for preparing gypsum cemented samples except that as shown in Chapter 5 the curing time for the biocemented samples was much longer. After curing the sample height and diameter were measured. The details on the various mix compositions and void ratios of the samples can be found in Appendix A1.

3.9.4 Standard Compression Triaxial Tests

All the tests were conducted in a temperature controlled room using a semi-automated triaxial system consisting of an instrumented Wykeham Farrance Tritech 100 kN compression test machine, two GDS pressure controllers, a water supply tank, amplifiers, and a computer as shown in Figure 3.15. These instruments were calibrated prior to testing and the calibrations were checked every six months. The motorized compression machine controlled the axial
deformation, which was measured externally by a displacement transducer (LVDT) mounted on the lower base platen. The two GDS devices controlled the pressure and volume change of the triaxial cell and sample pore pressure. The vertical load on the specimen was measured using both an internal submersible load cell and an external load cell. The use of the internal load cell eliminated the correction required for shaft friction of the loading piston during loading. The external load cell was included to provide an external check, and back up in the event of a failure of the internal device, and the loading piston was lubricated to minimize friction. The pore pressure transducer was connected to the base pedestal to measure the pore pressure at the bottom of the specimen. The tests were controlled using in-house developed data acquisition and control software.

Figure 3.15: Standard triaxial test equipment with bender elements

3.9.5 Testing Procedure

Triaxial tests have been performed on uncemented, biocemented and gypsum cemented Sydney sand samples. The standard procedure of the tests, after sample preparation consisted of three stages: saturation, consolidation and shearing. When the triaxial cell was assembled and filled with water a cell pressure of 10 kPa was applied and the sample allowed to drain from the atmospheric outlet. This was followed by the saturation stage. For uncemented and gypsum cemented samples a small pressure of about 5 kPa was applied to the base drain while the top drainage line was left open to the atmosphere. Water was permeated through the
samples until no further air bubbles could be detected at which point further drainage from the top was prevented. For the bio-cemented specimens it was observed that permeation caused calcite precipitate to be flushed out of the sample. While this did not appear to affect the cementation as the shear wave velocity of the cured samples remained essentially constant, the presence of the precipitate in the drainage lines was undesirable. Thus the permeation stage was not conducted for the majority of the bio-cemented samples. The back pressure and cell pressure were then increased together at the rate of 10 kPa/min, maintaining a constant effective cell pressure of about 10 kPa, until the back pressure reached 300 kPa. All the samples were saturated by maintaining a constant effective pressure until negligible change was observed in the pore volume and the pore pressure coefficient, $B$ was greater than 0.96. The $B$-value check was conducted by closing the top and the bottom drainage taps and increasing the cell pressure by 10 kPa ($\Delta \sigma_3$). The corresponding rise in pore pressure ($\Delta u$) was then recorded and the $B$ value calculated as $\Delta u/\Delta \sigma_3$. The specimen was considered sufficiently saturated if $B$ was more than 0.96.

Samples were then isotropically compressed by ramping up the cell pressure and measuring changes in pore volume and shear wave velocity. Shear tests were performed for effective cell pressures between 20 kPa and 500 kPa.

The samples were then sheared to failure, either drained or undrained, at a constant axial deformation rate of 0.001 %/min. If a pronounced shear plane developed shearing was terminated, otherwise tests were continued to axial strains of 20 %.

### 3.9.6 Shear Wave Velocity Measurement

Shear wave velocity measurements have been obtained using bender elements (2 piezoelectric ceramics) located at the top and bottom platens of the triaxial apparatus, as shown in Figure 3.13. Signals from the benders were continuously captured throughout the tests, during curing (cementation) of the sample, consolidation, and shearing phases. The results from these tests are discussed in Chapter 5. The equipment, method and interpretation procedure used in these tests has been described in detail by (Mohsin and Airey, 2003 and Airey and Mohsin, 2013) and only a brief overview is provided here.
3.9.6.1 Function Generator

Waveforms applied to the bender elements were generated by a function generator model HP33120A. It uses a direct digital synthesis technique to create stable, accurate output signals. It has four built in waveforms: sine, square, triangle and ramp. Besides these, user-defined waveforms can be downloaded or stored in its memory. The operating frequency range is 100 µHz to 100 kHz for triangle and ramp waveforms, and 100 µHz to 15 MHz for sine and square waveforms, respectively. In this study predominantly sine waves were used. A single sine wave was sent 100 times per second, and the traces were viewed on the oscilloscope. The amplitude was set at 10 V peak to peak, which was the maximum that could be generated by the function generator. The frequency of the sine wave was adjusted manually to maintain

\[ f \cdot t \approx 4 \]

where \( f \) is the frequency and \( t \) the wave travel time.

Figure 3.16: Function generator, model Agilent HP33120A.
3.9.6.2 Oscilloscope

A two channel Digital Oscilloscope, model DL1520L from Yokogawa, was used for this research. It has a maximum sampling rate of 200 MS/s, a maximum record length of 1M words, and a frequency bandwidth of 150 MHz. All channels have full ranges of 1 mV/div to 10 V/div. Data can be downloaded either in ASCII format or in Binary format. The GPIB interface was used for data communication in this research due to its ability to rapidly transfer data to the control software.

Signals from the function generator and from the receiver bender element were displayed on the oscilloscope. The last 256 readings (2.5 seconds) were stacked to obtain stable traces and these were downloaded to the controlling computer every time readings were written to file and when prompted manually.

A cross-correlation between the signal from the function generator and the received signal from the bender element was performed immediately after the waveforms were downloaded to the computer. Then the times of the maximum positive peak and the times of 5 other peaks in the cross-correlation signal were saved. Post processing allowed the time associated with the shear wave arrival to be determined and its evolution followed throughout the tests (Mohsin and Airey, 2003)

Figure 3.17: Digital Oscilloscope, model DL1520L from Yokogawa
3.9.6.3 Bender Element

In this study, bender elements were fabricated in house using Piezoelectric (PTZ) sheets of 0.5 mm thickness. The bender element dimensions varied slightly due to the use of different suppliers, but they were typically 13 mm x 10 mm x 0.5 mm. The Bender elements were encapsulated in epoxy resin and mounted into pucks which were located in the top and bottom end platens (Figure 3.13). The bender elements were mounted such that about two thirds of their length lies in the top and bottom platen of the triaxial, and the remaining one third protrudes into the specimens, that is about 4 mm.

The shear wave velocity, $V_s$ of the soil mass was obtained from the bender elements. The elapsed time, determined by cross correlation between input and output waveforms, together with the wave travel distance was used to compute the shear wave velocity, as shown in Equation (3.6).

$$v_s = \frac{L_{rt}}{t}$$  \hspace{1cm} (3.6)

where $L_{rt}$ is the distance between the centres of the transmitter and receiver bender elements (tip-to-tip distance plus half the distance the benders protrude into the specimen) and $t$ is the travel time of the signal from the transmitter to the receiver estimated from cross-correlation.

Knowledge of the shear wave velocity allows the small-strain shear modulus $G_{\text{max}}$ to be calculated using Equation (3.7).

$$G_{\text{max}} = \rho v_s^2$$  \hspace{1cm} (3.7)

where $\rho$ is the total density of the soil mass.

The shear wave velocity and shear modulus were determined post-test using the updated values of sample height and density that were appropriate at the time of the measurement.
3.9.7 Measurement of Calcite Precipitation

After the completion of the triaxial shearing stage, or the calcite content in bio-cemented samples were determined post-failure through acid washing. Each sample was split into three even sections, and from each 10 g of dry soil sample was taken. Samples were dried in an oven at 105 °C for 24 hours. The measured weight loss of the soil sample was used to estimate the carbonate content in the soil specimen. It was assumed that the weight loss was purely caused by dissolution of calcium carbonate. The calcite content was calculated as a ratio of the weight of calcite to the weight of the soil specimen before the acid wash test by using Equation (3.8)

\[
CaCO_3 = \left( \frac{(M_2 - M_3) - (M_4 - M_3)}{M_2 - M_1} \right) \times 100
\] (3.8)

where,

\[
CaCO_3 = \% \text{ of material soluble in hydrochloric acid}
\]

\[
M_1 = \text{mass of beaker in grams}
\]

\[
M_2 = \text{mass of beaker and test increment in grams}
\]

\[
M_3 = \text{mass of filter paper in grams}
\]

\[
M_4 = \text{mass of filter paper and residue in grams}
\]

The calcite content estimated by this procedure indicates the total carbonate content in the specimen. Some of this calcite may not be contributing to the cementation, being unattached to the grains. The same procedure has been used in all the tests and further microscopic studies would be helpful to confirm the amount of effective cementing material.

3. 9.8 Predicting Theoretical Calcite Precipitation

Calculations were carried out by assuming that every mole of urea used in preparing the samples was completely hydrolyzed and eventually disassociated to ammonium and
carbonate ions. Based on the batch analyses explained in Chapter 4, the specific urea hydrolysis rate was determined as 8 mM urea hydrolyzed per minute for every unit of biomass. The following equation (3.9) was used to predict the theoretical amount of calcite precipitated in every sample tested in the following chapters.

$$CaCO_3 = \frac{UC \times 16.7}{V_{max} \times CT \times BD} \times 100$$  \hspace{1cm} (3.9)

where,

- $CaCO_3 = \%$ of theoretical calcite precipitated
- $V_{max} = \text{maximum urea hydrolysis rate mM/min/OD}$
- $BD = \text{Biomass Density in absorbance unit}$
- $CT = \text{Curing time in min}$
- $UC = \text{Urea content in grams}$

### 3.10 Small Scale Foundation Tests

The main objectives of these tests were to demonstrate the ability to create bio-cemented soil columns using various soil mixing strategies and to demonstrate the potential of these techniques to improve the foundation performance. The procedure for creating the cemented soil columns was designed to be in accordance with deep soil mixing technology. The performance of the cemented columns has been assessed by placing a rigid foundation, with diameter 2.4 times that of the columns, on the soil surface and loading the footing to large displacements. The cemented columns fractured during loading and some additional tests were performed in which the foundations were repaired using the bio-cement. Details of the model test equipment, the methods used to form the columns and the testing procedure are described in this section.
3.10.1 Set-up

The apparatus shown in Figure 3.18 has been developed for the small scale foundation tests. It consists of a 600 mm diameter vessel that is 500 mm high, and is filled with Sydney sand. The column forming system consists of a vertical frame that enables the lowering and raising of the auger spinning motor, the auger, and a tube holder. In order to maintain experimental consistency and the reproducibility of column formation, a three level travel stop (L1, L2 and L3) mechanism has been used. This enables the column length and the rate of introduction of the cementation liquid and nutrients over the length of the column to be controlled accurately and consistently. The speed of the auger spinning motor is adjustable from 5 to 50 rpm. The shaft length of the auger and the blades are 350 mm and 35 mm, respectively.

Figure 3.18: Physical model setup for small scale footing test
3.10.2 Preparing Sand Bed

The Sydney sand used in this study was relatively uniform and clean from any debris. The physical properties of the sand used in this study have been given in Section 3.9.1. In order to study the effects of soil conditions, sand in the tank was backfilled by pouring at a fixed height and sand was prepared in three conditions; dry, partially saturated, and fully saturated. Tests were performed with different degrees of saturation in the tank to replicate the range of conditions in-situ and to explore how these would influence the amount of calcite precipitated and the response of the columns. It may be noted that Cheng et al. (2013) have shown that the cementation strength is affected by the degree of saturation and these test allowed these aspects to be investigated. Approximately, 178 kg of dry sand was poured into the tank at a fixed height of 500 mm using a bucket to achieve a relative density of 49% (loose). To prepare partially saturated sand, sand with 20% water content and mass of 218 kg was filled up to 400 mm in several layers and tamped lightly using a wooden block for each layer, as shown in Figure 3.19 (a). A target relative density of 66% (medium dense) was used and maintained throughout the testing program. For 100% saturated sand, water was continuously poured in, to the previously prepared sand bed until no visible air bubbles could be seen flowing out through the outflow drainage line, located at the bottom of the tank. When there were no bubbles seen, the drainage valve was closed and the sand was inundated with clean water and left overnight, as can be seen in Figure 3.19 (b).

![Figure 3.19: Techniques used to prepare soil for (a) wet (b) saturated conditions in the Physical Model test](image-url)
3.10.3 Model Auger Design

A model auger was designed to mix and produce the cemented columns. This auger was designed to a \( \frac{1}{10} \)th scale and was based on augers commonly used in the field. It is approximately 350 mm in length, with a diameter of 35 mm. The diameter was chosen in order to produce sample columns of 38 mm diameter with 200 mm and 100 mm lengths. There are 13 mixing blades fixed at a distance of 15 mm from each other, as shown in Figure 3.20 (a). These blades were arranged at 45° angles from the previous blade, as shown in Figure 3.20 (b). The main difference between the model auger and the full sized site auger was that the latter has grout injection ports placed near the toe of the auger. The model auger, which was attached to a revolving mechanism, was too small to allow for biocementation liquid entry through the top of the shaft. It was deliberately designed in this study without the injection point to demonstrate the feasibility of using mixing techniques to promote biocementation instead of injection technique. A funnel was used to manually add the biocementation liquid and the nutrients (urea and calcium chloride), as shown in Figure 3.21. This ensured that the treatment operation would be taking place under controllable environmental conditions. A variable speed electrically powered motor was used to propel the model auger in the confines of the more controlled laboratory environment.

![Laboratory scale model auger in (a) side and (b) plan view](image)

Figure 3.20: Laboratory scale model auger in (a) side and (b) plan view
3.10.4 Mould and Tube Extraction

In order to replicate the casing used to form cement columns during deep soil mixing in sandy soils PVC tubes were used. The internal diameter was 37.5 mm and the external diameter was 42 mm. PVC column lengths of 230 mm and 330 mm were used to mix and form cemented column lengths of 100 mm and 200 mm, respectively. A clamp was fabricated to hold the PVC tubes in place during mixing and to create a vertically aligned column every time. After mixing, the screws holding the tube were loosened and a collar with a handle was clamped to the tube to pull out the casing, that is the PVC tube as in Figure 3.22.
3.10.5 Creating Cemented Columns

Figure 3.23 shows the step by step procedure involved in mixing a column for a footing model test. Cemented soil columns of 38 mm in diameter and extending either 200 mm or 100 mm below the surface, have been created in the center of the confining vessel. A cross member was attached to the confining vessel to provide location and support for the column casing (PVC tube) and to guide the auger spinning motor up and down as shown in Figure 3.23 (a). To create a cemented column in dry soil, a PVC tube attached to the fixed frame is pushed into the tank through the guide to ensure the verticality of the column, as shown in Figure 3.23 (b).

Several trial mixes were carried out to find the best way to mix and to form uniformly cemented columns. Visual inspection was used to verify and validate the uniformity and the quality of the created cemented columns. This was normally done after the curing stage, where samples were simply extracted from the model test tank. In the case of mixing in dry sand, initially materials (e.g. gypsum, urea and calcium chloride) were poured from a funnel in one shot. Despite extensive operation of the mixing tool it was observed that soil at the bottom part of the tube was not properly mixed with the binding material. After the water (gypsum) or bacterial liquid (bio-cement) was poured in, the soil at the bottom part of the tube was not cemented. Figure 3.24 (a) and (c) show the cemented columns produced due to this technique. To avoid this problem, binding materials were added in three steps. While the auger is in the upper L1 position (position as shown in Figure 3.23 (c)), one third of the dry urea and calcium chloride powders, or gypsum, are carefully poured in using a funnel (as shown in Figure 3.23 (f)). The auger is then moved to the middle L2 position, as shown in Figure 3.23 (d) and soil is thoroughly mixed with the additives (gypsum/urea powder) at a medium speed. The auger penetration and withdrawal is controlled manually by rotating clockwise at a constant speed. After 30 seconds of mixing, the auger is lowered to the bottom L3 position as shown in Figure 3.23 (e), and mixing continues. The next third of the binder materials are added and the mixing procedure is repeated until the remaining urea and calcium chloride powders (or gypsum) are thoroughly mixed in. This is followed by pouring in the liquid, either the bacterial solution for the biocement or water for the gypsum, before ending with two more cycles (L2 and L3) of mixing at maximum speed.
Immediately after finishing mixing the auger is detached from its holder, the column is gently tamped to counter any loosening during auger withdrawal, and the PVC tube is pulled out. The column is then left in the vessel for 24 hours to allow for cementation to occur before loading.

Preparation of columns mixed in wet and saturated soil was also challenging. A similar procedure to that used for dry sand was also used to create cemented columns in wet and saturated soil, except that in this case liquid did not need to be added and the bacterial solution was injected using a syringe. The curing period was also extended from 24 hours to 36 hours for wet soil conditions and 48 hours when it was fully saturated. In the case of gypsum, mixing was carried out in less than two minutes as reactions occurred quickly in all the soil conditions. It was found that longer times were required to achieve sufficient strength in the bio-cemented columns formed underwater when the soil in saturated condition. In preliminary tests when the bacterial liquid was poured from the top reactions occurred immediately and calcite was precipitated. It was observed that the calcite could not be distributed evenly with this procedure. To avoid this problem, the bacterial solution was injected starting from the bottom of the column. This ensured that calcite was distributed uniformly during mixing all around the column. Figures 3.24 (b) and (d) shows samples which were well formed using the final technique.
Figure 3.23: Procedures for column mixing before the footing test
3.10.6 Model Footing Tests

Figure 3.25 shows the experimental setup and sequence of steps involved in a model footing test on biocemented and gypsum cemented sand. Prior to testing the cemented columns, the vertical and the horizontal frames used for mixing the columns have to be removed from the tank. This is followed by pushing in the tank below the support frame so that the shaft holding the load cell would be aligned right on the middle of the tank. A 90 mm diameter circular footing, 12 mm thick, was placed on the sand surface and the surface was leveled flush with the top of the cemented column (refer to Figure 3.25 (a)). The next step was manually driving down the shaft so that the load cell would slightly touch the ball bearing placed on top of the footing (as in figure 3.25 (b)). This is followed by placing the LVDT on the side of the load cell resting on a flat steel bar to accurately measure the settlement of footing during loading (as in figure 3.25 (c)). At this point of time the StrainSmart® software
is used to reset the already calibrated load cell and LVDT to zero before starting the test (Figure 3.25 (d)). Now, when it is ready, the switch button on the gear box was clicked to start the button on software and the switch button on the gear box was then turned on immediately (Figure 3.25 (e)). The footing has been vertically loaded to large a displacement at a constant deformation rate of 0.076 mm/min. The vertical loads, measured by a 250 kg capacity load cell, and displacement, measured by an LVDT transducer, were automatically logged at frequent intervals. Each test was run until there was a clear drop in the voltage reading, which indicated the failure of the cemented column. As soon as the loading test was completed, the cemented column was retrieved from the tank and the type of failure was observed (Figure 3.25(f)). This was followed by carrying out a carbonate test to quantify the amount of calcite precipitated in each sample. The detailed procedure of this test is described in Section 3.9.7. A series of tests with bio-cemented columns using different urea amounts, as well as with gypsum cemented columns with different gypsum amounts, have been conducted. Additional tests have been conducted without the cemented columns to measure the baseline performance of the natural sand.
Figure 3.25: Procedures involved in loading biocemented columns in a model test
3.10.7 Instrumentation

Two types of instruments were installed to monitor the performance footing model on biocemented Sydney sand. Each instrument was calibrated prior to taking readings and the instruments were connected to a data logger for the automatic recording of data. The system was able to simultaneously read the data from sixteen channels. An s-shaped load cell with an accuracy of ±0.01% of the full scale was placed between the loading shaft and the footing with a capacity of 250 kg to precisely measure the pattern of the applied load when transferred on to the biocemented column surface. An LVDT with an accuracy of 0.01 % of the full range (50 mm) was placed on top of the footing model to provide the settlement reading during loading. The soil was vertically loaded by a mechanically geared motor. The load cell and LVDT were both connected to the data acquisition system (Strain Smart 9000) as in Figure 3.26.

Figure 3.26: Data acquisition system (Model 5100B Scanner)
4.1 Introduction

Microbially Induced Calcite Precipitation (MICP) is a natural biochemical process that is closely dependent on the ability of the bacteria chosen and its limitations. According to Shirakawa et al. (2011), the choice of bacterial strain is an important factor to be considered for successful bio-cementation applications. There are different pathways that are involved in calcite precipitation. The first pathway involves the sulphur cycle, in particular sulphate reduction, which is carried out by sulphate reducing bacteria. A second pathway involves the nitrogen cycle, and more specifically, the oxidative deamination of amino acids, the reduction of nitrate and degradation of urea by ureolytic bacteria. The pathways leading to precipitation of calcite mentioned here are
generally found in nature, which accounts for the common occurrence of MICP and validates the observations by Boquet et al (1973) that under suitable conditions, most bacteria are capable of inducing carbonate. Due to its simplicity, the most commonly studied pathway is urea hydrolysis. The selection of Urease Producing Bacteria (UPB) is dependent on their ability to synthesize active urease. Bio-cementation using urea hydrolysis as the pathway involves two spontaneous biochemical reactions, urea hydrolysis and calcite precipitation as discussed in Chapter 2. Unlike other metabolic pathways, the hydrolysis of urea can be easily controlled and allows for the production of high concentrations of carbonate within a short period of time. Previous studies (Guo et al. 2009; Redden et al. 2013) have shown that the urea hydrolysis rate is the dominating factor that determines the amount of calcite that will eventually form. The larger the ureolysis rate, due to a higher concentration of urease enzyme, the more calcite will precipitate provided that sufficient urea and Ca$^{2+}$ ions present. The reason for this is that one of the calcite forming reactants, bicarbonate ion is produced through urea hydrolysis. Therefore, the more urea that gets hydrolyzed within a certain time period, the higher the amount of calcite that is produced. Also the time required for precipitation is important during practical bio-cementation as rapid reactions will result in clogging during injections of nutrients and exacerbate the difficulty of ensuring uniform cementation. This chapter discusses the results of experiments performed to assess the ability of Bacillus Megaterium to promote urea hydrolysis and produce calcite precipitation. The ureolytic activity reported in this chapter will be used to predict the theoretical (calculated) amount of calcite precipitated in Chapters 5 and 6 of this thesis. The specific objectives of this chapter are:

(1) To gauge the performance of B. megaterium by three indicators; growth rate, urease activity and urea hydrolysis (ureolysis) rate.
(2) To establish a correlation between the bacterial counts using optical density (OD) and the plate count technique to enable successful bacterial growth to be simply estimated.
(3) To find ureolytic kinetic parameters of B. megaterium using the Michaelis-Menten Model.
(4) To compare the potential of B. megaterium in producing urease in relation to other ureolytic bacteria.
4.2 Urea Hydrolysis Pathway

Of the many bacterially mediated processes that can produce cementation effects, bio-cementation via urea hydrolysis is the most attractive because it is simpler than iron and sulphate reduction processes, ammonification, nitrification, and many others (DeJong et al. 2013). Urea hydrolysis driven calcite precipitation has been widely studied due to its potential applications in bio-cementation. To produce effective field scale cementation, urease is required to function for an extended period of time at high concentrations of urea (CO(NH$_2$)$_2$), ammonium NH$_4^+$, and calcium Ca$^{2+}$, as well as across a wide range of temperatures. Especially when soil temperature can fluctuate from 15° to 45° within 24 hours in tropical regions with time and depth (Popiel et al. 2001). One of the earliest descriptions of urea hydrolysis was provided by Warner (1942) who described the reaction through equation 4.1. Later the same reaction was used in other applications including soil stabilization (Harkes, Paassen, Booster, Whiffin, & Loosdrecht, 2010; Stocks-Fischer, Galinat, & Bang, 1999), concrete crack remediation (Bang et al. 2001), and wastewater treatment (Hammes et al. 2003) using ureolytic microbes.

\[ CO(NH_2)_2 + H_2O \rightarrow 2NH_3 + CO_2 \] (4.1)

The breakdown of urea described by Reaction 4.1 is produced by a set of simultaneous reactions that start from urease catalyzing 1 mole of urea intra-cellularly to 1 mole of carbamic acid (NH$_2$COOH) and 1 mole of ammonia (NH$_3$) as described by 4.2.

\[ CO(NH_2)_2 + H_2O \rightarrow NH_2COOH + NH_3 \] (4.2)

Carbamic acid (NH$_2$COOH) spontaneously hydrolyses to form 1 mol of ammonia (NH$_3$) and 1 mol of carbonic acid (H$_2$CO$_3$) as in Reaction 4.3.

\[ NH_2COOH + H_2O \rightarrow NH_3 + H_2CO_3 \] (4.3)

The products in Reaction 4.3 dissociate in water in the following way:

\[ H_2CO_3 \leftrightarrow HCO_3^- + H^+ \] (4.4)

\[ NH_3 + H_2O \leftrightarrow NH_4^+ + OH^- \] (4.5)
Reactions (4.4) and (4.5) change the pH depending on the equilibrium that the reactants and products achieve. Hammes and Verstraete (2002) have investigated the series of events occurring during ureolytic calcification and emphasized the importance of pH and calcium metabolism during the process. The primary role of UPB has been ascribed to their ability to create an alkaline environment through various physiological activities. Therefore, when alkalophiles (microbes that grow optimally in high pH conditions) are introduced, this equilibrium will shift the bicarbonate ion (HCO$_3^-$) equilibrium, resulting in the formation of carbonate ion (CO$_3^{2-}$), according to Reaction 4.6.

$$HCO_3^- + H^+ + 2NH_4^+ + 2OH^- \leftrightarrow CO_3^{2-} + 2NH_3 + 2H_2O$$ \hspace{1cm} (4.6)

If Ca$^{2+}$ is present or introduced to the system, then calcium carbonate (CaCO$_3$) will be formed as given by Reaction 4.7:

$$CO_3^{2-} + Ca^{2+} \rightarrow CaCO_3$$ \hspace{1cm} (4.7)

Bacterial surfaces play an important role in calcite precipitation (Fortin et al. 1997). Due to the presence of several negatively charged bacteria, at a neutral pH, positively charged mineral ions can be bound on bacterial surfaces, favouring the cells as nucleation sites (Douglas and Beveridge 1998; Bauerlin, 2003). Generally, calcite precipitates develop on the external surface of bacteria cells by successive deposition (Pentecost and Bauld, 1988; Castanier et al. 1999) and bacteria can be embedded in growing calcite crystals (Rivadeneyra et al. 1998; Castanier et al. 1999).

In this process, a concentrated NH$_4^+$ salt solution is produced. This is a potential contaminant that may require treatment. The ammonium salt generation thus provides some limitations on the applicability of urea hydrolysis as an effective biocement forming process but is not considered in this thesis. The scope of this research is limited to demonstrating the performance of the bacteria to catalyze urea hydrolysis.
4.3 Ureolytic Bacteria

The types of microbes used in biocementation are believed to have a significant effect on the performance of this technique (DeJong et al. 2013). Previous studies have focused on a small number of microorganisms to precipitate CaCO$_3$ in soil. Even though a wide range of microorganisms are capable of precipitating calcite through urea hydrolysis, very little effort has been made to explore them. Studies have been limited to *Bacillus cohnii*, *Bacillus sphaericus*, *Bacillus subtilis* and *Sporasarcina pasteurii*. For example, *Bacillus sphaericus* (Achal & Pan, 2011) and *Sporasarcina pasteurii* (aka *Bacillus pasteurii*) (Burbank, Weaver, Williams, & Crawford, 2012) have been commonly used in research. However, none of these bacteria has been shown to produce high amounts of urease at an economical level. The most widely investigated bacteria, *Sporasarcina pasteurii* is an alkaliphilic bacterium found in soil, sewage and urinal incrustations (Whiffin, 2004). It is one of the most robust ureolytic bacteria and has been used in industrial applications such as in the bioremediation of cracks (Ramakrishna et al. 2005), the strengthening of concrete (Ramachandran, Ramakrishnan, & Bang, 2001) and biogrouting (van Paassen 2009). In this chapter the potential of *Bacillus Megaterium* to catalyze the urea hydrolysis is investigated and compared with the potential of other ureolytic bacteria. For the purpose of biocementation, this bacterium must fulfill at least four important criteria, it must be capable of economic production; it must have a high level of urease activity; it must be capable of being generated in a non-sterile environment; and it must be able to resist cell lysis (puncture). Urease activity and urea hydrolysis rate have been used in previous research papers in bio-cementation. Urease activity (mM urea/min) quantifies the rate at which the enzyme enables the breakdown of urea and specific urease activity is the urease activity per bacteria. The urea hydrolysis rate (day$^{-1}$) refers to the time it requires for the enzyme produced by bacteria to break down urea compound into ammonium and bicarbonate. They both describe the rate at which urea is de-synthetized and were used in this thesis.
4.4 Relationship Between Biomass and Optical Density

A variety of methods are available to assess the amount of bacteria. These include estimates of the mass of the bacteria (biomass), a count of the number of active live bacteria and indirect observation from water turbidity. Of these methods the most straightforward is the counting method in which the numbers of colony forming bacteria cells in a sample are measured directly after serial dilution. As this method takes at least 2 days, it is inappropriate for a rapid assessment and optical density is frequently used as a surrogate.

![Graph showing the relationship between bacteria cells and optical density (OD) of Bacillus Megaterium](image)

Figure 4.1: Relationship between bacteria cells and optical density (OD) of *Bacillus Megaterium*

The measured data on the relationship between colony forming units per milliliter (cfu/mL) and OD for *B. megaterium* are shown in Figure 4.1. The bacterial cells showed an almost linear relationship with a slope of \(20 \times 10^9\) cfu/mL/OD, however there appears to be significant scatter. In order to validate this relationship, a statistical analysis was conducted using student’s t test. A null hypothesis that both the techniques have identical mean bacterial count was made. At the 5 \% significance level, no significant difference between the sample’s means was found. Thus, the decision to accept the null hypothesis was made. This relationship allows the cell concentrations to be easily determined without tedious procedures being required. The relationship has been used in this chapter and the following chapters of this thesis.
The relationship between biomass and OD is shown in Figure 4.2. The dry bacterial mass (biomass) was determined by centrifuging 2 ml of the bacterial medium followed by drying at 60 °C for 24 hours. The cell concentrations were also monitored by measuring the optical density (OD) at 600 nm wave length. This relationship was represented by a linear regression line where, bacteria mass (mg/mL) = 3.91 x OD. Since the optical density of a bacterial solution can be easily determined using the spectrophotometer, the equation relating OD to mg/mL can provide a quick estimation of the biomass. Finally, a relationship between cfu/mL and biomass was established based on the previous two relationships as shown in Figure 4.3.

The bacterial count or biomass density for *B. megaterium* obtained in this study varies from values reported in the literature. These differences may be due to the techniques used in measuring the cell counts and differences in the morphological nature of the bacteria species. For example, in research conducted by Ng et al. (2013) using the same bacteria, 1.3 OD corresponded to approximately $5 \times 10^7$ cfu/mL. Compared with the present study, the biomass was 3 times less. However, this value is relatively larger than the results of 0.36 mg biomass/mL (1.0 OD) (Yoon et al., 1994) used to prepare a high density culture of *B. megaterium*. On the other hand, Dhani et al. (2013) have used the same type of bacteria for the surface treatment of a green building with a biomass of 3.6 mg/mL (0.58 OD) after the first 72 hours. This is approximately 7% less biomass for a given OD than the present study.

![Figure 4.2](image.png)

Figure 4.2: Relationship between biomass and optical density of *Bacillus Megaterium*
4.5 Growth Rate Parameters

Growth rate and urea hydrolysis data for *B. megaterium* have been obtained by carrying out batch experiments. A liquid medium rich in urea and glucose, as the energy and carbon sources respectively, was poured into Erlenmeyer flasks in an aerobic environment. Bacterial cultures were then introduced to the system. Samples were collected every half an hour to measure the bacterial concentration using the spectrophotometer. Urease activity was measured using conductivity meter assay during the lag and exponential growth phases as described in section 3.8.4. Specific urease activity was defined as the amount of urease activity per unit of biomass and was calculated as shown in Equation 3.5 in Chapter 3.

Cell absorbance was read at wavelength of 600 nm and the bacterial density (BD) (cfu/mL) in the solution was determined using the relationship given by Figure 4.1. Care was taken not to shake the samples as shaking the culture could cause cell clumping, which would produce inaccurate cell counts. Changes of pH were monitored using a digital pH meter with ±0.1 pH resolution throughout the batch experiments.

Growth rate parameters were obtained from batch experiments using bacteria which were transferred into the liquid medium directly from the swab stick (KWIK STIK™). The procedure
of inoculation of bacteria in liquid medium was explained in Section 3.4.2.2. One KWIK STIK™ was used for each experiment and the liquid medium was prepared using the recipe in Table 3.2. The experiments were repeated using a range of urea concentrations from 250 mM to 2 M. Experiments were conducted in triplicates for each urea concentration and the average results were used to study the bacterial growth. Growth curves were obtained by measuring optical density (OD) at regular exposure times. The cell count was determined from the OD measured with the spectrophotometer using the calibration chart, Figure 4.1. The growth curves of *B. megaterium* depicted in Figure 4.4 show a trend typical of many bacteria. There is a lag phase, a period of exponential growth and then a stationary (mature) phase. The growth curves were used to estimate the main growth parameters, lag phase duration (λ) and maximum specific growth rate (μ<sub>max</sub>).

![Figure 4.4: B. Megaterium growth curve showing the general relationship between changes in cell count and urea content](image)

The growth parameters were determined using the technique illustrated in Figure 4.5. The lag phase time was identified from the intersection of two straight lines fitted to the initial growth phase and the rapid growth phase. Normally, when bacteria are introduced into a new
environment, it takes some time before they start growing rapidly. This period is known as the lag phase when the bacteria are adjusting to the environment. Bacteria do not immediately reproduce, however, the cells are metabolically active and sometimes small increases in the number of cells are observed (Cooper and Lenski, 2000). During the lag phase bacteria are also synthesizing the enzymes and changes occur to factors such as pH, temperature, nutrients, oxygen and the water needed for cell division and population growth under their new environment. The lag phase recorded in this study was in the range of 5 hours to 8 hours. Following the lag phase is the log phase, in which the bacteria grow in a logarithmic (exponential) fashion. As the bacteria multiply, they consume available nutrients and produce waste products. The slope of the second straight line in Figure 4.5 gives the specific growth rate of these bacteria in cfu/mL/h units. The growth rate of B. megaterium for a given urea concentration in this study was in the range of 1.5 x 10^9 cfu/mL/h to 3.0 x 10^9 cfu/mL/h. Generally, when the nutrient supply is depleted, the bacterial growth will be inhibited and bacteria will start to decay. According to Norvick (1955), the bacterial decay rate will slowly pressure the bacteria to reproduce and go dormant in the face of declining nutrients concentrations and increasing waste concentrations.

![Figure 4.5: Growth curve of B. Megaterium used for estimation of lag time and the maximum specific growth rate](image-url)

Figure 4.5: Growth curve of B. Megaterium used for estimation of lag time and the maximum specific growth rate
In another study the growth of *B. megaterium* was characterized, in optimal conditions, by a growth rate of 0.093±0.005 AU/h and a lag phase of 5.6±0.2 hours when urea was substituted with other substrates (Periago et al. 2006). In this study, the average growth rate was 2.6 x 10^9 cfu/mL/h (≈ 0.13 AU/h ±0.005) and the lag phase was 7 hours, as shown in Figure 4.5. According to Periago et al. (2006), an increase of 40-60% in growth rate was observed when urea was used as substrate compared to nisin, carvacrol and thymol (the substrates used in the previous studies), showing that urea is a suitable substrate for this type of bacteria. Accurate estimation of lag time is essential in many areas of research and application. For example, sufficient lag time is required to control the rate of calcite precipitation without clogging of injection points. However, for the mixing technique used in this research the lag time is not so critical as clogging would not be an issue, but the lag time needs to be sufficient to allow mixing without significant precipitation occurring. This bacterium is suitable for applications in practice just like other bacteria. For example, there was a considerable lag time (12-20 days) before ureolysis began using *S. pasteurii* when researchers tried to stimulate groundwater ureolytic bacteria to grow and become ureolytically active (Tobler et al. 2011).

Previously, studies conducted by Parks, (2009) showed significant differences between the rates of growth in experiments carried out in the presence of calcium as opposed to those performed in the absence of calcium. *S. pasteurii* grown in the absence of calcium reached a maximum of 1.3 x 10^8 cfu/mL (≈0.007 AU/h) during the first 8 hours following inoculation. In the same research, cultures grown in the presence of calcium showed a decrease in bacterial counts (Parks, 2009). A similar decreasing trend was seen in *B. spharecius*, a type of ureolytic bacterium, in research conducted by Arunachalam et al. (2010). The growth curve for *B. spharecius* showed that the lag phase was 4 hours and the growth rate was 1.12 x 10^9 cfu/mL (≈0.056 AU/h). In contrast to these two bacteria, *B. megaterium* performed relatively consistently in all tests conducted in the presence of calcium ions in the batch experiments conducted in this study. Thus, the performance of *B. megaterium* will not be affected during the process of urea hydrolysis if the bacterial growth rate is maintained at optimum level.
*B. megaterium* produced in a three litre bio-reactor showed a higher specific growth rate of 1.64 ± 0.01/h, as compared to that achieved in shaking flasks which was 0.58 ±0.01/h (Rodriguez-Contreras et al. 2013). This suggests an improvement in fermentation conditions. This could have occurred because specific growth rate depends on many factors, like substrate concentration, competition, pH and end product concentration. It changes as the growth proceeds and in batch analysis, the environment changes as a function of time, substrate concentration and end product concentration. Moreover, the results of the experiments carried out in this study have shown similar growth rates to those reported by Rodriguez-Contreras et al. (2013). This is significant, as if B. megaterium is to be employed in field scale ground improvement it suggests that no additional changes in the culture conditions are needed to adapt the *B. megaterium* strain used in this study.

### 4.6 Urea Hydrolysis

The ability of *B. megaterium* to hydrolyze urea in this study was estimated from the change of conductivity of the liquid medium as its enzyme causes a conversion of non-ionic substrates (urea) to ionic products (NH$_4^+$ and CO$_3^{2-}$) during the process. The calibration protocols and the testing procedure have been explained in Section 3.8 of Chapter 3. Separate batch experiments were conducted to measure the influence of biomass concentration, calcium ion concentration and urea concentration during urea hydrolysis. Tests were conducted in 50 ml Erlenmeyer flasks using the conductivity meter to measure the chemical changes. Bacterial cells were germinated and grown at 30 °C using the growth medium specified in Table 3.2 for at least 24 hours prior to the start of batch experiments.

#### 4.6.1 The Effects of Biomass Concentration

The growth of bacteria was monitored using the spectrophotometer. The effect of biomass concentration on urea hydrolysis was measured using 6 M (∼300 g/L) urea in liquid media with a range of bacterial biomasses (0 – 15 x 10$^9$ cfu/mL). The conductivity value (units in mS) was monitored at various contact times (see Figure 4.6). The optimum biomass concentration in this
study was between $6 \times 10^9$ cfu/mL and $9 \times 10^9$ cfu/mL. However, initial bacterial concentrations of $3 \times 10^9$ cfu/mL and more than $12 \times 10^9$ cfu/mL show similar activities that are only slightly less than the optimum. These tests show that for a given amount of urea there is an optimum amount of bacteria which produces the greatest amount of ammonium and carbonate ions, indicated by the higher conductivity, and hence potentially the greatest amount of calcite. Fujita et al. (2000) report that the highest initial biomass concentration, exhibit the lowest rate of urea hydrolysis per absorbance unit. A similar trend is seen in the results from this experiment, as tests with the highest initial biomass concentration recorded the lowest urea hydrolysis per absorbance unit. This suggests that at some point, the system become oversaturated with organisms and ureolysis occurs at a rate independent of substrate concentration (zero order). In general, reactions catalyzed by enzymes are controlled by their concentration, thus in the case of excess substrate the rate of reaction becomes linear (Parks, 2009).

Figure 4.6: Effect of various concentration of bacterial biomass on urea hydrolysis of *B. megaterium* (Initial Biomass: cfu/mL $\times 10^9$) cultivated at 30°C, pH = 8.5. The standard deviation is indicated by means of error bars
4.6.2 The Effects of Calcium Concentration

Urease activity was tested with CaCl$_2$ concentrations ranging between 0 and 300 g/L. Approximately, $1 \times 10^9$ cfu/mL to $3 \times 10^9$ cfu/mL of bacteria were cultured and introduced into each batch experiment. This range of bacterial counts was chosen so that the influence of biomass could be reduced while studying the effect of calcium concentration. Urease activity was tested with CaCl$_2$ concentrations ranging between 0 and 300 g/L. The urea concentration was kept constant at 3 M ($\approx$150 g/L). For comparison, one of the experiments was conducted with no calcium chloride. Figure 4.7 shows the variation of conductivity with time in these tests. In all the tests containing the bacteria the conductivity increased when they were placed in the urea solution. After two hours, the conductivity was 75 % greater in experiments with calcium concentration similar to urea concentration (in 1:1 molar ratio) than with no calcium chloride. The experiments conducted with different concentrations of calcium chloride showed similar responses, with no clear trend in the maximum conductivity change achieved due to urea hydrolysis. However, the trend in conductivity changes was similar in the first two hours indicating an ongoing slow reaction during these hours. Some studies have reported a tendency of Ca$^{2+}$ to retard cell growth initially which is favourable for biocementation applications using injection techniques. However, this study shows that addition of CaCl$_2$ almost doubles the rate of reaction. In this study, almost 80 % of the urea was hydrolyzed when the CaCl$_2$ concentration was similar to the urea concentration. Previous studies with *B. sphaericus* have shown large increases (up to 10 times) in urease activity with calcium ions (Hammes, Boon, de Villiers, Verstraete, & Siciliano, 2003), whereas Whiffen (2004) with *B. pasteurii* reported that calcium at a high concentration (110-220 g/L) has a tendency to reduce urea hydrolysis depending on the calcium source. This study shows similar rates of urease activity for the range of calcium concentrations considered, with the greatest activity occurring with the maximum concentration used of 300 g/L. These results suggest that *B. megaterium* has high tolerance in adapting to high Ca$^{2+}$ concentrations. According to Tobler et al. (2011), a lower urea concentration will produce less carbonate and the pH will rise more slowly, which in turn will slow calcite precipitation. Furthermore, the claim that lower urea concentrations will induce longer lag times before calcite precipitation begins is arguable. For example, 1 day was reported in Ferris et al. (2004) and Mitchell and Ferris (2005) compared to only 7 hours in this study.
Figure 4.7: Effect of various concentration of calcium source on urea hydrolysis by *B. megaterium* (Initial Biomass = $1 \times 10^9$ cfu/mL) cultivated at 30°C, pH=8.5. The standard deviation is indicated by means of error bars.

### 4.6.3 The Effects of Urea Concentration

Five sets of batch experiments were carried out with various initial urea concentrations. The optimum number of bacteria were prepared initially using 1.5 M ($\approx$75 g/L) urea and added into all the five sets of batch experiments. Approximately $9 \times 10^9$ cfu/mL to $12 \times 10^9$ cfu/mL of bacteria cells were cultured and the absorbance reading of at least 0.45 was read. These ranges of bacteria were chosen so that the influence of biomass can be reduced while studying the effect of urea concentration. Experiments were conducted in the absence of calcium chloride, the initial liquid medium had a pH of 8.5 and a constant temperature of 30°C was maintained. Figure 4.8 shows the influence of the amount of urea on the rate of urea hydrolysis. The highest amount of urea (2 M) used in these experiments recorded the highest conductivity (maximum rate of urea hydrolysis) after 8 hours. This was followed by other experiments with lesser amounts of urea,
but even 0.25 M urea produces a significant increase in conductivity. The experiments with 3 M urea and without calcium chloride shown in Figure 4.7, achieved a conductivity of only 20 mS/cm, which was one quarter of the value achieved using 2 M urea. As higher urea concentrations are not expected to retard the reaction it suggests that the difference in the initial amount of bacteria used in the two sets of experiments is responsible for the different rates of urea hydrolysis. Another observation from Figure 4.8 is that with high urea concentrations the reaction occurs rapidly providing significant bacteria are available.

One of the criteria to be met when choosing suitable microbes for bio-cementation is that the bacteria must have high tolerance for urea and be able to survive in extreme alkaline conditions (alkaliphilic) because not all ureolytic bacteria are alkaliphilic. The use of enzymes from alkaliphilic microorganisms has been shown to have potential to create calcite precipitation for enhanced oil recovery purpose (Zajic et al. 1986). It is also interesting to note that under batch experimental conditions, the current bacteria will grow more, and produce the urease enzyme in the presence of sufficient urea. This suggests that the type of bacteria used in this study is dependent on the presence of urea for both the production of urease activity and also for cell growth unlike some other ureolytic bacteria. The change in conductivity in Figure 4.8 clearly shows that the bacteria were stimulated by an increase in urea concentration. This suggests that the urease enzyme was significantly induced by the presence of urea, similarly to the Proteus and Providencia species (Morsdorf et al. 1989; Rosenstein et al. 1981).
Figure 4.8: Effect of various concentration of urea on urea hydrolysis by *B. megaterium* (Initial Biomass = 9 x10^9 cfu/mL) cultivated at 30°C, pH=8.5. The standard deviation is indicated by means of error bars.

### 4.7 Urea Hydrolysis Rate

Much research has been focused on the details of the ureolytic bacteria performance within batch experiments. Despite the potential contribution of MICP to a range of engineering applications, little is known about the effects of the various parameters which control the rate of ureolysis. The enzyme urease drives the urea hydrolysis leading to the release of ammonium ions and bicarbonate. In this study, measurements of ammonium ion concentrations were used to calculate the rate of urea hydrolysis, which enabled a direct comparison to the microbial ureolysis rate reported in the literature. Figure 4.9 shows a comparison of the urea hydrolysis rates between *B. pasteurii* other studies and *B. megaterium* when OD was at least 0.45 (from this study). Depending on the experimental setup and culture conditions, experiments performed with *B. megaterium* and reported in literature have yielded fairly different results. To verify the ureolysis rate for *B. megaterium*, previously reported hydrolysis rates from experiments with *S. pasteurii*...
were used for comparison. The hydrolysis rate under lower urea concentration (6-33 mM) ranged between 0.05 and 0.9/day (Ferris et al. 2004; Mitchell and Ferris 2005; Dupraz et al. 2009). The rate obtained by Tobler et al. (2011), was 1.06 to 3.29/day and seems to be generally higher than 0.73 to 0.91/day obtained by Ferris et al. (2004) and Mitchell and Ferris (2005). This is because of using up to two orders of magnitude higher urea and Ca\(^{2+}\) concentrations. In this study, the maximum rate of urea hydrolysis was recorded as 4.18/day under the following conditions: a temperature 30 °C, biomass: 0.45OD (9 x 10\(^9\) cfu/mL), a pH of 8.5 and using 180 mM urea. For microbial ureolysis, the trend is for the rate to increase with increasing biomass (measured by optical density, OD) as shown in Figure 4.9. The results from this study shown in Figure 4.9 also show that there is a range of rates depending on amount of urea used, concentration of calcium ions present and the number of initial bacteria. Tobler et al. (2011) using \textit{S. pasteurii} demonstrated that urea hydrolysis was highly affected by biomass density and initial urea concentration. For example, the urea hydrolysis rates were up to a magnitude lower in 0.03 OD experiments (0.13-0.18/day) than those in experiments with approximately double the biomass, 0.07 OD (1.06-2.45/day). Other studies have also reported on the effect of biomass density on ureolysis rate (Dupraz et al. 2009) and so far significant difference in rates has been observed in this study when measured at OD of 0.45. Thus, the inconsistency of ureolysis rate found in this study compared with other reported values may well be due to other variable such as difference in the specific ureolytic activity of different lab cultures of bacteria.
Figure 4.9: Urea hydrolysis rate of ureolytic bacteria versus bacterial density

4.8 Ureolysis Kinetic Constant

In this study, the ureolytic kinetic constants of \textit{B. megaterium} were investigated by performing batch experiments at different urea concentrations. Since the kinetics of the majority of enzymatic reactions are well represented by the Michaelis-Menten model, the same model has been applied in this research.

To determine the urease kinetics parameters, bacterial cultures were grown in a series of 50 mL beakers. The liquid medium was prepared according to the recipe in Table 3.2. The beakers were inoculated with bacteria, which had been prepared the day before the test as described in section 3.4.2.2. The optical densities of the inoculating cells were measured at 600nm absorbance using the visual spectrophotometer.

The kinetic parameters were interpreted according to the conventional Michaelis-Menten model as in Equation (4.1).
\[ v_{\text{urea}} = \frac{v_{\text{urea, max}} S_{\text{urea}}}{K_m + S_{\text{urea}}} \]  

(4.1)

Where \( v_{\text{urea}} \) is specific urea hydrolysis rate (mM urea/min/A_600), \( S_{\text{urea}} \) is the urea concentration (mM), \( v_{\text{urea, max}} \) and \( K_m \) are the maximum specific urease activity (mM urea/min/A_600) and dissociation constant (substrate affinity) (mM), respectively. The constants, \( v_{\text{max}} \) and \( K_m \) were calculated by using the Lineweaver-Burk Equation shown in Equation. (4.2).

\[ \frac{1}{v_{\text{urea}}} = \frac{K_m}{v_{\text{urea, max}}} x \frac{1}{S_{\text{urea}}} + \frac{1}{v_{\text{urea, max}}} \]  

(4.2)

Apart from the amount and the type of bacteria, the hydrolysis rate in this study still depends on many factors. Figure 4.10 shows the dependence of urea concentration on specific urease activity rate. When the initial urea concentration was increased from 10 mM to 180 mM the specific urease activity rate was observed to increase asymptotically.

The optimal kinetic parameters, \( V_{\text{max}} \) and \( K_m \) are 8 mM urea/min/A_600 (2.05 mM urea/min/mg) and 40 mM, respectively (refer to Figure 4.11). A larger \( K_m \) value indicates a low affinity between the urea and bacteria cells. According to Hammes et al. (2003), \( K_m \) values for ureolytic bacteria range from 0.1 to 100 mM, whereas plant urease from jack bean possesses a \( K_m \) of 2.9 mM. A high specific urea hydrolysis is typical for bacteria with a high affinity for urea. This high affinity is very common for a high substrate turn over at low biomass. On the other hand, \( K_m \) and \( V_{\text{max}} \) values for urease from \textit{B. pasteurii} at pH 7.0 were estimated to be 41.6 mM and 3.55 mM urea/min/mg, respectively. When the reaction mixture was adjusted to a pH of 7.7, the kinetics constants decreased to 26.2 mM for \( K_m \) and 1.72 mM urea/min/mg for \( V_{\text{max}} \) demonstrating a higher affinity of the enzyme for urea at increased pH. According Hammes et al. (2003), for certain types of bacteria the urease activity may increase up to 10 fold in the presence of 30 mM calcium and apparently this can contribute to the type of crystals formed.
Figure 4.10: Effects of specific urea hydrolysis rate on the initial concentration of urea. *B. megaterium* cultivated at 30°C, pH = 8.5, (Initial Biomass = 5 x 10^9 cfu/ml). The standard deviation is indicated by means of error bar.

![Figure 4.10: Effects of specific urea hydrolysis rate on the initial concentration of urea.](image)

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Figure 4.11: Lineweaver-Burk plot of urea hydrolysis by *Bacillus Megaterium*

![Figure 4.11: Lineweaver-Burk plot of urea hydrolysis by Bacillus Megaterium](image)
The ability to hydrolyze urea is widely present among indigenous bacteria present in soils and groundwater environments (Fujita et al. 2000). The kinetics of calcium carbonate precipitation in response to the hydrolysis of urea has been extensively investigated (Ferris et al. 2004; Mitchell and Ferris 2005). However, in nearly all proposed models the very first ureolysis step has been over simplified to be first order with respect to urea concentration, neglecting any microbial involvement (Ferris et al. 2004; Dupraz et al. 2009; Tobler et al. 2009; Millo et al. 2012).

Reported values for $K_m$, urea vary between 26 mM for cell free extract at pH 7.7 (Stocks-Fisher et al. 1999) to 200 mM for a cell suspension (Whiffin 2004). According to van Paassen et al. (2009), the value for suspended cells of $B. pasteurii$ were 55 mM. Most studies on bio-cementation by urea hydrolysis reported the maximum hydrolysis rate varying between 0.002-0.16 mM urea/min (Bachmeier et al. 2002; Ferris et al. 2004; Stocks-Fisher et al. 1999). In particular, Al-Thawadi (2008) reported bacteria urease to be in the range of 11-28 mM urea/min and Whiffin (2007) suggested that plant and bacteria urease normally in the range of 4-18 mM urea/min. However, the required urease activity for effective bio-cementation as recommended by Whiffin (2004) was 10 mM urea/min and $B. megaterium$ in this study fulfills this recommendation.

### 4.9 Urease Activity

The observed lag phase duration of 7 hours in both growth and urease activity correspond well to a pH shift due to the hydrolysis of urea as shown in Figure 4.12. The amount of the enzyme present per cell (specific activity) reached the peak after 15 hours and remained nearly constant during the rest of the cultivation, which suggests that the enzyme was constitutively regulated. The same phenomenon was observed in several batch cultivations of $B. megaterium$ in this study. The optimum biomass recorded was 1.6 AU at the 30th hour ($\approx 32 \times 10^9$ cfu/mL) with growth rate of 0.21 AU/h ($\approx 4.2 \times 10^9$ cfu/mL/h). The urease activity was in the range of 12-15 mM urea/min and the specific urease activity was in the range of 8-10 mM/min/OD.
Whiffin (2004) reported that for *S. pasteurii* urease activity was 13.7 mM urea/min and the specific urease activity was in the range of 3 - 9.7 mM/min/OD. According to Whiffin (2004), *S. pasteurii* had a urease activity ten times higher than *P. vulgaris* under the same conditions. Previously, it was reported that, during nitrogen starvation, increases of 20 to 250 times were observed in specific urease activity in *B. megaterium* compared to *P. vulgaris* under all growth conditions (Kaltwasser et al. 1972). Thus, the increase in specific urease activity was found due to the lack of a nitrogen source such as urea. Hence, adding a sufficient amount of urea is essential to ensure a reasonable specific urease activity of this type of bacteria is maintained during the urea hydrolysis process.

Stabnikov et al. (2013) mentioned that the urease activity of isolated Bacillus strains was in the range from 6.2 to 8.8 mM hydrolyzed urea/min. Urease activities for other bacterial strains used for bio-cementation were also in similar ranges: from 5 to 20 mM hydrolyzed urea/min for *S. pasteurii* DSMZ 33 (Harkes et al. 2010); 2.2-13.3 mM hydrolyzed for *S. pasteurii* ATCC 11859 (Whiffin 2004); and more than 3.3 mM hydrolyzed urea/min for three Bacillus strains isolated from Australian soil and sludge (Al Thawadi and Cord-Ruwisch 2012). It was also shown by Qian et al. (2009) that stronger aggregates of calcite were formed at lower rates of urea
hydrolysis. Having urease activity in the range from 4.4 to 9.5 mM hydrolyzed urea/min ensured the strength of bio-cemented sand was from 32 to 35 MPa, however, the strength became lower at urease activities higher than 9.5 mM hydrolyzed urea/min (Whiffin 2004).

According to researchers involved in bio-cementation, ureolytic activity can be used to measure the precipitation of calcite (Muynck et al. 2008; Tittelboom et al. 2010; Bachmeier et al. 2002). Braissant et al. (2003) reported that the amount of urease produced was close to 1% of the dry cell dry weight. Thus, the specific urease activity obtained in this study, 8 mM hydrolyzed urea/min/OD after 24 hours will be used to predict the amount of calcite precipitated in the following chapters. Unlike in batch experiments where pH and temperature were controlled to provide a consistent performance of the bacteria, some variance in predicted calcite can be expected for experiments conducted in an open environment such as the triaxial and physical model tests.

4.10 Comparison of Urease Activity of Several Ureolytic Bacteria

So far, the extensive research on the feasibility of bio-cementation as an alternative ground improvement technology has been laboratory based. Prior to any full scale study of this technique, a detailed study on the potential of ureolytic bacteria to produce enough enzymes is necessary. The production of urease positive bacteria on a larger scale could become prohibitively expensive. Van Paassen (2011) estimated and used nearly 5 m³ of bacterial culture to bio-cement 100 m³ of sand. According to Whiffin (2004), the minimum level of urease activity required for the direct application in bio-cementation is 10mM/min/g dry biomass. Therefore, to achieve positive results for this application, a reliable and consistent high urease producing bacterium is vital and necessary.

However, there are many pathogens among urease producing bacteria (UPB). As shown in Figure 4.13, the most active urease producers are *Helicobacter pylori* with 25 mM urea/min g dry biomass (Lee and Calhoun 1997). These bacteria can infect the human stomach along with the opportunistic pathogens such as *Proteus vulgaris*, *Staphylococcus aureus* and *Pseudomonas*
*aeruginosa*. Meanwhile, it is known that active non-pathogenic producers of urease can be found among halotorent (able to tolerate high concentration of salt) and alkaliphilic Gram-positive, spore forming bacteria such as *Sporosarcina pasteurii* (formerly known as *Bacillus pasteurii*). Urease activity of *S. pasteurii* after 24 hours of growth was 13.7 mM hydrolyzed urea/min (Whiffin et al. 2007). *B. megaterium* tested in this study was grouped as a non-pathogenic bacterium with bio-safety Level 1. Compared to other pathogenic bacteria it produces a reasonable amount of urease approximately 8 mM urea/min/OD. Thus, it can be seen that among all the ureolytic strains tested, *B. megaterium* is one of the many available soil bacteria that could be utilized for bio-cementation.

Additionally, since temperature has an important impact on the growth and the activity of microorganisms, it also has a significant influence on bio-cementation. According to De Muynck et al. (2013), within a given range, an increase in temperature results in increased growth and activity of microorganisms. The latter can be attributed to the fact that chemical and enzymatic reactions proceed faster at higher temperatures. Several authors have reported increased ureolytic activities at higher temperature. Whiffin (2004) observed an increase of the specific urease activity of *B. pasteurii* of 0.04 mM urea hydrolyzed per minute with every degree of temperature increase in a range between 25 °C and 60 °C. Despite the fact that all the batch analysis in this study was conducted at 30 °C, we could expect a significant variance in its performance when applied in the field depending on the ground temperature.
Figure 4.13: Comparison of urease activities of currently tested bacteria with other urease producing bacteria (UPB). Activities are sourced from (a) Li et al. (2000) (b) Young et al. (1996) (c) Hansen and Solnik (2001) (d) Present study (e) Whiffin (2004). Error bars indicate the variability of urease activity under normal conditions of growth.

4.11 Summary

One of the objectives of this thesis is to investigate the potential of \textit{B. megaterium} to act as a catalyst for the production of bio-cement. This study examined the rates of urea hydrolysis when using a non-traditional bacteria for soil bio-cementation. Generally, urease producing bacteria (UPB) used for bio-cementation should be active in an environment with a salt present. Thus, halophilic, or halotolerant, and alkaliphilic UPB should be the preferable choice for the production of bio-cement. Therefore, the performance of \textit{B. megaterium} was evaluated under a high concentration of urea, Ca$^{2+}$ ions and various biomass concentrations.

It was successfully demonstrated that \textit{B. megaterium} was urea tolerant to a concentration of up to 3 M and the maximum rate of urea hydrolysis was 4.18 day$^{-1}$ at an optimum optical density (OD) reading of 0.45. It is a moderately alkaliphilic bacterium at a recorded optimum pH of 9.1. However, the level of urease activity was variable with respect to biomass concentration, suggesting that the enzymes were not constitutive (that is the amount of enzyme expressed per
cell is not constant) as indicated by the literature, therefore subjected to regulation (pH and temperature).

Overall, the results from batch analysis revealed that the growth of ureolytic bacteria introduced in this study can be easily stimulated to enhance rates of urea hydrolysis and can be used to induce calcite precipitation in this environment. The bio-safety level of the selected bacterium is also an important consideration. The safety level of B. megaterium is Level 1, indicating that only a minimum level of safety measures is required to work with this bacterium.

These findings suggest that B. megaterium has high potential in precipitating calcite through the urea hydrolysis process. Under normal batch conditions, B. megaterium can produce enough urease to cater for 10 mM urea/min activity required for bio-cementation. It was found that the potential urease capacity of this bacterium was 8 mM urea.min$^{-1}$.OD$^{-1}$ and sufficient for bio-cementation without additional processing. In spite of the laboratory performance, the desired urease activity for bio-cementation in field applications could still be obtained by concentrating the urease substrate and controlling other environmental factors, such as pH and temperature. Although B. megaterium clearly produces ten times more urease per cell than some other ureolytic bacteria, it is recommended that urease production be optimized before any field trial.

The production of urease positive bacteria for the purpose of in situ bio-cementation involves significant cost. This may limit the commercialisation of biocement applications for ground improvement. The key reason for this high cost is the labour, energy, equipment, and transportation costs involved when producing this bacteria by biotechnology. Thus, the ability to cultivate the organism and on site production with simple technology are highly desirable for cost minimization. Until now, to obtain reliable and constant high urease activity has only been achievable by cultivating urease positive cultures under sterile conditions. Recently, some attempts have been made to utilize plant derived urease to induce calcite precipitation. This could be an alternative approach to lower the cost of mass production of bacteria when applied in the field.

The enzymatic hydrolysis of urea presents a straightforward solution to calcite precipitation. The urease enzyme is common in a wide variety of microorganisms, and can be readily induced by adding an inexpensive substrate. According to Hamdan et al. (2013), the use of plant derived
urease to induce the carbonate cementation could avoid the generation of any toxic products, such as ammonium salt, when biocement is introduced in field applications.

The choice of bacteria is an important factor to be considered in sand bio-cementation. The quantity and the activity of an enzyme are unique to the bacteria producing it. The standard model that reasonably simulates enzymatic reactions used in this study was the Michaelis-Menten model. Results show that *B. megaterium* is capable of producing the desired level of urease activity for the experiments or modeling purposes. These results suggest that *B. megaterium* produces an enzyme that is similar to other microbial urease reported earlier in the literature review and should be considered for wider application. The kinetics parameters obtained using this model provide a reference point for any future research and field applications when it comes to selecting the type of bacteria. Continuous efforts should be made by researchers to explore the potential of different ureolytic bacteria in biocementation or in any other application in geotechnical engineering in the future.
5.1 Introduction

A series of unconfined compressive strength (UCS) tests and triaxial tests have been carried out to study the behaviour of biocemented Sydney sand. As has been discussed previously, a limited number of triaxial tests have been reported on bio-cemented specimens, but these studies have not thoroughly investigated the effects of calcite content and stress level. In this chapter, effective confining stresses ranging from 50 kPa to 500 kPa have been used in monotonic load tests to investigate the stress, strain and small strain stiffness behaviours of biocemented sand. These experimental data are also compared with further UCS and triaxial tests performed on gypsum cemented and uncemented specimens. In addition, almost all the available data discussed in the literature review were based on specimens created by the injection of cementitious solutions through sand. The injection process leads to heterogeneity in calcite distribution causing the samples to deform locally, and especially at lower calcite precipitation giving very small shear resistance for samples as a whole, when tested in the laboratory. The need for uniform and consistent biocemented samples arises from the challenges encountered by
previous researchers. Thus a mixing technique has been introduced in this thesis to overcome the problems with lack of homogeneity and to investigate whether this may have been influencing the results. It has been suggested that injection leads to calcite being deposited at the throats (contact points) between particles, and this is beneficial for cementation. Small strain stiffness or shear wave velocity has been suggested as a method for assessing the success of biocementation, however the lack of data on small strain stiffness of biocemented soil may delay the progress of this technique in future ground improvement work. Shear wave velocity measurements have been obtained from the triaxial tests to address this issue. Data obtained from this study were validated and compared with other existing models. It is intended that the data can be used as baseline measurements to check any data from future biocementation projects.

The discussion in this chapter is mainly focused and limited to the following:

1. The effect of curing time on the level of cementation and the corresponding amount of calcite/cement.
2. Comparison of the UCS strengths of bio-cemented and gypsum cemented specimens.
3. Comparison between the strengths of bio-cemented specimens in UCS and triaxial tests.
4. The influence of calcite content on the peak strength and stiffness for different confining pressures.
5. The effects of calcite and confining pressure on the small strain stiffness.

5.2 UCS Tests

The stress, strain responses from the UCS tests for cemented Sydney sand with various gypsum contents are shown in Figure 5.1. The UCS tests were conducted using a range of gypsum content from 5 % to 20 %. Detail information on the mix composition by the % weight of samples can be found at Appendix A1. Out of three identical samples tested only the one with the highest strength reported here with 5 % error margin. All the specimens show a very brittle response when tested using monotonic loading. Although this trend suggests consistency in sample preparation and shows significant increases in strength with increasing gypsum content, the results may have been affected by the test procedure. The samples were tested after having
been left to cure for 1 week in the laboratory and despite being wrapped in plastic film had partly
dried out before testing. Negligence during curing procedure might have influenced the degree of
saturation of the soil hence the strength of the affected. Cheng et al. (2013) have reported that
there is a significant relationship between the degree of the saturation and the cementation
strength. Some trial and error test was carried out during preliminary stage prior to actual test to
find out the optimum curing time. The low moisture content may have resulted in an additional
component of strength due to suction. However, this is not believed to be significant for samples
with low gypsum contents as the sand used in this research was uniformly sized. Another factor
that may have influenced the results was the difficulty in retaining water in the sand specimens
during preparation, which may have resulted in insufficient water being present for hydration of
the gypsum. Apart from that, to some extent the delay in mixing too could have affected the
hydration process as the nature of the gypsum which reacts relatively quickly in the presence of
water than any other type of binders was mentioned in studies conducted by Van Dreissche et al.
(2012). These factors may be responsible for the large increase in strength observed between 15
% and 20 % gypsum contents. That is, it may be a consequence of increased water retention due
to additional (gypsum) fines, which would also enhance any suction effect.

Figure 5.1: UCS test responses from gypsum cemented specimens

Similar UCS tests were conducted on bio-cemented Sydney sand to investigate the stress, strain
behaviour. Figure 5.2 shows increasing calcite precipitation leads to increased UCS strength.
Generally, the biologically cemented sand shows stiff and brittle behaviour during compression tests. According to DeJong et al. (2006), bio-cemented sand responds similar to naturally and artificially cemented sand at low confining pressure. Samples were back pressured using effective confining pressure of 50 kPa and 100 kPa. However, in comparison to gypsum, the calcite cemented specimens were more ductile than the gypsum cemented specimens. Figure 5.2 shows that only samples with calcite contents more than 3.33 % had responses similar to the gypsum cemented sand. There could be many reasons for the variance in responses of bio-cemented sand in comparison to gypsum cemented sand in this study. For example, most of the responses of the bio-cemented sand have been corrected for seating effects during data analysis. In part this has been required because specimen preparation did not always produce ends that were perfectly square and this may have influenced apparent ductility obtained. Seating effects were caused by the specimen ends not being perfectly square which led to difficulty in alignment of the seat in the top platen. This slight non-alignment is not believed to have significantly affected the strength. Also, in the case of samples with low calcite contents, tests may have been affected by the tendency of water (and cementing agents) to redistribute within the specimens during preparation (prepared by manual mixing) because of the low fines contents. Because of the low amount of fines there is limited water retention within the sand. There is a possibility that water could redistribute under gravity towards the specimen base, however this was not evident in the calcite distribution which were uniform throughout all specimens as shown in Table 5.1. However, there was less than 5 % variance in the calcite distribution in bio-cemented samples which suggests loss of water was not significant. Specimens were cut in three and the calcite contents measured using Equation 3.8. Typical results of UCS and triaxial test, shown in Table 5.1, indicate that the calcite distribution throughout the specimens was uniform, indicating the reliability of the mixing technique to form bio-cemented specimens with a wide range of cement contents. Similar to the gypsum cemented specimens, bio-cemented UCS specimens had also dried out to some extent before testing and this may also have contributed to some variance. For example, the bio-cemented samples may also have been influenced by suction effects, particularly at high calcite contents. Because of the fine cement particles increasing cementation will increase water retention and hence suction during drying. The suction could have contributed to the strength of the UCS specimens because they had partially dried out, but additional tests on saturated specimens would be required to confirm this possibility.
Basically Table 5.1 with results combining from both the UCS and triaxial test was compared to investigate the reliability of mixing technique in producing uniform calcite. Whereas Figure 5.2 was only aimed at showing the trend on how the increase in calcite precipitation could actually improve the UCS strength.

![Figure 5.2: UCS responses for bio-cemented specimens](image)

Table 5.1: Variance of calcite distributions in UCS and triaxial tests samples

<table>
<thead>
<tr>
<th>Test No/ID</th>
<th>Test Type</th>
<th>Average Calcite %</th>
<th>Top (%)</th>
<th>Middle (%)</th>
<th>Bottom (%)</th>
<th>Standard Variance (±%)</th>
<th>Sample Variance (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>3%B</td>
<td>UCS</td>
<td>1.33</td>
<td>1.52</td>
<td>1.14</td>
<td>1.34</td>
<td>0.19</td>
<td>3.6</td>
</tr>
<tr>
<td>B14</td>
<td>Triaxial</td>
<td>1.54</td>
<td>1.49</td>
<td>1.51</td>
<td>1.61</td>
<td>0.10</td>
<td>1.1</td>
</tr>
<tr>
<td>5%B</td>
<td>UCS</td>
<td>2.73</td>
<td>2.73</td>
<td>2.91</td>
<td>2.68</td>
<td>0.12</td>
<td>1.5</td>
</tr>
<tr>
<td>B10</td>
<td>Triaxial</td>
<td>2.61</td>
<td>2.94</td>
<td>2.79</td>
<td>2.79</td>
<td>0.16</td>
<td>2.7</td>
</tr>
<tr>
<td>10%B</td>
<td>UCS</td>
<td>5.33</td>
<td>5.52</td>
<td>5.21</td>
<td>5.26</td>
<td>0.15</td>
<td>2.3</td>
</tr>
<tr>
<td>B13</td>
<td>Triaxial</td>
<td>4.26</td>
<td>4.48</td>
<td>4.17</td>
<td>3.62</td>
<td>0.16</td>
<td>2.5</td>
</tr>
<tr>
<td>15%B</td>
<td>UCS</td>
<td>6.23</td>
<td>6.13</td>
<td>6.25</td>
<td>6.31</td>
<td>0.15</td>
<td>2.3</td>
</tr>
<tr>
<td>B16</td>
<td>Triaxial</td>
<td>6.98</td>
<td>7.09</td>
<td>6.90</td>
<td>6.91</td>
<td>0.11</td>
<td>1.1</td>
</tr>
</tbody>
</table>
Figure 5.3 shows a comparison between the UCS responses of gypsum and bio-cemented sand specimens. Although at low cement contents bio-cemented specimens are apparently much less stiff than the gypsum ones, there is a significant improvement in strength. Based on their strength ratios calcite is much more effective, approximately 1% calcite is equivalent to about 8% gypsum, whereas 9% calcite is equivalent to 20% gypsum. Hence, calcite is very effective for strength improvement with low amounts of cement. Relatively the UCS strength with 2% calcite is similar to the UCS strength with 5% gypsum.

The effect of gypsum/calcite content on UCS strength is illustrated in Figure 5.4. This Figure clearly shows the general trend for UCS strength to increase with the amount of calcite precipitated in the bio-cemented sand. A similar trend, also shown in Figure 5.4, was observed in the study conducted by Al Qabany and Soga (2013) except that they reported there was a problem in measuring the strength of weakly cemented (low calcite) samples due to
inhomogeneity. Weakly cemented samples tended to deform locally, giving only very small shear resistance, and some samples even collapsed immediately after loading was started with zero strength recorded. In contrast to that, weakly bio-cemented specimens in this study have shown significant strength improvements. The sand tested by Al Qabany and Soga (2013) is very similar in grading, shape and mineralogy to Sydney sand, indicating that the difference in response can be related to the method of specimen preparation. Al Qabany and Soga (2013) have used an injection method, whereas in the current study all the ingredients have been simply mixed together. For any injection strategy achieving uniform calcite precipitation is challenging and difficult to control, and this is particularly the case for low amounts of cement. Nevertheless, injection does allow a series of cementation episodes which enables very high strengths to be achieved (Cheng et al. 2012), strengths which are beyond those possible using simple mixing strategies. This study has shown that mixing leads to more homogeneous cementation and consistent strengths at low calcite contents. Mixing also leads to slightly higher strengths for all calcite contents when compared to the results reported by Al Qabany and Soga (2013), at least for up to 8 % calcite where comparison is possible.

The performance of the mixing technique varies depending on the type of cement. For example, much more gypsum was needed in this study to achieve any particular strength in comparison to calcite. The particulate nature of gypsum and its tendency to fill voids is responsible for it being less effective as a cementing agent. This has been reported in other studies (eg. Huang and Airey, 1988) where the addition of silty (gypsum) fines to the sand tended to fill void space without significant influence on the behaviour until transitional fines contents of about 25 %. Once sufficient gypsum is present it can fill voids and surround the parent sand particles creating a strongly cemented matrix. This is suggested to be at least part of the reason for the significant jump in strength for gypsum contents >15 % seen in Figure 5.4.
5.3 Curing

The process of cementation was monitored using bender elements to record the shear wave velocity during the curing stage when preparing the triaxial specimens. Figure 5.5 shows the shear wave velocity changes in gypsum cemented sand during curing. Sample curing is a sensitive procedure which can be affected by changes of temperature and humidity. Hence samples for UCS test were preserved in incubator. Unlike the UCS specimen preparation, the triaxial specimens were prepared within a rubber membrane sealed to the base pedestal of the cell. This meant that in this case no loss of moisture occurred after the sand mixture was placed in mould and the top platen placed on the specimen surface. This also restricted the supply of air/oxygen during the curing reaction. Following the curing stage specimens were saturated by pumping in water, and during saturation no significant changes in shear wave velocity were observed. Due to the automated process of monitoring the waveforms (Airey and Mohsin, 2013) and the large changes in shear wave velocity during curing it was not always possible to make sensible interpretation of the data post-test. One of the main reasons for this was the failure to adjust the input wave frequency during the overnight operation. This has resulted in only a small
number of tests for which reliable data of the curing process have been obtained. The data in Figure 5.5 show the rapid curing for gypsum contents greater than 10%. This is expected as the manufacturer states gypsum cures in approximately 55 minutes. Basic tests for gypsum such as fineness, density and setting time are determined in accordance to standard ASTM C472. However, for low gypsum contents there is more variability, and significantly greater curing times were recorded. The hydration of gypsum is believed to be a chemically mediated reaction and the slow reaction rate may prevent the temperature rise which normally accompanies the hydration reaction, effectively slowing the rate.

Figure 5.5: Shear wave velocity changes during curing of gypsum cemented specimens

Figure 5.6 shows the shear wave velocity changes during curing of the bio-cemented specimens. The rate of curing in the bio-cemented samples appears to be independent of the final amount of precipitate. The reactions to produce the bio-cement take about 12 hours (approx. 40000 seconds) which is at least two times slower than gypsum given amounts of nutrients of 5 to 15 %
similar to the amounts of gypsum. No significant changes in shear wave velocity were observed after 12 hours. Typically specimens were left for 24 hours before saturation. Although there is some scatter in the trend, shear wave velocity increases with the amount of precipitate.

![Shear wave velocity changes during curing of bio-cemented specimens](image)

Figure 5.6: Shear wave velocity changes during curing of bio-cemented specimens

Shear wave velocity data of selected gypsum and bio-cemented specimens are plotted in Figure 5.7 for comparison. The curing rate for the lowest calcite content of 1.88 % is almost similar to that for 5 % gypsum, but in terms of strength the UCS tests suggest 1.88 % calcite is equivalent to approximately 10 % gypsum. Even though it requires less cement to achieve the same strength, gypsum cures much more quickly than the biocement. Although it appears that curing of the bio-cemented specimens is independent of the amount of calcite it should be noted that no samples with low calcite contents, with UCS strengths less than 250 kPa were investigated. Thus it is not clear if low calcite contents would require longer curing times in similar fashion to the
low gypsum content specimens. Further, the specimens in UCS tests were cured for 1 week before testing whereas triaxial specimens were tested after approximately 24 hours. As discussed below there is more variability in strength for the triaxial specimens and in Chapter 6 it is shown that longer times were required for curing of columns underwater, and it is possible that curing may not be complete after 24 hours and there may be continuing, albeit small, changes in shear wave velocity.

Figure 5.7: Comparison of curing for gypsum and bio-cemented specimens.

5.4 Triaxial Stress-strain Responses

5.4.1 Uncemented Sand

Some preliminary triaxial tests were performed on uncemented Sydney sand. Tests were performed for different relative densities, drained and undrained and at different confining
stresses. To enable simple presentation and comparison between the various tests they are presented in terms of stress ratio \((q/p')\) versus axial strain. Results are typical of uncemented sand and show stress ratio rising to a peak before decreasing towards an ultimate or critical state value. The dashed line in Figure 5.8 shows the estimated critical state stress ratio, \(M = 1.35\) which corresponds to a friction angle of 32°. Some tests drop from the trend line suddenly down to the critical state. These were specimens that developed pronounced shear planes followed by rotation of the top platen.

The volume strains associated with the stress ratio responses from the drained tests in Figure 5.8 are shown in Figure 5.9. In all cases significant dilations were observed and were associated with stress ratios greater than the critical value as expected for uncemented sand (Bolton, 1986). Results and values found in this section is summarised in Appendix A1.

![Figure 5.8: Response of uncemented Sydney sand](image)

Figure 5.8: Response of uncemented Sydney sand
Bender elements were used in some of these tests to obtain the relation between small strain shear modulus ($G_{\text{max}}$) and mean effective stress ($p'$) for uncemented sand. Two of these tests (P1 and P2) are shown in Figure 5.10. During isotropic compression the responses from the two tests followed an identical relationship. It may be noted that one of these tests was performed using dry sand, and the other on a saturated specimen. The equation for the linear relation shown in Figure 5.10 (a) is given by:

$$G_{\text{max}} = 11.27 \, p'^{0.475} \tag{5.1}$$

Where $G$ is in MPa, and $p'$ is in kPa.

It may be noted that this relation covers a stress range of 10 kPa to 3000 kPa, significantly larger than captured in most existing published data and relations. For validation, data obtained in this study were compared with published data for sands using various empirical equations for $G_{\text{max}}$ and these are plotted in Figure 5.10 (b). $G_{\text{max}}$ is expressed as a function of void ratio $f(e) = (2.17-$
\( e^2/(1+e) \) and the mean effective stress \((p')\) as in Equation 5.2. Where A and n are material constants and are given in Table 5.2.

\[
G_{\text{max}} = A f(e) p'^n
\]  \hspace{1cm} (5.2)

Table 5.2: Constants proposed for empirical equation \(G_{\text{max}}\)

<table>
<thead>
<tr>
<th>A</th>
<th>n</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>5000</td>
<td>0.5</td>
<td>Shibuya and Tanaka (1996)</td>
</tr>
<tr>
<td>8400</td>
<td>0.5</td>
<td>Kokusho (1980)</td>
</tr>
<tr>
<td>7000</td>
<td>0.5</td>
<td>Hardin and Richart (1963)</td>
</tr>
</tbody>
</table>

The predicted responses of \(G_{\text{max}}\) from Shibuya and Tanaka (1996), Kokusho (1980) and Hardin and Richart (1963) are compared with the experimental data. As Sydney sand is similar in mineralogy, particle size and shape to Toyoura sand it is expected that the \(G_{\text{max}}\) relationship will also be similar, and as expected Figure 5.10 (b) shows that the established linear relation between \(G_{\text{max}}\) and \(p'\) in this study is very close to the published empirical equation proposed by Kokusho (1980) for Toyoura sand. However, the observed relation from this study for Sydney sand, obtained using bender elements, is slightly higher than the \(G_{\text{max}}\) values for other similar types of sand.
Figure 5.10: Variation of $G_{\text{max}}$ with $p'$ for uncemented sand
5.4.2 Gypsum Results

Gypsum cemented specimens with different cement contents were subjected to drained and undrained tests in most cases with an effective cell pressure of 50 kPa. The results in Figure 5.11 show the stress ratios achieved by the cemented specimens are much higher than for the uncedmented sand, shown in Figure 5.8, as expected. The peak deviator stress, and peak stress ratios tend to increase with the amount of cement. In some undrained tests especially on the more cemented specimens (cement contents of 15 % and 20 %) the stress ratio reached 3, the maximum possible in the triaxial apparatus. At this point specimens reached their unconfined strength and failure strengths of 650 – 1300 kPa in the range of the UCS strengths of gypsum specimens shown in Figure 5.4. After this, the specimens stress ratios dropped and the stress ratios reduced towards an ultimate state value. The ultimate critical state value is suggested to be identical to the uncedmented sand, M = 1.35. However, most drained specimens failed on pronounced shear planes and large parts were still moderately cemented at test completion so that there is uncertainty in the true ultimate friction angle. Other studies (Huang, 1994; Huang and Airey, 1998) in which gypsum have been used with carbonate sands have shown its presence does not influence the final frictional resistance, however these sands have significantly higher friction angles than Sydney sand.
The stiffness at small strains is shown in Figure 5.12. Data have been obtained from external measurement of displacement using an LVDT transducer. Data have been corrected for initial seating by assuming the steepest gradient could be extrapolated back to zero. In this process corrections to the strains were generally small. The data show a general trend for stiffness to increase with cement content, however, there is considerable scatter. For the stiffest specimen the estimated $G_{\text{max}} = (E/3)$ is approximately only one tenth of the value of $G_{\text{max}}$ measured from the bender elements, which are discussed below. It is believed that the bender elements are giving reasonable data and thus these small strain data shown in Figure 5.12 appear to be of little value.
Figures 5.13 and 5.14 show responses of gypsum cemented specimens in drained tests. Even small amounts of gypsum significantly increase the strength compared to uncemented sand. It may also be observed that the effect of small amounts of gypsum is much greater in triaxial tests than for UCS tests, shown in Figure 5.1. This could be related to differences in specimen preparation, although it is more likely to be due to the prevention of a tensile failure mode, because of the applied confining stress. Gypsum cemented specimens reached peak strengths at <1.5 % strain, whereas uncemented sand reached peak strength at axial strains between 2 and 5 %. The cementation prevents dilation which occurs almost from the start of shearing for the uncemented specimens. Dilation continues for uncemented specimens reaching a maximum of approximately 4 % at axial strains of greater than 10 %. The gypsum cemented specimens initially compress and start to deviate from linear deviator stress responses where dilation commences. The rate of dilation is then much greater than for the uncemented specimens, even though the densities are similar. The gypsum cemented samples form pronounced shear planes and soon after dilation ceases, but with continued shearing the deviator stress approaches the
same value as the uncemented specimens. The volumetric response of 10 % gypsum specimen is strange, and there appears to be some error in measurement. In general, these results are typical of the behaviour of artificially cemented specimens (eg Huang and Airey, 1988; Sharma et al. 2011; Lee et al. 2009; Asghari et al. 2003; Consoli et al. 2009; Clough et al. 1989 and many others).

Figure 5.13: Deviator stress, axial strain responses from drained tests (p’c = 50 kPa)
Figure 5.14: Volume strain, axial strain responses from drained tests ($p'_c = 50$ kPa)

Figure 5.15 shows the variation of $G_{\text{max}}$ with $p'$ for gypsum cemented specimens throughout the triaxial tests. This includes during isotropic compression to a mean effective stress of $p'_c = 50$ kPa and then drained shearing to large strains. The Figure shows the effect of gypsum content on the small strain stiffness, and that $G_{\text{max}}$ is approximately constant until close to failure. However, when looked at in detail $G_{\text{max}}$ increases slightly with $p'$ and then reduces as the cement starts to break down. Following the peak, the shear modulus drops significantly and approaches towards the uncemented response.
Figure 5.15: Variation of $G_{\text{max}}$ during compression and shear for Gypsum cemented specimens.
5.4.3 Bio-cemented Specimens

During sample preparation of the UCS and triaxial test specimens, sand was mixed with equal amounts, by mass, of calcium chloride and urea. Figure 5.16 shows the amount of calcite precipitate measured for both the UCS and the triaxial specimens post-test as a function of the amount of nutrients added during preparation. Each point in this figure represents a single test. Calcite precipitation obtained in this test can be counter checked using the urease activity value from Chapter 4 provided knowing the actual amount of nutrients used. For the UCS specimens there is a clear relation between the amount of urea added and the amount of calcite precipitate that was obtained. The triaxial specimens, on the other hand, show considerable scatter and variability in the amount of calcite measured at the end of the tests. Nevertheless, when checked for uniformity in calcite precipitation post-test, there was less than 5 % variability in the amount calcite in every sample. The calcite concentration was measured at three locations in each sample similarly to the UCS specimens, and some typical data have been included in Table 5.1.

Figure 5.16 shows a general trend that increasing the amount of the nutrients increases the amount of calcite, however, it has been very difficult to control and predict the amount of calcite in the triaxial specimens and this has influenced the presentation of data that follows. Although there are ways to estimate and control with quite accuracy the relation between mass of introduced reactants and final mass of products as claimed by Whiffin et al. (2007), it is deemed not suitable for the triaxial samples. Further, variability in the data could be the result of a change in procedure during saturation. In some early tests calcite was flushed out of the specimens during pumping of water to remove air for saturation and this could explain some of the lower results. However, in the majority of the tests saturation was achieved simply by pumping water into the specimens and there should have been no loss of nutrients or bacteria. There are several possible reasons for the variability in the amount of calcite obtained from UCS and triaxial tests. Apart from the curing time and the access to air that leads to drying out of samples, contamination of bacteria may also have affected the activity, particularly in the triaxial tests as no special measures were taken. Differences in the temperature and pH of the soil during sample preparation may also have contributed to differences in calcite precipitation.
Figure 5.16: Relation between amount of urea in mixture and calcite measured post-test

To study the effects of confining pressure and calcite, tests are presented below for different ranges of calcite. In all cases specimens were prepared in the same way so that differences in density are minor, although this was not closely controlled. At least three samples were prepared for each mix proportions. Figures 5.17 and 5.18 show the stress, strain and volume strain, axial strain results from a series of CID drained tests with different confining stresses for the lowest calcite contents, in the range from 1.5 % to 2.3 %.

The set of tests includes a specimen for which it is believed the membrane leaked as the cell and back pressures were equal. This has resulted in effectively a UCS test being performed on a fully saturated specimen. The peak deviator stress of 750 kPa is significantly higher than measured in the conventional UCS tests shown in Figure 5.4. Figure 5.4 would suggest UCS strength of about 300 kPa for a calcite content of 2 %. It has been shown by Cheng et al. (2013) that the UCS strength is influenced by the degree of saturation; however, Cheng et al (2013) report that reducing the degree of saturation leads to an increase in the UCS which is the opposite trend to that in the current study. Cheng et al (2013) also suggest the difference relates to the location of the calcite, with lower degrees of saturation leading to precipitation only at the particle contacts.
In the current tests all specimens were prepared in the same way with similar amounts of water so that there should not be a significant effect of saturation on the results.

The results shown in Figures 5.17 and 5.18 indicate a general trend for stiffness and strength to increase with confining stress. However, at a confining stress of 500 kPa the strength is less than at 200 kPa. This could be because of the lower calcite content in the more highly stressed specimen. It may also be noted that the volumetric response of the higher stressed specimen shows less compression and more gradual dilation, consistent with less effect of the cementation. Thus not only the lower calcite content, but also the increased stress level may be leading to breakdown of the cementation and influencing the behaviour. Nevertheless, the general patterns of behaviour are consistent with those expected of cemented specimens and similar to gypsum cement.

![Figure 5.17: Deviator stress, axial strain responses for bio-cemented specimens (1.5 % to 2.3 % calcite)](image-url)
Figure 5.18: Volume strain, axial strain responses for bio-cemented specimens (1.5 % to 2.3 % calcite)

Figures 5.19 and 5.20 show the effects of confining stress for a series of CID triaxial tests with calcite contents in the range of 2.8 % to 3.4 %. Another UCS test is available for a saturated test in this cement content range, as before as a result of membrane puncture. The UCS strength of 820 kPa is again significantly higher than would be expected from the UCS tests in Figure 5.4 which give a value of around 450 kPa for a calcite content of 3.4 %. However the results otherwise look reasonably consistent, showing an increase in strength and stiffness as confining stress increases. As for the lower calcite contents there is a trend for the rate of dilation to reduce with increasing stress level, although the effect is less pronounced for these more cemented specimens. The responses are generally similar to those of the lower calcite content specimens except that slightly higher strengths are observed with increasing calcite.
Figure 5.19: Deviator stress, axial strain responses for bio-cemented specimens (2.8 % to 3.4 % calcite).

Figure 5.20: Volume strain, axial strain responses for bio-cemented specimens (2.8 % to 3.4 % calcite)
Figures 5.21 and 5.22 show the effects of confining stress on the responses from CID triaxial tests for calcite contents between 4.2 % and 5 %. The effect of confining stress in these tests is similar to that discussed above. A notable feature is the greater variability of the specimen responses shown for confining stress of 50 kPa. In particular, most of these apparently well cemented specimens show lower initial stiffnesses and greater strain to peak than the specimens with lower cement contents. The peak for the specimen subjected to 500 kPa confining stress is below the 200 kPa specimen. This could be a result of the lower calcite content (4.2 % versus 5 %). The pattern of dilation, with rate of dilation reducing with confining stress, is similar to that for the lower calcite contents although it is obscured here by the variability in the axial strains. The strengths are generally higher than for specimens with lower cement contents, as expected.

The reasons for the greater variability could be related to possible lack of homogeneity of cementation, although as noted above variance in calcite content was low in all specimens, or due to the growth of regions of concentrated zones of calcite which reduce the influence of stronger quartz particles, or due to differences between the amount of calcite precipitated at contact points and within the pore spaces, or seating errors.

Figure 5.21: Deviator stress, axial strain responses for bio-cemented specimens (4.2 % to 5.0 % calcite)
Figure 5.22: Volume strain, axial strain responses for bio-cemented specimens (4.2 % to 5.0 % calcite)

Figures 5.23 and 5.24 shows the results of CID test with a confining stress of 50 kPa for 3 specimens with approximately 7 % calcite. As for the 4-5 % calcite specimens there is surprising variability. One of the specimens shows the largest strain to peak of all the calcite cemented specimens. This suggests that the calcite is not effectively contributing to cementation. However, why there should be such variability is unclear as essentially similar techniques were used in each case. Practically the results could not have been triplicated for validation here. This can be attributed to a series of reasons, so results presented in this chapter include considerable uncertainties which have to be investigated in future studies. Other studies (Cheng et al. 2013) have shown that high concentrations can retard or prevent reactions from occurring, and this may in part explain the variability of the strengths with high nutrient concentrations.
Figure 5.23: Deviator stress, axial strain responses for bio-cemented specimens (7% calcite)

Figure 5.24: Volume strain, axial strain responses for bio-cemented specimens (7% calcite)
Figure 5.25 shows the effect of varying calcite content on the deviator stress, axial strain responses for a confining stress of 50 kPa. This figure shows a trend of increasing strength and stiffness with increasing calcite content. Differences are small for calcite contents between 1.5 % and 3.0 % and there is no relation between strength and calcite content in this range. The strongest specimen with 7.32 % calcite has been shown as the other two tests shown in Figures 5.23 and 5.24 do not fit the trend, or expected behaviour.

![Diagram showing effects of calcite content on deviator stress, axial strain responses](image)

Figure 5.25: Effects of calcite content on deviator stress, axial strain responses for p’c = 50 kPa

Figures 5.26 and 5.27 show effects of varying amounts of calcite cementation in CID triaxial tests performed with effective confining stresses of 200 kPa and 500 kPa respectively. The responses show as expected that increasing calcite content increases both strength and stiffness. There is also a reduction in the effects of increases in the amount of calcite as the confining stress increases, which is evident by comparing Figures 5.25, 5.26 and 5.27. When the confining stress reaches 500 kPa the peak strengths are about double the ultimate strength, which corresponds to the strength of uncemented sand. At this confining stress, the influence of the
cementation on the peak strength is relatively low, however, the small strain stiffness which is
controlled by the cement is much greater than for uncemented sand. Nevertheless the specimen
with only 1.8 % calcite still had sufficient cementation that its strength was controlled by the
bonding at 500 kPa confining stress. This is significant as it shows that only relatively small
amounts of calcite cement are required to achieve the strengths needed for effective ground
improvement. At least for monotonic loading, the loss of stiffness before the peak is relatively
minor.

![Figure 5.26: Effects of calcite content on deviator stress, axial strain responses for $p'_c = 200$ kPa](image)

Figure 5.26: Effects of calcite content on deviator stress, axial strain responses for $p'_c = 200$ kPa
Figure 5.27: Effects of calcite content on deviator stress, axial strain responses for $p'_c = 500$ kPa

Bender elements were used in all the triaxial tests, but unfortunately $G_{\text{max}}$ could not be reliably interpreted from many of the tests because of poor signal quality. Most of the tests for which a reasonable variation of $G_{\text{max}}$ was obtained throughout the compression and shearing stages are shown in Figure 5.28. It can be seen that $G_{\text{max}}$ increases with the amount of calcite, as expected. The pattern is similar to that observed for the gypsum cemented specimens. $G_{\text{max}}$ increases slightly during isotropic compression. During shearing $G_{\text{max}}$ initially continues to increase reflecting the increase in the mean stress level. As the specimens approach failure the reduction in $G_{\text{max}}$ due to breakdown of cementation becomes greater than the increase associated with the rising mean stress. The reduction in $G_{\text{max}}$ accelerates as specimens approach failure, and often drops rapidly post-peak. Post-peak specimens approach towards the uncemented sand response, but due to remaining cementation in unfailed portions of the specimens $G_{\text{max}}$ does not reach the uncemented response.
Figure 5.28: Variation of $G_{\text{max}}$ with $p'$ for calcite specimens (1.8-2.2 % Black, 3-4 % Blue, 5 % Red)

Figure 5.29 summarises the data on peak strengths from the tests described above. Figure 5.29 shows the influence of the amount of cement and confining stress on the peak strength. This figure shows the significant effect of the calcite cement on the strength at low effective confining stresses and also that small amounts of cement still have an effect on the strength at confining stresses up to 500 kPa. In drawing a best fit line through the tests with confining stress of 50 kPa the 4 tests with high calcite contents that gave low peaks and low stiffnesses have been ignored. It is evident in Figure 5.29 that these points lie significantly below the trend from the other tests. Not shown on Figure 5.29 are the yield points from seven undrained tests. Of these, 5 tests, which had calcite contents between 3 and 4.4 %, reached yield (assumed to be similar to the peak strength in the drained tests) between deviator stresses of 1000 and 1400 kPa giving points that lie close to the failure line shown for the 50 kPa confining stress tests. The other two undrained tests (calcite contents 2.2 % and 2.8 %) yielded at low deviator stresses that would lie on a line passing through the lower 50 kPa confining stress points. These unusual low strengths, and the associated low stiffnesses suggest that the calcite is not uniformly distributed in these specimens. For the two undrained tests with low strengths the flushing out of some of the calcite during saturation is suspected, but for the higher cement contents the reasons are unclear.
Figure 5.29 shows that there is a very significant increase in strength between the UCS specimens and the specimens tested with 50 kPa confining stress. It is believed that this is primarily a consequence of the confining stress preventing a splitting failure mode from occurring. This is also significant in the context of ground improvement as it shows that with relatively low confining stresses the calcite is even more effective than has been indicated from UCS testing. The figure also shows comparison with UCS test data reported by Al Qabany and Soga (2013) for specimens produced by flushing bacteria and nutrient solutions through a similar sand with similar relative densities. These data lie slightly below the current study even though similar shear wave velocities have been reported as shown in Figure 5.30. Previous studies of bio-cementation have suggested there is a lower limit of about 2% calcite below which the cement is not effective. However, this is not evident in this study where more thorough mixing of soil and cement has occurred. As discussed above, this suggests that inhomogeneity in cementation occurring with the flushing technique is responsible for both low apparent strengths with weak cementation and the scatter in the data reported in previous studies (Kucharski et al. 1996; Whiffin et al. 2007). It is also acknowledged that the mixing technique cannot prevent inhomogeneity developing as evidenced by the low strengths of some of the specimens with high calcite contents, but at low calcite contents the mixing technique appears to produce stronger and more consistent specimens.
Figure 5.29: Summary of effects of cement and confining stress on peak strength

The values of shear wave velocity, measured after curing, for all the triaxial specimens are shown in Figure 5.30 (a). This shows a fairly consistent relation between the amount of cement and the resulting shear wave velocity, and that calcite is a much more effective cementing agent than gypsum. The greater effectiveness of the calcite cement is consistent with observations made by other studies (Al Qabany et al. 2011; Cheng et al. 2013; Ismail et al. 2002). These have also shown the calcite tends to be precipitated preferentially at the points of contact, whereas the particulate nature of the gypsum means that a significant amount is present in the void spaces where it may not contribute to the bonding of the sand particles. It should also be noted that increases in the amount of cement are associated with increases in dry unit weight (Duraisamy et al. 2014) which have been shown in many studies (Consoli, 2009; Huang and Airey, 1998) to lead to increases in strength and stiffness at constant cement content.
Another significant difference between the current study and most previous studies of biocementation is that specimens were prepared by mixing and cured with no further addition of nutrients or bacteria. In contrast, most prior studies have produced cementation by pumping solutions of nutrients and bacteria through uncemented sand. The results of one of these studies
(Al Qabany et al. 2013) are included on Figure 5.30 (a) for comparison. This shows that there is no significant difference in the shear wave velocity values between the two data sets, in this study produced by mixing, and in the earlier studies by pumping in solutions of nutrients and bacteria. The data from Al Qabany et al. (2013) were limited to calcite contents of 4.5 %, although higher calcite contents and stiffnesses have been reported in other pumping studies. Our results suggest that further increases in calcite content deviate from the linear relation suggested by Al Qabany et al. (2013). This is believed to be a consequence of additional calcite filling the pore space and not being as effective in cementing the grains. However, as noted above the production of higher calcite contents by mixing appears to result in non-uniform cementation and this may also be influencing the apparent trend.

Figure 5.30 (a) shows that the rate of increase of shear wave velocity reduces as the cement content increases. However, if the results are presented as shear modulus, $G_{\text{max}}$ versus cement content an approximately linear relationship is obtained as shown in Figure 5.30 (b). Similar linear relationships have been observed for other artificially cemented sands. From an earlier study of gypsum cemented carbonate sand (Mohsin and Airey, 2008) it was found that the shear modulus, $G_{\text{max}}$ could be predicted using Equations 5.3 and 5.4, where the contribution to $G_{\text{max}}$ of the cementation is provided by equation 5.3.

$$
\frac{G_{\text{max}}}{p_r}_{\text{cemented}} = \left( \frac{G_{\text{max}}}{p_r}_{\text{uncemented}} + \frac{G_{\text{max}}}{p_r}_{\text{cemented}} \right)
$$

(5.3)

$$
\frac{G_{\text{max}}}{p_r} = A (GC)^{0.08} \left( \frac{p'}{p_r} \right)^{0.01} e^{-2.42 \left( 1 + \frac{q}{p'} \right)^{0.02}}
$$

(5.4)

In these equations $p_r$ is a reference stress taken as 1 kPa, GC is the cement content as a percentage of the dry mass, $p'$ is mean effective stress, $e$ void ratio, $q$ deviator stress, and $A$ is a dimensionless constant.

The value of the constant $A$ for the gypsum cemented carbonate sand was found to be 16407 (Mohsin and Airey, 2008). To obtain reasonable fits for the data in Figure 5.30 (b), the value of $A$ would need to be two and six times bigger for the gypsum cemented quartz sand and calcite cemented quartz sand respectively. These results indicate both the importance of the sand particles, which is quartz as opposed to carbonate, and the greater effectiveness of the calcite
cement. The greater stiffness of gypsum cemented quartz sand compared with the gypsum cemented carbonate sand suggests that the strength and stiffness of the soil grains has a role in the overall cemented soil stiffness. As quartz is a hard, inert and durable mineral it would be expected to provide higher stiffness than friable carbonate soil grains. The greater stiffness with the calcite cement suggests that the location of the cement is important. It has been noted in previous studies that calcite tends to be precipitated on the particle grains and effectively forms bridges at the points of contact. Gypsum by contrast tends to fill the spaces between the grains and is as a result less effective in increasing the stiffness.

For the bio-cemented specimens the ratio of $G_{\text{max}}/\text{UCS}$ is in the range from 1400 to 2500 with no particular trend across the range of calcite contents investigated and an average value of 1750. For the gypsum cemented specimens there is a similar scatter and an average value of approximately 3000. The ratio for the gypsum cement appears to be higher because of the relatively low strength at least in comparison to the effect of the gypsum in increasing the stiffness. The ratio of stiffness to strength may be compared with other rocks and cemented materials. For example, Cheng et al. (2013) report values of $E_{\text{max}}/\text{UCS}$ in the range from 40 to 80 for bio-cemented sand treated with calcite using injection technique, and similar values have been reported for naturally cemented carbonate sand (Airey, 1993; Huang and Airey, 2008). These values appear very low compared to the values measured for the samples in this study, but this is believed to be because the other studies were measuring the initial tangent stiffness, which in this study has been shown to be significantly less than $G_{\text{max}}$. The ratios of $G_{\text{max}}/\text{UCS}$ observed in this study are similar to those reported for well cemented, hard rocks. This indicates yet again the effectiveness of the bio-cement in improving the stiffness of the cemented sand.

A comparison of the stress, strain responses of the bio-cemented and gypsum cemented specimens from drained triaxial tests conducted with an effective confining stress of 50 kPa is shown in Figure 5.31. The results are consistent with studies on a wide range of artificially cemented soils. It can again be seen that the calcite is more effective than gypsum, with the strength of calcite cemented specimens from the triaxial tests about three times that using gypsum for a similar mass of cementing agent. It has been noted above that the ratio of $G_{\text{max}}$ to UCS strength varies for gypsum specimens and that it tends to be lower at high cement contents. This same trend is evident in Figure 5.31 with the more strongly cemented gypsum specimens.
tending to have lower stiffness than the calcite cemented specimens with the same strength. This is believed to be related to the different modes of cementation created by calcite (crystals nucleating on particle surface) and gypsum (gypsum particles filling the void space).

Figure 5.31 Comparison of calcite and gypsum cemented specimens (p’<sub>c</sub> = 50 kPa)

5.5 Summary

Bio-cemented specimens have been produced by mixing sand, bacteria and nutrients and these have been compared with specimens cemented with gypsum. It has been found that mixing produces more uniform specimens than when bio-cement is created by pumping and that the strength and stiffness do not appear to be greatly affected by the method of sample preparation. Preparation by mixing is recommended to investigate the response of weakly cemented material. However, there are limitations to mixing if high calcite contents are required as calcite contents of greater than about 5% appear to be associated with variable cementation.
As noted in several studies calcite is a highly effective cementing agent, and for a given amount of cement gives higher strength and stiffness than other cementing agents. Results show that the built up of strength in UCS tests is similar or possibly slightly higher than samples treated using injection techniques. At the same time the problem of clogging in injection points has been avoided by using an ex-situ mixing technique, and this has been successfully demonstrated as feasible at laboratory scale.

The results from the triaxial tests have shown that up to calcite contents of about 5 % the approach of simply mixing the ingredients together is at least as good as injection based cementation from the perspective of improvement in engineering properties. Given the costs and need for complex staged injection to avoid clogging mixing appears to be a more cost effective way of introducing biocementation in situations where mixing is possible and only relatively modest cementation is required. Nevertheless, the current study indicates more research is needed to explore whether mixing can produce reliable cementation for higher calcite contents, and it is noted that multiple injection strategies have produced far greater cementation than from the single mixing process used in this study.

The patterns of behaviour observed in the triaxial tests of bio-cemented Sydney sand are very similar to those of specimens bound with gypsum. The results have been reasonably consistent throughout the laboratory tests conducted except for a few tests where membrane punctures and excessive seating errors led to unusual results. The triaxial test results have shown that the quantities of nutrients have an influence on the degree of cementation produced, however, there has been considerable scatter in the amount of calcite produced and it is difficult to predict the degree of cementation.

Use of automated shear wave velocity measurement has enabled variations in stiffness, and hence degree of cementation, to be monitored throughout the processes of curing, stress application and shearing. However, the large changes in shear wave velocity associated with curing have caused some difficulties in obtaining reliable data.
CHAPTER 6

PHYSICAL MODEL TESTS OF BIO-CEMENTED COLUMNS

6.1 Introduction

This chapter describes a series of static 1-g model footing tests performed on Sydney sand reinforced with cemented columns of bio-cemented and gypsum cemented Sydney sand. Model tests have been performed as they offer significant advantages in simulating complex systems under controlled conditions and simultaneously provide insight into the fundamental mechanisms involved. In the last five years, the biocementation process has been empirically scaled up from a laboratory sand column to a 100 m$^3$ field scale experiment, in which about 43 m$^3$ of sand was bio-cemented. Although a significant strength increase was obtained, the results were not completely satisfactory, as the calcite appeared heterogeneously distributed throughout the cemented sand body (Van Paassen et al. 2009, 2010). Apart from that, a scaled down test was carried out by Brian and Dejong (2009) to monitor the calcite distribution as well as the settlement of model footing. Results show uniform calcite formations with high concentration around injection points which suggests clogging. Therefore, the key parameters that control the in-situ distribution of calcite and the related
engineering properties in both naturally and artificially induced cemented sands appear to be insufficiently understood. As discussed previously the use of soil mixing can produce uniform cementation in laboratory specimens and can potentially produce uniform cementation in the ground. This chapter aims to investigate whether conventional deep soil mixing technology can succeed with biocementation, albeit at laboratory scale. The chapter also provides results from some preliminary laboratory model tests performed to investigate the ability of biocement to repair cemented soil columns. Al Tabbaa and Harbottle (2015) have explored the potential of self-healing system using biocementation application in foundations and other geotechnical structures. This could bring substantial savings in maintenance cost as well as enhance the durability and serviceability and improves the safety of structures and infrastructures. The main objectives of these tests are:

(1) To demonstrate the ability to form bio-cemented columns using an in-situ mixing technique in small scale model tests.

(2) To evaluate the performance of the model foundation on columns of bio-cemented Sydney sand and to compare this with gypsum cemented sand columns.

(3) To investigate the potential for healing and self-healing using biocementation when applied to repair cemented soil columns.

In this chapter, the use of MICP (Microbially Induced Calcite Precipitation) as an alternative to conventional cement in deep soil mixing is investigated. Small scale physical model tests are used to demonstrate the potential of in-situ mixed sand and biocementing agents to enhance foundation response to settlement. To enable the commercial application of biocementation in geotechnical works, extensive research has to be conducted to determine the optimum mixing conditions and to understand the effects of the biocement on engineering behavior. The addition of microbes and nutrients to soil can lead to significant enhancement in the engineering properties of bio-cemented soil, as demonstrated in the previous chapter from a series of UCS and triaxial tests. The results have shown that the general patterns of engineering behavior are similar for the bio-cemented and gypsum cemented samples where increases in strength and stiffness result from increases in the amount of cementing material. However, the amount of cementing material required to produce a certain cementing effect can vary widely and the calcite cement appear to be much more effective than gypsum in enhancing both stiffness and strength. It has been noted that the effectiveness of the cement also depends on the density of the sample and the confining pressure.
The tests described in this chapter have been performed to study whether the effects of soil mixing evident in laboratory element tests can be achieved in less controlled deep soil mixing model tests. Soil columns have been created in dry, wet and saturated sand beds to reproduce the field conditions, and the column mixing procedure has been designed to resemble the field process as closely as is practicable given the small model size. Results are presented to show the uniformity of the soil columns, the degree of improvement of strength and stiffness, and the ability of columns loaded to failure to be repaired using biocementation.

6.2 Test Program

The small scale model tests have been planned to show the ability of soil mixing to produce bio-cemented columns under a variety of soil moisture conditions. The response of a footing placed on the columns has then been used to compare the effectiveness of the bio-cemented columns through comparison with uncemented sand and gypsum cemented columns. The procedure developed to produce the cemented columns has been described in Chapter 3. The columns have a diameter of 38 mm and various lengths. The relatively small column diameter presented a number of challenges, firstly in achieving thorough soil mixing (discussed in Chapter 3) and secondly in terms of monitoring the cementation process. Initially it was planned to place bender elements in the columns to monitor and confirm the cementation process but this step could not be achieved. As such all measurements of column integrity and degree of cementation have had to be obtained post-test. A total of 40 column tests including 6 uncemented tests have been performed. Because of the time taken for curing of the cement in some soil conditions only a limited number of tests have been performed. The tests have concentrated on investigating the range of cementation possible with soil mixing and have been limited to a single diameter. Table 6.1 summarizes the testing program carried out to achieve the specific objectives of this study. Tests were performed for two column lengths, 100 and 200 mm, for sand that was dry, moist and saturated (submerged). For each of these combinations a range of gypsum and bio cementation levels were targeted that would be expected to have a significant impact on the footing response. Gypsum introduction in the auger set up is explained in detail in section 3.10.5 of Chapter 3. The details of soil preparation in model tank have been given in section 3.10.2 of Chapter 3.
Table 6.1: Testing programs in 1-g model experiments

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<thead>
<tr>
<th>Binder Type</th>
<th>Binder (%)</th>
<th>Column Dimension</th>
<th>Soil condition</th>
<th>Curing time (hrs)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Uncemented</td>
<td>NA</td>
<td>NA</td>
<td>Dry, wet and saturated</td>
<td>NA</td>
</tr>
<tr>
<td>Gypsum</td>
<td>5</td>
<td>D=38mm</td>
<td>Dry, wet and saturated</td>
<td>24</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>L=100mm &amp; 200mm</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>15</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Biocement</td>
<td>5.0 – 6.9</td>
<td>D=38mm</td>
<td>Dry, wet and saturated</td>
<td>24 (Dry)</td>
</tr>
<tr>
<td></td>
<td>7.0 - 8.9</td>
<td>L=100mm &amp; 200mm</td>
<td></td>
<td>48 (Wet)</td>
</tr>
<tr>
<td></td>
<td>9.0 – 9.9</td>
<td></td>
<td></td>
<td>72 (Sat)</td>
</tr>
</tbody>
</table>

Note: NA – not applicable

6.3 Column Repair Procedure

The physical model tests outlined above were extended to study the potential of bio-cement to repair damaged pile foundations (ground improved columns). As discussed below, loading of the footing led to all the cemented columns breaking into two or sometimes three sections, the upper break occurring approximately one footing diameter below the surface (see Figure 6.1). Three different strategies were investigated for repairing the damaged columns. In all cases investigated for repair the cemented columns were made by mixing 15% gypsum with the sand. To allow for later repair, a glass rod 8 mm in diameter was placed in the center of the columns after mixing in the gypsum, and was removed after 2 hours when the gypsum had set, leaving behind a hole in the center of the column for the full length of the cemented column, as shown in Figure 6.2. The column was then left to cure for at least 24 hours before the footing was loaded. The footings were loaded until a sudden drop in load indicated the breaking of the column. At this point, the load was removed and the column was repaired. The first repair technique involved injections of the bacterial solution (bacteria and nutrients)
around the cemented column in the vicinity of the break, as shown in Figure 6.3 (a). Five points that were equally spaced around the column were injected with 2 mL of the bio-solution, with each point at a distance of about 40 mm from the center of the column. The second repair technique simply involved pouring 10 mL of the biocement forming solution into the hole in the center of the column using a funnel, as shown in Figure 6.3 (b). The third repair technique also involved pouring 10 mL of nutrients (urea + CaCl₂) into the central hole, but in this third case, no bacteria were added. The third approach was used on columns that had already been repaired using the second method and had been reloaded to failure. The third technique was designed to assess whether residual bacteria from the previous repair effort could be reactivated by providing additional nutrients. Tests were performed with the sand surrounding the cemented columns in both the dry and saturated states.

Figure 6.1: Failed biocemented sample recovered from after test

Figure 6.2: Hole created in gypsum cemented sample prior to repairing
Figure 6.3: Foundation (column) repairing simulation using the (a) injection technique and (b) hollow casting technique

6.4 Model Test Results

The load versus displacement data from all the footing tests are presented in Figure 6.4. A logarithmic scale has been used for the load to enable easy comparison between the performance of the model footing with and without the cemented columns. The tests without the columns were continued up to large penetrations of 30 mm, which represents a settlement to diameter ratio of 0.3, to establish the ultimate capacity of the footings. In all tests the footings penetrated into the sand without any noticeable rotation of the footing. For the footings resting simply on the sand with no cemented columns the load continues to rise throughout the tests, as expected from the increasing confinement provided by the sand above the level of the footing. In most of the tests with the cemented columns a much stiffer foundation response can be observed in Figure 6.4 and the tests were terminated before reaching large displacements because there was a clear failure associated with a dramatic drop in load. There are also significant differences between the performance of the cemented columns in dry (part a and d) and saturated conditions (part c and f), and generally the results show the best performance is achieved with the gypsum cemented columns. More detailed discussion and interpretation of these trends are provided in the following pages.
Figure 6.4: Evolution of the average vertical pressure versus vertical displacement model footing for long (200 mm) (a, b, c) and short (100 mm) (d, e, f) cemented columns where parts a and d for dry sand, b and e for wet sand and c and f for saturated sand
6.4.1 Tests without Cemented Columns

Figure 6.5 shows in more detail the responses from tests on unreinforced sand. A range of responses have been obtained that depend on the relative density of the sand and the moisture conditions. Basically the relative densities in Figure 6.5 are referred to the dry, wet and saturated condition of the sand bed. The initial stiffness generally increases with relative density, however the response with relative density of 25\% lies significantly below the other curves, and the curve for relative density of 54\% is initially much less stiff than expected from the trend of the other tests. It is believed that in levelling the surface before placing the footing for the 54\% relative density a loose layer of sand was created on the soil surface. This explanation is consistent with the response at larger displacements where it approaches the curve for the 49\% relative density test. In addition for the tests with wet sand which was not saturated and had relative densities of 66\% and 73\%, suctions could have contributed to the effective stress and hence increased the resistance of the soil.

For shallow foundations, three failure modes; general shear, local shear and punching shear have been described by Chummar and Vesic (1973). The general failure mode is associated with dense sand and usually results in a clear peak in the load displacement response at relatively small normalized displacements. There is no evidence of this mode in the load, displacement responses and neither was there evidence of any significant heaving of the sand surface. The responses are generally consistent with local shear failure, which is expected when the relative density is less than 70\%. One of the difficulties with local shear failure is that there is no pronounced failure, the load continues to increase with penetration and identification of failure is problematic. Thus, based on the suggestion of Cerato and Lutenegger (2007) and as used in many studies, the failure load has been defined as the load producing a settlement of 10\% of the diameter of the foundation, which is 9 mm in these tests. This simple, but rather arbitrary rule has been used to identify failure in all the model tests and in the following discussion.
Consequently manual calculations have been performed using bearing capacity equations. In theory failure (settlement) of surface footing resting on homogeneous sand layers depends on the internal angle of friction ($\phi$) and the bearing capacity factor ($N\gamma$) (Lundgren and Mortensen, 1953). Therefore, with the known values of failure stress ($q_f$), effective unit weight ($\gamma'$) and foundation radius ($R$), the value of bearing capacity factor ($N\gamma$) can be found from equation (6.1).

$$N\gamma = \frac{q_f}{0.6\gamma'R}$$

The back calculated $N\gamma$ value for each test was used to determine the corresponding angle of friction from Terzaghi’s (1943) bearing capacity chart. These angles of friction, obtained for Sydney sand used in model footing test, can be compared with the triaxial test data from Chapter 5. The mobilized angles of friction estimated for the footing tests with loose, medium dense and dense sand are found to be close to the peak angles of friction determined from the triaxial tests for similar relative densities. For example, the back calculated angle of friction for loose sand from the model tests was in the range of 38° to 40°, whereas in the triaxial tests it was 36° to 39°. For medium dense sand the model test range was 41° to 43° compared to 40° for the triaxial.
sample. The critical state angle of friction in uncemented triaxial samples was 32°. These results are reasonable and thus provide some confidence in the interpretation of the footing tests with the cemented columns.

**6.4.2 Calcite Distribution Profile in Biocemented Columns**

The sand biocementing technique that is proposed in this chapter makes use of a model scale in-situ mixing procedure. As demonstrated in the previous chapter, mixing can overcome the major problem highlighted by previous researchers when using the injection technique to create biocement. This is that the injection technique when applied on site results in heterogeneous distribution of the calcite precipitation (Van Paassen, 2006; Whiffen, 2004; DeJong et al. 2009; Al-Thawadi, 2008; Cheng et al. 2013) as was detailed in section 2.6. Figure 6.6 shows the calcite profile in the bio-cemented Sydney sand columns obtained in the foundation model tests. In order to keep the material properties (unit weight, strength) of the cement columns similar to those used in the triaxial tests, the amounts of binder (% per dry mass) were also selected to be similar, but also sufficient bio-cement was used to ensure well cemented columns. The column with 15 % urea precipitated the highest amount of calcite, approximately 160 kg/m³ (8.7 % calcite) in wet soil. The least amount of calcite was recorded as 80 kg/m³ (5.9 % calcite) when using 5 % urea. The calcite contents were measured for the bottom, middle and upper thirds of the cemented columns. It can be seen that a consistent distribution of calcite along the cemented column has been produced in all the various soil moisture conditions tested. It should be noted that preliminary tests indicated that different curing times were required to produce well cemented columns, and that 3 days curing was required in saturated sand compared to only 1 day in dry sand. The calcite contents produced in these columns are comparable to those obtained in field tests from multiple injections. Van Paassen (2009) using the injection technique, produced non-uniform calcite contents that varied from 12 % (160 kg/m³) to 27 % (530 kg/m³) of the total dry weight. In contrast to this, a more uniformly distributed calcite was achieved with almost the same amount of calcite in this study. Hence, results from the model tests in this thesis show that bio-cemented columns can be produced using mixing techniques with improved uniformity of the calcite precipitation. However due to the small scale models used the method of introducing the bacteria has not exactly replicated the process used in typical field scale DSM methods and it is recommend that ups scaling be performed to confirm that uniform calcite can be achieved in
practice. In principle, this enables the strength of the bio-cemented columns to be directly correlated with the amount of calcite precipitated.

Figure 6.6: Actual calcite precipitation profile in (a) long (200 mm) and (b) short (100 mm) biocemented columns mixed in various soil conditions
6.5 Effect of Degree of Cementation on Bearing Capacity

The effect of degree of cementation on the bearing capacity of the cemented columns has been investigated using column lengths of 200 mm and 100 mm. In both cases, there are clear trends of an increase in vertical stress, for a given footing displacement, with an increase in cement content, as shown in Figure 6.7. The results also appear to show differences with moisture state. For example in Fig. 6.7 (a) the failure stress for the gypsum columns is lowest in the saturated sand and highest in the wet sand. This is believed to be primarily a consequence of the different relative densities in the different tests. It should be noted that the same sand test bed was used for all the columns and footing tests of a particular column length and moisture state (that is 3 biocemented columns, 3 gypsum cemented columns and a columnless test were performed in each sand bed). The sand in the vicinity of the columns was thus disturbed between tests, during extraction of the columns and during reforming the columns with different cement contents. This procedure could be expected to influence the footing response. However, the clear trend associated with each moisture condition evident in Fig. 6.7 (a) suggests that the responses were controlled by the density of the surrounding sand and the disturbance associated with reforming the columns has not had a major influence on the results.

The results for both the long and short columns show an increase in vertical stress at failure with an increase in the amount of cement. This trend is more pronounced for the gypsum cemented columns, longer columns and for the higher relative density sand beds. The bio-cemented columns give similar or slightly lower failure loads than gypsum cemented columns with the same percentage of cement. This is in marked contrast to the results from the UCS and triaxial tests which showed the calcite cement to be at least twice as effective as the gypsum. However, the triaxial test results also showed that when high calcite contents, greater than 5 %, were involved the strengths were often less than expected. In the column tests high nutrient concentrations were used to try and ensure effective biocementation and produce a high UCS. It is thus possible that lower calcite contents would have been just as effective, but this was not investigated and would require further tests to confirm.

It was noted after the tests that all the columns had broken into two, and the break occurred about one footing diameter, that is about 90 mm below the footing. This suggests that the difference
between the failure load with and without the columns should be related to the shear strength of the columns. If it is assumed that the deformation mechanism is unchanged by the column presence then it can be estimated that the columns with 15% gypsum require an extra load of between 750 N (\( \text{Id} = 0.25 \)) and 2000 N (\( \text{Id} = 0.7 \)) to produce failure. This is equivalent to shear stresses of 660 kPa to 1760 kPa on a shear plane passing horizontally through the columns.

Figure 6.7: The effect of degree of cementation on the bearing capacity using (a) 200 mm and (b) 100 mm length cemented columns
These values may be compared with deviator stresses at failure that vary from 300 kPa (UCS test) to 1800 kPa (triaxial with $\sigma'_c = 50$ kPa). It is difficult to directly compare these values with the estimated shear stresses as the effective confining stress acting on the column at failure depends on the relative density and the footing load, but the values appear to be within a reasonable range.

Figure 6.7 also shows that the failure load depends on the column length. This is also shown in Fig. 6.8 where the foundation stress is plotted against the foundation settlement. Comparison of the tests with L/D = 1 and L/D = 2 for the dry and wet moisture conditions shows that the longer piles fail at a greater load. For the columns in the saturated sand, which had the lowest relative densities the shorter column gives a slightly higher resistance, however in this case the columns did not contribute much to the footing resistance and did not fail (break in two) until much larger displacements. Assuming that all columns have the same strength, and they all fail by shearing at a similar depth, it can be inferred that there may be a greater confining effect preventing the failure of the longer columns. However, it is difficult to see how this could arise, and an alternative explanation is that the greater resistance is simply a consequence of the greater load being carried by the column, because of the increased friction and possibly end bearing associated with the longer columns.

Figure 6.8: The effect of 15% gypsum on bearing capacity improvement
Figure 6.9 shows the effects of the calcite cemented columns on the footing responses. Comparison with Fig. 6.8 shows that the mobilized footing stresses are significantly less than for the gypsum. However, unlike the gypsum columns the response is generally more ductile and apart from the wet test with L/D = 1 failure of the columns has not occurred at the designated failure deformation. If the maximum loads are compared (see Figure 6.4) it can be seen that the gypsum and calcite cemented columns fail at similar maximum loads, the only exception being for L/D = 2 columns in the wet sand. The more ductile response of the calcite columns suggest they have a lower stiffness, whereas the triaxial tests presented in Chapter 5 suggested the opposite. This in turn suggests that the preparation of the columns in-situ has influenced the strength and stiffness of the bio-cementation.

Figure 6.9 also suggests that the bio-cemented columns were more effective in dry soil than in wet and saturated conditions. This can be seen from the similar responses in dry and wet sand beds even though the dry sand had a lower relative density. It is suggested that the wet and saturated conditions reduce the effectiveness of the cementation, as also reported by Cheng et al, (2014). This could be a consequence of dilution of the nutrients and substrate preventing effective urea hydrolysis reactions, and from minimizing the pH rise that accompanies microbial activity in soil that facilitates calcite precipitation which is maximized when the pH is 8.5 to 9.0 (Stocks-Fisher et al. 1999).

Figure 6.9: The effect of 15 % urea (8-10 % calcite) on bearing capacity improvement
Alternatively, as suggested by Cheng et al, (2014) the wet and saturated conditions allow precipitation in the voids, whereas in dryer soil the water containing the nutrients is held at the points of contact encouraging precipitation where it is most effective. The column results tend to support the latter explanation as there is significant calcite precipitation in the columns but relatively low strengths.

### 6.6 Effect of Column Length on Bearing Capacity

The effect of the column length to diameter ratio on bearing capacity of the model footings is summarised in Figure 6.10. As discussed above, this figure indicates that the failure stress for the tests with bio-cemented columns increases with the amount of calcite precipitated and is apparently independent of the column length. For the gypsum cemented columns shown in Figure 6.10 (b) the same pattern is evident, except that the tests in the wet sand with L/D = 2 give significantly greater failure loads.
The effect of column length on reducing the settlement of the circular footing was studied at an applied vertical stresses of 30 kPa, 65 kPa and 25 kPa for dry, wet and saturated soil conditions, respectively. Note these were the stresses at failure for the footings without the column reinforcement. The settlement reduction from the cemented column was calculated using Equation (6.2).

\[
\text{% settlement reduction} = \left( \frac{\text{changes in settlement of cemented sand (mm)}}{\text{settlement in uncemented sand (mm)}} \right) \times 100
\]  

(6.2)

It can be seen from Figure 6.11 (a) that very similar settlement reductions are observed using 200 mm long gypsum and bio-cemented columns. Both cement types show the same trend of increasing settlement reduction with an increase in the amount of cement used. The bio-
cemented columns appear to be less successful in reducing settlement in the wet soil, but this may reflect the effects of disturbance and loosening of the wet sand and the much stiffer response in the uncemented wet soil. The settlement reductions associated with the 100 mm length columns shown in Figure 6.11 (b) are generally less than for the longer columns, and again there are no significant differences between the gypsum and bio-cement. As for the longer columns, the settlement reductions are least for the columns in wet sand, which are the tests with the higher relative density sand bed. It has been suggested that this trend could be a consequence of soil disturbance, but the consistent results also suggest that it is more likely to be the result of the higher stiffness associated with the denser sand. The lower settlement reductions of the 100 mm long columns are expected because the bases of the columns are in a region where the soil movements beneath the footing are predominantly downwards (Dijkstra et al, 2013). With increased cement content the columns become stiffer and are better able to transfer the load to the region beneath the actively deforming soil and this can explain the increasing settlement reduction with the more cemented columns.

In this study, more settlement reduction was demonstrated using 15% urea in a bio-cemented column in dry sand and less in wet sand. Similar settlement reductions have been reported by Martinez and Dejong (2009) using a 1-g model test. Although the findings on settlement reduction from this research accord with results in the present study, there are several questions that remain unanswered. These include the following: (1) does the ambient temperature influence the calcite precipitation and if so, to what extent will it affect the performance of the treated ground, (2) does the speed of the mixing auger influence the amount and the distribution of calcite, and (3) does the curing time influence the performance of the bio-cemented ground when a mixing technique is used on site.
Figure 6.11: Settlement reduction versus binder content in (a) long (200 mm) and (b) short (100 mm) columns
6.8 Effect of Hydrolysis Rate in Precipitating Calcite in Model Footing Test

Knowing the amount of urea and calcium chloride placed in the cemented columns the theoretical amount of calcite that could be precipitated was quantified using Equation (3.9). The actual calcite amounts precipitated have been shown in Figure 6.6. To enable comparison with the theoretical amount, the experimental data from all three soil conditions have been averaged. There is no apparent systematic difference between the amounts of calcite precipitated in the different moisture conditions. The predicted calcite amount slightly overestimates the actual calcite amount precipitated in the model tests, as can be seen in Figure 6.12. The trend of increasing calcite content with the increase of urea content used in the model tests is consistent with the trend obtained from the UCS and triaxial tests, however, there is surprisingly less scatter in the column tests than in the triaxial specimens.

![Graph](image-url)

**Figure 6.12:** Actual calcite precipitated versus predicted calcite in model columns.
6.9 Improvement Area Ratio Analysis

There used to be very limited attempts at analysis of the bearing capacity of single cemented columns in the literature (eg. Bouassida and Hadhri, 1995) and these are mostly focused on columns in cohesive clay rather than sand. Since then, robust research works have been carried out on cement improved columns in sand to increase bearing capacity by many researchers (eg. Al Tabbaa and Ayotamuno, 1999; Farouk and Shahien, 2013; Dijkstra et al. 2013). This includes the latest work by Al Tabba et al. (2013) on testing bearing capacity of carbonated columns produced by laboratory scaled augers in sand and gravels. For the purpose of comparison, the bearing capacity improvements produced by bio-cemented and gypsum columns are compared with other granular and cemented columns in this section. Out of many ground improvement techniques available, stone columns (also known as granular columns or granular piles) have been widely used to mitigate liquefaction. This ground improvement technique has been successfully applied to increase the bearing capacity and to reduce foundation settlement. In previously published research papers on ground improvement by stone and cemented columns, one of the key design parameters has been the improvement area ratio ($A_r$). This is useful in practice because it is common for multiple treated soil columns to be used. Equation 6.3 defines this parameter as the ratio of the cross sectional area of the improved column ($A_c$) to the cross sectional area of the foundation ($A_f$). This approach is normally used with grids of cemented columns. It is mentioned here as single column with a large surface footing is a special case of a large grid and there is little comparable research.

$$A_r = \frac{A_c}{A_f} \quad (6.3)$$

In this study the improvement area ratio was 17.8 % and this was constant for all the footing model tests carried out. The bearing capacity of saturated sand reinforced with biocemented and gypsum cemented column is presented in Table 6.2. These are compared with data from tests conducted by Bouassida and Porphaha (2004) who investigated the ultimate bearing capacity of clay reinforced by a group of end-bearing cemented soil columns. Comparison with clay is used here because of the limited data on cemented columns in compressible sands and is intended only to be indicative of the effects of the columns. In both studies failure occurred through the
cemented columns, however comparison is complicated by the different drainage conditions in
the two studies, and differences in UCS strength. Boussida and Porbaha (2004) reported UCS
strengths of their cemented columns ranging from 515 kPa to 715 kPa, whereas the UCS tests
results in this study were from 750 kPa to 950 kPa for bio-cement with calcite contents between
6 % and 9 % and 350 kPa for 15 % gypsum. These conforms with data from Chapter 5 which
shows that the calcite cement is at least twice as effective as the gypsum, which is the UCS
strength with 10 % gypsum is similar to the UCS strength with approximately 5 % calcite. For
example, 5 % calcite is produced by adding 10 % urea and 10 % calcium chloride to the bacterial
solution. On the other hand, Farouk and Shahien, (2013) stabilized loose sand using 16.8 %
Portland cement in columns achieved UCS strength about 2400 kPa which is 3 and 6 times the
strength achieved by biocement and gypsum cemented columns in this study. In a real scale
project, a deep mixed cement column of 0.5 m diameter with 20 % Portland cement and 22 %
area ratio has improved the bearing capacity of surrounding soil up to 520 to 650 kPa (Hosoya et
al. 1996). Interestingly the bearing capacity of columns in this study is approximately 2 times
higher than what was achieved by Farouk and Shahien (2013). Even though cemented columns
using Portland cement produced higher strength it has not as efficiently increased the bearing
capacity of sand as gypsum and biocement. According to Farouk and Shahien (2013), the bearing
capacity increase in loose sand was in the range of 3 % to 16 % depending on the improvement
ratio. As discussed above the strengths and stiffnesses of the bio-cemented columns do not
appear to be consistent with the data from the UCS tests and it is believed that the actual
strengths of the columns are less than indicated by the UCS tests.
Table 6.2: Bearing capacity results of columns tested in model footing tests

<table>
<thead>
<tr>
<th>Research</th>
<th>Improvement area ratio (%)</th>
<th>Cement type</th>
<th>% Cement</th>
<th>Column type</th>
<th>Bearing Capacity (kPa)</th>
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</thead>
<tbody>
<tr>
<td>Present study</td>
<td>17.8</td>
<td>Calcite</td>
<td>9.8</td>
<td>Floating</td>
<td>105</td>
</tr>
<tr>
<td>Present study</td>
<td>17.8</td>
<td>Gypsum</td>
<td>15</td>
<td>Floating</td>
<td>160</td>
</tr>
<tr>
<td>Bouassida and Porbaha (2004)</td>
<td>18.8</td>
<td>Portland cement</td>
<td>12</td>
<td>End-bearing</td>
<td>186</td>
</tr>
<tr>
<td>Farouk and Shahien (2013)</td>
<td>17.3</td>
<td>Portland Cement</td>
<td>16.8</td>
<td>Floating</td>
<td>80</td>
</tr>
</tbody>
</table>

The bio-cemented columns in this study have clearly demonstrated their potential to improve the bearing capacity and stiffness of the unreinforced loose sand. Although further studies may be needed to confirm the results, this study indicates that mixing of biocement in granular soil is expected to improve the bearing capacity as well as using other ground improvement technique such as stone columns.

Previously, the effect of improvement area ratio on settlement reduction in loose sand was investigated in research conducted by Farouk and Shahien (2013). As the improvement area ratio increased with various lengths of soil-cement columns the reduction in settlement of the foundations becomes more significant. About 70 % to 80 % of settlement reduction was measured in sand cemented with an improvement area ratio of 17.3 %. Similar reductions were measured in this study, when wet Sydney sand stabilized with cement column was subjected to a vertical stress of 65 kPa. It was also noticed that the same settlement reduction was observed
with normalized cemented column lengths of \( L/D = 1 \) and 2 when sand was treated with either 9.8 \% biocement or 15 \% gypsum. Based on the results obtained from this study and also reported by Farouk and Shahien (2013), it can be concluded that the effect of improvement area ratio in reducing settlement becomes relatively insignificant at \( L/D \) more than 1.

### 6.10 Column Repair Response

As discussed above, all the columns broke about one footing diameter below the tops of the columns. Figure 6.13 shows the response of the footings on the initial loading, in both dry and saturated sand, a distinct peak in the stress deformation response is observed. After unloading, two of the footings were reloaded to show the response without any repair. In each case, the stress increased to the value before unloading and then decreased with further deformation. Three methods of repair were then investigated using the bacterial solution to precipitate calcite and restore the columns. In the first repair method, in which the biocement is precipitated around the column the foundation stiffness is similar or less than for the failed reloaded column. This suggests that the biocement has not been effective in repairing the column; however, the effect of the biocement is evident at large settlements where the repaired column shows a higher resistance. In the second repair method, where the bacterial solution is poured into a central hole in the column, the solution can flow through the region of the break and calcite precipitated there can weld the two parts of the column back together. Evidence that this has successfully occurred can be inferred from the high stiffness and the higher resistance than the reloaded footing, as seen in Figure 6.13 (a) for the footing on repaired column 2. The effect of this repair approach does not appear to be so successful in saturated sand (Figure 6.13 (b)), although even in the saturated case, there does appear to be some benefit from the repair at large settlements. In the third repair method, the previously repaired column 2, which had been loaded to its breaking point, was repaired again by adding nutrients without any bacteria. The responses in Figures 6.13 (a) and 6.13 (b) show that after this repair (Repaired Column 3), the best foundation response was obtained, which suggests that additional calcite had been precipitated. This indicates that the potential for self-healing exists, provided the column has bacteria present at the time of construction and nutrients for the bacteria can be provided as and when required to heal cracks.
Figure 6.13: Stress, displacement responses from repaired model foundation tests in (a) dry sand and (b) saturated sand
6.11 Repair Potential

The challenges of producing uniform and strong biocementation have been noted in many studies, but to date, none of the methods proposed has proven to be suitable in field application. It has been shown in this study that simply mixing the bacteria, nutrients, and soil can produce uniformly cemented specimens, suggesting that in-situ and ex-situ mixing combined with dry soil mixing technologies may provide a viable method for the application of biocementation. However, it has been noted that achieving effective biocementation in saturated sand is more difficult than in dry sand and further study is needed to investigate whether longer curing periods will result in the same strength for the same amount of precipitated calcite. It was also observed that more nutrients were required to produce a given amount of calcite in saturated sand than in dry sand.

The effects of the repair techniques could also be investigated by extracting the columns from the sand after the loading tests. Two repair mechanisms could be identified. First, in the tests where the bacterial solution was injected around the pile, calcite precipitation on the surface of the column was evident, as can be seen in Figure 6.14 (a). This precipitation appeared to be primarily filling in cracks in the column surface created during the first loading. There was no evidence of cemented sand surrounding the column, but this may have been because this cementation was broken down during the loading of the repaired column. These observations suggest that the injection of a bacterial solution in the vicinity of cracks may be effective in sealing them and could prevent damage from water entry. Second, when the bacterial solution was poured through the central hole, it was able to weld the two parts of the damaged column together, as shown in Figure 6.14 (b). The shear plane exists below the red line. The virtual absence of the shear line after being repaired using bio-cement was represented by the red line. This observation suggests that there is potential for pile repair, providing a provision is made for tubes that can enable the bacterial solution to be directed to regions that require repair.
Self-healing strategies generally rely on the bacteria and nutrients being available when repair is required. This can be achieved by encapsulating cells of bacteria and nutrients that are then released if cracks occur. The third repair strategy involved simply adding more nutrients and reactivating bacteria from an earlier repair effort. The success of this approach suggests that the incorporation of bacteria in the original soil column may be beneficial for future repair and would require only the addition or release of nutrients to enable self-healing.

Although these tests have shown the ability of bio cementation to form and repair soil columns, the applicability of this approach to real foundations requires further study. In particular, the resilience of the bacteria when subjected to stress and construction environments needs to be demonstrated. Even for cementing agents that are well understood, it has been reported (Terashi, 2002) that strength parameters obtained from laboratory treatability studies are usually five times higher than those obtained in the field from deep soil mixing applications. In another research conducted by Massarsch (2005), static laboratory and field study on the deformation parameters of cemented columns reveals that results obtained from laboratory studies were two to three times higher than results obtained in-situ. However Al Tabbaa et al (2013) addressed that laboratory auger mixed results are much closer to those of field auger mixed results.
6.12 Summary

Differences between the behaviour of the bio-cemented columns and the cemented specimens investigated in UCS and triaxial tests (Chapter 5) may occur due to many factors, such as variations in degree of saturation, level of effective stress and the drainage conditions during shearing. At this stage of the research, these factors do not appear to be significant as the results from the small scale model tests show similar responses for the different moisture conditions investigated. It has been shown that calcite can be effectively precipitated in the small scale model tests which were designed to simulate, as closely as possible, the actual technique of deep soil mixing (DSM). Well cemented columns have been obtained which have produced increases in bearing capacity and reductions in settlement. It is believed that the results produced here should be more reliable and representative of actual field conditions than well controlled cylindrical specimen manufacture, and can provide correlation between the strength achieved and the amount of calcite precipitated at actual field condition. However, because the model tests were carried out at a single gravity (1g), they involved significantly lower stresses than what are typically encountered in the field. The numerical values of the parameters derived from the tests at higher stress levels may be different from those found here, but the overall patterns of behavior are expected to be similar.

The patterns of behavior observed in bio-cemented Sydney sand are very similar to those in specimens bound with gypsum and have been reasonably consistent throughout all the laboratory tests conducted. The laboratory tests have identified the quantities of nutrients required to produce a range of calcite contents and, hence, soil columns with a range of strength and stiffness. It has also been demonstrated that the mixing of soil, bacteria and nutrients can produce specimens with a uniform distribution of cement. Small scale footing tests have been performed on sand improved by bio-cemented columns, where the column creation has modeled the deep soil mixing process. These tests show that bio-cement could be an alternative to existing cementing agents.
Qualitatively, the results presented herein provide insight into the basic mechanisms that control the load versus settlement responses of the bio-cemented Sydney sand and the corresponding amount of calcite precipitated at different levels of cementation. However, the experimental focus of the study has limitations for predicting the response of bio-cemented columns in general. These limitations include only using one type of sand, a single grading, only a single column and a small and single size of circular footing. Milligan et al. (1986) and Adam and Collin (1997), in their studies comparing large and small scale tests on the behavior of granular soil, pointed out that the general mechanisms and behavior observed in the small model tests are reproduced at larger scales. Thus, an extension of the experiments to larger scale tests (with larger footing and different types) together with field tests needs to be carried out for various soil conditions. For example, different footings (size, shape, and depth), different characteristics of soil, and different particle size distributions of soil would be very useful to further validate the present findings.

The ability of biocement to repair broken soil columns, either by injection around the periphery of the column or by injection into a hole in the center of the column, has been demonstrated. The presence of bacteria can stimulate calcite precipitation when nutrients are provided, and this offers the potential for self-healing foundations. Only preliminary tests were performed in this study, and the promising results suggest this approach holds potential for repairing damaged below ground infrastructure.
CHAPTER 7

CONCLUSIONS AND RECOMMENDATIONS

7.1 Introduction

The works carried out in this research are briefly summarized, conclusions are drawn and some suggestions for future study are made in this chapter. Research was carried out in 3 phases, in line with the three main objective of this research. Bio-cemented samples were prepared by mixing techniques as an alternative to the injection techniques used by previous researchers. Batch analyses were carried out to gauge the performance of the chosen ureolytic bacteria (*B. megaterium*) in stimulating urea hydrolysis in terms of urease activity and hydrolysis rate. Unconfined compressive strength (UCS) and triaxial tests with bender elements were used to measure the engineering properties of the bio-cemented sand. Physical model tests were used to measure the feasibility of the proposed technique when applied in the field. Also the potential of bio-cement to repair foundations was investigated.
7.2 Urease Activity in Batch Analysis

One of the objectives of this thesis has been to investigate the potential of *B. megaterium* to act as a catalyst for the production of bio-cement. This study has examined the rates of urea hydrolysis using this non-traditional bacterium for soil bio-cementation applications. Generally, urease producing bacteria (UPB) used for bio-cementation should be active in typical geotechnical environments without sensitivity to salt concentration. Thus halophilic or halotolerant, and alkaliphilic UPB are preferable for the manufacture of bio-cement. The performance of *B. megaterium* was evaluated under the presence of high concentrations of urea, Ca$^{2+}$ ions and various biomass concentrations to determine its viability. Overall, the results from batch analyses revealed that growth of *B. megaterium* can be easily stimulated, producing high rates of urea hydrolysis and thus can be used to induce calcite precipitation in the geoenvironment. The bio-safety level of the selected bacterium is also an important consideration. The safety level of *B. megaterium* is at level 1 indicating that only a minimum level of safety measures are required in order to use this bacterium.

Overall the findings suggest that *B. megaterium* has high potential as a microbe for precipitating calcite through the urea hydrolysis process. Under normal batch conditions, *B. megaterium* can produce enough urease to cater for 10 mM urea/min activity required for bio-cementation. It was found that the potential urease capacity of this bacterium was 8 mM urea.min$^{-1}$.OD$^{-1}$ and sufficient for bio-cementation without additional processing. In spite of the good laboratory performance, the desired urease activity for bio-cementation in field application requires concentrating the urease substrate and the control of other environmental factors like pH and temperature. Although *B. megaterium* produced ten times more urease per cell than some other ureolytic bacteria, it is recommended that before its use in any field trial the factors controlling urease production should be optimised.

Hydrolysis of urea presents a straightforward solution to calcite precipitation for bio-cementation. The urease enzyme is common in a wide variety of microorganisms and can be readily produced for urea hydrolysis process by adding an inexpensive substrate. It has been shown that mixing can produce effective bio-cementation, but as one of the main concerns with mixing strategies is the viability of the bacteria the use of plant derived urease to induce the
carbonate cementation should be investigated in future studies. Research should also be carried out to reduce the impact of the by-products of the urea hydrolysis process (ammonium chloride in this study) on the environment.

### 7.3 Unconfined Compressive Strength (UCS) Test

A series of unconfined compressive strength (UCS) tests have been carried out to investigate the feasibility of mixing techniques in precipitating calcite. Although the first few trials were unsuccessful during the sample preparation stage due to excessive amounts of water used during mixing, by limiting the amount of water and increasing the curing time well cemented specimens have been obtained. Bacterial solutions were strictly prepared aseptically with controlled pH and temperature without compromising any standard procedure involved in UCS testing. Hence, the results obtained and reported in this thesis may not represent the actual performance of the bacteria on site due to the uncontrolled nature of the geo-environment.

Theoretically the UCS strengths achieved in this study with only very small amounts of calcite should be sufficient to mitigate liquefaction, which is a common issue in uniformly graded sandy soils. Results show that 1 % of urea (per dry mass of the sand), which produces approximately 0.5 % of calcite, is enough to produce cemented strengths of up to 150 kPa, sufficient to withstand static liquefaction. In order to achieve the same UCS value with other binders requires greater amounts of cementing agents. For example, 5 % calcite, 8 % Portland cement and 10 % gypsum have been shown to produce the same UCS value of 500 kPa (Ismail et al. 2002).

The UCS strengths measured in this study have shown that low amounts of biocement (calcite) can be far more effective. For example, the results show that approximately 1 % calcite is equivalent to about 8 % gypsum. In general the mixing technique has recorded slightly higher UCS strength in comparison with injection technique used by Al Qabany, et al (2013). The mixing technique leads to more homogenous samples, and significant UCS strengths for very low calcite contents which were not observed in samples created used injection, nevertheless some variance exists in the UCS strength results due to sample preparation and testing procedures. Mixing has been able to produce homogenous calcite distributions for up to 8% calcite, which have significant UCS strengths. Further strength increases would require subsequent injection phases, although this has not been investigated.
The results have shown that the degree of improvement in UCS strength is similar, and certainly not lower, than that achieved in samples treated using injection techniques. However, the mixing techniques investigated in this study mitigate the clogging issue present with injection and have successfully created MICP at the laboratory scale. The bio-cemented sand produced by using mixing not only achieved calcite precipitation, but it also produced a significant strength improvement, comparable to that achieved with multiple injections. The ability of mixing techniques to produce significant strength in one application makes this approach potentially very attractive for many ground improvement applications.

The maximum theoretically achievable amount of calcite precipitate was compared with the experimental data and generally this was slightly greater than measured. A correlation was established between UCS and calcite content, which was similar to previous studies conducted by other researchers. Thus, in principle, by controlling the amount of bacteria and nutrients, the amount of calcium carbonate (calcite) and the strength of the reinforced/stabilized sand body should be capable of being engineered.

### 7.4 Triaxial Compression Test with Bender Elements

A series of triaxial tests with bender elements were conducted using conventional and standard procedures. The stress, strain and volumetric responses, and the evolution of shear wave velocity during cementation and shearing of bio-cemented sand have been presented and compared with gypsum cemented sand samples. The effects of various cement contents and stress states have been shown and the effects of bio-cementation on small strain shear modulus have been investigated by monitoring shear wave velocity throughout the tests. Finally a simple model was proposed to predict the small strain stiffness modulus of bio-cemented Sydney sand.

Figure 5.29 in Chapter 5 has shown a summary of the influence of the cement content, cement type and confining pressure on the peak strength. This figure shows the significant effect of the calcite cement on the strength at low effective confining stresses and also that small amounts of cement still have an effect on the strength at confining stresses up to 500 kPa. This figure also shows comparison with UCS test data reported by Al Qabany et al. (2013) for specimens
produced by flushing bacteria and nutrient solutions through a different sand that had a similar grading and relative density. The data from Al Qabany et al. (2013) lie slightly below the current study even though similar stress and strain have been reported as shown in Chapter 5. It has been suggested that this is a consequence of the greater homogeneity in the mixed specimens, an effect which is most noticeable at very low cement contents.

Although there was an expected trend of increasing strength as the calcite content increased in the triaxial tests, some of the detailed trends have not been simple to explain and there is a need for further investigation before the field performance can be accurately predicted. Results obtained from triaxial tests in this and previous studies suggest that the relation between the strength and the amount of calcite precipitated depends on the degree of saturation during precipitation, type of sand grain, technique of introducing the bacteria and the uniformity of the calcite distribution. Bio-cemented specimens produced in the triaxial cell, where drying out was prevented and specimens were saturated before shearing, produced strengths 2 times more than partially dried specimens in unconfined compression. The mechanism responsible for this difference is unclear. Also in this study, bio-cemented specimens with greater than 5% calcite produced greater variability and often resulted in lower strengths than expected. Nevertheless, at lower calcite contents clear and correlated increases in strength and stiffness were observed. In general it was found that bio-cemented samples produced by mixing recorded higher strengths than samples produced by injection, provided the calcite content was below 5%. A calcite content of up to 10% could be produced by a single mixing process, but as noted further study is required to understand why the higher calcite contents did not result in the expected strengths. The results confirmed other studies of calcite cementation in showing that calcite are more efficient than gypsum in improving strength in sand. Triaxial tests, at a confining stress of 50 kPa, show that samples with 5% calcite produce similar strengths to 10% gypsum, and 2% calcite gives strengths similar to 5% gypsum. The triaxial tests confirm that simple mixing techniques can provide sufficient improvement in sand properties for consideration in ground improvement.

The data obtained from bender elements are consistent with previously established data for bio-cemented sand. This shows that there is no significant difference in the shear wave velocity values between the two data sets, in this study produced by mixing, and in the earlier studies by
pumping in solutions of nutrients and bacteria. However, the production of more than 4.5 % calcite by mixing appears to result in non-uniform cementation and this may be influencing the apparent trend. Linear relationships have been observed between small strain shear modulus and cement content in gypsum cemented sands by previous researchers and a similar trend is evident in this study. Improvements in the small strain stiffness modulus of bio-cemented sand, in similar fashion to gypsum cemented sand suggest that the bio-cement can perform adequately as an alternative binder to withstand dynamic load. At the same time, confidence in the stiffness allows more realistic prediction of ground movement when using biocement.

It was also challenging to test samples without disturbing the biogeochemical activity while in the triaxial machine. In the present study triaxial specimens were prepared in a partially saturated state as this ensured thorough mixing and produced homogeneous specimens. However, during saturation it was found that using conventional flow techniques to flush out the air the process also flushed out some of the cementing agents, and saturation was then performed by allowing no outflow. The extent to which this affected the cementation deserves further investigation. Further challenges were caused by the significant change in shear wave velocity during curing which led to poor signal quality from the bender elements and in many tests difficulty in interpreting the shear wave velocity when using the automated test procedure. Changes to the present data acquisition system to enable automation of the input bender frequency adjustment would assist in any further studies.

It has been difficult to control and predict the amount of calcite precipitated in the triaxial specimens. It is possible that there could be some error in the estimation of the measured calcite at the time the test was terminated. Due to insufficient curing time there may have some nutrients left over from uncompleted urea hydrolysis reactions and this may have compromised the calcite content measurements. The development of improved procedures are recommended to measure the calcite and this may resolve some of the scatter in the trends with cement content, and this could be further confirmed by microscopic analysis.

The level of cementation was monitored and measured using shear wave velocity during the curing stage in the triaxial tests. Although the results show that the rate of curing of bio-cemented specimens was independent of the final amount of calcite and the shear wave velocity increased with the amount of precipitate, all specimens were prepared in the same way with
similar moisture contents. The physical model tests where cementation occurred under water required up to 3 days to ensure well cemented columns were produced and additional monitoring of curing of specimens with higher initial moisture content suggested that this increased the delay before cementation occurred. These observations suggest there is a need for greater investigation of the curing process to enable it to be predicted under less controlled field conditions. Apart from the curing time and the access to air that leads to drying out of samples, contamination of bacteria may also have affected the activity, particularly in the triaxial tests as no special measures were taken to ensure biologically clean conditions. Differences in the temperature and pH of the soil during sample preparation may also have contributed to differences in calcite precipitation.

7.5 Physical Model Foundation Tests at Single Gravity

More than 40 laboratory model test were carried out in this thesis to study the bearing capacity and the settlement behavior of circular model foundations placed on Sydney sand reinforced with single small diameter cemented columns. Cemented columns were produced by mixing sand and cementing agents in-situ within a laboratory test chamber. Both gypsum and bio-cemented columns were produced in sand with various moisture conditions and relative densities.

Unlike the UCS and triaxial test specimens, cemented columns were produced by simulating in-situ deep soil mixing technique at reduced scale in the laboratory. Columns of 38 mm in diameter and up to 200 mm long were successfully created using an in-situ mixing technique that was developed and refined in this study to produce uniformly cemented columns in dry, moist and saturated sand.

Homogenous distributions of calcite were demonstrated using the mixing technique developed, and these have allowed well cemented columns to be produced. In contrast to previous reports, results from the model tests in this thesis show that bio-cemented columns can be produced using mixing techniques with improved uniformity of calcite. This enables the strength of the bio-cemented columns to be directly correlated with the amount of calcite precipitated. Also the amounts of calcite precipitated in the physical model tests were comparable with field tests.
which have used multiple injection technique. One of the major contributions of the present study is that it suggests that biocementation can be applied through the deep soil mixing method.

Significant variance in the increase of vertical stress at failure was recorded for columns treated in soil with different moisture condition. The bearing capacity of bio-cemented columns in moist sand is higher than columns mixed in dry and saturated sand. These differences are believed to be primarily the result of differences in the relative densities in the different test moisture conditions. The failure stress of the biocemented column increases with cement content and is independent of the effect of column length.

The potential of the chosen urease producing bacteria (UPB) was also proven when it was used to repair columns in model tests using healing mechanisms. Unlike the conventional strategy where bacteria and nutrients are supplied to produce the repair, in this thesis there was an attempt to revive the bacterial activity by just supplying nutrients to the already existing bacteria in the cemented column. The success of this approach suggests that the incorporation of bacteria in the original soil column may be beneficial for future repair and would require only the addition or release of nutrients to enable self-healing. Survival of bacteria inside the biocemented column and other ground structures also need to be studied in detail (the test took only a few days in this study), such that the efficiency of this self-healing potential can be further evaluated.

In the future there should be work on small stiffness measurement carried out in order to measure the performance of bio-cemented columns more accurately. This will provide better understanding on the behavior of the cemented column and explain the uncertainties of data in triaxial tests. Therefore it is suggested to equip the physical model tank with more measuring instruments for use during model tests. For example bender elements are suggested here to measure the shear wave velocity and hence degree of cementation of the columns. Apart from that it is also recommended to place a load cell below the footing model in order to measure the load transmitted to the cemented column more accurately. Further tests could also be performed with lower calcite contents, as the model tests described in Chapter 6 all had more than 5.9 % calcite, whereas the triaxial tests suggest significant strength and stiffness gains occur with much lower calcite contents.
7.6 Summary

The proposed urease producing bacteria (UBC) *Bacillus Megaterium* was found suitable for biocementation. It fulfills all the 4 important criteria outlined in the literature review. It also fulfills an additional criterion which is it has some ability to withstand mixing pressures. Results in Chapter 4 clearly show that the bacillus can produce enough urease in extreme soil conditions such as an alkaliphilic state (pH 8.5 to 11). Also proven was the survival of these bacteria in high concentration of urea. Currently the dependency of researchers to a certain type of urease producing bacteria which precipitates calcite can be reduced. Additionally, the monopoly and the dependency of only a few regular chemical pathways of producing calcite such as denitrification process and iron reducing process can be reduced too. Hence calcite precipitation using urea hydrolysis pathway which has potential towards CO$_2$ sequestration can be popularised.

Its resistance towards cell-lysis (rupture) is proven as mixing was successfully demonstrated at all levels of testing in this thesis. Unlike other types of UBCs, the advantage of *bacillus megaterium* is that its thick wall enables it to survive in high osmotic pressures as well as kinetic pressures due to mixing.

Basically the bio-cement acts as a cementing agent and the stress, strain behavior is relatively similar with other traditional binders. Results of triaxial tests of biocemented specimens in Chapter 5 show a positive stress, strain and stiffness improvement behavior in comparison to gypsum. Only a small amount of calcite approximately 5% is required to achieve the same strength of 10% gypsum cemented sample.

Another significant difference between the current study and most previous studies of bio-cementation is that specimens were prepared by mixing and cured with no further addition of nutrients or bacteria. In contrast, most prior studies have produced cementation by pumping solutions of nutrients and bacteria through uncemented sand multiple times.

The greater stiffness with the calcite cement suggests that the location of the cement is important. It has been noted in previous studies that calcite tends to be precipitated on the particle grains and effectively forms bridges at the points of contact. Gypsum by contrast tends to fill the spaces between the grains and is as a result less effective in increasing the stiffness.
One of the achievements of the present study was at low calcite content (less than 2 %) the mixing technique appears to produce stronger and more consistent specimens. However, more research is needed to explore whether mixing can produce reliable cementation for higher calcite contents using single nutrient application.

After successful application of biocementation with higher calcite content, the byproduct of urea hydrolysis process which is ammonium salts concentration has a major impact on the performance of the treatment too. Therefore detail studies need to be conducted in this regards for prevention of any harmful effect to the environment.

The patterns of behaviour observed in bio-cemented Sydney sand are very similar to those in specimens bound with gypsum and have been reasonably consistent throughout all the laboratory tests conducted. The laboratory tests have identified the quantities of nutrients required to produce a range of calcite contents and hence soil columns with a range of strengths and stiffnesses. It has also been demonstrated that mixing of soil, bacteria and nutrients can produce specimens with a uniform distribution of cement. An increase in shear wave velocity during curing of the cementing agents has been observed, but more careful study of all parameters that affect the wave velocity are required for this to provide a reliable bio-cementation indicator. This study has shown the potential for soil improvement with bio-cements, but further tests are required in the future to more closely model the soil mixing process and to produce bio-cemented columns under field conditions. A limited number of studies have been conducted on small strain stiffness response of bio-cemented sand. Therefore it is suggested more experiments have to be conducted in the future to validate the data and the reliability of the model proposed here in this chapter.

It is also important to validate the calcite distribution through macroscopic imaging technique. The clear view of the cementation offers some insight into the micro mechanisms of deformation as do the small changes in the porosity and the cement distribution. Cement distribution map could be used to confirm the reduction in the degree of cementation in the region of localized deformation.

The shallow foundation model tests have revealed potential benefits and key issues to address for utilizing bio-cementation as a ground improvement technique. From a geotechnical point of
view, bio-cementation has a great potential for a variety of applications, but needs to be optimized for each case. Bio-cementation of the loose sand beneath the footing resulted in a significant increase in capacity of the footing as compared to an untreated case. Calcite bonds transfer load at the micro scale which enables the bio-cementation treated soil to sustain larger loads than the untreated soil. The brittle nature of the bio-cemented sample was observed through the shear failure at the depth of one diameter below the surface. The cementation gradient (calcite distribution) measured and observed along shear plane upon dissection supports the notable reduction in settlement of footing which was loaded until failure in all the soil conditions.

The stiffness gradient is controlled by the physiochemical processes during transport of nutrients and microbes as well as the biological processes. The key factors for upscaling bio-cementation include but are not limited to: uniformity of microbe concentration; nutrient and calcium accessibility; treatment system; flow rate; solution pH; microbe activity, and monitoring technique. Many of the problems encountered in the scaled shallow foundation experiment exemplify potential problems encountered in the field and require further study.
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APPENDICES

Appendix A1: Triaxial test results of bio-cement and gypsum cemented sample

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<th>Cement Type</th>
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<th>$p'_c$</th>
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Note: ND - not detected
## Appendix A2: Physical model test results of biocement and gypsum cement columns

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