Biocementation of sand in geotechnical engineering

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To the dearests

My mother and father: Mitra and Ali Agha

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SUMMARY

In geotechnical engineering, conventional soil improvement techniques – including both mechanical and chemical stabilization methods – have potential drawbacks such as high cost, high energy consumption and sometimes negative environmental influences. However, the rapid development of biotechnology has provided opportunities for innovation in soil improvement methods. In recent years, a promising approach, the so-called microbial geotechnology, has been attempted. This study contributes to the development of biocement and its application to soil improvement. Using the microbially induced carbonate precipitation (MICP) process, calcium carbonate and other types of minerals can be generated and used to increase the shear strength and reduce the permeability of soil. The primary objective of this research is to study the mechanisms for the optimization of the MICP process to increase the efficiency of calcite precipitation. For the first time, this study has showed that the urease activity in samples with bacterial cells was $70 \pm 3\%$ more effective than that with a supernatant or intact cultural liquid. This can be explained by the presence of urease-inactivating protease in the supernatant. In addition, an increase in the efficiency of cell aggregation in sand by 86% has been achieved after pretreating the sand with an addition of Ca^{2+} to the bacterial cells. This study also shows that cell aggregation on the surface of sand leads to a significant increase in crystallization of calcite.

In addition to the use of calcium-based biocement, a new variation of biocement through the microbially induced iron salts precipitation was also studied. The iron-based biocement consists of UPB, ferrous and urea solution and the iron is produced by microbial activity through a reduction process. Experiments with ferrous cations provided by chemical reagents were also studied. The data on the sand biocemented with iron-based biocement have shown a reduction in the hydraulic conductivity of the sand from 10^{-4} m/s to 10^{-7} m/s. There is also an increase in the unconfined compressive strength (UCS) of 402 kPa when the precipitated iron to sand ratio is 6% (w/w). The precipitated iron hydroxide in the sand grains has been identified from the FESEM images and EDX analysis.

Two applications of biocement for soil improvement have been studied using model tests. The first is for the erosion control of dikes and the second is for dust control or wind erosion of soil. Dike models of 150 mm tall with a slope angle of 30 were made using sand and

SUMMARY

treated using calcium based biocement. The biocement was applied using either the percolation or the spray method. The treated dike models were tested in a hydraulic flume. The model tests indicate that the stability of the dike models is affected by the amount of biotreatment received or the precipitated calcium carbonate to sand ratio (w/w). The dikes were shown to be stable against overtopping flow when the models were treated to achieve a calcium carbonate to sand ratio of 4.5% (w/w) using the spray method or 2.8% (w/w) using the percolation method, respectively. The performance of the dike is also affected by the methods of treatment which result in different distributions of the precipitated calcium carbonate in the dike.

The study of the use of biocement for dust control and wind erosion was carried out using a wind tunnel. Fine sand was used to simulate dust. The sand was put on a 550 mm 400 mm plate with a depth of 50 mm as a model. The surface of sand was sprayed using biocement solution of different concentrations. The model was put in the wind tunnel and the change in the soil mass of the soil model after subjecting to a horizontal wind of 36 km/h was measured. Similar tests on soil treated using calcium chloride solution and water were also carried out for comparison. The test data show that the rate of mass losses for bio-treated sand with a calcium carbonate to sand ratio of 0.1% (w/w) decreased by 85% 14 days after treatment. The CaCO_3 analysis data show that almost 96% of calcium ions were precipitated on the surface of the soil models. The study shows that the use of biocement is much more effective for dust control than the use of calcium chloride solution and water.

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CHAPTER 1 INTRODUCTION

1.1 INTRODUCTION

In geotechnical engineering practice, soil at its natural state is often less than ideal for its intended engineering purposes. As a result, some forms of ground treatment have to be carried out to enhance its mechanical properties such as shear strength, hydraulic conductivity, compressibility, stiffness etc. Conventional soil improvement is usually performed mechanically or chemically. In some rare cases thermal and electrical stabilization techniques have also been used. For chemical improvement methods such as grouting, cement or chemical products are injected into or mixed with soil to increase the shear strength or reduce the hydraulic conductivity of soil. The general view of these methods, such as soil stabilization using Portland cement, is that they are harmless/clean in spite of the energy intensive, carbon-producing manufacturing process for cementation materials (DeJong et al., 2012). These methods involve the quarrying of large volumes of raw materials and associated land destruction, coupled with a high pH (DeJong et al., 2012). Therefore, it can be said that the conventional ground improvement methods, mostly the chemical methods are expensive, require many injection wells for treating large volumes and sometimes environmentally unfriendly.

An alternative approach is to use microbial processes to improve the engineering properties of soil. Soil improvement through the microbial methods is environmentally friendly due to use the natural and biogeochemical processes. It showed significant reduction in embodied energy and carbon emissions, relative to cement-based techniques. In addition, the treatment process may also causes less disturbance to soil and environment. The applications of microbial soil improvement methods can be divided into two categories (Ivanov and Chu 2008): biocementation and bioclogging. Biocementation improves the shear strength of soil by forming cementation materials in soil and bioclogging reduces the hydraulic conductivity of soil by filling the pores in soil.

One of the microbial processes is the precipitation of calcium carbonate or calcite which can occur through a microbial activity called microbially-induced calcium carbonate precipitation (MICP) (Stocks-Fischer et al. 1999, Mitchell and Santamarina 2005, Yang et al. 2007, Ivanov

and Chu 2008, DeJong et al. 2010). MICP can be either a natural or an engineered process. The natural MICP process has been found in rocks in many parts of the world under various environments, such as marine water, fresh water, and soils (Ehrlich 1998). The engineered MICP process is controlled by different factors and through different mechanisms (Cacchio et al. 2003, Yang et al. 2007, Qian et al. 2010). Calcium carbonate crystals can be precipitated in soil when the amount of calcium and carbonate ions in solution exceeds its solubility. The urease producing bacteria (UPB) was used in the form of either bacterial cells (Whiffin et al. 2007) or enzyme urease (Yasuhara et al. 2011). The role of UPB in the MICP process is to provide the conditions for precipitation of calcium carbonate crystals (Bachmeier et al. 2002). Apart from changes induced in the environment through microbial activity, bacterial cells have been shown to be excellent nucleation sites for growing minerals due to the presence of several negatively charged groups on the cell wall, resulting in positively charged cation ions bound on bacterial surfaces (Douglas and Beveridge 1998, Ehrlich 1998). Many investigations confirmed the precipitation of calcium carbonate on the bacterial cell surface (Stocks-Fischer et al. 1999, Fujita et al. 2000, Warren et al. 2001, Hammes and Verstraete 2002, Stabnikov et al. 2011).

Based on previous studies, various factors such as adsorption (Van Paassen et al. 2006, Stabnikov et al. 2011), calcium dosage (Muynck et al. 2009), and urease activity affecting the biocementation process regarding to improve the application of this process. However, many other factors that may enhance calcium carbonate crystallization such as increasing adsorption of bacterial cell and urease activity, and immobilization of bacterial cell and effect of pretreatments of sand and cells have not been studied yet. Thus, the present study contributes to investigate the mechanisms and affecting factors to improve the efficiency of MICP with treatment conditions including pretreatment of cells, urease activity, and sand grains with various cations. Later, the results showing improvement in efficiency were used to study two applications of MICP in geotechnical engineering.

Besides calcium carbonate crystallization, soil grains can also be cemented with agents containing magnesium, iron, manganese and aluminum which are crystallized in the form of carbonates, silicates, sulfides and hydroxide (DeJong et al. 2006, Ivanov and Chu 2008). Dniker et al. (2003) reported that iron hydroxides, depending on its crystallization, can be an important cementing agent in soils. Little work has been done to study the mechanisms and potential application of iron-based bioclogging and biocementation as the iron-based biogROUT

leads to the precipitation of ferric hydroxide. This study attempted to contribute on optimization of this process while the ferrous cations provided through chemical reagents. Thus, the application of iron-based biocement which consisted of UPB and ferrous and urea solution was adopted in this study. In addition, formation of cemented concretions of iron hydroxides can be detected in soil having high pH or redox potential where microbial ferrous cation is produced through the activity of iron-reducing bacteria (IRB) (Weiss et al. 2005). Therefore, this study describes the feasibility of using a sustainable, cheap and readily available source of ferric cation in the form of iron ore for the iron-based biocementation. In the new method, the source of iron for iron-based biocement is produced by microbial activity through a reduction process. Further on, the effect of iron-based biocement on biocementation or bioclogging with ferrous cations provided through microbial production or chemical reagents evaluated by measuring the shear strength and the hydraulic conductivity of soil.

Most studies on the application of calcium-based biocement have carried out in the laboratory scale (Mitchell and Santamarina 2005, DeJong et al. 2006, Whiffin et al. 2007, Bang et al. 2008, Ivanov and Chu 2008, DeJong et al. 2010, Stabnikov et al. 2011). It has been shown that precipitation of calcium carbonate crystals within the soil matrix will form bridges between the sand grains which result in enhancing the mechanical properties of soils (Whiffin et al. 2007). Thus, a number of studies have considered MICP for reduction of hydraulic conductivity (Lawrence and Moores 1972, Whiffin et al. 2007) by building ponds or impermeable crusts (Stabnikov et al. 2011). The previous studies of Chu et al (2011a) have indicated that biocementation in sand could be applied to dikes. The slopes of dikes and dunes can become unstable due to a combination of seepage and external loading (Vaníček and Vaníček 2008). Therefore, the present study contributes to provide the sufficient degree of cementation for stabilizing loose sand of dikes through MICP process using the model tests. Two methods of applying biocement solution, namely the percolation method and the spray method were adopted and compared. Test models were treated with various calcium carbonates to sand ratio (% w/w) and the stability of the bio-treated model dike was examined in the hydraulic flume under various head pressures. After flume test, the geotechnical properties of specimens from dried model dikes were evaluated through the depth. The improvement in engineering properties of bio-treated models using two different methods was evaluated using unconfined compressive strength, bending strength and

hydraulic conductivity. In addition, the uniformity of treatments in different application methods was also evaluated by calcium carbonate analysis.

The second application of MICP in this study is to increase the cohesion in sand for dust control purpose which has been initially studied by Bang et al. (2009). However, the published results did not cover all application because of the selected sand type and size range. Also, the size of samples was small and not adequate to show field application. Therefore, in the present study the relationship between biocementation and rate of generated dust at different environmental conditions, such as temperature in four time intervals after curing in two non-cohesive sand-size ranges, were studied. The application of biocement solution as a dust suppressant for road and desert was studied. The surface of a layer of sand was treated using the spray method with different calcium carbonates to sand ratio (% w/w), water and just calcium chloride. The effectiveness of bio-treatment to wind erosion was evaluated by carrying out model tests in a wind tunnel.

1.2 OBJECTIVES

The specific objectives of the thesis are:

- 1) To identify the mechanisms and conditions for enhancing the MICP process for soil improvement. The effect of saline-washed suspension of bacterial cells and liquid supernatant containing urease on adsorption of cations on sand surface as well as employing cations on adsorption of bacterial cell prior to MICP was studied.
- 2) To study the use of iron based biocement for soil improvement. The mechanisms and conditions for production of iron-based biocement using three different sources of ferrous, namely microbially produced ferrous, ferrous sulfate and a combination of both were investigated.
- 3) To establish a method for using the MICP approach to enhance the ability of a dike against erosion. The relationship between the calcium carbonate to sand ratio (w/w) and enhanced engineering properties of soil was assessed.
- 4) To develop a method for using the MICP approach to reduce dust concentration and wind erosion of road or desert sand using model tests.

1.3 SCOPE

The scopes of work were confined to experimental studies to understand further the mechanisms and application of biocementation and bioclogging for sand. The former studies was used to establish the optimized conditions for using the microbial method for biocementation and bioclogging in sand using both calcium and iron based biocement. The product of this establishment was expected to enable development of microbial approaches for production of strengthened soil. In this study, the applicability of microbial methods was fused with the knowledge of microbiology, hydraulics and soil mechanics using empirical approaches. Therefore, through the thesis model tests were carried out. Tests were used for further investigation on optimization of mechanisms and conditions for enhancing the MICP process as a consequence of pre-treatment on sand. Later, the achievements for enhancing the MICP process were used to establish the use of the biocementation and bioclogging methods as a model tests for practical applications.

1.4 OUTLINE

This thesis is organized into seven chapters including this chapter.

Chapter 2 presents literature review on new and conventional methods of soil stabilization in which comparisons of different approaches are made. The mechanisms of new approaches in stabilization of soil and their applications are also presented.

Chapter 3 describes the mechanisms of MICP. Various factors such as cell aggregation by bioagents, cation pretreatments, urease activity in the presence of cations and the effect of grain size on biocementation were investigated.

Chapter 4 presents the study on iron-based biocementation and bioclogging with different source of ferrous cation and the effect of iron-based bioclogging and biocementation on the hydraulic conductivity and the shear strength of sand samples. The data obtained from production of three different methods of iron-based biocement production with three different iron sources are presented.

Chapter 5 illustrates the study on the application of calcium-based biocement in erosion control of dikes. Data from test models on six soil models treated with different percentages of calcium carbonate to sand ratio (w/w) are presented and used to identify the best application and calcium carbonate content of MICP for dike erosion control.

Chapter 6 presents a study to use the microbial method for dust control. Results on test models using a wind tunnel on soil treated with two different calcium chloride to sand ratios are presented.

Chapter 7 gives the conclusions of the research. Further research recommendations are also given in this chapter.

CHAPTER 2 LITERATURE REVIEW

2.1 COMMON METHODS FOR SOIL STABILIZATION

There are many methods for soil improvement. A summary and classification of different soil improvement techniques are presented in Table 2.1 (Chu et al. 2009). Ground improvement methods have been classified into five categories. The first category (A) is ground improvement without admixtures in non-cohesive soils or fill materials. This category is further divided into five methods of application: dynamic compaction, vibro compaction, explosive compaction, electric pulse compaction, and surface compaction (including rapid impact compaction). These are mainly mechanical methods.

The second category (B) is ground improvement without admixtures in cohesive soils and is divided into seven methods of application, which consist of replacement/displacement, preloading using fill or vacuum, dynamic consolidation, electro-osmosis or electro-kinetic consolidation, thermal stabilization, and hydro-blasting compaction. The principles of these methods are mostly excavation or displacement, dynamic compaction with vertical or horizontal drains, vacuum pressure, DC current, heating or freezing the soil, and a combined wetting and deep explosion action.

The third category (C) is ground improvement with admixtures or inclusions, which are applied by seven methods including vibro replacement or stone columns, dynamic replacement, sand compaction piles, geotextile confined columns, rigid inclusions, and geosynthetic reinforced columns. Principles of this category are based on the use of piles, rigid or semi-rigid bodies or columns, blasting, closed-bottom geotextil-lined cylindrical holes, and the use of bamboo, timber, and other natural products.

Table 2.1. Classification of ground improvement methods (after Chu et al. 2009)

Category	Method	Principle
A. Ground improvement Without admixtures in non-cohesive soils or fill materials	A1. Dynamic compaction	Densification of granular soil by dropping a heavy weight from air onto ground.
	A2. Vibro compaction	Densification of granular soil using a vibratory probe inserted into ground.
	A3. Explosive compaction	Shock waves and vibrations are generated by blasting to cause granular soil ground to settle through liquefaction or compaction.
	A4. Electric pulse compaction	Densification of granular soil using the shock waves and energy generated by electric pulse under ultra-high voltage.
	A5. Surface compaction (including rapid impact compaction).	Compaction of fill or ground at the surface or shallow depth using a variety of compaction machines.
B. Ground improvement Without admixtures in cohesive soils	B1. Replacement/displacement (including load reduction using light weight materials)	Remove bad soil by excavation or displacement and replace it by good soil or rocks. Some light weight materials may be used as backfill to reduce the load or earth pressure.
	B2. Preloading using fill (including the use of vertical drains)	Fill is applied and removed to pre-consolidate compressible soil so that its compressibility will be much reduced when future loads are applied.
	B3. Preloading using vacuum (including combined fill and vacuum)	Vacuum pressure of up to 90 kPa is used to pre-consolidate compressible soil so that its compressibility will be much reduced when future loads are applied.

	B4. Dynamic consolidation with enhanced drainage (including vacuum)	Similar to dynamic compaction except vertical or horizontal drains (or together with vacuum) are used to dissipate pore pressures generated in soil during compaction.
	B5. Electro-osmosis or electro-kinetic consolidation	DC current causes water in soil or solutions to flow from anodes to cathodes which are installed in soil.
	B6. Thermal stabilization	Change the physical or mechanical properties of soil permanently or temporarily by heating or freezing.
	B7. Hydro-blasting compaction	Collapsible soil (loess) is compacted by a combined wetting and deep explosion action along a borehole.
C. Ground improvement with admixtures or inclusions	C1. Vibro replacement or stone columns	Hole jetted into soft, fine-grained soil and back filled with densely compacted gravel or sand to form columns.
	C2. Dynamic replacement	Aggregates are driven into soil by high energy dynamic impact to form columns. The backfill can be sand, gravel, stones or demolition debris.
	C3. Sand compaction piles	Sand is fed into ground through a casing pipe and compacted by vibration, dynamic impact, or static excitation to form columns.
	C4. Geotextile confined columns	Sand is fed into a closed bottom geotextile lined cylindrical hole to form a column.
	C5. Rigid inclusions	Use of piles, rigid or semi-rigid bodies or columns, premade or formed in-situ

	C6. Geosynthetic reinforced column or pile supported embankment	Use of piles, rigid or semi-rigid columns/inclusions and geosynthetic girds to enhance the stability and reduce the settlement of embankments.
	C7 Microbial methods	Use of microbial materials to modify soil to increase its strength or reduce its hydraulic conductivity.
	C8. Other methods	Unconventional methods, such as formation of sand piles using blasting and the use of bamboo, timber and other natural products.
D. Ground improvement with grouting type admixtures	D1. Particulate grouting	Grout granular soil or cavities or fissures in soil or rock by injecting cement or other particulate grouts to either increase the strength or reduce the hydraulic conductivity.
	D2. Chemical grouting	Solutions of two or more chemicals react in soil pores to form a gel or a solid precipitate to either increase the strength or reduce the hydraulic conductivity of soil or ground.
	D3. Mixing methods (including premixing or deep mixing)	Treat the weak soil by mixing it with cement, lime, or other binders in-situ using a mixing machine or before placement
	D4. Jet grouting	High speed jets at depth erode the soil and inject grout to form columns or panels
	D5. Compaction grouting	Very stiff, mortar-like grout is injected into discrete zones and remains in a homogenous mass so as to densify loose soil

	D6. Compensation grouting	Medium to high viscosity particulate suspensions is injected into the ground between a subsurface excavation and a structure in order to negate or reduce settlement of the structure due to ongoing excavation.
E. Earth reinforcement	E1. Geosynthetics or mechanically stabilized earth (MSE)	Use of the tensile strength of various steel or geosynthetic materials to enhance the shear strength of soil and stability of roads, foundations, embankments, slopes, or retaining walls.
	E2. Ground anchors or soil nails	Use of the tensile strength of embedded nails or anchors to enhance the stability of slopes or retaining walls.
	E3. Biological methods using vegetation	Use of the roots of vegetation for stability of slopes.

The fourth category (D) is ground improvement with grouting-type admixtures, divided into six methods of application based on chemical stabilization. The purpose is to inject chemical substances into soils to improve their geotechnical properties (Karol 2003). The physiochemical and mechanical properties of any chemical grout, which are important in selection for a specific job, include gel time control, sensitivity, toxicity, low initial viscosity, water solubility, and economic factors (Karol 2003). The most common classes of chemical grouts include silicates, lignins, resins, acrylamides, urethanes, and biopolymers such as polyhydroxybutyrate, xantan, chitosan, polyglutamic acid, and sodium alginate (Momemi et al. 1999). Conventional chemicals used for the cementation effect consist of portland cement, lime, sodium silicate, calcium chloride, acrylates, acrylamides, and polyurethanes (Karol 2003). High cost and restriction of use, toxicity, effect on the environment, and pollution of ground water are the weaknesses of this method. In this category, the slurry grouting is a conventional cementing method for strengthening soil properties (Arulrajah et al. 2009). Portland cement is most commonly used, owing to its fineness as compared to other cement types.

The last category (E) is earth reinforcement, divided into three methods of application including geosynthetics or mechanically stabilized earth (MSE), ground anchors or soil nails, and biological methods. The principles of methods are based on tensile strength of various steel, geosynthetic materials, embedded nails or anchors, and roots of vegetation.

Use of microbial methods to modify soil to increase its strength or reduce its hydraulic conductivity is a new approach in the third category (ground improvement with admixtures or inclusions). Microorganisms can interact with soil and sediment particles in numerous ways. Injection of nutrients for motivating bacteria in bioremediation studies in the 1950s indicated biochemical conversions and clogging of pores. The study of Vandervivers and Baveye (1992a) showed that bacteria have the ability to reduce the hydraulic conductivity by accumulation of their cell bodies or exopolymers. In addition, microbial binding does have a significant effect on the shear strength of soil (Meadows et al. 1994, Wood et al. 1995). The application of biological processes in geotechnical engineering was reported by Mitchell & Santamarina (2005), and in parallel the US National Research Council (NRC, 2006) recognized biogeotechnical engineering as an essential research topic for the 21st century (DeJong et al. 2012). Various researchers studied the microbial stabilization of soil as a cost-effective and environmentally friendly method for alternative application of conventional methods of soil stabilization. The mechanisms and two applications of microbial stabilization of soil were reviewed in the present study.

2.2 MICROBIAL STABILIZATION OF SOIL

Mitchell (1975) discussed critically the essential roles of chemistry in improving the behavior of fine-grained soils and the dependence of macro-scale performance of soil on the micro-scale conditions. This can be explained by the effect of soil improvement techniques on changes between soil particles and resulted in stabilization of soil for construction. An example was mentioned by DeJong et al. (2013) as some ground improvement methods specifically target chemical changes in the clay fabric to stabilize soils for construction (e.g. pozzolanic changes). Mitchell and Santamarina (2005) presented a new opportunity to exploit many chemical processes through microbial activity. Microbes are ubiquitous near the ground surface (more than 10^{12} microbes per kg of soil), and their population decreases between 2 and 30m to 10^{11} to 10^6 microbes per kg of soil, almost regardless of mineralogy, pH, and

other environmental factors (Mitchell and Santamarina 2005, DeJong et al. 2012). Microbial activity provides the opportunity to manipulate soil and improve the soil property using natural or stimulated process. Subsequently, Ivanov and Chu (2008) reported that interactions between microorganisms and soil or sediment particles can occur in the following ways: microbial production of particle binding material (biocementation) and microbial production of pore-filling material (bioclogging).

2.3 BIOCEMENTATION

Biocementation as defined by Ivanov and Chu (2008) is a process to enhance the strength and stiffness properties of soil and rocks through microbial activity or products. On the other hand, natural cement creation in the earth's crust was studied as chemical deposition and chemical processes with the assistance of weathering. Examples of natural cementing could be seen in treated material such as conglomerate, breccia, sandstone, siltstone, shale, limestone, gypsum (Ivanov and Chu 2008), and the formation of ferric hydroxide in the pores of sand. Silica, hydrous silicates, and hydrous iron oxides were mentioned as the facilitated agents in the cementation of sand.

2.3.1 Microbial-induced calcium carbonate precipitation (MICP) process

Calcite precipitation can be induced by several MICP processes. The main categories of the calcite precipitation process include hydrolysis of urea, photosynthesis, sulfate reduction inducing dolomite precipitation (Warthmann et al. 2000), denitrification (Van Paassen et al. 2009, Hamdan et al. 2011b), iron reduction inducing ankerite, other mixed mineral precipitation (Roden et al. 2002, Crawford and Williams 2011), and other nonspecific pathways (DeJong et al. 2012).

Photosynthesis could promote carbonate precipitation with the aid of fungi, algae, and other biogeochemical agents (Ehrlich 2002). The calcification is the result of calcareous plants, autotrophic nutrient acquisition physiologies in nutrient-deficient environments. For sulfate reduction, the dissolution of gypsum and removal of sulfate by sulfate-reducing bacteria could eliminate the inhibitors for carbonate formation. These activities increase the resultant pH and offer mechanisms that favor dolomite precipitation. A nitrogen cycle involves ammonification, nitrate reduction, or urea hydrolysis. These three mechanisms are all capable

of producing calcium carbonate with the same by-products, i.e. ammonia and carbon dioxide (Castanier et al. 1999, Stocks-Fischer et al. 1999).

Van Passen et al. (2010) compared the potential process and concluded that calcite conversion by a urease production process was significantly higher than the other approaches such as aerobic oxidation, sulfate reduction, and denitrification. This is supported by the fact that this study was investigated using the mechanisms of MICP and its applications.

The ability of indigenous bacteria for hydrolyzing urea is widely distributed in soils and ground water systems. Specific urea hydrolyzing bacteria have been isolated from different natural or man-made environments, for example, ground water (Elliott 1986), limestone caves and loamy soil, building materials (Badora et al. 1998), air (Muynck et al. 2010), among many others. Lloyd and Sheaffe (1973) showed that, in six different soil types from Australia, 17–30% of the total bacterial populations, including aerobes, micro-aerophiles, and anaerobes, could hydrolyze urea rapidly.

Urease-producing bacteria (UPB) can produce urease for catalyzing the hydrolysis of urea. UPB uses urea as an energy source and converts it into carbon dioxide (or carbonate) and hydroxide (Kucharski et al. 2005, Mitchell and Santamarina 2005, Ivanov and Chu 2008, Chu and Ivanov 2009). Urea is hydrolyzed according to Reactions 2.1 to 2.3. Generation of ammonia and carbonic acid is the result of decomposition of carbonate ions (Reaction 2.1). Equilibrium of carbonic acid and ammonia molecules in water leads to an increase in the pH environment (Reactions 2.2 and 2.3) which endorses the precipitation of calcite.

The typical urease-positive bacteria are genera *Sporosarcina*, *Clostridium* and *Desulfotomaculum* (Kucharski et al. 2005). The aerobic bacteria are preferable as they release CO_2 from cell respiration, and CO_2 production is paralleled by the pH rise due to ammonium production. The species of *Sporosarcina* (previously *Bacillus*) *pasteurii* was identified as common alkaliphilic aerobic soil bacteria with high urease activity (Kucharski et al. 2005, DeJong et al. 2006, DeJong et al. 2010).

Synthesis of urease is raised 20 to 25 fold in poor nitrogen environments (Mobley et al. 1995). Also, the rate of microbial precipitation was faster than chemical precipitation of calcium carbonate (Stocks-Fischer et al. 1999), and the precipitation is correlated with cell growth of *Sporosarcina*. The effect of different minerals such as Ni^{2+} in activation and structural integrity of urease was studied (Mobley et al. 1995, Al-Thawadi 2011). Various types of calcium carbonate minerals are precipitated by the microbial method. For example, it is mentioned that *Acinobacter sp.*, *Deleyahlophia sp.*, and *E.coli* can form vaterite (Sanchez-Moral et al. 2003), aragonitic sherulites (Rivadeneira et al. 1993), and calcite (Bachmeier et al. 2002) respectively. Moreover, slime-producing bacteria and *Myxococcus xanthus* form magnesium calcite spherulites and dumbbells (Ivanov and Chu 2008). Limited studies have also explored the precipitation of other minerals (Weaver et al. 2011). Further study was carried out in this area and is presented in Chapter 4.

2.3.2 Mechanisms of biocementation

The biocementation process is catalyzed by bacteria or their enzymes. DeJong et al. (2006) studied controlling ureolysis by aerobic microbes to raise the pH in a super saturated solution, forcing precipitation of calcite within granular porous media. Furthermore, they have measured both increased stiffness, using S-wave velocity measurements, and increased undrained strength. Kavazanjian et al. (2009) studied various microbiological processes. i) Precipitation, mineral transformation, biofilm and biopolymer growth to attain beneficial changes in engineering properties of soils in short time frames, ii) carbonate precipitation, microbial transformation of smectite to illite, and biopolymer plugging. Rebata-Landa et al. (2006) conducted research on survivability to accommodate grain size and depth of burial, clogging for the controlled modification of hydraulic conductivity, and gas generation for bulk stiffness control.

Creating uniform cementation is essential for bio-mediated soil improvement to be used for civil infrastructure applications (DeJong et al. 2009). When microbes are injected into the subsurface, the microbial cells are filtered by the soil matrix. Filtration of cells generally results in a log-linear reduction of microbe concentration along the injection path. The degree of cementation corresponds directly to the stiffness of the soil; therefore, a cementation gradient results in a stiffness gradient. Laboratory results indicate that a push-pull injection process (DeJong et al. 2009) may effectively counteract the gradient of microbial concentration, resulting in more uniform cementation.

Using a grid of injection/extraction wells, the microbes and nutrients are injected through the soil by inducing an artificial hydraulic gradient. Subsequently, nutrients are injected using a reversed artificial hydraulic gradient. The reversal of injection direction provides more nutrients for consumption by the smaller microbe concentration, and fewer nutrients for the large microbe concentration near the initial injection source. This process improves spatial distribution of the treatment, and, therefore, cementation uniformity across the treated zone (DeJong et al. 2009, DeJong et al. 2010).

Adsorption of bacterial cells onto soil particles surface and movement of bacterial cells in porous soil depends on size, surface charge (zeta-potential), and surface hydrophobicity of soil particles and bacterial cells, as well as the concentration of protons and other ions in environment (Harvey et al. 1989, Jacobs et al. 2007, Risse and Faucette 2009). Coating of sand surface with calcium, ferric, or aluminum cations enhances significantly the adhesion of bacterial cells to sand grains (Harvey et al. 1989, Lukasik et al. 1999).

Studies on adsorption have been carried out for bank filtration of river water, transport of indigenous bacteria in sandy aquifers, or for the remediation of contaminated groundwater (Hendricks et al. 1979, Chen and Strevett 2001, Chen and Strevett 2002, Jada et al. 2006, Jacobs et al. 2007, Wand et al. 2007). Investigation on adsorption of bacterial cells to sand grains during the process of biocementation is one of the crucial factors.

Investigation of the nucleation site of cells for crystallization of calcite (Kucharski et al. 2005, DeJong et al. 2006, Stabnikov et al. 2011) shows the balanced Reactions 2.4 to 2.7 for calcite precipitation (DeJong et al. 2006):

Moreover, precipitation of calcite on the cell surface as a consequence of deposition of calcium ions on the surface of negatively charged cells, are shown in Reactions 2.8 and 2.9 (Kantzas et al. 1992).

2.3.3 Factors affecting biocementation

Mitchell and Santamarina (2005) mentioned the ability of microorganisms to move through pore spaces and attach to sand grains is the basis of biocementation and one of the factors that influence the cementation in soil matrix. Ranjard and Richaume (2001) found that the most suitable microorganisms for microbial geotechnical engineering application are bacteria in view of the small size and large physiological diversity. Bacteria are the main microorganisms in soil (Janssen 2006) with a typical diameter of 0.5 μm to 2 μm to pass through the pores of the soil matrix. Further investigation has shown volumetric increments of cell size after calcite precipitation compared to time of injection (DeJong et al. 2006). Still further investigation on the modification of bacterial cell size with the presence of divalent and trivalent cations is presented in Chapter 3 of this study.

The concentration of reagents plays a critical role for biocementation as shown in Reactions 2.1 and 2.5. It is mentioned by Nemati et al. (2003) that a solution containing equal parts of both reactants would provide better conversion to calcite. The study of De Muynck et al. (2010b) illustrated the weight gain of limestone specimens due to carbonate precipitation with increased concentrations of reagent. High concentrations (0.5 to 1.0 M) compared to low concentrations (0.05 to 0.25 M) of urea and calcium chloride however possessed relatively low efficiency in calcite formation (Nemati and Voordouw 2003, Okwadha and Li 2010).

The urease enzyme is produced by UPB and released into the environment. The urease is only active at a certain range of pH. The urease activity of alkalotolerant bacteria, such as *S.pasteurii*, has an optimum pH value of 8 (Stocks-Fischer et al. 1999) or between 8.7 and 9.5 (i.e., 9.1, 9.3, and 8.7–9.5) (Dupraz et al. 2009).

Moreover, the effect of chemicals such as Ca^{2+} (Hammes et al. 2003a), Ba^{2+} , citrate (2–20 Mm) (Smith et al. 1993), Fe^{3+} and Al^{3+} (50mM) (studied on Chapter 3), and Na2EDTA-detiothreitol (0.1–5 mM) (Smith et al. 1993) on enhancing the activation of urease was studied. In contradiction, ions such as Na^+ , K^+ , Cl^- , Br^- (2–20 mM), and chemicals such as

nitrate or acetate (2–20mM) did not show effects on the urease activity while Li^+ , Mg^{2+} , Zn^{2+} , and I^- act as inhibitors for urease (Smith et al. 1993).

Van Passen (2009) mentioned negligible activity of urease activity at temperatures below 5°C. Whiffin (2004) found that urease activity increased proportionally at temperatures between 25°C and 60°C. However, most of the common UPB used in the existing MICP treatments (i.e., *S. pasteurii*, *B. megaterium*) are of a mesophilic type with the optimum growth temperatures ranging from 30°C to 45°C. It is also suggested to select UPB that are optimum at soil temperature. The soil temperature varies with latitude, altitude, incident solar radiation, moisture content, conduction, type of soil, depth of soil, etc. (Salomons and Förstner 1980, Ok et al. 2011).

Okwadha and Li (2010) studied the influence of bacterial cell concentration to support higher hydrolysis of urea and provided sufficient cementation reagents to increase the rate of calcite precipitation through the MICP process. In addition, as shown in Reactions 2.8 and 2.9, bacterial cells served as nucleation sites for calcite to precipitate in a biochemical reaction (Stocks-Fischer et al. 1999, Stabnikov et al. 2011). Moreover, bacterial cell characteristics consist of metabolic activity and oxygen supply. The fraction of attached cells, ionic nutrient treatment flushes, as well as time between batches of injection also play key roles in MICP (Kantzas et al. 1992, DeJong et al. 2006).

It is obvious that the number of bacteria significantly reduced by travelling through porous media and adsorption is the most often suggested mechanism. Physical factors (porous media, temperature, flow velocity), chemical factors (ionic strength, ion species, pH), the nature of the sand, and bacterial population are well known for their impact on bacterial adsorption (Harvey 1993, Jacobs et al. 2007). However, far too little attention has been paid to adsorption of cations on sand surface (one of the concerns of MICP) which can coat the sand particles and enhance the adsorption of bacteria. Bacterial surfaces are negatively charged and can absorb a wide range of cation cations from solutions (Wightman and Fein 2004). Attaching of cells to sand grains, called immobilization of bacterial cells, is one of the crucial affecting factors on biocementation process. The method of coating sand with cation hydroxide has been previously shown to provide an even and durable method to adsorb microorganisms very effectively. In bacterial transport in porous media, the total interaction energy needed by microbial particles to adhere and attach themselves to solid surfaces is

explained by the classic Derjaguin–Landau–Verwey–Overbeek (DLVO) theory, which is composed of the repulsive electrostatic forces and the attractive Van Der Waals forces (Okwadha and Li 2010). Mentioned reagents such as cation hydroxide, KCl, and CaCl_2 increased the ionic strength of the suspending solution, reduced the thickness of the electrical double layer, and caused more adsorption of bacteria to the sand surface (Tan et al. 1991, Lukasik et al. 1999). Van Paassen et al. (2006) studied immobilization of bacterial cells by divalent cations, preferably from the group consisting of calcium and magnesium during the process of adsorption of MICP to increase the rate of precipitation. Subsequently, it is well known that the bond between trivalent cations and sand particles could be stronger than divalent ones. High ionic strength increases electrical double layer (EDL) compression by decreasing EDL repulsive forces leaving attractive Van Der Waals forces to dominate, and in the process promotes bacterial adhesion and attachment to the substratum (Faibish et al. 1998, Okwadha and Li 2010). Therefore, trivalent cations were suggested and studied in the present study (Chapter 3) to enhance cell adsorption of sand through the process of MICP. In addition, general bacterial cells moved furthest in coarse sand and least in fine sand in the same suspending solution. The greater movement of bacteria in coarse sand was attributed to the low adsorption capacity of the coarse sand (Tan et al. 1991). One of the objectives in adsorption research concerned in the present study (Chapter 3) was the effect of sand size on adsorption of bacterial cell on coated sand.

Furthermore, even distribution and in-place fixation of cells were achieved by the proper application method (Harkes et al. 2010). Although a high salinity solution encourages flocculation and promotes the adsorption of bacteria (Torkzaban et al. 2008), a low salinity solution has its own advantage for homogenous distribution and movement of bacteria through the soil matrix (Harkes et al. 2010). The injection method affects the composition of calcite: Martinez (2011) found that the stopped-flow injection method (injection of 1.5 pore volume of reagent, followed by 2.5 hours of rest period) presented better uniform cementation and distribution of cementation fluid. At the same time, continued use of the injection method provided abundant calcite precipitation near the injection point which decreased farther from the injection point. Barkouki (2010) also confirmed the same results by numerical modeling. Positive relationships between repeated injection of reagents or number of treatments to the soil and increasing composition of calcite were also investigated (Muynck et al. 2010).

The pore-water chemistry (with a capability to carry Ca^{2+} and/or HCO_3^-), the presence of previous carbonate substrate, as well as hydraulic conductivity, texture, composition, and stabilization of the soil or sediment were studied as inhibiting or facilitating soil factors to control the process of biocementation (Hall et al. 2004, Mozley and Davis 2005).

High concentration of calcium or organic concentration in injection solutions act as fertilizers and inhibitor respectively (DeJong et al. 2009). Therefore, underrating the aqueous chemistry is crucial prior to field experiments. However, the efficiency of biocementation is affected by the properties of the soil matrix and the total volume of the injected solution (Kantzas et al. 1992, DeJong et al. 2010).

Based on the literature, the factors affecting the process of microorganisms in biocementation also include the temperature, nutrient concentration, time, oxygen content, soil density, and pH (Ragusa et al. 1994, Stavropoulou et al. 1998, Wang and Lu 2005, Jeong et al. 2006, Xiao et al. 2006, Ahmed and Hussain 2008).

Microscopy investigations demonstrate that the calcite structure varies with treatment formulation (Al Qabany et al. 2011); cementation occurs preferentially at particle contacts; calcite precipitation occurs directly on or around individual microbes and their aggregates; and cementation breakage during shearing occurs within the calcite crystals (DeJong et al. 2009) which were investigated with different factors in Chapter 3 of this study.

2.3.4 Biological reaction of other potential microbial processes

Besides MICP, there are some other potential microbial processes that can lead to biocementation as summarized in Table 2.2. These processes include the binding of soil particles with sulfides of cation produced by sulfate-reducing bacteria, and carbonates of cations produced due to hydrolysis of urea. Moreover, the application of both iron-reducing bacteria (IRB) for ferrous cation production and UPB for hydrolyzing urea is a new approach which was studied in this project as presented in Chapter 4.

Table 2.2. Possible microbial processes that can lead potentially to biocementation (after Ivanov and Chu 2008)

Physiological group of microorganisms	Mechanisms of biocementation	Essential conditions for biocementation	Potential geotechnical applications
Sulfate-reducing bacteria	Production of undissolved sulphides of cations	Anaerobic conditions; presence of sulfate and carbon source in soil	Enhance stability for slopes and dams
IRB	Production of undissolved ferrous cation and precipitation of iron salts through urease process	Anaerobic conditions; presence of iron and carbon source in soil; UPB	Enhance the strength of sand
Denitrifying bacteria	Reduction of nitrate to nitrogen gas (N ₂)v	Organic compounds and urea	Mitigate liquefaction potential of sand; enhance stability for retaining walls, embankments and dams; increase bearing capacity of foundations

2.4 BIOLOGGING

Ivanov and Chu (2008) defined the term of bioclogging as the process to reduce the hydraulic conductivity of soil and porous rocks due to microbial activity or products. It could be used to reduce drain channel erosion, form grout curtains to reduce the migration of heavy cations and organic pollutants, and prevent piping of earth dams and dikes. The result of dissolving the high concentration of organic matter in water and passing it through the soil matrix indicated microbial clogging to block the spaces of soil texture (Mitchell and Nevo 1964, Taylor and Jaffé 1990). Microbial clogging is considered problem in wastewater recycling by

sprinkler irrigation, septic tank filters, sand filtration, and in bioremediation processes. Researchers, however, have been focusing on the advantages of microbial clogging as an environmentally friendly approach in appropriate situations to minimize seepage (Taylor and Jaffé 1990, Vandevivere and Baveye 1992 a, Kim and Fogler 2000).

Different possible microbial processes that may lead to bioclogging are summarized in Table 2.3. These processes include a formation of an impermeable layer brought on by algae and cyanobacterial biomass; slime in the soil induced by aerobic and facultative anaerobic heterotrophic bacteria, oligotrophic microaerophilic bacteria, and nitrifying bacteria; and production of undissolved sulfides of cations by sulfate-reducing bacteria.

The preferred bacteria for bioclogging include *Clostridium*, *Eubacterium*, *Peptococcus*, *Streptococcus* and *Lactobacillus* (Bezuijen et al. 2007). For example, experiments showed a 22% reduction in hydraulic conductivity through the introduction of algae into soil samples (Boulangier and Tokimatsu 2006). The efficiency of transporting microbial cells into soil dependent on factors such as the cell sizes, cell surface, and physical properties was demonstrated in these experiments (Murphy and Ginn 2000).

The precise location of the leak is critical for the injection of nutrients. The nutrient composition allows microorganisms present in the soil or structure to ferment and grow. The organic acids formed during fermentation of energy are in turn digested by other bacteria. The sealing of leaking construction pit landfills, reduction of drain channel erosion, reduction of the movements of contaminants, and control of the erosion of dams and dikes can be useful applications of bioclogging (Ivanov and Chu 2008).

The production of a ferrous cation solution, ferric salts, and hydroxides due to the activity of IRB through microbial production of ferrous cation showed bioclogging processes (Ivanov et al. 2009). Ferric cation reduction was previously considered by scientists to be an abiological reaction initiated by reducing conditions. Isolation of iron-reducing microorganisms in the late 1980s showed that this important component of the iron cycle was largely mediated by microbial processes (Lovely et al. 1991). It is known that microbial respiration with ferric oxides as the terminal electron acceptors is an important process in anoxic environments, and that nearly all the ferric cation reduction in these environments is enzymatically catalyzed (Lovley 1997)

Table 2.3. Microbial processes that can lead potentially to bioclogging (after Ivanov and Chu 2008)

Physiological group of microorganisms	Mechanisms of biocementation	Essential conditions for biocementation	Potential geotechnical applications
IRB	Production of ferrous cation solution and precipitation of undissolved ferrous and ferric salts and hydroxides in soil	Anaerobic conditions changed for aerobic conditions; presence of ferric minerals	Density soil on reclaimed land sites and prevent soil avalanching Reduce liquefaction potential of soil
Algae and cyanobacteria	Formation of impermeable layer of biomass	Light penetration and presence of nutrients	Reduce of water infiltration into slopes and control seepage
Aerobic and facultative anaerobic heterotrophic slime-producing bacteria	Production of slime in soil	Presence of Oxygen and medium with ratio of C:N>20	Avoid cover for soil erosion control and slope
Oligotrophic microaerophilic bacteria	Production of slime in soil	Low concentration of oxygen and medium with low concentration of carbon source	Reduce drain channel erosion and control seepage
Nitrifying bacteria	Oxidize ammonium to nitrite and nitrate	Presence of ammonium and oxygen in soil	Reduce drain channel
Sulfate-reducing bacteria	Production of undissolved sulphides of cations	Anaerobic conditions; presence of sulfate and carbon source in soil	Form grout curtains to immobilize heavy cations and organic pollutants

Laboratory results have shown that IRB, *Shewanella alga*, act as catalysers for reduced amorphous ferric cation by the order of 20 and 1000 compared to goethite (FeOOH) and hematite (Fe_2O_3) respectively. IRB reduced ferric to ferrous cation and formed iron hydroxide to bind soil particles together by cementation in high pH or redox potential.

Reduction of iron depends on several factors such as crystalline structure, surface area, cell/mineral contact, free energy of ferric cation and humic acids (Nealson and Saffarini 1994, Zachara et al. 1998, Zachara et al. 2002). For example, increasing the crystal structure of the ferric oxide reduced the microbial reduction. Amorphous ferric cation has the largest surface area followed by goethite and hematite (Roden and Zachara 1996, Urrutia et al. 1998) (Figure 2.1).

Figure 2.1. Basic of microbial ferric cation reduction by IRB (after Lovley 1997)

Ferric cation can undergo oxidation easily to form iron hydroxides. Ferric hydroxide ($\text{Fe}(\text{OH})_3$) is the dominant product of ferric oxidation (Weiss et al. 2005). Poorly crystalline iron hydroxides with larger and more reactive surfaces were observed due to low temperature and the presence of contaminants in nature. Several factors affect aggregation of iron hydroxides such as size and structure of crystal structure, pH, path of formation of the iron hydroxide, and characteristics of organic and ionic solution of soil (Roden and Zachara 1996). High pH, small crystal size, and translocation of iron and re-crystallization were found to aid in the formation of stable iron hydroxide (Fontes 1992).

2.5 APPLICATION OF BIOCEMENTATION IN GEOTECHNICAL ENGINEERING

Various applications of MICP have been investigated by researchers which can be categorized in this study according to its application in geotechnical and geoenvironmental engineering. Other applications such as remediation/stabilization of cracks in concrete, stabilization of dams and embankments, and increased cone tip resistance were studied as complementary and not presented in this study. Table 2.5 shows the application of calcium-based and iron-based biocementation. Research has provided insights into MICP from micrometer- to meter-length scales (Figure 2.2).

Table 2.4. Comparisons between applications of MICP in soil improvements

Applications	Calcium-based biocement	Iron-based biocement
Geotechnical Engineering	Water-Impermeable crust (Stabnikov et al. 2011); Surface treatment (Muynck et al. 2008); Liquefaction resistance (Van der Ruyt and Van der Zon 2009, Burbank et al. 2011, Burbank et al. 2012, Montoya 2012, He et al. 2013). Strength and stiffness (Métayer-Levrel et al. 1999, Whiffin et al. 2007, DeJong et al. 2009, DeJong et al. 2010, Chu et al. 2012 b, Chu et al. 2013); Dust control (Bang et al. 2008, Bang et al. 2011, Meyer et al. 2011) and wind erosion ; Controlling erosion in coastal area and rivers. Green engineering and energy saving (Chu et al. 2012 a); Stabilization of dams and dikes (studied in Chapter 5).	Iron + UPB for strengthening (Ivanov et al. 2009, Ivanov et al. 2012).

One-dimensional column (studied in Chapter 3), two-dimensional flow (4), and three-dimensional test models (studied in Chapter 5 and 6) have enabled enquiry into treatment uniformity, formulation optimization, and self-equilibrating ability, as well as a demonstration of conceptual ideas about soil improvement after iron-based or calcium-based biocementation (DeJong et al. 2012) (Figure 2.2).

Figure 2.2. Overview of up scaling of MICP: (a) urease enzyme structure housed within microbes; (b) *Bacillus pasteurii* (UPB); (c) bacterial impression within precipitated calcite (d) structure of precipitated calcite; (e) MICP-cemented sand grains; (f) CT scan of MICP-cemented sand; (g) MICP-cemented triaxial specimen; (h) modeling of MICP triaxial compression test; (i) 1D column tests; (j) radial flow treatments ; (k) MICP impermeable skin for retention basin; (l) MICP for controlling the erosion of dikes (studied in Chapter 5); (m) MICP treatment of 100 m³ sand; (n) field trial (after DeJong et al. 2012)

2.6 COMPARISON OF CONVENTIONAL AND MICROBIAL METHODS

The conventional ground improvement methods that compete with microbial techniques are typically cement-based techniques. The general view of these methods, such as soil stabilization using Portland cement, is that they are harmless/clean in spite of the energy intensive, carbon-producing manufacturing process for cementation materials (DeJong et al., 2012). These methods involve the quarrying of large volumes of raw materials and associated land destruction, coupled with a high pH (DeJong et al., 2012). Therefore, one key advantage of microbial stabilization methods is their potential for a significant reduction in embodied energy and carbon emissions, relative to cement-based techniques (DeJong et al., 2012). In addition, microbial methods use natural and biogeochemical processes to improve soil, which makes them non-toxic and environmentally friendly, whereas chemicals are used in the

chemical stabilization methods. An example of the toxicity of chemical stabilization methods was shown in a case study in Japan in 1974 when acrylamide grout leached into waterways. The acrylamide grout caused five substantiated cases of water poisoning (Karol, 2003).

In addition, microbial methods – specifically MICP – may enable improvement over larger distances, owing to their low viscosity and injection pressure requirements. They can be deployed beneath and around existing structures without any disruptions. These general attributes make microbial methods potentially favorable for many ground improvement projects. Therefore, based on related studies and the characteristics mentioned above, microbial soil improvement technologies provide opportunities to address important issues, such as climate change, energy, food, shelter, infrastructure, urbanization, sustainability, waste management, safety, water availability and economic stability, compared to conventional soil improvement methods.

There are also disadvantages associated with microbial stabilization. Microbial processes can sometimes be slower than mechanical and chemical processes. Additionally, environmental issues include pH, temperature, concentration of electron acceptors and donors, concentrations and diffusion rates of nutrients and metabolites should be considered which means that operators must be competent in the technical aspects of microbiology. Subsequently, the construction of conventional methods are relatively easy and don't involve careful monitoring, while the application of microbial methods are complicated and growing conditions should be carefully monitored. Nevertheless, it should be mentioned that the MICP process is not an entirely green technology. During the hydrolysis of urea by UPB through MICP, the by-products (such as ammonia) may cause environmental concerns, such as toxic effects on the health of humans (atmospheric nitrogen deposition), vegetation and aquatic organisms, leading to the eutrophication and acidification of sensitive ecosystems and the discoloration of stones (Camargo et al., 2005; Sutton et al., 2008; Tobler et al., 2011; Dhami et al. 2013). Furthermore, there has often been public resistance to microbial methods using exogenous organisms. Due to this complexity, the sustainability analyses to date are limited and there are issues that must still be addressed.

It is known that soil improvement by the microbial precipitation of calcite requires less carbon than cement stabilization. However, due to the very limited field applications, the actual costs (energy required for manufacturing urea and calcium chloride, for injecting the

improvement media into the ground, for treatment of the by-products, etc.) are largely unknown. The first estimation of material costs (e.g. urea, calcium) by Ivanov & Chu (2008) was about US\$5/m³ (range from 0.5 to 9 US\$/m³). Later, DeJong et al. (2012) reported a range from US\$25–75/m³ all the way to US\$500/m³ as the total cost of MICP treatment (materials, equipment and installation) in saturated soils. Based on recent work by Cheng (2012), the cost of applying microbial calcite precipitation involves four major parts: enzyme production, expenditure of chemical reagents (urea and CaCl₂), waste products treatment and equipment. Enzyme production is the first cost and includes labor, equipment, operation, chemicals, sterilization and transport of the culture from the biotechnology company to the site of use. The cost of chemicals for the cultivation of bacteria is considered one of the major expenses, and economical alternatives are needed for the medium ingredients that account for as much as 60% of the total operating costs (Kristiansen, 2001). Cheng & Cord-Ruwisch (2012) also found that, like many other ground treatment processes, delivery is a major cost. The use of inexpensive materials, such as corn steep liquor or lactose mother liquor (Achal et al., 2009a,b, 2010; Mitchell et al., 2010) and the activation of indigenous ureolytic bacteria (Burbank et al., 2012), may help to lower the cost of treatment. Moreover, if processes can be developed that enable biotreatment to be delivered more economically, then a strong potential exists for its successful adoption.

New findings by Cheng & Cord-Ruwisch (2012) have shown that materials and costs could be reduced by more efficient cementation. They discussed the optimal balance of substrates for various applications, which could be used to increase the economic feasibility, reduce the production of unwanted by-products and increase the long term efficacy of these biominerals. Their cost estimation based on efficient cementation covers a wide range, the lower half of which is competitive with conventional ground improvement techniques such as deep soil mixing, jet grouting and chemical grouting. After considering the general attributes of microbial processes, DeJong et al. (2012) identified and evaluated 24 different applications in a qualitative manner using the criteria of cost, implementation, probability of success and social acceptance. The applications and their approximate ‘ranking’ are summarized and presented in Table 2.4. Note that, due to the very limited field applications, challenges for the actual cost (energy required for manufacturing urea and calcium chloride, injecting the improvement media into the ground and treating the unwanted by-products) are largely unknown. Therefore, the adoption of microbial methods in the industry is expected to take some time.

Table 2.5. Evaluation of application alternatives and their potential, considering implementation feasibility, probability of success, cost / viability, and social acceptance (after DeJong et al. 2012)

Application	Implementation Easy: 5 Difficult: 1	Probability of success High: 5 Low: 1	Cost/viability Economics:5 Expensive: 1	Societal acceptance High:5 Low: 1	Total Scour out of 20
Structural repair	5	5	3	5	18
Erosion control	4	5	4	5	18
Immobilization of contaminants	5	4	4	5	18
Dust mitigation	4	5	4	5	18
Ground improvement for rural roads	5	5	3	4	17
Shallow carbon sequestration	5	3	4	5	17
Leak management	4	3	4	5	16
Ground improvement for urban road subgrading	5	3	3	4	15
Soil liquefaction mitigation (MICP)	3	5	3	3	14
Ground improvement for ash ponds	1	4	4	5	14

Chapter 2- Literature review

Soil liquefaction mitigation (biogas)	3	3	3	3	12
De- desertification	1	5	1	5	12
Enhanced water/oil/gas recovery	1	3	3	5	12
Sediment weakening by fluidisation	3	2	3	3	11
Underground creation (pipeline)	3	4	1	3	11
Stabilisation of sinkholes	1	3	2	5	11
Landfills as new energy resource	3	4	1	2	10
Construction products (bricks)	2	4	1	3	10
Water storage	3	2	2	2	9
De-swelling of clays	1	1	1	4	7
Deep carbon sequestration	1	1	1	3	6
Underground creation (tunnel)	1	1	2	1	5

2.7 CONCLUDING REMARKS

The feasibility of microbial calcium carbonate precipitation is well established in the literature. A quantity of prior studies has concentrated on the mechanisms of biocementation such as adsorption (Van Paassen et al. 2006, Stabnikov et al. 2011), calcium dosage (Muynck et al. 2009), and urease activity. However, many other factors that may enhance calcium carbonate crystallization such as increasing adsorption of bacterial cell and urease activity, and immobilization of bacterial cell and effect of pretreatments of sand and cells have not been studied yet. The present study investigates the mechanisms and affecting factors to improve the efficiency of MICP with treatment conditions including pretreatment of cells, urease activity, and sand grains with various cations. In addition, the results showing improvement in efficiency were used to study two applications of MICP in geotechnical engineering.

Some previously published work has focused on microbial calcite precipitation for purposes other than strength development (Castanier et al. 1999, Stocks-Fischer et al. 1999, Fujita et al. 2000, Bang et al. 2001, Bachmeier et al. 2002, Hammes et al. 2003a, Muynck et al. 2005, Ivanov and Chu 2008, Hamer et al. 2009, Fujita et al. 2010, Van Tittelboom et al. 2010, Chu et al. 2013). Several previous studies that have considered strength have only achieved minor improvements that are useful only as consolidation or patching treatments for existing material (Tiano et al. 1999, Castanier et al. 2000, Ramachandran et al. 2001, Rodriguez-Navarro et al. 2003). Numerous prior studies also have achieved significant strength production through MICP (Whiffin 2004, Army 2005, Whiffin et al. 2007, Bang et al. 2011).

A number of studies have considered MICP for reduction of hydraulic conductivity (Lawrence and Moores 1972, Whiffin et al. 2007) by building ponds or impermeable crusts (Stabnikov et al. 2011). The present study contributes the sufficient degree of cementation for stabilizing loose sand of dikes through microbial calcite precipitation. The formation of bulks or crusts (Chu et al. 2012 b) indicates a novel area for application of MICP. Direct measurement of unconfined compressive strength, hydraulic conductivity, bending strength, calcium carbonate and test models on hydraulic flume were used for evaluation of the effectiveness of microbially induced soil improvement

There have been recent experimental attempts to study the effects of biocementation on sand dust suppressions (Bang et al. 2008, Bang et al. 2011, Meyer et al. 2011). However, the published results did not cover all application because of the selected sand type and size range. Also, the size of samples was small and not adequate to show field application. Therefore, in the present study the relationship between biocementation and rate of generated dust at different environmental conditions, such as temperature in four time intervals after curing in two non-cohesive sand-size ranges, were studied. Note that the literature review for common methods of this application is presented in the Chapter 6.

Moreover, little work has been done to study the mechanisms and potential application of iron-based bioclogging and biocementation (Chu and Ivanov 2009, Chu et al. 2011). Therefore, this is a novel area for study. The iron-based biocement consisted of the application of a microbial source of iron or a chemical source of iron and urea, with UPB, for the precipitation of ferric hydroxide due to enzymatic hydrolysis of urea. However, the cultivation of IRB for production a microbial source of iron, the methods of applying and a number of treatments still requires focus.

CHAPTER 3 MECHANISMS OF MICROBIALLY-INDUCED CALCIUM CARBONATE PRECIPITATION

3.1 INTRODUCTION

It is known that urease suspension or bacterial cells were used for microbially-induced calcium carbonate precipitation (MICP) in sand (Harkes et al. 2010). It has been contemplated that bacteria may be used without having to isolate the urease from the bacteria (Van Paassen et al. 2006). The MICP process takes place in soil at two major stages:

- 1) Adsorption of urease or UPB on soil particles;
- 2) Enzymatic hydrolysis of urea and formation of calcium carbonate crystals.

Physical factors (porous media, temperature and flow velocity), chemical factors (ionic strength, ion species and pH), the nature of the sand, and bacterial population are well known for their impact on bacterial adsorption (Harvey 1993, Jacobs et al. 2007). However, far too little attention has been paid to adsorption of cations on sand surface which is one of the concerns of MICP that can coat the sand particles and enhance the adsorption of bacteria. Microbial metabolic activity and cation cations influence the timing, rate, and location of calcium to form carbonate mineral precipitate (DeJong et al. 2009). Recent developments in MICP have heightened the need for more research on adsorption of urease or UPB which is hypothetically one of the important factors of MICP. It could affect MICP by the quantity of adsorbed urease or cells on sand. Studies were made to investigate the effect of cations on adsorption of cells in the process of MICP.

The objectives of the study presented in this chapter are:

- 1) To examine the effect of saline-washed suspension of bacterial cells and liquid supernatant containing urease on adsorption of cations on sand surface.
- 2) To determine the influence of employing divalent and trivalent cations such as Ca^{2+} , Fe^{3+} , and Al^{3+} on adsorption of bacterial cell by size distribution and urease, prior to MICP.
- 3) To examine the feasibility of employing various concentrations of Ca^{2+} with bacterial cells prior to MICP.

3.2 MATERIALS

The Changi Beach sand was used in all experiments with the grain size distribution shown in Figure 3.1. Sand properties are also shown in Table 3.1.

Table 3.1. Sand properties

Specific gravity	Medium size (mm)	Coefficient of uniformity	Coefficient of curvature	Maximum void ratio	Minimum void ratio
2.65	0.4	3.28	0.53	0.916	0.533

Figure 3.1. Grain size distribution for Changi Beach sand

The coarse, medium and fine portions in sand were separated using sieves for having diameters from bigger than 1.2 mm, 1.2 to 0.6 mm, and below than 0.2 mm to study the effect of particle size on biocementation. The porosity of each fraction was calculated based on the mass of sand within that fraction, the specific gravity of sand, and the total volume of sand. The calculated porosity according to the volume of sand under the experimental conditions was 45.2%, 41.5%, and 42.8% for the fine (diameter < 0.2 mm), medium (diameter from 0.2-0.6 mm), and coarse (diameter > 1.2 mm) fractions of sand, respectively. The sand specimens were prepared with dry densities of 1540, 1510, and 1457 kg/m³.

The cultural liquid of UPB, *Bacillus sp.* VS1, was isolated from the sand and grown in a liquid medium consisting of Tryptic Soy Broth DIFCO™, 30 g/l; urea, 20 g/l; MnSO₄·H₂O, 12 mg/l; NiCl₂·6H₂O, 24 mg/l; and phenol red, 10 mg/l. The rod shaped bacteria was alkalophilic, halotolerant, gram-positive, spore-forming, and aerobic (Stabnikov et al. 2011). Aerobic conditions for cultivation of UPB were created by pumping in air.

The change of the pH of the bacterial suspension is an indicator for bacterial growth. Figure 3.2 shows the dynamics of pH during cultivation of UPB. Another indicator for bacterial growth is phenol red which is yellow at the pH 6.8, but gradually changes to a red/bright pink to purple color at a pH above 8.2. Urease activity results in a pH increase, so microbial producers of urease must be tolerant to alkaline pH.

Figure 3.2. Dynamics of pH during cultivation of UPB after 5 days

The hydrolysis of urea liberates ionic products from non-ionic substrates according to the following Reaction:

In this study, urease activity was determined by the conductivity method. The urease reaction involved the hydrolysis of non-ionic substrate urea to ionic products thus generating a proportionate increase in conductivity under standard conditions (Appendix A) (Lawrence

and Moores 1972, Grunwald 1984). Conductivity was recorded over five minutes under a standardized condition of 1.5 M urea at 25°C. The rate of conductivity increases (mS/min) was converted to the urea hydrolysis rate (mM urea hydrolyzed /min) by calculation of the mole of urea to the mole of ammonium chloride considered in 30 minutes time. Moreover, biomass concentration and OD₆₀₀ (Optical Density) were measured under standard conditions (Appendix B). The concentration of biomass for bacterial culture in this study was 8 g/L corresponding to a cell concentration of approximately 10^8 cells/ml.

3.3 EXPERIMENTAL PROCEDURE

3.3.1 Treatment of sand with cations

Sand samples in a 50-ml syringe (28 mm in diameter and 120 mm in length as shown in Figure 3.3) were treated with water (as a control) or solutions of calcium, ferric, and aluminum cations in the experiments. The aliquots of 25 ml (approximately one pore volume of sand) of 50 mM freshly prepared solutions of aluminum chloride, ferric chloride, or calcium chloride with the pH of 3.6, 1.8, and 5.8, respectively, were injected from the bottom of the sand sample to the top and incubated for 1 hour. The solution was then drained off by gravity and the sand was washed three times by the injection of 25 ml deionized water from the top to the bottom as shown in Figure 3.3.

Figure 3.3. Sand samples in syringes treated from injection point of syringe (bottom)

The average pH of the water after the third washing was 6.5, 6.9, and 7.2 for the samples pre-treated with Al^{3+} , Fe^{3+} , and Ca^{2+} cations, respectively. In the control test, 25 ml of deionized water was used instead. After the sand was washed, 25 ml of different bioagents were injected from the bottom to the top and incubated for 2 hours.

The concentrations of cations in the solution before and after adsorption were determined using elemental analysis by ICP-OES (Inductively Coupled Plasma-Optical Emission Spectrophotometer, Perkin Elmer, 2000V). An ICP source consists of a flowing stream of argon gas ionized by an applied radio frequency field typically oscillating at 27.1 MHz which is inductively coupled to the ionized gas by a water-cooled coil surrounding a quartz “torch” that supports and confines the plasma. A sample was prepared through the digestion method with the mixing of 2 g of the crushed sample with 50 ml of 2.5% acetic acid and was shaken for 12 hours, following with filtration of the solution and the measurement of calcium concentration.

3.3.2 Instability of Urease activity in different bioagents, treatment of bioagents and treatment of sand with bioagents

After 48 hours of batch cultivation, 500 ml of intact bacterial suspension (cultural liquid) with a biomass concentration of 8 g dry biomass/l was separated into two fractions using centrifugation with 1000 g for 20 minutes. One fraction was centrifuged bacterial biomass and was re-suspended in 500 ml of 1% solution of NaCl. Another fraction was 500 ml of liquid supernatant-containing urease. The protease and urease of all three bioagents was measured. Protease is an enzyme which breaks peptide bonds and hydrolyzes the entire enzyme in cells. Hypothetically, by increasing the concentration of protease, the efficiency of biocementation decreases. To determine the activity of protease in the present study, Sigma’s non-specific protease activity assay may be used as a standardized procedure which was proposed by Cupp-Enyard (2008a). In this assay, casein acts as a substrate and when the protease digests casein, the amino acid tyrosine is liberated along with other amino acids and peptide fragments. Then Folin’s reagent primarily reacts with free tyrosine to produce a blue-colored chromophore, which is quantifiable as an absorbance value on the spectrophotometer. The activity of protease samples can be determined in terms of units, which is the amount in micromoles of tyrosine equivalents released from casein per minute from the standard curve.

This study followed by another study which during the MICP process with either bacterial cells or the urease suspension, the protease, urease and calcium carbonate precipitation was measured.

The size distribution of cellular aggregates was made by the following samples: 1) before and after treatment of cells with solution of CaCl_2 and 2) after aluminum, ferric, and calcium ions on cellular aggregation in different size of sand grains. The size distribution of cellular aggregates was measured using Mastersizer 2000 (Malvern Instruments Ltd, UK). Moreover, the particle size distribution of the bacterial cells and the maximum size of 90% of the particles (d_{90}) have been used to characterize the changes of the cell sizes during bio-treatment.

In another study, the aliquots of 25 ml of three different bioagents such as a) intact suspension of bacterial cells with a biomass concentration of 8 g dry biomass/l; b) centrifuged intact bacterial suspension which was washed with a biomass concentration of 8 g dry biomass/l; or c) supernatant of centrifuged intact bacterial suspension (solution of urease), were injected from the bottom of the sand sample to the top and incubated for 2 hours. The suspension/solution was then drained off by gravity. Different microbial solutions were injected from the bottom to the top of the samples.

3.3.3 Microscopy investigation

The structure of the sand samples was observed using a SEM (Scanning Electron Microscopy) (Zeiss EV050, UK). Sand samples were prepared in 2% glutaraldehyde for 2 hours. Then, they were washed 3 times with a 0.1 M sodium cacodylate buffer for 20 minutes and dehydrated step-wise in 50, 70, 85, and 95% (v/v) solutions of ethanol for 10 minutes. Samples were kept in 100% ethanol until dried with the critical point dryer Polavon E3100 (Quorum Technologies, UK), and then sputter-coated with Au-Pd using Emitech SC7620 (Quorum Technologies, UK).

The thermal FESEM (Field Emission Scanning Electron Microscopy) was also used with a JSM-7600F to combine ultra-high resolution imaging with optimized analytical functionality. FESEM is a microscope that works with electrons (particles with a negative charge) instead of light. These electrons are liberated by a field emission source, and the object is scanned by electrons according to a zig-zag pattern. In addition to FESEM, EDX (Energy Dispersive X-

ray) analysis was also used for chemical analysis. The intensity of backscattered electrons generated by electron bombardment can be correlated to the atomic number of the element within the sampling volume. Hence, qualitative elemental information can be revealed.

3.4 RESULTS AND DISCUSSION

3.4.1 Treatment of sand with cations

Concentrations of cations in the solution before and after adsorption to sand grains were determined using elemental analysis by ICP-OES. The efficiency of adsorption of cations on sand for different fractions is shown in Table 3.2.

Table 3.2. Efficiency of cations adsorption on sand grains surface

Solution for the treatment of sand	Efficiency of cation sorption, % of initial quantity for sand grains with the following sizes		
	0.2 mm-0.6 mm	0.6 mm-1.2 mm	>1.2 mm
Solution with Ca^{2+}	6	3	2
Solution with Fe^{3+}	49	55	14
Solution with Al^{3+}	24	29	14

The efficiency of sorption for trivalent cations (Al and Fe) was significantly higher than divalent (Ca). The efficiency of ferric and aluminum cation was 95% and 90% more than calcium in sample of 0.6 mm grains. This can be explained by an extra positive charge of trivalent cations compared to divalent ones to significantly increase the strength of the attachment to sand grains. Increasing the strength of attachment in Fe^{3+} cations can be explained by the higher molecular weight of Fe^{3+} compared to other cations.

Specific surface area properties were quantified and qualified by means of a complete gas sorption analyzer based on the BET (Brunauer, Emmett, and Teller) theory by using the Autosorb-1-C (Quantachrome instruments, USA). The results of Autosorb-1-C for specific surface area indicated $9.216\text{E}+00 \text{ m}^2/\text{g}$, $2.990\text{E}+00 \text{ m}^2/\text{g}$, and $3.721\text{E}+00 \text{ m}^2/\text{g}$ for coarse, medium, and fine sand grains, respectively. Significantly high surface areas for grains larger than 1.2 mm with the factor of three among fractions were found. Although, the highest surface area for grains larger than 1.2 mm was measured by BET, only 0.6 mm grains

showed the highest adsorption for trivalent cations after treatment. The explanation of the results can be the pore size between 1.2 mm grains, which hypothetically did not hold cations and were subsequently washed out by the solution.

3.4.2 Instability of urease activity in different bioagents

Intact bacterial suspension (cultural liquid) was separated using centrifugation to two bioagents: saline-washed suspension of bacterial cells and liquid supernatant-containing urease. As Figure 3.4 shows, the use of suspension of washed bacterial cells was more effective than that of supernatant and intact cultural liquid by 72% and 67% in terms of urease activity.

Figure 3.4. Changes of urease activity in different solutions (Chu et al. 2014)

The effect of increased urease activity of bacterial cells after dilution of the bacterial suspension with saline solution was known and could be explained by an increased release of urease from the cells under the presence of NaCl by Harkes et al. (2010).

The higher biocementation efficiency of washed bacterial cells in comparison with supernatant and non-centrifuged cultural suspension could be explained by the presence of protease activity in supernatant and non-centrifuged cultural liquid and the absence of this

activity in the suspension of washed bacterial cells. Protease activity in supernatant and intact bacterial suspension was within the range 0.1–0.2 μ moles of tyrosine/ml.min and could hydrolyze protein molecules of urease (Figure 3.5). This activity was not detected in the suspension of washed bacterial cells. This may be one of the reasons for the higher biocementation activity of washed bacterial cells in comparison with supernatant.

Figure 3.5. Correlation between urease and protease activities during growth of *Bacillus* sp. Strain VS1 (Chu et al. 2014)

The presence of protease can be used to explain that urease activity was not only lowest but also most unstable in supernatant. The urease activity decreased in supernatant from 0.50 to 0.17 mmol NH_4^+ /l.min for 60 minutes; while, the reduction of urease activity for the washed bacterial suspension for the same time was from 1.30 to 0.75 mmol NH_4^+ /l.min (Figure 3.5). Inhibition of urease activity in the solution of urea could be possible due to the increase of pH, according to Reaction 3.1.

The difference in the reduction rate of the urease activity (Figure 3.4) and in the rate of calcium carbonate production due to the urease activity (Figure 3.6) could be due to the removal of protease adsorbed on the fresh precipitate of calcium carbonate as well as the buffering of pH in the solution of urea and calcium chloride during the formation of calcium

carbonate. The above findings about activation of urease activity have important practical implications. It means that bioclogging and biocementation of soil can be better performed using a concentrated bacterial suspension or an intact bacterial suspension rather than the supernatant or filtrate containing only dissolved enzymes. That is the reason for using washed cells for the two applications of calcium-based biocement in Chapters 5 and 6.

Figure 3.6. Changes of calcium carbonate precipitation rate, g/l·min (Chu et al. 2014)

However, there will be no difference between inhibitions of urease in supernatant or bacterial suspension in this case. Considering the fast reduction of urease activity in the urea solution as shown in Figure 3.6, the optimal duration of biocementation could be shorter than 1 hour. Hence, the reduction of the calcium carbonate precipitation rate in the solution of both urea and calcium chloride was not too fast, as can be seen in Figure 3.6. The activation time for biocementation continued for about 2 hours.

Percentages of precipitated calcium carbonate to the added quantity of calcium in CaCl_2 were 73, 55, and 49% for the saline-washed suspension of bacterial cells, intact cultural liquid and liquid supernatant, respectively. From the results of urease activity, it could be concluded that low urease activity is even better for biocementation because of a low urea hydrolysis rate leading to higher availability of urea, longer hydrolyzing time processes, and subsequently stronger calcium carbonate aggregates.

According to the results of this section, the depth of bacterial cells penetration in soil depends on the rate of cell retention (adsorption) by the soil particles and the hydraulic conductivity of soil. When the saturated hydraulic conductivity of fine sand was about 10^{-4} m/s and the time of complete adsorption of bacterial cells on sand was about 1 hour, as in the case of this study, the calculated depth of bacterial cells penetration for one cycle of the sand treatment was about 36 cm. Therefore, to increase the depth of bioclogging and biocementation, the sand must be treated with bacterial suspension either continuously for a presented period of time or using the injection of bacterial suspension at different depths from the surface.

3.4.3 Effect of cations on bacterial cells adsorption

The uptake of bacterial cells from the suspension by the sand particles with sizes between 0.2 and 0.6 mm was determined by measuring the depletion of cells (optical density at 600 nm, OD_{600}) and urease activity from a bacterial suspension. Adsorption of cells on the sand was completed in 60 minutes (Figure 3.7).

Figure 3.7. Adsorption of bacterial cells on sand: curve 1, adsorption was measured by OD_{600} ; curve 2, adsorption was measured by urease activity of cells (Chu et al. 2014)

As shown in Table 3.3, pretreatment of sand with Ca^{2+} , Fe^{3+} , or Al^{3+} solution increased adsorption of bacterial cells by $31 \pm 6\%$ (mean $\pm$ standard deviation) in comparison with the control, which was treatment of sand with water.

Table 3.3. Efficiency of bacterial cells adsorption on sand grains surface after pretreatment of sand with cations and control treatment of sand with water (Chu et al. 2014)

Solution for the treatment of sand	Efficiency of cells sorption, % of initial quality for sand grains with the following sizes		
	0.2 mm-0.6 mm	0.6 mm-1.2 mm	>1.2 mm
Water	70	68	65
Solution with Ca^{2+}	96	94	82
Solution with Fe^{3+}	90	89	80
Solution with Al^{3+}	92	90	84

The effect of the size of sand grains within the range of 0.2 to 2 mm and the specific surface of grains on the efficiency of bacterial cells adsorption was not significant according to the results of Table 3.3, in contradiction of 75% higher surface area for grains larger than 1.2 mm by BET results. This can be explained by the fact that efficiency of bacterial cells adsorption depends mainly on the number of positively charged sites created by cations adsorbed on the sand grain surface.

It is known that the coating of sand surface with cations significantly enhances adhesion of bacterial cells to the sand grains (Harvey et al. 1989, Lukasik et al. 1999). This could be due to the increased density of the positively charged sites at the sand grain surface, which attract negatively charged sites of bacterial cell surface. In this study, pretreatments of sand with trivalent cations, Fe^{3+} and Al^{3+} , or divalent cation Ca^{2+} enhanced the adsorption of bacterial cells for almost the same value, $31 \pm 6\%$, notwithstanding that trivalent cations bridged between sand and bacterial cells could have 1.5 times stronger net bonds compared to the salt bridges created by divalent cations. This means that the cation-enhanced bacterial cell adsorption is due to the increase in the number of the positively charged sites, but not due to the strength of the bonds between cations and the surfaces.

3.4.4 Size distribution of cell aggregates with cations

Size distribution of cells before and after pretreatment with divalent and trivalent cations was investigated using SEM and Mastersizer 2000. Unattached cells of *Bacillus* sp. before pretreatment were observed by using SEM images (Figure 3.8a). The influence of cations as a

pretreatment on aggregation of bacterial cells (Figure 3.8b) compared to separated cells was confirmed by SEM images.

a **b**

Figure 3.8. Cells of UPB under SEM arrows show (a) unattached cells of *Bacillus* sp. before and (b) after pretreatment with the solution of calcium chloride (Stabnikov et al. 2011)

Further investigation of size distribution of cell aggregation of 0.6 to 1.2 mm sand grains before and after pretreatment with 50 mM of aluminum, ferric chloride, and calcium chloride using Mastersizer 2000 (see Appendix C), was shown in Figure 3.9. The sizes of 90% of the cells (d_{90}) before and after pretreatment with aluminum and ferric chloride increased from 6.5 μm to 13 μm .

Figure 3.9. Results of d_{90} of cell aggregation before and after pre treatment with cations

However, as Figure 3.9 shows, a comparison of the results of pretreatments with 50mM trivalent and divalent cations indicated that the sizes of 90% of the cell (d_{90}) were raised from 12 μm by trivalent cations to 48 μm for Ca^{2+} . This could be explained by the higher efficiency of calcium chloride on cell aggregation and the rate of precipitation compared to trivalent cations.

Moreover, FESEM images of bio-treated sand confirmed the hypothesis that bacteria adhere to sand grain surfaces, and crystals of calcium carbonate grow and attach to sand surfaces (Figure 3.10), finally producing a clogging or cementation effect. This fact supported the importance of aggregation of bacterial cells using pretreatment with cations. The results of the investigation of FESEM images for special parts of the sample treated with MICP are shown in Figure 3.10. Precipitation of calcium carbonate (spectrum 1) was clearly observed on the surface of the sand (spectrum 2). Hence, as Figure 3.10 (b) shows, the chemical

analysis of the spectrums also confirmed the precipitation of calcium carbonate by showing the calcium, carbon, and oxygen as the three highest elements. Silicon and oxygen showed the highest value in chemical analysis of spectrum 2.

Figure 3.10. FESEM results (a) SEM images and (b) EDX analysis for chemicals from spectrum 1 and spectrum 2 which indicate calcium carbonate and silicate sand respectively

Further observation of precipitated calcium carbonate by SEM showed precipitated crystals of calcium carbonate as a result of adhesion of cells on the surface of the sand. Crystals of calcium carbonate on the surface or between the pores were observed as a result of MICP in Figure 3.11 (a).

Figure 3.11. SEM images of (a) calcium carbonate crystals and (b) precipitated calcium carbonate on the surface of sand grains

Hence, no significant diversity between cell aggregation after pretreatment with Fe^{3+} or Al^{3+} was observed. No significant correlation between size of sand grains and cell aggregation was found.

3.4.5 Size distribution of cell aggregates by various concentration of calcium chloride

As shown in Appendix C (Figure C1a), the average size of cell aggregates was about $1\ \mu\text{m}$ and more than $10\ \mu\text{m}$ for aggregates formed in the absence and presence of cations, respectively. Figure 3.12 shows $d_{(90)}$ of aggregated cells after pretreatment with various concentrations of calcium chloride solution. In the presence of calcium chloride, aggregates with sizes up to $400\ \mu\text{m}$ could be observed (Appendix C). These large cell aggregates were immobilized on the top layer of sand since they could not penetrate through the pore system. The immobilization of the cells in the top layer is responsible for the formation of the calcite crust on the surface.

Figure 3.12. Formation of cell aggregates after pretreatment with various concentrations of calcium chloride solutions.

The formation of a biocement-treated crust on the top layer of sand could be due to the treatment of the cultural liquid with $100\ \text{mM}\ \text{CaCl}_2$ solution, which stimulated the formation of cell aggregates. Hence, increasing the concentration of CaCl_2 as a treatment with the factor of 10 is shown in Figure 3.12. The average cell aggregate size does not demonstrate significant change after treatment with $0.1\ \text{mM}$ and $1\ \text{mM}$ of calcium chloride. Moreover,

increasing the concentration of calcium chloride to 10 mM and 100 mM (Figure 3.12) increased 70% of the size of cell aggregates.

3.4.6 Efficiency of urease activity on biocementation by pretreatment with cations

The results of efficiency of urease activity after pretreatment with cations and incubation with intact suspension, supernatant, or bacterial cells on different grain sizes are shown in Tables 3.4, 3.5, and 3.6 respectively.

Table 3.4. Efficiency of urease activity after incubation with intact suspension, in sand grains after pretreatment of sand with cations

Solution for the treatment of sand	Efficiency of urease activity after intact suspension, % of initial quantity for sand grains with the following sizes		
	0.2 mm-0.6 mm	0.6 mm-1.2 mm	>1.2 mm
Water	33	40	30
Solution with Ca^{2+}	40	38	55
Solution with Fe^{3+}	41	41	54
Solution with Al^{3+}	60	42	52

There was no significant correlation between pretreatment of grains with divalent or trivalent cations and incubation of intact suspension (Table 3.4) or supernatant (Table 3.5) on efficiency of urease activity as shown. Subsequently, comparing the results of sand grain size pre-treated with divalent and trivalent cations on efficiency of urease activity did not illustrate significant correlation.

Table 3.5. Efficiency of urease activity after incubation with supernatant in sand grains after pretreatment of sand with cations

Solution for the treatment of sand	Efficiency of urease activity after supernatant, % of initial quantity for sand grains with the following sizes		
	0.2 mm-0.6 mm	0.6 mm-1.2 mm	>1.2 mm
Water	38	29	32
Solution with Ca^{2+}	43	38	25
Solution with Fe^{3+}	43	23	36
Solution with Al^{3+}	41	35	35

Consequently, urease activity showed minimum 50% increment after incubation with bacterial cells compared to supernatants and intact suspension (Tables 3.5 and 3.6). Pretreatment of sand with Ca^{2+} , Fe^{3+} , or Al^{3+} solution increased efficiency of urease activity after incubation with bacterial cells by 52% in comparison with the control (Table 3.6).

Table 3.6. Efficiency of urease activity after incubation with bacterial cells in sand grains after pretreatment of sand with cations

Solution for the treatment of sand	Efficiency of urease activity after cells, % of initial quantity for sand grains with the following sizes		
	0.2 mm-0.6 mm	0.6 mm-1.2 mm	>1.2 mm
Water	45	28	58
Solution with Ca^{2+}	93	92	97
Solution with Fe^{3+}	100	95	93
Solution with Al^{3+}	91	93	90

Comparing the results of trivalent and divalent cations illustrated strong evidence of significant efficiency for urease activity in samples pre-treated with Fe^{3+} and bacterial cells. It could be explained by adsorption of cells by cations and remaining cations on the surface of sand sample. The same effect of cations in increasing the activation of urease by the addition of 30 mM Ca^{2+} up to ten fold is mentioned in literature (Hammes et al. 2003a). This means that cation-enhanced bacterial cell adsorption is due to the increase in the number of positively charged sites, but not due to the strength of the bonds between cations and surfaces.

Bachmeir et al. (2002) indicated that the immobilization of urease activity have been shown to stabilize enzyme activity for longer periods. Therefore, by increasing the adsorption of bacterial cell, the immobilization potential of cells of *S.pasturii* inside the porous media has been shown (Figure 3.10). Pretreatments of sand with 50 mM trivalent cations, Fe^{3+} and Al^{3+} , or divalent cation Ca^{2+} enhanced adsorption and immobilization of bacterial cells (Table 3.3). Bacteria attached to the sand granules are supposed to provide the nucleation sites where calcite is precipitated due to the hydrolysis of urea, an increase of pH (Stocks-Fischer et al. 1999), and, probably, transformation of bicarbonate to carbonate. SEM images of MICP-treated sand (Figure 3.10) showed adsorption of cells to sand grain surface, crystals of

calcium carbonate grow and attach to sand surface and finally clogging or cementation effect is produced (Stabnikov et al. 2011)

3.5 CONCLUSIONS

The conclusions of the study presented in this chapter are as follows:

- 1) It was found that urease activity in samples with the suspension of bacterial cells was $70 \pm 3\%$ more effective than that of supernatant and intact cultural liquid.
- 2) The increased urease activity of washed cells suspension as compared with supernatant can be explained by the presence of urease-inactivating protease in supernatant.
- 3) The efficiency of adsorption of bacterial cells by 0.6 mm sand grains sand after pre-treated with ferric and aluminum cation was 95% more than that of calcium. This can be explained by an extra cation of trivalent cations compared to divalent ones to significantly increase the strength of the attachment to sand grains.
- 4) The efficiency of cell aggregation in sand is increased by 86%, 45% and 63% respectively after sand was pretreated with bacterial cells with Ca^{2+} , Fe^{3+} , or Al^{3+} solutions.
- 5) Pretreatments of sand with trivalent cations, Fe^{3+} and Al^{3+} , or divalent cation Ca^{2+} enhanced adsorption of bacterial cells of sand by $31 \pm 6\%$ (mean $\pm$ standard deviation) in comparison with control, which was treatment of sand with water.
- 6) Pretreatment of sand with the solution of cations increased the efficiency of urease activity after incubation with bacterial cell by 52% in comparison with the control which was sand treated with water (Table 3.6).
- 7) Low urease activity in this experiment could be even better for biocementation because low urea hydrolysis rate leads to stronger calcium carbonate aggregates under high pH conditions.

CHAPTER 4 IRON-BASED BIOCEMENT AND BIOCEMENTATION

4.1. INTRODUCTION

It was shown in Chapter 3 that the microbial-induced calcium carbonate precipitation (MICP) process can be used for soil improvement. Çabalar (2007) and Ivanov and Chu (2008) also reported that biocementation in sands can be facilitated by using an agent such as hydrous iron oxide. The iron salts precipitated can be considered a clogging and cementing compound called iron-based biocement. There are two reasons for the use of iron-based biocement. The first reason is annual production of thousands of tons of iron salts. The second is that natural sandstone often originates from sand saturated with ferric oxides or hydroxides (Pettijohn et al. 1987). Therefore, iron salts precipitation through iron-based biocementation could be a new method.

Little work has been published on the mechanisms and potential applications of iron-based biocementation (Ivanov et al. 2009, Chu et al. 2011, Ivanov et al. 2012). Therefore, in the first method discussed in this chapter, calcium chloride used in the MICP process was replaced with ferrous sulfate for precipitation of iron salts as a biocementation method. In the present study, innovative biocement production by the addition of urea and ferrous sulfate solution after the application of UPB to sand samples was carried out and the possibility of increasing the shear strength of soil in the presence of ferrous cation was explored. In another study by Ivanov et al. (2012), the new method using EDTA ferric monosodium was adopted instead of the use ferrous sulfate to stabilize the ferrous cations. The present study compares the results of both studies.

Furthermore, the idea of using a cheap and readily available source of ferric cation in the form of iron ore is proposed. In the new method, the source of iron for iron-based biocement is produced by microbial activity through a reduction process. Ivanov et al. (2004) showed that iron ore was reduced by iron-reducing bacteria (IRB) in an anaerobic environment and produces dissolved ferrous cations. This chemical reaction is called fermentation in which complex organic matter, glucose, cellulose and amino acids are partially oxidized and result in the production of organic acids, alcohols, hydrogen and methane. These fermentation

products can be subsequently oxidized with ferric cation serving as the terminal electron acceptor. Hence, microorganisms are able to reduce ferric cation to ferrous cation and accumulate the energy (Lovley 2000, Lovley et al. 2004). Therefore, a novel area of study is created by investigating the mechanisms and potential applications of iron-based biocement with the microbial production of ferrous. The cultivation of IRB to provide a microbial source of iron with the sand treatment condition includes the concentration of iron and a number of treatments that require focused study. Up to date, no data has been published on the use of microbial production of ferrous cation for the reduction of hydraulic conductivity of soil.

In addition, to decrease the environmental impact and cost of iron-based biogROUT with ferrous sulfate, a biocement production method consisting of a combination of ferrous sulfate and microbially produced ferrous cations is introduced as a third method.

The objectives of the study presented in this chapter are as follows:

- 1) To examine iron-based biocement production using UPB, ferrous sulfate and urea solution.
- 2) To study the feasibility of microbial production of ferrous cation and identify factors affecting the production of ferrous cation by appropriate microbial activities.
- 3) To study the possibility of the production of iron-based biocement with the application of UPB with a combination of ferrous sulfate and microbially produced ferrous cations and urea solution.
- 4) To evaluate the effects of different iron-based biocements on the hydraulic conductivity of sand.

4.2 ESTABLISHMENT OF IRON-BASED BIOCEMENT SOLUTION

In the present project, three different types of iron-based biocement solution are studied. Three methods with three different sources of iron cations comprising ferrous sulfate (set I), microbially produced ferrous cation (set II) and a combination of ferrous sulfate and microbially produced ferrous cation (set III) were used. The biocement solutions needed to be optimized according to the general required conditions for the precipitation of cations through urease activity, such as high pH. Therefore, the establishment of the iron-based biocement

solution with these three methods was studied first. As the pH of ferrous cation solution is in the acidic phase, the different biomass concentrations of UPB for increasing the pH of biocement solution, as well as for increasing the precipitation rate, needed to be checked. Moreover, the effect of using diluted (with 1% NaCl in neutral pH) or concentrated UPB was studied. Therefore, the pH of the solution containing different sources of iron produced by IRB, ferrous sulfate and a combination of ferrous sulfate and microbially produced ferrous cations after the addition of diluted or concentrated UPB was examined and the rate of precipitated cations in the biocement solution.

4.2.1 Ferrous sulfate

The first method for production of iron-based biogrout was designed by the use of ferrous sulfate instead of calcium chloride. Similar to the precipitation of calcium carbonate in the presence of hydroxide and carbonate ions through MICP, iron salts can be precipitated based on the following Reactions:

In this method, UPB was applied before the solution of ferrous sulfate ($\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$, molecular weight of 278 g from SIGMA) and urea for the production of iron salts. 100 ml of intact bacterial suspension (*Bacillus sp.*) was used in the experiment. This is the same as described in Section 3.2.2 of Chapter 3. The bacterial suspension was separated into two fractions using centrifugation with 1000 g for 20 minutes. Half of the samples were treated with centrifuged bacterial biomass which was re-suspended in 100 ml of 1% solution of NaCl for applying as diluted UPB with a pH around 7. The other half was treated with intact bacterial suspension as concentrated UPB with a pH around 9. Based on the results of *Bacillus sp.* in Chapter 3, the efficiency of bacterial adsorption to sand grains is increased by using cells. However, as the pH of ferrous sulfate and urea is acidic, it was assumed that the intact bacterial suspension with a pH around 9 increases the pH of the solution for a higher precipitation of iron salts.

Since the pH of ferrous sulfate and urea solution is acidic (3-4) and the activation of urease activity occurs in the basic phase (8-9.5), it was important to monitor the pH of the solution

after mixing with UPB. In the first experiment using the first method, 30 ml of diluted or concentrated UPB was mixed with 30 ml of 7.5 mM ferrous sulfate and 15 mM urea solution. The results of pH in this experiment are shown in Figure 4.1. Note that the pH of ferrous sulfate and urea solution before the addition of UPB was basic (3.1). The increases in pH in the samples treated with diluted or concentrated UPB are different. The highest pH for diluted UPB was measured in the basic phase (8) while, under the same conditions, the pH for concentrated UPB was neutral (7) after 12 hours. Moreover, the fast trend in increasing the pH from the acidic phase of ferrous sulfate to the basic phase can be seen in the samples treated with concentrated UPB.

Figure 4.1. Effect of diluted or concentrated bacterial culture on pH of the biogrout solution of ferrous sulfate and urea

The data in Figure 4.1 shows that the basic pH of the solution after the addition of diluted UPB is applicable as the biocement solution. The second experiment with the solution of ferrous sulfate and urea was conducted to examine the change in pH of the solution of ferrous sulfate and urea after adding a different biomass concentration of UPB. This can be used to determine the optimum biomass concentration for the highest production of iron salts. A stoichiometric concentration of urea and ferrous sulfate according to Reaction 4.1 and ferrous sulfate with a concentration of 7.5 mM was prepared for the test. Afterwards, measurements of the pH and the amount of precipitation using TSS (Total Suspended Solids) were carried out as described in Appendix D. The results of pH are presented in Figure 4.2.

Figure 4.2. Effect of different biomass concentration on pH of the solution of ferrous sulfate and urea

Figure 4.2 shows the pH results after the addition of different biomass concentrations of UPB to the solution of ferrous sulfate and urea. Increasing the biomass concentration raised the pH of the ferrous sulfate and urea solution, which means that increasing the ions of hydroxide, is expected to increase the iron salts.

Figure 4.3. Effect of biomass concentration on precipitated rate of iron salts

A higher pH in the biocement solution increased the rate of release of hydroxide ions, which in turn increased the rate of iron salts (ferric hydroxide) precipitation. The rate of precipitated cation was measured by the addition of various biomass concentrations of UPB. As shown in Figure 4.3, 0.4 g/l of biomass concentration gave the highest rate of precipitated cation. Therefore, this is the biomass concentration used on sand samples treated with ferrous sulfate later in the study.

As shown in Figure 4.3, increasing the biomass concentration of UPB raised the rate of precipitation. The maximum rate of precipitation was obtained using a biomass concentration of 0.4 g/l. Based on theoretical calculations of the mass of ferrous sulfate and precipitated salts, almost 70% of the ferrous cations precipitated. The rate of precipitation during the time intervals of the experiments was measured and the results are shown in Figure 4.4.

Figure 4.4. Precipitated rate of iron (%) over time

As shown in Figure 4.4, the rate of precipitation was significantly higher during the first thirty minutes. Although precipitation of iron salts occurred over the course of the experiment, the rate reduced drastically after the first hour. On the other hand, the precipitation of iron salts decreased with a decreasing concentration of UPB (Figure 4.3). Rapid precipitation of iron is not desirable and could result in clogging and precipitation only in the top part of the soil layer. Thus, a slower reaction time between the bacterial suspension and chemicals was selected as this allows the chemical solution to fully penetrate the ground

before precipitation commences. Therefore, similar to the mechanisms studied in Chapter 3 to improve the precipitation rate through MICP, applying the reagent solution two hours after the application of UPB provides time for the retention of cells in sand grains and could result in increased precipitation of iron salts in deeper layers.

4.2.2 Microbially produced ferrous cation

The second method for the production of an iron-based solution is to use a cheap and readily available source of ferric cation in the form of iron ore. This also decreases the cost of the iron source for biogrout. According to Ivanov et al. (2004), iron ore is reduced by IRB in an anaerobic environment to produce dissolved ferrous cations in the presence of a carbon source through fermentation. This fermentation process produces organic acids, alcohols, hydrogen and methane (see Appendix E) (Lovley 2000, Lovley et al. 2004). The microbially produced ferrous cation could then be used instead of ferrous sulfate as a source of cation ions in biogrout.

For this experiment, IRB was selected from anaerobic sludge from the Jurong Wastewater Reclamation Plant. The dry sludge contained up to 3% (w/w) of IRB. It was stored at 4°C before being used as inoculums to cultivate enrichment cultures with the medium listed in Table 4.1. The composition of the medium for cultivation of IRB was chosen based on the standard method proposed by Eaton and Franson (2005).

Table 4.1. Composition of medium for cultivation of IRB

Chemicals	Concentration (g/l)
Sodium bicarbonate (NaHCO_3)	2.5
Ammonium chloride (NH_4Cl)	0.25
Sodium phosphate (Na_2HPO_4)	0.6
Potassium chloride (KCl)	0.1
Acetate or peptone as a carbon source	1.5
Iron ore	1
Trace elements solution	10 ml
Trace vitamins	10 ml
Anaerobic sludge	100 ml

The trace elements solution listed in Table 4.1 consisted of 10 mg of calcium pantothenate, niacin, P-aminobenzoic acid, thiamine, riboflavin, pyridoxine, cobalamin, thioctic (α-lipoic acid), folic acid and biotin prepared in 1 L of distilled water, filter sterilized and stored in darkness at 4°C. The composition of the trace vitamins in Table 4.1 was reported by Eaton and Franson (2005) to include a mixture of MgSO_4 (3g), MnSO_4 (0.5 g) and NaCl (1g) plus 0.1 g of FeSO_4 , CaCl_2 , CoCl_2 and ZnSO_4 mixed with 0.01 g of CuSO_4 , $\text{AlK}(\text{SO}_4)_2$, H_3BO_3 and NaMoO_4 in 1 l of distilled water.

The mixture described in Table 4.1 was purged with nitrogen gas to remove the oxygen and create anaerobic conditions, sealed with the original plastic cap and wrapped with aluminum foil before being placed in a shaker. The mixture was then placed in dark conditions at a controlled temperature of 25°C. The mixture was left for 10 days.

To check the activation of IRB and ferrous cation production, the oxidation reduction potential (ORP) of the mixture was measured over time as shown in Figure 4.5. It can be seen that the ORP decreased (or reduction potential increased) and that ferric cation was continuously reduced to ferrous. The initial ORP value of the mixture was approximately 60 mV. After two days the ORP value dropped to 0 mV and subsequently to -375 mV at day ten.

Figure 4.5. Measurement of the ORP during the IRB cultivation over time

To check the ferrous cation production by IRB activity, the ferrous cation concentration in the mixture described in Table 4.1 was measured over time during the cultivation of IRB. The results are shown in Figure 4.6. A modified Phenathroline method proposed by Clesceri et al. (1998) was used for determining the total and dissolved ferrous cation content (Appendix F). The ferrous cation concentration during the cultivation of IRB was 142.1 mg/l after nine days. However, as shown in Figure 4.6, the ferrous cation concentration dropped to 136 after ten days. The drop in the ferrous cation concentration can be explained by the reduction in the carbon source.

Figure 4.6. Dynamics of ferrous cation concentration during ten days in the cultivation of IRB

Carbon sources such as glucose and cellulose were used as electron donors. Dissimilatory ferrous cation reduction occurred when iron ore was used as an external electron acceptor in bacterial metabolism. As shown in Figure 4.6, the results of the experiment using glucose ten days after cultivation show a reduction in ferrous cation concentration. This indicates the necessity of adding glucose as a carbon source after ten days for the production of ferrous.

Since glucose has a higher solubility, it was easier for the IRB to break the molecules of glucose during fermentation, thereby resulting in a shorter time interval for increasing the concentration of ferrous. A close examination of the different carbon sources indicated that

the start of the growth phase for the sample with glucose was about three days, while the samples with cellulose (such as tissue paper) took more than five days after preparation of the samples. Therefore, glucose is recommended for the microbially production of ferrous cation in a shorter time even though it is more expensive than cellulose.

Figure 4.7. (a) Oxidation of ferrous cation on water surface: bubbles formed within sample and (b) production of the layer of reddish brown substance

During the fermentation process, a brown foamy layer and gas bubbles formed on the surface of the samples (Figure 4.7 a). As shown in Figure 4.7 (b), the formation of a reddish brown substance on the surface of the water indicated the oxidation of ferrous. In addition, gas bubbles (Figure 4.7 a) were observed within the sand sample once a significant amount of ferrous cation was detected. In another experiment with soil, it was found that the concentration of ferrous cation in the pore water through the soil matrix was higher than the concentration in the top layer of water. This is due to the presence of dissolved oxygen in the top layer which causes the ferrous cation to oxidize to ferric cation and the concentration of ferrous cations to decrease.

Next, environmental factors impacting IRB activity were considered such as temperature, concentration of iron ore and concentration of energy source. To evaluate the effect of environmental conditions on ferrous cation production, a new set of samples (II.1-9) with various formulations in concentration of carbon and iron source were prepared in three repetitions to place under temperatures of 8°C, 25°C and 50°C. The iron ore and carbon source were added to a 500 ml plastic container with the solution listed in Table 4.2. Since this experiment was designed to evaluate the effect of temperature on ferrous concentration,

cellulose was used as the carbon source instead of glucose to reduce the cost. In addition, 50 ml of culture liquid of IRB and 400 ml of water were added to the container. The container was then sealed and shaken to mix its contents.

Table 4.2. Testing program for combinations of iron and cellulose for microbially production of ferrous cation

Sample	Iron ore (g/l)	Cellulose (g/l)
II.1	1	1
II.2		3
II.3		5
II.4	2	1
II.5		3
II.6		5
II.7	3	1
II.8		3
II.9		5

The mixture was placed into a PVC pipe lined with a layer of transparency, purged with nitrogen gas and left to cure for 28 days. The above-mentioned steps were repeated for the other formulations. The results of these experiments are shown in three different charts.

Figure 4.8 shows the ferrous cation concentration at room temperature (25°C) versus time. As Figure 4.8 shows, after 21 days at 25°C, samples II.9 and II.4 show increments in ferrous cation concentration to 1086.46 and 815.27 g/l, respectively. Samples II.1, II.6, II.7 and II.8 show significantly lower concentrations of ferrous. It is observed that although the concentration of ferrous cation in samples II.1, II.6, II.7, II.8 and II.9 increased, the concentration of ferrous cation in samples II.2, II.3, II.4 and II.5 decreased (Figure 4.8). The drop in the ferrous cation concentration can be explained by the reduction in the anaerobic environment (as the most important factor on ferrous cation production by IRB) for these samples.

Figure 4.8. Concentration of microbially produced ferrous cation (mg/l) by IRB at 25°C

Figure 4.9 shows the concentration of microbially produced ferrous cation through IRB activity versus time at 8°C. The results of this experiment show a significant reduction in ferrous cation concentration by decreasing the temperature from room temperature (25°C).

Figure 4.9. Concentration of microbially produced ferrous cation (mg/l) by IRB at 8°C

As shown in Figure 4.9, the ferrous cation concentration for all samples in set II decreased at 8°C. This can be explained by the decreased IRB activity. For example, sample II.9, which had the highest amount of cellulose, also showed the highest amount of ferrous cation at 10 mg/l.

Lastly, Figure 4.10 shows the concentration of microbially produced ferrous cation through IRB activity versus time at 50°C. The results of these experiments show a significant increase in ferrous cation concentration by increasing the temperature from room temperature (25°C).

Figure 4.10. Concentration of microbial ferrous cation (mg/l) produced by IRB at 50°C

As shown in Figure 4.10 at 50°C and 28 days, samples II.9 and II.3 have concentrations of 1139 and 860 mg/l for ferrous, respectively. It is observed that all of the samples show a drop in ferrous cation concentration. In this case, the maximum concentration of ferrous cation is 1892 mg/l for sample II.9. The drop in the ferrous cation concentration can be explained by the depletion of the carbon source or transport of oxygen to the samples. By comparing the results from different temperatures, it is evident that as the temperature goes up, the amount of microbially produced ferrous cation in the samples increases by decreasing the dissolved oxygen in the solution. Therefore, as the highest ferrous cation concentrations at 25°C and

50°C are 1149 and 1892 mg/l, respectively, preparation of sand samples should be conducted at 50°C for maximum microbial activity for ferrous cation production.

The results of ferrous cation production as a consequence of IRB activity show the effect of IRB treated with the highest mass of cellulose (5 g). It can be concluded that the amount of iron ore does not affect aggregation. Therefore, the activity of IRB and optimum environmental conditions such as anaerobic environment, temperature and energy source are the affecting factors in the production of microbial ferrous. The microbially produced ferrous cation activity was used in the experiments on iron-based bio-treatments. As the highest IRB activity was measured at the highest temperature, the samples were placed at 50°C for higher microbial ferrous cation precipitation.

In another experiment, the microbially produced ferrous cation was used for the establishment of iron-based biogrout with microbially produced ferrous cation and urea with the application of UPB. For the experiment, based on the results of cellulose as an energy source (Figure 4.10), a solution of microbially produced ferrous cation with a final ferrous cation concentration of 2 g/l and urea (0.1 M) was prepared. Next, 30 ml of diluted and concentrated bacterial cells (as described in Section 4.1.2) were added to the mixture of microbially produced ferrous cation and urea. Measurements of the pH and the amount of precipitation using TSS were carried out (Appendix D). The results in Figure 4.11 show that the pH of the iron-based biogrout with the use of microbially produced ferrous cation was almost above 9 after an hour. Note that the pH of the microbially produced ferrous cation and urea solution before the addition of UPB was 6.4.

As shown in Figure 4.11, the high pH of the solution indicates the possibility of hydrolyzing urea by UPB and the precipitation of iron salts due to high pH requirements for urease activity. However, the solution of microbially produced ferrous cation and urea shows almost the same range of pH after the addition of diluted or concentrated UPB, which indicates the applicability of the iron-based solution using microbially produced ferrous.

Figure 4.11. pH of solution with microbially produced ferrous cation during the precipitation of iron salts

Figure 4.12. Effect of biomass concentration on precipitation rate of iron salts with the microbially produced ferrous cation

Subsequently, the precipitation rate of the solution treated with various biomass concentrations of diluted UPB was measured. As shown in Figure 4.12, increasing the biomass concentration of UPB raised the rate of precipitation and the maximum precipitation was obtained from a biomass concentration of 0.5 g/l. The results of various biomass concentrations of UPB after 12 hours showed that almost 25% of the microbially produced ferrous cation precipitated as iron salts. The effect of the higher pH in the samples treated with microbially produced ferrous cation and UPB did not lead to a significantly higher rate of precipitation. The reason for the lower rate of precipitation than ferrous sulfate could be inaccessibility, low concentration or instability of the microbially produced ferrous cation for precipitation as iron salts.

4.2.3 Combination of ferrous sulfate and microbially produced ferrous cation

In the method for production of iron-based biocement solution using ferrous sulfate, the cost of the ferrous sulfate was too high and the microbially produced ferrous cation was time-consuming, especially when using cellulose. In addition, there was not enough ferrous cation produced (maximum concentration of 2 g/l). Thus, the third method for production of iron-based biogrout is the combination of ferrous sulfate and microbially produced ferrous cations. The idea for this combination method was based on the fact that when ferrous cations produced by IRB activity are applied to the ground they can be oxidized by hydroxide ions to form ferric/ferrous hydroxide, which can clog soil pores. The precipitation of ferric hydroxide requires availability of hydroxide and ferric cations. In iron-based biocement in the presence of UPB, hydroxide ions could be produced as a consequence of hydrolyzing the urea and the salts of iron could precipitate. This new approach is the third method examined in this study and would decrease the cost of ferrous sulfate, and increase the stability of ferric chelates.

The possibility of combining ferrous sulfate and microbially produced ferrous cation was investigated. Set (III) of samples was prepared to examine changes in the pH of a solution with a combination of half ferrous sulfate and half microbially produced ferrous cation (final concentration of 2 g/l) mixed with urea after the injection of UPB over 12 hours. The experiments were conducted using diluted and centrifuged bacterial cells as described in Section 4.2.1. The solution of ferrous cation and urea was applied with final ferrous cation and urea concentrations of 2 g/l and 0.1 M, respectively. Note that as the maximum value of microbially produced ferrous cation using IRB was measured for 2 g/l based on microbially activity with cellulose as the carbon source (Figure 4.10), the final concentration of this

section was also set at 2 g/l with the combination of the two iron sources. Figure 4.13 shows the results of pH for the combination of ferrous sulfate and microbially produced ferrous cation as an iron source. Note that the pH of the microbially produced ferrous cation and urea solution before the addition of UPB was 6.4.

Figure 4.13. pH of biogrout with the combination of ferrous sulfate and microbially produced ferrous cation during the precipitation of iron salts

As shown in Figure 4.13, the results of the diluted UPB treated with a combination of ferrous sulfate and microbially produced ferrous cations with urea show a gradual increase in pH from 6.4 to 9.1 after 12 hours. It seems that the presence of microbially produced ferrous cation is the reason for the initially higher pH (6.4) than in the solution of urea with only ferrous sulfate.

Figure 4.14. Effect of biomass concentration on precipitated rate of iron salts with the combination of ferrous sulfate and microbially produced ferrous cation

In addition, the rate of iron salts precipitation for the iron-based solution with the combination of ferrous sulfate and microbially produced ferrous cation and diluted UPB was measured using the TSS method (Appendix D). Data obtained from the tests of TSS are shown in Figure 4.14. Comparing the results of TSS (shown in Figure 4.14) to the initial concentration of ferrous cation (2 g/l) concluded the efficiency of third method. The results show that almost 56% of the ferrous cations were precipitated as ferrous/ferric hydroxides after 12 hours.

4.3 PROCEDURE FOR SAND SAMPLES PREPARATION

The results of Section 4.2 for the establishment of iron-based biocement solution were used in the current section for the preparation of sand samples. The sand samples were examined for soil improvement tests after the application of iron-based biogROUT. Specifically, the effect of each type of biocement with various sources of ferrous cation on unconfined compressive strength (UCS) and hydraulic conductivity was studied.

Sand from Changi Beach was used in all of the experiments in this section. The basic properties of Changi Beach sand are described in Table 3.1 in Section 3.2.1 of Chapter 3. The sand was sieved and prepared. It was found to have a mean grain size (D_{50}) of 0.55 mm and fine content of about 4% (w/w). According to the Unified Soil Classification System (ASTM: D2487), it is a medium grained, poorly graded, clean sand. The size distribution of the sand ranges from 0.075 mm to 1.18 mm. The sand placed in the experiments had a porosity of about 40%.

According to the results of Figure 4.3, 4.12 and 4.14, almost 70% of the ferrous cations were precipitated as iron salts by the use of ferrous sulfate. In contrast, the values for microbially produced ferrous cation and by the combination of the two methods are 25% and 56%, respectively. Therefore, UC tests were conducted for the samples treated with ferrous sulfate (Appendix G). Hydraulic conductivity was measured for sand specimens treated with microbially produced ferrous cation or the combination of ferrous sulfate and microbially produced ferrous cations.

For all of the hydraulic conductivity tests in this section, with different iron sources the sand samples were treated with the same percent of ferrous cation to sand ratio of 1.5 (w/w). Constant-head hydraulic conductivity tests were performed using a flexible-wall triaxial apparatus (Appendix H). Identical soil specimens with a height-to-diameter ratio of 1:1 and a diameter of 50 mm were used. The soil specimens were firstly saturated by applying bottom and cell pressure as explained in the tables in each sub-section. The degree of saturation (B value) for all samples was 95%. Note that two repetitions were carried out for each specimen of each iron-based biocement method.

4.3.1 Ferrous sulfate

Sand sample preparation for iron-based biocement with ferrous sulfate was carried out based on the results in Section 4.2.1. Ten samples (set I) were prepared by placing 100 g of Changi Beach sand inside a PVC split-mould with a rubber membrane coating at the bottom. The height-to-diameter ratio of the specimens was 2:1 with a 38 mm internal diameter. Ferrous sulfate (1 M) was mixed with urea (2 M) according to Reaction 4.1 and various formulations of percent of ferrous sulfate to sand ratio (w/w). The final percent of ferrous sulfate to sand ratio (w/w) is listed in Table 4.3. The process of applying iron-based biogrout was as follows: a) 20 ml of bacterial culture of UPB was manually poured on all treated sample and b) the solution of ferrous sulfate and urea was manually poured on the sand samples two hours after applying bacterial culture in the first batch of injection.

Table 4.3. Testing program for dried samples treated with ferrous sulfates for UC test

Sample	Ferrous sulfate % (w/w)	Urea (M)	Number of treatments	Precipitated cation (% w/w)
I.1	0.2	0.03	1	0.14
I.2	0.3	0.1	1	0.2
I.3	0.5	0.1	1	0.4
I.4	1	0.2	1	0.7
I.5	1.5	0.3	2	1.1
I.6	2	0.4	2	1.4
I.7	2.5	0.5	2	1.8
I.8	3	0.6	3	2.1
I.9	3.5	0.6	3	2.5
I.10	4.5	0.8	3	3
I.11	6	1	3	4
I.12	9	1	3	6

Two or three treatments were set for the samples treated with a ferrous sulfate to sand ratio (w/w) above 1%. Note that the second and third batches of injection were carried out after six and 24 hours, respectively. The bio-treated samples were left to cure at ambient temperature (25°C) for two days after the last injection. Afterward, the samples were placed in an oven at 60°C for one day and classified as oven-dried samples. Note that, based on the results of the

establishment of iron-based biogrout using TSS, almost 70% of the ferrous sulfate precipitated as ferric hydroxide with the use of diluted UPB.

As shown in Table 4.4, two samples (I.13 and I.14) treated with ferrous sulfate to sand ratios of 2.5% and 3.5% (w/w) respectively remained at ambient temperature (25°C) for another day and were classified as wet samples. UC tests were carried out to determine the shear strength of the treated oven-dried and wet sand samples. The UC tests were conducted for the iron-based bio-treated sand columns according to test procedures D5084 and ASTM 2003.

Table 4.4. Testing program for wet samples treated with ferrous sulfates for UC test

Samples (I)	I.13	I.14
Ferrous sulfate (%w/w)	2.5	3.5
Urea (M)	0.5	0.6
Number of treatments	2	3
Precipitated cation (% w/w)	1.8	2.5

Subsequently in the set I of experiments another three samples (I.13, 14 and 15) was prepared for testing of hydraulic conductivity considering the final ferrous sulfate to sand ratio of 0.5, 1 and 1.5 % (w/w) to be comparable with the results of microbially produced ferrous cation and the combination of two sources. The testing program for samples was shown in Table 4.5. The method and procedure of treatment was same as samples treated for UCS but with the different concentration in the solution of urea and ferrous sulfate. Hydraulic conductivity tests were conducted according to the standard hydraulic conductivity testing (Appendix H). The detail of confining cell pressure and top pressure for hydraulic conductivity tests of each sample were reported in Table 4.5.

Table 4.5. Testing program of sand samples treated with ferrous sulfate for hydraulic conductivity tests

Samples (I)	I.15	I.16	I.17
Ferrous sulfate (%w/w)	0.5	1	1.5
Urea (M)	0.3	0.3	0.3
Confining pressure	110	130	120
Top pressure	85	105	95

4.3.2 Microbially produced ferrous cation

The impact of IRB activity on soil improvement is studied in this section. Based on the results of Section 4.2.2, the highest production of ferrous cation through IRB activity and fermentation was 2 g/l. For the experiment, three samples from set II (II.11, II.12 and II.13) with microbially produced ferrous cation and urea as the sources of cation and carbonate respectively were prepared by the formulation shown in Table 4.5. Next, Changi Beach sand was placed in a PVC cylindrical pipe lined with a layer of transparency. The height-to-diameter ratio of the specimens was 1:1 with a 50 mm internal diameter. The process for the application of iron-based biogrout was as follows: a) 20 ml of bacterial cells of UPB was poured on the sand samples (the preparation was described in Section 4.3.1) and b) two hours after applying bacterial cells, 40 ml of the solution of microbially produced ferrous cation and urea was added (the details of the concentrations of the three samples are in Table 4.6). However, as the concentration of microbially produced ferrous cation was low (2 g/l), increasing the number of treatments were considered to achieve the desired ferrous cation to sand ratio (% w/w). The application of iron-based biogrout for each treatment was set daily. The samples were treated and kept at room temperature. They were used for hydraulic conductivity test (Appendix H) five days after the last application of biogrout. The confining cell pressure and top pressure for the hydraulic conductivity tests of each sample are reported in Table 4.6. As the rate of precipitation in samples treated with the combination source of ferrous sulfate and microbially produced ferrous cation was low, samples for UC tests were not prepared.

Table 4.6. Testing program of sand samples treated with microbially produced ferrous cation

Samples	II.11	II.12	II.13
Microbially produced ferrous cation (%w/w)	0.5	1	1.5
Urea (M)	0.3	0.3	0.3
Confining pressure	100	100	110
Top pressure	75	75	85

4.3.3 Combination of ferrous sulfate and microbially produced ferrous cation

To study the effect of the combination of ferrous sulfate and microbially produced ferrous cations on soil properties, three sand samples were prepared based on the results of Section 4.2.3. Set (III) of the experiment in this section consisted of three samples (III.1, III.2 and III.3) with different percentages of ferrous sulfate and microbially produced ferrous cation in the biogROUT. The purpose of this was to examine the differences in the results of the hydraulic conductivity tests. Note that a total ferrous cation to sand ratio of 1.5% (w/w) was set for all samples, but with various proportions of ferrous sulfate and microbially produced ferrous cation. The experiments were carried out at room temperature. The two components of liquid biocement were applied sequentially as follows: (a) 20 ml of diluted bacterial cells was prepared (the procedure is described in Section 4.3.1) and (b) two hours after the injection of bacterial cells, 40 ml of urea solution containing a specific concentration of the combination solution of ferrous sulfate and microbially produced ferrous cation was prepared as shown in Table 4.7. Like the samples prepared with microbially produced ferrous, the samples in these experiments were treated several times to achieve the desired ferrous cation content.

Table 4.7. Testing program of sand samples treated with combination of ferrous sulfate and microbially produced ferrous cation

Samples	III.1	III.2	III.3
Total ferrous cation (% w/w)	1.5	1.5	1.5
Microbially produced ferrous cation (%)	70	50	30
Ferrous sulfate (%)	30	50	70
Urea (M)	0.3	0.3	0.3
No. of treatments	6	9	13
Confining pressure	100	100	110
Top pressure	75	75	85

After treatment, the samples were left for five days until the tests to measure hydraulic conductivity were carried out. As the rate of precipitation in the samples treated with the combination source of ferrous sulfate and microbially produced ferrous cation was low, UC tests were not conducted. The confining cell pressure and top pressure for the hydraulic conductivity tests of each sample are reported in Table 4.7.

4.4 RESULTS AND DISCUSSIONS

4.4.1 Ferrous sulfate

Iron-based biogrout treated samples with urea and ferrous sulfate with a prior application of UPB were prepared according to various ferrous sulfate to sand ratios (% w/w). As shown in Table 4.3, samples I.1 to I.12 were prepared with ferrous sulfate to sand ratios of 0.2% to 9% (w/w). The impact of iron-based biogrout with various ferrous sulfates to sand ratios (% w/w) was examined using UCS. Note that, based on the results of the establishment of iron-based biogrout using TSS, almost 70% of the ferrous sulfate precipitated as ferric hydroxide with the use of diluted UPB. Therefore, the results of the UC tests are presented with the content of precipitated cation in sand (% w/w) for iron-based biocement. As Figure 4.15 shows, samples I.1 and I.2 treated with precipitated cation to sand ratios below 0.35% (w/w) were not enough to result in any improvement. As shown in Figure 4.15, biocement was observed on the surface of the columns. The attachment of bacteria on the surface of the sample and the consequent bond to ferrous cations is the reason for the formation of a crust on the surface of sample I.2.

Figure 4.15. Sample I.2, treated with precipitated cation to sand ratio of 0.2% (w/w)

Figure 4.16 shows the sand columns of samples I.5 to I.12 treated with precipitated cation to sand ratios from 1.1% to 6% (w/w). Figure 4.16 shows the iron-based biocement treated samples before the UC tests. The impact of increasing the percent of precipitated cation to sand ratio can be seen in Figure 4.15 and 4.16.

Figure 4.16. Samples treated with precipitated cation to sand ratio from 1.1 to 6 % (w/w): (a) I.5, (b) I.6, (c) I.7, (d) I.8, (e) I.9, (f) I.10, (g) I.11 and (h) I.12

As shown in Figure 4.16, a homogenous structure was observed in the iron-based biocement treated samples by increasing the content of precipitated cations in samples I.10 to I.12. The color of the sand columns might be attributed to the ferrous sulfate to sand ratio (w/w) in which a higher percent results in a more reddish color. As the top and bottom surfaces of the sample were more exposed to oxygen in the ambient air, they appear to be more reddish brown than the rest of the sample. The samples were oxidized by means of adjusting the air flow into the oven to the maximum. This may have only allowed the ferrous cation on the surfaces of the sample to oxidize, but not the ferrous cation within the sample to completely oxidize as the oxygen concentration was too low in the core.

No UC test was carried out for samples I.1 and I.2. All of the samples were trimmed for the UC test. Specimens with a height of 20 or 30 mm were prepared depending on the availability. However, sample I.3 to I.6 treated with a precipitated cation to sand ratio of below 1.4% (w/w) could stand after trimming but the UCS was low. A UC test was commenced with a value of 10 kPa as the weakest sample. The samples after the UC test are shown in Figure 4.17.

Figure 4.17. Sample I.9 and I.10 after UC test

Figure 4.18. Results of UC test for iron-based bio-treated sample with various precipitated cation to sand ratio (% w/w)

The results of the UC tests for various precipitated cation to sand ratios (w/w) are shown in Figure 4.18. Note that the specimens for the UC tests were trimmed depending on the availability of samples. The specimens show a brittle structure, which is the reason for the percent value of axial strain. UC tests were conducted to record the improvement in the strength of the iron-based bio-treated samples, but did not show the exact value of axial strain. Hydrolyzing urea by UPB and production of carbon dioxide as gas was one of the reasons for the different behaviors of the bio-treated samples in the UC tests. If the gas dissolved in the water inside of the sand samples, the results showed an increase in pH and the production of carbonate ions which by the presence of ferrous cations, can precipitate as a cation salts; if the gas went out of the sample, it could cause pores in the structure of the sample, which was one of the reasons for the different axial strain shown in Figure 4.18. In addition, the different behaviors of bio-treated sand samples can be explained by the fact that UCS is a factor of density. Low cementation through iron-based biocementation can explain the quantity of unfilled pores in samples I.8 to I.10 with a weak structure (Figure 4.17). In comparison, increasing the precipitation of metal salts results in a higher UCS value in samples I.11 and I.12 with a brittle structure. This can be seen exclusively in the bio-treated samples as the precipitation of cation salts is a factor of the distribution, fixation and activity of bacteria. Additionally, the weak or brittle structure of specimens affected the trimming process and UC test as the samples did not trim to a perfect shape and a smooth surface. Therefore, the space between the load ring and the surface of the sample is another potential reason for the value of axial strain. In conclusion, gas production through the biocementation process, low precipitation of cation salts between sand grains and the trimming process are the major reasons for the different behaviors of the sand samples.

The data in Figure 4.18 shows the higher value of UCS for bio-treated samples with a higher precipitated cation to sand ratio (w/w). The highest UCS value was 402 kPa and was obtained from sample I.12, which was treated with a precipitated cation to sand ratio of 6% (w/w). However, as shown in Figure 4.16, samples treated by less precipitated cation to sand ratios (I.4 to I.8) showed abundant precipitation of salts near the surface or injection point. Therefore, higher improvement was observed on the surface and decreased with increasing distance from the injection point. That is one of the reasons for the low values on the UC tests.

To further investigate the impact of various precipitated cation to sand ratios on the strength of bio-treated samples, the results of the UC tests (S) for iron-based bio-treated sand are plotted against the content of precipitated cations (F, % w/w) in sand. This data is presented in Figure 4.19 and the R^2 (coefficient of determination) value indicates how well data points fit a line. As shown in Figure 4.19, the curve for the iron-based biogrout (F, %) was fitted by the following Equation:

$$S = 63.354 F \text{ kPa} \quad (R^2 = 0.93) \quad (4.1)$$

As shown in Figure 4.19, samples I.5 to I.7 were treated with precipitated cation to sand ratios of 1% to 1.75% (w/w) and showed UCS values ranging from 50 kPa to 80 kPa. Increasing the precipitated cation to sand ratios from 2% to 3% (w/w) in samples I.8 to I.10 resulted in a range from 120 kPa to 155 kPa. By increasing the precipitated cation to sand ratios in samples I.9 and I.10, it seems the composition of additive precipitated cations between the sand grains became high enough to improve the soil properties. Samples I.11 and I.12 were treated with precipitated cation to sand ratios of 4.5% and 6% (w/w), the highest in the experiment, and showed UCS values of 360 kPa and 402 kPa, respectively.

Figure 4.19. Effect of precipitated cation in the treated sand on the UCS of dried samples

Figure 4.20 shows a comparison of the USC results for wet samples I.13 and I.14 and dried samples I.7 and I.9 which have similar precipitated cation to sand ratios (% w/w). The results illustrate that the UCS values are higher for dried samples than wet samples.

Figure 4.20. Effect of precipitated cation on the UCS of wet and dried samples

Figure 4.21 also shows a comparison between the results of wet samples in the present study and those by Ivanov et al. (2012). The samples used in the study by Ivanov et al. (2012) were treated with ferric monosodium.

As shown in Figure 4.21, the UCS values of the samples treated with ferrous sulfate are lower than the UCS values for samples treated with ferric monosodium. The higher UCS value of samples treated with ferric monosodium can be explained by the extra positive available ion in each molecule of ferric cation compared to ferrous. This extra ion increased the UCS value of wet samples up to 50%, as shown in Figure 4.16. Moreover, Ivanov et al. (2012) improved iron-based biogROUT by using ferric chelates to ensure the stability of ions at circumneutral pH and reported the low pH of ferric chloride and sulfate solution. However, in their study using EDTA, which is a potent chelator from the aminopolycarboxylates group, the UCS values of the samples were still lower than of the chemical grouts.

Figure 4.21. UC test results for wet bio-treated samples, (✕) is the results of this study and (◆) is the results of Ivanov et al. (2012)

A small portion of biotreated sand was observed under the stereo-microscope to investigate microscopic levels of change in the particle surface roughness. As shown in Figure 4.22, these micro-level investigations indicated that the iron-based biocemented sand appear to be bound together by the brownish precipitate, which presumably is precipitated ferric hydroxide from the hydrolysis of urea by UPB in the presence of ferrous sulfate solution. It can be seen in Figure 4.22 that there are precipitated cations on the surface of the sand grains as well as in the channels between them. As shown in Figure 4.22, the bio-treated sand is in a large clump held closely together; this is unlike clean sand in which each sand grain is separated with less cohesion. Figure 4.22 also shows a close-up of the dried gel on the edge of the sample that holds the sand grains together. Subsequently, piece of sand samples was observed under SEM for further micro-investigations of the pores between the sand grains. Pores between sand grains and filling of pores can be seen.

As Figure 4.23 shows, precipitated cations were observed between the sand grains. It can be seen that the bio-treated sand grains by precipitated cation have a different texture than the clean sand. However, unlike the control sample, the surfaces of the sand grains seem to be unevenly coated by iron salts and this is what makes the texture of the sand grains seem

rougher than the control sample. In addition, a small piece of sand samples was observed under FESEM/EDX (described in Section 3.3. of Chapter 3) for further micro-investigations and chemical analysis of the material filling the pores between the sand grains.

Figure 4.22. Stereo microscopy of sample I.6

Figure 4.23. SEM image of biotreated sand with ferrous sulfate, arrows shows bridges between the sand grains with precipitated cation

Figure 4.24. FESEM/EDX analysis of (a) coating of sand grains and filling of pores with precipitated material and (b) parts of precipitated material and element analysis with the white dots of each part of sample and the arrows of precipitated iron (round) and sulfur (cubic)

Figure 4.24 (a) shows the EDX analysis of the elements on the surface of sand grains. According to Figure 4.24 (a), the Si element (sand) presents on the edge of the sample and these samples seem to have a layer of material coating the surface of the sand grains and filling the pores between them. EDX analysis for precipitated material indicated that iron and sulfur precipitated through iron-based biocement (Figure 4.24b). From the results of EDX analysis, it can be certainly concluded that this material is the dried precipitated metal cation that is responsible for the binding of the sand grains. Zoom in on one of the pores filling with precipitated materials (Figure 4.24b) indicated that the most precipitated salts were ferric hydroxide. However, the precipitation of sulfur between the iron salts was observed.

As shown in Figures 4.23 and 4.24, coating of sand grains as well as filling pores by iron salts can be seen. Increasing in particle surface roughness lead to increase frictional resistance of sand and lead to increases in the angle of internal friction. Decreasing void ratio after biocementation is confirmed in micro level investigations by stereo-microscopy (Figure 4.22) and SEM (Figure 4.23) which lead to increase in angle of friction (ϕ). The observation and microscopic investigations for binding sand grains and filling the pores between sand grains are confirmed by increasing the UCS. It is known that the ferric hydroxide produced by the hydrolysis of ferric salts can form different kinds of particles and colloids (Meyer et al. 2000). The precipitated metal cation in the sand from the iron-based biogrout looked like gel but not like crystals (Figure 4.23). It is likely that this gel-like material has better clogging properties than crystals in MICP.

Therefore, in another experiment, the samples treated with ferrous sulfate (Table 4.4) in the iron-based biogrout were examined using triaxial cells to measure the hydraulic conductivity. As shown in Figure 4.25, the data illustrates the effect of precipitated cation to sand ratios of 0.35%, 0.7% and 1.05 % (w/w) in the bioclogging process on the hydraulic conductivity.

As shown in Figure 4.25, a precipitated cation to sand ratio of 1.05% (w/w) reduced the hydraulic conductivity from 5.2×10^{-4} m/s for the clean sand to 7.1×10^{-7} m/s. Thus, it can be concluded that the iron-based biocementation process not only affects the stiffness and shear strength of the soil, but also reduces the hydraulic conductivity of sand.

Figure 4.25. Results of hydraulic conductivity in iron-based biocement treated samples with ferrous sulfate

Comparing the results of UCS shows that increasing the precipitated metal to sand ratios from 2% to 6% in samples I.8 to I.12 resulted in a range of 120 kPa to 402 kPa. The calculation of stiffness for samples also shows the higher value for sample I.12 than I.8. Therefore, it can be said that biocementation affects the stiffness in iron-based bio-treated samples which is a factor of precipitated metal. However, in another study of iron-based biogROUT with ferric monosodium, Ivanov et al. (2012) reported that a precipitated cation content of 1.15% (w/w) reduced the hydraulic conductivity of sand to 9.0×10^{-7} m/s.

In conclusion for iron-based biocement with ferrous sulfate, samples treated with precipitated cation to sand ratios below 0.3% (w/w) did not show significant improvement in shear strength, which can be explained by the low precipitation of iron salts and high void ratio. Hence, increasing the precipitated cation to sand ratio (w/w) resulted in a significant increase in the UCS value up to 402 kPa for a precipitated cation to sand ratio of 6% (w/w). Observations and UC tests showed that treatments with a higher precipitated cation to sand ratio may lead to deeper penetration of chemicals, which is the reason for homogenous treatment. However, as reported by Ivanov et al. (2012) in the study of iron-based biogROUT

with ferric monosodium, the UCS values for iron-based bio-treated samples with ferrous sulfate were lower than those obtained for sand treated with cement or other chemical grout. The results of hydraulic conductivity testing for samples treated with a precipitated cation to sand ratio of 1.05% (w/w) indicate a reduction in hydraulic conductivity to the value of 7.1×10^{-7} m/s, while the UCS value for the same dried sample was 61 kPa. Thus, based on the results of iron-based biocement with ferrous sulfate, it can be concluded that the process of bioclogging shows better improvement than biocementation.

4.4.2 Microbially produced ferrous cation

Set II of bio-treated samples with microbially produced ferrous cation to sand ratios of 0.5%, 1% and 1.5% (w/w) was prepared. Figure 4.26 (a) shows the dried sample treated with UPB and the solution of dissolved microbially produced ferrous cation with urea.

Figure 4.26. Sample II.13 treated with (a) microbially produced ferrous cation dried sample and (b) wet sample after hydraulic conductivity test

Low precipitation of metal was expected based on the experiments on the establishment of iron-based biogrout with microbially produced ferrous cation and that is the reason soil samples did not show significant strength. As shown in Figure 4.26 (b), the wet samples were too weak to stand. The samples were examined by hydraulic conductivity testing and the results are shown in Figure 4.27. It is noted that the hydraulic conductivity of clean sand was 5.2×10^{-4} m/s using a rigid wall apparatus.

Figure 4.27. Results of hydraulic conductivity in clean and biotreated sand with microbial produced ferrous

Figure 4.27 shows the average hydraulic conductivity (k_v) values measured using a triaxial cell for samples II.11, II.12 and II.13 treated with precipitated cation to sand ratios of 0.15%, 0.25% and 0.34% (w/w) respectively based on the results of Section 4.2.2. The results indicate a reduction in the coefficient of hydraulic conductivity. The results in Figure 4.27 indicate that the precipitation of ferric hydroxide reduced the coefficient of hydraulic conductivity from 5.2×10^{-4} m/s for clean sand to 1.6×10^{-6} m/s for sample II.13.

Image obtained by stereo-microscopy shows more clogging of sand grains in comparison with the clean sand but do not show significant changes between the sand grains, as shown in Figure 4.28. The production of ferrous cation through IRB activity and the possibility of produced ferrous cation in the bioclogging of sand samples were previously mentioned in the study by Ivanov and Chu (2008) which examined IRB in an anaerobic environment. The microbially produced ferrous cation was then mixed with urea and applied after the bacterial cells of UPB for iron-based bioclogging and biocementation. However, as the concentration of microbially produced ferrous cation was 2 g/l, the bioclogging process was dependent on the higher number of injection, which is the reason for the low precipitation of iron salts. A high number of injection are one of the limitation factors for the application of microbially produced ferrous.

Figure 4.28. Bioclogging in sample II.13 treated with microbially produced ferrous cation (57x)

4.4.3 Combination of ferrous sulfate and microbially produced ferrous cation

The results of the iron-based biocements with the same content of ferrous sulfate or microbially produced ferrous cation to sand ratios (0.5%, 1% and 1.5 % w/w) were presented in the previous two Sections (4.4.1 and 4.4.2). The results of the samples treated with ferrous sulfate to sand ratios of 1.5% (w/w) indicate a precipitated cation to sand ratio of 1.05% (w/w), a UCS value of 61 kPa and a reduction in the hydraulic conductivity from 5.2×10^{-4} m/s for clean sand to 7.1×10^{-7} m/s. However, the results of the samples treated with a microbially produced ferrous cation to sand ratio of 1.5% (w/w) indicate precipitated cation to sand ratio of 0.375% (w/w) and, as a consequence, reduction of hydraulic conductivity to 1.6×10^{-6} m/s.

In another study, Ivanov et al. (2012) reported the problem of low pH in the ferric chloride or sulfate solution for iron-based biocement. The authors suggested improving the iron-based biogrout by using chelates for ferric cations or using microbially produced ferrous cation to ensure the stability of ferric chelates at a circumneutral pH. Based on the results, a third method for iron-based biocement consisting of a combination of ferrous sulfate and microbially produced ferrous cation was established. Increasing the pH of ferrous cation and urea solution in the third method indicates the stability of the ferrous cations in the solution

and can accomplish the requiring conditions for hydrolyzing urea. Thus, the third method of iron-based biocemet using microbially produced ferrous cation and ferrous sulfate was introduced. The results of the new iron-based biogrout which consisted of the application of combined microbial ferrous cation and ferrous sulfate mixed with urea for the precipitation of ferric hydroxide due to the enzymatic hydrolysis of urea by UPB were presented in this section. Note that the final ferrous cation concentration of the bio-treated sample was measured as 2 g/l.

The results of iron-based bioclogging with three formulations of the content of microbially produced ferrous cation and ferrous sulfates with the same final ferrous cations to sand ratio of 1.5% (w/w) (see Table 4.7) were presented. Note that the rate of precipitated cation in these three samples was different depending on the ratio of microbially produced ferrous cation and ferrous sulfate (Table 4.7). Figure 4.29 shows sample III.2 before and after hydraulic conductivity testing. Note that the hydraulic conductivity of clean sand was 5.2×10^{-4} m/s using a rigid wall apparatus. The results of hydraulic conductivity testing for samples III.1, III.2 and III.3 with precipitated cation to sand ratios of 0.6%, 0.75% and 0.95 % (w/w) are shown in Figure 4.30.

Figure 4.29. Samples III.2 treated with the combination of half ferrous sulfate and half microbially produced ferrous cation when (a) dried sample and (b) wet sample after hydraulic conductivity tests

Figure 4.30. Results of hydraulic conductivity in bio-treated samples with combination of ferrous sulfate and microbially produced ferrous cation

As shown in Figure 4.30, bioclogging of the sample treated with a combination of half ferrous sulfate and half microbially produced ferrous cation (III.2) and a precipitated cation to sand ratio of 0.72% (w/w) indicates a reduction in the hydraulic conductivity to 7.7×10^{-7} m/s. The results of hydraulic conductivity testing of the sample treated with a 70% of ferrous sulfate and 30% of microbial ferrous cation (III.3) shows a reduction in the hydraulic conductivity to 6.0×10^{-7} m/s. Therefore, the results of hydraulic conductivity testing indicate bioclogging in all three samples treated with a combination of ferrous sulfate and microbially produced ferrous cation

The results of hydraulic conductivity testing for the samples treated with the third method of iron-based biogrout illustrate that microbially produced ferrous cation can combine with ferrous sulfate and become involved in the process of iron-based bioclogging. The microbially produced ferrous cation shows successful application after combining with ferrous sulfate to increase the pH of the solution (Figure 4.12) and, as a consequence, cause a significant reduction in the hydraulic conductivity through the precipitation of iron salts.

The application of microbial ferrous cation for bioclogging has the process of cultivation of IRB and is time-consuming with specific conditions for ferrous cation production. On the other hand, the high cost of using ferrous cation through chemicals such as ferrous sulfate or ferric monosodium is mentioned before (Ivanov et al. 2012). From the results of the present study, it can be concluded that part of the biogrout solution could be replaced by using a chemical source of ferric cation and the other part could be replaced by microbial ferrous. Note that the hydraulic conductivity of an iron-based precipitated cation to sand ratio of 1.05% (w/w) with ferrous sulfate was 7.1×10^{-7} m/s, while using 30% microbially produced ferrous cation and 70% ferrous sulfate reduced the hydraulic conductivity to 6.0×10^{-7} m/s.

As an alternative to bioclogging with ferrous sulfate (in the present study) or ferric monosodium, the third method using the combination of ferrous sulfate and microbial activity of IRB to produce ferrous cation was introduced as an economical and green method using a cheap and accessible source of iron ore. The pH of the solution for the new method of iron-based biogrout was in the range of UPB activity for hydrolyzing urea (pH=9). This can be explained by the existence of microbially production of dissolved ferrous. The results of hydraulic conductivity tests for the third method of iron-based biogrout for a sample treated with a precipitated cation to sand ratio of 0.9 % (w/w) showed a reduction in hydraulic conductivity up to 6.0×10^{-7} m/s. In comparison, Ivanov et al. (2012) reported a hydraulic conductivity of 9.0×10^{-7} m/s for a sample treated with a precipitated cation to sand ratio of 1.15% (w/w) using ferric monosodium. Therefore, based on the results of this study and the study by Ivanov et al. (2012), the application of chelates for stabilizing ferrous cations using either chemical ferric chelators or the microbial ferrous cation is recommended.

4.5 CONCLUSIONS

The conclusions of the study presented in this chapter are as follows:

- 1) The data obtained from the iron-based biocement solution with ferrous sulfate indicates 70% of precipitated cations.
- 2) The IRB activity in the fermentation process indicates the possibility of using microbially produced ferrous cation for production of 25% of precipitated cations. Low concentration of microbially produced ferrous cations because of the specific fermentation process in production of microbial ferrous cation can explain the lowest rate of precipitated cation compared to ferrous sulfate.
- 3) The data obtained from the establishment of an iron-based biocement solution shows the possibility of the combination of ferrous sulfate and microbially produced ferrous. The samples showed 56% of cations precipitated.
- 4) Samples treated with iron-based biocement with a precipitated cation to sand ratio below 1% (w/w) did not show significant improvements in shear strength. When the precipitated cation to sand ratio increased to 6% (w/w), the UC strength increased to 402 kPa.
- 5) The UC strength for the wet biocement treated sample with the precipitated cation to sand ratio of 2.5% (w/w) was 80 kPa. The UC strength for the biocement treated sample at dry condition was at 125 kPa.
- 6) Precipitation of iron salts was seen between sand grains in the SEM images and confirmed by the element analysis using the EDX.
- 7) Sand samples treated with different sources of ferrous cation using different types of iron-based biocement showed different values of reduction in the hydraulic conductivity. The hydraulic conductivity of sample treated with ferrous sulfate and a precipitated cation to sand ratio of 1.05% (w/w) was 7.1×10^{-7} m/s.
- 8) Samples treated with microbially produced iron in iron-based biocement with precipitated cation to sand ratio of 0.375% (w/w) did not show much UC strength. Nevertheless, there was still a reduction in hydraulic conductivity to a value of 1.6×10^{-6} m/s.
- 9) The process of bioclogging in sand treated with the third type of iron-based biocement at a cation to sand ratio of 0.9% (w/w) reduced the hydraulic conductivity of sand from 10^{-4} m/s to 6.0×10^{-7} m/s.

- 10) Iron-based biocement is not recommended for field applications because of its low UCS. However, the results of this study demonstrated that 1% of cation salts in sand was sufficient to reduce the hydraulic conductivity to 10^{-7} m/s as the bioclogging was performed mainly in the channels between sand particles, as shown by previous studies (Ivanov et al., 2012; DeJong et al., 2010). For example, samples treated with iron-based biogrout through microbial ferrous cations did not show any strength improvement; however, when they were involved in the process of bioclogging, they showed a significant reduction in hydraulic conductivity through the precipitation of iron salts. Therefore, it is necessary to mention that the uncertain behavior and low strength of bio-treated samples with low cation salts could affect the engineering properties of structures constructed/treated with iron-based biocement and this should be considered as a critical issue during design.

CHAPTER 5 APPLICATION OF CALCIUM-BASED BIOCEMENT FOR EROSION CONTROL OF DIKES

5.1 INTRODUCTION

Water-induced surface erosion is considered one of the most common causes for the failure of river or sea dikes. Rainfall, wave action, turbulent water currents and overtopping are some of the natural causes of erosion. Based on recent flood events resulting in dike failures, including the River Elbe flood (2002), the New Orleans flood (2005) and the Mississippi flood (2008), overtopping is one of the major factors causing important morphological changes (Briaud et al., 2008). The overtopping caused erosion and, consequently, led to the failure of dikes. In dike erosion, erodibility can be defined as the relationship between the velocities of the water flowing over the soil. Therefore, the amount of erosion is dependent on the erodibility of the dike material and the depth and duration of the overtopping flow (Tinney and Hsu, 1961; Chinnarasri, 2000). As overtopping represents a risk to both people and riverine infrastructure, erosion control of dikes is an important aspect of assessing structure stability and safety. It is a challenge to geotechnical engineers to develop innovative methods to enhance the ability of dikes against overtopping in ways that can be constructed speedily and conveniently.

Studies have been performed using various methods to protect dikes against erosion by introducing the cementation between individual soil particles (Knudsen 1984, Chen and Anderson 1986, Clopper and Chen 1987, Ralston 1987, Pilarczyk 1988, Ragusa et al. 1994, USACE 1996, Stavropoulou et al. 1998, Hanson et al. 1999, Plate 2002, Ahmed and Hussain 2008). As shown in Table 5.1, Chu et al. (2009) have summarized the types of river or coastal protection structures according to the materials used. The disadvantages of these methods include a high time consumption, high maintenance costs, heavy or special construction equipment requirements harm to the environment and, in some cases, limitations in scale of dikes show the space for developing new technology. Moreover, considering that two or more methods are often used in the protection of coastal infrastructure and their long length, a small improvement in the design could result in significant savings.

Table 5.1. Classification of dikes and coastal structures according to materials used (after Chu et al. 2009)

Type	Construction method
Earth-fill dike or levee	1). Using compacted soils 2). Using cement mix soils or bagged soil 3). Using dumped rocks
Masonry and concrete	1). Using cast-in-place or precast concrete walls 2). Using precast concrete panels 3). Using roller compacted concrete
Steel sheet piles or bored piles	1). Driven steel sheetpile wall 2). Contiguous bored pile or prefabricated sprung piles
Geotextile or geosynthetic materials	1). Geo-tube filled with concrete mortar, sand or clay 2). Rubber dam 3). Geo-bag or geo-container 4). Geo-mattress
Prefabricated concrete segment	1). Concrete caissons 2). Semi-circular concrete caissons 3). Steel or concrete suction piles or caissons 4). Tongtu assembly method
Mix types	Dike construction involving the use two or more of the above methods

Beside from techniques summarized in Table 5.1, biocementation may be a potential method for erosion control of dikes. Therefore, in this chapter, a study that used biocementation for erosion control of dikes is presented. The mechanisms of calcium-based biocement for increasing the rate of calcium carbonate precipitation were presented in Chapter 3. It was established that washing the bacterial cells could remove the protease in the solution of bacterial cells but the urease activity was not reduced. As a consequence, by addition of urea and calcium chloride solution to the bacterial cell, the rate of calcium carbonate precipitation was not reduced. Based on this, the *S. pasteurii* bacterial cells after washing with a solution of 1% NaCl were used with the urea and calcium chloride solution for the treatment of the sand used in the dike. The application of microbially-induced calcium carbonate precipitation (MICP) for erosion control of river or sea dikes was studied using model tests carried out in a

hydraulic flume. The models were treated using the MICP process with different targeted percentages of calcium carbonate to sand ratios (w/w) of 2.5 to 7.5%. Two different treatment methods, namely the percolation method and the spray method, were used.

The objectives of the study were as follows:

- 1) To establish a method for using the MICP approach to enhance the ability of a dike against erosion.
- 2) To assess the change in the properties of the soil in the dike after the MICP treatment.

5.2 MATERIALS

Seven dike models were used in the experimental study. The models were made using Ottawa sand in a transparent plastic box. As shown in Figure 5.1, the dimensions of the models were 530 mm long, 300 mm wide and 150 mm high. Dry sand was manually deposited in a loose state, in a single layer, in a manner with a homogenous distribution for the whole dike. A bulk density around 1600 kg/m^3 was achieved. A specific volume and mass of sand was deposited in a plastic container with a specific length, height and width to provide homogenous deposition. The void ratios of the models before treatment were in the range of 0.56 to 0.58. A brief description of the typical engineering properties of Ottawa sand is shown in Table 5.2. This information was obtained from the MSDS (material safety data sheet) from the U.S. Silica Company. The medium particle size of the sand was around $400 \mu\text{m}$, as shown in Figure 5.2.

Table 5.2. Properties of Ottawa sand

Description	Medium size (mm)	Chemical Content	Typical Specific Gravity	Bulk density at loose state
Ottawa sand	0.40	99.5+% SiO_2	2.65	1600 kg/m^3

After the models were prepared, the body or surface was treated with UPB cells and a solution of urea and calcium chloride. The procedure for cultivating UPB was described in Section 3.2.1 of Chapter 3.

Figure 5.1. (a) Schematic of test models with the size characteristics (b) and the transparency wall and base

Figure 5.2. Particle size distribution curve

Next, 0.6 L of cultivated UPB suspension was centrifuged at 1000 g at 4°C for 20 minutes to separate the bacterial cells from the culture liquid. The bacteria solution was re-suspended in 1% NaCl solution before it was supplied to the test models. A solution of 0.75 M CaCl_2 plus 1.5 M urea was supplied to all treated samples to provide a source of calcium cations and carbonate. Based on the biological and chemical Reactions (see Chapter 2, Reactions 2.4 and 2.5) of MICP, the targeted calcium carbonate to sand ratio (% w/w) was calculated from the concentration of calcium chloride and number of treatments. The targeted calcium carbonate to sand ratios of 2.5%, 5% and 7.5% (w/w) were adopted. Two different methods of application were used, namely percolation and spray. The major difference between the two methods is the recirculation of biocement solution in the percolation method.

In the percolation method, the models were treated using a tubing system and re-circulating the biogrout. The models were treated using Masterflex® 9640-16 L/S peroxide-cured silicone tubing with an inner diameter of 3.1 mm. The tubing system consists of three separate tubes (one for bacterial suspension, one for urea/Ca solution and one for re-circulation of urea/Ca) for each model test. Two Masterflex® easy load peristaltic pumps (L/s@0.0006 to 3400 ml/min) were used in the experiments to deliver and re-circulate the bio-solution. The tubes were located on the crest and middle part of the dike and the bio-solution dripped on the models using the Masterflex® pump (1). Two holes were constructed on the bottom corner of a transparent plastic box to control the water table inside of the box with the help of valves. After full saturation of the model dike, extra bio-solution exceeding 1 cm was collected in the dish below the box. In addition, a Masterflex® pump (2) was provided to re-circulate the urea/Ca solution from the collecting dish to the top of model at a rate of 17 ml/min. Through designing this method it was considered that the percolation of bio-solution covered half part of the model dike while the re-circulating tube covered the other half (as shown in Figure 5.3). However, in the next treatment the location of tubes changed. Therefore, both sides get treatment.

There were five sequential treatments for the biocementation of sand using the percolation method in test models #1, #3 and #5, depending on the targeted calcium carbonate to sand ratio (2.5%, 5% or 7.5% w/w). Every treatment included the following operations: 1) bacterial cells of UPB was injected on the surface of the test model ; 2) effluent was re-circulated at a rate of 17 ml/min for all treatments using a Masterflex® easy load peristaltic

pump (L/s[®] 0.0006 to 3400 ml/min) with Masterflex® 9640-12 silicone tubing for 24 hours; 3) liquid was drained off from the box; 4) calcium chloride and urea solution was injected into the model and re-circulated for three days at a rate of 17 ml/min for all treatments using the same pump and a new set of tubes; and 5) liquid was drained off from the box. Figure 5.3 shows the preparation of model tests for the percolation group, while Figure 5.4 shows the models treated by the percolation method. Note that the supply rates and times of the treatments were adjusted so that no liquid was above the sand surface.

Figure 5.3. An image and sketch of treatment of test models #1, #3 and #5 treated with the percolation method

In the spray method, the bacterial cell suspension was mixed with 11 g/L (0.1 M) of CaCl_2 before spraying. The following two sequential treatments were applied to tests #2, #4 and #6 depending on the targeted calcium carbonate to sand ratio (% w/w). Every treatment included the following operations: 1) culture liquid with added CaCl_2 solution was sprayed on the surface of the test models and two hours were allowed to pass; and 2) calcium chloride and

urea solution was sprayed on the surface of the test models. The test models were manually sprayed using a 500 ml handheld plastic spray bottle fitted at approximately 500 mm above the sample. All applications were performed and maintained at room temperature. Each spray supplied a dosage of about 0.09 kg/m^2 of dry biomass.

Figure 5.4. Images of test models prepared using the percolation method (a) test model #1, (b) test model #3, and (c) test model #5.

The sprays of the solutions containing 2, 1.5 and 1 L of calcium chloride and urea on the surface of the model of the dike provided dosages of approximately 12.9, 9.7 and 6.5 L/m^2 of calcium, respectively. The average supply rate for the bacterial suspension and chemical solution was 0.7 ml/cm^2 per hour (or 7 L/m^2 per hour). Figure 5.5 shows the test models treated with the spray method.

Figure 5.5. Images of test models prepared using the spray method (a) test model #2, (b) test model #4, and (c) test model #6.

The quantity of solution varied during the treatments (percolation or spray) depending on the targeted content of calcium carbonate as follows:

- 1) Tests #1 and #2: 1.75 L of culture liquid and 2 L of calcium chloride and urea were used for the first treatment; and 1 L of culture liquid and 1.25 L of calcium chloride and urea were used for the second treatment.
- 2) Tests #3 and #4: 1.75 L of culture liquid and 2 L of calcium chloride and urea solution were used for the first and second treatments; 1.25 L of culture liquid and 1.5 L of calcium chloride and urea solution were used for the third treatment; and 0.75 L of culture liquid and 1 L of calcium chloride and urea solution were used for the fourth treatment.
- 3) Tests #5 and #6: 1.75 L of culture liquid and 2 L of calcium chloride and urea solution were used for the first to third treatments; 1.25 L of culture liquid and 1.5 L of calcium chloride and urea solution were used for the fourth and fifth treatments; and 0.5 L of culture liquid and 0.75 L of calcium chloride and urea solution were used for the sixth treatment. The details of the treatments are summarized in Table 5.3.
- 4) Test #7 was used as a control and thus was not treated.

Table 5.3. Testing program for preparation of model tests

Test models		#1	#2	#3	#4	#5	#6	#7
No. of treatments		2	2	4	4	6	6	0
1 st treatment	Cells (L)	1.75	1.75	1.75	1.75	1.75	1.75	-
	Urea/Ca (L)	2	2	2	2	2	2	-
2 nd treatment	Cells (L)	1	1	1.75	1.75	1.75	1.75	-
	Urea/Ca(L)	1.25	1.25	2	2	2	2	-
3 rd treatment	Cells (L)	-	-	1.25	1.25	1.75	1.75	-
	Urea/Ca (L)	-	-	1.5	1.5	2	2	-
4 th treatment	Cells (L)	-	-	0.75	0.75	1.25	1.25	-
	Urea/Ca (L)	-	-	1	1	1.5	1.5	-
5 th treatment	Cells (L)	-	-	-	-	1.25	1.25	-
	Urea/Ca (L)	-	-	-	-	1.5	1.5	-
6 th treatment	Cells (L)	-	-	-	-	0.5	0.5	-
	Urea/Ca (L)	-	-	-	-	0.75	0.75	-
Method of treatment		P	S	P	S	P	S	-
Target of calcium carbonate content (%)		2.5	2.5	5	5	7.5	7.5	

S = spray and P = percolation

As shown in Chapter 3, the rate of urease activity is not constant during treatments. Therefore, the activation of urease and, consequently, the calcium carbonate precipitation, need to be checked during the bio-treatments. The pH of the effluent after applying the calcium chloride and urea solution shows the activity of bacterial cells for hydrolyzing urea. Therefore, the rate of hydrolyzing of urea during recirculation of calcium chloride and urea solution in three days was measured. 20 ml of effluent was taken and the pH was measured 2, 24, 48 and 72 hours after drip of biogROUT.

Figure 5.6. pH of effluent after applying calcium chloride and urea solution in third treatment

Figure 5.6 shows the results of pH at different time intervals after the application of calcium-urea solution for test #5. It is apparent from the data that there is an increment in pH from the time of application to two hours later, but then the pH decreases with time. It is known that the higher the pH of the biogROUT the higher the hydrolyzation of urea and, as a consequence, the higher the precipitation of calcium carbonate. Hence, the rise in pH after applying the calcium chloride and urea solution was unavoidable because of the bacterial reaction. The pH then decreased with time due to the inactivation of UPB because of a lack of energy. In this study, the decrease in pH was used as a sign indicating the time required for a new batch of treatments.

After the required cycles of treatments were performed, the liquid was drained off from the test models and they were left to dry outdoors for five days before the flume tests. For tests

#3 and #5, the highest volume of solution was used for the first and second treatments to provide the highest level of bio-reagents for biocementation of the surfaces and walls of the test models (0-50 mm). A lower volume of solution was used for the third to sixth treatments. Tests #4 and #6 were treated with the same concentration as tests #3 and #5 respectively so the method of treatment (percolation or spray) could be compared.

5.3 TESTING METHODS

Tests were carried out to evaluate the ability of the models against erosion. The tests were conducted using a flume in the Hydraulic Laboratory, as shown in Figure 5.7 (a). The flume had a height of 2 m, a width of 0.3 m and a length of 10 m. A high-speed camera (Olympus) was used to record the process of erosion for both the untreated sample and test model #1. A different camera (Microsoft) was used for the rest of the tests, as shown in Figure 5.7 (b). During the tests, water flow was created from left to right (Figure 5.7 c) with different flow rates. The flow rate started at 2 L/s and was subsequently increased to 4, 6, 8, 12, 16, 18 and 22 L/s, as shown in Figure 5.7 (d). The water depth started at 150 mm, which is the same as the height of the dike.

After the flume tests, the engineering properties of the soil in the models were examined using an unconfined compressive (UC) test, four-point bending test and hydraulic conductivity test. As shown in Figure 5.8 (a), specimens were taken from three different locations in the test models treated using the percolation method, namely the crest (A), middle (B) and base (C). Figure 5.8 (b) also shows specimens which were taken from the crust of bio-treated model tests using the spray method. Three duplications for the UC test and calcium carbonate content and four duplications for the four-bending test were conducted to represent the vertical soil properties and calcium carbonate content of 0-50, 50-100 and 100-150 mm, respectively. For this study on the effect of calcium carbonate content on bioclogging and biocementation using two methods of applying the biocement solution, sand samples were collected from various spaces in the different length of three locations, A (20, 35, 50 mm), B (60, 85, 100 mm) and C (120, 135 and 150 mm). The calcium carbonate content in the treated sand was then determined using ICP-OES (as described in Section 3.3.1 of Chapter 3). Three duplications for experiments were repeated to check the repeatability. The mean values and standard deviations of the measurements were also determined.

Figure 5.7. Experimental setup: (a) hydraulic flume; (b) video camera; (c). application of water flow; and (d) side view of the test model in the flume.

Figure 5.8. Schematic of UCS and hydraulic conductivity specimen's preparation from different locations of tests treated by (a) the percolation and (b) the spray method

5.4 RESULTS AND DISCUSSION

5.4.1 Test #7, untreated test model

Test model #7 was untreated and tested using a flow rate of 2 L/s in the flume test, as shown in Figure 5.9. The water depth was 150 mm, which is the same as the height of the model. The water flow caused overtopping and had an erosion effect on the dike. Figure 5.9 shows a series of photos taken at different times. It can be seen that the dike eroded within 15 seconds.

Figure 5.9. Images of untreated sand dike under flow at different times: (a) before flow started; (b) when water reached the peak of the dike; (c) when erosion started; and (d) after erosion

5.4.2 Test #1

Test model #1 was treated with a targeted calcium carbonate to sand ratio of 2.5% (w/w) by the percolation method. A flow rate of 2 L/s was used for the flume test. The water depth was 150 mm and overtopping took place as shown in Figure 5.10 (b). Figure 5.10 shows a series of photos taken at different times. It can be seen that the dike started to erode from the toe within four minutes. The results of test model #1 show that the water level rose (Figure 5.10 a and b) and that erosion took place at the toe in four minutes (Figure 5.10 c and d). Test model #1 was not strong enough against erosion and the dike collapsed within five minutes. However, the extent of erosion was much smaller than in test model #7 for the untreated test model. Thus, treatment with a target ratio of 2.5% (w/w) calcium carbonate to sand by the percolation method resulted in some improvement. However, it is still not adequate for dike stabilization.

Figure 5.10. Images of test model #1 treated by percolation method, (a) while the flow rate was 2 L/s: water flow rise in test model ($t=35s$), (b) peak point of water level ($t=200s$), (c) collapsing point ($t=240s$), (d) after sample collapsed ($t=250s$), (e) failure ($T=283s$) and (f) analyzing the results

5.4.3 Test #2

Test model #2 was treated with a targeted calcium carbonate to sand ratio of 2.5% (w/w) by the spray method. Flow rates of 2, 4, 6 and 10 L/s were used for the flume test. The test model was tested in flume for three days under each flow rate except 10 L/s for 6 hours as shown in Figure 5.11. After that the test model failed. The water was kept to be 15 mm above the top of the dike as minimum and increased up to 70 mm through different flow rates. During the test, water seeped through a crack on the upstream slope into the body of the dike. The seeped water caused erosion inside the body of the dike ten minutes after the test, as shown in Figure 5.11 (b). Figure 5.11 shows a series of photos taken at different times.

Figure 5.11. Images of test #2 treated by the spray method while the flow rate was changed from 2 to 10 L/s: (a) starting point 2 L/s, (b, c, d, e, f and g) 2 L/s, (h) 4 L/s, (i) 6 L/s and (j) 10 L/s.

The results of test model #2 illustrate the effectiveness of the biocement treatment for the crust via the spray method. The dike started to erode inside at 10 minutes and 16 seconds, as shown in Figure 5.11 (b). The dike showed more erosion within first twenty four hours under the same flow rate (2 L/s). After three days the flow rate was increased to 4 L/s and the impact of erosion increased, as shown in Figure 5.11 (g). After three days the flow rate was increased to 6 L/s and the erosion hole became bigger, as shown in Figure 5.11 (h). Subsequently, six hours after increasing the flow rate to 10 L/s, spreading erosion inside the dike under the protective layer led to a decrease in the resistance of the model and it collapsed in the form of a clump. Although laboratory experiments showed water erosion is caused by the infiltration of water due to the hollow shape, the biocement treatment can improve the strength of the dike and prevent it from collapsing. As shown in Figure 5.12, the failure could be explained by the decrease in the friction resistance of the base of the dike due to the reduction in the contact area at the base. However, a closer picture of this sample, as shown in Figure 5.12, confirms that the formation of a protective layer on the surface was effective in protecting the dike from erosion.

Figure 5.12. Formation of a protective layer in test #2 and the positions of samples for lab tests

After the flume tests, the soil was air dried and used for UC and hydraulic conductivity tests. As the bio-treated specimens were mainly in the crust for models treated using the spray method and the crust was too thin (10 mm) for a UC test, a four-point bending test was used to measure the maximum bending strength of the specimens. Ideally, four-point bending tests should be conducted following the ASTM standard D6272-10 with the use of two support spans (as shown in Figure 5.13) in which the support-span-to-depth ratio should be 16:1 and the maximum width of the specimen should be one quarter of the support span. In this case, the support spans were 80 mm and 60 mm. The specimens were cut and polished using sandpaper into roughly the following dimensions, depending on the availability of soil samples: a) 5 mm thick, 100 mm long and 20 mm wide (support span of 80 mm); or b) 4 mm thick, 80 mm long and 15 mm wide (support span of 60 mm).

Figure 5.13. Experiment setup for four-point bending test

For this experiment, the loading ring of the UC test was employed. The loading rate was calculated (ASTM D6272-10 (ASTM, 2010)) based on the ratio of support spans for a load span of one third or one half of the support span as presented in Equations 5.1 or 5.2, respectively:

$$R = 0.185ZL^2 \quad (5.1)$$

$$R = 0.167ZL^2/d \quad (5.2)$$

Where R is the rate of cross head motion (mm/min), L is the support span (mm), d is the depth of the beam (mm) and Z is the rate of straining of the outer fibers (mm/mm min). Note that Z shall be equal to 0.01.

The loading span was one third or one half of the length of the supported span. The round loading was 6 mm in diameter (5 to 8 mm as specified in ASTM). The experimental setup and schematic photo are shown in Figure 5.13. Based on the results of the four-bending tests, the maximum bending strength can be calculated as the maximum fiber stress (S) based on Equations 5.3 or 5.4 for a load span of one third or one half of the support span, respectively:

$$S = PL/bd^2 \quad (5.3)$$

$$S = 3PL/4bd^2 \quad (5.4)$$

Where S is the stress in the outer fiber throughout the load span (KPa), P is the load at a given point on the load-deflection curve (N), L is the support span (mm), b is the width of beam (mm) and d is the depth of beam (mm).

The results of four-pint bending test are shown in Table 5.4. It can be seen that the maximum bending strengths are in the range of 223-387 kPa and the average is 312 kPa. As clean sand has no bending strength at all, the average bending strength of 312 kPa is a good indication of the biocementation effect.

Table 5.4. Maximum bending strengths for the specimens of the crust of test model #2

Specimen	A1	A2	A3	A4	Average.
Maximum bending strength (kPa)	387	348	289	223	312

The hydraulic conductivity of the specimen from the bio-treated crust (location A1) of test model #2 with a 50 mm diameter was measured with the use of the triaxial cell (Appendix H). As the bio-treated models were mostly hard and brittle in location A and weak in locations B and C, it was difficult to trim the samples into perfect cylinders without cracks as needed for the triaxial cell. A special procedure was adopted to solve this problem. The samples were wrapped using impermeable modeling clay, as shown in Figure 5.14. More details are given in Appendix I.

Figure 5.14. Experiment setup for hydraulic conductivity test

Figure 5.14 shows a sample for the hydraulic conductivity test. A confining pressure of 200 kPa was used. Two GDS pressure volume controllers capable of applying pressure and measuring volume at the same time were used to apply pressures of 175 kPa at the bottom of the specimen and 170 kPa on the top. The hydraulic conductivity of the bio-treated samples from the crust in test model #2 treated by the spray method was, on average, reduced from an initial value of 4.0×10^{-4} to 3.2×10^{-7} m/s.

5.4.4 Test #3

Test model #3 was treated with a targeted calcium carbonate to sand ratio of 5% (w/w) by the percolation method. The flow rate started at 2 L/s (Figure 5.15 a and b) and was increased to 4 (Figure 5.15 c), 8 (Figure 5.15 d, e and f) and 16 L/s (Figure 5.15 g, h and i). The test was performed for four days for each flow rate, resulting in a total of twenty days. The water was kept at a minimum height of 15 mm above the top of the dike and gradually increased to 90 mm as the flow rate increased.

A series of photos was taken at different times and selected photos are shown in Figure 5.15. Overtopping caused erosion on the left corner of test model #3 at 9 minutes and 28 seconds, as shown in Figure 5.15 (b). There was a failure, as shown in Figure 5.15 (b), after the water reached the peak of the test model when the flow rate was set to 2 L/s. This localized failure did not expand until the end of the test, as shown in Figure 5.15 (j). As shown in Figure 5.15 (c), the flow rate increased to 4 L/s after four days.

Figure 5.15 Images of test #3 treated by the percolation method while (a) the flow rate was 2 L/s at starting point, (b) 2 L/s, (c) 4 L/s, (d) 8 L/s, (e) 16 L/s and (f) crack in surface of sample after experiment at day 16.

After the flume experiment, the test model #3 was air dried. As shown in Figure 5.16 (b), the test model was cut vertically and then horizontally into two pieces. Specimens were then taken from different locations in A, B and C, as shown in Figure 5.8 (a), for soil testing. UC tests were carried out to assess the shear strength of the bio-treated samples. The UC tests were conducted using a compression testing machine (Appendix G). As shown in Figure 5.16 (a) and (b), body cementation was observed in the test model #3. The depth of cementation can be explained by the treatment method on the homogenous distribution of bio-reagents.

There was no formation of a permeable crust on the top surface of the samples such as seen in model dike #2.

Figure 5.16. (a) Air dried, (b) preparation of specimens for UC tests and hydraulic conductivity tests of test model #3

As shown in Figure 5.17 (a and b), columns were prepared from dried test model #3 for UC testing. The specimens measured 15 mm in diameter and 30 mm in height, depending on the availability. A loading rate of 0.4 mm/min (or 2% strain) was used for the tests. Three UC tests were conducted for each location (A, B and C) to represent the whole strength of the specific depth, as shown in Figure 5.16.

As shown in Figure 5.17 and Table 5.5, the UCS results for test model #3 treated by the percolation method range from 185-810 kPa. The average UCS for location A (0-50 mm) of the test model was 722 kPa. The average UCS for locations B (50-100 mm) and C (100-150 mm) were 368 and 225 kPa, respectively. The results of the UC tests for different locations explain the resistance and performance of test model #3 in the flume when 90 mm of water was above it.

Figure 5.17. (a) Column preparation before and (b) after UC test and the results of UC tests for all specimens of test model #3

Table 5.5. Results of UC test for the specimens of test model #3

Specimens	UCS (kPa)			
	1	2	3	Average
A	610	745	810	722
B	510	338	257	368
C	278	211	185	255

As shown in Figure 5.17 and Table 5.5, there was a large variation in the UCS values for samples taken from the same zone in location B. Based on the observations, this could, to a degree, be attributed to the non-uniformity in the treatment of the models through the surface percolation of bio-reagents by the percolation method which resulted in breaking at the weakest part of the bar for some specimens or, alternatively, to the disturbance to the samples during sample preparation.

Subsequently, the hydraulic conductivity of specimens with a diameter of 50 mm from locations A, B and C of test model #3 was measured using the triaxial cell (Appendix H). The preparation process is explained in Appendix I. The details of the hydraulic conductivity test are shown in Table 5.6.

Table 5.6. Properties and the results of hydraulic conductivity test for the specimens of test model #3

Specimen	A4	B1	C1
Confining pressure (kPa)	210	180	210
Bottom pressure (kPa)	180	150	180
Top pressure (kPa)	185	155	185
Coefficient of hydraulic conductivity (m/s)	8.2×10^{-7}	4.1×10^{-6}	7.1×10^{-6}

As shown in Table 5.6, the hydraulic conductivity of the bio-treated sand taken from the crust of test model #3 was reduced from an initial value of 4.0×10^{-4} to 8.2×10^{-7} m/s. The hydraulic conductivity of the specimens from locations B and C was reduced from the initial values to 4.1×10^{-6} m/s and 7.1×10^{-6} m/s, respectively. A comparison of the results of hydraulic conductivity for locations A, B and C of test model #3 did not show significant reduction between layers. However, it showed a 10^{-2} m/s reduction compared to untreated

sand. It can be concluded that the hydraulic conductivity of the bio-treated sand is not adequate for the impermeable layer, but it is practical for controlling erosion due to water movement when the strength of the model is sufficient for 90 mm of water above the dike.

However, as the test model showed failure on part of its surface during the flume test, it can be concluded that a higher number of treatments is required to increase the calcium carbonate content. A comparison between test models #1 and #3 indicated an increase in the calcium carbonate content within the soil matrix and in the stability of the test model after being in the flume with a flow rate of 16 L/s. Increasing the precipitated calcium carbonate to sand ratio provided more precipitation on the surface of the sand grains and in the channels between them, which also improved the body of the test model. Overall, test model #3 showed more improvement than test model #1 in the sand characteristics by biocementation in the body and surface of the samples, but this improvement was not adequate to protect the whole test model from erosion.

5.4.5 Test #4

Test model #4 was treated with a targeted calcium carbonate to sand ratio of 5% (w/w) by increasing the number of treatments (Table 5.3) through the spray method. A series of photos was taken at different times and select photos are shown in Figure 5.18. The flow rate started at 2 L/s (Figure 5.18 a and b) and was increased to 4 (Figure 5.18 c), 8 (Figure 5.18 d), 12 (Figure 5.18 e) and 16 L/s (Figure 5.18 f). Test model #4 was performed for four days for each flow rate, resulting in a total of twenty days. The water was kept at a minimum height of 15 mm above the top of the dike and gradually increased to 110 mm as the flow rate increased.

Test model #4 showed an improved performance in the resistance to erosion compared with test model #2. A 20-30 mm thick crust was observed on the surface, as shown in Figure 5.18 (h). Less treated sand was observed below the crust, as visible in Figure 5.18 (b) by the difference in color. There was a crack, as shown in Figure 5.18 (h), which likely formed during the installation of the test model. Increasing the flow rate to 12 and 16 L/s resulted in an increase in the size of the crack, as shown in Figures 5.18 (e) and 5.18 (f). However, the crack did not show further expansion or lead to the failure of the test model.

Figure 5.18. Images of test model #4 treated by the spray method while the flow rate was (a and b) 2 L/s, (c) 4 L/s, (d) 8 L/s, (e) 12 L/s, (f) 16 L/s, (g) after experiment and (h) bio-treated crust under water in the flume

After the flume experiment, the test model #4 was air dried and cut along the crack (Figure 5.19). There was a crust formed by biocementation along the cross-section of the cut, as shown in Figure 5.19 (a). The thickness of this layer varied from 10 to 23 mm, as shown in Figure 5.19 (b). For further investigation, the UCS and hydraulic conductivity of the bio-treated crust (0-23 mm) was measured. The location of the crust specimens used for the UC and hydraulic conductivity tests can be seen in Figure 5.19 (c).

Figure 5.19. (a and b) Formation of crust on the surface of test model #4 and (c) location of specimens for lab testing

UC tests were conducted on three specimens (A1, A2 and A3). The specimens had a diameter of roughly 11 mm and a height of 22 mm, depending on the availability. Small samples with a small diameter had to be used as the cementation zone was only 10-23 mm thick. Nevertheless, the UC test provided a way to assess the degree of improvement of the sand given the fact that clean sand would not even stand as a column. The increase in the number of treatments in test model #4 resulted in stronger walls and a thicker crust compared to test model #2. The results of the UC test of the protective crust treated by the spray method on the surface of test model #4 are shown in Table 5.7. The measured UCS values for the crust (0-23 mm) are 582, 612 and 668 kPa.

Table 5.7. Results of UCS for the specimens of test model #4

Specimen	A1	A2	A3	Average
UCS (kPa)	668	612	582	620

As shown in Table 5.7, the average UCS value was 620 kPa. Afterward, to explain the results of UCS, three specimens were taken from the crust to measure the calcium carbonate content using ICP-OES. The calcium carbonate analysis exhibited that the average of calcium carbonates content with the rate of 2.96 % (w/w). Therefore, the average results of UC tests indicated improvement in the stability of test model #4 toward the water pressure by increasing the higher number of treatment and subsequently increasing the targeted calcium carbonate to sand ratio (%w/w) compared to test model #2.

For further investigation, another sample from the location A4, as shown in Figure 5.19 (c), was prepared for hydraulic conductivity testing (Appendix H). This sample had a diameter of 38 mm. The preparation process used to shape the sample is described in Appendix I. A confining pressure of 160 kPa was used for the hydraulic conductivity test. A pressure of 130 kPa was applied to the top of the specimen. The results indicate that the hydraulic conductivity of the sample was reduced from the initial value of 4.0×10^{-4} to 3.0×10^{-7} m/s.

5.4.6 Test #5

Test model #5 was treated with a targeted calcium carbonate to sand ratio of 7.5% (w/w) by increasing the number of treatments to six (Table 5.3) through the percolation method. A series of photos was taken at different times and selected photos are shown in Figure 5.20. The flow rate started at 2 L/s (Figure 5.20 a and b) and was increased to 6 (Figure 5.20 c), 12

(Figure 5.20 d) and 18 L/s (Figure 5.20 e) for four days each and to 22 L/s (Figure 5.20 f) for fourteen days. The test model was kept in the flume for a total of thirty days. The water was kept at a minimum height of 15 mm above the top of the dike and gradually increased to 140 mm as the flow rate increased. No failure or erosion was observed for the test model #5.

Figure 5.20. Images of test model #5 treated by the percolation method while the flow rate was: (a) and (b) 2 L/s, (c) 6 L/s, (d) 12 L/s, (e) 18 L/s and (f) 22 L/s.

After the flume experiment, the soil in test model #5 was air dried and cut using tools in preparation for the UC and hydraulic conductivity tests. The locations of the specimens are marked in Figure 5.21. UC tests were conducted on three specimens from level A (A1 to A3), B (B1 to B3) and C (C1 to C3). The dimensions of the specimens were 25 mm in diameter

and 50 mm in height. The results of the UCS tests are shown in Figure 5.21 and Table 5.8 and range from 372-1234 kPa. The average values are 1107 kPa for A, 502 kPa for B and 452 kPa for C.

Figure 5.21. (a) Samples taken from test model #5 and results of UCS for specimens from test model #5

Table 5.8. Results of UCS test for the specimens of test model #5

Specimens	UCS (kPa)			
	1	2	3	Average
A (0-50 mm)	1234	1128	960	1107
B (50-100 mm)	660	458	387	502
C (100-150 mm)	512	473	372	452

Afterward, to explain the results of UCS and hydraulic conductivity in the model test #5, three specimens were collected from the UCS specimens to represent the calcium carbonate content of three locations, A (20, 35, 50 mm), B (60, 85, 100 mm) and C (120, 135 and 150 mm) to analyze the calcium carbonate content with UCS. As shown in Figure 5.22, the average results of ICP-OES indicated a higher percent of calcium carbonate content in location A than the other two locations. In addition, based on the results of UCS specimens as shown in Table 5.8 and the results of ICP-OES, it can be concluded the relationship between highest calcium carbonate content and highest UCS value.

Figure 5.22. Results of calcium carbonate content to sand ratio (% w/w) with UCS as well as depth in the specimens collected from three locations A, B and C of test model #5

The percent of calcium carbonate precipitation in locations B and C shows almost the same range. The calcium carbonate analysis exhibited that about 1.4 times less crystals were precipitated in locations B and C compared to the location A. Hence, it can be conclude that the most of total value precipitated in the top 50 mm, which is the reason for the significantly higher average UCS value (1107 kPa) in the specimens from the crest than locations B or C. Note that the specimens for calcium carbonate content were collected from the UCS and hydraulic conductivity samples. However, calcium carbonate content of samples from the same place shows a variety range for calcium carbonate content which can be explained by the effect of adsorption of bacterial cells as well as availability of cations which is required for precipitation of calcite crystals.

In addition, the relationship between the results of the ICP and UC tests of specimens in crest, middle and base locations in test #5 was also established. From this study, a linear relationship between UCS (S) and the percent of precipitated calcite to sand ratio (C) treated by the percolation method is established as shown in Figure 5.23 which indicated that there is a good agreement between the two.

Figure 5.23. Relationship between calcium carbonate content to sand ratio (% w/w) and UCS for the specimens of model test #5

Subsequently, hydraulic conductivity tests were conducted on samples taken from A1, B1 and C1, as shown in Table 5.9. The samples were prepared as described in Appendix I with a diameter of 50 mm. The results of hydraulic conductivity testing for these specimens are shown in Table 5.9 and range from 1.1×10^{-7} m/s to 1.7×10^{-7} m/s.

Table 5.9. Properties and results of hydraulic conductivity for the specimens of test model #5

Specimen	A1	B1	C1
Cell pressure (kPa)	210	110	110
Bottom pressure (kPa)	180	80	80
Top pressure (kPa)	185	85	85
Coefficient of hydraulic conductivity (m/s)	1.1×10^{-7}	1.4×10^{-7}	1.7×10^{-7}

Subsequently, the relationship between the results of the ICP and hydraulic conductivity tests of specimens in three locations in test #5 treated by the percolation method was also established. From this study, a linear relationship between hydraulic conductivity (H) and the calcium carbonate to sand ratio (C) treated by the percolation method is established as shown in Figure 5.24.

Figure 5.24. Relationship between calcium carbonate content to sand ratio (% w/w) and hydraulic conductivity for the specimens of model test #5

5.4.7 Test #6

Test model #6 was treated with a targeted calcium carbonate to sand ratio of 7.5 % (w/w) using the spray method. The performance of the test model was examined in a flume with flow rates of 2 (Figure 5.25 a), 6 (Figure 5.25 b), 12 (Figure 5.25 c), 18 (Figure 5.25 d) and 22 L/s (Figure 5.25 e and f). The model remained in the flume for four days each for flow rates of 2, 6, 12 and 18 L/s and 14 days for 22 L/s, resulting in a total of 30 days. The water was kept at a minimum height of 15 mm above the top of the dike and gradually increased to 140 mm as the flow rate increased. As shown in Figure 5.25, no signs of failure or erosion were observed during the flume test under the different flow rates.

Figure 5.25. Images of test model #6 while the flow rate was: (a) 2 L/s, (b) 6 L/s, (c) 12 L/s, (d) 18 L/s and (e and f) 22 L/s

Therefore, the bio-treatment was considered adequate. Only the top 60 mm of soil was adequately treated by using the spray method, which can be explained by the method of applying biogrout and the formation of a protective layer on the surface of the model. The formation of a bio-treated crust partially decreased the penetration of the biocementation solution through the lower layers of the test model. After the flume tests, the test model were air dried and cut for further testing, as shown in Figure 5.26. The specimens used for the UC tests were taken from the top 30 mm of location A and location B (30-60 mm). Small samples with a small diameter had to be used as the cementation zone was only 30 mm thick. Nevertheless, the UC test provided a way to assess the degree of improvement of the sand given the fact that clean sand would not even stand as a column.

Figure 5.26. Formation of crust on the surface of test model #6 and untreated sand beneath the crust and locations of specimens for lab tests

The UC results are shown in Table 5.10 and Figure 5.27. The measured UCS values range from 189-1521 kPa. The average UCS value for the top 30 mm was 1332 kPa and for the 30 mm below the average was 180 kPa.

Table 5.10. Results of UC test for the specimens of test model #6

Specimens	UCS (kPa)			
	1	2	3	Average
A (0-30 mm)	1345	1129	1521	1332
B (30-60 mm)	229	189	123	180

Figure 5.27. Results of UC test for specimens from test model #6

To compare the two methods for applying biogROUT on the distribution of calcium carbonate content, sand samples were collected from the bio-treated sand in first 30 mm of crust (A) and second 30 mm (B). These samples were then prepared for ICP-OES testing in order to measure the calcium carbonate content. The results were read on the screen and are presented with the results of UCS and depth from the top of model dike #6 in Figure 5.28. In addition, the relationship between the results of the calcium carbonate content and UC tests of specimens in test model #6 treated by the spray method was also established. From this study, a linear relationship between UCS (S) and the calcium carbonate to sand ratio (C) treated by the spray method is established as shown in Figure 5.29 which indicated that there is a good agreement between the two.

Figure 5.28. Results of calcium carbonate to sand ratio (% w/w) and UCS as well as depth in the specimens collected from two locations A and B of test model #6

Figure 5.29. Relationship between calcium carbonate content to sand ratio (% w/w) and UCS for the specimens of test model #6

The results of ICP-OES, as shown in Figure 5.28, indicated that the highest percent of calcium carbonate was in location A which formed a crust. Note that the average value precipitated in the 30-60 mm was 8 times less than top 30 mm, which is the reason for the

significantly higher average UCS value (1332 kPa) in the specimens from this layer than from location B.

Subsequently, the hydraulic conductivity of samples taken from locations A1 (0-30 mm) and B1 (30-60 mm) was measured. Using the spray method resulted in only 60 mm of bio-treated sand, as shown in Figure 5.26. The diameter of the samples was 38 mm. The samples were prepared as described in Appendix I and the details of the test are shown in Table 5.11.

Table 5.11. Properties and results of hydraulic conductivity tests for the specimens from test model #6

Specimen	A1	B1
Cell pressure (kPa)	210	110
Bottom pressure (kPa)	180	80
Top pressure (kPa)	185	85
Coefficient of hydraulic conductivity (m/s)	1.0×10^{-7}	1.7×10^{-7}

From the results in Table 5.11, it can be seen that the hydraulic conductivity of the bio-treated sand taken from location A (0-30 mm) was reduced from the initial value of 4.0×10^{-4} to 1.0×10^{-7} m/s. The coefficient of hydraulic conductivity for location B (30-60 mm) was reduced to 1.7×10^{-7} m/s.

Comparison between the ICP results for the different locations (A, B and C) in test models #5 and 6 indicates that a 40% increment in the precipitation of calcium carbonate in location A (0-30 mm) of the model was achieved when treated by the spray method. The ICP results for location B and C also show significantly higher calcium carbonate content about 70% and 100% respectively when using the percolation method than the spray method. In addition, this was confirmed by the observations. The comparison of relationship between the results of the ICP and UC tests for two application methods was also established. The results of calcium carbonate content and UCS from specimens of model dikes treated using the percolation method and the spray method was also summarized separately in Figure 5.30.

The average of data obtained from ICP-OES values and UCS for model tests treated using spray methods (tests #4 and #6) indicate the amount of calcium carbonate content on the bio-

treated sand in crust. In another application method, the average of data obtained from ICP-OES values and UCS for model tests (#3 and #5) treated using the percolation method specify the amount of calcium carbonate content on the different locations of bio-treated model.

Figure 5.30. Effect of calcium carbonate content to sand ratio (% w/w) on UCS in different method of application of biocement solution; (●) is the results of the percolation method and (■) is the results of the spray method.

From the results of this study, a linear relationship between UCS (S) and the calcium carbonate to sand ratio (C) is established for the results of each application method and compared as shown in Figure 5.30. The average UCS results from the model tests illustrated that there is a good agreement between the specimens in both methods. As shown in Figure 5.30, the curve for the UCS (S_s) of specimens treated using the spray method (C %) was fitted by the following Equation (5.5) :

$$S_s = 302.8C - 18.22 \text{ kPa} \quad (R^2 = 0.996) \quad (5.5)$$

In addition, the curve for the UCS (S_d) of specimens treated using the drop method (C, %) was fitted by the following Equation (5.6) :

$$S_d = 386.2C - 72.93 \text{ kPa} \quad (R^2 = 0.938) \quad (5.6)$$

In addition, the relationship between the results of the ICP and UC tests without considering the application method for this study and another study by Chu et al. (2014) was also established. Data obtained from ICP-OES values in Figures 5.22 and 5.28 indicate the amount of calcium carbonate content on the bio-treated sand in various locations. In another study by Chu et al. (2014), sand samples were treated with cells of *Bacillus sp.* and a biocementing solution containing 0.75 M of calcium chloride and 1.5 M of urea, incubated for 24 hours, and then dried at 60 °C until the weight became constant. The samples were then used for unconfined compression tests. From this study, a linear relationship between UCS (S) and the calcium carbonate to sand ratio (C) is established as shown in Figure 5.31. The average UCS results from the tests for models #5 and #6 are added to Figure 5.31 and there is a good agreement.

Figure 5.31. Effect of calcium carbonate content to sand ratio (% w/w) on UCS; (◆ is the results of this study.

Moreover, there is also a relationship between the hydraulic conductivity values (H) and the calcium carbonate to sand ratio (C) as shown in Figure 5.32 based on the data by Chu et al. (2014). The average hydraulic conductivity values for the three specimens from test # 5 and #6 are also plotted in Figure 5.32. A good agreement based on R^2 is also achieved.

Figure 5.32. Effect of calcium carbonate content to sand ratio (% w/w) on hydraulic conductivity while (■) is the results of this study.

5.4.8 Discussion

The UC test results for the models #3 to #6 indicate that the method of application of biocement solution (spray or percolation) and the calcium carbonate to sand ratio (% w/w) have a significant effect on the UC strength of the bio-treated soil. The mechanisms of the percolation method for avoiding the crust formation can be divided by sequential injection of bacterial suspension and cementation solution as explained earlier by Whiffin et al., (2007) and Al-Thawadi (2008). The percolation method is considered to be the main factor in increasing the depth cementation (Figures 5.16, 5.21 and 5.22) of test models compared to the spray method. The major difference between the percolation method and the spray method is the recirculation of biocement solution. In the percolation method, the recirculation of biocement solution was carried out for three days and the conditions for cells and reagents to have the highest contact with the sand grains in the models were provided. However, in the spray method, the spraying of biocement solution for each treatment was carried out and the conditions for calcium carbonate precipitation on the surface of models were provided. This explains the differences in the UC test results for the specimens from models #3 and #5. It can be concluded that using the percolation method, a relatively more uniform treatment can be achieved. Moreover, the results also show that the higher the calcium carbonate to sand ratio, the higher the strength and different calcium carbonate content during MICP led to different levels of bioclogging or biocementation of sand. The highest shear strength came

from the crust resulting from the spray method and this was also where the calcium carbonate to sand ratio was the highest.

The data in Figure 5.17 and 5.21 shows the behavior of prepared columns from model tests #3 and #5, treated with calcium-based biocement. The stiffness calculated by the slope of curve. Comparing the results of UCS shows that increasing the precipitated metal to sand ratios from 1.5% to 3% in samples of model test #5 resulted in a range of 452 kPa to 1107 kPa. The calculation of stiffness for samples also shows the higher value by increasing the precipitated metal as shown previously by other researchers (DJong et al. 2012). Therefore, it can be said that biocementation affects the stiffness in calcium-based bio-treated samples which is a factor of precipitated metal.

Further investigation of the results of hydraulic conductivity testing of specimens taken from the models indicated that bio-treatment not only increased the stiffness and shear strength of the soil, but also significantly reduced the hydraulic conductivity of the sand. Test model #5 treated by the percolation method showed a reduction in the coefficient of hydraulic conductivity by three order of magnitude in the crust, middle and base (Figure 5.24). In contrast, in the test model #6 treated by the spray method, the reduction in hydraulic conductivity was only significant in the impermeable crust (location A).

From the results of calcium carbonate content using ICP-OES, it was observed that using the spray method almost 4 times more crystals of calcium carbonate on the top 30 mm of the model while using the percolation method shows 2.8 times more of the calcium carbonate crystals was in the top 50 mm of the bio-treated models compared to the untreated model dikes. Therefore, the spray method increased the crystals of calcium absorption just in the upper layer and resulted in the formation of an impermeable crust. Although the results of the percolation method exhibited the highest strength and calcium carbonate content in the first 50 mm of model dikes, the relative homogeneous calcium carbonate and strength between 50 mm to 150 mm has been found. The calcium carbonate content analyses showed about 1.4 times less crystals were precipitated in the first 50 mm of model test treated by the percolation method. In addition, using the percolation method exhibited almost 1.5 times more crystals of calcium carbonate in the depth of model dikes in locations B and C in comparison with model tests treated by the spray method which shows distribution of bio-solution through the percolation method.

5.5 CONCLUSIONS

Six test models were prepared and tested using a hydraulic flume to establish a method for using the MICP approach to enhance the ability of a dike against erosion. Application of biocement solution by two methods was studied. The first was the percolation method and the second the spray method. The conclusions of the study presented in this chapter are as follows:

- 1) The model treated using the percolation method with a targeted CaCO_3 to sand ratio of 2.5% (w/w) did not show significant improvement in shear strength. By increasing the targeted calcium carbonate to sand ratio to the 5% to 7.5% (w/w) range using the percolation method, the stability of the dike against overtopping can be improved. The highest UCS value obtained for the soil treated by the percolation method was 1234 kPa.
- 2) After treating the model with a targeted calcium carbonate to sand ratio of 2.5% (w/w) using the spray method, a hard crust was formed on the surface which increased considerably the resistance of the dike against erosion. Using the four-point bending test, the crust was measured to have a maximum bending strength of 312 kPa. However, the thin crust was brittle and prone to cracks. Water entered the cracks and caused erosion inside the dike during a model tests.
- 3) Increasing the target of calcium carbonate to sand ratio in test models treated by the spray method increased the thickness of the bio-treated crust up to 30 mm. However, the sand zone below the crust remained untreated. The formation of the crust prevented cells and calcium ions from penetrating deeper into the soil. The ICP-OES test results indicate that almost 4 times more crystals of the calcium carbonate were in the bio-treated crust.
- 4) The SEM images of the treated soil indicated that calcite formed through the MICP process filled the pores in the sand. This helped to reduce the hydraulic conductivity and increase the stiffness and shear strength of sand. The test results showed that the hydraulic conductivity of sand was reduced from an order of 10^{-4} m/s to 10^{-7} m/s when an average of 2 kg/m^2 of calcium are precipitated in the sand.
- 5) The percolation method results in a relatively more uniform cementation in the model. Nevertheless, the calcium carbonate content in the top 50 cm was still higher than the soil below shown by ICP-OES test results. Thus, the method for applying the biocement solution and the calcium carbonate to sand ratio are the two most important factors affecting the effect of the MICP treatment for the model dikes.

CHAPTER 6 APPLICATION OF BIOCEMENTATION FOR DUST CONTROL OF SAND

6.1 INTRODUCTION

Dust is an environmental hazard. Dust that is commonly encountered includes dust from agricultural fields, road beds, disturbed land, mineral extraction and construction and industrial sites. Ground erosion by wind and dust generation from unpaved roads, dune movement in deserts, dust storms in arid and semi-arid lands as well as the emissions and dispersion of agriculture, construction, transportation and mining are a serious geotechnical and environmental concern due to soil loss and air and water pollution.

According to the USA Environmental Protection Agency (EPA), environmental parameters such as particle size, soil moisture and soil strength affect the quantity of dust generated (Epps and Ehsan 2002). There are a number of biological and chemical methods for the stabilization of exposed soil surfaces by aggregation of fine particles and reduction of the rate of aggregate loss. Some patents have been issued on methods to control wind erosion of soil and dust suppression. Piechota et al. (2004) defined the "effectiveness" of dust suppressants as the ability to maintain soil particles on the surface under erosive forces, such as wind. However, common dust suppressants have disadvantages such as high cost, short-lived results, environmental toxicity (negative effect on plants and water pollution), high equipment maintenance, leaching with rain, corrosivity, etc. Therefore, there is a need to develop new methods for dust control.

An alternative method for dust suppression is biocementation using non-pathogenic and urealytic bacteria with calcium chloride and urea solution (Çabalar 2007, Bang et al. 2008, Ivanov and Chu 2008, DeJong et al. 2010, Meyer et al. 2011). Biocementation is based on the precipitation or crystallization of insoluble compounds in porous soil using the enzymatic activity of microorganisms. Several experimental studies have been performed using biocement for sand dust suppression (Bang et al. 2008, Bang et al. 2011, Meyer et al. 2011). However, more research is required. In the present study, experimental work was carried out to establish the relationship between the amount of biocement used and the rate of dust generation under different environmental conditions, such as temperature and curing time.

Two sets of experiments were conducted. The first set used sand with particles too large to be suspended by turbulent flow, such as road sand. The second set used sand with fine particles, such as desert sand. The developed method was then compared to two common methods for dust control namely water and chemical stabilization (calcium chloride) with bio-treated samples.

The objective of the study was to establish a method for using the MICP approach to reduce wind erosion and dust concentration against erosive force of wind.

6.2 COMMON METHODS FOR DUST CONTROL

The impact of wind and traffic as mechanical factors, climate change as a natural factor and human activities as secondary factors are parameters that affect the formation of dust in the air (Piketh et al. 2000, Munson et al. 2011). The basic processes of particle movement by wind can be categorized as follows (Bagnold 1941, Livingstone and Warren 1996):

- 1) Creeping: Creeping is the rolling of sand grains (larger than 0.5 mm) on surfaces. The creeping of the soil particles starts without detachment from the surface. About 5-25% of sand particles are moved through creeping.
- 2) Saltation: Saltation is the “leap” of a grain along a surface, forced by wind. It mainly occurs in medium and fine classes of sand (0.1-0.5 mm) as a process of a sudden uplift at a vertical angle and a return to the same surface at a flat angle (Harvey 1993). High-speed cine-photography has shown that saltating grains impact the surface at an angle with the horizontal at about 10° and, on average, rebound at 25°, although the latter depends on the particle size and on the configuration and slope of the bed (Harvey 1993). Saltation is responsible for the majority (50-70%) of particle movements.
- 3) Suspension: Suspension is a splash of surface grains when a saltating particle hits the ground. The movement of particles that are smaller than silt or clay (100 µm) or less for particles in air) is called the suspension mode (Maurer et al. 2006). It is well established that the threshold velocities for grain transport decrease with the decreasing diameter of sand particles, but increase again for particles less than about 80 µm in diameter (Pillay

and Kutty 2005). These fine dust particles are therefore not easily entrained by wind alone (Harvey 1993). Suspended particles are lifted and can be carried many kilometers downwind. Suspension is responsible for 30-40% of particle movement. The distance of grain movement due to suspension is generally less than that due to saltation, but the particles remain in the air for a longer time.

The control of dust is strongly related to the gradation of soil particles. Saltation and suspension of particles are the reason for dust generation. That is the reason why studies on the mechanisms or controlling of dust generation focus on particles between 100 and 400 μm . Based on the saltation and suspension movement of particles, one of the approaches to encounter the force of the erosive source of wind is aggregation of fine particles. In their 2004 study, Piechota et al. stated that dust suppressants indicate the binding of fine particles from suspension, while erosion control technologies aim to reduce the effective force of wind. Therefore, a “crusted surface” - even with a weak crust - significantly reduces the rate of erosion and, subsequently, can bind fine particles and suppress dust. A crusted surface can also protect the underlying less cohesive particles from the impact of aerodynamic and mechanical forces (Harvey 1993, Morgan et al. 1998, Noller et al. 2011). Each of the common methods varies in its mechanism for the formation of a crust to aggregate the soil particles, such as increasing surface tension, increasing tensile force or lowering vapor pressure (evaporation), by which it provides dust abatement. In the present study, the term “effectiveness” for each dust suppressant refers to the effectiveness of the methods with regard to time after spraying the suppressant solution. For example, Sanders et al. (1997) reported that the effectiveness of the 2.3 L of calcium chloride in m^2 of road surface after 4.5 months of study was two times less than untreated surface. The methods of dust suppression are widely used and have been described in previous studies (Cowherd et al. 1988, Kirchner 1988, Palmer et al. 1995, Sanders et al. 1997, Bolander and Yamada 1999, Gebhart et al. 1999, Jones and Mitchley 2001, Fay et al. 2012). The conventional methods for controlling dust can be classified as follows: a) agronomic methods (consisting of establishing vegetative cover), b) surface penetration methods, c) admixture methods (consisting of synthetic polymer products and biopolymers) and d) surface blanket methods (consisting of aggregates, prefabricated membranes or meshes and bituminous).

From the above common methods, surface penetration was selected as the control in this study. Thus, the mechanisms and subcategories of this method will now be explained.

Surface penetration is the most common and accessible of the methods and consists of applying a liquid to the soil surface that penetrates deep into the soil and forms a crust on the surface. Examples of this category include the use of:

- 1) Water: Water is the most common, short-term, low cost and uneconomical solution (Gebhart et al. 1999, Epps and Ehsan 2002, Piechota et al. 2004, Thompson and Visser 2007). It is an extremely temporary solution and considered as low cost method for construction sites and unpaved roads where surfaces are disturbed only for short periods of time. For long-term benefits, the use of expensive equipment is required for reapplication at a frequency dependent on the environmental conditions which makes it uneconomical. Previous studies indicate the creation of a tensile force between the sand grains by the existing water due to the annular ring of water surrounding each grain and the accompanying negative pore-water pressure. The tensile force is a function of surface tension, which depends on grain shape and moisture content. A maximum limiting value of the negative pressure is the vapor pressure of the soil-water solution. For example, the vapor pressure is very high in clay soils. However, in fine sand, the vapor pressure is reduced to zero when the soil dries, leaving loose, unbound sand particles (Gebhart et al. 1999, Epps and Ehsan 2002, Piechota et al. 2004, Thompson and Visser 2007). Therefore, the low vapor pressure and low tensile force are the limitation factors, especially in relatively hot and arid climates. In some cases, to reduce the tensile force due to drying, the area is covered with geosynthetic material after water treatment, which can be expensive.
- 2) Organic non-petroleum products: These products include lignin sulfonate derived from paper processes, tall (pine) oil which is a by-product of wood pulp, and vegetable derivatives from sucrose mixed with glycerides and molasses (Piechota et al. 2004, Jones 2011). These products encapsulate the soil particles and create a binding network throughout the treated area. The advantage of these products is immediate use. The main limitation is that they are water-soluble binders and therefore susceptible to leaching from the soil during heavy rainfall.
- 3) Organic petroleum products: These products are derived from petroleum and include oils, solvents, cutback solvents, asphalt emulsions, dust oils and tars (Piechota et al. 2004, Yan and Hoekman 2012). Petroleum-based products are not water-soluble, but

they are prone to evaporation (Piechota et al. 2004, Yan and Hoekman 2012). Petroleum products do not form a strong bond. They remain soft and exhibit the properties of wax when cured.

- 4) Electro-chemicals: These consist of chemicals such as sulfonated oils, enzymes and ammonium chloride with a boundary for use with certain types of soil.
- 5) Chemical stabilization: These include the use of a solution of CaCl_2 or MgCl_2 . The idea behind using chemicals is due to their hygroscopic properties and their ability to absorb moisture from the air (Kett et al. 2010, Edvardsson et al. 2012). Samples treated with chemicals show a high affinity for water and the ability to bind aggregate particles together. Epps and Ehsan (2002) and Rushing and Newman (2010) showed the higher effectiveness of chemical stabilization than water using a weight of 111 g/mol of CaCl_2 , which was expected to penetrate to a deeper depth and result in a longer protection time. Moreover, it was found that the greater the atmospheric humidity, the greater the amount of moisture absorbed by CaCl_2 at the same temperature. This ability could be used to suppress dust (Epps and Ehsan 2002). Furthermore, chemical stabilization results in a significant increase in the surface tension of water molecules between soil particles. This helps retain particles in treated areas by increasing the adhesive force between them. However, the vapor pressure of a solution of salts is always lower than water, which results in a slower rate of evaporation compared to water treatment. Moreover, the positively charged ions (Mg^{2+} and Ca^{2+}) can flocculate the negatively charged clay particles by electrostatic forces and further reduce dust production (Kett et al. 2010, Edvardsson et al. 2012). The long-term efficiency of chloride salts is somewhat limited because the material is water-soluble and will leach from the soil with prolonged exposure to rainfall. Other limitations of chemical stabilization are the high cost, corrosivity and high maintenance.

The limitations and advantages of the common surface penetration methods indicate there are many factors to consider when selecting a dust control method. The ideal method should be environmentally compatible, easily applied, require minimal maintenance equipment, reasonably effective at controlling dust, not degradable by common environmental elements, be relatively harmless or inconvenience to adjacent residents and be cost competitive (Thompson and Visser 2007). Moses et al. (2012) reported that a road surface with a moisture

content of 0.5% generates approximately 82% more dust than a road surface with 15% moisture content. Increasing the moisture content is one of the reasons for repetitions of common treatments. It is well known that increasing the strength of the surface of the soil can be a solution for decreasing the repetitions of treatment needed to increase the moisture content. However, environmental conditions, soil type and material costs lead to choosing treatments with a higher binding strength and effectiveness. An ideal treatment is one that forms a protective crust, increases the surface tension of water surrounding the soil particles, resists leaching due to a large ionic radius, demonstrates low vapor pressure and thus low evaporation, is effective in different environmental conditions and retains its ability without repeated application. In summary, more research is required on a new method to overcome the limitations of dust suppressants.

6.3 WIND TUNNEL DESIGN

Studies of wind erosion require the generation of wind. Thus, a wind tunnel was designed for use in this study. A wind tunnel for use in dust control and wind erosion studies needs to control a number of physical factors such as wind speed and particle size (Raupach and Leys 1990, Zobeck et al. 2003). Raupach and Leys (1990) found that the results of a rectangular cross-section wind tunnel were more reliable due to air stream homogeneity. The most important requirement for the initiation of vertical particle uplift in a wind tunnel is the establishment of a wind profile with specific wind speeds. Due to rotor blade movement, irregularities in the wind tunnel construction or induced swirl at the tunnel entrance, undesired turbulence can be induced in the airstream. Therefore, it is crucial to minimize the effects of turbulence by installing a diffuser at the tunnel entrance to achieve an accurate wind speed.

Considering the above factors, the wind tunnel for the present study was designed as shown in Figure 6.1. The design was similar to Pietersma et al. (1996), White (1996) and Ferreira and Fino (2011). The tunnel had a height of 510 mm, a width of 400 mm and a length of 4000 mm. It was composed of two segments. The first part was 1000 mm long and consisted of a fan, diffuser and tail. The second part of the main body was 3000 mm long. To ensure a consistent flow of air, all openings (between segments or doors) were sealed off with duct tape. The entrance of the tunnel was funnel-shaped and the end was open to decrease air

turbulence and promote a homogeneous wind velocity through the tunnel. A medium-pressure axial fan with a diameter of 180 mm and rotor blades with a constant wind speed was used driven by a 0.3 KW and maximal 50 Hz electric motor. At the top of the main segment was a window for putting samples into the tunnel for testing. Because saltating particles move relatively short distances during wind events, measurements of the horizontal sand flux indicate the amount of wind erosion near the measurement sites. The sand samples were placed 1250 mm downstream of the fan.

Figure 6.1. Images of the wind tunnel set up and a schematic illustration

The selected wind speed was 40 km/h with a duration of six minutes. The wind speed was selected to represent the wind strength used in previous studies by Bang et al. (2011) on the effectiveness of biocement compared to common methods on mass loss. The wind speed at the top of the sand sample was measured using an anemometer AIRFLOW LCA 6000

(Airflow Developments Limited, UK). The device had a measurement range of 0.72–108 km/hr and was inserted vertically to measure wind speed.

The wind tunnel was also used to assess the effectiveness of bio-treatment on dust emission. The gathered dust emissions were investigated using an isokinetic filter sampler together with a vacuum pump. The isokinetic filter sampler contained a round-shaped filter holder, a conic inlet and a 2 mm membrane filter between them (Figure 6.2). A small vacuum pump was attached by a flexible tube to the outlet of the filter holder. A flow speed of 10 to 16 L/min was used, which is a factor of the filter resistance and the maximum output of the vacuum pump. The whole filter construction, which consisted of four inlets, was placed 2450 mm downstream and, as Figure 6.1 shows, at a height of 200 mm from the surface of the sand samples.

Figure 6.2. Iso kinetic filter sampler was used in this study to measure airborne.

6.4 MATERIALS

A thin layer of sand (50 mm) was placed over a plastic plate measuring 550 mm long and 400 mm wide, as shown in Figure 6.3. Five series of samples using two different types of sand, namely river sand (RS) and fine sand (FS), were used. The sand was loosely packed at its initial state.

Figure 6.3. Schematic of models with the size characteristics

The river sand contained 95% SiO_2 . The typical engineering properties of river sand from the MSDS are shown in Table 6.1. The mean particle size of river sand was around 0.4 mm, as shown in Figure 6.4. The void ratios of the river sand were in the range of 0.53 to 0.56.

Table 6.1. Properties of river sand

Description	Grain Shape	Mean size (mm)	Chemical Content	Typical Specific Gravity	Bulk Density (kg/m^3)
River sand	Round	0.4	95+% SiO_2	2.65	1500

Figure 6.4. Particle size distribution curve of river sand (RS)

The fine sand had a mean particle size around 0.1 mm. Fine sand was chosen to study the effectiveness of biocementation on suspension-prone dust particles, mostly in the range of desert sand. The basic engineering properties of the fine sand are shown in Table 6.2. The grain size distribution curve of the fine sand is shown in Figure 6.5

Table 6.2. Properties of fine sand

Description	Grain Shape	Mean size (mm)	Typical Specific Gravity	Bulk Density (kg/m ³)
Fine sand	Round	0.1	2.6	1450

Figure 6.5. Particle size distribution curve of fine sand

After the models were prepared using clean sand, selected FS1, FS2, RS1 and RS2 models were treated with cells of urease-producing bacteria (UPB) and a solution of urea and CaCl₂. The procedure for the cultivation of UPB was described in Section 3.2.1 of Chapter 3. 100 ml of cultivated UPB suspension was centrifuged at 1000 g at 4°C for 20 minutes to separate the bacteria cells from the culture liquid. As discussed in Chapter 3, urease from *Bacillus sp.* VS1 cells can be used under the general conditions (presence of calcium ions) required for the crystallization of calcium carbonate through MICP. It has been established that the rate of urease activity is not constant during cementation. In addition, pretreatment of cells by the use of cations can significantly enhance the adsorption of cells to sand grains and

immobilized urease. Therefore, in this study the supply of 100 ml of diluted UPB with 1% NaCl was stirred with 200 ml of urea and CaCl_2 solution for 10 seconds before spraying. The control samples were prepared by spraying 1.36 L/m^2 of solution consisting of one of the following: 1) water (RS4 and FS4) or 2) a calcium chloride to sand ratio and sodium chloride to sand ratio of 1.25% (w/w) and 1% (w/w), respectively (RS3 and FS3). The control samples were prepared in the same way as the bio-treated samples. This allowed a comparison to be made between biocement and the two other common methods.

Table 6.3. Testing program for preparation of model tests

Samples		Treatment	UPB (g/l)	NaCl (% w/w)	CaCl_2 (% w/w)	Urea (% w/w)	Total solution of water (L/m^2)
River sand (RS)	Fine sand (FS)						
RS1	FS1	Biocement	8	1	0.125	0.17	1.36
RS2	FS2		8	1	1.25	1.7	1.36
RS3	FS3	Chemical	-	1	1.25	-	1.36
RS4	FS4	Water	-	-	-	-	1.36
RS5	FS5	Untreated	-	-	-	-	-

The models were manually sprayed onto the surface of the sand samples using a 500 ml handheld plastic spray bottle. The spray was done at approximately 200 mm above each model. The models were maintained at an outdoor temperature with an average humidity of 75%, unless otherwise noted. Sample RS5 and FS5 were used as untreated.

For each set of bio-treatment in this experiments, calcium chloride to sand ratios of 0.125%, 0.625% and 1.25% (w/w) were adopted with the same biomass concentration of UPB. Each spray supplied a dosage of about 0.004 kg/m^2 of dry biomass. The maximum concentrations of CaCl_2 and NaCl used in this study for the bio-treated samples were 1.25% (54 mM) and 1%, respectively. This was approximately 36 times less than the present allowable concentration (38%) for commercial use (Rushing and Newman 2010). In addition, the concentrations of NH_4Cl (1%) and urea (1.7%) were 14 times lower than the urea content allowed (43%) for commercial use as a frost protectant pesticide with no evidence of adverse chronic effects.

6.5 DEFINITION OF MEASURING PARAMETERS

In this study, both wind erosion and dust concentration were studied. The term wind erosion potential highlights the loss of material from the soil, whereas the term dust concentration emphasizes the suspension of particles in the atmosphere. This distinction is made as wind erosion and dust control are sometimes considered separately in practice.

6.5.1 Wind erosion potential

River sand and fine sand samples were assessed on their potential to resist wind erosion by measuring the mass loss during a wind tunnel test. This method was used in previous wind tunnel studies (Epps and Ehsan 2002, Bang et al. 2011, Meyer et al. 2011). Quantification of biocementation was accomplished by weighing the model samples before and after the test. The mass of soil displaced from the sample was considered an indication of the anticipated performance for saltating grains and was used to determine the relative effectiveness of the dust suppressant.

In this study the tests indicated the resistance and effectiveness of bio-treatment at time intervals of 2, 4, 7 and 14 days after curing. The time interval of two days was selected to judge the immediate performance. It should be pointed out that each test was conducted three times to check the repeatability. The average of the three tests was reported. From the test data, the most efficient concentration of calcium chloride for surface treatment was determined. The effect of temperature on bio-treatment and chemical stabilization was also examined by placing the sand samples at two different temperatures (40° and 50°C). These temperatures were selected based on the temperature of arid land and deserts. The tests also show the behavior of calcium chloride in the bio-treated and chemical-treated sand samples.

6.5.2 Dust concentration

The concentration of fine sand particles in the suspension state within the testing sequence called dust concentration was recorded using an isokinetic filter dust detector sampler together with a vacuum pump. This method has been used by researchers such as Rushing and Newman (2010) for recording dust concentration. In the present study immediately before the fan in the wind tunnel was activated, the dust detectors were turned on. Dust from the samples was sucked onto the filter paper where it was collected after the test. The

collection time for the mitigated dust to be absorbed to the filter paper was 60 minutes at 16 km/h. Samples of FS with identical conditions were tested at time intervals of 2, 4, 7 and 14 days after curing. The data was converted to the specific aerosol production rate ($\text{mg}/\text{m}^3/\text{s}/\text{m}^2$) by considering 3600 seconds as the time and 0.22 m^2 as the surface area of the samples.

6.6 TESTS ON RIVER SAND

6.6.1 Wind erosion potential

The wind erosion potential was assessed by measuring the mass losses of samples after wind tunnel experiments performed 2, 4, 7 and 14 days after curing the surface of the test model by the spray method are shown in Table 6.4.

Table 6.4. Mass loss (%) for different tests of bio-treated and control models

Series / Test#		Time (days)	RS1	RS 2	RS3	RS4
1	1-1	2	36.89	17.18	50.1	82.14
	1-2	4	37.04	15.87	94.4	93.7
	1-3	7	38.79	15.11	93.1	95.1
	1-4	14	39.78	14.84	94.4	89.14
2	2-1	2	37.14	16.78	43.24	83.3
	2-2	4	38.94	16.87	92.2	89.9
	2-3	7	36.1	14.01	92.45	89.17
	2-4	14	37.18	14.53	91.8	90.1
3	3-1	2	39.75	17.8	47.5	79.1
	3-2	4	41.98	16.9	82.14	82.8
	3-3	7	36.87	14.25	83.4	88.85
	3-4	14	38.95	14.1	85	96.1

The average mass loss of the three tests for all treated samples for the different time intervals after curing is shown in Figure 6.6.

Figure 6.6. Results of mass loss in all treated models

As shown in Figure 6.6, the results of RS3 and RS4 indicate significant correlation between sand loss and time after curing. The mass losses for chemical-treated samples with calcium chloride show almost the same trend as with water four days after curing. However, the water content in the presence of chloride salts as a result of its hygroscopic characteristics was not adequate to reduce the mass losses for chemical-treated soil longer than two days after curing.

As shown in Figure 6.6, bio-treated samples with a calcium chloride to sand ratio of 1.25% (w/w) show approximately 17% mass loss two days after curing and around 15% fourteen days after curing. Bio-treated samples yielded higher sand losses in the early days after treatment and a reduction in the percent of sand loss was observed seven days after treatment. The same results were obtained fourteen days after curing, whereas the opposite results were obtained for the control samples (RS3 and RS4). The data suggests that the erosion potential of bio-treated samples decreased when a higher concentration of calcium chloride was applied. The application of a bio-treatment with a calcium chloride to sand ratio of 0.125% (w/w) indicated an average 38% reduction in mass loss across all intervals. Therefore, based on the results shown in Figure 6.6, a negative correlation was found between bio-treatment

and mass loss for all time intervals after curing. Regarding the percent of mass loss, the bio-treated samples (RS1 and RS2) show 85% more cohesion on the surface of the sand than control samples (RS3 and RS4).

Figure 7.6 (a and b) shows that noticeable sand particles aggregated on the surface of the bio-treated samples (RS1 and RS2), forming thin plates of bio-treated sand (compared to Figure 6.7 b and c). However, the size and thickness of the sand plates in bio-treated samples with less calcium chloride (Figure 6.7 b) were not as big as those of the samples treated with a higher percent of calcium chloride (Figure 6.7 a).

Figure 6.7. Aggregated sand plates (arrows) two day after curing in bio-treated and control models (a) bio-treated with calcium chloride to sand ratio of 1.25% (w/w), (b) bio-treated with calcium chloride to sand ratio of 0.125% (w/w), (c) water treated and (d) chemical treated

Subsequently, the sand plates were collected and analyzed using ICP-OES to determine the calcium carbonate content. Figure 6.8 shows the results of calcium carbonate content in a 2 mm thick sand plate from bio-treated samples.

Figure 6.8. Results of ICP-OES for content of calcium carbonate in bio-treated samples with calcium chloride to sand ratio of 0.125 (RS1) and 1.25% (RS2) (w/w)

As shown in Figure 6.8, the ICP-OES data indicates 96% precipitation of calcium on the surface of samples RS1 and RS2. From the results of ICP-OES less than 0.01% and 0.1% of calcium carbonate to sand ratio (w/w) precipitated between sand grains. This explains the formation of plates of calcium carbonate on the surface of bio-treated samples.

Part of the bio-treated aggregated plates from RS2 was collected and imaged using SEM. As shown in Figure 6.9 (a), rod-shaped sand grains in close contact with precipitated salts were observed. Further investigation of Figure 6.9 (b) shows the clear precipitation of crystals of calcium carbonate. These microscopic images explain the formation of sand plates on the surface of bio-treated samples. However, based on the ICP-OES results (Figure 6.8) indicating the presence of calcium after MICP process, it can be concluded that the SEM images show the precipitated calcium carbonate between sand grains. The calcium carbonate crystals fill the pores between the sand grains and aggregate in the layers of sand toward the erosive force of wind.

Figure 6.9. SEM images of the bio-treated sand (a) arrows show the calcium carbonate between two sand grains, and (b) closer view of the same location, arrows show the precipitated calcium carbonate

Increasing the content of calcium chloride resulted in the formation of thick sand plates due to the precipitation of calcium carbonate crystals between sand grains, as shown in Figure

6.10. Biocement helps the formation of the insulating film of the treatment become thicker and establish a denser sand plate, resulting in more aggregated particles.

a **b**

Figure 6.10. (a) Bio-treated model with calcium chloride to sand ratio of 0.125 % (w/w) seven days after curing and after wind tunnel testing with arrows show broken sand plates and, cracks on the surface in comparison to (b) chemical treated model with no sand plates on the surface

The observation of cracks in the bio-treated samples indicates the formation of sand plates, which were not observed in the samples treated with water or chemicals. The equivalent mass loss of bio-treated samples RS2 and RS1 was related to the microbial activity in the production of calcium carbonate between sand grains.

Figure 6.11. Mass loss (%) of bio-treated and control models (a) two days and (b) four days after curing

The effectiveness of the different treatments four days after curing is shown in Figure 6.11. As the lifecycle cost of treatment to preserve a specific area compared to initial material cost is one of the main issues in erosion potential, the results of the bio-treated and control samples are gathered in Figure 6.11. An 86% reduction in mass loss was found in the bio-treated samples compared to the common methods two days after treatment. Note that bio-treated samples were treated with 1.25% calcium chloride and 1% NaCl with 1.36 L/m² water and cells of UPB, while chemical-treated samples were treated with the same chemicals. The water-treated samples were treated with 1.36 L/m² of water. This could suggest that the bio-treated method shows the highest effectiveness of the three treatments.

As each of the bio-treated dust suppressants evaluated in this study offers some degree of successful dust mitigation compared to the conventional methods, the field application of bio-treatment needs more consideration in regard to the CaCl_2 to sand ratio (% w/w). The concentration of CaCl_2 , which led to various percentages of calcium carbonate, is a crucial factor relating to the cost and effectiveness of treatments. Therefore, the maximum and minimum measured values for erosion potential among the bio-treated samples were reported. The bio-treated sample with 1.25% calcium chloride lost 20% of its mass fourteen days after curing, while decreasing the concentration of calcium chloride by a factor of ten resulted in a 31% mass loss.

6.6.2 Calcium chloride in the surface penetration method and biocement

Various environmental factors affect the effectiveness of dust suppression. Temperature is one of the crucial environmental factors in dust generation, especially in arid lands. Samples from two groups of common and new dust suppressants, namely chemical (RS3) and bio-treatment (RS2), both with a calcium chloride to sand ratio of 1.25% (w/w), were chosen and placed at 40 °C and 50 °C immediately after curing to simulate the conditions of arid lands and deserts, respectively. The results of wind tunnel testing in intervals of 2, 4, 7 and 14 days after treatment are presented in Figure 6.12 to show the effect of microbial activity on mass loss. The bio-treated sand samples showed the smallest mass loss - approximately 15% - two days after curing and more or less stayed the same over fourteen days. The samples treated with chemicals experienced the highest mass loss - almost 43% - two days after treatment, which increased to 93% four days after treatment and stayed in the same range over fourteen days. In general, in the days immediately after treatment, samples treated with chemicals yielded lower mass losses (40-50%), while the opposite was observed four days after treatment and onward. The reason for this effect is the decreasing hygroscopic characteristics of calcium chloride which absorb and retain the moisture. The results show a minor effect of temperature on mass loss in chemical-treated samples, which is the consequence of a lower concentration of calcium chloride.

Figure 6.12. Results of mass loss in chemical and bio-treated models

The results of chemical and bio-treated samples prove that CaCl_2 as a hygroscopic agent attracts and retains moisture from the atmosphere, increases the water content of the surface, retards the evaporation of moisture and tightens the soil. The results are the same as those reported in the prior study by Epps and Ehsan (2002). However, in the present study, precipitation of calcium carbonate crystals between sand grains (Fig 6.9 a and b) was the reason for the effectiveness of the bio-treated samples at various temperatures.

6.7 TESTS ON FINE SAND

From the common methods for dust control, chemical suppression was chosen for comparison with the results of bio-treatment. All samples were treated with the same method (spray) and the same volume of water (1.36 L/m^2) to achieve the same moisture content. Therefore, only the effectiveness of the treatments will be considered. The concentration of fine sand particles in a suspension state within the testing sequence was recorded using an isokinetic filter dust detector sampler with a vacuum pump. The collection time for the mitigated dust to be absorbed to the filter paper was 60 minutes at 16 km/h. Note that the average results of the three repetitions of each test are shown in Figure 6.13. The data was

converted to the specific aerosol production rate ($\text{mg}/\text{m}^3/\text{s}/\text{m}^2$), by considering 3600 seconds as the time and 0.22 m^2 as the surface area of the model samples.

Figure 6.13. Results of dust emission on water, chemical and bio-treated models

As Figure 6.13 shows, a comparison of the results two days after curing shows a 17% and 70% reduction in dust concentration for the chemical-treated and bio-treated samples, respectively. The effectiveness of bio-treated samples in the reduction of dust concentration two days after treatment with calcium chloride to sand ratios of 1.25% and 0.125% (w/w) were 70% and 50%, respectively. However, the results of dust concentration stayed more or less the same over the fourteen days after curing for the bio-treated samples, while the control samples are the same as untreated sand. The dust concentration for bio-treated sample FS2 fourteen days after curing shows an 80% reduction in comparison with the common methods.

As shown in Figure 6.14, the formation of a thin layer of calcium carbonate plates on the surface of the bio-treated samples can explain the performance of the bio-treated samples in the wind tunnel tests. The sand plates were identified by the cracks on the surface of the sand samples. However, the thin layer of sand plates broke under the erosive force of wind, as shown in Figure 6.14, and untreated sand showed beneath the sand plates.

Figure 6.14. Formation of sand plates and broken sand plates as shown with arrows on the surface of models FS1 after wind tunnel test two days after curing

To determine the effect of bio-treatment on the mass loss of FS samples, the mass losses of the control and bio-treated samples after wind tunnel testing at different time intervals are shown in Figure 6.15. The highest and lowest mass loss values fourteen days after curing were 18% and 7% for the bio-treated samples treated with calcium chloride to sand ratios of 0.125% and 1.25% (w/w), respectively.

Figure 6.15. Results of mass loss of models treated for dust emission measurements

As shown in Figure 6.15, the FS samples treated with chemicals generally lost a larger amount of mass than the bio-treated FS samples. Comparing the water-treated samples to the chemical-treated samples seven days after treatment confirms the 30% greater effectiveness of chemical treatment in the reduction of dust. The results of this study suggest that the effect of bio-treatment is stronger on mass loss than on dust emission. Moreover, as dust concentration is one of the consequences of wind erosion, it could be concluded that increasing the strength between the sand particles on the surface leads to a reduction in the detachment of fine particles and, therefore, lower dust concentration.

Figure 6.16 shows the surfaces of sand samples treated with a calcium chloride to sand ratio of 1.25% (w/w) at different time intervals after curing.

Figure 6.16. Surfaces of sand samples treated with calcium chloride to sand ratio of 1.25% (w/w) (a) at spraying time, (b and c) two and seven days after curing (before wind tunnel test) and (d and e) two and fourteen days after wind tunnel test respectively

The surface of FS3 two days after curing before (Figure 6.16 b) and after (Figure 6.16 d) wind tunnel testing shows the low effectiveness of chemical treatment and the disturbance to the surface. It can be said that, because of the low concentration of CaCl_2 in the chemical-treated sample, the hygroscopic (water absorbance) effect of calcium chloride is not adequate to bind fine particles together. Figure 6.16 (c) shows the surface of the sample seven days after curing but before the wind tunnel test, while Figure 6.16 (d) shows the same sample after wind tunnel testing.

The surfaces of bio-treated sand samples with calcium chloride to sand ratios of 0.125% and 1.25% (w/w) at different time intervals before and after wind tunnel testing are shown in Figures 6.17 and 6.18, respectively.

Figure 6.17. Surfaces of bio-treated sand samples with calcium chloride to sand ratio of 0.125% (w/w) (a) at spraying time, (b and c) two and fourteen days after curing (before wind tunnel test) and (d and e) two and fourteen days after wind tunnel test respectively

The surface of bio-treated sand samples with a calcium chloride to sand ratio of 0.125% (w/w) two days after curing before (Figure 6.17 b) and after (Figure 6.17 d) wind tunnel testing shows that the reduction in the effectiveness of bio-treated dust suppressants starts at the edges of the samples. This can be explained by the lower amount of treatment at the edges compared to the center. The bio-treated samples show broken plates of sand and the highest mass loss along the edges. However, by increasing the calcium carbonate content to sand ratio (% w/w), the bio-treated sand layer and as a consequence stabilized layer becomes thicker, as shown in the images two (Figure 6.18 b) and fourteen (Figure 6.18 d) days after curing. An integrated sand plate of precipitated calcium carbonate was observed on the surface of both samples FS1 and FS3 and the fine particles remained under the forming sand plate.

Figure 6.18. Surfaces of bio-treated sand samples with calcium chloride to sand ratio of 1.25% (w/w) (a) at spraying time, (b and c) two and fourteen days after curing (before wind tunnel test) and (d and e) two and fourteen days after wind tunnel test respectively

Based on visual comparison of the forming sand plates, it was concluded that the bio-treated FS samples indicated more aggregation (Figure 6.13) than the RS samples (Figure 6.9). Fewer cracks were observed in the integrated plates of the bio-treated sand than in the bio-treated RS samples.

The dosage of applied dust suppressants, 20 g CaCl_2/m^2 , 22 g urea/ m^2 , and 7 g of bacterial biomass/ m^2 , total 49 g/ m^2 , was significantly smaller than dosages of any other dust suppressant published in literature. For example, about 360 g/ m^2 of sulfolignin, 750 g/ m^2 of cement or 1200 g/ m^2 of bituminous materials have to be used for the dust control on the roads and airfield, as it was calculated from the related manual (Army 2005). The wind tunnel test results and other observations from this study indicate that the effectiveness of the bio-treatment method for dust control depends on many variables, such as the percent of precipitated calcium carbonate and tensile strength. The results of this study for the effectiveness of bio-treated specimens through MICP are in line with the results of Bang et al (2011). Bang et al. (2009) reported the potential use of *S.pasteurii* as a long-term, environmentally-friendly approach using a microbial process to efficiently suppress dust particles. In previous studies, biocement showed enough strength to improve soil properties through the precipitation and crystallization of calcium carbonate (as discussed in Chapter 5). The characteristics of biocement suggest the idea of applying UPB cells with a solution of CaCl_2 and urea as an effective method of dust suppression. This study continues the investigation of the MICP process and identifies dust control as one of its potential applications in geotechnical engineering. Considering the degradation time and mechanical strength of dust suppression discussed earlier by Yang et al. (2009), it is necessary to note that the degradation of biocement occurs before the crystallization of calcium carbonate. This means that once the precipitated calcium carbonate takes its place between the sand grains, the tensile force is increased and, due to the formation of sand plates, fine particles remain below the bio-treated sand plates (Figure 6.18).

Regarding the selection of dust suppressants for different applications, it is necessary to mention that MICP could overcome the deficiencies and disadvantages of the conventional methods, such as being environmentally compatible, not degrading in common ways, being relatively harmless to vehicles and resisting leaching. For example, Epps and Ehsan (2002) reported that magnesium chloride or petroleum-based products are not appropriate options for agricultural areas because of their effect on crops. Bio-treatment can be applied to

agricultural areas without causing problems for plants. Moreover, considering the critical disadvantages of using 38% CaCl_2 , such as corrosion to vehicles, the need for application equipment and creation of a slippery surface (Bolander 1999, Roads 2005, Monlux and Mitchell 2007, Schilling et al. 2009), they can be resolved by the application of 1.25% CaCl_2 through bio-treatment, through which more effectiveness and less limitation can be achieved. Also, one of the limitations of non-petroleum products, such as lignin sulfonate, is that they are completely destroyed or significantly reduced in the presence of heavy rain because of the solubility of lignin sulfonate in water (Bolander 1999, Santoni et al. 2002)

6.8 CONCLUSIONS

The conclusions of the study presented in this chapter are as follows:

- 1) The results indicated that the reduction of wind erosion and dust concentration was dependent on the formation of sand plates on the surface of the sand samples which was a result of precipitation of calcium carbonate as indicated by the SEM images and the ICP-OES results.
- 2) The results of calcium carbonate analysis using ICP-OES exhibited less than 0.01% and 0.1% of calcium carbonate to sand ratio (w/w) precipitated between sand grains in bio-treatment sand with a calcium chloride to sand ratio of 0.125% and 1.25% (w/w). This explains the formation of plates of calcium carbonate on the surface of bio-treated samples.
- 3) The results of wind erosion tests for river sand (RS) fourteen days after the treatment indicated that the application of bio-treatment with a calcium chloride to sand ratio of 0.125% and 1.25% (w/w) reduced the wind erosion potential by 62% and 85%, respectively as compared to that of clean sand.
- 4) Bio-treatment with a calcium chloride to sand ratio of 1.25% (w/w) reduced the wind erosion of typical fine sand (FS) by 70% fourteen days after curing.
- 5) Dust concentration for bio-treated samples with a calcium chloride to sand ratio of 1.25% (w/w) fourteen days after curing indicated an 80% reduction in comparison with common methods.
- 6) Under high temperature conditions (40 and 50 °C), the bio-treated samples show less wind erosion potential than the test sections treated with the calcium chloride during the test period.

CHAPTER 7 CONCLUSIONS AND RECOMMENDATIONS

7.1 CONCLUSIONS

1) Optimization of calcium carbonate precipitation

Three options exist for the use of bacterial urease for the MICP process: (i) use of washed bacterial cell, (ii) use of supernatant containing urease and (iii) use of intact suspension. Urease activity was 50% higher after incubation with cells compared to that using supernatants or intact suspension. Consequently, biocementation of sand with the suspension of bacterial cells was more effective than the treatment of sand with the supernatant containing urease. The increased urease activity of washed cells suspension as compared with supernatant can be explained by the presence of urease-inactivating protease in supernatant (Figure 3.7). The difference in the reduction rate of urease activity in the solution of urea or in the solution of urea and calcium chloride could be due to: 1) the removal of protease adsorbed on fresh precipitate of calcium carbonate and 2) the buffering of pH in the solution of urea and calcium chloride during formation of calcium carbonate. Moreover, as the concentration of urease activity in the solution of bacterial cells is higher than supernatant, the immobilization of bacterial cell means the immobilization of urease activity. The results exhibited better performance of biocementation using a concentrated bacterial suspension or intact bacterial suspension rather than centrifuged or filtrate containing only dissolved enzymes. This is the results of study on optimization of calcium carbonate precipitation.

Urease activity of cells of bacterial strain *Bacillus sp.* VS1 in this study was sufficient to hydrolyze 90 g of urea/L of biocement solution for approximately 9.3 h. The urease concentration tested in this study was in the range of 2.3 mM urea/min which was considered low according to other studies (Whiffin 2004). Whiffin (2004) recommended the application of low urease concentration. The application of the low concentration of urease with a higher number of applications in this research could produce significant strength in stabilization of dikes. Moreover, low concentration of urease with higher incubation time (14 days) was studied as a dust suppressant and could produce significant surface strength against erosive force of wind.

2) Optimization of application for iron-based biocement

The precipitated iron salts can be considered as a clogging and cementing compound called iron-based biocement. In the present study, innovative biocement production by the addition of urea and ferrous cation solution after the application of UPB to sand samples was carried out and the possibility of increasing the shear strength of soil in the presence of ferrous cation was explored. The source of ferrous cation in iron-based biogrout could be (i) microbial ferrous cation produced using iron-reducing bacteria (IRB), (ii) ferrous sulfate and, (iii) the combination of ferrous sulfate and microbially produced ferrous cations.

Samples treated with iron-based biogrout through microbial ferrous cation did not show any strength improvement but when they were involved in the process of bioclogging, they have shown significant reduction on hydraulic conductivity to 10^{-7} m/s through the precipitation of 1% of cation salts. The bioclogging was performed mainly in the channels between the sand particles as have shown by previous studies. Microscopic images produced by FESEM and EDX analysis revealed the presence of iron precipitate in sand pores (Figure 4.23 and 4.24). Subsequently, sand samples treated with ferrous sulfate and UPB exhibited significant increase of unconfined compressive (UC) strength. Drying and repeating applications of UPB and solution of urea and ferrous sulfate on sand greatly enhanced the strength of sample treated with targeted precipitated cation to sand ratio of 6% (w/w) to 402 kPa. However, in another study by Ivanov et al. (2012), the new method using EDTA ferric monosodium was adopted to instead use ferrous sulfate to stabilize the ferrous cations. It should be point out that in the method using ferric monosodium because of the relatively extra charge of ferric cation compared to ferrous, the precipitated products might be more in comparison with ferrous sulfate, and accordingly different cementation behavior was achieved.

3) Crust formation

Methods to construct test models in sand, based on an MICP approach with the lowest target of calcium carbonate to sand ratio (2.5% w/w) treated by the spray method have shown formation of 10 mm protective crust (Figure 5.12). The thickness of crust formation increased to 20 and 60 mm by increasing the targeted calcium carbonate to sand ratio of 5 and 7.5% w/w respectively. This can suggest that by increasing the number of treatment significant improvement could produce. However, the sand zone

below the crust remained untreated after the spray method. The test results indicated that the hydraulic conductivity of sand was reduced from the order of 10^{-4} m/s for clean sand to 10^{-7} m/s when an average 2.5 kg/m^2 of calcium were precipitated in sand through the spray method. The method of spraying biocement solution showed significantly minor calcium carbonate content compared to the percolation method in the depth of test models, determined by the results of ICP-OES test. Moreover, crystals of calcium carbonate are stabilized in the top 30 mm of the test model up to 50% more when treated by the spray method. Therefore, the findings on the hydraulic flume and geotechnical parameters after the bio-treatment of dikes using the spray method confirmed the potential application of engineered crust formation in erosion control of dikes as well as previous studies such as water-impermeable crust (Stabnikov et al. 2011).

4) Optimization of the percolation method in application for dikes

Test models were prepared to establish a method using the MICP process and the percolation method for enhancing the ability of a dike against erosion. The test model treated with a targeted calcium carbonate to sand ratio less than 2.5% (w/w) using the percolation method did not show significant strength in its crest, middle or base. However, increasing the targeted calcium carbonate to sand ratio upper than 5% and less than 7.5% (w/w) improved the sand properties up to the base (100-150 mm) of the dikes. Moreover, the calcite formed through the MICP process filled the pores in the sand and resulted in a substantial reduction in the hydraulic conductivity as well as an increase in the stiffness and shear strength of sand. Thus, the MICP process for enhancing the ability of a dike against erosion using the percolation method and the results of hydraulic flume, geotechnical parameters and calcium carbonate content analysis from this research exhibited the application of MICP process for improvement of unsaturated soil.

The results also show that the cementation depth is a factor of penetration depth of bacterial cells and cementation solution. This improvement was attributed by use of applying biocement solution through the percolation method. The percolation method results in homogeneous body cementation throughout the test model as well as a significantly higher calcium carbonate content in the layers from 100-150 mm compared to the spray method. Therefore, the method for applying the biocement solution and the calcium carbonate to sand ratio may be the most important factors affecting the results of UCS and hydraulic conductivity testing of dikes.

5) Optimization of application for dust suppressants

Two different data collection systems, namely wind erosion and dust concentration were used in this study to provide data and improve laboratory wind tunnel testing assessment of two different sand samples. Small quantity of less than 0.1% (w/w) calcite precipitation can cause bio-aggregation of sand as shown in the experiments of this study. The results of wind erosion measurement for bio-treated samples indicated an alternative approach with the concentration of approximately 36 times less than commercial concentration of calcium chloride and 85% higher effectiveness. Moreover, dust concentration data indicate that there is a substantial reduction in fugitive dust emission with application of biocement dust suppressants. The results indicated that the reduction of wind erosion and dust concentration was dependent on the formation of sand plates (Figure 6.7) on the surface of the sand samples. SEM images and data obtained from ICP-OES of the sand plates showed the formation of calcium carbonate between sand grains. The results also showed the effect of different calcium carbonate to sand ratios (% w/w) on the formation of sand plates.

7.2 SIGNIFICANCE OF RESEARCH FINDINGS

The urease enzyme and cell findings of this study contribute to the literature on the practical implications of MICP. It means a better performance of biocementation using a concentrated bacterial suspension or intact bacterial suspension rather than centrifuged suspension or a filtrate containing only dissolved enzymes. The results of the study on the optimization of calcium carbonate precipitation also contribute to the application of a low concentration of urease with a higher number of applications which could significantly increase strength for the stabilization of dikes. The MICP process for enhancing the ability of a dike against erosion using two method (namely the spray and percolation methods) contributes to the applications of MICP for improving the geotechnical parameters of unsaturated soil from both a research and practical engineering perspective. The results of this research, including the series of hydraulic flume tests, UCS tests, hydraulic conductivity tests and calcium carbonate content analyses will help researchers and engineers to understand and estimate the conditions and behaviors of dikes after bio-treatment.

This study has shown that the different methods for applying biocement solutions have different capacities for soil improvement. The improved understanding of the differences between the spray and percolation methods will help engineers to use the appropriate characteristics and parameters for applying biocement solutions to solve soil improvement problems. The differences between the characteristics of soil improvement (such as crust formation or depth treatment), UCS and calcium carbonate content (higher concentration in the crust) for the spray and percolation methods have been shown to be a major factor for practical design. The proposed methods in this work have shown applicability in practical tests, such as the percolation irrigation method. In addition, the findings on the geotechnical parameters of bio-treated specimens with the spray method confirm the potential application of engineered crust formation in erosion control of dikes. It should be pointed out that the most suitable method and targeted calcium carbonate content will vary depending on the application and can be adjusted to achieve the desired results. Therefore, the calcium carbonate content can provide important information to engineers when choosing an application method based on geotechnical problems.

The proposed MICP using a low concentration of urease with a higher incubation time (14 days) has been shown to be a dust suppressant and could produce a significant surface strength against the erosive force of wind. Therefore, this study contributes to the feasibility of biocementation of soil surfaces using a low dosage of biocement, about 100 g/m² of soil surface, which could be used for the following activities: (1) dust suppression in arid and semi-arid areas and (2) during industrial activities accompanied by dust formation.

In order to apply other cations than calcium in microbial improvement techniques, a novel area of study is created by investigating the mechanisms and potential applications of iron-based biocement. The precipitated iron salts can be considered a clogging and cementing compound called iron-based biocement. Thus, this study significantly contributes to innovative biocement production by the addition of urea and ferrous cation solutions after the application of UPB to sand samples. The source of ferrous cations in iron-based biogrout could be chemical reagents, such as ferrous sulfate. However, this research aims to decrease the environmental impact and cost of iron-based biogrout using chemical reagents by introducing an alternative biocement production method using microbial ferrous cations. The microbial ferrous cations are produced using iron-reducing bacteria (IRB). The possibility of increasing the shear strength of soil in the presence of ferrous cations was explored. The UCS

and hydraulic conductivity test results show that the proposed method is applicable for the reduction of the hydraulic conductivity of soil. It is necessary to mention that the uncertain behaviors and low strength of samples treated with low cation salts would affect the engineering properties of structures constructed/treated with iron-based biocement and this issue should be considered during design.

7.3 RECOMMENDATIONS

- 1) Strains of halophilic and alkaliphilic urease-producing bacteria with low protease activity have to be selected to ensure stability of biocementation rate. This was not investigated in this study and required further investigation.
- 2) If the time consumed by biocementation for some real geotechnical engineering application needs to be shortened due to engineering consideration, either a higher amount of starting volume of the culture liquid can be used or the urease of the culture liquid could be concentrated using nanofiltration technique. Nevertheless, the mechanism of the involvement of intracellular urease in the biocementation process was not evaluated in this study and requires further investigation.
- 3) Immobilization of bacterial cells by pretreatment with the solution of divalent and trivalent cations was studied. Further investigation on immobilization of cells without extra application of cations is considered worthwhile.
- 4) In the field application, the percolation method could be improved by use of alternative surface percolation devices such as pumping in the biocement solution through a hole and pumping out or percolation irrigation equipments. An additional advantage may be achieved with the biocement solution system by reuse of bacterial cells via recirculation. Because bacterial cells and in general urease is the most expensive consumable ingredient in the biocementation, the recirculation of bacterial cells represents an additional cost saving by decreasing the number of treatment.
- 5) Applicability of bio-treatment for dust control has to be examined for each individual case depending on the required mechanical strength of the treated soil and taking into account high sensitivity of biochemical reactions to environmental conditions. There are several problems exist in the system that has not been yet fully addressed as follows: 1) the application of MICP as a dust suppressant method in the present study only considered the surface change, and no field testing was completed to verify the

laboratory results. The biogROUT could be applied on the surface with two normal methods as conventional did, firstly by spraying and secondly mixing with layer of surface; 2) the effectiveness of new approach requires examination of both frequency of reapplication for longer time such as one year; 3) devices for accurate strength on the strength of surface treatment should be developed and studied; 4) for the application of bio-treatment as a dust suppressant, the technology of low cost dry biocement as a stable and an efficient geotechnical material has to be developed and considered worthwhile.

- 6) Significant reduction of hydraulic conductivity and lower cost of iron-based biocement by microbial production of ferrous cation with IRB are the reasons for using the potential geotechnical applications of the iron-based biocement which is not evaluated in this study and recommended for pilot test in future work. However, the urban geotechnical applications of iron-based biocement could be as follows: 1) to form grout curtains to reduce the migration of pollutants after accidental spill or leakage of toxic pollutant into permeable soil; 2) to diminish piping of earth dams and dikes; 3) to construct the reservoirs and ponds; 4) seepage control; 5) to fix leakages of ground water in underground constructions; 6) reduce corrosion of the pipelines; 7) land reclamation; 8) the sealing of the percolationpings in the tunnels; 9) the reduction of the liquefaction potential of soil. None of the applications of iron-based biocement was evaluated in this study and required further investigation.
- 7) During the hydrolysis of urea by UPB through MICP, the by-products (such as ammonia) may have toxic effects on human health (atmospheric nitrogen deposition) and the environment, leading to the eutrophication and acidification of sensitive ecosystems and discoloration of stones (Sutton et al., 2008; Tobleret al., 2011; Dhami et al., 2013). Therefore, additional research is necessary to overcome or manage these by-products. Various factors, such as fate and transport, are essential for the management of by-products. Additionally, it is important to verify the stability of treatment by the presence of by-products with time. Degradation of treatment can be categorized as geotechnical mechanical loading (e.g. an earthquake) or chemical/biochemical processes (e.g. dissolution by carbon dioxide rich waters) that may change the rate of chemical processes (Dhami et al., 2013). Due to the complexity of biogeotechnology, the observational method, rather than specific tests, may be appropriate for verification of the treatment. Further investigation regarding by-product management and treatment verification with time for various applications is recommended.

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APPENDICES

Appendix A	Ammonium Determination by a Modified Nessler Analysis
Appendix B	Optical Density and Dry Biomass Density
Appendix C	Particle size distribution
Appendix D	Microbial produced ferrous cation
Appendix E	Quantitative test for determination of ferrous cation concentration
Appendix F	Total Suspended Solids (TSS) dried at 103–105°C
Appendix G	Determination of unconfined compressive strength
Appendix H	Hydraulic conductivity test
Appendix I	Preparation of irregular by-shaped for hydraulic conductivity test

APPENDIX A

Ammonium determination by a modified Nessler analysis

Measuring the urease activity of solutions was done by checking the conductivity of different concentrations of NH_4Cl . Figure A1, A2 and A3 show the conductivity and concentration with R^2 value. By the calibration curve of three ranges of concentrations of NH_4Cl and conductivity, the required equation for calculation of urease activity was found. Concentration of NH_4^+ in sample was calculated by conductivity and nearest value of conductivity in one of the calibration curves. According to calculated concentration of NH_4^+ , concentration of urea and subsequently by considering time and dilution rate the urease activity was calculated.

Figure A1. Calibration curve for conductivity of 0.25-1M of NH_4Cl (ms/cm)

Figure A2. Calibration curve for conductivity of 0.025-0.1M of NH_4Cl (ms/cm) Figure A3. Calibration curve for conductivity of 0.001-0.01 M of NH_4Cl (mS/cm)

A linear relationship exists between absorbance and ammonium concentration (mM) in the range of each test mM NH_4^+ , which is expressed by the following Equations:

Appendix A

$$y = 0.0094x - 0.0216 \quad (R^2 = 0.9997) \quad (A1)$$

$$y = 0.0065x - 0.0027 \quad (R^2 = 0.999) \quad (A2)$$

$$y = 0.0048x - 0.0001 \quad (R^2 = 0.996) \quad (A3)$$

APPENDIX B

Optical density (OD) and dry biomass density

Dry Biomass density of grown culture was determined by measuring the optical density at absorbance of 600 nm. The procedure of this experiment is to prepare 4:1 bacterial suspension which was diluted by fresh medium. Then dilute this solution with fresh medium as described in Table B1 and Figure B1 below. After that measuring the optical density, biomass concentration via TSS was carried out.

Table B1: dilution of bacterial suspension and fresh medium for OD and biomass

Bacterial suspension	Fresh medium
5	2.5
5	5
5	10
5	20

Figure B1. Standard curve of dry biomass density versus optical density at 600 nm of the grown culture

Appendix B

A linear relationship exists between dry biomass density and optical density (600 nm) in the range of 0 – 1.22 mg/mL, which is expressed by:

$$Y = 1.0825x - 0.1181 \quad (R^2 = 0.992) \quad (B1)$$

APPENDIX C

Particle size distribution

Further investigation on size distribution of cells aggregation of 0.6 to 1.2 mm sand grains before and after pretreatment with 50mM of aluminum, ferric chloride and calcium chloride using Mastersizer 2000, was shown in Figure C1.

Figure C1. Formation of cell aggregates (a) before and after treatment of cultural liquid with (b) aluminum (c) ferric chloride and, (d) calcium chloride.

Size distribution of cells aggregation before and after pretreatment with different concentrations of calcium chloride using Mastersizer 2000, was shown in Figure C2.

Figure C2. Formation of cell aggregates after pretreatment with concentration of (a) 0.1 mM; (b) 1 mM; (c) 10 mM and; (d) 100M calcium solution.

APPENDIX D

Total suspended solids (TSS) dried at 103–105°C

A well-mixed sample is filtered through a weighed standard glass-fiber filter. Insert disk with wrinkled side up in filtration apparatus. Apply vacuum and wash disk with three successive 20-mL portions of reagent-grade water. Continue suction to remove all traces of water, turn vacuum off, and discard washings. Remove filter from filtration apparatus and transfer to an inert aluminum weighing dish. Dry in an oven at 105 to 110°C for 1 h. The increase in weight of the filter represents the total suspended solids (Tress 1978).

$$\text{Total suspended solid (mg)} = ((A - B) \times 1000) / (\text{sample volume, ml})$$

Where *A* is weight of filter plus dried residue (mg) and *B* is weight of filter (mg).

APPENDIX E

Microbially produced ferrous cation

APPENDIX F

Quantitative test for determination of ferrous cation concentration

A modified Phenanthroline Method was used for determination of total and dissolved ferrous cation content. Three molecules of phenanthroline chelate each atom of iron to form an orange-red complex. The colored solution formed obeys Beer's Law and its intensity is independent of pH from 3 to 9.

For calibration curve, the stock solutions of sample with different known concentrations was prepared (2.5, 5, 10, 25, 50, 100, 120 mg/l) and tested as shown in Table E1. Then 2.5 ml of stock solution with 5 ml of phenanthroline and 2.5 ml of ammonium buffer solution were mixed. After that, the mixture was diluted to 25 ml with distilled water. The absorbance of solution was measured using the Shimadzu UV/V spectrometer. For different concentrations of stock solutions of sample this procedure must be repeated.

Table E1. Iron concentration vs. absorbance

Iron conc. (mg/L)	0	2.5	5	10	25	50	100	120
Absorbance	0	0.053	0.105	0.204	0.501	1.006	1.979	2.29

Figure E1. Plot of calibration iron, concentration vs. Absorbance

After the calibration curve was prepared (Figure E1), the total and dissolved ferrous cation of each sample was measured. At first 0.2 HCl was added to 10 ml of centrifuged sample and wait for at least 30 minutes. These samples filtered through 0.45 μm membrane filter. Subsequently, 2.5 ml of filtered sample was added into tube containing 5 ml of phenantroline and 2.5 ml of ammonium buffer solution. Then dilute this sample to 25 ml with distilled water and shake vigorously and were prepared as shown in Figure E2. Further dilution should be performed when necessary. Color intensity was measured using UV/Vis spectrophotometer. Ferrous cation content from iron calibration curve was determined by the method described by Greenberg et al. 1992.

Figure E2. Prepared solution for measuring ferrous cation concentration using UV/Vis spectrophotometer

APPENDIX G

Determination of unconfined compressive strength

The test procedure of D2166 according to the ASTM 2003 is followed. The schematic layout of triaxial apparatus is shown in Figure G1. The unconfined compressive strength (UCS) q_u is the load per unit area at which the cylindrical specimen of a cohesive soil falls in compression. The compression device was a compression testing machine, electronically controlled, with the capability of infinite rates of strain and stress loads (Figure G1). In this test method, UCS is taken as the maximum load attained per unit area or the load per unit area at 15 % axial strain, whichever is secured first during the performance of a test. Shear strength (S_u)—for UCS test specimens, the shear strength is calculated to be 1/2 of the compressive stress at failure.

The axial strain, ε , to the nearest 0.1 %, for a given applied load was calculated, as follows:

$$\varepsilon = \Delta L / L_0 \times 100$$

Where ΔL is length change of specimen as read from deformation indicator or computed from the electronic device (mm) and L_0 is initial length of test specimen (mm).

Figure G1.UCS apparatus

The average cross-sectional area, A , for a given applied load was calculated, as follows:

$$A = A_0 / ((1 - \varepsilon / 100))$$

Where A_0 is initial average cross-sectional area of the specimen (mm^2) and ε is axial strain for the given load (expressed as a decimal).

The compressive stress, σ_c , to three significant Figures or nearest 1 kPa (0.01 ton/ft²), for a given applied load, was calculated as follows:

$$\sigma_c = (P/A)$$

Where P is given applied load (kN) and A is corresponding average cross-sectional area (mm^2).

APPENDIX H

Determination of hydraulic conductivity

The idea of this test method is to establish steady-state flow condition in a cylindrical specimen by maintaining a pressure gradient at two ends. The tests were conducted in a triaxial cell test. The test procedure D5084 according to the ASTM 2003 is followed with the method reported by Carpenter and Stephenson (1986). For example 45 kPa and 40 kPa at bottom and top of specimen, respectively, were targeted by two GDS machines, thus provided a pressure difference of 5 kPa. However, only 2-3 kPa could be maintained during the tests. A radial confining pressure of 50 kPa was kept around the specimen by another GDS, in order to avoid the effect of permeate flow along the interface between the ring and sample, which usually occurs in ordinary hydraulic conductivity test (Chu et al. 1992, Briaud et al. 2008). The volume of effluent was read directly from the top GDS. Once steady-state flow conditions were obtained, the intrinsic hydraulic conductivity can be calculated (CRD-C 163-92, 1992). The schematic of hydraulic conductivity test and the equipment is shown in Figure H1.

Figure H1. Saturated triaxial apparatus and schematic diagram of hydraulic conductivity test

Calculation of hydraulic conductivity was based upon Darcy's law as follows,

$$k_v = 1.63qL / (A\{(p_1 - p_2) - p_c\}) \times R_t \times 10^{-4}$$

Where k_v is hydraulic conductivity (m/s), q is mean flow rate (ml/min), L is specimen height (mm), A is mean cross-sectional area (mm²), $(p_1 - p_2)$ is pressure difference (kPa), p_c is static pressure corrector (kPa) and R_t is temperature correction factor.

APPENDIX I

Preparation of irregular by-shaped specimens for hydraulic conductivity test

As shown in Figure I1, specimens were wrapped using impermeable modeling clay to make uniform cylinders. Mean cross sectional area of the sample can be calculated by removing the sample and measuring the weight of water in the hole after test. Procedures for hydraulic conductivity tests of specimens having an irregular cross section are shown in Figure I1.

Figure I1. Procedures for hydraulic conductivity tests of specimens having an irregular cross section

Publications

Journal Paper

1. Chu, J., V., Ivanov, **M., Naeimi**, V., Stabnikov, & H., Liu, (2014). "Optimization of calcium-based bioclogging and biocementation of sand." *Acta geotechnical*, Volume 9, Issue 2, pp 277-285.
2. Chu, J., V., Ivanov, V., Stabnikov, J., He, B., Li, & **M., Naeimi**, (2012). "Biocement: green building- and energy-saving material." *Advanced Materials Research*, Vol. 347– 353, pp. 4051– 4054.
3. Stabnikov, V., **M., Naeimi**, J., Chu, & V., Ivanov, (2011). "Formation of water impermeable crust on sand surface using biocement." *Cement and Concrete Research*, Vol. 41, pp. 1143-1149.

International Conf

1. **Naeimi**, M., J., Chu, V., Ivanov, & V., Stabnikov, (2012). "Improvement of engineering properties of granular soil using biocement." Ground Improvement and Ground Control, Wollongong, Australia.
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