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A Dissertation Submitted in Partial Fulfillment of the Requirements for the Degree of Doctor of Philosophy in Engineering

Experimental Investigation on the Effect of Biogrouting by Using Different Calcium Sources on the Improvement and Performance of Poorly Graded Fine Sand

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Abstract

Nowadays, it has become the establishment of infrastructure in the lands previously was considered inappropriate an order cannot be avoided, as a result of the rapid growth of cities. To improve the engineering properties of these lands, various techniques have been applied, such as vibroflotation, dynamic compaction, and composite foundations. Recently, an innovative and sustainable technique called Microbial Induced Calcite Precipitation (MICP) has emerged for soil improvement.

Microbial Induced Calcite Precipitation (MICP) is commonly carried out by injecting chemical solutions (e.g., urea and calcium source) and bacteria (e.g., Sporosarcina pasteurii, B. megaterium) to the soil matrix where treatment is required several times. MICP researches were aiming to improve various engineering properties of the soil such as reduction of soil hydraulic conductivity, improvement in the stiffness and strength of sandy soil, liquefaction mitigation, the remediation of cracks in building materials.

A number of factors must be considered to enable the use and control of the MICP process in field applications, including the concentrations of bacteria solution, the concentrations and type of the chemical solutions, in addition to methods to introduce the bacteria and these chemical solutions to the soil, pH, etc.

The objective of this study is to evaluate the performance of the Microbial Induced Calcite Precipitation as a technique to improve and enhance the very fine poorly graded sandy soils, and expand knowledge about improving a very soft soil by using MICP technique. Furthermore, to investigate the efficiency of MICP treatment by using a new cementation solution consists of remnants of the food industry.

Firstly, experimental study to explore the effectiveness of the MICP technique for improving the engineering properties of the poor graded fine sandy soil was conducted. The influence of factors such as grain size distribution and initial water content of untreated sand on the effectiveness of the MICP technique was investigated. The results

indicate that MICP is effective for this type of sand. The results also demonstrate that the use of MICP is more effective for sand with an an initial water content of 0 (i.e., dry) with respect to increasing the strength, while the MICP is slightly better for sand with an initial water content of 100 (i.e., saturated) for the purpose of decreasing the permeability.

Secondly, experimental study to explore a new source of calcium chloride was conducted. The new source was the egg shells. It has been exploring the feasibility of using a new source of calcium in improving the engineering properties of two different types of fine sand (river and silica sands). Also, explore the impact of the sand type on the results of MICP. The results indicate that the use of calcium chloride made of egg shells has the same effectiveness of that of pure calcium chloride (analytical grade) in the cementation solution. This was demonstrated clearly by measure the Precipitated Calcium Carbonate content, where the same amount of Calcium Carbonate of both calcium sources was precipitated. But it was slightly higher in the river sand.

In both cases (river and silica sands samples) the use of cementation solution contained calcium chloride made of egg shells has a significant effect on the permeability, but the effect was greater in the silica sand samples. Also, the effect of using cementation solution contained calcium chloride made of egg shells was exactly the same effect of using cementation solution contained analytical grade of calcium chloride. Finally, from SEM images, the calcium carbonate type in all cases was the calcite and the size of crystals almost the same. But the crystals type of calcite was changed according to the type of sand.

Finally, an experimental study to explore the effect of the use of six types of calcium sources in the cementation solution, two concentrations of cementation solution as well as two types of soils on the efficiency of MICP technique was conducted.

Keywords: microbial-induced calcite precipitation sand soil improvement permeability strength

摘 要

由于当前城市快速发展，不可避免地穿越一些不适宜基础设施建设的不良地基。因此，需采用多种多样的地基处理技术，如振冲法、强夯法和复合地基法等以改善不良地基的工程特性。近年来，在地基处理领域兴起了一种新型、可持续发展的技术——微生物固化技术（MICP）。

微生物固化技术（MICP）通过向土体内多次注入化学溶液（譬如尿素和钙源）和细菌（譬如巴氏芽孢杆菌）。目前，对于 MICP 技术的研究主要改善土体的工程特性，如减小渗透性、提高土体的刚度和强度、提高抗液化性能及建筑材料裂缝修补等。

MICP 技术应用于实际工程时需要考虑多种因素，包括菌液的浓度、化学溶液的浓度及类型以及向土体内注入细菌和化学溶液的方法以及溶液的 pH 值等。

本文主要评估 MICP 技术改善不良级配砂土，以及将 MICP 技术用于改善超软土体。此外，使用食品废弃物制备胶结溶液进行 MICP 处理以研究 MICP 技术的有效性。

首先，开展试验研究 MICP 技术在改善级配不良砂土工程特性的有效性。基于砂土颗粒级配及未固化砂土初始含水量等因素探讨各因素对 MICP 技术有效性的影响。试验结果表明，MICP 技术用于处理该种类型不良砂土是有效可行的。当未固化砂土初始含水量为零（即处于完全干燥状态）时，MICP 技术在改善砂土的强度方面更为有效；当砂土初始含水量为 100%（即完全饱和状态）时，MICP 技术在减小砂土渗透性方面效果较好。

其次，开展试验研究一种新型的氯化钙——钙源取自鸡蛋壳。分别采用该新型钙源以及商业氯化钙（分析纯）作为钙源固化 2 种不同类型的细砂（即河砂和硅砂）以验证该新型钙源用于微生物固化技术的可行性。同时，分析砂土类型对 MICP 技术的影响。通过分析 MICP 过程中产生的碳酸钙晶体量，可以发现使用 2 种钙源可产生相同量的碳酸钙晶体，表明由鸡蛋壳制备的氯化钙溶液与纯氯化钙（分析纯）

制备的溶液有相同的效果。然而在分析土体类型的影响时，发现河砂中微生物过程诱导产生的碳酸钙晶体量较高。

由鸡蛋壳制备的氯化钙溶液均会显著影响微生物固化河砂和硅砂试样的渗透性，但对于硅砂试样的影响更为显著。此外，使用鸡蛋壳制备的氯化钙溶液与商业氯化钙（分析纯）制备的氯化钙溶液有相同的效果。由扫描电镜（SEM）图可知，所有研究的工况中细菌诱导产生的碳酸钙晶体均为方解石，且晶体尺寸几乎相同，然而方解石晶体类型因固化砂土类型不同而变化。

最后，基于 6 种不同钙源、2 种胶结液浓度以及 2 种类型土体开展微生物固化技术有效性试验研究。

关键词： 微生物诱导碳酸钙沉积　砂土　土体改良　渗透性　强度

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1 Introduction

1.1 Background

Cementation is an important method for soil improvement. Cementation, whether naturally occurring or added by man-made activities, has been shown to increase the ability of sands to resist failure when subjected to static or dynamic loading. Natural cementation happens because of bonding by clay particles, chemical deposition or weathering by-products. Artificial cementation mostly includes the injection of colloidal silica grout, Portland cement, or gypsum. In general, a diversity of chemical, jetting, and permeation grouting techniques are used to distribute the artificial grouts in the subsurface. Historically, some artificial grouts have caused environmental concerns due to detrimental effects on groundwater quality from grout constituents (Karol, 2003)[1]. In addition, the limited injection distance for chemical grouting or super-jet grouting is another concern when designing for ground improvement.

Recently, bio-mediated methods have been developed as promising alternatives for ground improvement. As a new approach, microbial induced calcite precipitation (MICP) soil improvement method has attracted great interest. MICP soil improvement broadly refers to “a chemical reaction network that is managed and controlled within soil through bacteria activity and whose byproducts alter the engineering properties of soil” (DeJong et al., 2010)[2]. Main processes involved in MICP soil improvement methods include biocementation, bioclogging and biogas production. The main effects of the MICP treatment include increasing the stiffness and shear strength of sandy soil, reducing the permeability of sand, decreasing the compressibility of clayey soil, and increasing the liquefaction resistance of sand.

Geometric compatibility between the microbes and soil particles is essential to the

effectiveness of the MICP treatment. The preferred size of bacteria usually 0.5 to 3 micrometers in length because this size allows the bacteria to move freely through the pores between the soil particles. Most of the MICP methods may be suitable for sand. However, as pore sizes of clay may be too small for the delivery of bacterial cells, the application of MICP methods in clay is not an easy task. Therefore, a small number of researches were conducted on MICP application in clay.

Recent researches have shown that many types of bacteria have the ability to produce biocementation. These include urease producing bacteria, iron reducing bacteria, nitrifying bacteria, oligotrophic microaerophilic bacteria, sulfate reducing bacteria, and dimorphic phytase-active yeast for the production of calcium-phosphate precipitation (DeJong et al., 2006; Ivanov and Chu, (2008); Chu et al., 2009; Roeselers and van Loosdrecht, 2010)[3-6]. The MICP process has been identified as the most effective approach for biocementation and bioclogging. Several groups of organisms can induce MICP through their metabolic processes. The most common one is urease producing bacteria. It produces MICP through hydrolysis of urea. Heterotrophic organisms, like sulfate-reducing bacteria, can also induce MICP by reducing sulfate to H_2S and releasing HCO_3^- if dissolved gypsum ($CaSO_4 \cdot 2H_2O$) presented in the environment. When there is a calcium ion at a time of hydrolysis process results in the production of calcium carbonate and ammonium (Stocks-Fischer et al., 1999; Fujita et al., 2000)[7-8].

Numerous factors may influence the effectiveness of MICP. These factors include urease activity, distribution and fixation of bacteria, chemical solutions type and concentration, retention time, properties of parent soil (including size, textures, interaction with water, etc.), pH, temperature, etc. The effects of biocementation and bioclogging of soil are mainly assessed by measuring the unconfined compressive strength and coefficient of permeability of soil respectively.

1.2 Benefits and Application Fields of MICP

MICP researches were aiming to improve various engineering properties of the soil. For example Nemati et al. (2005)[9] investigated the reduction of soil hydraulic conductivity, Rong et al. (2012)[10]; Van Paassen (2009)[11] and Whiffin et al., (2007)[12] targeted improvement in the stiffness, DeJong et al. (2010)[2] focused on reducing the permeability.

MICP has been proposed for several geotechnical engineering applications, these applications involve enhancement in the durability of cementitious materials, highly durable bricks, repair of limestone monuments, sealing of concrete cracks and improving sand properties.

The researchers proposed the following applications:

- Reduction in foundation settlement (DeJong et al., 2010)[2].
- Strengthening of concrete and remediation of cracks (Achal et al., 2011)[13].
- Soil stabilization prior to tunneling construction (DeJong et al., 2009)[14].
- Wastewater treatment (Hammes, 2003)[15].
- Improvement in the stiffness/strength of sandy soil (Rong et al., 2012; Van Paassen, 2009; Whiffin et al., 2007)[10-12].
- Liquefaction mitigation (Montoya et al., 2012)[16].
- Reduction in soil permeability (Nemati et al., (2005)[9]; Dennis and Turner, (1998)[17]; Seki et al., (1998)[18]).
- Microbially enhanced oil recovery (Nemati et al., 2005)[9].
- Erosion control and dust control (Meyer et al., 2011)[19].
- Slope stabilization (DeJong et al., 2009)[14].
- Piping prevention for dams, levees (DeJong et al., 2009)[14].

1.3 Significance of this Study

An intensive study on the effect of Microbial Induced Calcite Precipitation technique on the engineering properties of poorly graded fine sand has been conducted. Furthermore, a new source of calcium through the use of industrial waste is obtained. This study drives the adoption of MICP technology on a large scale by reducing the cost of materials used in it.

1.4 General Objective

The general objective of this study is to evaluate the performance of the Microbial Induced Calcite Precipitation (MICP) as a technique to improve and enhance the very fine poorly graded sandy soils, and expand knowledge about improving a very soft soil by using MICP technique.

1.5 Specific Objective

To evaluate the efficiency of MICP treatment, the comparison between the engineering properties of the soils before and after treatment will be conducted. To achieve this purpose, the following objectives will be targeted:

- To investigate the efficiency of MICP treatment as soil improvement technique on very fine poorly graded silica sand and fine river sand.
- To investigate the effects of the MICP soil improvement technique on compressive strength and permeability of the very fine poorly graded silica sand and fine river sand.
- To investigate the efficiency of MICP treatment by using a new cementation solution consisting of remnants of the food industry.
- To examine the relationship between calcite content and enhanced engineering properties in the MICP treated very fine poorly graded silica sand and fine river sand.

- To identify the effects of soil particle sizes and voids on microbial cementation.

1.6 Scope of this Study

The scope of this study is focusing on using Microbial Induced Calcite Precipitation (MICP) method, which is considered as a ground improvement technique to enhance the engineering properties of soils. The applicability of microbiological processes to soil improvement will likely depend on a variety of factors, comprising the type of microbial metabolism desired, interactions with other microbes existing in the environment, soil type, available nutrients, depth below ground surface, pH, temperature, pressure, concentration of ions, and the availability of oxygen and other oxidants. A series of tests will be conducted to investigate the effect of several parameters on bio-cementation, including the grain size, urease activity, calcite content, distribution of cementitious materials, types of nutrients.

1.7 Organization of this Dissertation

This dissertation comprises a total of seven chapters.

Chapter one includes a brief background of this study of the soil improvement technique, benefits and application fields of MICP, the significance of this study, the main and specific objectives, the scope of this study, and the dissertation organization.

Chapter two reviews the literature and previous work on the existing ground improvement technologies, specifically the emergence of this new viable and feasible technology of microbial induced calcite precipitation in the construction industry, especially its application in the geotechnical aspects, some key parameters to mechanisms of MICP, and the mechanisms of soil improvement by MICP.

Chapter three is about bacteria cultivation method. Besides the traditional method of preparation of the solution, the new method involves the use of remnants of the food industry, sample preparation method and the experimental setup for the different type of

soil properties tests; the experimental method is also discussed in this chapter.

Chapter four discusses the feasibility of using microbial induced calcite precipitation to improve the engineering properties of poorly graded fine sand. The influence of some factors such as grain size distribution, the initial water content of untreated sand and curing conditions on the effectiveness of the MICP technique are investigated.

Chapter five is about the exploration the influences of calcium chloride type and sand type on microbially induced carbonate precipitation. Calcium chloride from a new source was made. This chapter investigates and compares the effects of the new and old calcium sources and types of soil, on the calcium carbonate content, the crystal formation, the strength and permeability.

Chapter six is about the investigation of the influences of calcium sources on microbial induced carbonate precipitation. This chapter aims to study the effect of four different calcium sources, the concentration of the cementation solution, the soil type, and the use of cementation solution which consists of a combination of calcium sources, in the calcium carbonate content, the crystal formation, pH, the porosity and the strength.

Chapter seven concludes the significant results and recommendations for future research achieved by the author.

2 Literature Review

2.1 Introduction

This chapter presents the general overview of the literature relevant to MICP technique in the construction industry, especially the geotechnical aspects (soil improvement) performed by different researchers. This chapter begins with a description of the traditional ways to ground improvement, and then covers the emergence of the new technique (MICP). An overview of urease producing bacteria strain and its production in previous researches and describing biochemical reactions related to MICP treatment are some targets of this chapter. The most important key factors which have a significant impact on the MICP will be discussed briefly. At the end of this chapter, the mechanisms of soil improvement by MICP (Biocementation, Bioclogging, Biogas) and investigating the ability to use MICP technique to improve diverse building materials will be discussed.

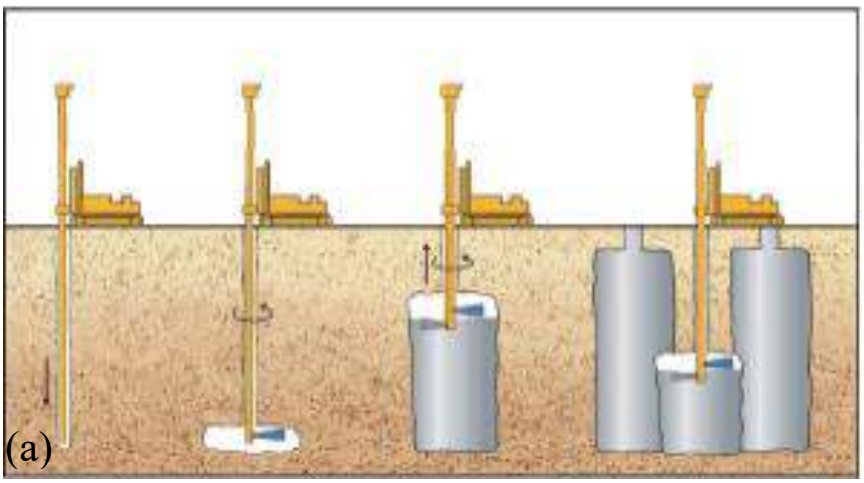
2.2 Ground Improvement

Because of the rapid growth of cities in the present days, it cannot be avoided to construct infrastructure in week soils. The problematic soils are generally described by low strength and high compressibility (Huat, 2006; Kazemian et al., 2011; Ho and Chan, 2011)[20, 21, 22]. Therefore, development of the problematic soils is prone to considerable geological and engineering risks, including liquefaction of loose sediments, excessive settlement of the embankment or foundation, soil erosion, debris flow, and catastrophic landslide.

Therefore, to improve the engineering properties of these lands, stabilization of soil (ground improvement) may be required to face these risks. Before and during construction, soil stabilization is often applied from the surface by using various

techniques such as dynamic compaction, installing nails, sheets or piles, or mixing the soil with lime or cement (Karol, 2003)[1] (Figure 2-1). When stabilization of soil mass is required deeper in the underground, these surface techniques are insufficient and strengthening techniques, such as vibroflotation, deep mixing cement, composite foundations, ground freezing or chemical grouting are being used (Figure 2-2).

Figure 2-1 Shallow stabilization (dynamic compaction)



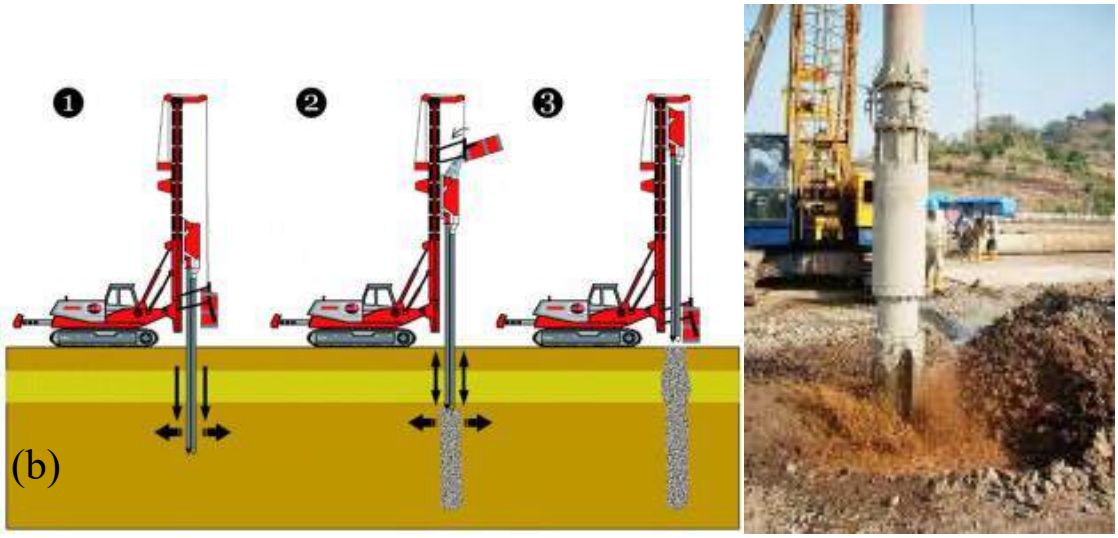


Figure 2-2 Deep stabilization (a) chemical grouting (b) vibroflotation

There are several limitations of traditional methods of ground improvement. In deep-mixing method, the radius of action of the mixing equipment is limited. Freezing is a temporary solution and is useful only during the construction process. The chemical grouts injection process requires high pressures because of their high viscosity or fast hardening time. Moreover, most of these methods are expensive, containing chemicals with a significant impact on the environment (DeJong et al., 2006)[3], the need for large equipment to be implemented, disturbing urban infrastructure. Because of all these limitations, the traditional methods are not suitable for treating large volumes of soil.

Recently, an innovative and sustainable technique called Microbial Induced Calcite Precipitation (MICP) has emerged for soil improvement.

2.3 Microbially Induced Carbonate Precipitation (MICP) (Emergence and Development)

With the growing awareness of environmental hazards as a result of human activities, environment-friendly technologies in improving the soil become an urgent need. Using the biological activity to improve soil properties can meet the requirements of society for ground improvement in a natural manner.

In nature, many types of bacteria can catalyze chemical reactions in the suitable subsurface resulting in precipitation of inorganic minerals, which change the physical and mechanical properties of the soil. One of these chemical reactions is microbially induced calcium carbonate precipitation.

A significant portion of the inorganic carbon on the earth surface is of biogenic origin (Ehrlich, 1996)[23]. Precipitation of calcium carbonate occurs when the amount of calcium and carbonate ions in solution exceeds the solubility product, i.e. the solution gets oversaturated.

To simulate microbially induced calcium carbonate precipitation in the nature, special types of bacteria have to be injected and transported over a substantial distance into the porous material, accompanied by injecting of chemical solutions. Transport of bacteria (and hence bacterial activity) is limited in fine-grained soils. And because the bacteria have a typical size of 0.5 μm to 5 μm, they cannot be transported through silty or clayey soils, consequently, their activity cannot be used to induce carbonate precipitation in these soil layers (Mitchell and Santamarina, 2005) [24].

The beginning was slow, and very few studies have been done by precipitating calcite inside a soil to prepare artificially cemented soils (Ismail et al., 2002)[25]. Akili and Torrance (1981) [26] carried out the first study based on the precipitation of calcite cement from calcite-enriched water, through evaporation. They pumped the calcite-enriched water continuously into the dry sand in a container with a diameter of 200 mm. After three months of treatment, the amounts of calcium carbonate depositions were not significant. Akili and Torrance (1981)[26] made another attempt, they found that the use of chemical reaction to create calcite was working successfully, but their application was limited to measuring the increase of cone resistance, and they did not try to use the calcite in the testing of soil elements. Cailleau (1982)[27] and Molenaar and Venmans (1993)[28] developed another method based on circulating a supersaturated solution of calcium

carbonate through a porous deposition. In 2001 in Australia, the microorganism was used to harden and reinforce sand, and renovate monuments (Van Paassen, 2011)[29]. Then, Starting from the sand and bacteria, the Australian research group worked to make a column of sand stone (Kucharski et al., 2006)[30]. Van Paassen (2011)[29] reported that despite the fact that most of the biogrouting tests were carried out in sand, the first experimental application was performed in coarser material by drilling company Visser and Smit Hanab. MICP technique was used to solve leakages problem at Amsterdam-Paris high speed railway system in 2005, at that time MICP technique was called Bio Sealing (Van Beek et al., 2008)[31].

Over the years before 2008, as a new soil improvement technique, MICP was applied in a laboratory scale and mainly focused with sands. It can be seen from Figure 2-3 that within five years of laboratory experiments, it has been scaled up from 0.01 m to 1.0 m laboratory column samples (one-dimensional) and from 1.0m^3 up to the size of 43 m^3.

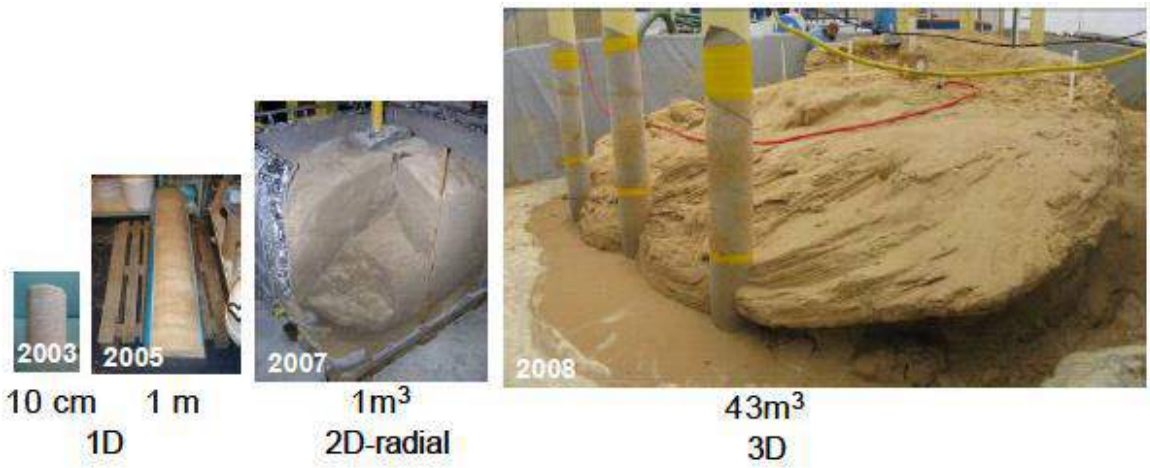


Figure 2-3 Ground improvement method based on MICP was scaled up from laboratory column to a 100 m³ experiment (Van Paassen, 2011)[29].

Many researchers such as Vandevivere and Baveye (1992)[32] , Baveye et al. (1998)[33] , Le Métayer-Levrel et al. (1999)[34] , Mitchell and Santamarina (2005)[24] ,

Lian et al. (2006)[35], DeJong et al. (2010)[2] , Harkes et al. (2010)[36] , Meyer et al. (2011)[19] , Yasuhara et al. (2011)[37], Achal, et al. (2011)[38], Rong and Qian (2012)[39], Ivanov et al. (2012)[40] , Neupane et al. (2013)[41] , Soon (2013)[42] , Cheng et al. (2014)[43] , Eryürük et al. (2015)[44], Sharma and Ramkrishnan (2016)[45] have focused on using MICP technique to improve the engineering properties of soft ground and building material.

2.4 Microbially Induced Carbonate Precipitation (MICP) Using Urease Producing Bacteria

Calcium carbonate ($CaCO_3$) is considered to be one of the most common minerals on the earth. It is naturally present in different environments, like fresh water and sedimentary rock, including marble, limestone, calcareous sandstone (Klein and Hurlbut, 1998; Hammes et al., 2003)[46, 47]. The precipitation of calcium carbonate is ruled by four parameters: the concentration of calcium, the concentration of carbonate, the pH of the environment, and the presence of the nucleation sites (Hammes et al., 2003)[47].

Several groups of bacteria can induce MICP through their metabolic processes. One of these groups of bacteria is photosynthetic bacteria; it produces MICP by utilizing dissolved CO_2 in water and thus shifting the equilibrium of HCO_3^- and CO_3^{2-}, while increasing pH value (McConnaughey and Whelan, 1997; Ehrlich, 1998)[48, 49]. Heterotrophic bacteria, like sulfate-reducing bacteria, can also induce MICP by reducing sulfate to H_2S and releasing HCO_3^- if dissolved gypsum ($CaSO_4 \cdot 2H_2O$) presented in the environment. MICP can also be produced by bacteria involved in the nitrogen circle. Hydrolysis of urea is the most easily controlled and widespread reaction that occurred in these bacteria, which results in the production of carbonate ions and ammonium (Mobley and Hausinger, 1989; Stocks-Fischer et al., 1999; Fujita et al., 2000)[7, 8, 50]. MICP then occurs in the presence of calcium ions. Reactions involved in the three groups of bacteria are shown in Table 2-1.

Urea hydrolysis was promoted by bacteria through urease production, which is an enzyme that catalyzes the hydrolysis of urea into carbon dioxide and ammonia. Sporosarcina pasteurii, which is one type of alkalophilic urease-producing bacteria, is usually adopted in biocementation treatment, due to its high activity, non-repressed by NH_4^+, and its non-pathogenicity (Whiffin, 2004)[51]. As shown in Table 2-1, Sporosarcina pasteurii uses urea as energy sources, releasing ammonium and carbonate/bicarbonate as byproducts, also, creating an alkaline environment for the preparation of MICP. The negatively charged cell surfaces might attract the free calcium cation, as a result of this process, calcite could be formed by using cells as the nucleation center.

Table 2.1 Reactions involved in three major groups of organisms than can produce MICP (after McConnaughey and Whelan, 1997; Ehrlich, 1998; Mobley and Hausinger, 1989; Fujita et al., 2000) [8, 48-50]

MICP producing bacteria	Reactions involved
Photosynthetic bacteria	$CO_2 + H_2O \rightarrow (CH_2O) + O_2$ $2\ HCO_3^- \leftrightarrow CO_2 + CO_3^{2-} + H_2O$ $CO_3^{2-} + H_2O \rightarrow HCO_3^- + OH^-$ $Ca^{2+} + HCO_3^- + OH^- \rightarrow CaCO_3 + 2H_2O$
Heterotrophic bacteria	$CaSO_4 \cdot 2H_2O \rightarrow Ca^{2+} + SO_4^{2-} + 2H_2O$ $2(CH_2O) + SO_4^{2-} \rightarrow HS^- + HCO_3^- + CO_2 + H_2O$ $Ca^{2+} + HCO_3^- + OH^- \rightarrow CaCO_3 + 2H_2O$
The bacteria involved in the nitrogen cycle (hydrolysis of urea, this study)	$CO(NH_2)_2 + 2H_2O \rightarrow 2NH_4^+ + CO_3^{2-}$ (Hydrolysis of urea) $2NH_3 + 2H_2O \rightarrow 2NH_4^+ + 2OH^-$ (pH increase) $H_2CO_3 + 2OH^- \rightarrow HCO_3^- + H_2O + OH^-$ $HCO_3^- + H_2O + OH^- \rightarrow CO_3^{2-} + 2H_2O$

	$Ca^{2+} + CO_3^{2-} \rightarrow CaCO_3$	(Precipitation of Calcite)

The overall conductivity of the solution increases at a rate linearly proportional to active urease presence because hydrolysis of urea can liberate ionic products (NH_4^+ and CO_3^{2-}) from non-ionic substrates (urea, $CO(NH_2)_2$) (Hanss and Rey, 1971; Grunwald, 1984)[52, 53]. Determination of urease activity can thus be transferred to measuring the increase of conductivity in the bacteria sample, in the presence of urea.

2.5 Overview of Urease Producing Bacteria Strain and Its Production in Previous Researches

The researchers used sporosarcina pasteurii extensively in their research, especially strain ATCC 11859, due to its tolerant and alkali-philic characters (Ferris et al., 1997; Bang et al., 2001; Bachmeier et al., 2002; Mitchell and Ferris, 2005; DeJong et al., 2006; Whiffin et al., 2007; Mortensen et al., 2011)[3, 12, 54-58]. Furthermore, it can produce high urease activity, not repressed by NH^{4+}, while being non-pathogenic (Whiffin, 2004)[51]. Urease activity of different strains of S. pasteurii used in these references varies from 5 to 20 mM urea/min (Harkes et al., 2010)[36]; 2.2 to 13.3 mM urea/min (Whiffin, 2004)[51]; average 11.3 mM urea/min (Cheng, 2012)[59].

Some other isolated Bacillus strains of urease producing bacteria have urease activity varied from more than 3.3 mM urea/min (Al-Thawadi et al., 2012)[60]; 8.3±0.1 mM urea/min for strain VS1, and 9.0±0.0 mM urea/min for stain VUK5 (Stabnikov et al., 2013)[61].

The factors which could affect the production of urease producing bacteria includes, calcium concentration, urea concentration, pH value, temperature, soil type, etc. (Fujita et al., 2000; Stocks-Fischer et al., 1999; Van Paassen, 2009; Whiffin, 2004)[7, 8, 11, 51]. pH/urease activity vs. time in bacteria cultivation and long-term urease activity in

preserved urease producing bacteria sample are shown in Figure 2.4a and 2.4b respectively (Whiffin, 2004)[51].

As shown in Figure 2-4a, pH value was observed to increase from 7 to 9 plus during the first 24 h of cultivation; urease activity and biomass of bacteria kept increasing, yet the highest specific urease activity (urease activity/biomass) occurs at about 8 h. It has also been reported that increase in temperature up to 60°C results in an increase in urease activity (Van Paassen, 2009; Whiffin, 2004)[11, 51].

A few types of known commercial bacteria are used by researchers for their ability to produce a urease enzyme, such as Bacillus megaterium; largely found in natural tropical soil (soon et al. 2014)[62].

Some have utilized B. megaterium in other applications of MICP such as improving concrete strength as well as its durability (Achal et al. 2011)[13] such as Siddique et al. (2008)[63] who used Bacillus megaterium and showed improvement in compressive strength in cement mortars. B. pasteurii was also employed in some of the previous studies (Qian et al., 2009, 2010)[64-66].

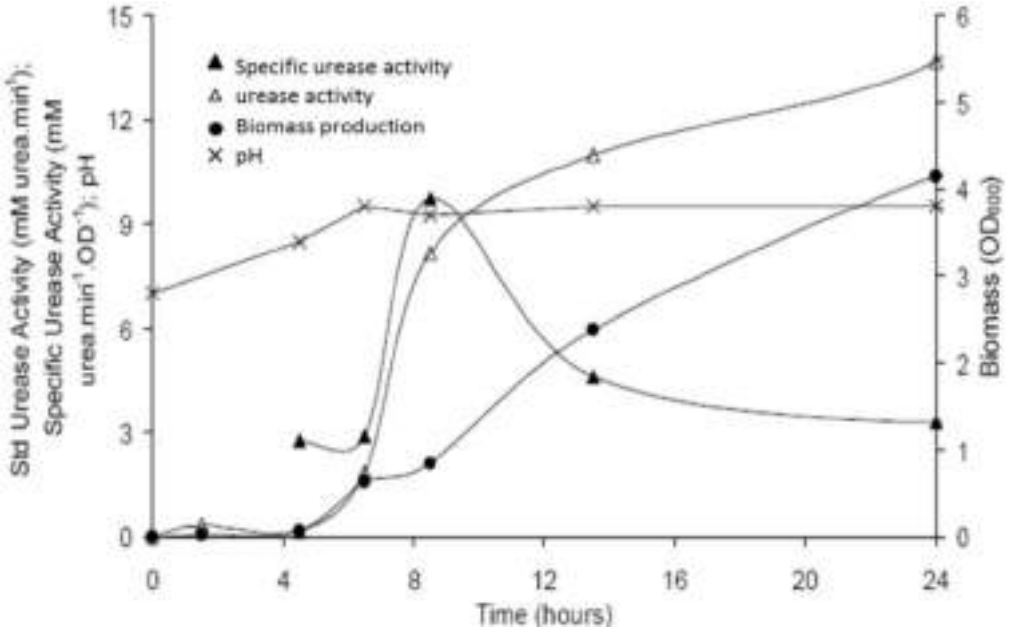
Besides the family of Bacillus sp., there are other bacteria types which possess similar traits but may differ in terms of their enzymatic activity which is one of the important factors to be considered when choosing the best bacterium for MICP.

Rebata-Landa (2007)[67] has proven that by using Pseudomonas fluorescens he was able to produce urease enzyme and promote the precipitation of calcite. (P. fluorescens is a mesophilic, non-spore-forming species that exists naturally in sediment.) On another note, Cheng and Cord-Ruwisch (2012)[68] used Bacillus sphaericus (MCP-11) in order to develop a process for bacteria immobilization which was found to be effective in unsaturated conditions by using the surface percolation method.

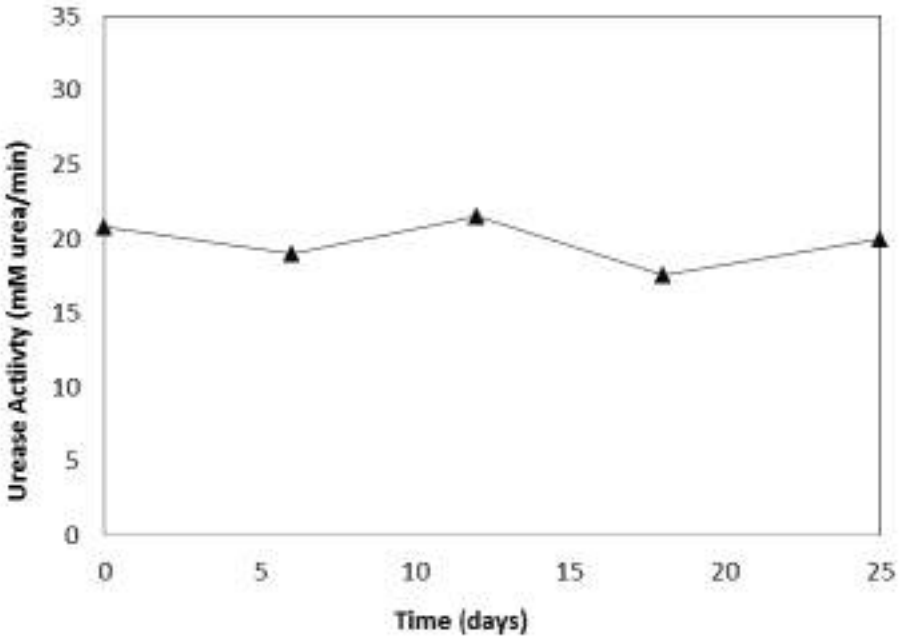
2.6 Key Parameters to Mechanisms of MICP

2.6.1 Bacteria Types

The urease activity of the bacteria, usually, it is influenced by the type of bacteria used.



(a)



(b)

Figure 2-4 Factors influencing urease activity of UPB strain S. pasteurii, a) pH value, b) time (after Whiffin, 2004)[51]

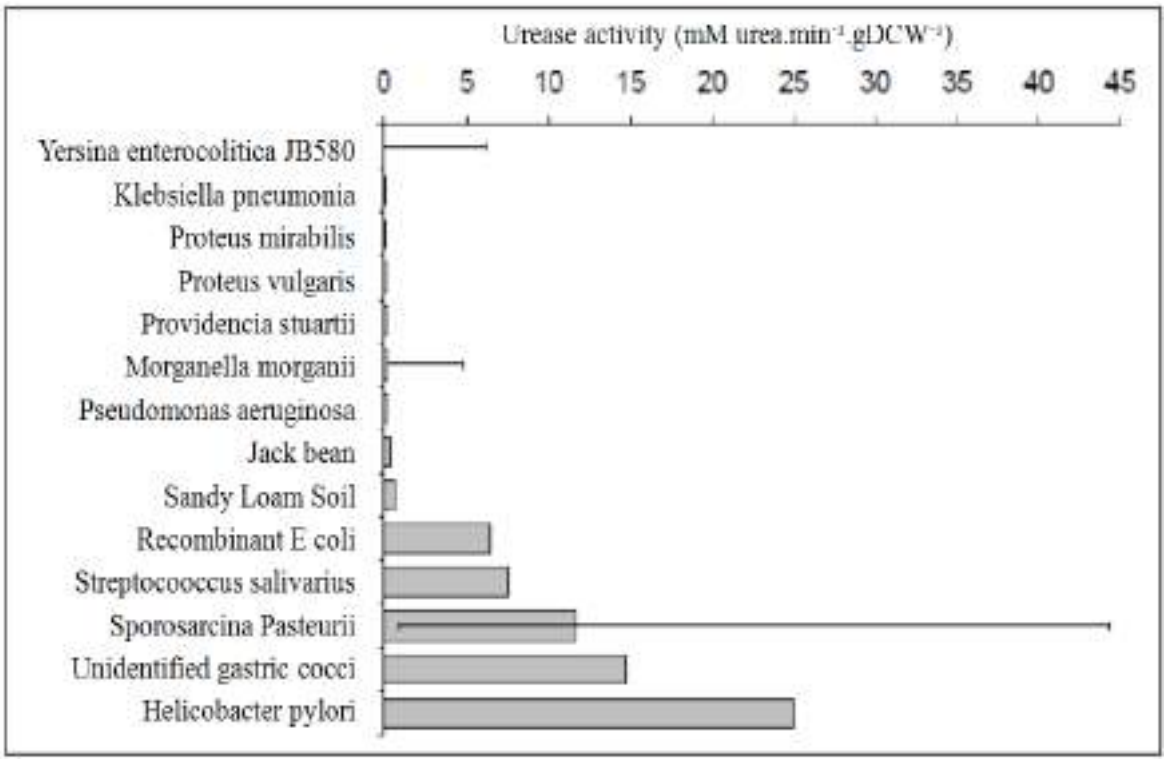


Figure 2-5 Comparison between *S. pasteurii* with regards to its specific urease activity against other types of microorganism as summarized by Whiffin (2004)[51]

Therefore, the type of bacteria has a significant effect on the efficiency of MICP (Okwadha and Li, 2010)[69].

The bacteria types that are suitable for MICP application should be able to produce catalyst urea hydrolysis. The researchers who worked on microbially induced calcium carbonate precipitation focused on the microbial characteristics, e.g. metabolic pathway and the type of microorganism to improve the efficiency of the treatment (De Muyncka et al. 2010)[70]. The typical types of bacteria are Bacillus Pasteurii, Desulfotomaculum, Clostridium and Spoloactobacilus (Kucharski et al. 2012)[71]. Bacillus Pasteurii is a more

common type of bacteria used in the researches to precipitate calcium carbonate in the soil through conversion of urea to ammonia and carbon dioxide in the presence of calcium ion (Le Métayer-Levrel et al., 1999; Hammes, 2003)[15, 34]. Sharma and Ramkrishnan's study (2016)[45] intended to experimentally analyze the effectiveness of use of microbial induced calcite precipitates for improving the shear strength parameters of two different types of fine soils. For this process, they used Bacillus Sporosarcinai to catalyze the calcite precipitation. Other types of Bacilli used in MICP, for instance, B. pasteurii in concrete and soil improvement (Whiffin et al., 2007)[12]. Lee (2014)[72] investigated that the performances of bio-mediated soil improvement by using B. megaterium to trigger calcite precipitation in different types of soils. Also, Jiang et al. (2016)[73] used B. megaterium to quantify the ureolytic efficiency of a urease-producing bacterium and purified urease enzyme in the oxic and anoxic conditions.

2.6.2 Urease Activity

In spite of urease activity is confirmed in urease producing bacteria cultivation, it is important to note that the optimization of urease activity does not necessarily lead to the success of MICP formation. As shown in Figure 2-6, Cheng and Cord-Ruwisch (2014)[43] established a mathematical model to predict the calcite precipitation amount presenting along the depth of a saturated sand column at different urease activity of bacteria.

With fixed penetration rate, the predicted homogeneity of the precipitated calcite distribution along the depth of a specimen improves as the urease activity decreases. Consequently, using urease producing bacteria with low activity could lead to a more uniform cementation within sand. Such observation, which is stronger MICP can be formed at lower urea hydrolysis rate was also made by Qian et al. (2009)[64]. Such assumption was also confirmed by the results getting from other researchers (Fidaleo and Levecchia 2003; Martinez et al., 2013)[74, 75], in which the sets having lower

urea-hydrolysis rate resulted in a more uniform MICP formation, providing other setting to be the same in control.

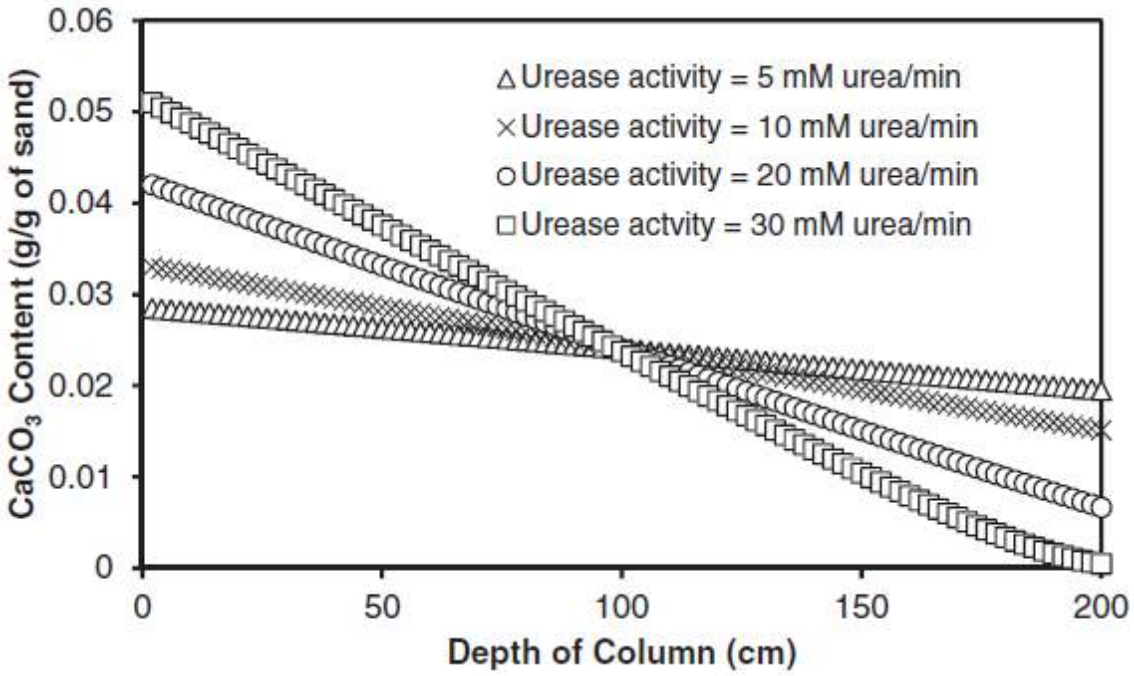


Figure 2-6 The amount of precipitated calcite as a function of depth under specified urease activities with constant penetration rate as predicted using model described in Cheng and Cord-Ruwisch, 2014[43]

2.6.3 Bacteria Concentration

To certainly increase the amount of calcite precipitated from MICP process, high bacterial cell concentration must be supplied to the soil sample (Okwadha and Li 2010)[69]. The rate of urea hydrolysis is directly proportional to the concentration of bacteria. To commence the urea hydrolysis, a high concentration of bacteria is needed to produce more urease per unit volume. Van Paassen (2009)[11] reported that bacterial cells were excellent sites for growing minerals throughout the creation of rock. Lian et al. (2006)[35] identified from 30 SEM images that nucleation of calcite took place at bacteria cell walls. Also, it has been reported that the higher concentration of bacteria near the

contact surfaces among the particles directly results in increased calcite precipitation in the region (Montoya, 2012)[76].

2.6.4 Urease Stored in Cell and Out of Cell

The location of urease has an effect on the MICP formation. According to Qian et al. (2010)[65], use of extracellular polymeric substances isolated from bacteria cells inhibited MICP crystallization, as the organic molecules combined with calcium ions, which prevented calcium ion from participating in nucleation or reacting with carbonate ions to form calcium carbonate. Furthermore, bacterial cells could accelerate crystal nucleation, as their charged surface supplies energy towards nucleation. Figure 2-7a and 2-7b shows calcium carbonate crystal formed in extracellular polymeric substances solution and the solution of urease producing bacteria cells, which observed as global and cubic shape respectively. Such observation indicated that urease producing bacteria cells acted as a nucleation site during MICP formation.

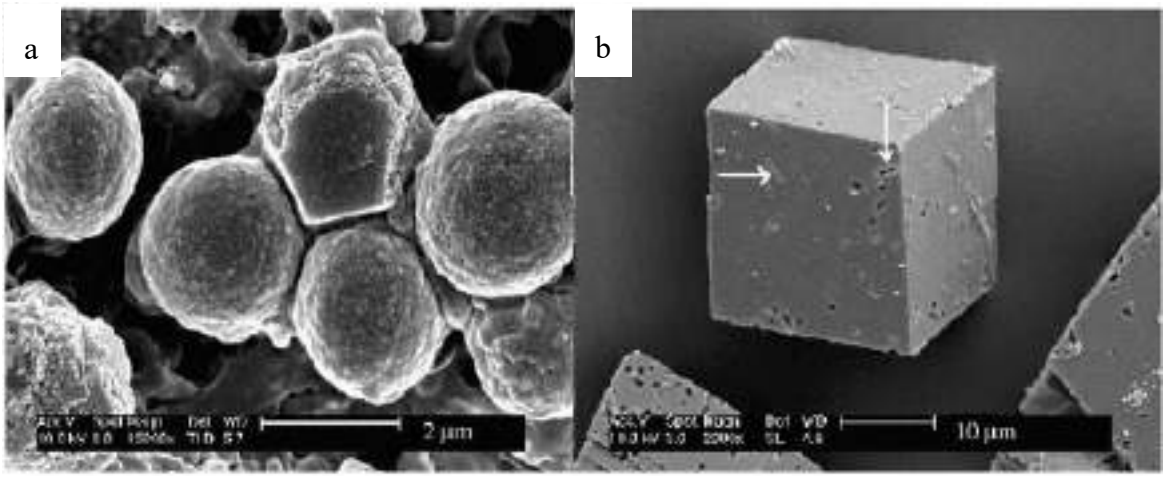


Figure 2-7 SEM images of calcite crystal forms in (a) Extracellular Polymeric Substances solution and (b) Urease Producing Bacteria cells solution (Bacterial cells are marked with white arrows in 2-7b) (Qian et al., 2010)[65]

2.6.5 Ureolysis under anaerobic condition

The urease-producing bacteria types are supposed to be aerobic. Therefore, their

growth and performance are expected to be inhabited without the presence of oxygen. Many experiments were performed on ureolysis rate of Sporosarcina pasteurii under both aerobic and anaerobic conditions (Parks, 2009)[77]. Figure 2-8 shows remaining urea molecule and calcium concentration over time in anaerobic medium in one of the experiments. The decreased concentration of both parameters proved its capability of ureolysis without the presence of oxygen. It was found that the rate of ureolysis in anaerobic was similar to that under aerobic condition when calcium is inclusive in the media yet no external electron acceptor offered. However, the rate of the growth of anaerobic bacteria is slower than that of aerobic bacteria.

Ghosh et al. (2006)[78] used anaerobic bacteria within cement-sand mortar/concrete to develop bioconcrete material. The results of their study showed that compressive strength of concrete and mortar of cement-sand increased significantly.

2.6.6 Bacteria Size

Geometrical compatibility of urease-producing bacteria is very important for soil treatment through injection of the microorganisms (Soon et al., 2014)[62]. The size of bacteria potentially influences bacterial calcification. Bacteria size typically ranges between 0.5 and 3.0 μm (Mitchell and Santamarina, 2005)[24], also bacterial spores, stress resistant resting stages of some species, can be as small around 0.2μm. Due to microorganism's small size, it is moving freely in the pore spaces of grained materials, either by self-propelled movement or by passive diffusion. But the length of microbial cellular filaments can be up to 100 μm, which can be an obstacle in penetration of filamentous microorganisms into soil. Mitchell and Santamarina (2005)[24] noticed that when soil pores become saturated with bacteria of 1 μm size such as Sporosarcina pasteurii (could reach approximately 108 bacteria cell / ml), it could also cause space limitation. Transport of bacterial cells and staying within the interconnected porous

network of a soil mass is organized through cell shape, cell size, cell surface roughness and electrical interactions, and cell physiological state (Murphy and Ginn, 2000)[79].

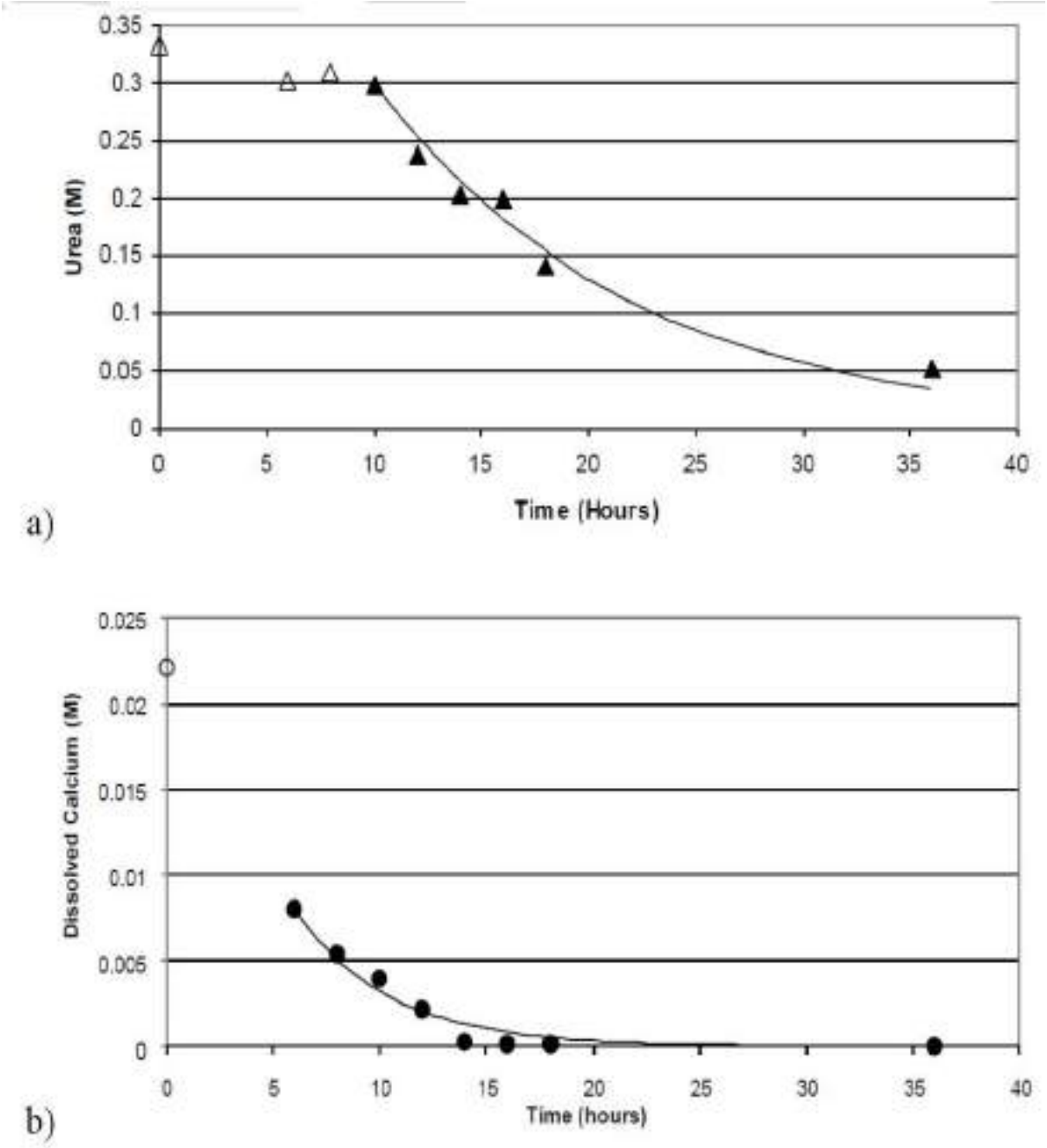


Figure 2-8 One set of experiment data for S. pasteurii in anaerobic medium; (a) urea, (b) dissolved calcium concentration (Parks, 2009)[77]

2.6.7 Distribution and Fixation of Bacteria in Soil

Usually, in order to get good results from MICP treatment, the urease-producing bacteria should be distributed regularly and fixed in place when they are injected into the soil. Harkes et al. (2010)[36] developed a procedure based on a two-phase injection process to enhance fixation and distribution of bacterial cells and their enzyme activity in sand. They found that injection of bacteria suspension, and then injection of one pore volume of high salinity fixation fluid could retain practically all bacteria cells in the soil particles. Harkes et al. (2010)[36] used high-salinity and low-salinity solutions in his study. It was found that a 0.05M $CaCl_2$ solution can stimulate adsorption and flocculation of the bacterial cells, whereas the use of a low-salinity solution, such as fresh surface water, resulted in stimulating transport and remobilization of these cells.

2.6.8 Soil Particle Size

The main restriction on bacteria transport is the size of soil pores. The size of soil pores should be sufficient to allow the bacteria cells pass and move from one pore space to another (Mitchell and Santamarina, 2005)[24]. Holtz and Kovacs, (1981)[80] reported that the size of the pore throat is dependent on the smaller portion of particles in the soil, and can be estimated as 20% of the soil particle size, this percentage is equivalent to 10% passing in a mechanical sieve. This provides an approximate limit of the lower bound on treatment by injection which depends on the particle size relative to the bacteria size. Rebata-Landa (2007)[67] determined that the most favorable range of soil particles sizes for MICP reactions is between 50 to 400 μm. Whereas, finer particles sizes are not favorable for the activity of bacteria and movement, as it obstructs movement of the bacteria itself and transport of chemical solutions. These situations need a balanced relationship between the pore structure characteristics and the bacteria size. A

compatibility relationship between bacteria-soil type illustrates the dimensional boundaries of compatibility is presented in Figure 2-9.

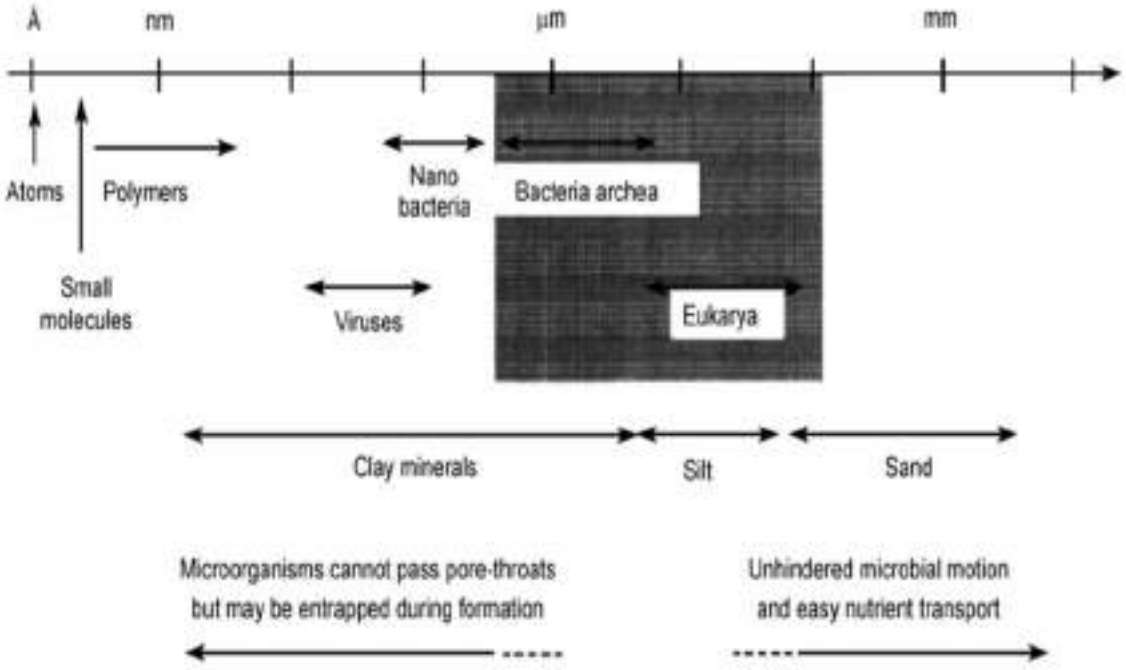


Figure 2-9 Comparison of typical sizes of soil particles and bacteria, geometric limitations, and approximate limits of various treatment methods (Rong et al., 2012)[81]

2.6.9 Nutrients

In cell growth and metabolite production, the selection of the nutrients and determination of its concentrations in the cultivation media is a very important step. The bacteria are the only organism in MICP system, which in turn needs an energy source to continue to precede metabolic processes and thus the continuation of the precipitation. Nutrients are the energy sources for bacteria, and which are provided to bacteria through both culture stage and soil treatment stage. Several studies have shown that the urease-producing bacteria can grow in a wide variety of complex and synthetic media. Wu et al. (1997)[82] reported that, for better precipitation of carbonates, the experimental studies on the reduction of soil hydraulic conductivity of improved biomass growth in soil with dextrose-nutrient solution have appeared a positive correlation between attached

microbial biomass and the soil hydraulic conductivity. The common nutrients for bacteria include CO_2, N, P, K, Mg, Ca, Fe, etc. (Mitchell and Santamarina, 2005)[24], but these nutrients have a high cost. To meet the demand, low cost media is required for the production of urease, other alternative nutrients to reduce the cost and protect the environment have been proposed. Lactose mother liquor and corn steep liquor, two industry waste, were studied by Achal et al. (2009)[83] and Achal et al. (2010)[84] respectively. The urease-producing bacteria such as S. Pasteur prefer protein based media as a nutrient source to grow well (Morsdorf and Kaltwasser, 1989)[85].

2.6.10 Chemical Solutions

In order to achieve successful treatment by microbial induced calcite precipitation in the soil, in addition to bacteria, chemical solutions need to be injected to the location where improvement is required. The chemical solutions and additives used in the experiments included calcium sulfate, calcium chloride, calcium acetate, calcium nitrate, ammonia, alcohol, and iron hydroxide. In most of the studies, urea-calcium based cementation media were used to precipitate calcium carbonate. When the low quantity of the chemical solutions is added, the precipitation of calcium carbonate is mostly gone in the particle-particle contact, which is sealing the micro channels in the soil and works as bioclogging. While the chemical solution is added at a higher quantity, the precipitation is in the pores creating high strength and works as biocementation. The effect of the concentration of calcium salts and urea, on the efficiency of the bioclogging treatment was examined by De Muynck et al. (2010)[86]. They found that the waterproofing effect for a calcium dosage of 17 g Ca^{2+}/m^2 in the absorption of water was very close to that of untreated specimens, while concentration of 67 g Ca^{2+}/m^2 resulted in a decrease of the rate of the absorption of water about 50%. Also, Nemati et al. (2005)[9] reported that the injection of mixture of urea and calcium chloride many causes reduced permeability in

soil media, while increases in reactant concentrations up to a certain level (urea and $CaCl_2$•$2H_2O$ concentrations: 36 and 90 g/L, respectively) increased the quantity of produced $CaCO_3$. Zhang et al. (2014)[87] studied the effects of calcium sources on microbially induced carbonate precipitation, the results illustrate that the samples using $Ca(CH_3COO)_2$ as the calcium source have a higher strength and a more uniform distribution in the pore of soil than those using $CaCl_2$ or $Ca(NO_3)_2$. The crystal type of the MICP of the samples treated with $Ca(CH_3COO)_2$ is chiefly aragonite, while that of the others is chiefly calcite, as shown in Figure 2-10. They believed that $Ca(CH_3COO)_2$ was an appropriate alternative calcium source to replace $CaCl_2$ for the MICP technology applied in the reinforced concrete structures.

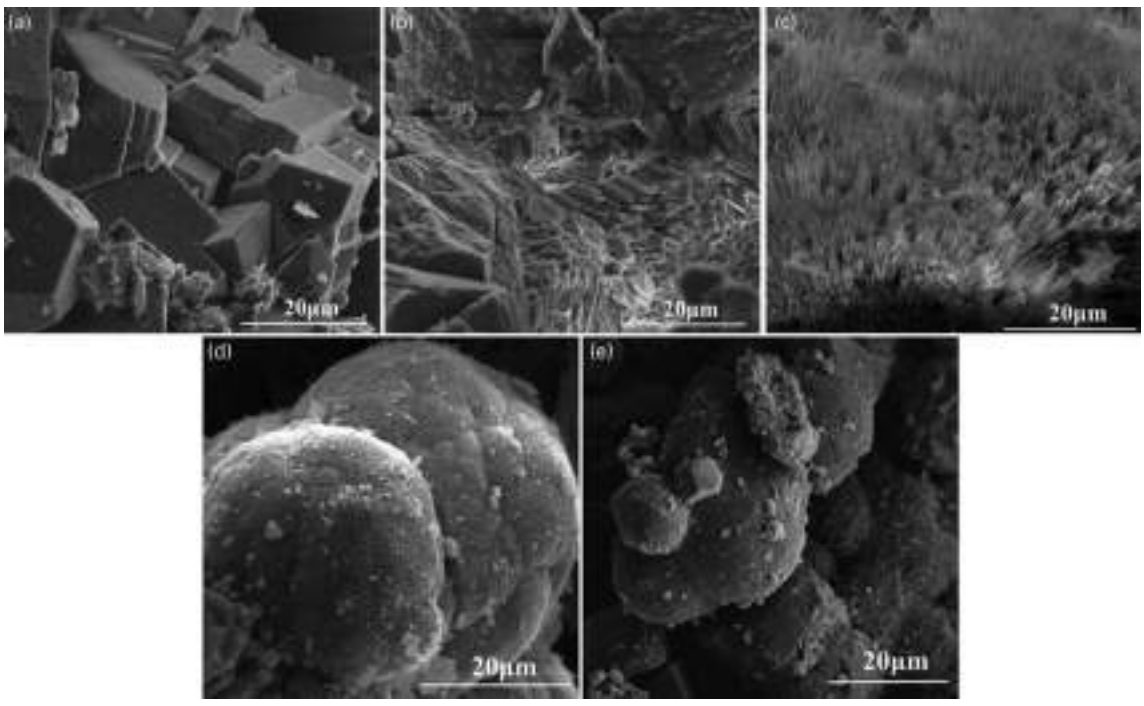


Figure 2-10 Presented images (SEM) of biogrouted samples of different calcium sources: (a) calcite (chloride sample); (b) calcite (nitrate sample); (c) aragonite (acetate sample); (d) vaterite; (e) vaterite. (Zhang et al., 2014)[87]

Yu et al. (2015)[88] examined the influence of using barium hydrogen phosphate on the MICP treatment. Their results show that the cementing mechanism of the bio-phosphate cement is that barium hydrogen phosphate particles by microbial precipitation can form large agglomerates with each other and interact with quartz sand to produce van der Waals bonds in sandstones. Gawwad et al. (2016)[89] studied the effect of different $MgCl_2$ concentrations on the mechanical properties of bio-mortar. They concluded that the presence of $MgCl_2$ in cementation solution leads to change in the crystal type and morphology of microbial precipitated mineral. Also, the presence of $MgCl_2$ leads to the retardation of microbial precipitation rate, producing little content of carbonate containing phases. Other researchers studied the effect of magnesium as substitute material in MICP, they used magnesium chloride as additive to the chemical solutions to delay the reaction rate and to enhance the amount of carbonate precipitation (Yasuhara et al., 2011, Yasuhara et al., 2012, Neupane et al., 2013, Neupane et al., 2015, Putra et al., 2016)[37, 41, 90-92].

2.6.11 pH

The urease activity is also affected by the pH. Using cell (Sporosarcina pasteurii) free extract, Stocks Fisher et al. (1999)[7] found an optimum rate at pH 8.5. Within a certain pH range, below and above this optimum pH the urease activity decreased, forming a bell-shaped inhibition curve.

This graph clearly indicates that the cell free extract is quite sensitive to changes in pH, especially on the acid side, whereas the whole cells in suspension show a negligible effect of pH from 6 to 8.5. One reason to the decrease in urease activity seems to be that the different cells used are protecting the enzyme from acidity (Van Paassen, 2009)[11].

The carbonate ions concentration is concerning to the pH at MICP process. A microbial process which leads to an increase of both pH and the concentration of

dissolved inorganic carbon is the utilization of organic acids (Braissant et al., 2002)[93].

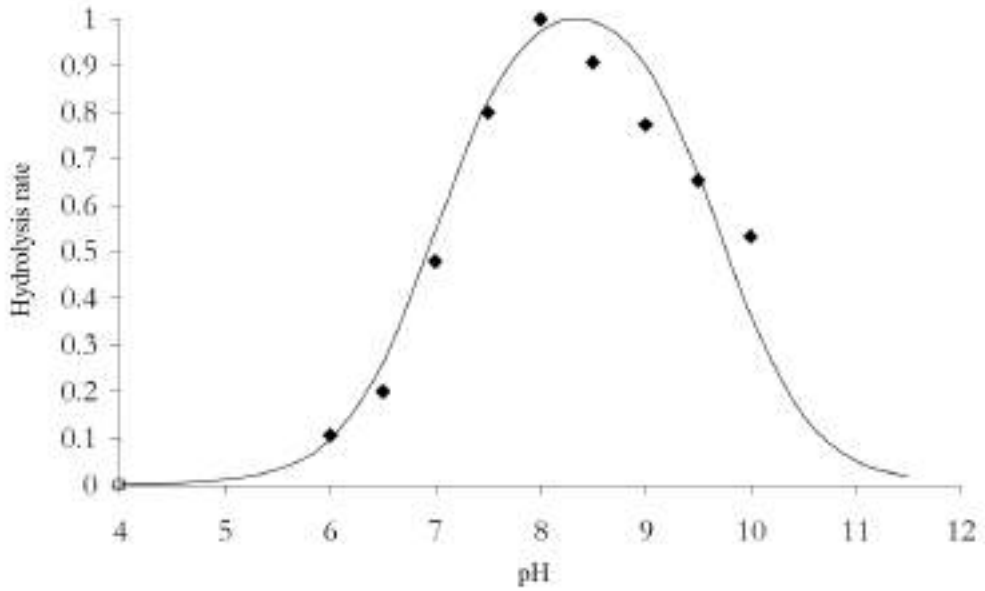


Figure 2-11 Effect of pH on urease activity measured on cell free extract (Stocks-Fischer et al., 1999)[7]

DeJong et al. (2006)[3] reported that the bacterial ureolytic activity could produce ammonium ion and bicarbonate ions and thus increase the pH, which results in calcium carbonate production, the local rise in pH often reasons the microbes themselves to help as nucleation sites for crystallization. When ammonia is used to form calcium carbonate precipitate, the pH is controlled between 8 and 11 (Popescu et al., 2014)[94]. Ferris et al., (2004)[95], Fujita et al., (2004)[96], Harkes et al., (2010)[36] performed studies using S. pasteurii to investigate the series of events happening during ureolytic calcification asserting the importance of pH. Overall, with the exception of a few groups of bacteria that prefer acidic media, urease-producing bacteria usually has an optimum pH of near 8. It is also stated that the microbial ureases might be irreversibly denatured at pH values below 5 (Mobley et al., 1995)[97].

2.6.12 Treatment Techniques

The sequence for the injection of the cementation solution for the MICP process

may as well be known as a treatment technique that varies among many kinds processes reported in literature. The different techniques give various outcomes depending on the parameters that many have set to achieve each of their aims. The most important factor in achieving an equable calcium carbonate precipitation throughout the soil mass is the uniform distribution and fixation of the bacterial cells. Several MICP injection strategies have been investigated. The most famous technique in the beginning of this MICP technology was through the gravitational method whereby the cementation solution is flushed from their injection points either from top to bottom or from the bottom to the top of the samples. Yasuhara et al. (2011)[37] explained that the solutions consisting of urea and calcium chloride were injected into the soil specimens for approximately 30 minutes and the same amount was injected another 4 or 8 times at an interval of 2 hours. After the final injection, the samples were then cured for 24 hours under a stagnant condition. Also, some researchers provided these cementation solutions in a sterilized condition prior to injecting them into the soil specimens with the exception of urea as it is sensitive to high temperatures. As such, it was sterilized by means of sterile injection passing through 0.2 μm filter (Mountassir et al., 2014)[98]. Another technique is mixing of the bacterial cell and cementation solutions together before injection. This led to immediate flocculation of bacteria and crystal growth. This technique may be taken into account for treatment of surfaces, very coarse grained materials and mixed in place applications (Le Métayer-Levrel et al., 1999)[34]. This technique is probably not suitable for the fine or medium sand because this technique leads to quick clogging of injection point and its surrounding pore space (Whiffin et al., 2007)[12]. The single-phase injection is another strategy which has been conducted by Shahrokhi et al. (2015)[113], this strategy achieved improvements in stiffness and strength, but not significantly affects the drainage capacity. To avoid crystal accumulation around the injection points, the strategy of two-phase injection was conducted, this strategy implemented through the injection of the bacterial

suspension at the beginning of treatment, followed by the injection of a fixation fluid (high salt content). This procedure has successfully retained 100% of urease activity in the sand column (Whiffin et al., 2007)[12]. Another strategy to achieve the same goal is staged-injection which has been examined by Tobler et al. (2012)[99].This injection strategy consisted of injecting the bacterial suspension over the course of 30 minutes, followed by an injection of cementation fluid also over the course of 30 minutes with these steps being repeated five times. It was explained that during the initial observation of the experiments, with every injection cycle, more calcite precipitates were formed and located on the fracture surfaces of the samples.

The effects of stopped-flow injection and continuous injection on the uniformity of calcium carbonate formation in the sand column were studied by Martinez et al. (2011)[100]. They found that stopped-flow injection method offered better uniform cementation. On the other hand, continuous injection method promoted abundant calcium carbonate precipitation near the injection point, but the calcite content decreased with the distance from the injection point. Barkouki et al. (2011)[101] obtained the same results from the numerical modeling. The stopped-flow injection is capable of distributing cementation fluid equally in the sand column before the composition of calcium carbonate.

2.6.13 Temperature

Temperature has a significant effect on the hydrolysis rate and precipitation of calcium carbonate by bacteria. Between 5 and 35ºC, a rise of 10ºC causes an increase of the urease activity by a factor (Q10) of 3.4 (Figure 2-12). Below 5 ºC, no urease activity was measured (Van Paassen, 2009)[11]. Urease-catalyzed ureolysis is temperature dependent and the optimum temperature ranges from 20 °C to 37°C. Actually, it has been reported that an increase in temperature will result in an increase in urease activity up to a

temperature of 60°C (Whiffin, 2004)[51].

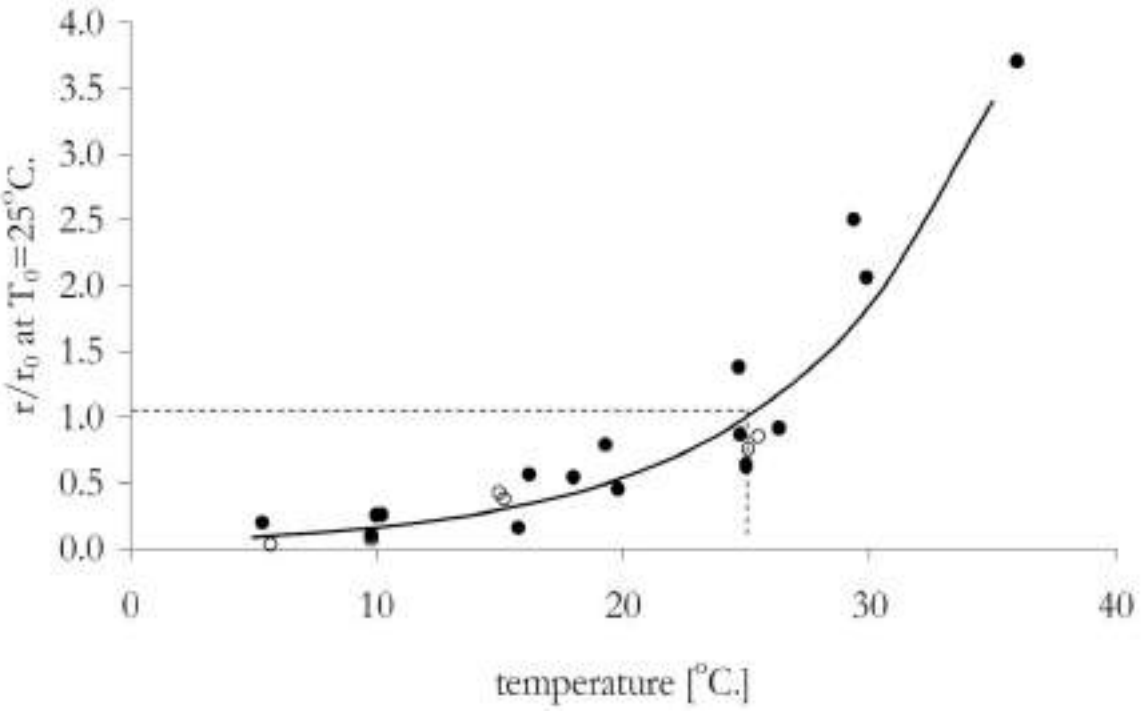


Figure 2-12 The effect of temperature on urease activity was measured in experiments where a constant amount of bacteria was supplied with 0.5 M urea (●) and 0.5M urea and CaCl2 (○) and the temperature was varied (Van Paassen, 2009)[11].

With respect to urease enzyme, Sahrawat (1984)[122] suggested that the optimum temperature for urease activity lies at approximately 60 °C. Urease activity increased with increasing temperature from 10 °C and reached the peak at 60 °C. Similarly, other studies performed by Whiffin and Van Paassen[11, 51] described that an increase in temperature will result in an increase in urease activity up to a temperature of 60°C. Although the urease activity reaches the peak at 70ºC, most of the MICP treatments were performed at room temperatures (i.e. 20 ºC -30ºC). This is because most of the urease-producing bacteria used in the existing MICP treatments (i.e. S. pasteutii, B. megaterium) are of mesopilic type with the optimum growth temperatures ranging from

30 ºC - 45ºC.

2.6.14 Number of Chemical Solutions Treatment times

The number of chemical solution treatment injections during the improvement period is called treatment times. The number of injections per day depends on the concentration of the chemical solutions. Different treatment times can be used (one time, two times, four times) in an injection cycle. There is some evidence showing that a multiplying number of injections during improvement duration is very significant to make a hardened soil and to control the hydraulic conductivity of the soil. Many studies on the effect of repeated injection on the carbonate precipitation on limestone and decrease in permeability of sand were conducted. The limestone treated for second and third times experienced additional 36% and 33% of weight gains, respectively (De Muynck et al., 2010)[86]. The decrease in permeability for sand possessed the same trend, where the first injection contributed to an approximately 65% of reduction (Nemati et al., 2005)[9]. The second and third re-injection of bacteria culture and reagent contributed to another 12% and insignificant reductions, respectively. The introduction of urease enzyme directly into the sand delivered a greater reduction in hydraulic conductivity, i.e. 28 % and 7% of diminution for second and third treatments, respectively. Al Qabany et al. (2011)[102] reported that the samples which were treated with a lower chemical concentration with more injection times were stiffer when dissolved in acid.

2.6.15 Flow Rate, Pressure and Direction

According to previous studies carried out on the sand, the typical cementation solution flow pressure ranges from 0.1 to 0.3 bar (Martinez et al., 2011; Nemati et al., 2005; Whiffin et al., 2007)[9, 12, 100]. The flow rate of bacteria suspension and cementation solution should be treated differently. Martinez et al. (2013)[75] suggested a lower flow

rate or stopped flow for bacteria suspension because higher flow rate resulted in lower bacteria retention, and more bacteria may be flushed out with a high flow rate of bacterial suspension.

The preferred infiltration rate of cementation solution is controversial. The input rates of some experiments and their efficiency published in the literature are shown in Table 2-2 (after Al Qabany et al., 2011)[102]. According to Al Qabany (2011)[102], doubling the input rate of cementation solution (from 0.042 to 0.084mol/L/h) led to nearly 50% reduction of cementation efficiency, regardless of its concentration. The results of DeJong et al. (2006)[3], Rebata-Landa (2007)[67] and Whiffin et al. (2007)[12] show that cementation efficiency was similar at different flow rates up to 0.042 mol/L/h at diverse cementation solution concentrations. An upper limit of cementation flow rate (which is 0.042mol/L/h in this case) might then be concluded, under which the cementation efficiency could be guaranteed.

Table 2-2 Input rates of cementation solution in literature (after Al Qabany et al., 2011)[102]

	DeJong et al. (2006)	**Whiffin et al. (2007)**	**Rebata-Landa (2007)**	**Al Qabany et al. (2012)**	
Infiltration rate (mol/L/h)	0.0025	0.0088	0.042	0.042	0.084
Concentration (M)	0.1	1.1	0.25	0.1/0.25/0.5	
Efficiency (%)	92	88	95	>90	50

However, high infiltration rate of cementation solution might be preferred in large scale treatment. Cheng and Cord-Ruwisch (2014)[103] proposed a model to predict the trend of cementation in depth according to different filtration rate. Higher infiltration rate

of cementation solution resulted in a more homogeneous cementation while the lowest infiltration rate only allows partial cementation because of the clogging effect.

Opposite flow direction (from bottom to top) of bacterial and cementation solution is preferred to achieve a more uniform cementation because of the plugging effect near the injection source (Martinez et al., 2013)[75]. However, it might not be practical for the application in situ.

2.7 The Mechanisms of Soil Improvement by MICP

The Calcium carbonate precipitation is responsible for improving the engineering properties of the treated soil through three mechanisms, biocementation, bioclogging and biogas.

2.7.1 Biocementation

Biocementation is defined as an improvement of soil strength and stiffness properties of soils and rocks by the production of binding materials among soil particles by bacterial activities (Ivanov and Chu, 2008)[4].

Attention on the improvement of the soil the properties using biocementation is growing quickly in recent years. Biocementation is produced by many types of bacteria, for examples, urease-producing bacteria, iron-reducing bacteria, sulfate-reducing bacteria, nitrifying bacteria, oligotrophic microaerophilic bacteria, and dimorphic phytase-active yeast (which could produce calcium-phosphate precipitation) etc. (DeJong et al., 2006; Ivanov and Chu, 2008; Chu et al., 2009; Roeselers and van Loosdrecht, 2010)[3-6] , listed in Table 2-3. The widely applied method up to now is to produce microbial carbonate precipitation by urease-producing bacteria.

The mechanism of strength improvement contributed by calcium carbonate

precipitated was explained by DeJong et al. (2010)[2]. Calcium carbonate precipitation leads to decrease in the voids (porosity), subsequently changes the overall properties. Also, the distribution of calcium carbonate within the void of the soil is critical.

Table 2-3 Potential microbial processes that can seek potentially to biocementation (Ivanov and Chu, 2008)[4]

Types of bacteria	**The mechanism of biocementation**	**Necessary conditions for biocementation**	**Geotechnical applications**
Sulfate-reducing bacteria	Production of undissolved sulfides	presence of carbon and Sulfate source in soil; anaerobic conditions	Improve stability of dams and slopes
Ammonifying bacteria	Formation of undissolved carbonates in soil as a result of increase of pH and release of Carbon dioxide	Presence of dissolved metal salt and urea	Mitigate the liquefaction potential of sand; Improve the stability of the embankments, dams, and retaining walls; Increase the bearing capacity of foundations.
Iron reducing bacteria	Production the solution of ferrous and precipitation of undissolved ferrous, hydroxides and ferric salts in the soil	Presence of ferric minerals; Anaerobic conditions changed for aerobic conditions	Densifying soil on reclaimed land sites and prevents soil avalanching. Mitigate the liquefaction potential of the sand.

Uniform distribution leads to the calcite precipitated on the surface of soil particles

uniformly at an equal thickness. As a result, the bonding formed by precipitated calcite to adhere the particles of soil is relatively small, and consequently insignificant improvement in soil properties. Another type of distribution is preferential distribution, which refers to a condition in which the calcite only precipitated at particle-particle contacts. This is the preferred distribution as all calcite precipitated contributes directly to the improvement in soil properties. Unfortunately, bio-geo-chemical processes do not naturally optimize for soil engineering properties. For that reason the preferential distribution is not viable, also the uniform distribution is not viable and there is a third type of distribution is the actual distribution, which is an intermediate state between the former two types of distribution.

Biocementation mechanisms have been proposed for several geotechnical engineering applications. DeJong et al. (2010)[2] reported that the imagined applications may include liquefaction mitigation through cementation of subsurface, building settlement reduction through increasing bearing capacity for foundations, safety of dams (upstream injection of technique would plug erosive piping), soil stabilization prior to tunneling would reduce disruption and increase efficiency, barring of erosion (treatment would increase resistance to erosive forces of water flow), slope stabilization (treatment could provide additional stability needed to prevent failures),

Montoya et al. (2012)[16] investigated the effect of MICP treatment on excess pore pressure generation and deformation in the sand beneath the structure (Liquefaction Mitigation) using dynamic centrifuge models of a structure founded on saturated sand. They reported that the treated soils through MICP showed a significant reduction of excess pore pressures in addition to more than a 50% reduction of vertical strains beneath the structure compared to the untreated soil. Cheng et al. (2013)[104] investigated the geotechnical properties of sand biocemented under different degrees of saturation. The results indicate that when the MICP treatment is performed under a low degree of

saturation, higher soil strength can be obtained at similar $CaCO_3$ content. The results also confirmed the potential of biocementation for soil improvement in many geotechnical engineering applications, including liquefaction mitigation, slope stabilization, and reinforcement of subgrade.

Rong et al. (2012)[10], Van Paassen (2009)[11], Whiffin et al. (2007)[12] confirmed the potential of biocementation for improvement in the stiffness/strength of sandy soil.

Kalantary and Kahani (2015)[105] investigated the ability to manage time and location when calcium carbonate precipitating in sandy soil. The results showed good ability of this method to control time and location of biological precipitating. Also, the unconfined compressive strength of sandy soil in Caspian Sea coast increased up to 400 kPa. The ability to manage time and location of calcium carbonate precipitating indicates that this method can be potentially used in many applications of civil engineering such as liquefaction mitigation, control of soil erosion, immobilizing of pollutions in soil.

Besides the applications of MICP in the field of geotechnical engineering, recent advances, however, indicate the potential use of this technique for the remediation of cracks in building materials, strength improvement and self-healing of cementitious materials. Biological mortar is one of these applications, the goal of the biological mortar is to avoid some of the chemical and physical incompatibility problems of frequently used repair mortars with the essential material, particularly in the case of brittle materials (Le Metayer-Levrel et al., 1999; Orial et al., 2002)[34, 106]. The mixture, which gave the best results, consists of one part of bacterial paste, one part of chemical solutions and two parts of limestone powder. This technique was tested successfully on a small scale on figurines of the Amiens Cathedral and on a portal of the church of Argenton-Château (France). Another application is remediation of cracks in concrete. Ramachandran et al. (2001)[107] investigated the microbiological remediation of cracks in concrete. They filled out samples of cracked concrete with bacteria, cementation media and sand. The treated

samples showed a compressive strength and stiffness value higher than those without treatment.

Besides the external application of bacteria in the case of remediation of cracks, microorganisms have also been applied to the concrete mixture. The researches were mainly focused on the consequences of this addition on the material properties of concrete, i.e. strength and durability. Ramachandran et al. (2001)[107] explained that at low concentrations of bacteria, the compressive strengths of mortar cubes increased from 55 MPa for untreated cubes to 65 MPa for those treated. While using high concentrations of bacteria, it resulted in a decreased strength due to the disintegration of the bacteria with time, making the concrete more porous. However, the increase of the resistance of concrete towards alkali, sulfate, freeze thaw attack and drying shrinkage was observed as a result of the presence of bacteria.

As an extension to the Ramachandran's abovementioned research, Jonkers and Schlangen (2007)[108] used the bacteria as self-healing agents for the autonomous remediation of cracks in concrete.

2.7.2 Bioclogging

Clogging method in geotechnical engineering refers to filling the soil voids with grouts and thus controlling hydraulic conductivity of soil (Karol, 2003)[1]. Ivanov and Chu (2008)[4] defined the bioclogging as a reduction of hydraulic conductivity of soil or porous rock by generating pore-filling materials by bacteria processes. This method uses chemical grouting. Also, appropriate types of bacteria may be employed for the same purpose. Bioclogging in the soil may arise from accumulation of bacterial cells in the pore space, biofilm development, or production of undissolved inorganic sulphides/carbonate/ferrous matters (Baveye et al., 1998; Guo et al., 2013; Ivanov and Chu, 2008; Vandevivere and Baveye, 1992) [4, 32, 33, 109].

An overview of bioclogging method is shown in Table 2-4, including a selection of bacteria group, clogging mechanism, essential conditions for bioclogging, and potential geotechnical applications (Ivanov and Chu, 2008)[4]. Bioclogging may be widely used for barriers to stop/divert subsurface transport of contaminants, reactive barriers that treat/clean groundwater as it flows, groundwater protection through immobilizing heavy metal/organic materials before contamination of aquifers, seepage control of the reservoir/drain channel, dam protection by reducing piping effect, used to create subsurface facilities for storage of liquefied natural gas, carbon sequestration (used to create subsurface facilities for storage of CO_2), etc. (DeJong et al., 2006; Ivanov and Chu, 2008)[3, 4].

2.7.3 Biogas

Biogas is a term pointing to gas bubbles produced by microbial processes to reduce the degree of saturation in soil. For the bubbles formed that generated of biogas, must be within a specific size range and distributed uniformly within the pore fluid in order to meaningfully reduce pore fluid compressibility (DeJong et al., 2010)[2].

Liquefaction mitigation is one of the potential applications that may be realized by desaturation through biogas production. Denitrifying bacteria are ubiquitous in the subsurface and therefore denitrification offers the potential for bio-stimulation of indigenous microorganisms. Also, in comparison to other processes, denitrification does not produce toxic end products, may be cost effective since nearly 100% utilization of electron donor is possible, does not require the addition of potentially harmful exogenous organic materials such as urea, is thermodynamically more favorable.

Microbial denitrification may also have applicability for sequestration of radionuclides and metal contaminants. The potential for sequestration of radionuclides and metal contaminants through microbially induced carbonate precipitation (MICP) is

tremendous. Co-precipitation of radionuclides and metal contaminants with carbonate via denitrification has the potential to be a preferred method for in-situ remediation of radionuclide and metal contaminants for many of the same reasons with respect to MICP for ground improvement (Hamdan, 2013)[110].

Li (2014)[111] developed a new method that combines biogas generation in situ with biosealing of the biogas bubbles in sand utilizing a small quantity of biocement. Biogas bubbles were produced in the form of nitrogen gas during the bacterial denitrification process by denitrifying bacteria Acidovorax sp. DN1 in the fully saturated sand.

Table 2-4 3 Potential microbial processes that can seek potentially to bioclogging [4]

Physiological group of microorganisms	Physiological group of microorganisms	Essential conditions for bioclogging	Potential geotechnical applications
Algae and cyanobacteria	Formation of impermeable layer of biomass	Light penetration and presence of nutrients	Reduce of water infiltration into slopes and control seepage
facultative anaerobic heterotrophic slime-producing	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 oxygen and medium with low concentration of carbon source	Reduce drain channel erosion and control seepage
Nitrifying bacteria	Production of slime in soil	Presence of ammonium and oxygen in soil	Reduce drain channel
Sulphate-reducing bacteria	Production of undissolved sulfides of metals	Anaerobic conditions; presence of sulfate and carbon source in soil	Form grout curtains to reduce the migration of heavy metals and organic pollutants
Ammonifying bacteria	Formation of undissolved carbonates of metals in soil due to increase of pH and release of CO_2	Presence of urea and dissolved metal salt	Prevent piping of earth dams and dikes
Iron-reducing bacteria	Production of ferrous solution and precipitation of undissolved ferrous and ferric salts and hydroxides in soil	Anaerobic conditions: changed for aerobic conditions; presence of ferric minerals	Prevent piping of earth dams and dikes

3 Methods and Materials

3.1 Introduction

Material and methodology adopted in the experiment are introduced in this chapter. The cultivation of Sporosarcina pasteurii bacteria, namely Urease-Producing Bacteria (UPB), that have been used in the experiment is explained in Section 3.2. Cementation chemical solution which is one of the most important factors that affect the effectiveness of biocementation is described in Section 3.3. The types and properties of sand engaged in this study are described in Section 3.4. Preparation of the sand specimens for the MICP treatment and extraction of the specimens after the end of treatment are described in Section 3.5. The system of MICP test and the MICP procedure are described in Section 3.6. Physical, Mechanical and other engineering properties measurement tests on soil specimens, including unconfined compression (UC) test, permeability test, pH test, optical density (OD600) test, calcium carbonate content measurement and microscopy investigation (SEM), by which the calcium carbonate bonds and their distribution within the sand after treatments can be obtained, are presented in Section 3.7.

3.2 Cultivation of Bacteria

3.2.1 The bacteria type and first prepare

The strain of urease-producing bacteria used in this study was Sporosarcina pasteurii (ATCC® 11859™), also known by name (Bacillus pasteurii) (Figure 3-1), was purchased from the American Type Culture Collection (ATCC). Selection criteria of this bacterial strain included its ability to catalyze urea by producing a urease enzyme (DeJong et al.,2006; DeJong et al.,2010; Ivanov and Chu, 2008; Buikema, 2015)[2-4, 112], it can survive in extreme environmental conditions because of acceptance of the high concentration of salt, acceptance of high pH (Whiffin, 2004)[51] and finally it is safe and

non-pathogenic (Eryuruk et al.,2015) [44].

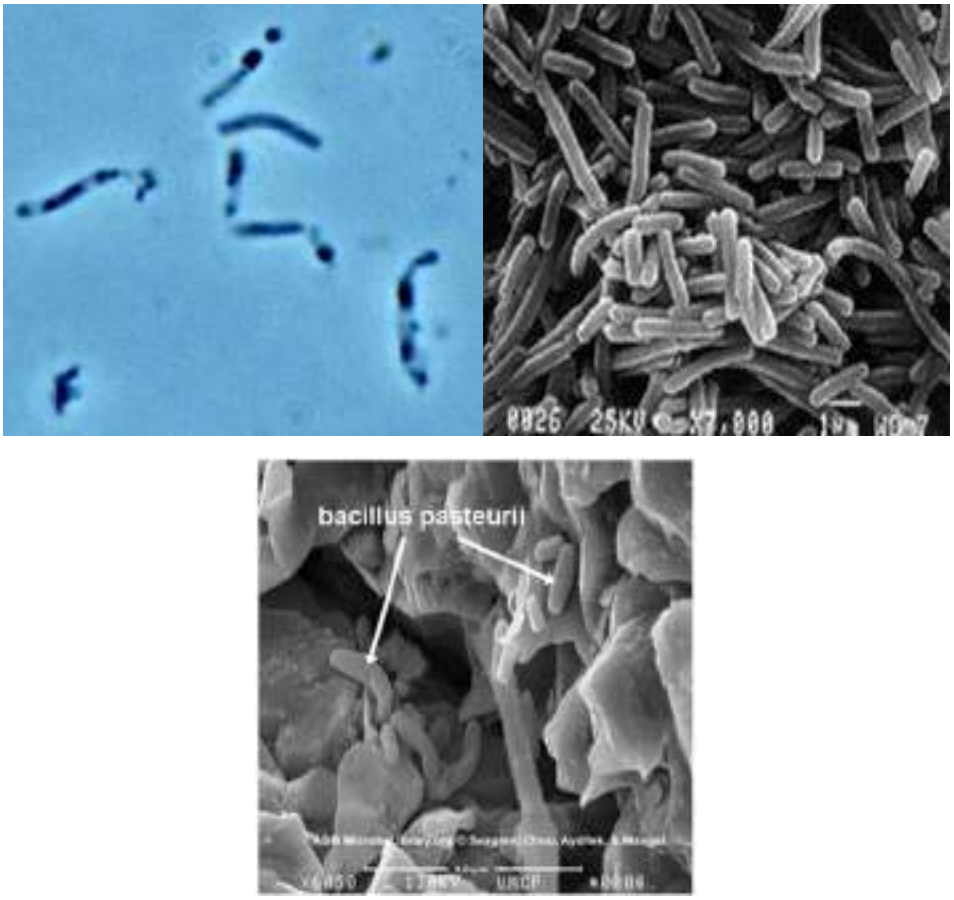


Figure 3-1 Sporosarcina pasteurii cells

The procedure for the first time bacteria preparation after open vial is that:

Using a single tube of #1376 NH_4-YE medium broth (20 g L^{-1} of yeast extract powder, 10 g L^{-1} of $(NH_4)_2SO_4$ and 15 g L^{-1} Tris buffer ($NH_2C(CH_2OH)_3$) in pH 9.0) (5 to 6 ml) (Figure 3-2), withdraw approximately 0.5 to 1.0 ml with a Pasteur or a 1.0 ml pipette. Rehydrate the entire pellet. Then transfer this aliquot back into the broth tube, mix well. Then transfer 1.0 ml of the suspension to the second tube of broth. From the second tube, use several drops to inoculate a slant and/or plate if desired. The cryoprotectant used in the freeze-drying procedure may inhibit growth in the primary tube, hence the immediate transfer is needed. Finally, incubate all tubes and plates at 30°C for 48 to 72 hours.

Figure 3-2 Ingredients of #1376 NH4-YE medium broth

After growth in tubes and plates, bacteria were harvested and inoculated in NH4-YE liquid media (same components) in a shaking incubator at 170-180 rpm and a constant temperature of 30ºC ± 2. The Sporosarcina Pasteurii grew to early stationary phase before harvesting at a concentration of approximately (2×10^8 cells/ml) (OD600= 2). Abiotic (without bacteria) bottles of media were every time prepared and incubated under the similar conditions such as controller samples to check for pollution and to make certain that the growth obtained in the remaining inoculated liquid media was indeed only Sporosarcina Pasteurii.

3.2.2 Stock Culture Preparation

The clean work environment is very important when preparing bacteria, therefore, the wash of the conical flasks and oven-drying under 105ºC must be the first step in preparing the culture of bacteria to ensure that they are all clean without any particle of dust or dirt. After that, the nutrient broth including three main chemical materials [i.e. extract yeast, $C_4H_{11}NO_3$, and $(NH_4)_2.SO_4$] must be prepared. A stock solution of broth was made in 250 ml conical flasks. The conical flasks were divided into three groups. The first group is containing 2 g of the yeast extract powder dissolved in 80 ml of

deionized water, the second group consist of one 250 ml conical flask containing 1 g of $(NH_4)_2SO_4$ was dissolved in 10 ml of deionized water for each conical flask of yeast extract, the third group is consisting of one 250 ml conical flask which contains 1.57 g of Tris is dissolved in 10 ml of deionized water for each conical flask of yeast extract. Then, the flasks were shaking by hand until the powder dissolved totally. Then, all the conical flasks were sealed with plastic cover and paper and wrapped by rope tightly (Figure 3-3).

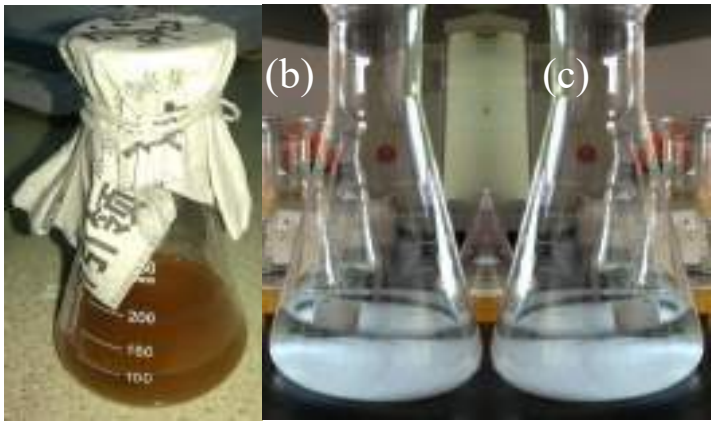


Figure 3-3 #1376 NH4-YE medium broth (a) yeast extract (b) $(NH_4)_2SO_4$ (c) Tris

Then, all conical flasks of these three types of solutions were sterilized at 121ºC for 30 minutes in an autoclave machine (Figure 3-4). After the end of the sterilization process in the autoclave, all conical flasks were put aside to cool down to room temperatures before use.

After that, all solutions placed into super-clean work table (Figure 3-5) for the purpose of adding Sporosarcina Pasteurii bacteria that have been prepared earlier.

Now, the flasks are opened up by removing the plastic cover and a fire source is used to burn the opening of the conical flask to ensure the absence of any other type of bacteria. Then add 10 ml of $(NH_4)_2SO_4$ solution and the same amount of Tris solution to each conical flask of yeast extract solution. Finally, add 4-5 ml of bacteria solution to NH_4-YE broth.

After that, the flasks contained a combination of solutions developed in a shaking

incubator at a temperature (30°±2° C) and shaken at (170-180) rpm (Figure 3-6). After 2-3 days, the bacterial solution became turbid (muddy color). The flasks of bacterial solutions stored in the refrigerator at a temperature of (4°C) until the start time of laboratory experiments and to be used in the preparation of bacterial solution at the next time.

Figure 3-4 Autoclave device and nutrient broth after the end of sterilization process

Figure 3-5 Super-clean work table

Figure 3-6 The flasks of bacterial solution in the shaking incubator

3.3 Cementation Chemical Solutions

Urea and salt contains the calcium ions considered as a substantial ingredient for evolving calcite precipitation. Many different equimolar combinations of cementation chemical solutions for the MICP treatment are comprised of urea $CO(NH_2)_2$, calcium chloride anhydrate $CaCl_2$ and calcium acetate monohydrate $C_4H_6CaO_4.H_2O$. They were investigated as cementation solutions in this study (Figure 3-7).

Figure 3-7 The ingredients of cementation chemical solutions for the MICP treatment

The equimolar urea-calcium chloride and urea-calcium acetate cementation combinations were prepared by dissolving the urea and the calcium salt in deionized water. Different concentrations of cementation chemical solutions were adopted during the injection process for the purpose of feeding the bacteria. The molecular weights of the cementation chemical solutions and molarity were prepared using Eq. (3-1). This Equation is for the analytical grade (AR) chemicals that adopted in part of this research. The molecular weights and concentrations of urea, calcium chloride anhydrate and calcium acetate monohydrate summarized in Table 3-1.

$$m = M_w \times C \times V \quad (3\text{-}1)$$

Where:

m : the mass of solute in grams (g) that must be dissolved in volume V of solution to make the desired molar concentration (C).

M_w : the molecular weight in g/mol

C : the molar concentration in mol/L (Molar or M)

V : the volume of solution in liters (L) in which the indicated mass (m) of solute must be dissolved to make the preferred molar concentration (C).

Table 3-1 Composition and concentration of cementation chemical solutions

Composition	Concentration (M)	Content g/l
Urea	0.5	30
$(CONH_2)_2$	1.0	60
Calcium chloride anhydrate	0.5	55.5
$CaCl_2$	1.0	111
Calcium acetate monohydrate	0.5	88
$C_4H_6CaO_4.H_2O$	1.0	176

In the second part of this research, different types of calcium sources were adopted. Although the use of calcium chloride and calcium acetate in this part, too, but they

have been prepared in the laboratory using the remnants of the food industry (i.e. egg shells) as a source of calcium.

To prepare the calcium chloride solution has a concentration of 1M from the egg shells, the egg shells were crushed and mixed with hydrochloric acid (the concentration was 2M) for 24 h inside a reactor of glass with the volume of 20 liters (Figure 3-8).

Figure 3-8 Egg shells reaction with the hydrochloric acid inside the glass reactor

Mixing ratio was 1 gram of egg shells for every 10 ml of hydrochloric acid. The reaction occurs according to the following equation:

$$CaCO_{3\ (egg\ shells)} + 2HCl \longrightarrow CaCl_2 + H_2O + CO_{2\ (gas)} \qquad (3\text{-}2)$$

After the end of the reaction, 0.5 g/l of sodium hydroxide (NaOH) was added to the solution for the purpose of making pH=7. Then, impurities were removed from the solution using a filter paper (Figure 3-9).

The calcium acetate solution with a concentration of 1M from the egg shells was prepared, the egg shells were crushed and mixed with acetic acid (the concentration was 2M) inside the same glass reactor that previously mentioned.

Figure 3-9 The egg shells solution ($CaCl_2$) before and after filtration by filter paper

Also, mixing ratio was 1 gram of egg shells for every 10 ml of acetic acid. And the reaction occurs according to the following equation:

$$CaCO_{3\ (egg\ shells)} + 2CH_3COOH \longrightarrow Ca(CH_3COO)_2 + H_2O + CO_{2\ (gas)} \quad (3\text{-}3)$$

The solution needs 4 g/l of sodium hydroxide (NaOH) for the purpose of making the pH=7. And again, impurities were removed from the solution using a filter paper.

3.4 Types and Properties of the Used Sands

Two types of sand were used in this study, the first type was silica sand, The ISO sand sample was obtained from the Xiamen Company ISO Standard Sand in China. Table 3-2 tabulated the composition of the ISO sand samples achieved from the mechanical sieving method. According to the Unified Soil Classification System, the ISO sand classified as well graded sand (SW).

Table 3-2 Composition of the ISO sand

Composition	Value (%)
Gravel	8
Silica Sand	89
Silt	3
Clay	0
USCS	SW
Mineral Morphology	Silica (Quarts)
Shape	Angular

After removing the gravel and silt, silica sand was milled by using an electric grinder, then re-sieving the output sand from the milling process by the mechanical sieving method. The particle size of 0.1 mm was selected for the current study. The second type of sand was the alluvial sand. The specimen was obtained from a site in Yangtze riverbank in Hankou Wuhan China. Both were poorly graded fine sand, the silica sand was white in color, while the river sand was grayish (Figure 3-10). A summary of the basic physical properties is shown in Table 3-3.

Figure 3-10 The two types of untreated sand: river sand (left side) and silica sand (right side)

Table 3-3 Physical properties of untreated sand

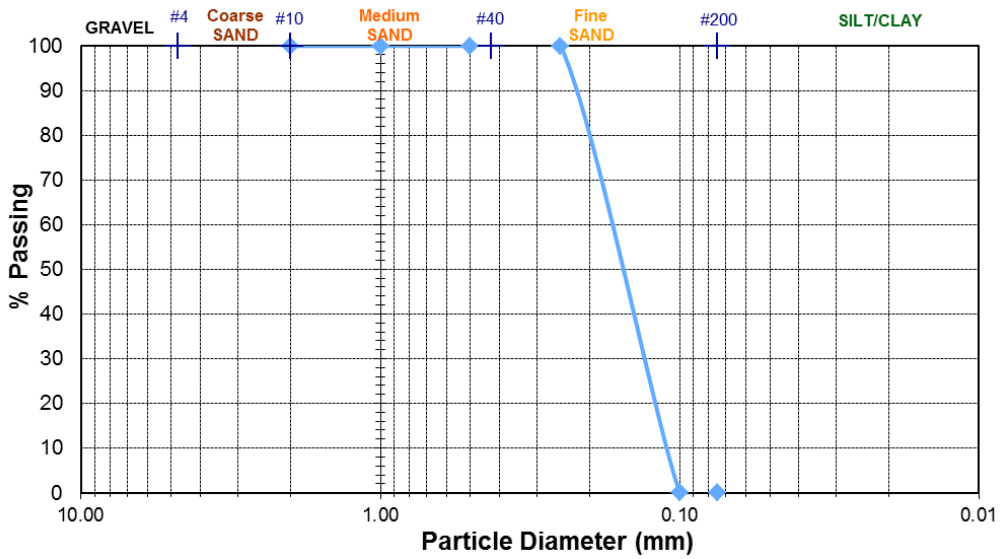
Properties	River Sand	Silica Sand
Unified Soil Classification (USCS)	Poorly graded sand	Poorly graded sand
Dry Density (DD)	1.4 g/cm^3	1.6 g/cm3
Original Unconfined Compressive Strength, UCS (kPa)	-	-
Original Saturated Hydraulic Conductivity, k_{sat}	5.9×10^{-5}m/s	1.9×10-5m/s
Shape	Angular	Angular

The gradation curves are shown in Figure 3-11, using mechanical sieving method following ASTM C136/C136M-14 and D6913-04(2009). Soil specimens were oven-dried to a constant weight before the test. The specimens were shaken for 10 minutes using a sieves shaker. The stack of sieves was arranged as following screen size: 2 mm, 1 mm, 0.5 mm, 0.25 mm, 0.1 mm, 0.075 mm and pan receiver (Figure 3-12). The results are shown in Figure 3-11 where: d10 = 110 μm, d50 = 185 μm for the silica sand, d10 = 107 μm, d50 = 180 μm for the river sand. It can be seen that the two types of sand are approximately similar to each other, regarding the size distribution.

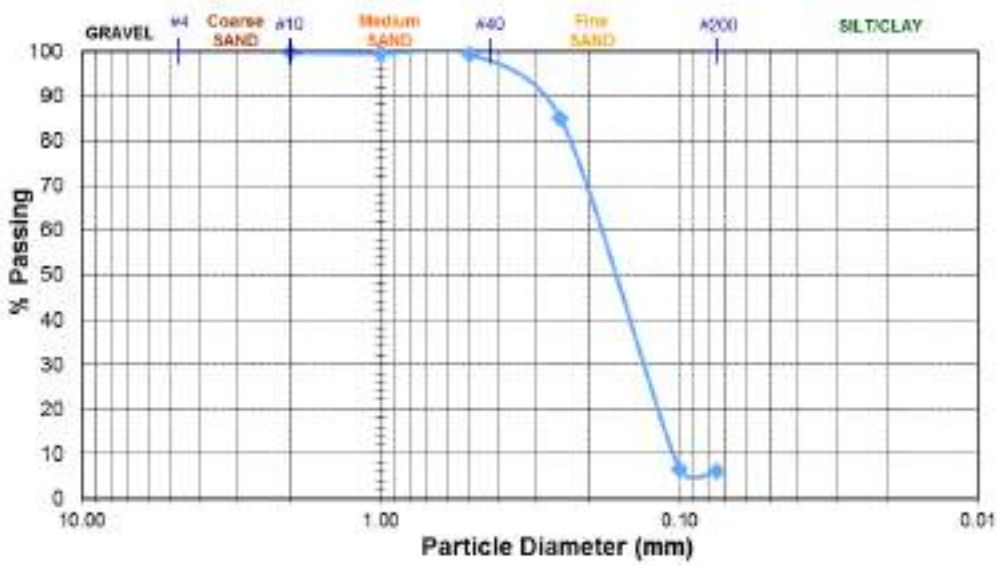
3.5 Preparation and Extraction the Specimens

River and Silica poorly graded sand were the types of soils used in this study as highlighted earlier. The poorly graded sand column was prepared by cutting polyvinyl chloride (PVC) pipe with a diameter of 37 mm into 150 mm high using an electric saw. Then, closed bottom of a PVC column by rubber stopper size 8# after making a hole in it, and to close this hole another small rubber stopper size 00# was used (Figure 3-13). The sand was packed into PVC pipe and compacted prior to laboratory tests by beating lightly on the outer wall of the pipe; this was suggested by Shahrokhi et al. (2015) [113]. The total height of the soil specimen was 80 mm. To prevent soil from driving out and keeping the

soil particles into PVC column, a porous stone with approximately 5 mm thickness was placed at the bottom of the sand sample (Figure 3-13). Another porous stone was placed at the top of the soil surface work as filter layers



(a)



(b)

Figure 3-11 Particle size distribution of the (a) Silica sand and (b) River sand

Figure 3-12 Mechanical sieves shaker

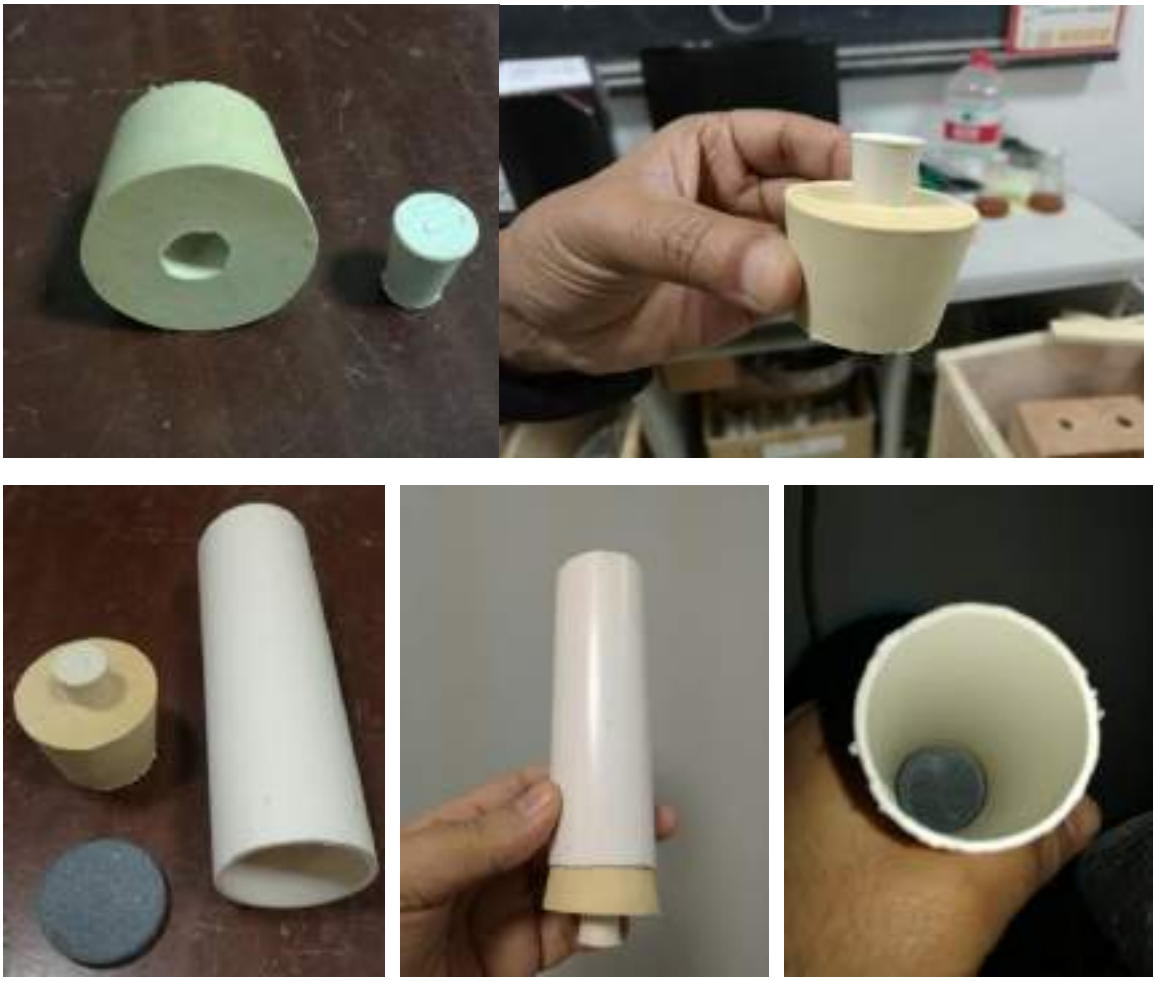


Figure 3-13 Preparation of the PVC column

After the end of MICP treatment, the soil specimen was extracted from the PVC column through cutting the side of PVC column longitudinally by soldering pencil and push the sample of soil out of it (Figure 3-14), and prepared for testing (i.e. UCS test, calcium carbonate test and Scanning Electronic Microscopy , etc.). More details will be mentioned in the following chapters.

Figure 3-14 Taking out soil specimen from the PVC column

3.6 MICP Procedure

In general, the system of MICP test consisted of a polyvinyl chloride (PVC) column sealed with a perforated rubber stopper and the hole in the rubber stopper was sealed by another rubber stopper (as described in the previous section), syringe for injection solutions and beaker (50 ml) to collect the effluent (Figure 3-15).

Microbial induced carbonate precipitation for soil treatment was conducted using gravity-induced downward precipitation. Initially, the sand columns were flushed with two porous volume tap water, then flushed with one porous volume bacterial solution, followed by 3 hours of retention time to allow the cells of bacteria installs itself in the soil granules. At this stage, the samples were flushed with a cementation solution. The MICP reaction time was 12 and 24 h depending on the concentration of the cementation solution. The whole tests were conducted at a temperature of 30°C±2°C. The step of flushing with cementation solution was repeated many times. When the treatment was finished, the soil specimens were put in the oven at a temperature of 60°C for 7 days. More details in the following chapters will be described.

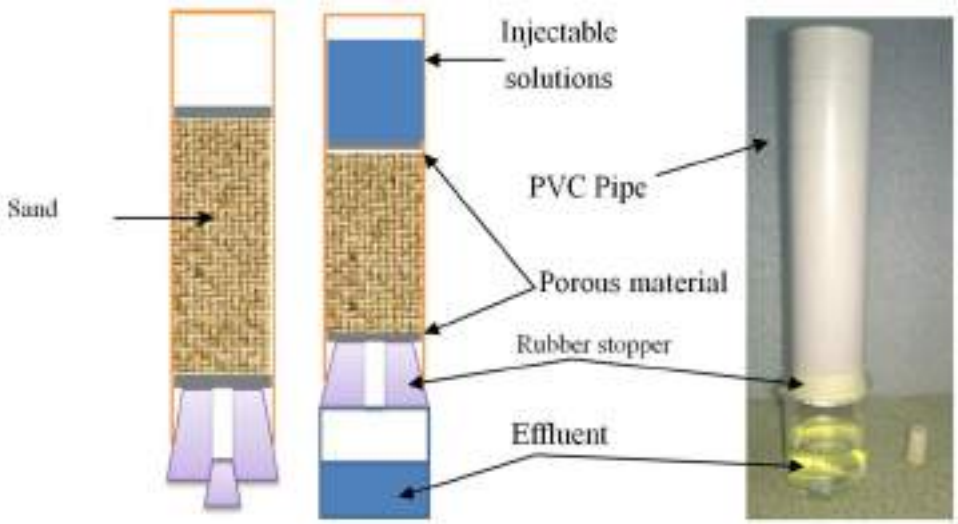


Figure 3-15 The system of MICP test

3.7 Physical and Mechanical Properties Tests

3.7.1 Unconfined Compressive Strength

The unconfined compressive strength (UCS) is a measure of the strength of the material. It can be defined as the maximum axial compressive stress that a sample with cylindrical shape of the material can endure under unconfined conditions (i.e. the confining stress is zero). Because the compressive stress in this test is applied only along

the longitudinal axis of the sample, it is also known as the uniaxial compressive strength of a material. The main aim of this test is to quickly obtain a measure of compressive strength for the type of soils which have adequate cohesion to permit testing in the unconfined condition (D2166/D2166M−13).

The unconfined compressive strength test covers the determination of the unconfined compressive strength of cohesive or cemented soil, using strain-controlled application of the axial load. This test provides an approximate value of the strength in terms of total stresses.

The unconfined compressive strength test is valid only with cohesive materials which will not drive out the water from the soil because of deformation or compaction during the loading portion of the test and which will retain substantial strength after removing the confining pressures, for instance, clays or cemented soils. Powdery soil, fissured materials, peat, and sand cannot be tested by this method to obtain valid unconfined compression strength values.

The device used in my work shown in Figure 3-16 was TSZ-3 machine, which consists of Compression Device, Deformation Indicator, Dial Comparator and Computer.

Figure 3-16 TSZ-3 machine to conduct unconfined compressive strength

Unconfined compression strength (UCS) tests were conducted according to the procedure described in ASTM D2166/D2166M (ASTM, 2013) with ratios of diameter-to-height 1:2. Unconfined compression (UC) tests were conducted using cylindrical sample size 37 mm×80 mm, at a strain rate of 1 mm/min (Figure 3-17). When specimen size was limited in some sand biocementation samples, UC tests were conducted with small specimens of roughly 10mm×20mm in dimension at a loading rate of 0.4 mm/min (Li, 2015)[114], to adopt the special sample size requirement. The highest value of the compressive force per unit area, which the specimens might bear, was indicated to as the UCS of the soil.

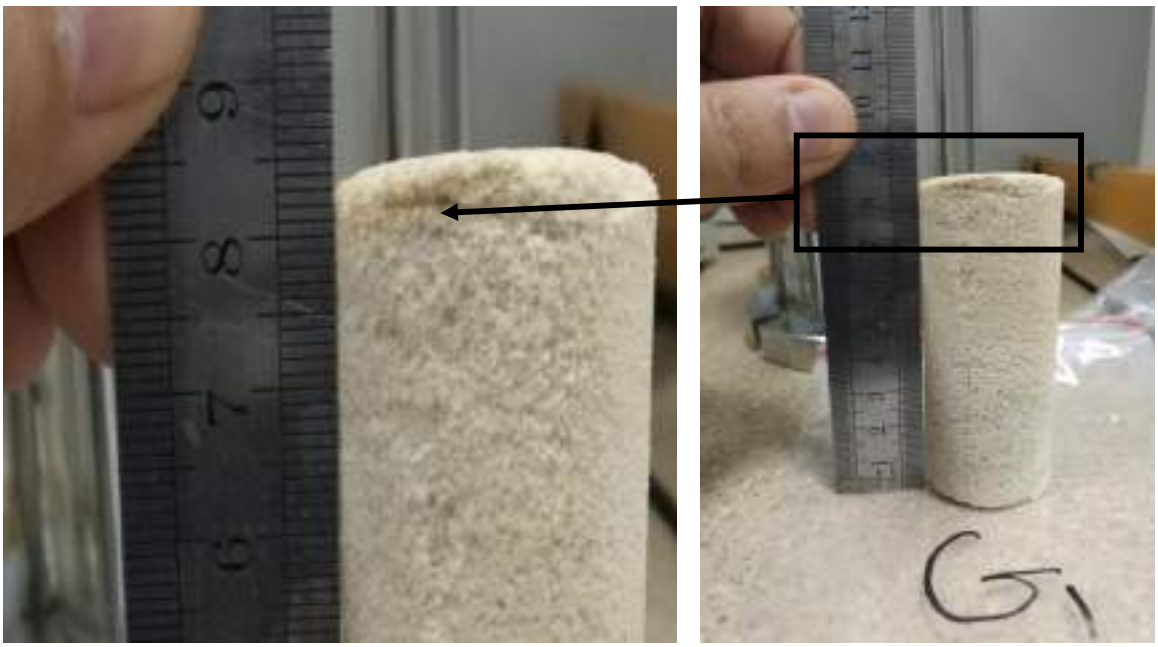


Figure 3-17 Soil sample

3.7.2 pH

During MICP treatment and after every cementation chemical solution injection process, pH measurement was carried out on the collected effluents of each sample. The measurements were performed using a pen type pH meter (MODEL PH-009(I)) (Figure

3-18). At the first time of using the device, the calibration was conducted using pH buffer powder to ensure the accuracy of the measurement. During the use of the device, flushing with distilled water was conducted before taking the reading of pH for the next sample.

Figure 3-18 Pen type pH meter

3.7.3 Optical Density (OD600)

OD600 is an abbreviation representing the optical density of measured samples at a 600 nm of wavelength. This device is used to measure the concentration of bacterial cells in a liquid (bacterial solution) (Figure 3-19).

OD600 is desirable to Ultraviolet-Visible (UV-Vis) spectroscopy when measuring the concentration of bacterial solution because at this wavelength, the cells will not be killed as they are under too much Ultraviolet-Visible light. Measurement of OD_{600} or turbidity is not a direct measurement of bacterial numbers, but an indirect measurement of cell biomass that comprises both dead and living cells (Al-Salloum et al., 2016)[115].

3.7.4 Permeability Test

In this research, two types of permeability test were conducted, Constant Head Test for river sand before treatment and Falling Head Test for river sand after treatment and silica sand before and after treatment.

Figure 3-19 Spectrophotometer (OD600)

The constant head permeability test is a common laboratory testing method used to determine the permeability of granular soils like sands and gravels containing little or no silt. The constant head permeability test procedure used for this research was according to ASTM D 2434 standard. TST-70 permeameter was used to conduct the test (Figure 3-20). The test involves the flow of water through a column of cylindrical soil sample under the constant pressure difference. The constant water level was adjusted above a few centimeters of the top of the soil. The permeability of sand was determined by opening the bottom outlet. The amount of water flow for a convenient time interval was collected. The coefficient of permeability for a constant head test is given by Eq. (3-4). The test was conducted four times 3min, 6min, 9min and 12 min. The average saturated permeability of the sand sample was taken.

$$k = qL / Ah \tag{3-4}$$

Where:

k: Coefficient of permeability in cm/s;

q: Discharge in cm^3/s;

L: Length of soil sample column in cm;

A: Cross-sectional area of the soil column in cm^2;

h: Head difference causing flow in cm.

Figure 3-19 TST-70 permeameter (Constant Head Test device)

Falling head test is a laboratory measurement of the coefficient of permeability of saturated fine grained soils with intermediate and low permeability soils. The permeability for the specimen was measured using falling head test directly from the water head variance in the reservoir by Eq. (3-5). Areas a and A were designed to be the same for the convenience of measurement (Li, 2015)[114]. It must be mentioned that this method might not be very accurate.

$$k = 2.3 \times (aL / At) \times \log(h_u / h_L) \quad (3\text{-}5)$$

Where:

k: Coefficient of permeability;

a: The cross section area of the standpipe;

L: Length of soil sample column in cm;

A: The cross section area of the soil column;

h_u: The upper water level in the standpipe;

h_L : The lower water level in the standpipe;

t: Required time to get a head drop of Δh (h_u-h_L).

The time of water dropping within a specified distance along the upper volume of the tube; over the soil specimen; was recorded. These procedures were repeated three times, and the average saturated permeability of soil was calculated.

3.7.5 Calcium Carbonate Content Measurement

To determine the precipitated calcium carbonate ($CaCO_3$) in the soil specimens, the specimens were crushed, oven-dried and the weights (M_{dry}) were recorded. The dry soil was soaked for 24 h in HCl solution (1 M) to dissolve precipitated $CaCO_3$, then the specimens were washed with water many times to ensure removal of the acid and dissolved $CaCO_3$ and drained, and finally oven-dried at a temperature of 105°C. The weights were recorded again ($M_{residual}$). The difference between the weights before and after this process is considered to be the weight of the $CaCO_3$ precipitated in the specimens (Rebata-Landa, 2007)[67] (Equation 3-6).

$$M_{calcite} = M_{dry} - M_{residual} \quad (3\text{-}6)$$

Figure 3-20 The samples soaked in hydrochloric acid

3.7.6 Microscopy Investigation

In order to study the calcium carbonate bonds and their distribution within the sand after treatments, the scanning electron microscope (SEM) was conducted. Additionally, the SEM provides a good comprehension of the bonding between the calcite crystals and the sand particles. SEM images of samples are produced by scanning a focused beam of electrons across the surface of the samples. Scanning electron microscope uses vacuum conditions and uses electrons to form an image. Water must be removed from the samples since water would evaporate in the vacuum (Goldstein et al., 2012)[116]. A sample with approximate dimensions of 1cm×1cm×1cm was prepared, and then the sample was oven-dried at a temperature of 105°C for 24 h (Figure 3-21). An FEI Quanta 200 ESEM/VPSEM scanning electron microscope was used to conduct this test (Figure 3-22). The samples were coated with gold and carbon using a BAL-TEC / SCD 050 sputter coater (Figure 3-23). This test was conducted in the Central Laboratory at Huazhong University of Science and Technology.

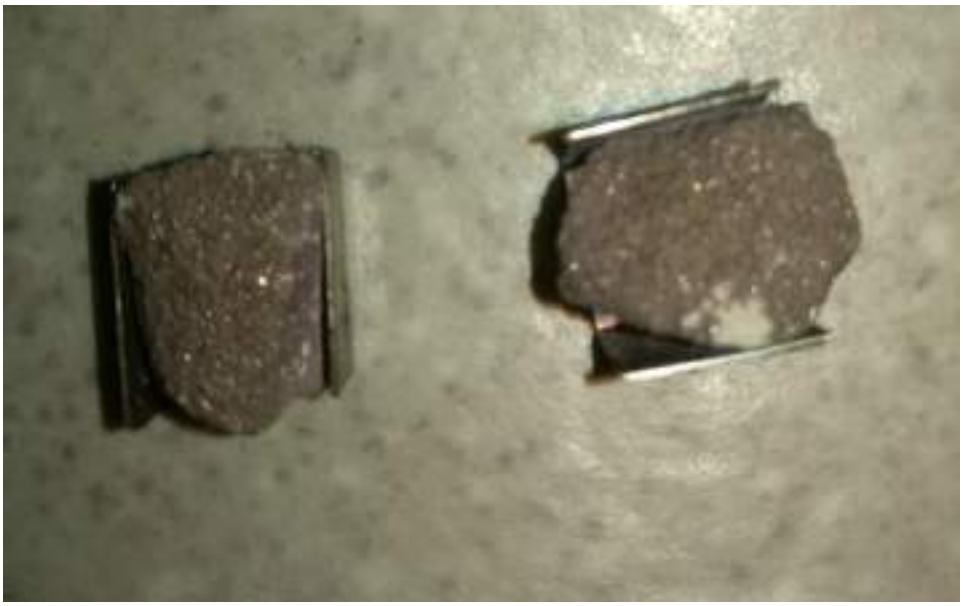


Figure 3-21 The sample used with SEM test after coated with gold

Figure 3-22 FEI Quanta 200 ESEM/VPSEM scanning electron microscope

Figure 3-23 BAL-TEC / SCD 050 sputter coater

4 Improving Poorly Graded Fine Sand with Microbial Induced Calcite Precipitation

4.1 Introduction

In the present days, it has become the establishment of infrastructure in the problematic soils an order cannot be avoided. To improve the engineering properties of these types of soils, various techniques have been applied, such as composite foundations, dynamic compaction, freezing, mixing the soil with lime or cement and vibroflotation. Recently, an innovative and sustainable technique called Microbial Induced Calcite Precipitation (MICP) has emerged for soil improvement. This chapter aims to explore the effectiveness of the MICP technique for improving the engineering properties of the poor graded fine sandy soil. The influence of factors such as grain size distribution and initial water content of untreated sand on the effectiveness of the MICP technique was investigated. Set of laboratory tests were conducted, including optical density (OD600), calcium carbonate content, unconfined compressive strength, and soil permeability.

4.2 Experimental setup

4.2.1 Preparation of Soil Specimens

In this study, the poorly graded sand column was prepared by packing the dry silica sand (with a unit weight of 16 kN/m^3, porosity of 37.67 %, and pore volume of about 33 ml) into a polyvinyl chloride (PVC) column, until reaching the height of 80 mm and 37 mm of diameter (more details in the previous chapter). The coefficient of permeability of the untreated silica sand was approximately 1.9×10^{-5} m/s.

4.2.2 Bacterial Culture and Cementation Solution

The ureolytic bacterium used in the current study was Sporosarcina pasteurii (ATCC 11859). The ATCC 11859 was cultivated under sterile aerobic batch conditions in a yeast extract medium (20 g/l yeast extract, 10 g/l ammonium sulfate, 0.13 M Tris buffer, pH = 9) (more details in Section 3-2). The optical density OD600 of the bacterial culture varied between 1.8 and 1.9. The bio-cementation was conducted using highly concentrated cementation solution consisting of equal moles of anhydrous calcium chloride (1 M, 111 g/l) and urea (1 M, 60 g/l). Both of them (urea and calcium chloride) were analytical grade (AR), following Van Paassen et al. (2009)[11], Al- Thawadi et al. (2012)[60] and Cheng et al. (2014)[43].

4.2.3 The Steps of MICP Treatment

Microbially induced carbonate precipitation for soil treatment was conducted using gravity-induced downward precipitation at a flow rate of 0.150 l/h. Initially, the sand columns were divided into two groups. The first group was dry sand columns, and the second group was saturated sand columns. The two groups were flushed with 33 ml bacterial culture, followed by 3 hours of retention time and then repeated flushes with 33 ml cementation solution. The MICP reaction time was 24 h with a highly concentrated cementation solution. The flushing with 33 ml cementation solution was repeated every 24 h. The whole tests were performed at a temperature of 30°C±2°C. After 7 days of reaction, the soil specimens were put in the oven at a temperature of 60°C. After 7 days, half of the soil samples were flushed with 1 L of tap water and then submerged in a basin containing tap water for 24 h, until the curing finished.

4.2.4 $CaCO_3$ Content

To determine the amount of precipitated $CaCO_3$ in the soil specimens. The

procedures described in Section 3.7.5 were adopted. The samples were crushed with hammer, oven-dried and the dry weights were recorded. The dry soil was soaked in HCl solution to dissolve precipitated $CaCO_3$, then washed with water and finally oven-dried and the weights were recorded. The difference between the weights before and after soaking in HCl considered being the weight of the $CaCO_3$ precipitated in the specimen.

4.2.5 Permeability, Unconfined Compressive Strength and Calcium Carbonate Content

The permeability tests were conducted using the falling head method according to ASTM D5856–15 before and after the MICP treatment (more details in Section 3.7.4). These tests determined the reduction in permeability and the relation with the amount of forming $CaCO_3$ crystals (i.e., gram/gram calcite per sand).

Also, the unconfined compression test (UCT) was conducted at a constant loading rate of 1.5%/min in accordance with ASTM D2166/D2166M-13 (ASTM 2013) (more details in Section 3.7.1). These tests were conducted to establish the relationship between the strength of the soil samples and its $CaCO_3$ content and crystal formation. Before the UCT test, half of the soil samples were washed with 1 L of tap water and then submerged in basin contains tap water for 24 h, followed by an air dried process at 30°C for 24 h.

4.2.6 Microscopy Investigation

The scanning electron microscope (SEM) helps to study the calcite bonds and their distribution within the treated sand. Moreover, the SEM provides a good understanding of the bonding between the calcite crystals and the sand particles. An FEI Quanta 200 ESEM/VPSEM scanning electron microscope was used to conduct this test. The samples were coated with gold and carbon using a BAL-TEC / SCD 050 sputter coater (more details in Section 3.7.6).

4.3 Results and Discussion

4.3.1 Effect of using poorly graded fine sand

For the purpose of examining the effectiveness of the MICP technique to improve the engineering properties of poorly graded fine sand (only the sand remaining on the sieve of 0.1 mm) and verifying the feasibility of using this technique for this type of soil, the experiment was conducted using crunched silica sand. After conducting sieve analysis for crunched silica sand, the sand particles remained in the 0.1 mm sieve were collected and used to prepare the sand column specimens.

After preparing the samples by packing the sand into a PVC pipe and bacteria and cementation solution for seven days, and after the completion of curing stage, different tests were conducted. Figure 4-1, Figure 4-2 and Figure 4-3 show the results of those tests. The test results show that the MICP technique effectively improved the engineering properties of the poorly graded fine sands. The OD600 test for the effluent showed that the soil was able to retain a high concentration of bacteria cells, approximately 88.33% of initial concentration of bacteria cells, which is consistent with the content of precipitated calcium carbonate.

The test for the calcium carbonate content showed that the ratio of the weight of calcium carbonate to that of the sand in the samples was approximately 0.29 on average. The high concentration of precipitated calcium carbonate showed a significant effect on the test results of unconfined compressive strength and permeability. The unconfined compressive strength reached up to 3000 kPa, while the reduction of permeability reached up to 90%. All these results showed high efficiency of the MICP method to improve the engineering properties of the poorly graded fine sands.

4.3.2 Initial Degree of Saturation

Figures 4-1 and 4-2 show a comparison of measured strength between saturated and

dry treated soils. It can be seen that the initial water content has a significant impact on the effectiveness of bio-cementation. For similar bacteria concentration (OD600 = 1.8) and similar retention time, the dry sand samples (i.e., S = 0) had greater unconfined compressive strength than those of the saturated sand samples (i.e., S = 100). This occurred because the sand particles in the dry sand columns had the ability to absorb more bacteria cells than those in the saturated sand columns. This explanation was based on the values of OD600 (0.210 and 0.528 for dry and saturated sand columns, respectively) of the first effluent.

The same phenomenon was illustrated when testing soil samples in a state of saturation (i.e., unconfined compressive strength was conducted on the soil samples flushed with water as the last step of curing and before the UCT test). However, the unconfined compressive strength for samples in the dry test was much higher than that in the saturated test. This might be because of the additional cementation effect produced by the calcium salt. The additional strength due to calcium salt may disappear after the samples become wet again, and thus this effect needs to be further studied in the future.

Figure 4-1 also shows that the dry soil samples had higher failure strain than saturated soil samples. The value of the strain corresponding to the highest unconfined compressive strength was 0.060 and 0.026 for dry and saturated sand columns, respectively, which means that the strain of dry soil samples was three times that of saturated soil samples.

Even when the comparison was made between the strains in both cases (dry and saturated sand samples) at the same value of unconfined compressive strength, it is found that the strain of dry soil condition was always greater. For example, for the unconfined compressive strength of 2400 kPa, the strain was 0.047 and 0.023 for dry and saturated sand columns, respectively.

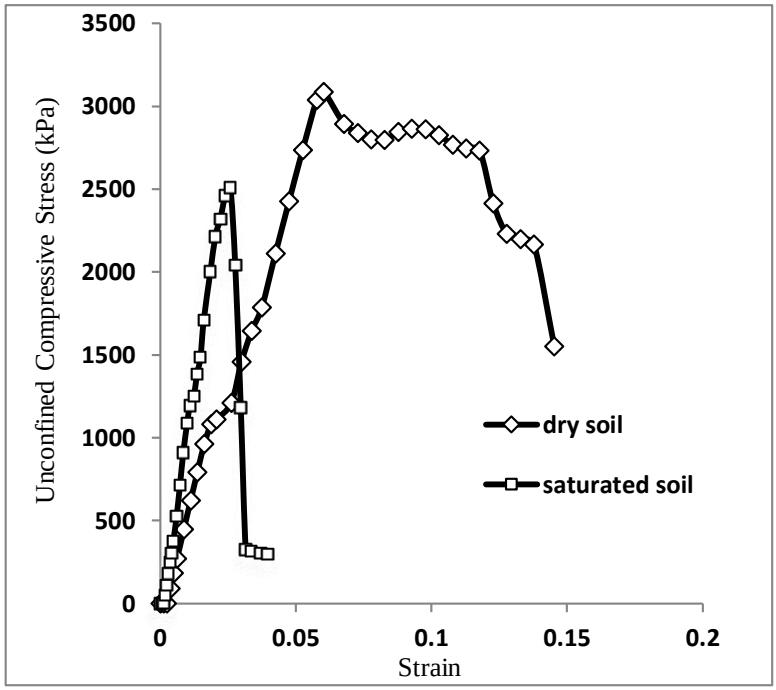


Figure 4-1 Stress-strain curves of treated sand with different initial water content

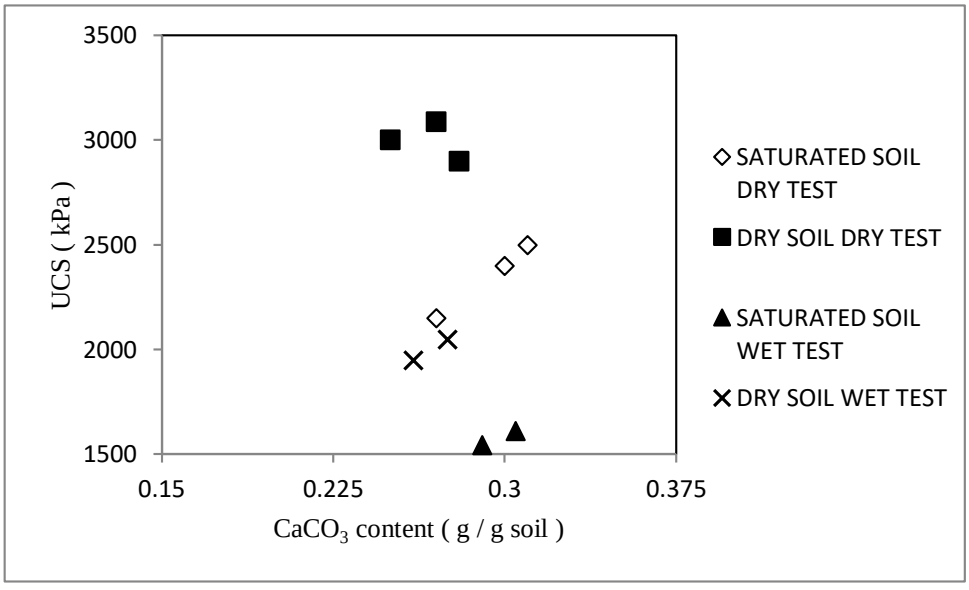


Figure 4-2 Relationship between strength and calcite content for dry and saturated samples

4.3.3 Coefficient of Permeability

The coefficient of permeability of the cured specimens (still contained in molds) was

determined using the falling-head permeability test. Yasuhara et al. (2011)[37] reported that more treatment cycles using high concentration solution of urea and $CaCl_2$ produced a greater reduction in coefficient of permeability of sand. Al Qabany and Soga (2013)[117] reported that, for four injection cycles, high concentration cementation solution (i.e., ranges from 0.1 to 1.0 M) produced a quicker and greater reduction in coefficient of permeability. The formation of calcium carbonate precipitation near the particle-particle contacts reduces the pore throats and restricts water flow.

Ferris et al. (1997)[54] noticed a reduction of 15% to 20% in permeability, while Whiffin et al. (2007)[12] noticed a reduction from 22% to 75% in permeability. However, it is imaginable that the permeability could be reduced further with more treatment.

In addition, Figure 4-3 shows a comparison of measured permeability between saturated and dry treated soils. It can be seen that the initial water content has a significant impact on the effectiveness of bio-clogging. In general, saturated sand samples showed higher calcium carbonate content than the dry soil samples, which is reflected in the permeability values, where the decrease in the permeability of saturated sand samples was slightly greater than those of dry samples.

After all, in both cases (saturated and dry samples) the use of MICP technique has a significant effect on permeability, where the average decreases in the permeability of the dry and saturated sand samples were approximately 87% and 90%, respectively, after seven treatment cycles.

4.3.4 Optical Density and Cell Concentration of Bacteria

The OD600 value is an indicator and measure of bacterial content and soil matrix's ability to retain bacteria. Figure 4-4 shows the comparison of OD600 for the effluent No. 1, 4, and 7 (i.e., the effluent of the first, fourth, and seventh day) for both dry and saturated sand samples, which have been treated with a solution of bacteria having initial

OD600 of 1.8. This Figure illustrates that OD600 for the first effluent was 0.528 and 0.210 for saturated and dry sand samples, respectively, and this means that the dry soil was able to retain more bacteria cells. In spite of that, the initial OD600 was 1.8 for both types of samples, but the dry sand was able to retain 1.59, while the saturated soil retained just 1.27. On the other hand, the OD600 of the effluent of the fourth and seventh day was very low in general, despite that the dry sand showed higher values than saturated sand, which is because the dry sand retained more bacteria at the first day and as a result was able to lose more bacteria in the following days.

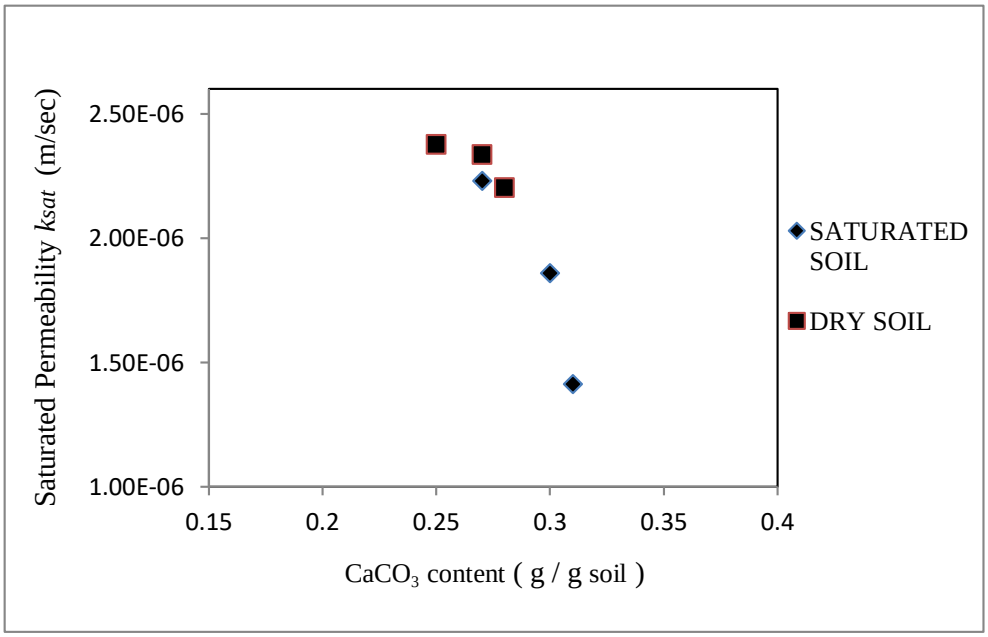


Figure 4-3 Relationship between permeability and calcite content for dry and saturated samples

Lastly, Figure 4-5 shows the initial bacteria cell concentration before treating the sand (bacteria solution) and bacteria cell concentration retained inside the dry and saturated sand after first chemical solution treatment. Following Ramachandran et al. (2001)[107], bacteria cell concentration can be calculated as follows:

$$Y = 8.59 \times 107 \times (\mathrm{OD600})^{1.3627} \qquad (4\text{-}1)$$

Where, Y is the bacteria cell concentration (= 1.9×10^8, 1.6×10^8, 1.12×10^8 cells/ml) of the initial bacterial solution, retained bacteria in the dry and saturated sand,

respectively.

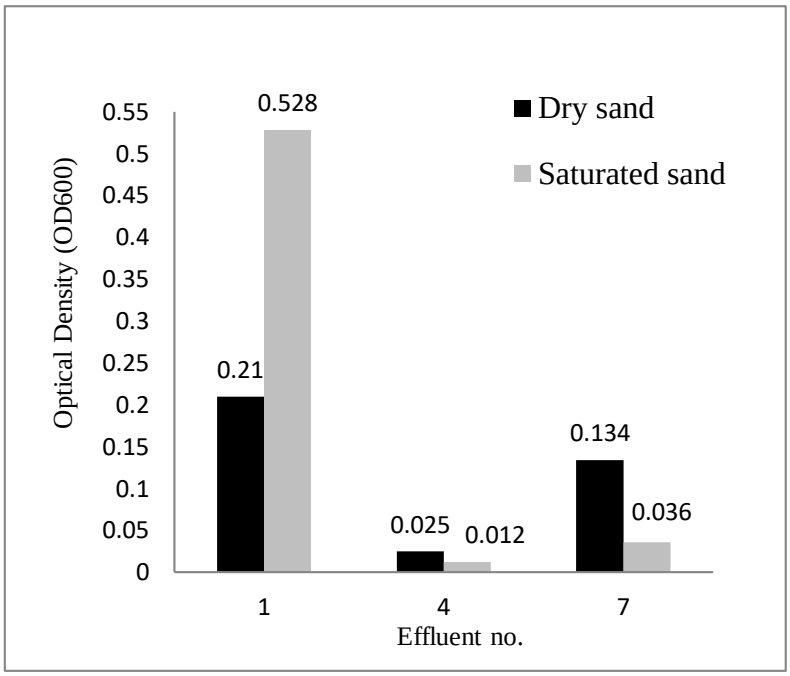


Figure 4-4 Relationship between OD600 and effluent number for dry and saturated samples

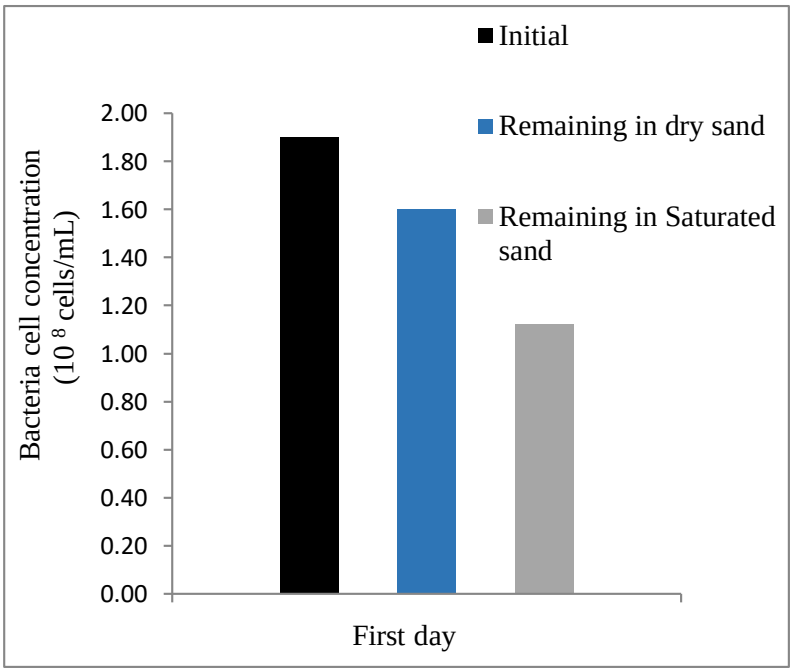


Figure 4-5 Microbe concentration at first day (cells/ml)

4.3.5 Scanning Electron Microscopy (SEM)

To investigate the impact of particle size and initial degree of saturation on the crystal formation of treated samples, SEM analysis was conducted and the results are shown in Figure 4-6. The $CaCO_3$ measurements and SEM images indicate that the amount of $CaCO_3$ precipitated in saturated samples was slightly higher than that in dry samples. It was also indicated that the crystals produced in saturate samples were relatively small in size (about 5-10 μm in diameter) and almost fully covered the surface of the sand grains (see Figure 4-6, right column), where the crystals could not provide strength development like the dry case. On the other hand, the samples treated at dry condition produced smaller amounts of crystals, but larger size (15-20 μm, see Figure 4-6, left column), which can close the gap between the adjacent sand grains and thus promote strength development.

4.4 Conclusions

The results presented in this study revealed that using the MICP technique can improve the engineering properties of poorly graded fine sand. However, the efficiency of MICP in improving the soil properties varied significantly depending on the treatment conditions. The dry sand samples (i.e., initially dry sand before adding the bacteria solution) were found to yield greater unconfined compressive strength relative to the saturated sand samples (i.e., initially saturated sand before adding the bacteria solution). Moreover, the dry sand samples yielded higher failure strains relative to the saturated sand samples. The results also showed that the decrease of permeability of dry sand was relatively smaller than that of saturated sand. Also, the curing condition had a significant impact on the compressive strength, where the treated sand samples that soaked in water during the last step of curing showed a greater decrease of compressive strength, as compared to the treated sand samples with curing that does not include soaking in water.

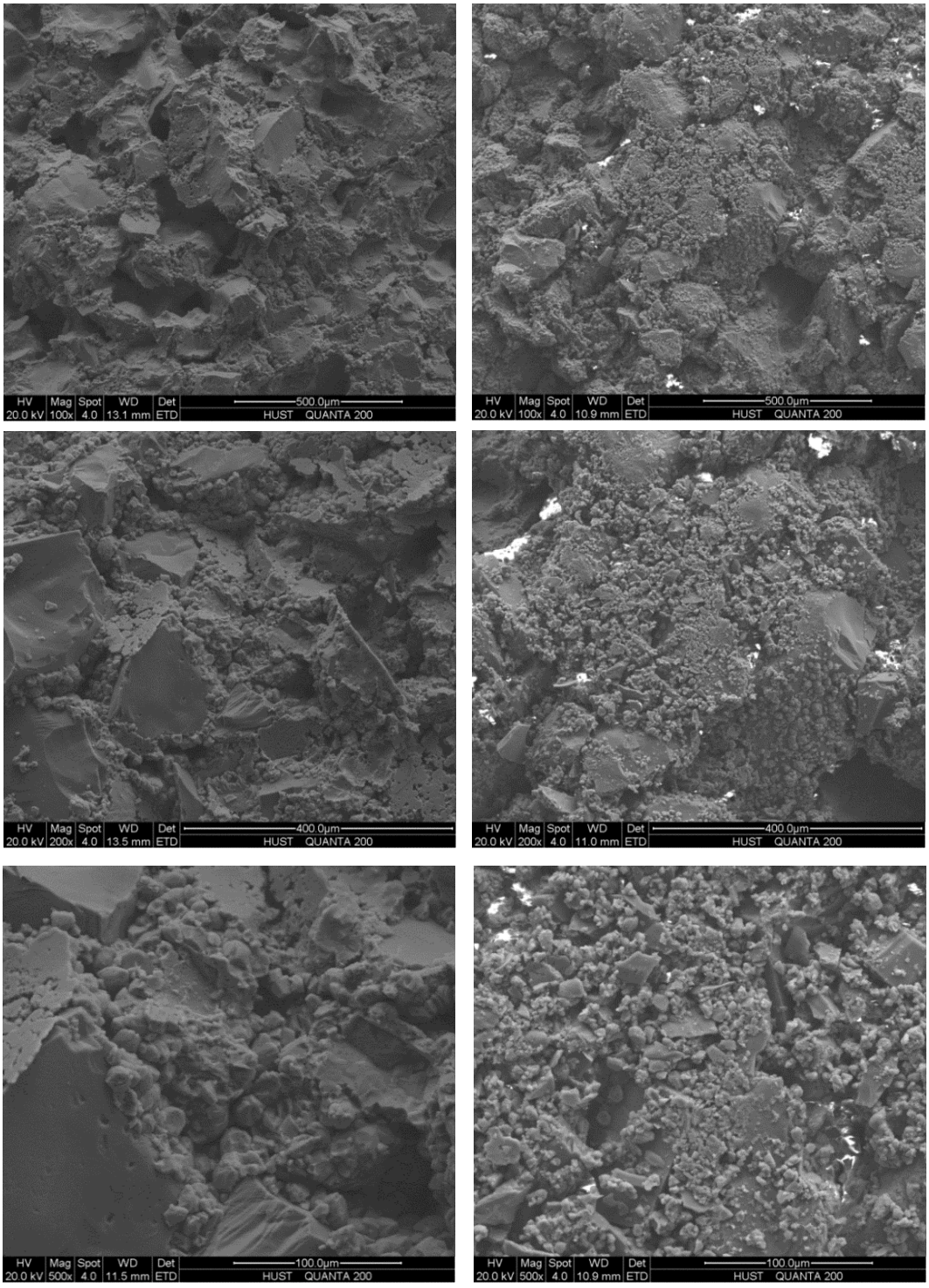


Figure 4-6 SEM of $CaCO_3$ crystals formed at different initial degree of saturation of dry sand (left) and saturated sand (right)

5 The Effect of Using the Eggshell as Calcium Chloride Source and Sand Type on Microbial Induced Carbonate Precipitation

5.1 Introduction

Microbial Induced Calcite Precipitation (MICP) is commonly carried out by injecting chemical solutions (e.g., urea and calcium source) and bacteria (e.g., Sporosarcina pasteurii, B. megaterium) to the soil where treatment is required. Ureolytic bacteria catalyze the hydrolysis of urea to produce ammonium and carbonate ions, which interact with calcium ions (e.g., calcium chloride and calcium acetate) to form calcite or aragonite that precipitates throughout the soil matrix (Zhang et al., 2014)[87].

$$(NH_2)_2CO+3H_2O \longrightarrow 2NH_4^+ + HCO_3^- + OH^- \quad (5\text{-}1)$$

$$CaCl_2 + HCO_3^- + OH^- \longrightarrow CaCO_3\downarrow + H_2O + 2Cl^- \quad (5\text{-}2)$$

The researchers were investigating the feasibility of using the MICP method for the purpose of improving the engineering properties of granular soil, where it gave encouraging results with respect to cohesion, internal friction angle, stiffness, strength and permeability (Ivanov and Chu, 2008; Le Metayer-Levrel et al., 1999) [4, 34].

All former researchers used pure urea and calcium source (analytical grade) as a cementation solution in their research, and this contributed directly to raising the cost of using the MICP method as a means to improve the engineering properties of sandy soils. This was somewhat, one of the reasons of delayed the adoption of this technology on a large scale to improve the soil.

This chapter aims to explore a new source of calcium substitute for pure calcium,

which is currently used in MICP research. The new source, which was chosen for this research was the egg shells. It has been exploring the feasibility of using a new source of calcium in improving the engineering properties of two types of soil (river and silica sand). The research investigated and compared the effects of the new and old calcium source and types of soil, in the calcium carbonate content, the crystal formation, the strength and permeability. Set of laboratory tests were conducted, including calcium carbonate content, unconfined compressive strength, soil permeability and microscopy investigation (SEM).

In this Chapter, Section 5.2 discusses the materials and methods of preparation of soil specimens, bacterial culture and cementation solution besides laboratory tests. Section 5.3 includes results and discussion. Section 5.4 drew some conclusions.

5.2 Materials and Methods

5.2.1 Preparation of Soil Specimens

Two types of poorly graded sand were selected for the current study. The first type was fine silica sand which remaining on the sieve with an opening size of 0.1 mm (Figure 3-11 a). While the second type was river sand taken from the Yangtze river bank (Figure 3-11 b). The poorly graded sand column was prepared by packing the dry sand (with a unit weight of 16 kN/m^3 and14 kN/m^3, porosity of 37.67 % and 48.67 %, and void volume of about 33 ml and 43 ml, for silica sand and river sand respectively) into a polyvinyl chloride (PVC) column of 80 mm high and 37 mm inner diameter. The coefficient of permeability of the untreated sand was approximately 1.9 × 10^{-5} m/s and 5.9×10^{-5}m/s for silica sand and river sand respectively.

5.2.2 Bacterial Culture and Cementation Solution

The ureolytic bacterium used in the current study was Sporosarcina pasteurii (ATCC

11859). The ATCC 11859 was cultivated under sterile aerobic batch conditions in a yeast extract medium (20 g/l yeast extract, 10 g/l ammonium sulfate, 0.13 M Tris buffer, pH = 9) (more details in Section 3.2). The optical density OD600 of the bacterial culture varied between 1.0 and 1.3.

The bio-cementation was conducted using two types of cementation solution, the first one is a highly concentrated cementation solution consisting of equal moles of pure anhydrous calcium chloride (1 M, 111 g/l) and urea (1 M, 60 g/l), following Al- Thawadi et al. (2012)[60] and Cheng et al. (2014)[43]. While the other was prepared from urea (1 M, 60 g/l) and the egg shells were used as a calcium source (more details in Section 3.3)

5.2.3 MICP Procedure

Microbial induced carbonate precipitation for soil treatment was conducted using gravity-induced downward precipitation. Initially, the sand columns were divided into two groups. The first group was silica sand columns, and the second group was Yangtze river sand columns. The two groups were flushed with 33 ml for silica sand and 43 ml for Yangtze river sand bacterial culture, followed by 3 hours of retention time to allow the cells of bacteria install themselves in the soil granules.

At this stage, each group was divided into two subgroups. The first subgroup was flushed with a cementation solution made of $CaCl_2$ (egg shells) and urea, while the second subgroup was flushed with a cementation solution made of pure $CaCl_2$ and urea. The MICP reaction time was 24 h with a highly concentrated cementation solution. The flushing with cementation solution was repeated every 24 h. The whole tests were performed at a temperature of 30°C ± 2°C. After 4 days of flushing with cementation solution, the treatment finished and the soil specimens were put in the oven at a temperature of 60°C for 7 days.

A B

Figure 5-1 The soil samples after treatment A. Yangtze river sand B. Silica sand

5.2.4 $CaCO_3$ Content

To determine the amount of precipitated $CaCO_3$ in the soil specimens, the procedures described in Section 3.7.5 were adopted. The samples were crushed with hammer, oven-dried and the dry weights were recorded. The dry soil was soaked in HCl solution to dissolve precipitated $CaCO_3$, then washed with water and finally oven-dried and the weights were recorded. The difference between the weights before and after soaking in HCl considered being the weight of the $CaCO_3$ precipitated in the specimen.

5.2.5 Permeability, Unconfined Compressive Strength

The permeability tests were conducted using Constant Head Test for river sand before treatment and Falling Head Test for river sand after treatment and silica sand before and after treatment (more details in Section 3.7.4). These tests determined the reduction in permeability and the relation with the calcium source and soil type.

Also, Unconfined Compressive Strength (UCS) tests were conducted according to the procedure reported in ASTM D2166 (ASTM, 2013) on bio-cemented soil specimens with selected diameter-to-height ratios of 1:2 (more details in Section 3.7.1). The samples were flushed with deionized water (about four pore volumes) and dried at 60°C for 24

hours prior to UCS measurements. These tests were conducted to establish the relationship between the strength of the soil samples and its calcium source and soil type.

5.2.6 Microscopy Investigation

In order to study the calcium carbonate types, bonds, and their distribution within the sand after treatments, the scanning electron microscope (SEM) was conducted (more details in Section 3.7.6).

5.3 Results and Discussion

5.3.1 The Visual Examination

Before starting treatment of soil samples with a bacterial culture and cementation solution, the visual examination was conducted on the cementation solution made from eggshells, and compared with the cementation solution consisting of the analytical grade material, for the purpose of ascertaining the existence of initial effectiveness reliable later when beginning the actual treatment.

The examination was conducted through the use of two cups of glass. 25 ml of cementation solution containing urea and calcium chloride (analytical grade) was put in the first cup of glass, while the second contained 25 ml of cementation solution consisting of urea and calcium chloride which is made of eggshells. Then 5 ml of the bacterial culture was added to each of the two cups. The two cups were stored for a period of 24 h to give the bacteria ample opportunity to precipitate calcium carbonate.

Figure 5-2 illustrates this process. It is obvious that the bacteria were able to produce calcium carbonate in both cementation solutions, and almost the same amount (the lower part of the solution in white).

The next step in this examination was to make a comparison between the weights of calcium carbonate precipitated from the activity of bacteria in the former two types of cementation solution. This step is performed by drying the content of the two cups in an

oven at 100 °C. After the drought (Figure 5-3), the weights of the two cups with precipitate (M_2) were recorded. The difference between the weight of the two empty cups (M_1) and the weight of the two cups with precipitate (M_2), is considered to be the weight of precipitated calcium carbonate ($M_{calcite}$).

$$M_{calcite} = M_2 - M_1 \quad (5\text{-}3)$$

This examination indicated that the weight of precipitated calcium carbonate was 1.88 g and 1.82 g in each of the solutions containing calcium chloride made from egg shells and the solution containing the pure calcium chloride, respectively. This shows that the cementation solution made from egg shells has the same efficiency as cementation solution made from pure materials (analytical grade).

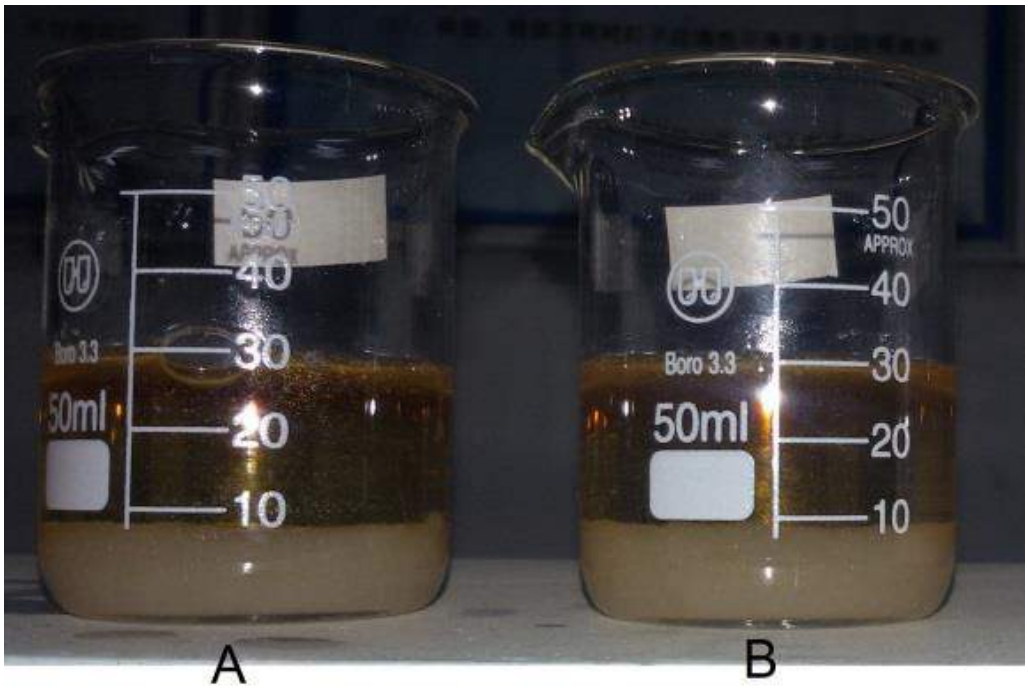


Figure 5-2 The calcite precipitation from two different calcium sources. A. $CaCl_2$ (egg shells) and B. $CaCl_2$ (analytical grade)

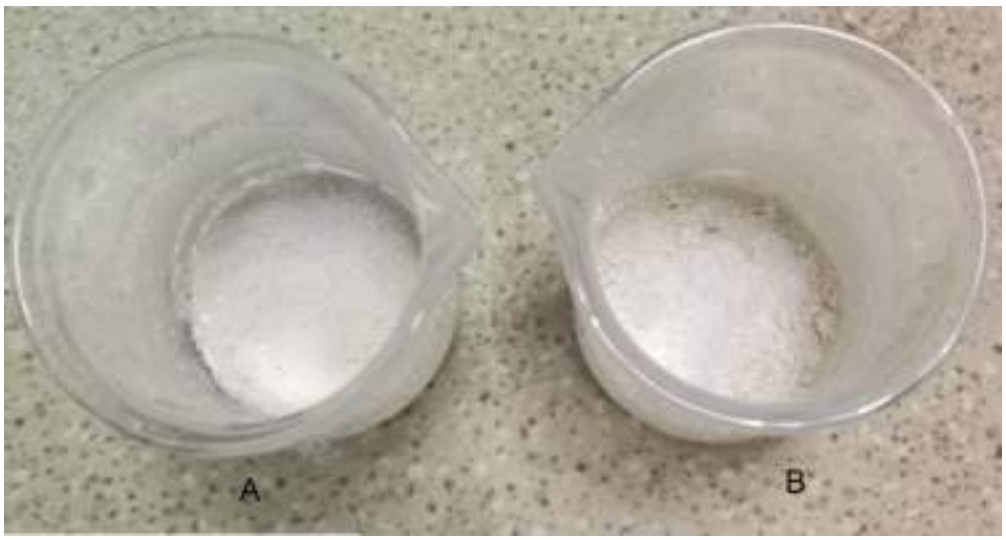


Figure 5-3 The calcite precipitation from two different calcium sources after drying.

A. $CaCl_2$ (egg shells) and B. $CaCl_2$ (analytical grade)

5.3.2 Calcium Carbonate Content

The calcium carbonate content (i.e., gram/gram calcite per sand) of the samples of the two types of soil which were treated with two different calcium sources is shown in Figure 5-4.

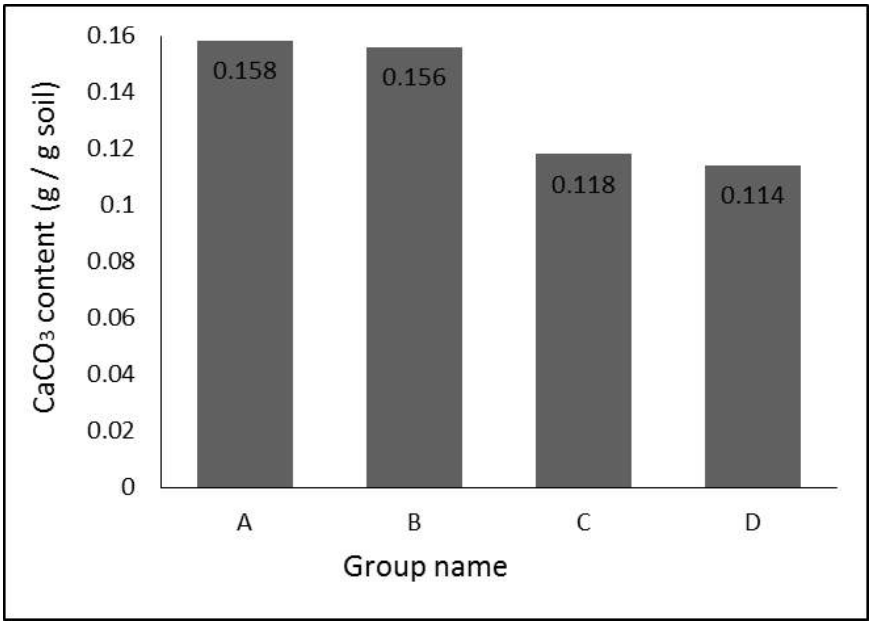


Figure 5-4 Effect of Calcium chloride type and soil type on the calcium carbonate content

The two groups A and B are of river sand, the group A was treated with cementation solution composed of calcium chloride which is made of egg shells and urea, while group B was treated with cementation solution composed of pure calcium chloride (analytical grade) and urea.

After four times of treatment, calcium carbonate content was 0.158 and 0.156 in group A and group B, respectively. These values showed that the bacteria cells were able to produce the same amount of calcium carbonate in both types of cementation solution. The same thing happened with silica sand groups C and D, where group C has treated with the same procedure of group A treatment, while group D was treated with the same procedure of group B treatment. After four times of treatment, calcium carbonate content was 0.118 and 0.114 in group C and group D, respectively. Figure 5-4 also showed that the calcium carbonate content of river sand (groups A and B) was generally slightly higher than the calcium carbonate content of silica sand (groups C and D). This can be interpreted as the result of different soil types.

5.3.3 Coefficient of Permeability

Bioclogging is a process where the soil void is filled with the product from biomicrobial-induced biochemical process. The clogging of soil as a result of the formation of calcium carbonate precipitation near the soil particles contacts restricts water flow through soil, and hence reduces its hydraulic conductivity. Vandevivere and Baveye (1992)[32] and Abdel Aal et al. (2010)[118] found that the hydraulic conductivity of soil reduced significantly through the accumulation of biomass and production of exopolymeric substances. Al Qabany and Soga (2013)[117] reported that, for four injection cycles, the high concentration cementation solution produced a quicker and greater reduction in the coefficient of permeability.

The falling head permeability test was used to determine the coefficient of

permeability of the cured specimens (still contained in molds). The two groups A and B are of river sand, while groups C and D are of silica sand, the groups A and C were treated with cementation solution composed of calcium chloride, which is made of egg shells and urea, while the groups B and D were treated with cementation solution composed of pure calcium chloride (analytical grade) and urea. The groups control 1 and control 2 were for river sand and silica sand without treatment, respectively. Figure 5-5 shows the effect of calcium source and soil type on the permeability.

From Figure 5-5, it can be seen that in the river sand samples (groups A and B) the effect of using cementation solution contained calcium chloride made of egg shells was exactly the same effect of using cementation solution containing analytical grade of calcium chloride on the coefficient of permeability, where the average coefficient of permeability and the average decreases in the permeability of group A and group B were 3.23×10^{-5} m/s, 3.35×10^{-5} m/s and 45%, 43%, respectively .

The same thing is repeated when talking about the silica sand samples (groups C and D), where the average coefficient of permeability and the average decreases in the permeability of group C and group D were 2.1×10^{-6} m/s, 2.01×10^{-6} m/s and 87%, 88%, respectively.

After all, in both cases (river sand and silica sand samples) the use of cementation solution contained calcium chloride made of egg shells has a significant effect on the permeability, but the effect was greater in the silica sand samples, where the average decreases in the permeability of the silica sand and river sand samples were approximately 87% and 45%, respectively.

5.3.4 Uniaxial Compressive Strength

To investigate the impact of the calcium source and soil type on the strength of treated samples, unconfined compressive strength test was conducted. From Figure 5-6, it

can be seen that in the river sand samples with calcium chloride (egg shells) and calcium chloride (analytical grade) (groups A and B, respectively), the unconfined compressive strength values were very close (331kPa, 380kPa). The same thing was observed for samples of silica sand with calcium chloride (egg shells) and calcium chloride (analytical grade) (groups C and D, respectively), where the values were (638 kPa, 648 kPa).

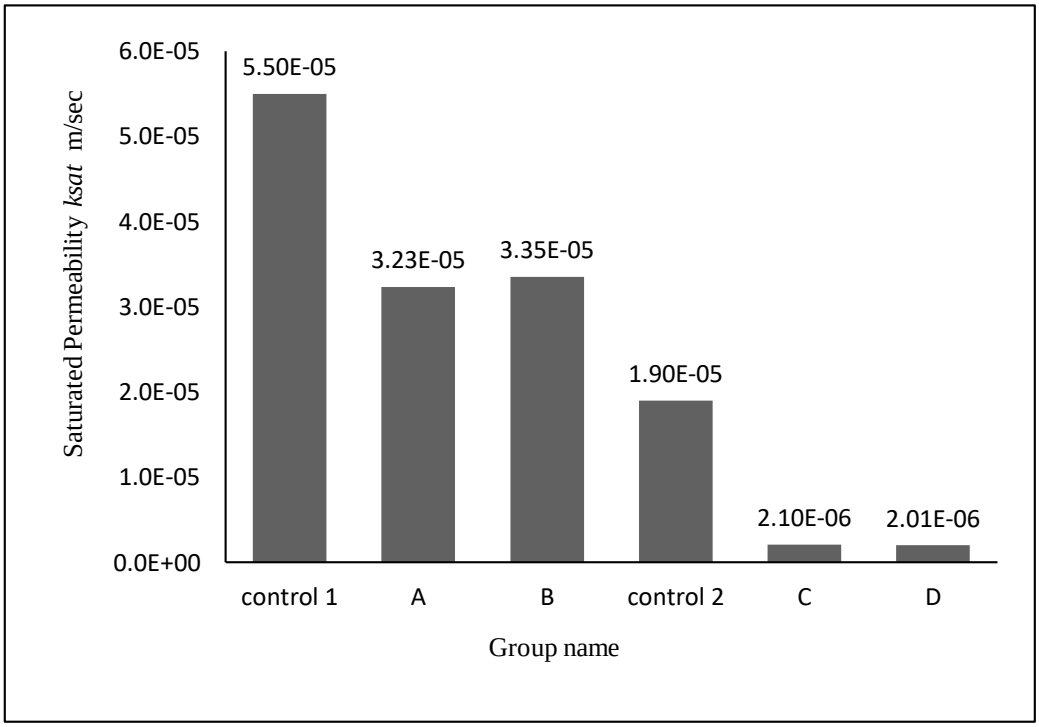


Figure 5-5 Effect of calcium source and soil type on the permeability

Also, it can be seen that the sand type has the greater role in the difference in the values of the unconfined compressive strength. For the same type of calcium chloride source, the value of unconfined compressive strength of silica sand samples was approximately two times higher than that of river sand samples.

Generally, the effect of using cementation solution contained calcium chloride made of egg shells was exactly the same effect of using cementation solution containing analytical grade of calcium chloride.

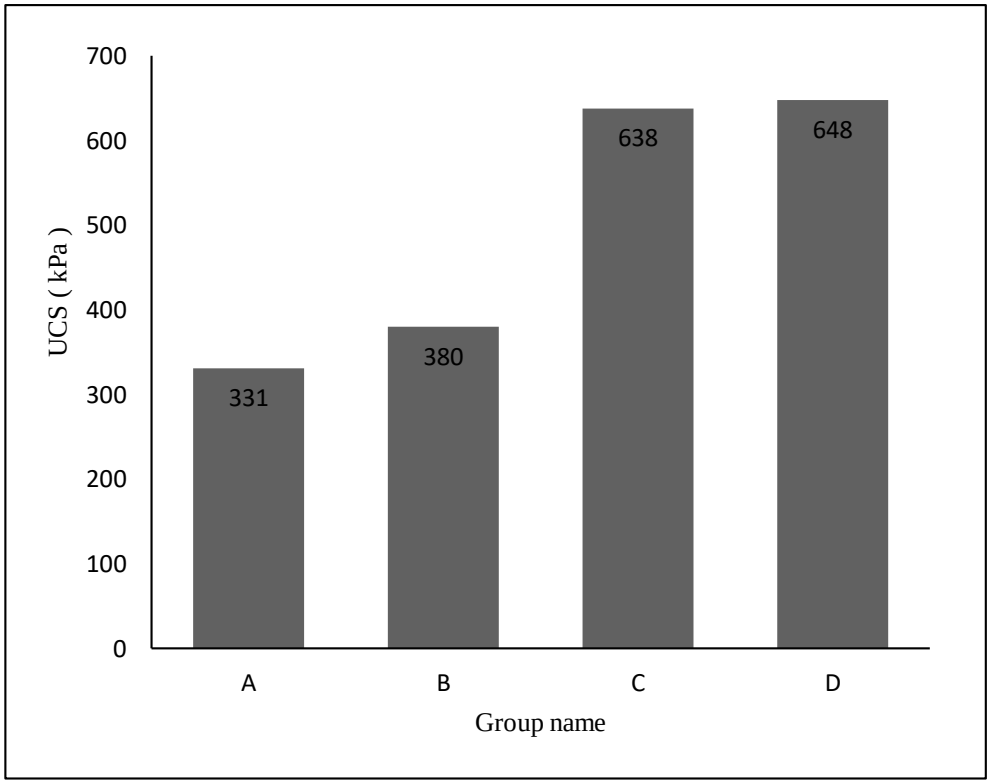


Figure 5-6 Effect of calcium chloride source and soil type on the strength

5.3.5 Scanning Electron Microscopy (SEM)

To investigate the impact of the calcium source and soil type on the crystal formation of treated samples, SEM analysis was conducted. The SEM results shown in Figure 5-7 A-D correspond to the river sand with the calcium chloride (egg shells) sample, river sand with the calcium chloride (analytical grade) sample, silica sand with the calcium chloride (egg shells) sample and silica sand with the calcium chloride (analytical grade) sample, respectively.

Generally, the calcium carbonate crystal types include calcite, aragonite, and vaterite. And calcite is the most common and steadiest among them. From Figure 5-7 A-D, it was clear that the calcium carbonate type in all cases was the calcite and the crystal size was almost the same (about 8-14 μm in diameter).

For the river sand samples, rhombohedral crystals with smooth surfaces could be

observed as a characteristic for calcite (Figure 5-7 A and B). While for the silica sand samples, the hexahedral crystals with rough surfaces as a characteristic for calcite could be observed in the case of the samples treated with cementation solution composed of calcium chloride (egg shells) and urea (Figure 5-7 C), and the hexahedral crystals with smooth surfaces of the samples treated with cementation solution composed of calcium chloride (analytical grade) and urea (Figure 5-7D). This means that the same type of cementation solution produced two different forms of calcite crystals (rhombohedral crystals, hexahedral crystals), depending on the types of treated soils.

This leads us to the conclusion that the calcium chloride type had little effect on the crystals formed compared to the significant impact that resulted from changing the type of soil.

5.4 Conclusions

The results presented in this study revealed that using the cementation solution composed of calcium chloride, which is made of egg shells and urea with MICP technique can improve the mechanical engineering properties of the fine sand (river and silica sands), exactly like the cementation solution composed of calcium chloride (analytical grade) and urea with MICP.

The bacteria cells were able to produce the same amount of calcium carbonate in both types of cementation solution, but it was in the river sand samples slightly higher than that in the silica sand samples. Also, in both types of sand, the use of cementation solution contained calcium chloride made of egg shells has a significant effect on the permeability, but the effect was greater in the silica sand samples. Moreover, for the same type of calcium chloride source, the type of sand has played a major role with respect to unconfined compressive strength, where the unconfined compressive strength of silica sand samples was approximately two times higher than that of river sand samples.

Additionally, the calcium chloride type had little effect on the crystals formed compared to the significant impact that resulted from changing the type of soils.

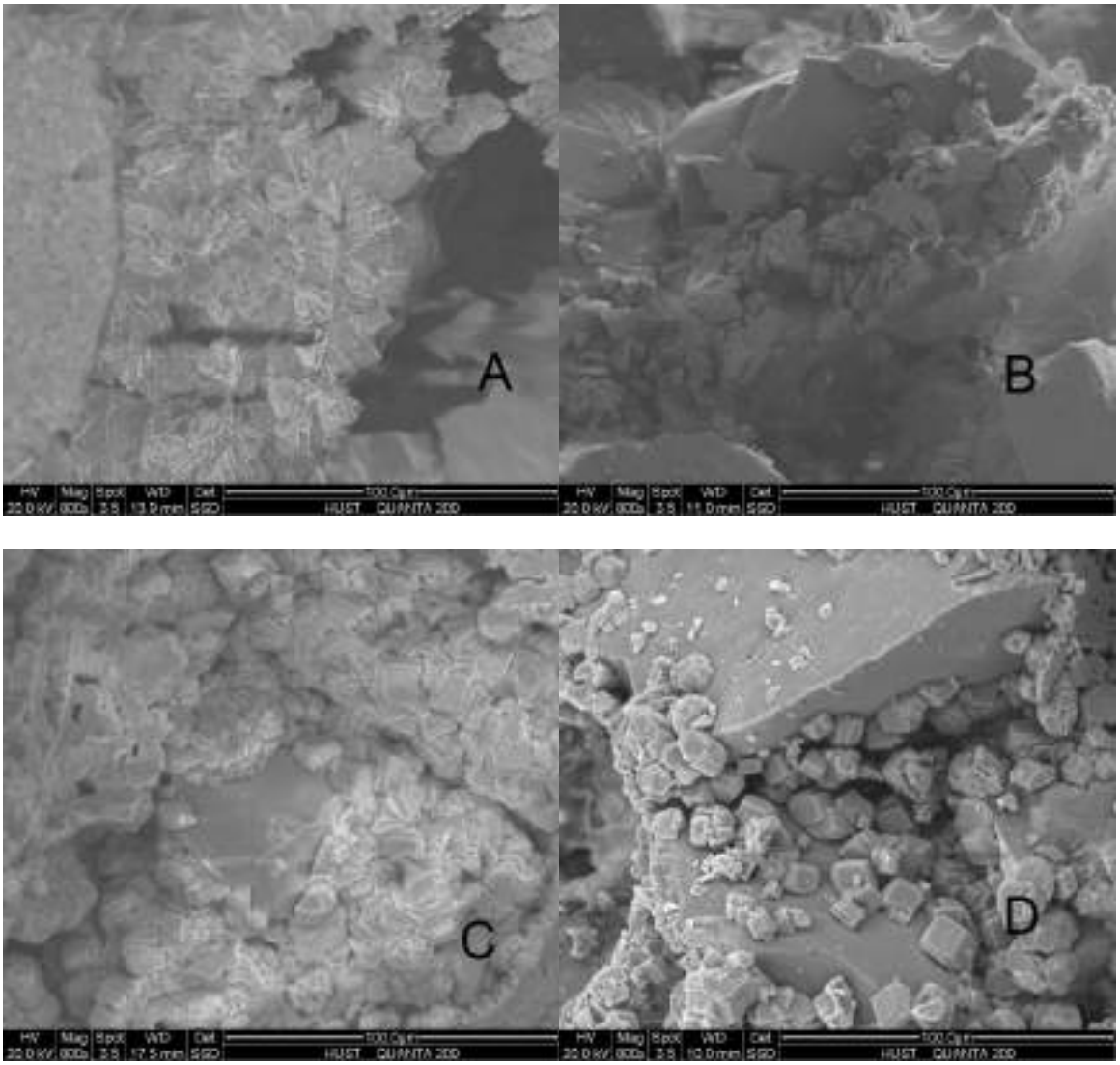


Figure 5-7 $CaCO_3$ crystal images (SEM) of treated samples of different calcium sources and soil types

6 Influences of Calcium Sources on Microbial Induced Carbonate Precipitation

6.1 Introduction

MICP technology is relatively one of the new technologies emerge in the field of soil improvement. Therefore, this technique needs more intensive research before it becomes usable widely.

This chapter aims to study the effect of calcium source type, the concentration of the cementation solution, the soil type, the use of cementation solution consisting of a combination of calcium sources, in the calcium carbonate content, the crystal formation, pH, the porosity, and the strength.

Set of laboratory tests were conducted, including pH, porosity, calcium carbonate content, unconfined compressive strength and microscopy investigation (SEM). In this chapter, Section 6.2 discusses the materials and methods of preparation of soil specimens, bacterial culture and cementation solution besides laboratory tests. Section 6.3 presents the results and discussion. Section 6.4 draws conclusions.

6.2 Materials and Methods

6.2.1 Preparation of Soil Specimens

The specimens were prepared in the same manner described in Section 5.2.1. Two types of poorly graded sand were packed into a polyvinyl chloride (PVC) column of 80 mm high and 37 mm inner diameter. The first type was fine silica sand which remains in the sieve with an opening size of 0.1 mm. While the second type was river sand taken

from the Yangtze river bank (more details in Section 5.2.1).

6.2.2 Bacterial Culture and Cementation Solution

The ureolytic bacterium used in the current study was Sporosarcina pasteurii (ATCC 11859). The ATCC 11859 was cultivated under sterile aerobic batch conditions in a yeast extract medium (20 g/l yeast extract, 10 g/l ammonium sulfate, 0.13 M Tris buffer, pH = 9) (more details in Section 3.2). The optical density OD600 of the bacterial culture varied between 1.4 and 1.5. The bio-cementation was conducted using four types of cementation solution, the first one is two concentrations of cementation solution consisting of equal moles of pure anhydrous calcium chloride (1 M, 111 g/l; 0.5 M, 55.5 g/l) and urea (1 M, 60 g/l; 0.5 M, 30 g/l). The second type is two concentrations of cementation solution consisting of equal moles of pure calcium acetate monohydrate (1 M, 176 g/l; 0.5 M, 88g/l) and urea (1 M, 60 g/l; 0.5 M, 30 g/l). The third type was prepared from urea (1 M, 60 g/l) and calcium chloride made of eggshells (1 M). The fourth type was prepared from urea (1 M, 60 g/l) and calcium acetate made of eggshells (1 M) (more details in Section 3.3).

6.2.3 MICP Procedure

Microbial induced carbonate precipitation for soil treatment was conducted using gravity-induced downward precipitation. Initially, the sand columns were divided into two groups. The first group was silica sand columns, and the second group was Yangtze river sand columns. The two groups were flushed with 33 ml for silica sand and 43 ml for Yangtze river sand bacterial culture, followed by 3 hours of retention time to allow the cells of bacteria to install themselves in the soil granules. At this stage, each group was divided into six subgroups depending on the type of cementation solution. The first subgroup was flushed with a cementation solution made of $CaCl_2$ (eggshells) and urea, the second and third subgroups were flushed with a cementation solution made of pure $CaCl_2$ and urea (1 M, 0.5 M), the fourth subgroup was flushed with a cementation

solution made of $C_4H_6O_4Ca$ (eggshells) and urea. While the fifth and sixth subgroups were flushed with a cementation solution made of pure $C_4H_6O_4Ca$ and urea (1 M, 0.5 M). In addition to the previous groups, other two subgroups of Yangtze river sand were prepared, the first was flushed with a cementation solution made of a mixture of calcium chloride and calcium acetate and urea (1 M), while the second was flushed with a cementation solution made of calcium chloride and urea (1 M) and calcium acetate and urea (1 M) in alternating order. The MICP reaction time was 24 h for cementation solution with concentration of 1 M and 12 h with a concentration of 0.5 M. The flushing with cementation solution was repeated every 24 h for cementation solution with concentration of 1 M and 12 h with a concentration of 0.5 M. The whole tests were performed at a temperature of 30°C±2°C. After 4 days of flushing with cementation solution, the treatment was stopped and the soil specimens were put in the oven at a temperature of 60ºC for 7 days. The variables and details of the experimental combinations are tabulated in Table 6-1.

6.2.4 $CaCO_3$ Content

To determine the amount of precipitated $CaCO_3$ in the soil specimens, the procedures described in Section 3.7.5 were adopted. The samples were crushed with hammer, oven-dried and the dry weights were recorded. The dry soil was soaked in HCl solution to dissolve precipitated $CaCO_3$, then washed with water and finally oven-dried and the weights were recorded. The difference between the weights before and after soaking in HCl considered being the weight of the $CaCO_3$ precipitated in the specimen.

6.2.5 Unconfined Compressive Strength

Unconfined Compressive Strength (UCS) tests were conducted according to the procedure reported in ASTM D2166 (ASTM, 2013) on bio-cemented soil specimens with

selected diameter-to-height ratios of 1:2 (more details in Section 3.7.1). The samples were flushed with deionized water (about four pore volumes) and dried at 60°C for 24 hours prior to UCS measurements. These tests were conducted to establish the relationship between the strength of the soil samples and its calcium source and soil type.

Table 6-1 Combinations of MICP treatment

Type of soil	Type of Cementation solution	Combination abbreviation
Yangtze river sand	$CaCl_2$ (eggshells) and urea (1M)	A
	$C_4H_6O_4Ca$ (eggshells) and urea (1M)	B
	$CaCl_2$ (AR) and urea (1M)	C
	$C_4H_6O_4Ca$ (AR) and urea (1M)	D
	CaCl2 (AR) and urea (0.5M)	E
	$C_4H_6O_4Ca$ (AR) and urea (0.5M)	F
	($CaCl_2$+ $C_4H_6O_4Ca$) and urea (1M)	G
	($CaCl_2$ or $C_4H_6O_4Ca$) and urea (1M) (alternate)	H
Silica sand	$CaCl_2$ (eggshells) and urea (1M)	I
	$C_4H_6O_4Ca$ (eggshells) and urea (1M)	J
	$CaCl_2$ (AR) and urea (1M)	K
	$C_4H_6O_4Ca$ (AR) and urea (1M)	L
	$CaCl_2$ (AR) and urea (0.5M)	M
	$C_4H_6O_4Ca$ (AR) and urea (0.5M)	N

6.2.6 Microscopy Investigation

In order to study the calcium carbonate types, bonds, and their distribution within the sand after treatments, the scanning electron microscope (SEM) was conducted (more details in Section 3.7.6).

6.3 Results and Discussion

6.3.1 Porosity

The porosity can be defined as a measure of the volume of voids in the soil to the total volume of the soil. These voids may be among grains of sand, silt, clay and organic matters in the soil or cavities of the soil. Usually, this open space is filled with air or water or both. Hence, the precipitation of calcium carbonate in the pore space will result in a reduction of the pore space volume.

The porosity was calculated by measuring the difference between the mass of the sample. Where the volume of water contained in a saturated column of known volume can indicate porosity. The mass of saturated sample minus the oven-dry mass of the sample, divided by the density of water, gives the volume of water. The volume of water divided by the original volume of the sample gives porosity.

$$n = (M_{sat} - M_{dry}) / (\rho_w V_t) \quad (6\text{-}1)$$

Where:

n : *porosity;*

M_{sat} : *mass of saturated sample;*

M_{dry} : *mass of the dry sample;*

ρ_w : *density of water;*

V_t : *volume of sample.*

The untreated sand column sample was prepared at same initial density for the

purpose of comparing the porosity of the samples before and after treatment. The pore volume of the specimens decreased as a result of the MICP treatment. The decrease in porosity of treated samples were from 48.67% (for all the river sand specimens before treatment) to 44.91%, 44.31%, 45.11%, 44.63%, 44.52%, 45.31%, 45.12%, 44.01% for groups A - H respectively, and from 37.67% (for all the silica sand specimens before treatment) to 34.95%, 34.33%, 34.33%, 35.06, 34.23%, 34.57% for groups I - N respectively. Table 6-2 shows the porosity of soil specimens before and after cementation by MICP technique and the amount of the decrease at the end of treatment duration relative to the original porosity.

Table 6-2 Porosity before and after treatment

Specimen group	Initial porosity %	Final porosity %	Decrease in porosity %
A	48.67	44.91	7.75
B	48.67	44.31	8.98
C	48.67	45.11	7.34
D	48.67	44.63	8.36
E	48.67	44.52	8.57
F	48.67	45.31	6.93
G	48.67	45.12	7.34
H	48.67	44.01	9.60
I	37.67	34.95	7.22
J	37.67	34.33	8.86
K	37.67	34.33	8.87
L	37.67	35.06	6.92
M	37.67	34.23	9.14
N	37.67	34.57	8.23

It was clear that there is diversity in the decrease of porosity between the samples. This is due to the different concentrations and types of soil and cementation chemical solutions used in the treatment. Also, it can be interpreted as a result of the number of bacterial cells that have been absorbed into the soil particles. Where it is, in general, the samples which absorbed a greater number of bacteria cells were capable to form a greater amount of calcium carbonate compared with samples that absorb less amount of bacteria cells.

6.3.2 pH

MICP method has many types of effects in the treated soil, one of these effects is the increases of pH of the environment near the bacteria cells. The local pH rise can be achieved by the production of ammonia resulting from the enzymatic hydrolysis of urea, known as urease activity. During MICP treatment, pH was measured in all groups. pH measured for the specimens treated with cementation chemical solutions 0.5M twice per day, while, the specimens treated with cementation chemical solutions of 1M were once per day. Figure 6-1 presents the variation in pH of effluent over time.

The initial value of pH for bacteria suspension was 8.4. The first effluent pH value was oscillating between 8 and 8.2, while, the effluent pH value after that fluctuated around 7, which achieves the requirements of downstream biological treatment processes. This rate of decreasing can be caused by a reduction in the rate of enzyme activities or by the accumulation of organic complexes (Hamdan, 2013)[110]. Nevertheless, a lower ureolysis rate can be beneficial for the distribution of chemical solutions and result in more uniform calcium carbonate precipitation given a uniform distribution of microbes (Martinez et al., 2013)[75].

Figure 6-1 shows that the pH values were not greatly affected by soil type, or the type of cementation chemical solutions and its concentration.

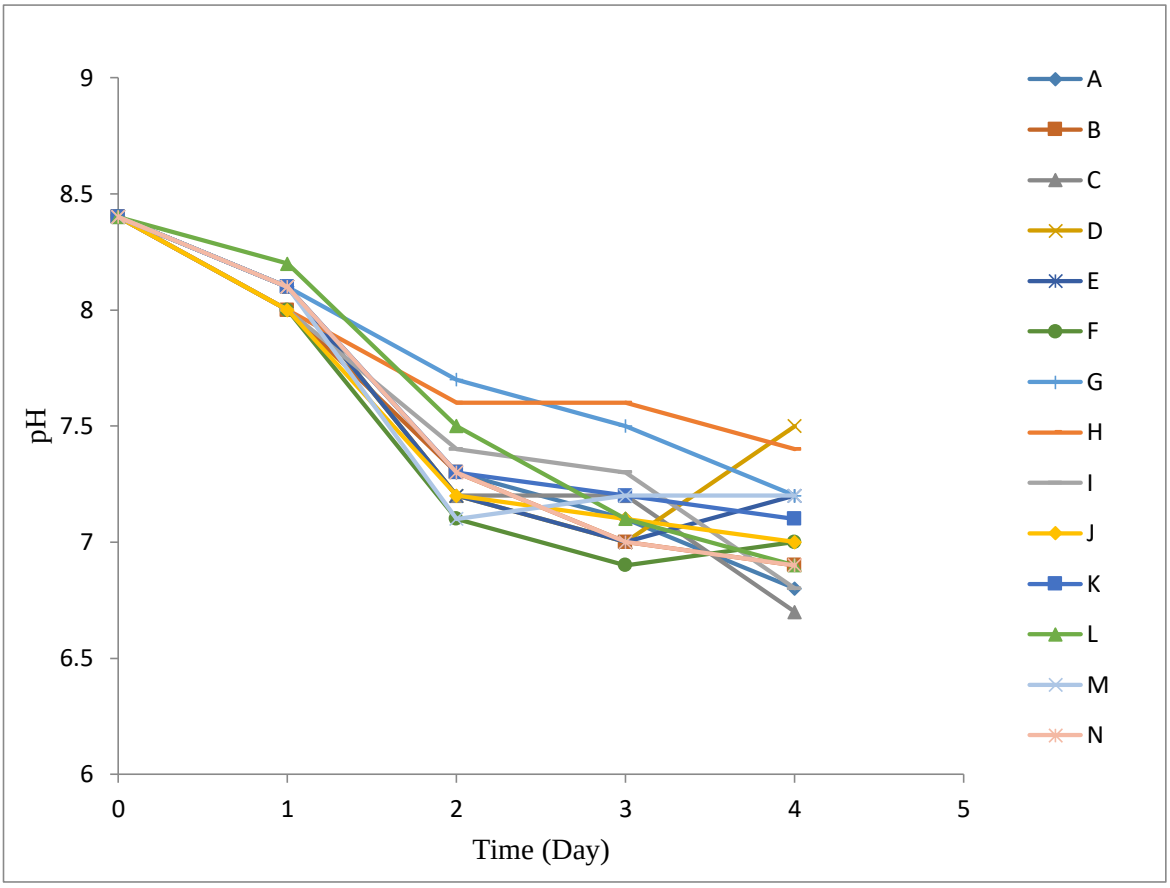


Figure 6-1 Variation of pH over time

6.3.3 Calcium Carbonate Content

The calcium carbonate content (i.e., gram/gram calcite per sand) of the samples of the two types of soil which were treated with different calcium sources are shown in Figure 6-2.

Figure 6-2 shows that the river sand samples (groups A- H) presented greater ability in precipitation of calcium carbonate from those shown by silica sand samples (groups I-N). This happened regardless of the type, source and concentration of calcium. Where the highest calcium carbonate content of the river sand was 0.173 in group B and less content was 0.155 in group A, while higher calcium carbonate content of silica sand was 0.131 in the group N and less content was 0.116 in group K. In general, the average

content of calcium carbonate for all groups of river sand was 0.165, and the average content of calcium carbonate for all groups of silica sand was 0.136.

After the end of treatment with two types of calcium chloride (1M), calcium carbonate content was 0.155, 0.163, 0.125 and 0.116 in groups A, C, I, K, respectively. These values showed that the bacteria cells were able to produce approximately the same amount of calcium carbonate in the river sand samples (groups A and C), and the same thing in silica sand samples (groups I, K) for both types of cementation solution. The same thing happened with the groups treated two types of calcium acetate, where the calcium carbonate content was 0.173, 0.162, 0.120 and 0.122 in groups B, D, J, L, respectively.

Also, Figure 6-2 shows that the groups E, F, M, N, which were treated with a cementation solution of calcium chloride and urea or calcium acetate and urea concentration of 0.5 M, presented calcium carbonate content slightly higher than the calcium carbonate content of the corresponding groups of the same type of soil which were treated with a cementation solution concentration of 1 M.

The average calcium carbonate content of samples treated with cementation solution concentration of 0.5 were 0.170 and 0.120 for river sand and silica sand respectively, compared with the average calcium carbonate content of samples treated with cementation solution concentrate 1M which were 0.163 and 0.120 for river sand and silica sand respectively.

From the above, it is clear that the type and source of calcium in the cementation solution did not have a great significance in the precipitated amount of calcium carbonate. The significant influencing factors on the precipitated amount of calcium carbonate were the soil type (especially the porous of soil), and the concentration of the cementation solution.

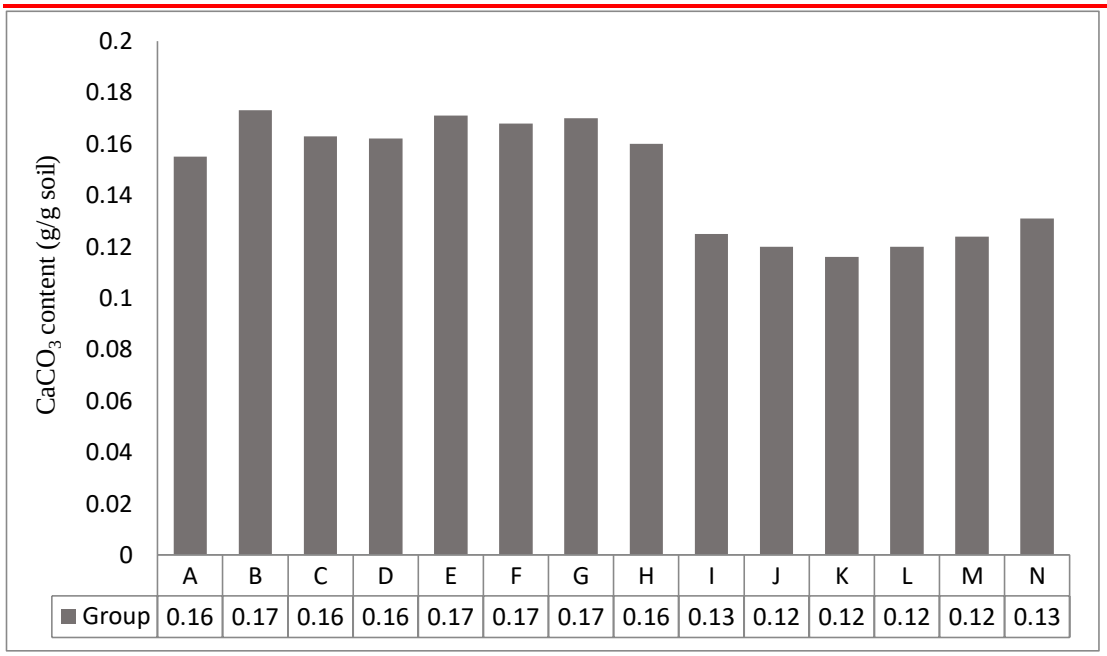


Figure 6-2 Effect of calcium source and soil type on the calcium carbonate content

6.3.4 Failure Mode Analysis of Sandstone

Typical failure modes of treated specimens under uniaxial compression are shown in Figure 6-3. It can be seen that major inclined shear cracks formed along the specimens with the cracking plane inclined 90° with respect to the horizontal plane and the specimen cannot burst into pieces after failure (longitudinal splitting), while the second type of failure is simple shear. The weak regions for all specimens with the second failure mode mainly concentrated on the one-third of the bottom of the sample.

According to Bobet and Einstein (1998)[119], under a uniaxial stress, pre-existing microcracks with appropriate dimensions and directions with respect to the maximum principal stress get closed when the applied compressive stress reaches a particular level known as the crack-closure stress. When the local tensile strength becomes less than the tensile stresses produced by compression at the edges of the pre-existing flaws, then reproduce new cracks from those edges and propagating cracks, known as wing cracks. These wing cracks align themselves to parallel to the maximum principal stress (Bobet and Einstein 1998)[119]. This indicates that when microstructure of a specimen does not

hinder the propagation of wing cracks, the specimen fails in axial splitting mode (Figure 6-3 a). However, when wing crack propagation along the maximum principal stress is constrained because of the existing microstructure, coalescence of nearby wing cracks or wing cracks in close proximity generated from the edges of the appropriately oriented microcracks takes place in order to release the strain energy in the form of shear fracture (Figure 6-3 b) resulting in higher unconfined compressive strength than that generally showed by axial splitting.

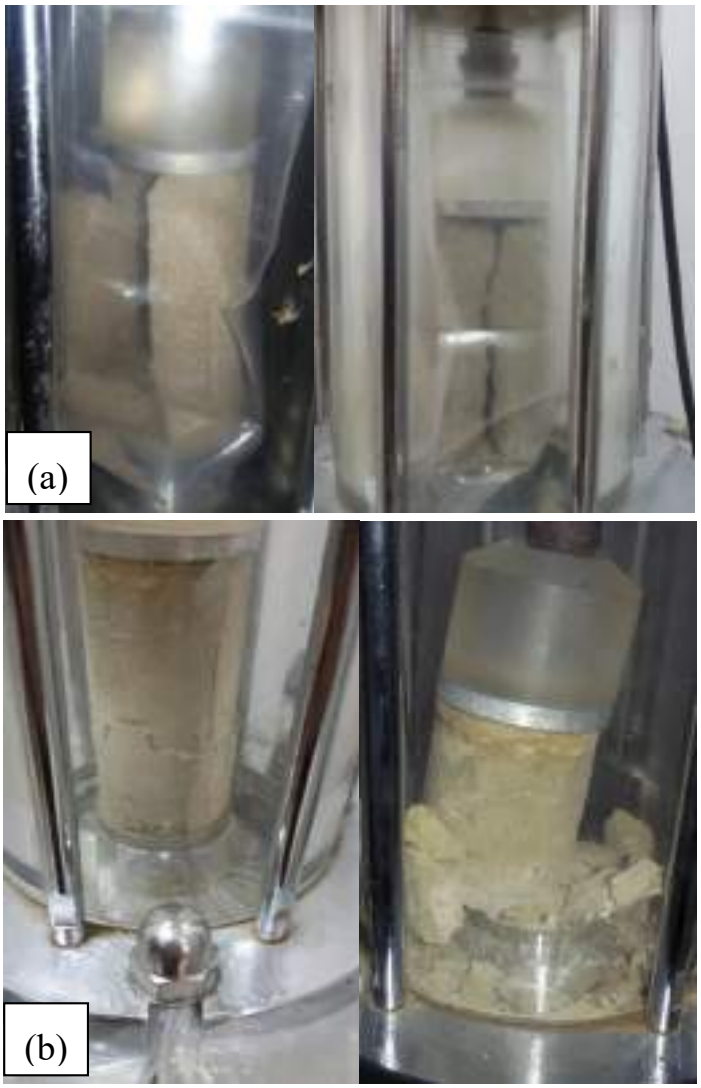


Figure 6-3 Typical failure modes of treated specimens under uniaxial compression.

(a) axial splitting (b) shear fracture

6.3.5 Unconfined Compressive Strength of Treated Soil

The effect of MICP on the strength of treated soil under different experimental procedures was determined by applying UCS test. The UCS values were determined as the highest deviatoric stresses.

The UCS of the samples of the two types of soil which were treated with different calcium sources is shown in Figure 6-4.

Figure 6-4 shows that the treated silica sand samples became stronger than the treated river sand samples, regardless of the type and concentration of the cementation solution used for treatment, where the average unconfined compressive strength was 656.17 and 442.31 in the silica sand and river sand samples, respectively.

The samples in groups A, B, I and J that have been treated by using a cementation solution containing calcium source (calcium chloride and calcium acetate) made of eggshells showed the results were very close to those of the samples C, D, K and L which have been treated with a cementation solution containing pure calcium chloride and calcium acetate (AR). This corresponds with the results that have been discussed in Section 5.3.4. Except group J, these groups showed strength less than that of group L.

Anyway, this result was expected because calcium acetate made of egg shells that were used in treating the sample J was left salt deposits on the upper surface of the sample, shortly after the end of the injection process, and with repeated injections, the amount of salt increases on the surface (Figure 6-5). This led to slow penetration of the cementation solution into the soil, and consequently, heterogeneity in the distribution of calcium carbonate sediments. This happened with group J and did not happen to group L because of the low permeability of silica sand (group J) compared with the permeability of river sand (group L).

Also, the results shown in Figure 6-4 indicate that the samples using calcium acetate (groups B, D, F, J, L and N) as the calcium source possess a slightly higher strength than

those groups using calcium chloride (groups A, C, E, I, K and M).

Except for group J, these groups showed strength less than the strength of group I, for the reasons which have been reported previously. These results agreed with the findings of Zhang et al.(2014)[87].

Also, Figure 6-4 showed that the samples in groups E, F, M and N that have been treated with a cementation solution of concentration 0.5 M have higher strength than those in the other groups which have been treated with a cementation solution of concentration 1 M. This indicates that the input chemical concentration potentially has an effect on the precipitation pattern.

Kunst and Rapoport (1995)[120] reported that the bacteria growth under a salt stress condition had a negative effect on the production of hydrolysis enzyme. Calcium chloride and calcium acetate, which are the main components in the cementation solution, are salts that may contribute to the salinity of cementation solution. Therefore, it is not recommended for the MICP treatment using a very high concentration of cementation solution.

Finally, for the purpose of exploring the effect of using different types of calcium sources at the same time for soil treatment, two groups G and H were prepared. Group G was treated by using a mixture containing calcium acetate and calcium chloride and urea of concentration 1 M. While group H has been treated using alternating injections of two cementation solution, the first one contains calcium chloride and urea while the second one contains calcium acetate and urea.

The results showed that the strength of the groups G and H did not differ much from the strength of the other groups that have been using one type of calcium sources in treating them. In general, group G showed greater improvement in strength compared to group H.

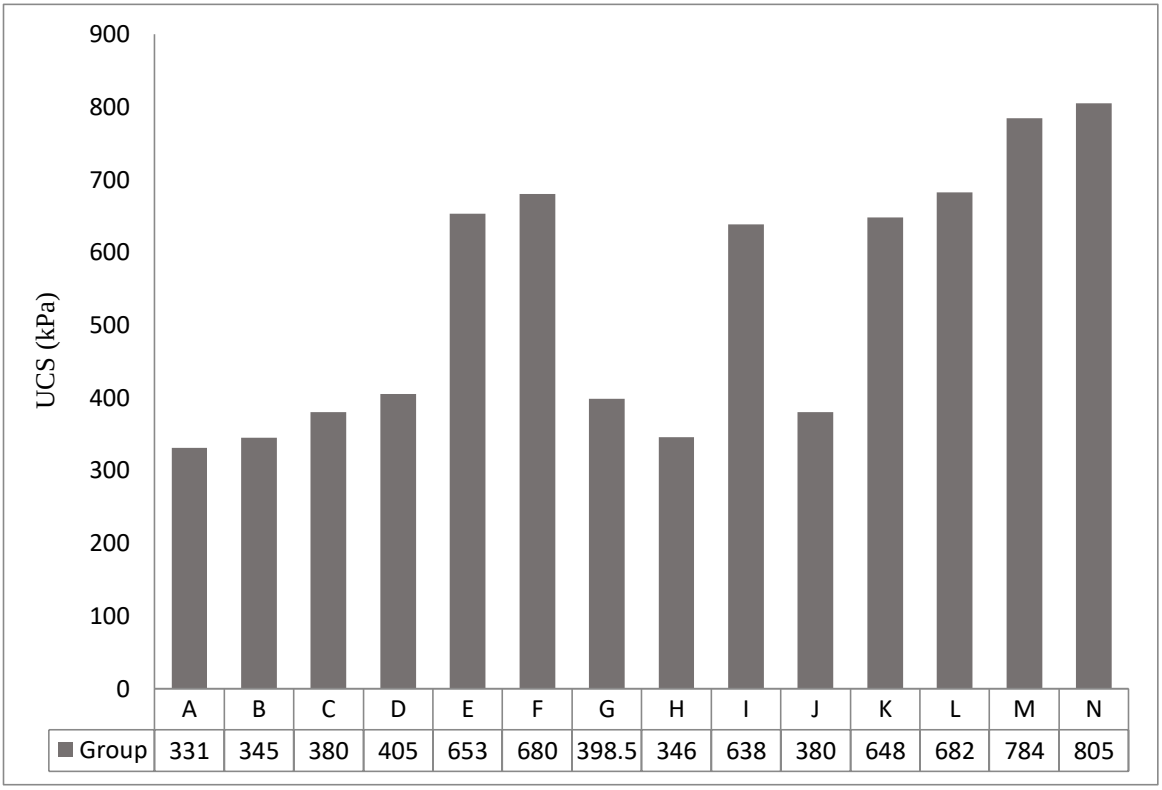


Figure 6-4 Influence of calcium source, cementation solution concentration and soil type on the strength

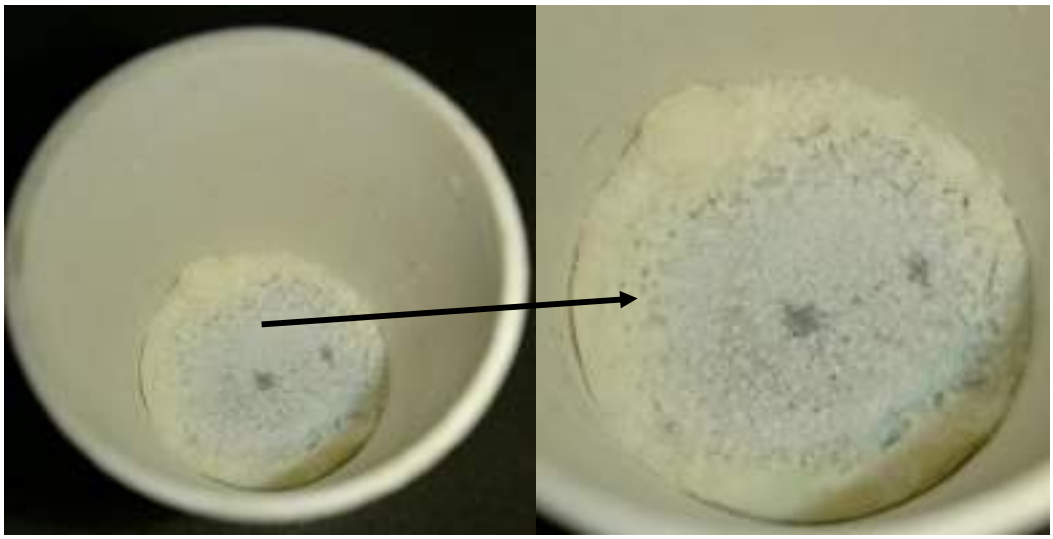


Figure 6-5 The salt deposits on the upper surface of the samples of group J

6.3.6 Scanning Electron Microscopy (SEM)

Figure 6-5 shows the image of the electron microscope of the samples of river sand and silica sand in groups A, C, I and K. In general, the results of the image showed a behavior quite similar to what has been discussed in Section 5.3.5.

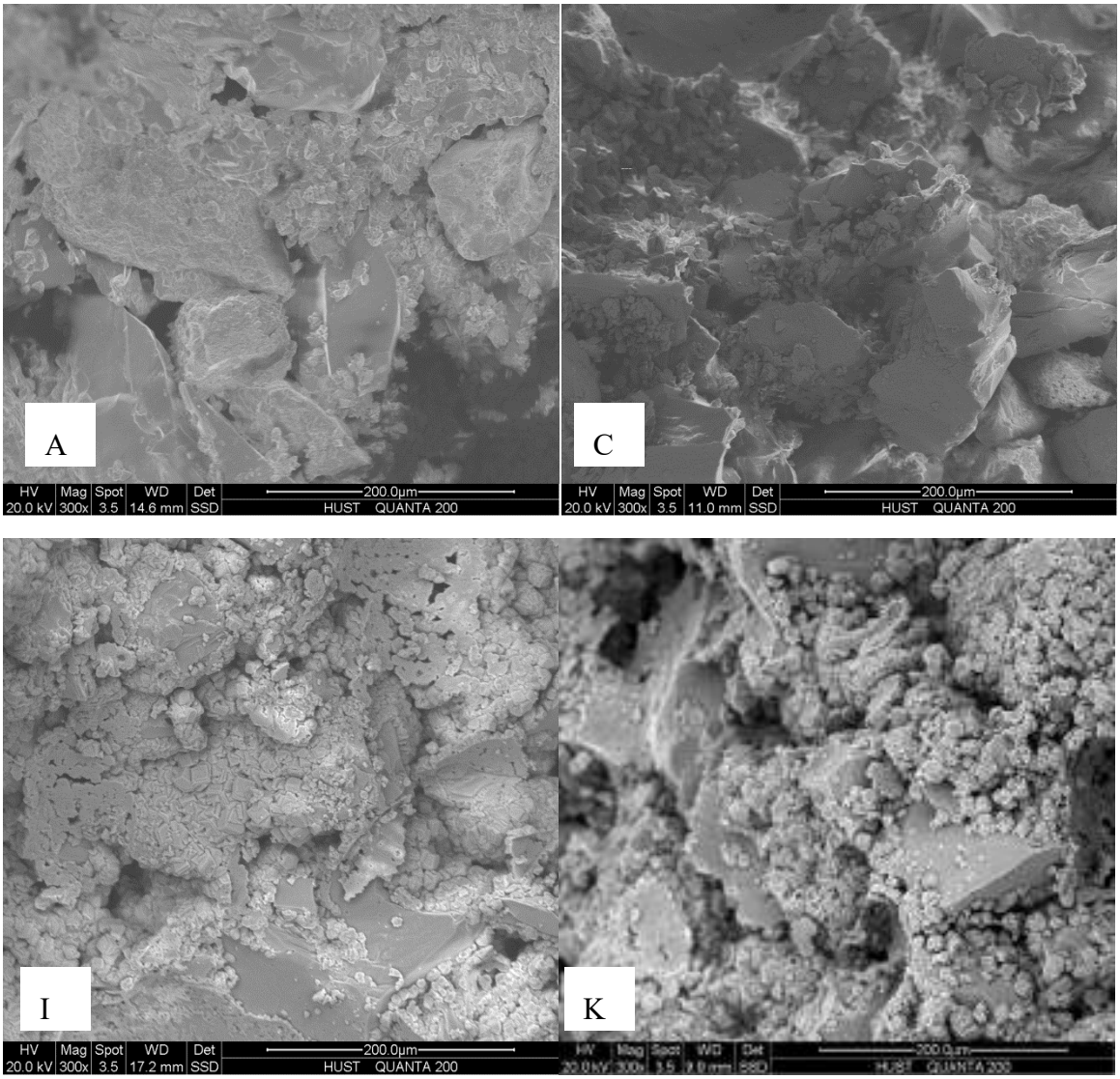


Figure 6-5 Samples treated with 1M of $CaCl_2$ (eggshells and AR) cementation solution and different soil types.

From SEM images in Figure 6-6, the sand granules in groups M and E were fully covered with calcium carbonate. The strength of the groups M and E were higher than

that of other groups although the calcium carbonate content does not change by a large margin. This can be explained by that the strength of the cemented column was due to the point-to-point contacts of calcium carbonate crystals, and the calcium carbonate crystals formed by the concentration of 0.5 M solution was distributed regularly and more homogeneous than the crystals of concentration 1 M , therefore, the calcium carbonate crystals of concentration 1M covered the sand particles but the point-to-point contacts binding the particles together was weaker. Also, rhombohedral crystals were observed in group E, while in group M hexahedral crystals with smooth surfaces were observed.

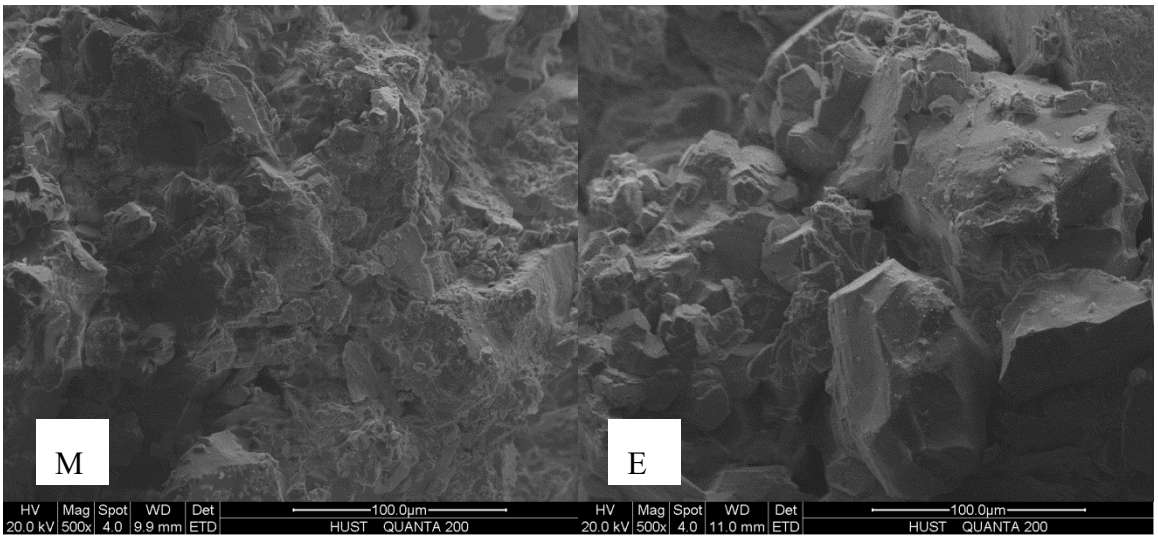


Figure 6-6 Samples treated with 0.5 M of $CaCl_2$ (AR) cementation solution and different soil types.

Figure 6-7 shows the image of the electron microscope of the groups B and J. These groups were treated with cementation solution contain calcium acetate made of eggshells and urea.

In previous researches, the crystal type of the MICP of the samples treated with calcium acetate is mainly aragonite. The acicular morphology is the typical characteristics of the aragonite (Zhang et al., 2014)[87] (Figure 6-8a). De Muynck et al.(2008)[121] discovered that the crystal morphology of calcium carbonate can be

influenced by the composition of cementation solution. Using calcium acetate as the calcium source, the calcium carbonate crystal was spheroid (Figure 6-8b).

The SEM results shown in Figure 6-7 correspond to the calcium acetate sample (vaterite). The crystal is a circulatory shape with smooth surfaces. It should be noted that the mineral morphology of the acetate sample in this study is very different from the 'spheroid' one. This change in mineral morphology may have resulted from the addition of calcium hydroxide to the eggshell during the process of making calcium acetate.

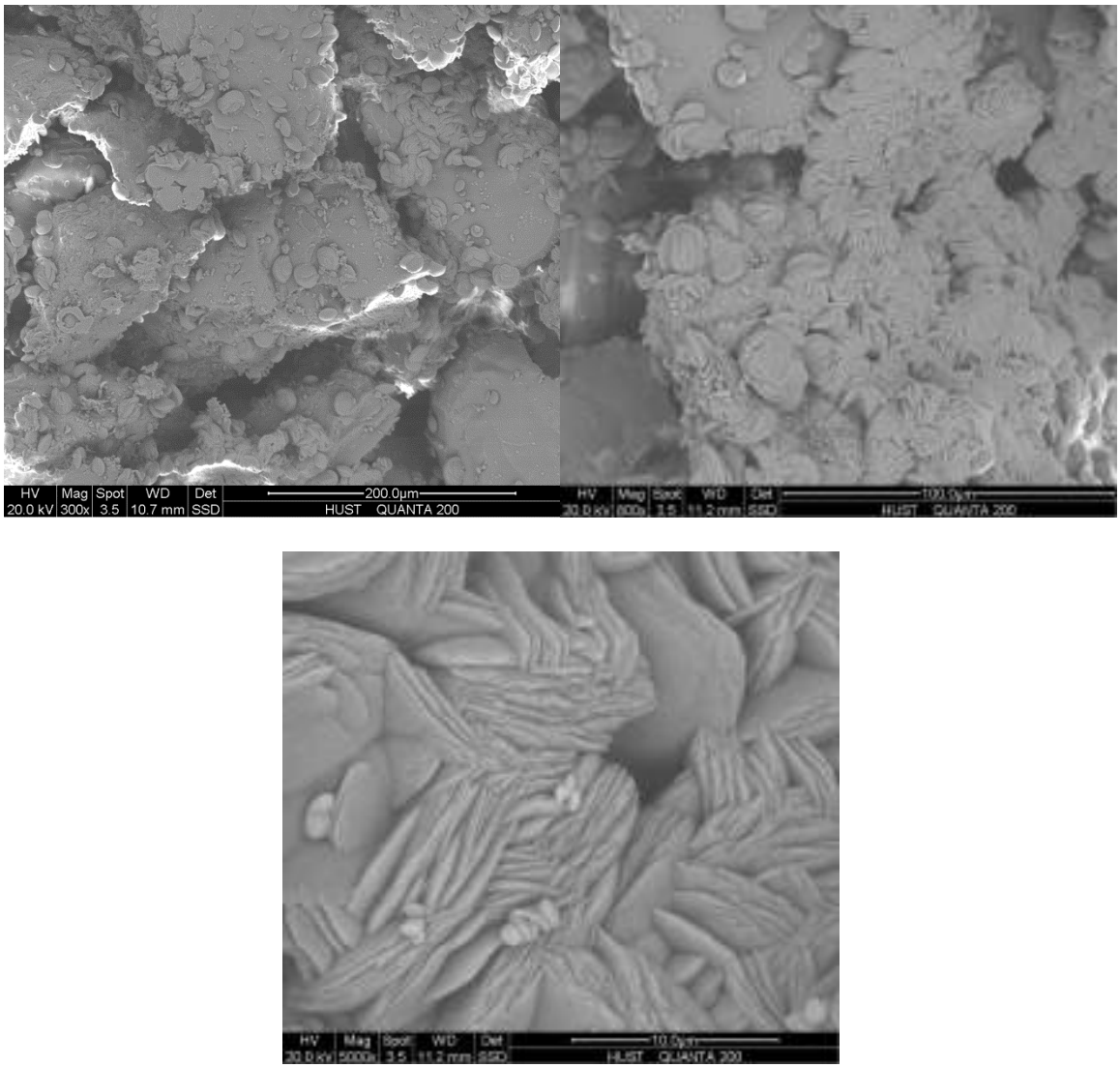


Figure 6-7 Samples treated with 1M of calcium acetate (eggshells) cementation solution and

different soil types.

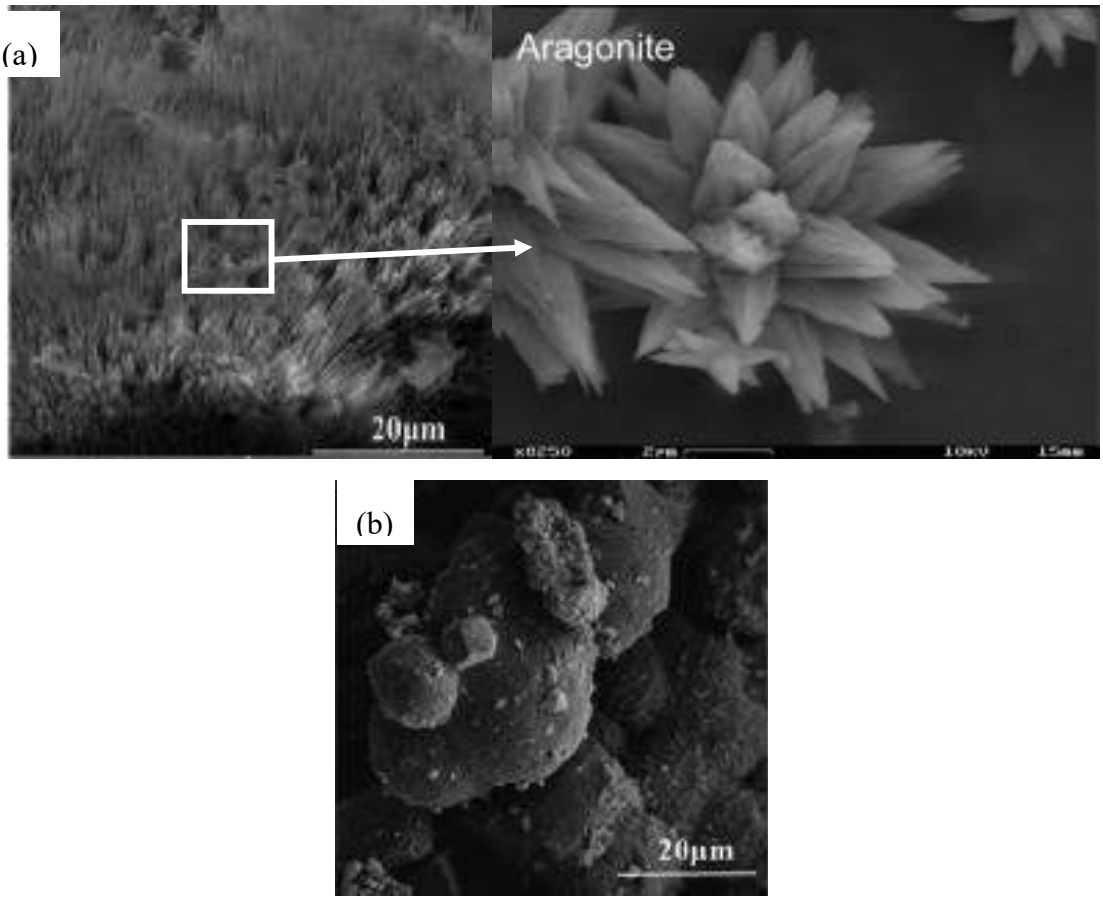


Figure 6-8 SEM images of treated samples using the calcium acetate as a calcium source.

a) aragonite (Zhang et al., 2014)[87]; b) vaterite (De Muynck et al., 2008)[121]

6.4 Conclusions

The results presented in this study revealed that using the cementation solution composed of calcium acetate, which is made of egg shells and urea with MICP technique can improve the mechanical engineering properties of the fine sand (river and silica sands), exactly like the cementation solution composed of calcium acetate (analytical grade) and urea with MICP.

The bacteria cells were able to produce the same amount of calcium carbonate in both types of cementation solution, but it was in the river sand samples slightly higher than that in the silica sand samples. Also, the type of calcium chloride had little effect on

the crystals formed compared to the significant impact that resulted from changing the type of soils, while calcium acetate type had a significant impact on the mineral morphology of the calcium carbonate crystals. In the case of very fine soils, use of calcium acetate as a source of calcium is not preferred.

The concentration of the cementation solution has a significant impact in increasing the effectiveness of MICP. The use of low concentrations provides a greater improvement in the engineering properties of the treated soil. The pH values were not greatly affected by soil type or the type of cementation chemical solutions and its concentration.

The specimens with shear fracture had higher unconfined compressive strength than that generally showed by axial splitting.

The use of a cementation solution containing variety mixtures of calcium sources did not show any noticeable difference in improving the properties of treated sand compared with sand treated with a cementation solution containing one type of calcium.

7 Conclusions and Recommendations

7.1 Summary of Conclusions

Microbial induced calcite precipitation (MICP) is a very complex process, particularly when it occurs between sand particles for improving mechanical and engineering properties of soil. Many factors may affect this process. This study investigated several factors, including the type of sand, the particle size of sand, initial degree of saturation, type of calcium source in cementation chemical solutions, the concentration of cementation chemical solutions, mixtures of different sources of calcium, reaction time, and curing conditions.

The following important conclusions can be drawn, based on the current study:

1. Using the MICP technique can improve the engineering properties of poorly graded fine sand.
2. The curing condition had a significant impact on the compressive strength, where the treated sand samples that soaked in water during the last step of curing showed a greater decrease of compressive strength, as compared to the treated sand samples with curing not soaked in water.
3. Using the cementation solution composed of calcium acetate or calcium chloride, which is made of egg shells and urea with MICP technique can improve the engineering properties of the fine sand (river and silica sands), exactly like the cementation solution composed of calcium acetate or calcium chloride (analytical grade) and urea with MICP.

4. The bacteria cells were able to produce the same amount of calcium carbonate in both types of cementation solution, but it was in the river sand samples slightly higher than that in the silica sand samples.
5. The concentration of the cementation solution has a significant impact in increasing the effectiveness of MICP.
6. The type of sand has played a major role with respect to unconfined compressive strength.

7.2 Recommendations

Although some findings are obtained from this study, there are some deficiencies in the experimental works. The following are some recommendations which can be taken into account in future study in this field.

1. A study using a pressurized injection system should be implemented, which should be considered for further experimental works such as the introduction of the push injection system or push-pull injection system which might improve the homogeneity of precipitated calcium carbonate and thus improve the homogeneity of the whole sample.
2. A cheaper nutrient is best when the technique needs to be applied in-situ; a cost-effective nutrient for the bacteria would be beneficial.
3. A study using industrial grade materials (i.e. urea, hydrochloric acid, acetic acid) to prepare the sources of calcium would be advantageous for the biocementation process to be implemented in the site. Cost-effective chemical solutions would be beneficial.

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12 – 04 - 2017

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1. Jawad F, Zheng J J. Improving Poorly Graded Fine Sand with Microbial Induced Calcite Precipitation. British Journal of Applied Science & Technology, 2016, 17(2): 1-9.
2. Jawad F, Zheng J J. Review of the Factors That Influence on the Microbial Induced Calcite Precipitation. Civil and Environmental Research, 2016, 8(10): 69-76.
3. Jawad F, Zheng J J. Influences of Calcium Sources and Type of Sand on Microbial Induced Carbonate Precipitation. International Journal of Advances in Engineering & Technology, 2017, 10(1): 20-29.
4. Jawad F, Zheng J J. Influences of Calcium Sources, Concentration of the Cementation Solution and Type of Sand on Microbial Induced Carbonate Precipitation. International Journal of Civil, Structural, Environmental and Infrastructure Engineering Research and Development, 2017, 7(2): 23-32.