MONASH University

Bio-grouting Improving Engineering Behaviour of Coarse Granular Columns

By

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A thesis submitted in fulfilment of the requirements for the degree of Doctor of Philosophy

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This thesis is dedicated to…

The memory of my mother, Ghania, for her infinite love, passion and encouragement, May her soul rest in peace!

To my wife, Sarah, through good and bad times, your love and extensive support have been omnipresent in this important milestone of my life, for which I am eternally grateful.

To my son, Daniel, and to my daughter, Elina

Executive summary

Bio-grouting by means of microbial induced calcite precipitation (MICP) has received significant research attention over the last decade as it has the potential to outweigh existing ground improvement techniques and mitigate environmental concerns with currently used materials. Although bio-grouting process and its implication on the improvement of soil engineering properties have been extensively studied over the past decade at laboratory scale, it has only been applied to fine and medium sand with minimal geotechnical applications. It has also yet to be utilised in field applications as it still needs to be optimised for a large-scale use. Several physical and environmental parameters that have a significant effect on the amount and efficiency of calcium carbonate precipitation in a soil matrix, need to be understood to enable a controlled use of bio-grouting and to scale up the method from the laboratory to field applications. A lack of knowledge also exists concerning the suitability of bio-grouting of coarser materials and whether the bio-grouting process can be achieved in the same way as with fine and medium sands. In addition, an efficient improvement of the macro-mechanical behaviour of coarse sand treated by various amounts of bio-cement materials requires an in-depth understanding of its microstructure. This thesis is tailored to investigate a suitable bio-grouting methodology to improve the engineering properties of coarser materials, to study physical and environmental factors that may be affecting the performance of bio-grout treated coarser materials, to study the soil microstructure changes at a different level of bio-cementation, and to mitigate bulging that may occur during loading of sand columns installed in soft clays. A multi-soil lift strategy with options of up to four soil lifts was undertaken to test the applicability of a bio-grout process to cement coarser materials columns. It was found that a single soil lift treatment strategy can lead to a very high increase in strength and stiffness compared to using multi-soil lifts. Various reagent phases (2, 4, 6 and 12 phases) consisting of alternately percolating solutions containing bacterial

suspension and cementation solution through the soil column, has also been investigated. It was shown that using a 4-reagent phase treatment strategy gave a high unconfined compressive strength. The thesis reveals that bio-grouting was strongly influenced by the choice of cementation solution concentrations, reaction times and temperatures. Higher strength and time-efficient bio-cementation of coarse sand columns were achieved over 24 hours incubation time with an equimolar cementation solution (1M) at 20°C temperature. It was seen that an increase of biochemical treatment cycles was associated with increased deposition of calcium carbonate ($CaCO_3$) and consequently an increase in compressive strength. Furthermore, the bio-cemented coarser materials retained reasonable porosity and permeability, which should allow dissipation of pore water pressure if required. The study also established a correlation between the gain in strength and stiffness of the bio-cemented coarse sand with the increase in the amount of deposited $CaCO_3$, initial relative density and dry density. In addition, this study showed that a gap-graded particle size distribution can improve the UCS of bio-cemented coarser granular materials. Furthermore, the microscopic investigations using X-ray computed tomography (XCT), scanning electron microscopy (SEM), and energy dispersive spectroscopy (EDS) were linked to the macro-mechanical changes, thus providing unique insight into the causation of the changes. Several common soil properties (calcium carbonate content, dry density, void ratio, and porosity) were successfully identified using the XCT technique. Last but not least, it was found that placement of an *ex-situ* bio-cemented coarse materials column substantially reduced its vertical strains compared with un-cemented sand column and kaolin clay. A further reduction of about 11% in the vertical strain was observed when a bio-cemented column was created *in-situ*. Bulging was also significantly reduced by 62% to 75% following *in-situ* bio-cementation and mostly occurred in the bottom section of the bio-cemented column, where bio-cementation was less evident.

Declaration

This thesis contains no materials which has been accepted for the award of any other degree or diploma at any other university or equivalent and that, to the best of my knowledge and belief, this thesis contains no materials previously published or written by another person, except where due reference is made in the text of the thesis.

Aamir Mahawish

Thesis including published works declaration

In accordance with Monash University Doctorate Regulation 17.2 Doctor of Philosophy and Research Master Regulation the following declaration are made:

I hereby declare that this thesis contains no materials which has been accepted for the award of any other degree or diploma at any university or equivalent institution and that, to the best of my knowledge and belief, this thesis contains no materials previously published or written by another person, except where due reference is made in the text of the thesis.

This thesis includes **six** original papers published in peer-reviewed journals, and **one** paper is currently under review. The core theme of the thesis is Geomechanics. The ideas, development and writing up of all the papers in the thesis were the principle responsibility of myself, the student, working within the Department of **Civil Engineering** under the supervision of **Professor Abdelmalek Bouazza and Associate Professor Will Gates**.

In the case of *Chapters 2 to 8* my contribution to the research work involved the following:

Thesis Chapter	Publication Title	Status *(published, in press, accepted or returned for revision)*	Nature and % of student contribution	Co-author Name (s) Nature and % of Co-author's contribution	Co-authors, Monash student Y/N
2	**Biogrouting Coarse Materials Using Soil-Lift Treatment Strategy**	*Published*	(70%) development of ideas; establishment of the methodology; Experimental work; Data analysis; Writing up and revisions.	1) Prof. Malek Bouazza (20%) Financial support; Input into manuscript; Revision 2) Dr Will Gates (10%) Input into manuscript; Revision	**No**
3	**Strengthening Crushed Coarse Aggregates Using Biogrouting**	*Published*	(70%) development of ideas; establishment of the methodology; Experimental work; Data analysis; Writing up and revisions.	1) Prof. Malek Bouazza (20%) Financial support; Input into manuscript; Revision 2) Dr Will Gates (10%) Input into manuscript; Revision	**No**
4	**Factors affecting the bio-cementing**	*Published*	(70%) development of	1) Prof. Malek Bouazza (20%)	**No**

			(70%) development of ideas; establishment of the methodology; Experimental work; Data analysis; Writing up and revisions.	Financial support; Input into manuscript; Revision	
	process of coarse sand			2) Dr Will Gates (10%) Input into manuscript; Revision	
5	Improvement of Coarse Sand Engineering Properties by Microbially Induced Calcite Precipitation	*Published*	(70%) development of ideas; establishment of the methodology; Experimental work; Data analysis; Writing up and revisions.	1) Prof. Malek Bouazza (20%) Financial support; Input into manuscript; Revision 2) Dr Will Gates (10%) Input into manuscript; Revision	No
6	Effect of particle size distribution on the bio-cementation of coarse aggregates	*Published*	(70%) development of ideas; establishment of the methodology; Experimental work; Data analysis; Writing up and revisions.	1) Prof. Malek Bouazza (20%) Financial support; Input into manuscript; Revision 2) Dr Will Gates (10%) Input into manuscript; Revision	No
7	Unconfined compressive	*Published*	(70%) development of ideas;	1) Prof. Malek Bouazza (20%) Financial	No

	strength and visualization of the microstructure of coarse sand subjected to different biocementation levels		establishment of the methodology; Experimental work; Data analysis; Writing up and revisions.	support; Input into manuscript; Revision 2) Dr Will Gates (10%) Input into manuscript; Revision	

I **have renumbered** sections of submitted or published papers in order to generate a consistent presentation within the thesis.

Student signature: ~signature~ **Date:** 23/01/2020

The undersigned hereby certify that the above declaration correctly reflects the nature and extent of the student's and co-authors' contribution to this work. In instances where I am not the responsible author I have consulted with the responsible author to agree on the respective contributions of the authors.

Main Supervisor signature: Prof Abdel Malek Bouazza   Date: 23/01/2020

List of Publications

The results obtained from the research conducted by the candidate have already been summarised and published in the journal and conference papers listed below. It is worth noting that all the journal papers were published in high quality journals in the field. In addition, a manuscript written

based on the outcomes of the recent stone column experiments is currently under review and it is presented herein as the eight chapter of this thesis

<u>Peer reviewed journal papers</u>

1. Mahawish, A., Bouazza, A. and Gates, W. P. 2016. Bio-grouting coarse materials using soil-lift treatment strategy. *Canadian Geotechnical Journal*, 53(12), pp.2080-2085.

2. Mahawish, A., Bouazza, A. and Gates, W. P. 2017a. Effect of particle size distribution on the bio-cementation of coarse aggregates. *Acta Geotechnica*, 13(4), pp. 1019-1025.

3. Mahawish, A., Bouazza, A. and Gates, W.P. 2018a. Factors affecting the bio-cementing process of coarse sand. *Ground Improvement*, 172 (1), pp. 25-36.

4. Mahawish, A., Bouazza, M. and Gates, W. P. 2018b. Improvement of coarse sand engineering properties by microbially induced calcite precipitation. *Geomicrobiology Journal*, 35(10), pp. 887-897.

5. Mahawish, A., Bouazza, M. and Gates, W. P. 2018c. Strengthening crushed coarse aggregates using bio-grouting. *Geomechanics and Geoengineering Journal*, 14(1), pp. 59-70.

6. Mahawish, A., Bouazza, A. and Gates Will, P. 2019. Unconfined compressive strength and visualization of the microstructure of coarse sand subjected to different bio-cementation levels. *Journal of Geotechnical and Geo-environmental Engineering*, 145(8), pp.04019033.

Conference Papers

1. Mahawish, A., A. Bouazza, and W. P. Gates. 2017. Microstructure of bio-cemented coarse sand. In Proc., *Int. Conf. on Piled Foundations and Ground Improvement Technology*, 482–491. New York: DFI.

2. Mahawish, A., Bouazza, A., & Gates, W. P. 2018. Improvement of soft soils using bio-cemented sand columns. *Springer Series in Geomechanics and Geoengineering*, (216849), 822-825. https://doi.org/10.1007/978-3-319-97112-4_184.

Journal paper under review

1. Mahawish, A., Bouazza, A. and Gates Will, P. 2019. Biogrouted stone columns in soft clay. *Canadian Geotechnical Journal*.

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Last but not least, I would like to thank my family: my wife, Sarah and my brothers and sisters for supporting me spiritually throughout my life in general. Special thanks go to The Higher Committee for Education Development in Iraq for giving me this opportunity to complete my PhD.

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Chapter 1: Introduction

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1.1 Background

The scarcity of land for the construction of new infrastructures, especially in urban and metropolitan areas has created a significant pressure on land demand; consequently, land prices have risen dramatically in recent years. The building and construction industry has, therefore, increasingly considered developing available lands that have often been ignored because of poor ground engineering properties. These sites include lands in low-lying areas, wastelands, saturated fine to medium silty sand deposits and creek lands having deep deposits of soft saturated marine and estuarine clays deposits. Such lands are generally characterised by undesirable geotechnical properties for foundations, including variable thickness, very low strength and high compressibility. Use of concrete piles to transfer loads to lowermost better strata, or ground improvements techniques are very often the solutions adopted to allow the construction process to progress. Ground improvement refers to a technique that improves the engineering properties of a weak soil mass such as shear strength, stiffness and permeability. Ground improvement has been developed into a sophisticated tool to support foundations for a wide variety of structures. It often reduces direct costs and saves construction time if it is appropriately applied. A range of ground improvement techniques has been employed to artificially improve soil properties including, two of the main traditional ground improvement techniques that are mechanical compaction and chemical grouting. Mechanical compaction is conducted using heavy machinery; thereby, it consumes a large amount of energy and causes soil disturbance. Soils in the surrounding space can also be affected. By contrast, most of the artificial chemical grouts (e.g., chemical based on acrylamides, lignosulfonates, and polyurethanes, with an exception of sodium silicate) are considered toxic and are deemed to be harmful to the natural environment and human health (DeJong et al. 2010). Furthermore, the use of Ordinary Portland Cement in the improvement process has a very high carbon footprint on the

environment, as its production results in the release of significant amounts of greenhouse gases. For example, the production of one ton of Portland cement produces about one ton of carbon dioxide. Also, it was recently reported that cement production formed about 5 % of global carbon dioxide emissions (IPCC, 2013). Thus, it is important that the construction industry plays a significant role in improving the environment role by supporting green technologies.

Some of the ground improvement methods currently used have been practised for many years. Others techniques are being developed to provide sustainable and cost-effective approaches to replace conventional techniques.

In the past two decades, the use of stone columns in soft soils to improve their bearing capacity has greatly increased throughout the world. Typically, stone columns consist of vertical reinforcement introduced by constructing a column of densely packed stones to strengthen the weak local soil. The placement of stone columns in a specific grid pattern allows the soil mass to behave like a homogenous layer with improved density and stiffness. This process leads to the enhancement of the load-bearing capacity and minimises the settlements of the treated ground compared to the untreated ground. Stone columns, installed using the vibro-replacement method, is one of the most common improvement techniques for the foundation of embankments or structures on soft soils. The inclusion of coarse gravel or coarser crushed aggregates, which has higher strength, stiffness and permeability than the natural soft soil, improves the bearing capacity and the stability of embankments and natural slopes, reduces total and differential settlements, and accelerates soil consolidation. Native soft soils do not lend themselves to compaction through vibration alone and therefore are partially replaced by granular materials in a regular pattern, where the cavities are constructed using vibratory probe accompany by a water jet as shown in figure 1.1. After installation of a group of stone columns in cohesive soils, a composite material that has lower compressibility, higher

strength and higher permeability than natural soils alone, is created. Also, stone columns may be used in cohesive soil to reduce the magnitude of total and differential settlement, improve stability, reduce lateral spreading and increase the rate of consolidation. However, stone columns tend to slump or bulge excessively under loading if sufficient lateral resistance is not provided by the surrounding soil (Highes and Withers 1974). While stone columns transmit some load to the soil by shear stresses (along with the column-soil interface) and end bearing (at the column base), the predominant load transfer mechanism is lateral bulging into the surrounding soil as depicted in Figure 1.2. The passive resistance of the surrounding soil dictates the column performance under load. Generally, the column bulging will be highest close to the top of the column where the overburden pressures are lowest (McCabe et al., 2007). Thus, it is essential to seek sustainable, cost-effective and eco-friendly techniques for improving and modifying the performance of granular columns in mitigating excessive radial expansion during loading.
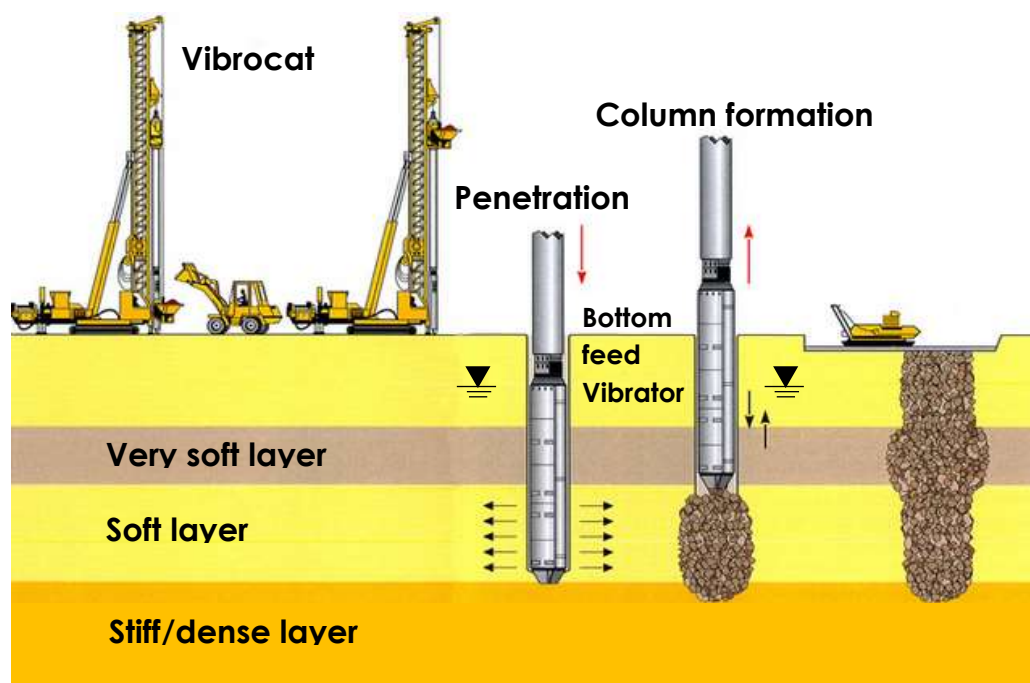
Figure 1.1. Installation of dry vibro stone columns (updated based on imaged obtained from http://www.zealchontesting.com/sale-8426042-zcq75-vibro-replacement-stone-columns.html).
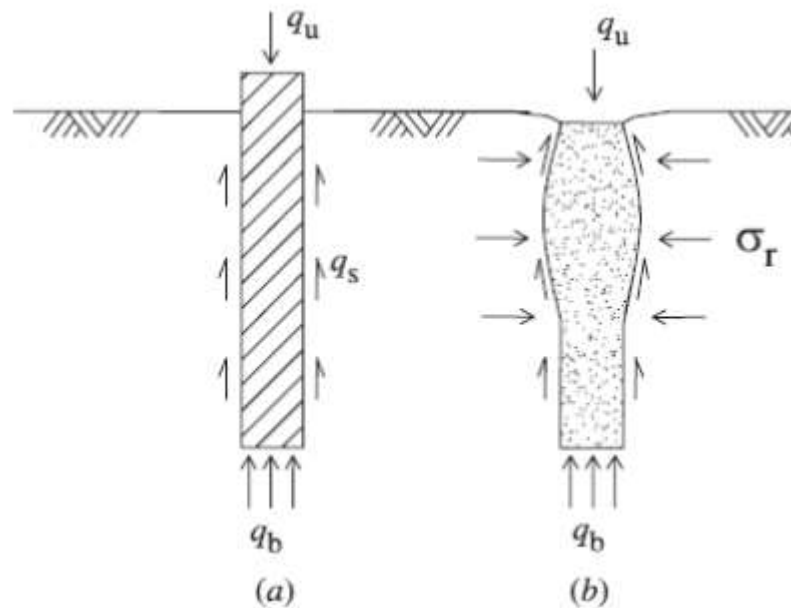
Figure 1.2: Mechanisms of load transfer for (a) a rigid pile and (b) a stone column (after Hughes and Withers 1974).

1.2 State of the art

Bio-grouting, based on biogeochemical processes such as microbial induced calcite precipitation (MICP), can offer a more sustainable strengthening/stabilising option. The enzymatic activities of microorganisms, such as *Sporosarcina pasteurii*, cause the precipitation of calcium carbonate within a soil matrix and binds granular particles together at inter-particle contacts leading to increased strength and stiffness of a given soil (Mitchell and Santamarina 2005, DeJong et al. 2006, Mortensen and DeJong 2011, Montoya 2012, Stabnikov et al. 2015, Terzis & Laloui, 2018, 2019, Dejong & Kavazanjian, 2019). This approach of soil stabilisation has been suggested to be used in numerous geotechnical applications. However, it is yet to be optimised and used to improve the engineering properties of coarser materials with the view to be used as sand/stone columns installed within weak soils.

1.2.1 An optimisation of bio-grouting technique for soil improvement

There are several key factors, including calcium concentration; concentration of dissolved inorganic carbon (DIC); pH; availability of nucleation site that need to be taken into account to achieve the target cementation. These factors govern precipitation of calcium carbonate (Castanier et al. 1999, Kile et al. 2000, Hammes and Verstraete 2002). The concentration of carbonate ion is mostly dependent on the saturation of DIC and solution pH. Besides, the presence of calcium ion is vital to precipitate calcium carbonate in the presence of a nucleation site (bacterial cells for instance). Furthermore, other parameters have also effects on the performance of calcite precipitation, such as geometric compatibility of bacteria, treatment conditions (reagent concentration and injection/percolation strategy), biological and environmental factors (bacterial cells concentration, salinity, oxygen availability, temperature, and incubation time)(Rivadeneyra et al. 2004, De Muynck et al. 2010, Mortensen et al. 2011, Pepper et al. 2011). Bacterial cells are considered the main factor in bio-grouting process as urease-producing bacteria are driven calcium carbonate precipitation through catalysing ureolysis by urease enzyme. Hence, the key point to choosing a bacterial strain is to be capable of producing urease enzyme, non-pathogenic, alkalophilic and halophilic, of tolerating high ammonium concentration and also have a high negative zeta-potential to promote adhesion for divalent cations (Dick et al. 2006, De Muynck et al. 2007). Numerous bacteria were utilised to trigger calcium carbonate precipitation; examples include Bacillus sphericus in enhancing the durability of concrete (De Muynck et al. 2008, Van Tittelboom et al. 2010), Bacillus Megaterium to improve concrete strength and durability (Achal et al. 2009, 2011). However, the most prominent bacterial strains is *S. Pasteuri* (formerly known *Bacillus Pasteuri*), which has been extensively used in most of the current research on concrete or soil and was deemed to be highly urease producing bacteria (Whiffin et al. 2007, Raijiwala et al. 2009, Sarda et al. 2009, DeJong et al. 2010, Mortensen and DeJong 2011, Gomez et al. 2014).

The enzyme urease produced by bacteria is analogous to any other enzymatic reactions where it depends on environmental temperature. As temperature increases from 10 $^{\circ}$C to 60 $^{\circ}$C, the urease activity increases resulting in an increase in the precipitation rate of calcium carbonate (Ng et al. 2012). However, it was found that most urease enzymes typically worked within an optimum temperature range of 20 $^{\circ}$C to 37 $^{\circ}$C (Mitchell and Ferris 2006, Okwadha and Li 2010) and it was completely stable at 35 $^{\circ}$C (Dhami et al. 2013). Thus, changing or controlling the in-situ temperatures to suit MICP in field situations may be difficult. Consequently, for MICP to be suitable for engineering applications, where there are spatial and temporal variabilities in soil temperatures in the field (Barry Macaulay et al. 2013, Singh et al., 2015), requires more research effort. Incubation time is also postulated to have a significant impact on the applications of the bio-grouting process. Incubation time is the amount of time that the urease enzyme requires to catalyse the hydrolysis of the urea. This factor also requires understanding before scaling up the process.

Additionally, bacterial cells themselves, whether live or dead, can function as nucleation sites for calcite crystals (Schultze-Lam et al. 1996) by attracting divalent cations to the wall of the bacterial cells (Greenfield 1963, Stocks-Fischer et al. 1999, Van Lith et al. 2003, Aloisi et al. 2006). Bacterial cells may flush from a soil matrix if an improper injection method is used. A practical method is required to induce adequate calcite precipitation and to obtain a better distribution of calcite crystals within a soil matrix. Different bacterial fixation methodologies and their effectiveness in MICP-treated granular soils have been reported by several researchers (Le Metayer-Levrel et al. 1999, Whiffin et al. 2007, Harkes et al. 2010, Cheng and Cord-Ruwisch 2012, Tobler et al. 2012, Al Qabany and Soga 2013, Shahrokhi-Shahraki et al. 2015). None of them seemed to have been successfully tested to improve coarser materials, such as very coarse sand with D_{50} 1.6 mm. One technique that was suggested to stabilise coarse granular soils was to a mix of bacterial suspension with cementation solution before they were injected into the soil

matrix (Le Metayer-Levrel et al. 1999). However, this protocol caused the inhomogeneous formation of small clumps or masses of calcite throughout the matrix. Another approach was based on a two-phase injection strategy, where bacterial cells suspension were injected first, followed by the injection of 50mM calcium chloride solution (Whiffin et al. 2007). This protocol seemed to have had a positive effect on the bacterial cell fixation within fine to medium sand. However, the main drawback associated with this protocol was that it necessitated the use of 0.5M calcium concentrations, much of which was flushed after the bacterial cells fixation process. This protocol has also not been tested with coarser materials. Another point to note is that this process can compromise the application of the MICP method, when it is scaled up to the field as using too much of calcium without inducing calcium carbonate precipitation can have a significant effect on the cost of the MICP process. Other strategies to note were staged injection of bacterial suspension followed by cementation solution, with or without incubation time for bacterial suspension and cementation solution to intermix (Harkes et al. 2010). Incubation time is necessary to facilitate several reactions occurring between microorganisms and reagents solutions (Al Qabany et al. 2011, Rong et al. 2012). This approach led to a more homogeneous distribution of calcite crystals within the specimen and improved immobilisation of bacterial cells within sand grains (Tobler et al. 2012). As a modification for the protocol above (staged injection), bacterial suspension and cementation solution were sequentially percolated using multiple thinner layers (lesser volume) of the bacterial suspension and cementation solution followed by incubation time (retention time)(Cheng and Cord-Ruwisch 2012). A final methodology to note is the single-stage injection where cementation solution is injected simultaneously with injection of bacterial suspension into the sand. This was considered to be an ineffective method due to rapid clogging of the injection points which restricted the injection of more fluids (Shahrokhi-Shahraki et al. 2015). Hence, the strategies discussed above cannot be directly used with coarser materials, typically used in stone columns as special attention is required to

obtain a better cementation distribution within a long coarse sand column with retention of high compressive strength. Thus, a new inspired bio-grouting strategy to enhance coarser materials is needed.

Soil type, sieve analysis and soil classification, relative density and geometric compatibility (porosity and pore volume) have an important influence on MICP-treated soil. Kim et al. (2013) concluded that biological activity in poor graded sand was more pronounced than in well-graded sand and indicated that more uniform voids among soil particles resulted in more active calcium carbonate deposition based on laboratory test results. However, there is a lack of understanding related to relative density, dry density and gradation of bio-cemented soil. Another important point is soil-bacteria compatibility as the bacteria sizes are in the range of 0.5-3mm (Mitchell and Santamarina 2005, Madigan et al. 2008). Many types of soil have pore throats, which can be used by microorganisms to travel through soil matrices. However, some soils can have a restriction on microbial transport as the pore throat sizes within the soil matrix are too small to let microbes pass from one side to another (Mitchell and Santamarina 2005). Figure 1.3 shows a geometric comparison of soil size-bacteria relationship; it can be seen that MICP soil improvement can be employed in a wide range of soils, especially coarser materials. DeJong et al. (2010) stated that moving toward coarser soils tends to have unhindered microbial motion and is easy to provide necessary nutrient for microorganisms. Besides, the effectiveness of MICP-treated soil can be decreased as a result of bio-cementation, especially in case of low pore throat soils.
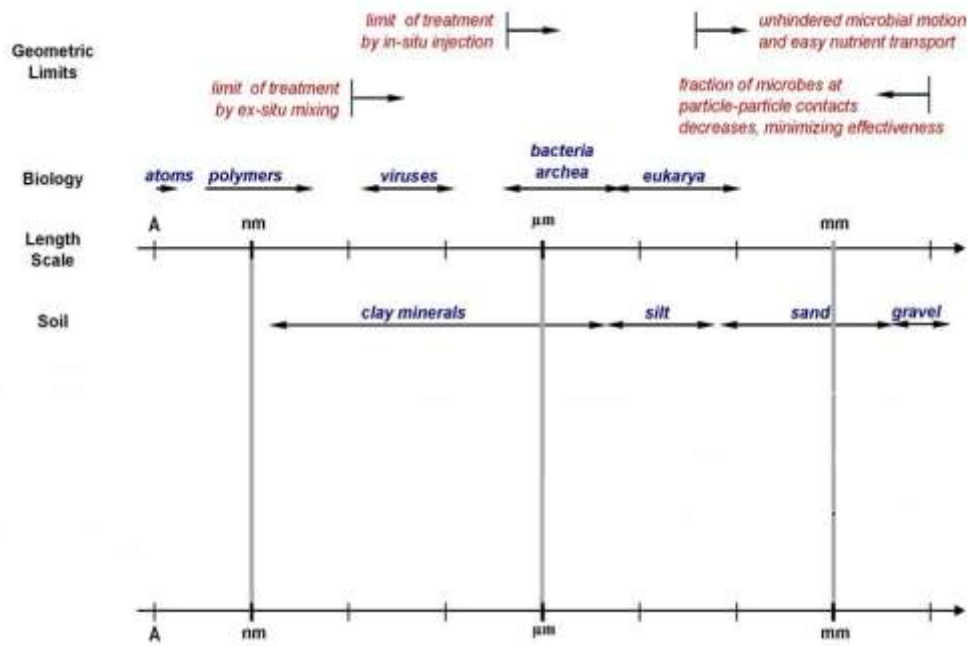
Figure 1.3: Comparison of bacterial cells sizes and soil grains sizes and geometric limitations, (DeJong et al. 2010, extended from Mitchell and Santamarina 2005)

Selecting ideal cementation solution concentrations is also important for calcite precipitation as it is considered to be a critical control factor for improving the strength of bio-treated soils. Nemati et al. (2005) and DeJong et al. (2009) indicated that a chemical optimisation of the cementation solution could also contribute to obtaining uniform calcite precipitation. Nemati et al. (2005) indicated that a better conversion to calcium carbonate could be accomplished by utilising equimolar concentrations of both reactants (urea and $CaCl_2$). This latter observation is supported by the study of Whiffin (2004), who also reported that the deposited calcite proportionally increased with an increase in equimolar cementation solution concentrations. A significant increase in soil strength and a decrease in permeability were also reported at high cementation solution (1M) when investigating the enzyme of urease (Yasuhara et al. 2011). However, a decrease in calcite precipitation was seen when using cementation solution concentrations above 1.5M, due to inhibitory effects of high salinity on microbial activity and calcite precipitation (Nemati and Voordouw 2003,

Nemati et al. 2005, De Muynck et al. 2010). Also, the crystals of deposited calcium carbonate can become larger as an increase of cementation solution concentrations can lead to a reduction in precipitation uniformity in the soil media (Al Qabany et al. 2012). By contrast, other studies found that the precipitation of calcium carbonate at lower cementation solution concentrations is more efficient than when higher reagent solution concentrations are used (Nemati et al. 2005, De Muynck et al. 2010, Okwadha and Li 2010, Al Qabany et al. 2011, 2012). The increased efficiency was attributed to a gradual reduction in the permeability with lower cementation solution concentrations (Al Qabany et al. 2012) and can contribute to preventing clogging near injection point by promoting more homogeneous cells and ultimately metabolic activity distribution in porous media (Phillips et al. 2013). Application of a greater number of injections of relatively low cementation solution concentrations was suggested by Al Qabany et al. (2012) to induce the same amount of calcium carbonate precipitation and at the same time attain high soil strength. On the other hand, the effect of non-equimolar (e.g., disproportionate amounts of calcium and urea) cementation solutions has also been studied. For instance, Yasuhara et al. (2011) stated that using non-equimolar cementation solutions resulted in an increase of UCS and a decrease in permeability of a biotreated sand. Okwadha and Li (2010) investigated various non-equimolar cementation solutions and concluded that a higher amount of deposited calcium carbonate was associated with a higher cementation solution concentration. Thus, although, a lot of research work has been conducted to investigate the effects of cementation solutions on fine to medium sand, there is still uncertainty about selecting the most adequate cementation solution concentration that can be adopted for practical use. A lack of knowledge also exists concerning the proper cementation solution concentration that would be used with coarser materials and whether the bio-cementation process can be achieved in the same way as with fine and medium sands as was discussed in previous studies (Al Qabany and Soga 2013, Cheng et al. 2017), because more

calcium carbonate precipitation may be required to attain a targeted strength.

1.2.2 *Microstructure evolution of the bio-cemented granular columns*

Experimental investigations indicated that preferential condition of calcium carbonate precipitation could not be achieved practically because the bio-grouting process is not naturally optimised to suit the goals of geotechnical practitioners (DeJong et al. 2010). However, there are fractures of deposited calcium carbonate located in neighbouring of inter-particle contacts that can promote bio-cementation within the porous matrix of soils, as shown in Figure 1.4. This may be associated with the physiological characteristics of bacterial cells that incline themselves to attach on smaller surfaces (this is yet to be microscopically investigated). More calcium carbonate precipitation may be observed near the narrow areas in a pore matrix of soil as a result of microbe agglomeration with increasing concentration (increasing the number of bacterial cells flushes). It was observed experimentally that more calcium carbonate precipitated near the injection points as bacteria tend to agglomerate near these parts of soil columns, due to narrowing the available pore passages as a result of bio-cementation and high ionic charges (Fujita et al. 2008; DeJong et al. 2010). It is worth noting that the effect of bacterial cells flushes on the microstructure changes of bio-cemented soils has not been microscopically validated. Microscopic investigations of MICP-treated soils have been mainly focused on the selected bio-cemented fractures using scanning electron microscopy (SEM), which provided a minimal picture concerning the global microstructure changes but it established a basis for future development. Also, previous studies mainly focussed on fine to medium sand. Soils with different characteristics including particle size, such as very coarse sand are yet to be microscopically investigated. Furthermore, linking the macroscopic mechanical behaviour of a bio-cemented material with a calibrated advanced microscopic tool such as X-Ray computed tomography (XCT) may prove a more reliable method to evaluate changes

in material engineering properties (Tagliaferri et al. 2011; Terzis and Laloui 2018). XCT is a non-destructive 3D imaging technique capable of carefully describing and analysing internal structures of soils, to a resolution of less than a micron. Therefore, the intrinsic microscopic global mechanism of bio-grouting treated coarser materials, such as very coarse sand remain to be investigated using an advanced microscopic tool.
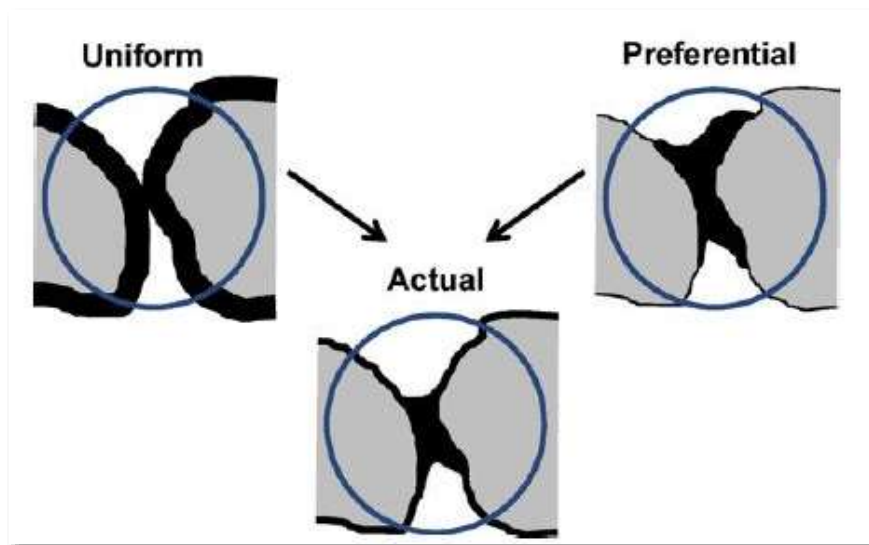
Figure 1.4: Illustration of calcite distribution alternatives within pore space (DeJong et al. 2010).

1.2.3 *Potential geotechnical applications of the bio-grouting process*

The bio-grouting process has been suggested for a wide range of applications to a variety of important geotechnical problems including the stability improvement of retaining walls, embankment, and dams; mitigating of erosions in soils, dams and pavement surface; an active stabiliser for water sources such as channels, aquaculture ponds, or reservoirs in sandy soils; suppressing of dust by binding soils grains on open surfaces and an immobilisation of sand; control of erosion in coastal areas and protection of buildings near rivers; mitigation of liquefaction potential of fine sand; encapsulation of weak soil such as clay, and in marine environments (Mitchell and Santamarina 2005, DeJong et al. 2006, Whiffin et al. 2007, Ivanov and Chu 2008, Van der Ruyt and van der Zon 2009, DeJong et al.

2010, Harkes et al. 2010, van Paassen 2010, Bang and Bang 2011, Mortensen and DeJong 2011, Stabnikov et al. 2011, Montoya 2012, Weil et al. 2012, Chu et al. 2012, Stabnikov et al. 2013, Ivanov et al. 2015, Stabnikov et al. 2015). It has also been used empirically to resolve several practical geotechnical concerns. In 2004 for instance, the permeability of sandy soils in Rotterdam port in Holland was significantly reduced using the bio-grout approach (Wang et al. 2017). In 2010, a layer of gravel subjected to horizontal drilling and laying of gas pipes remained stable after being treated by the bio-grouting process (Van der Star et al. 2011). There were also groundwater seepage problems in Canada that were successfully tackled using bio-grouting process (Wang et al. 2017). Similarly, a dam seepage blocking in Austria was achieved with this method (Zhang 2010). In China, bio-grouting has also been used to repair cracks and stop seepage that appeared in a building basement and an adjacent underground garage (Jia et al. 2013). Bacterial suspension along with cementation solution was injected for this purpose (Jia et al. 2013). Van Paassen (2011) performed a large-scale bio-grouting test on the sand with a volume of 100 m^3 and found that 43 m^3 of the sand body was successfully bonded by bio-cementation (Figure 1.5).

Figure 1.5. Setup for large-scale biogrout experiment to strengthening 100 m^3 sand (Van Paassen et al. 2009; Van Paassen 2011).

Gomez et al. (2014) presented a field-scale application of bio-grouting to improve the erosion resistance of loose sand deposits and provide surface stabilisation for dust control and future revegetation. Three test plots were treated with a bacterial culture and nutrient solutions at varying concentrations, and the fourth test plot served as a control. Dynamic cone penetration testing and calcite content measurements indicated improvement to a depth of approximately 28 cm near the targeted depth of 30 cm. The results suggest that further optimisation of solutions and techniques could render bio-grout viable for larger-scale applications. The results from this field study are promising and demonstrate the potential of bio-grouting as an effective soil improvement method. However, many problems still need to be studied further to enable up-scaling of this method to the field applications. It is also worth noting that bio-grouting to strengthening sand/stone columns is yet to be investigated.

1.3 Research gaps

Bio-grout as a soil improvement has focused primarily on fine to medium sands; coarser soils have not been investigated. It is of particular interest to geotechnical engineers to utilise bio-grouting as a soil improvement technique with other types of soils, for example, coarser materials, such as very coarse sand and crushed aggregate. Also, while the bio-grouting process is well documented in terms of procedures and materials that are required to conduct a successful application for bio-grout within a specific type of soils such as fine sand and medium sand, there are no clear methodologies for applying this technique to coarser materials such as very coarse sand and crushed aggregates. Also, no previous studies have tested the effectiveness of bio-grout treated coarser materials using destructive laboratory tests like unconfined compressive strength. Furthermore, optimisation of bio-grout treated coarser materials through methodologies and conditions that are more realistic is necessary to achieve a better distribution of deposited calcium carbonate within the pore throat of soils, and consequently, gaining high soil strength and stiffness. Thus, one of the

targets of this study is to access the applicability of the bio-grout method to stone columns with the view to adopt the process in the field.

It is also worth mentioning that there are no or limited studies which have investigated the microstructure of bio-cemented coarse granular materials. Last but not least, no studies have been conducted yet, on bio-grouting applied explicitly to enhance the behaviour of coarse granular piles under loading. Improving the engineering properties of coarser materials can provide a baseto scale up the bio-grouting process to be used in the stone column applications.

1.4 Scope of Research

1.5 Aims and Specific Objectives

The main aims of this thesis are framed to establish methods that can be used with coarser materials, to investigate practical factors affecting the performance of bio-grouting treated coarse materials, to optimise the method to be used with coarse materials such as very coarse sand and aggregates and to mitigate bulging that may occur during loading of stone columns installed in soft clay. The main aims of this research are divided into three main phases that drive the research into the final aims:

1.5.1 Phase one

➢ To investigate the possible biochemical treatment strategies that may be used with coarser materials.

➢ To investigate factors affecting the performance of the bio-grouting process of coarse materials.

➢ To optimise the bio-grouting process for coarse materials.

1.5.2 Phase two

➢ To link the macroscopic mechanical behaviour of a bio-cemented coarse material with a calibrated microscopic tool such as X-Ray

computed tomography (XCT) to provide a more reliable method to evaluate changes in material engineering properties.

1.5.2 Phase Three

➤ To develop an effective, economical and eco-friendly method for constructing and installing coarse sand columns treated by MICP technique in soft clay. Installation of MICP-treated granular material columns needs to be compatible with existing granular column installation technique.

1.6 Thesis layout

The general layout of the thesis is comprised of three main phases, as shown in figure 1.6 and the black oval on each phase provides the chapter number related to the research outcomes of each phase. Each phase also is collectively represented by one or a series of chapter(s) that generally demonstrate(s) and summarise(s) the potential outcomes of that phase. The following chapters are the main ribs of the thesis.
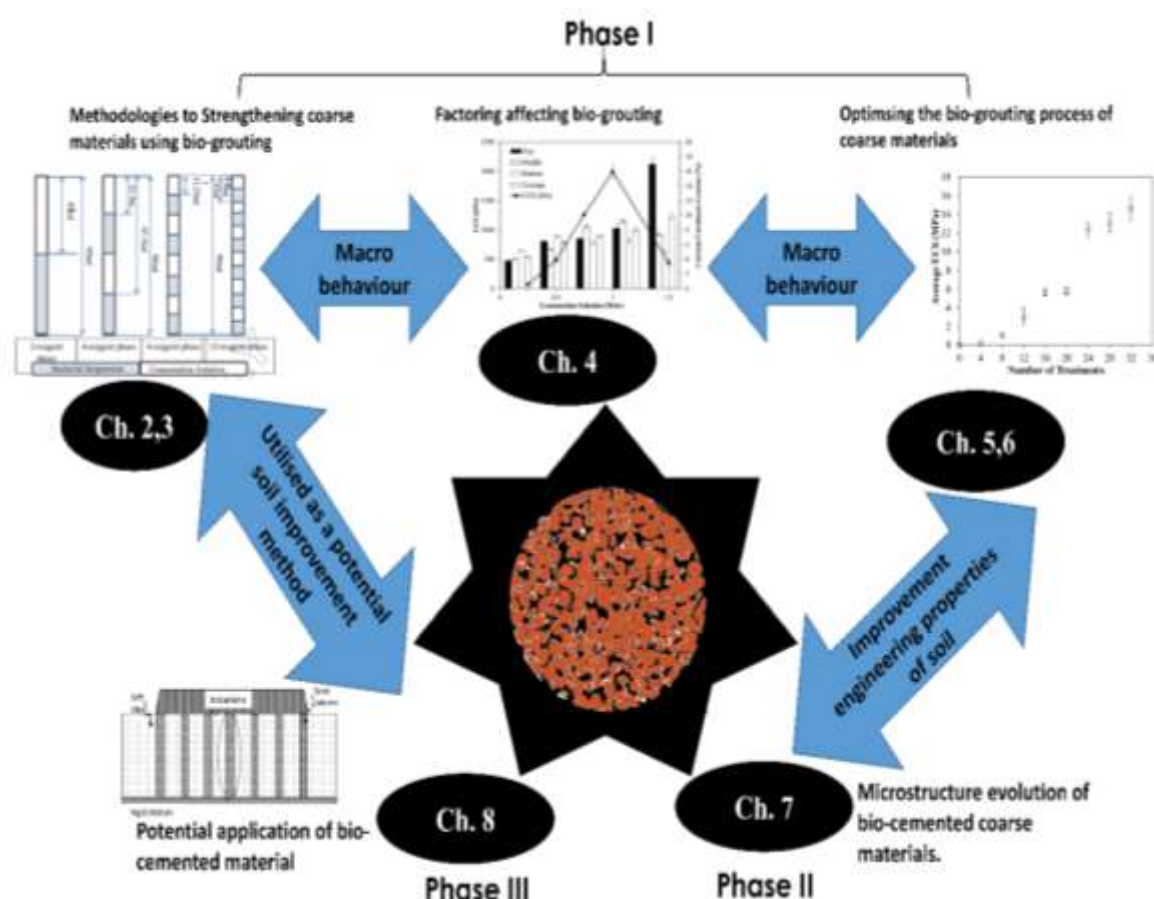
Figure 1.6: Scope of the study and the associated thesis chapter numbers.

Chapter 1: Introduction

An overview of the thesis, objectives and the potential outcomes of the research study is presented in this chapter. The contents of other chapters of the thesis are also briefly described at the end of the first chapter.

Chapter 2: Biogrouting Coarse Materials Using Soil-Lift Treatment Strategy

A description of the adopted treatment methodologies that have a potential effect on the MICP treated coarser materials is given. This chapter includes the test methodologies and results, including a discussion of test observations.

Chapter 3: Strengthening crushed coarse aggregates using bio-grouting

A further description of two typical strategies to apply bio-grouting is provided. The first protocol is based on using a multi-reagent phase strategy,

and the second is a multi-soil lifts strategy to treat crushed aggregate column materials. This chapter includes the test methodologies and results, including a discussion of test observations.

Chapter 4: Factors affecting the bio-cementing process of coarse sand.

A description is provided of the most practical factors affecting strength behaviour of bio-cemented coarser materials, including temperature, incubation time, cementation solution concentrations. This chapter includes the test methodologies and results, including a discussion of test observations.

Chapter 5: Improvement of coarse sand engineering properties by microbially induced calcite precipitation.

A description of important factors that participate in optimising bio-grouting method for practical purposes is presented. These factors include the number of biochemical treatments, amount of deposited calcium carbonate, initial relative density, dry density and porosity of the soil. This chapter also consists of the test methodology and results, and discussion of test observations.

Chapter 6: Effect of particle size distribution on the bio-cementation of coarse aggregates.

This chapter investigates the effect of a range of particle size distributions on bio-cemented coarse material as part of optimising the bio-grouting to be used with coarser materials. Unconfined compression tests (UCS) are conducted on the bio-cemented soil columns, and the precipitated calcium carbonate is also acid quantified after the completion of the UCS tests. The implications of the current study are investigated and discussed in this paper.

Chapter 7: Unconfined Compressive Strength and Visualization of the Microstructure of Coarse Sand Subjected to Different Biocementation Levels

This chapter explores the use of laboratory-based X-Ray computed tomography (XCT) to track microstructure changes in the amount and distribution of calcium carbonate along a bio-cemented sand sample associated with its macro-mechanical behaviour. Other soil characteristics such as porosity, dry density, void ratio and pore volume that could change due to the bio-grouting process were also identified and analysed in the current study.

Chapter 8: Biogrouted stone columns in soft clay

The chapter examines the efficacy of bio-grouting granular columns when installed in very soft soils to mitigate excessive radial column expansion during loading. Staged loading of the granular columns (un-cemented and bio-cemented) is conducted to replicate the staged construction process typically encountered in projects such as in embankments and similar.

Chapter 9: Conclusions and Recommendations

The conclusions and recommendations of this thesis are summarised herein. This chapter also includes the area for future works.

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Chapter 2: Biogrouting Coarse Materials Using Soil-Lift Treatment Strategy

Table of Contents

Biogrouting Coarse Materials Using Soil-Lift Treatment Strategy

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Abstract

This paper investigates the feasibility of using a soil-lift bio-grouting treatment strategy to improve the mechanical properties of coarse sand with the view of applying it to stone columns/sand piles and rammed earth columns type of applications. A two-phase percolation approach was adopted in this study that included percolating a bacterial suspension *Sporosarcina Pasteurii* in the first phase and a cementation solution in the second phase. This process was repeated every two treatments. The study reveals that an increase in the number of soil-lifts negatively influenced the mechanical properties of the bio-cemented coarse sand. However, the minimum strength and stiffness achieved (2.8 MPa) in this study was sufficient to mitigate slumping of a soil column that may occur during installation or excessive radial expansion. Furthermore, it is shown that a single lift treatment can lead to a very high increase in strength and stiffness (up to 8.9 MPa and 2.3 GPa, respectively). However, calcite distributions within bio-cemented soil columns piles were quite heterogeneous with increasing number of soil lift treatments. Soil-lift treatment can be seen as a practical strategy that can be used to inject treatment liquids in deeper depths, such as in soil columns piles.

2.1 Introduction

The ground can be improved by many different techniques including densification through blasting, vibration and compaction, pre-compression, addition of admixtures including lime and cement, installation of stiffening columns and several other methods (Mitchell 1981, Mitchell and Jardine 2002, Karol 2003, Gniel and Bouazza 2009, 2010, Chu et al. 2013). Recently, biologically mediated processes have been used for ground improvement to mitigate liquefaction, erosion, and improve hydraulic and strength properties of granular soils (Bouazza et al. 2009, Stabnikov et al. 2011). In particular, microbial induced calcite precipitation (MICP) by urea hydrolysis has been shown to be a viable ground improvement alternative which can mitigate soil liquefaction potential, stabilize coastal sand dunes and slopes, suppress dust, increase bearing capacity of foundations and strengthen soft marine clays (DeJong et al. 2006, Whiffin et al. 2007, Bang et al. 2009, DeJong et al. 2010, Chou et al. 2011, Mortensen and DeJong 2011, Paassen 2011, Yasuhara H et al. 2011, Chu et al. 2012, Montoya 2012, Rong et al. 2012, Al Qabany and Soga 2013, Cheng et al. 2013, Soon et al. 2013, Ivanov et al., 2015).

Ureolysis-driven MICP relies mainly on urease-producing bacterial strains such as *Sporosarcina pasteurii*. MICP occurs by adding urease-producing bacteria into a soil matrix to promote catalysis of urea hydrolysis as described by the following equations (Hammes and Verstraete 2002):

$$CO(NH_2)_2 + H_2O \xrightarrow{\text{Urease Enzyme}} CO_2 + 2NH_3 \qquad \uparrow pH \qquad (2.1)$$

$$2NH_3 + CO_2 + H_2O \longrightarrow 2NH_4^+ + CO_3^{2-} \qquad\qquad (2.2)$$
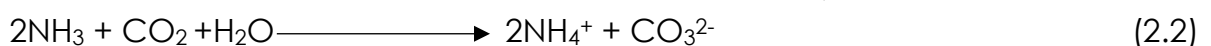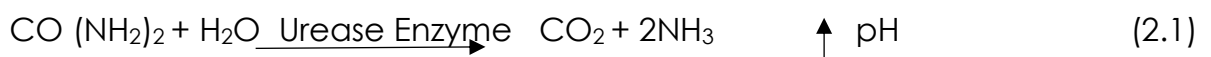
As can be seen in equation 2, urea hydrolysis produces an excess of dissolved carbonate ion. In the presence of excess calcium ions, calcium carbonate is precipitated spontaneously by the reaction given in Eq. (3)

$$Ca + CO_3^{2-} \longrightarrow CaCO_3 \qquad\qquad \downarrow pH \qquad (2.3)$$

Deposited calcite on the immobilised bacterial cell surfaces creates cementitious bridges between loose soil particles leading to soil solidification and increase in strength and stiffness (Whiffin et al. 2007, DeJong et al. 2010, van Paassen et al. 2010, Mortensen et al. 2011, Chu et al. 2012, Al Qabany and Soga 2013).

This paper focuses on the possibility of using a bio-grouting process based on MICP to increase the stiffness and strength of coarse granular soils with the view of applying it to sand piles/stone columns or rammed columns commonly used to improve weak soil sites. The objective of this study is to show the feasibility of treating a coarse sand column using gravity-driven percolation process combined with soil-lift treatments strategy. Various soil-lift strategy (1, 2, 3, 4, and 5 soil lifts) are undertaken to test its applicability to MICP- treated coarse sand columns. The effect of soil-lift strategy on dry unit weight, calcite precipitation content and strength and stiffness of bio-cemented coarse sand columns is discussed and analysed.

2.2 Materials and Methods

2.2.1 Bacterial suspension and cementation solution

Urease producing bacteria used was *Sporosarcina Pasteurii* (ATCC® 11859). A urea hydrolysing bacterium was grown at 30 °C in an ammonium-yeast extract medium (20 g/L yeast extract, 10 g/L $(NH_4)_2SO_4$ and 130 mM tris buffer (pH=9.0). The individual recipes were autoclaved separately and then mixed together post sterilization. A group of 1 litre bottles containing a growth medium were inoculated with two-percent of *Sporosarcina Pasteurii* stock culture and incubated aerobically at 30 °C in a shaking water bath (200 rpm). The bacterial cells were harvested at a final optical density (OD_{600}) of 3.0-3.5 using WPA CO 8000 spectrophotometer (BioChrom Ltd), equivalent to 3.8×10^8 - 4.7×10^8 cells/ml based on equation 2.4 (Ramachandran et al. 2001).

Number of bacterial cells= $8.59 \times 10^7 \times OD_{600}^{1.3627}$ (2.4)

An enzyme urease activity of 21.45 mM/min was used in this study. The bacterial cells were harvested at the beginning of the stationary growth phase, in which the size of a population of bacteria remained relatively constant to ensure that no further growth of bacterial cells could occur in the granular columns during the prescribed retention time (incubation time). The reason for this was to ensure that same number of bacterial cells was used in each sand column due to the lack of required nutrient to grow more bacterial cells. The harvested microorganisms were stored at 4°C in the 1 litre bottles for a maximum of two weeks. Ureolytic process was driven by using urea-calcium chloride cementation solutions. The concentration of cementation solution recipes were 1M of urea and 1M of calcium chloride.

2.2.2 Sand

The sand used in this study has an average grain size of 1.6 mm. It is classified as poorly graded coarse sand (SP) according to the Unified Soil Classification System (ASTM 2006). Maximum and minimum dry densities measurements were conducted in accordance with ASTM D4253 and ASTM D4254, to control compaction of the sand column model. Summary of the sand properties (specific gravity (G_s), coefficient of curvature (C_c), coefficient of uniformity (C_u), the medium grain size (D_{50}), maximum and minimum void ratios (e_{max} and e_{min}) are presented in Table 2.1. The sand particles were typically sub-angular in shape and were deemed to be representative of the stone aggregate used for full scale stone columns (Gniel and Bouazza 2009, 2010).

Table 2.1: Coarse sand characteristics

Soil Type	Classification	G_s	C_c	C_u	D_{50} (mm)	e_{max}	e_{min}
Grade 8/16	SP	2.64	0.97	1.35	1.60	0.84	0.55

2.2.3 Experimental set-up and treatment methodology

Figure 2.1 shows a typical column used in the current study. It is made of a split mould Poly Vinyl Chloride (PVC) pipe, 220 mm high and with an internal diameter of 51 mm when assembled together. A wire mesh and filter paper were placed at the bottom of the column to minimise the loss of sand grains during preparation and treatment. The columns were positioned vertically with fully open top and control valve at the bottom. Soil placement was based on the use of various soil lifts and included the option of using 1, 2, 3, 4, and 5 lifts, respectively. Dry coarse sand was pluviated into the specimen mould in individual lifts. Each lift was compacted to a relative density of 60% due to their sub-angular particle shape and poor grading, using a vibratory compaction method. After placement of each soil lift, tap water was flushed through to expel air from the pore matrix.

A two-phase biochemical treatment protocol was adopted in this study and included sequential percolation of bacterial suspensions and cementation solutions from the top of the columns (Cheng 2012). The process was initiated by alternating percolation of a quarter pore volume (20 ml) of bacterial suspension and a quarter pore volume (20 ml) of cementation solution within the first lift in the case of one soil lift strategy. A 24-hour retention time was chosen to achieve better intermixing between bacteria and substrates. The second biochemical treatment included only 40 ml of the cementation solution and the same aforementioned retention time was used. The previous processes were considered as two biochemical treatments and repeated up to 16-time, which was equivalent to 32-biochemical treatment. For instance, a three-soil lift was achieved by dividing the total specimen length (102 mm) by three resulting in a lift thickness of ~33 mm. Five biochemical treatments for each lift was considered to obtain a reasonable improvement of the coarse sand. Once the first soil lift was emplaced it received 5- biochemical treatments based on the determined pore volume of the soil lift. The second lift received also 5 biochemical treatments, but this also means that lift 1 received an

additional 5 biochemical treatments. This process was repeated until the final lift (lift 3), which itself received 22 biochemical treatments, resulting in the number of biochemical treatments (referred as NOT) shown in Figure 2.2 for each lift. A similar protocol was adopted for the other soil-lift columns. After completing the biochemical treatments of the soil lifts, the soil columns were percolated with deionized water to remove any residual chemicals from the pore throat of the coarse sand. The split mould was removed after 24 hours, and then the bio-cemented coarse sand columns were left to cure at a 30 °C temperature for two weeks to accelerate the bio-cementation process and obtain acceptable samples within a reasonable timeframe for experimental works. All the mechanical tests reported in this paper were conducted in a controlled room temperature (20±2 °C). The samples for UCS test were then taken out their split moulds and the absences of the flat surfaces were overcome by using sulphur capping to obtain plane surfaces within 0.05 mm.
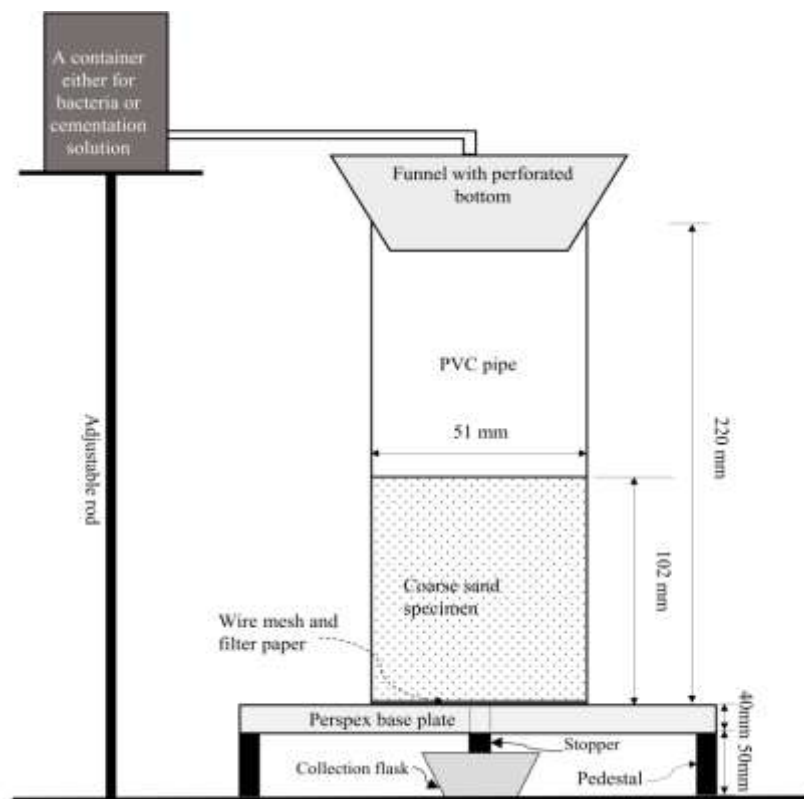
Figure 2.1. Schematic setup of experimental.
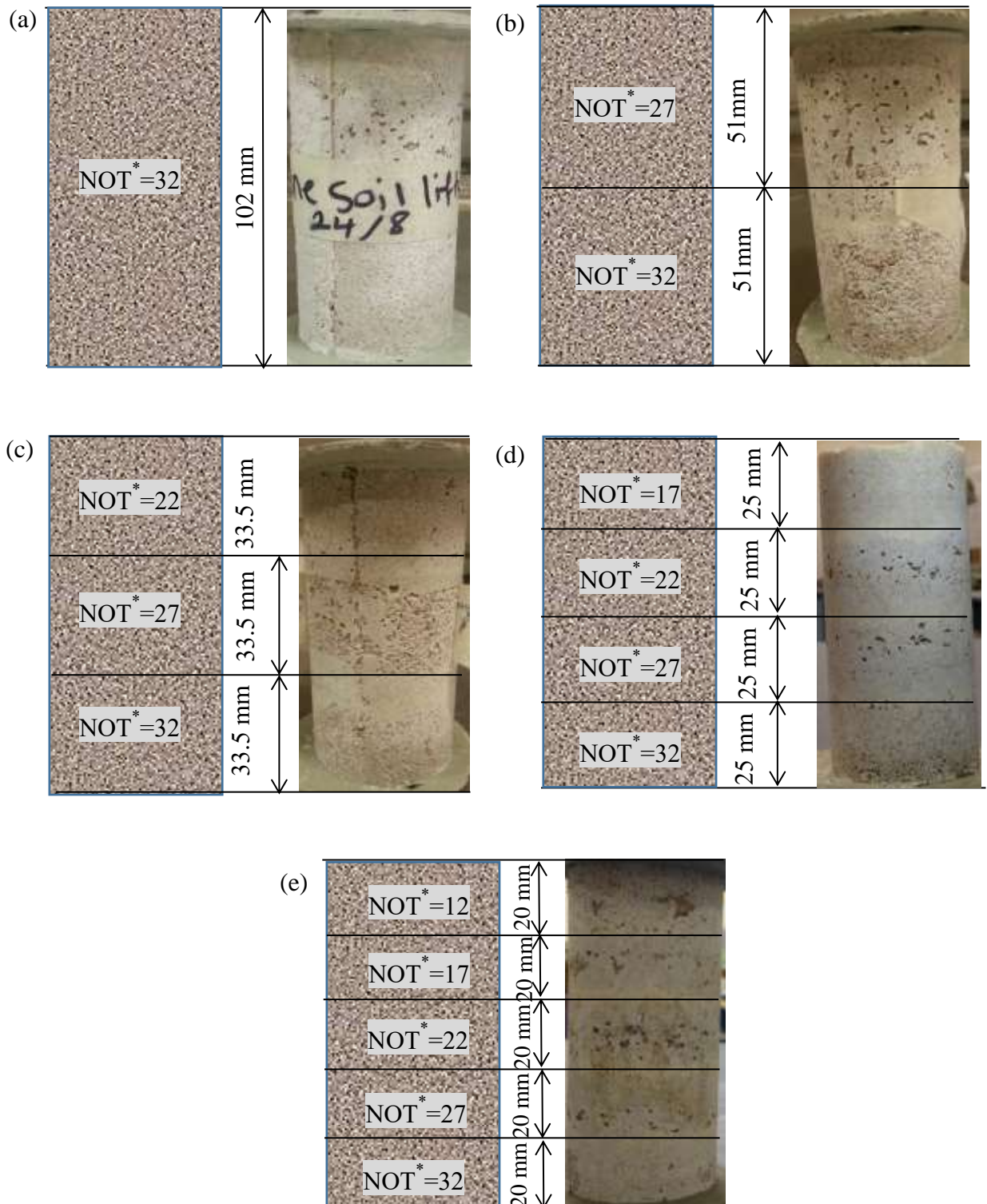
Figure 2.2. Layout of MICP-treated coarse sand using various soil lifts strategy (* NOT refers to Number of Treatments): (a) treatment of one soil-lift; (b) treatment of two-soil lift; (c) treatment of three-soil lift; (d) treatment of four soil lift; (e) treatment of five-soil lift.

2.2.4 Unconfined Compressive Strength (UCS) Tests

After completion of the biochemical treatments and curing periods, the bio-cemented coarse sand columns were subjected to UCS test to quantify any possible increase in strength and stiffness. An Instron 4204-50kN constant-displacement mode UCS machine and laser extensometer were used to conduct the UCS tests. All the tests were carried out at a constant rate of 1%/min to monitor the propagation and observation of the shear failure in the samples. Calcite precipitation was quantified after completion of the UCS tests.

2.2.5 Calcite content

Gravimetric hydrochloric acid (HCL) washing technique was used to measure the amount of calcite precipitation in each column. Sub-specimens were collected from parts (top, middle and bottom) of the biocemented specimens after completion of the UCS tests. They were dried in an oven at 105 °C for 24 hours and then their masses were measured. Next, the specimens were rinsed with 2M HCl to dissolve precipitated calcite and flushed with deionised water to allow the dissolved salts to be rinsed out from the specimens which then were left to dry for 24 hours. The difference between mass before and after acid wash was taken as the mass of calcium carbonate. The percentage of calcium carbonate was obtained by dividing the mass of calcium carbonate by the mass of coarse sand specimen.

2.3 Results and discussion

2.3.1 Influence of soil-lift treatment strategy on calcite content of bio-cemented coarse sand

Figure 2.3, shows the relationship between the amount of calcite precipitation in three regions (top, middle and bottom) of the bio-cemented coarse sand columns against the number of soil lifts. Even though the coarse samples were similarly treated in terms of number of biochemical treatments

(e.g. up to 32-treatments), they show a variation in the amount and distribution of calcite precipitation. A visual investigation of these specimens was conducted and calcium carbonate was observed as a white deposition on the external surfaces of the coarse sand columns (see Figure 2.2). Calcite precipitation was more abundant toward the bottom portions in all specimens with the exception of the one-soil lift specimen, indicating that treating soil lifts technique resulted in a more heterogeneous distribution of calcite within the pore throat of the coarse sand. This is because the number of treatments was less in the upper compared to the lower parts of the soil (i.e.an increase in number of soil lifts resulted in less number of treatments for soil lifts near to the surface of the specimens)

It can be clearly seen from Figure 2.3 that calcite at the bottom of the (3-5) soil lift specimens was significantly higher than in the top part of these specimens and was twice the precipitated calcite content in the upper part of the five-lift soil column. Calcite precipitation content was in the range of approximately 21-22% per weight of dry coarse sand. In addition, Calcite precipitated mostly near the percolation point in the case of the one-soil lift due to the repeat of the percolation process which resulted in the adherence of the bacterial cells to the solid surfaces located near the percolation points. Utilizing one soil lift column with high calcium chloride concentration can lead to increase in ionic charges, and tend to promote bacterial adhesion (Faibish et al. 1998, Foppen and Schijven 2006) due to bacterial cells having a high negative zeta potential that are attracted to soil's particles under physical infiltrations (Dick et al. 2006). The average weight of calcite precipitation was 71.60g, 71.00g, 70.90g, 68.20g and 68.00g, respectively in specimens which have undergone one-lift, two-lift, three-lift, four-lift and five lift treatments. The efficiency of soil-lift columns strategy with regards to the amount of calcium used varied between 45% and 47% depending on the lift strategy used.
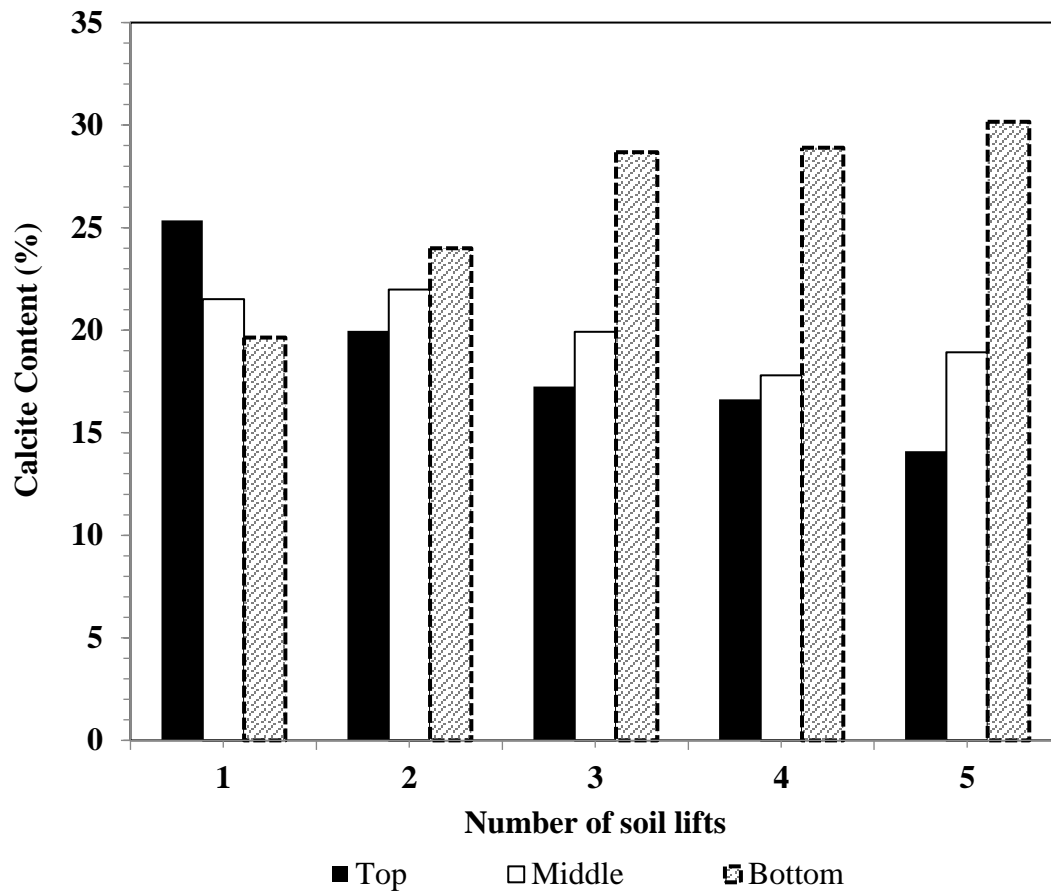
Figure 2.3. Effect of number of lifts on the amount of calcite precipitation of bio-cemented coarse sand.

2.3.2 Effect of soil-lift treatment strategy on strength and stiffness of biocemented coarse sand

Figure 2.4, shows the effects of soil-lift treatment strategy on the compressive strength and stiffness of specimens treated by the MICP method. The experimental results indicate that an increase of soil lifts negatively affected the strength and stiffness of bio-cemented coarse sand samples. It seems that the interaction between soil lifts resulted in the creation of regions with weak resistance, which eventually led to weaker specimens. Shear failures were observed to originate from the interface between the lifts (see figure 2.5), and were primarily dependent on the more calcite-calcite contacts as

it was seen that a thin layer of calcium carbonate resided onto surfaces of the soil particles (DeJong et al. 2010). Also, the calcite deposited was a relatively weaker than the coarse sand particles due to the heterogeneous distribution of the calcite. Furthermore, because each lift was vibrated in place, this process might have also resulted in a capillary break which in turn could have affected how the treatment solutions penetrated between lifts. Notwithstanding the above, it can be seen from Figure 2.4 that the strength and stiffness of bio-cemented coarse sand were improved despite the fact that strength and stiffness decreased as the number of lifts increased. The one soil lift treatment technique achieved the highest strength and stiffness (8.8MPa and 2.27GPa, respectively).
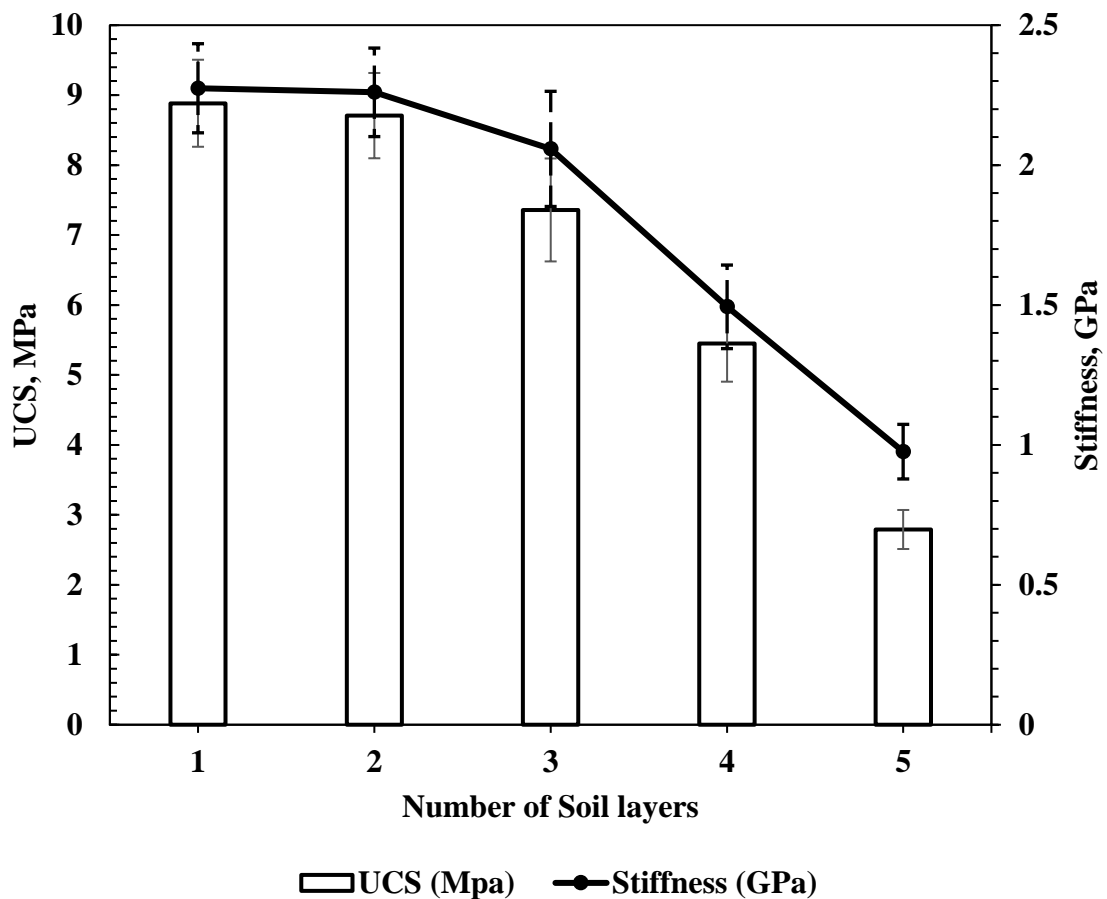
Figure 2.4. Effect of Number of soil layers on the strength and stiffness of bio-cemented coarse sand.
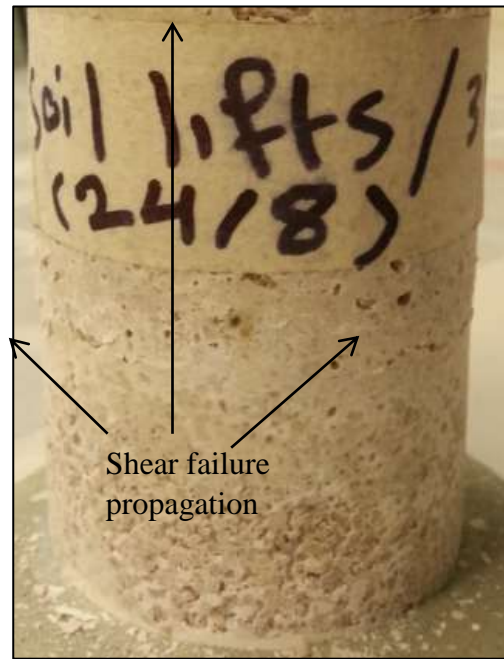
Figure 2.5. Shear failure propagation at the interface of three-soil lift column.

2.3.3 *Effect of soil-lift treatment strategy on dry density of coarse sand bound by microbial activities*

Figure 2.6, illustrates variations of the unconfined compressive strength and stiffness with dry unit weight. Strength increased with an increase in dry unit weight and a similar trend was observed for stiffness of bio-cemented coarse sand samples. A maximum strength and stiffness was achieved when the dry unit weight was approximately 19kN/m^3. This behaviour is well known in the case of using artificially cemented soils as the strength increases with an increase of cement content and unit weight. By contrast, van Paassen et al. (2010) stated that high variances were observed in the correlations of dry unit weight with strength and stiffness. They reported that part of these variances were due to differences in the initial dry unit weight (van Paassen et al. 2009). The core samples that were taken from a large-scale (100 m^3) experiment were tested by conducting UCS and showed peak strength varying significantly from 0.7-12.4MPa (van Paassen et al. 2010). The current study showed that a considerably high strength and stiffness using MICP-treated coarse sand can be achieved under various number of soil-lift treatment strategies. It is worth noting that dry unit weight increased with

decreasing soil-lift number due to some deposited calcite flushing out of the columns. This is due to the vibratory compaction process adopted in this study to achieve the target relative density. Vibratory compaction has led to breakage of the bonds between particles and some of calcite crystals were consequently flushed out when applying treatments to the subsequent lifts.
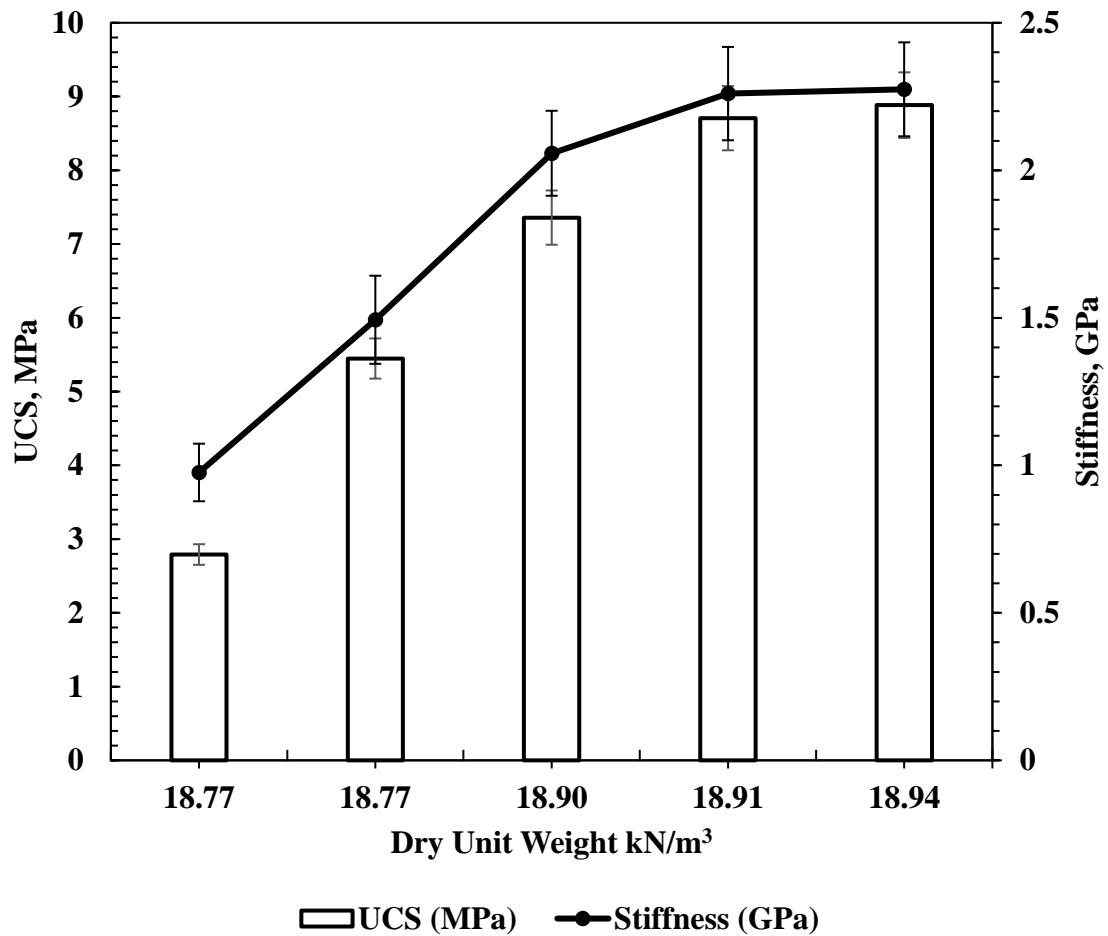
Figure 2.6. Effect of dry unit weight on the strength and stiffness of bio-cemented coarse sand.

2.4 Conclusions

MICP method faces many challenges in geotechnical application especially when it is considered as a potential enhancement to engineering properties of deeper soils when stone columns/sand piles or rammed columns are used. Although, bacterial suspension and cementation solution used in the MICP method have a low viscosity, the main practical difficulty resides on how to inject these solutions to the places where improvement is needed. To overcome these complexities, this study experimented utilizing various number of soil lifts. The study reveals that an increase of soil-lift number has a negative influence of the mechanical properties of the bio-cemented coarse sand. However, the improvement obtained was substantially greater than reported in other published studies in which soils with an average grain size less than 1 mm were used. The minimum strength and stiffness achieved in this study is enough to mitigate slumping of a soil column that may occur during installation or excessive radial expansion during loading and improve rammed columns capacity. Based on the findings from this study, the following conclusions can be drawn

1. Single soil lift column gave more homogenous calcite distribution than multiple soil lifts columns.
2. The main weaknesses in the multiple soil lifts columns were associated with interface between soil lifts.
3. Multiple soil lifts columns resulted in good strength and stiffness improvements in coarse sands and the maximum strength and stiffness was achieved by using one soil lift.
4. Strength and stiffness was dependent on the overall mass of the bio-cemented materials.

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Chapter 3: Strengthening crushed coarse aggregates using bio-grouting

Table of Contents

Strengthening crushed coarse aggregates using bio-grouting

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ABSTRACT

This paper focuses on using urea hydrolysis as a bio-grouting process to increase the strength of crushed aggregates commonly used in stone columns. Various reagent phases (2, 4, 6 and 12 phases) consisted of alternately percolating solutions containing bacterial suspension and cementation solution through the soil column. In addition, a multi-soil lift strategy with options of up to four soil lifts was undertaken to test the applicability of bio-grout to cement crushed aggregate columns. While the average amount of calcium carbonate precipitation was roughly unchanged in both techniques, the distribution within the crushed aggregate columns was heterogeneous. However, the distribution of the precipitated calcium carbonate is almost uniform in crushed aggregates treated by a two-soil lift strategy and a four-phase treatment strategy. It is also deducted that both techniques can be combined to gain a uniform calcium carbonate and strength along a long sand/stone column. Furthermore, a one-soil lift resulted in higher strength than using multi-soil lifts, and a maximum strength of approximately 2.3 MPa was achieved using 4-reagent phase treatment strategy. Scanning electron microscopy and electron dispersive spectroscopy analysis validate that calcium carbonate was deposited as white crystals on the surface of the crushed aggregate particles.

3.1 Introduction

An important feature in geotechnical engineering applications is the bonding between soil particles to prevent shear failure, unacceptable deformation or even fluid flow. For soils with poor strength, different soil improvement techniques are usually used to improve soil particle bonding (Mitchell 1981, Mitchell and Jardine 2002, Karol 2003, Gniel and Bouazza 2009, 2010, Chu et al. 2013). Among these various techniques, chemical grouting is often used to reduce hydraulic conductivity or increase the strength and stiffness of a given soil. However, chemical grouts [e.g. chemicals based on acrylamides, lignosulfonates, polyurethane, etc.] are expensive or may have significant adverse impacts on human and environmental health (Karol 2003, Ivanov and Chu 2008, DeJong et al. 2010). Biogrouting may prove to be an alternative approach of soil improvement. It was adopted recently to increase shear strength and reduce permeability of soils (Whiffin 2004, DeJong et al. 2006, Whiffin et al. 2007, Van Paassen 2009, Al Qabany et al. 2012, Mahawish et al. 2016, 2017, 2018a, 2018b). Bio-grouting is a novel biogeochemical technology based on precipitation of insoluble and inorganic compounds in a soil pore matrix using enzymatic activities of natural microorganisms, augmented with the planned addition of chemical reagents. Microbial-induced calcium carbonate precipitation (MICP) is currently the most widely studied and applied bio-grouting (bio-cementation) technology because it is mainly dependent on urease-producing (ureolytic) bacterial cells reacting with a controlled cementation solution (the solution of calcium chloride salts and urea) (Ivanov and Chu 2008, De Muynck et al. 2010, DeJong et al. 2010, Stabnikov et al. 2015). Ureolytic bacteria have a major impact in the increase of the concentration of dissolved inorganic carbon (as $CO_2(g)$ and CO_3^{2-}) and increase solution pH through urea hydrolysis (ureolysis). Ureolytic bacterial cells are nucleation sites for calcium carbonate crystallisation to create numerous micro gradients of pH and carbonate ions in places surrounding bacterial cells as a result of ureolysis (Gollapudi et al. 1995, Ivanov and Chu 2008, Stabnikov

et al. 2015). Bio-cementation can be utilised to cement cohesionless soils, such as sand and gravel. A soil can be bio-cemented by introducing foreign bacteria, or by stimulating native bacteria. In both cases, percolation of cementation solution into the soil enhances bio-cementation. Calcium carbonates are recognised as among the most abundant minerals in the earth crust. Calcium carbonate forms in various areas as a natural phenomenon, such as in rocks and in environments such as marine water, fresh water and terrestrial environment (Castanier et al. 1999, Hammes and Verstraete 2002, Whiffin 2004). Ureolysis induces calcium carbonate crystallisation and formation of a bond bio-product based on the following biochemical reactions (Hammes and Verstraete 2002):

$$CO\,(NH_2)_2 + H_2O \xrightarrow{\text{Urease Enzyme}} CO_2 + 2NH_3 \tag{3.1}$$

$$2NH_3 + CO_2 + H_2O \xrightarrow{\hspace{2cm}} 2NH_4^+ + CO_3^{2-} \qquad \uparrow \ \text{pH} \tag{3.2}$$
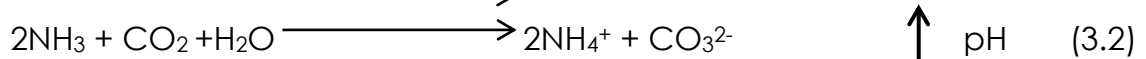
As can be seen in Equation (3.2), urea hydrolysis produces an excess of dissolved carbonate ion. In the presence of excess calcium ions, calcium carbonate is precipitated spontaneously by the reaction in Equation (3.3):

$$Ca + CO_3^{2-} \xrightarrow{\hspace{2cm}} CaCO_3 \qquad \downarrow \ \text{pH} \tag{3.3}$$

The pH with an arrow up in Equation (3.2) indicates that the pH increases with urea hydrolysis, and then decreases to pH ~8.3 after precipitation of sufficient calcium carbonate as presented in Equation (3.3) with an arrow down. While many microbes are known to exist in soil environments, the required bacterial strains have to be capable to produce the urease enzyme in sufficient quantities, be non-pathogenic, alkalophilic and halophilic, tolerate high ammonium concentrations and also have a high negative zeta-potential to promote the electrochemical attraction of divalent cations (Dick et al. 2006, De Muynck et al. 2007). The most prominent bacterial cell studied for bio-cementation is Sporosacina pasteurii, which has been extensively used to drive precipitation of calcium carbonate within soil and concrete (Whiffin et al. 2007, Sarda et al. 2009,

DeJong et al. 2010, 2013, Mortensen and DeJong 2011, Gomez et al. 2014). Bio-cementation has been considered for use in numerous geotechnical applications such as a liquefiable fine sand deposit, dust suppression, protection of coastal areas, slope stability, subgrade reinforcement and many more (DeJong et al. 2006, 2010, Mortensen and DeJong 2011, Montoya 2012, Weil et al. 2012, Stabnikov et al. 2015). However, application of bio-cementation in stone columns (i.e. as used in vibro-replacement) has not been considered yet, especially for the specific case where a sand/stone column may suffer from bulging, general shear failure and slip-failure. MICP has been proven to mitigate slumping of sand columns that may occur during excessive radial expansion (Mahawish et al. 2016). Following on this previous work, the aim of this paper is to present an evaluation of the application of MICP to enhance engineering properties (compressive strength) of crushed coarse aggregate columns and to quantify the amount of calcium carbonate precipitation since the intention of the global research programme is to eventually develop rigid columns suitable for improvement of soft and weak soils. Two typical strategies to apply bio-grouting are proposed; the first protocol is based on using a multi-reagent phase strategy and the second is a multi-soil lifts strategy to treat crushed aggregate column materials. Both strategies are expected to provide an insight about the distribution of calcium carbonate precipitation and its effect on unconfined compressive strength (UCS) values of coarse materials.

3.2 Materials and methods

3.2.1 Bacterial Suspension and Cementation Solution

Urease producing bacteria used was *Sporosarcina pasteurii* (ATCC® 11859), an ureolytic bacterium grown at 30 °C in an ammonium-yeast extract medium (20 g/L yeast extract, 10 g/L $(NH_4)2SO_4$ and 130 mM tris buffer (pH=9.0). The individual components of growth medium were autoclaved separately and then mixed together post sterilization. A group of 1 litre

bottles containing growth medium were inoculated with two-percent of *S. pasteurii* stock culture and incubated aerobically at 30 $^{\circ}$C in a shaking water bath (200 rpm). The bacterial cells were harvested at a final optical density, at 600 nm (OD$_{600}$), of 3.0-3.5 using WPA CO 8000 spectrophotometer (BioChrom Ltd), assumed to be equivalent to 3.8 x 10^8 - 4.7 x 10^8 cells/ml based on equation 3.4 (Ramachandran et al. 2001).

Number of bacteria= 8.59 x 107 x OD600 1.3627　　　　　　　　　　(3.4)

An enzyme urease activity of around 19.38-21.45 mM urea hydrolysed per min was used in this study based on the maximum optical density of the harvested bacterial cells. Urease activity was based on an electricalconductivity assay (using labCHEM-CP device).

The bacterial cells were harvested at the beginning of the stationary growth phase, in which the population of bacteria remained relatively constant when percolated through the granular columns to ensure that minimal cell growth occurred during the prescribed retention time (incubation time). The reason for this was to ensure that the roughly same number of bacterial cells was used in each crushed aggregate column due to the lack of required nutrient to grow more bacterial cells. The harvested microorganisms were stored at 4 $^{\circ}$C in the 1 litre bottles for a maximum of two weeks. The ureolytic process was driven by using urea-calcium chloride cementation solutions. The concentration of cementation solution recipes were 1M of urea and 1M of calcium chloride.

3.2.2　Crushed aggregate and soil column specimen preparation

Stone columns usually compose of angular and subangular granular material that generally has an average particle size of between 25 and 75 mm in diameter, and a typical stone column ranges from 0.4 to 1.2 m in diameter. This leads to column diameters with average particle size ratios of 5–50. The model crushed aggregate columns used to imitate stone columns in the current laboratory investigations were scaled to a ratio of about 5. Pakenham Blue Metal (Old Basalt) crushed aggregate with an average

particle size of 9.9 mm was used to prepare cylindrical specimens for the bio-cementation treatments and compression tests. It is classified as poorly graded crushed aggregate (GP) according to the Unified Soil Classification System (ASTM 2006). Maximum and minimum dry density measurements were conducted in accordance with ASTM D4253 (ASTM 2014a) and ASTM D4254 (ASTM 2014b) and were 1.49 and 1.62 g/cm^3, respectively, to control compaction of the crushed aggregate column model. Summary of the soil properties (specific gravity (G_s), coefficient of curvature (C_c), coefficient of uniformity (C_U), the median grain size (D_{50}), and maximum and minimum void ratios (emax and emin) are presented in Table 3.1. As crushed rock aggregate is typically used to form stone columns, with a relative density ranging between 60% and 100%, soil columns used were prepared by a vibration in assembled split moulds to achieve a target relative density of 60% (equivalent to a dry density of 1.56 g/cm^3). The soil columns have a length to diameter aspect ratio of 2:1. The columns are made of a split mould polyvinyl chloride pipe, 220 mm high and with an internal diameter of 51 mm when assembled together. A wire mesh and filter paper were placed at the bottom of the column to minimise the possible loss of crushed aggregates particles during preparation and treatment. The columns were positioned vertically with fully opened upper surface and a drainage control valve was connected at the base. After placement and compaction of crushed aggregates in the columns, tap water (200% pore volume (PV) of aggregate columns (180 mL)) was flushed through it to expel the extra air from the pore matrix. This step was done by closing the bottom valve, percolating deionised water until ponding on top of the specimen, then opening the bottom valve; the percolation of water was continued until reaching to two PVs.

Table 3.1: Properties of crushed aggregate.

Soil Type	Old Basalt
Classification	SP
G_s	2.78
C_c	1.011
C_u	2.14
D_{50} (mm)	9.90
e_{max}	0.86
e_{min}	0.72
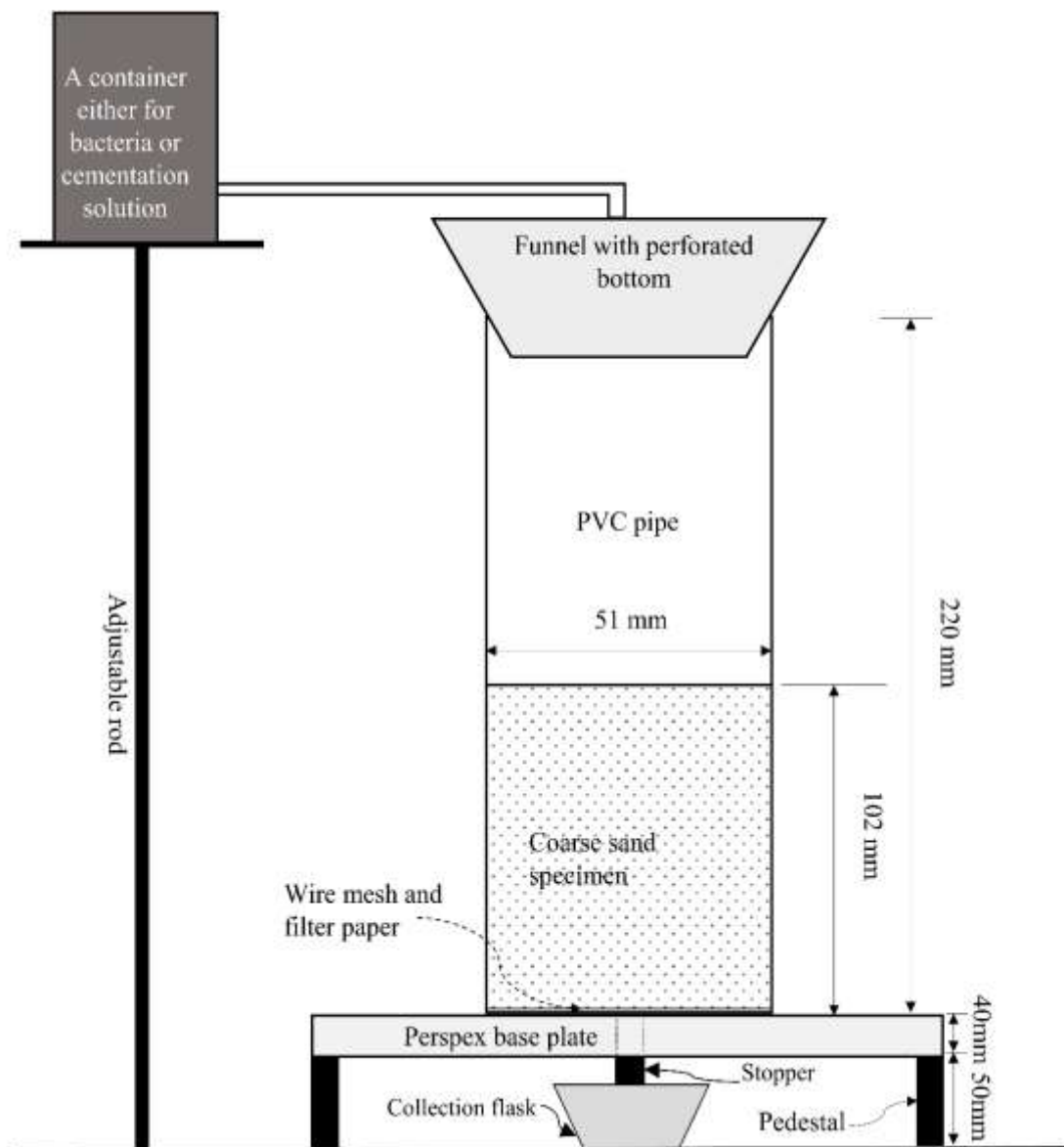
Figure 3.1. Diagram of the setup for coarse crushed aggregate column.

3.2.3 Soil placement and treatment strategy

3.2.3.1 Multi-reagent phase strategy

A percolation process was used for bio-grouting of crushed aggregate columns. To achieve a percolating flow, a gravity-driven system was developed (Figure 3.1). The percolator was made by perforating a small plastic bottle with a 1 mm dia. hole. The percolator had a diameter less than the diameter of the mould, allowing it to rest on the top edge of the mould. An external tube running from either a bacterial suspension container, or a cementation solution bottle (urea and calcium chloride) was installed above the percolator to keep a stable flow rate of 1 L/h. The multi-reagent phase strategy involved placement of crushed aggregates into the soil columns as one lift, which were then packed up to the required relative density. The amount of bacterial suspension and cementation solution in each treatment cycles was determined based on the PV (pore water capacity) of the crushed aggregate columns. The strategy consisted of the following steps:

(i) Flushing cementation solution (100% PV mL of crushed aggregate columns) to promote high ionic strength within the pore matrix. The divalent calcium ions increase the ionic charge at the particle surfaces. Urea plays a significant role in providing a suitable environment for the bacterial cells as they have a high affinity for urea. This step was applied once before the beginning of the treatment cycles.

(ii) After the cementation solution volume has been drained out from each specimen, the bottom valve was closed and percolation of a lesser volume of bacterial suspension and cementation solution without ammonium-YE media were alternated to achieve a multi reagent phase strategy (2, 4, 8 and 12 phases) as outlined in Figure 3.2. The amount of liquids percolated is equivalent to 100% of the PV of the crushed aggregate columns.

(iii) Incubation (retention time) for 24 h at 20 ± 2°C, allowing the percolated liquids to facilitate the occurrence of reactions between microorganisms

and reagents solutions. When incubation time ended, the bottom valve was opened to discharge the liquids waste (effluent) and prepare the pore matrix for the next treatment.

(iv) This treatment step included percolation of fresh cementation solution (100% PV, mL) only and allowed ureolysis and $CaCO_3$ deposition to take place for same retention time (24 h). The effluent was also discharged after finishing the incubation time for the purpose mentioned in the previous step.

Steps ii and iv were considered as two biochemical treatment cycles and were repeated until reaching the required number of treatment cycles (i.e. 20 treatment cycles). As an example, eight-reagent phase strategy comprised of alternating percolation of small amount of bacterial suspension (11.25 mL) and small amount of cementation solution (11.25 mL) until having eight reagent phases in the first treatment cycle. The second treatment cycle only involved percolating of one PV of cementation solution (90 mL) and 24-h incubation time was given before and after this treatment cycle to allow ureolysis to take place. The previous processes were performed until reaching 20 biochemical treatment cycles. A similar technique was applied with other crushed aggregate columns. The only difference was the number of reagent phases as shown in Figure 3.2. After completing the biochemical treatment cycles of the multi-phase reagent crushed aggregate columns, the columns were percolated with deionised water to remove any residual chemicals from the pore throat of the crushed aggregate. The split mould was removed after 24 h, and then the bio-cemented crushed aggregate columns were left to cure at 20 ± 2°C for 2 weeks to obtain acceptable samples within a reasonable timeframe for experimental works. The samples for UCS test were then taken out their split moulds and the absence of flat surfaces was overcome by using sulphur capping to obtain plane surfaces within 0.05 mm. All the mechanical tests reported in this paper were conducted in a controlled room temperature (20 ± 2°C).
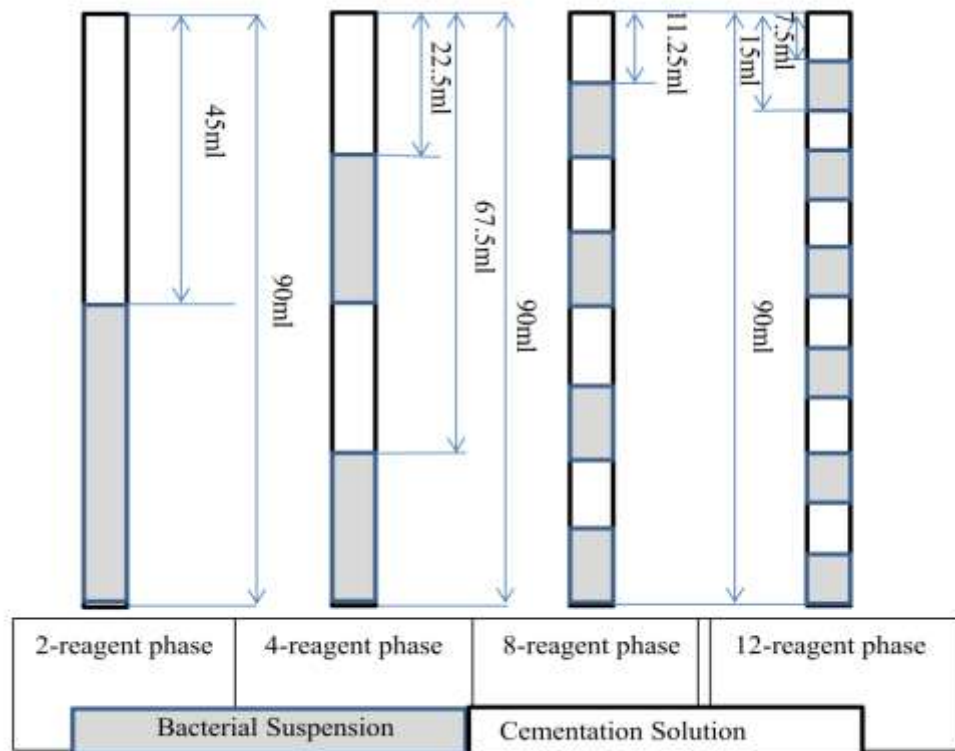
Figure 3.2. Summary of protocols adopted of reagent phase strategy.

3.2.3.2   Multi-soil lift strategy

The soil placement strategy developed was based on the use of as many as four soil lifts (layers) where the crushed aggregate columns in this strategy were treated layer by layer and for each crushed aggregate lift (layer), two reagent phases were used for treatment. After placement of each crushed aggregate lift, tap water (equivalent to two PVs of the crushed aggregate lift) was flushed through it to expel the extra air from the pore matrix. The above-described two-phase reagent treatment strategy (Figure 3.2) was adopted to treat each crushed aggregate lift (Figure 3.3). The process of multi-soil lift strategy was basically done as follows:

 (i) Flushing cementation solution (100% PV mL of crushed aggregate columns) to promote high ionic strength within the pore matrix. The divalent calcium ions increase the ionic charge at the particle surfaces. Urea plays a significant role in providing a suitable environment for the bacterial cells

as they have a high affinity for urea. This step was applied once before the beginning of the treatment cycles. (ii) The process was initiated by determining PV of each crushed aggregate lift, and the first treatment cycle included alternating percolation of a half-PV of bacterial suspension and a half-PV of cementation solution within the first lift.

(iii) A 24-h incubation time was chosen to achieve better intermixing between bacteria and substrates.

(iv) After draining out the effluent of first cycle, the second treatment cycle involved only one PV of the cementation solution and the same aforementioned incubation time was adopted. Steps ii and iv were considered as two biochemical treatment cycles and were repeated until reaching the required number of biochemical treatment cycles in each soil lift as shown in Figure. 3 (i.e. 20 treatment cycles in the case of one soil lift column). However, the number of biochemical treatment cycles that was applied in each lift depended on the number of soil lift used. As the number of soil lifts increased, the number of biochemical treatment cycles for the top regions of crushed aggregate columns decreased. The targeted number of treatments in each crushed aggregate column was 20 cycles, which were divided by its number of soil lifts. The reason for instance, a four-soil lift column was achieved by dividing the total specimen length (102 mm) by four, resulting in a crushed aggregate lift with a thickness of ~25 mm. The total number of biochemical treatment cycles were set to be 20 cycles, which was also divided by the number of soil lifts (i.e. four lifts in this case) resulting in five biochemical treatment cycles in each placed crushed aggregate lift. Once the first soil lift was placed it received five treatment cycles. The second lift also received five treatment cycles, but this also means that lift 1 received an additional five biochemical treatment cycles. This process was repeated until the final lift (lift 4), which itself received also five treatment cycles, resulted in the number of treatment cycles (NOT) shown in Figure 3.3 for each lift. The protocols of other soil lift columns were similar to the four-soil lift column with different soil lift thickness and number of treatment cycles in each lift and are indicated in Figure 3.3.
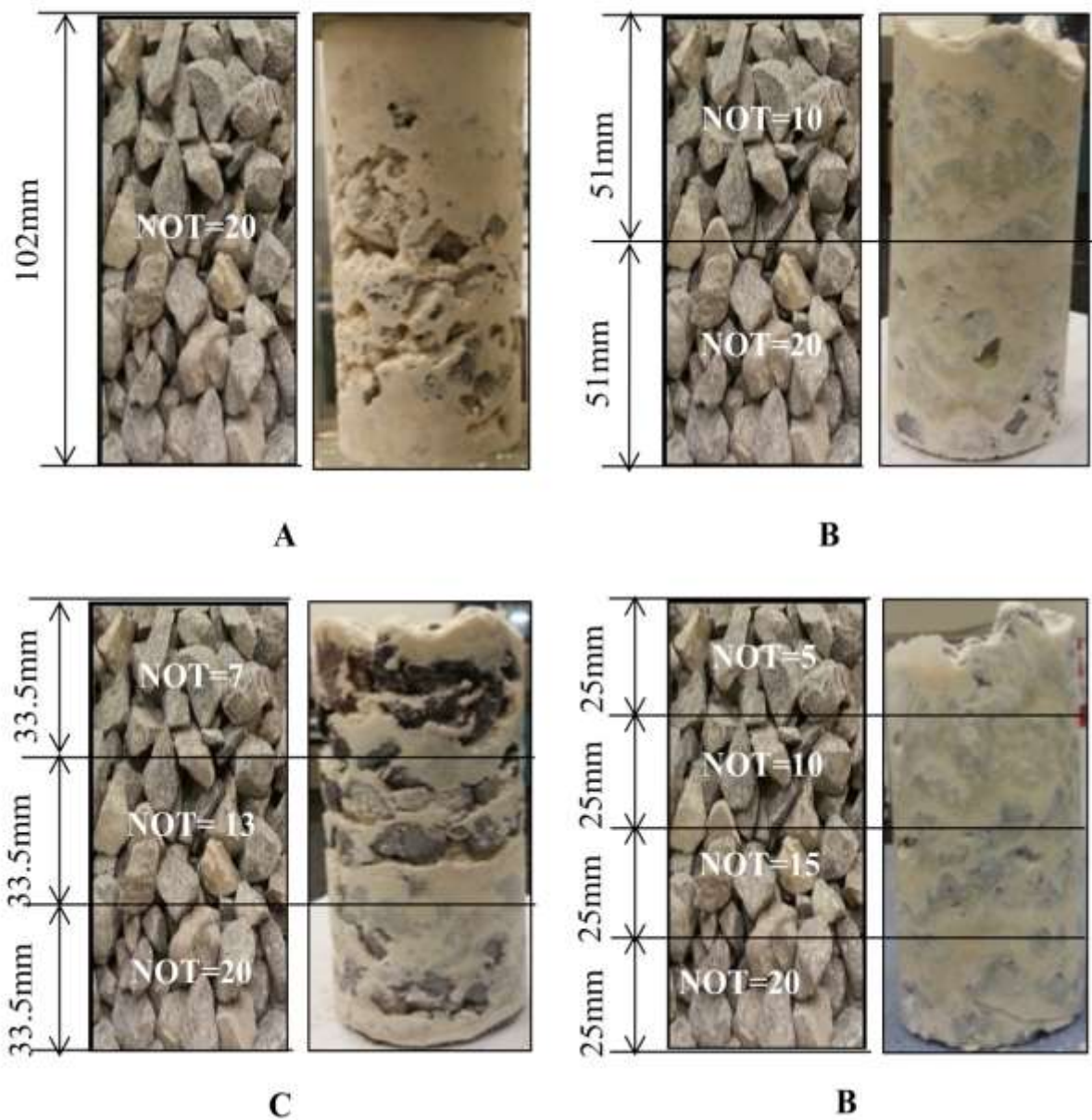
Figure 3.3. Layout of MICP-treated crushed gravel using various soil lifts strategy (NOT refers to number of treatments, A: treatment of one-soil lift, B: treatment of two-soil lifts, C: treatment of three-soil lifts, D: treatment of four-soil lifts).

3.2.4 UCS tests

After completion of the biochemical treatments and curing periods, all bio-cemented crushed aggregates columns were subjected to standard UCS tests to quantify any possible increase in compressive strength. All the tests were carried out at a constant rate of 1%/min to monitor the propagation and observation of the shear failure in the samples. Calcium carbonate precipitation was quantified after completion of the UCS tests.

3.2.5 Calcium carbonate content

Gravimetric hydrochloric acid (HCl) washing technique was used to measure the amount of calcium carbonate precipitation in each column. Sub-specimens were collected from parts (top, middle and bottom within around 15 mm of each part) of the bio-cemented samples after completion of the UCS tests. They were dried at 105°C for 24 h and then their masses were measured. Next, the samples were rinsed with 2M HCl to dissolve precipitated calcium carbonate and flushed with deionised water to allow the dissolved salts to be rinsed out from the samples which then were left in the oven to dry for 24 h. The difference between mass before and after acid wash was taken as the mass of calcium carbonate. The percentage of calcium carbonate was obtained by dividing the mass of calcium carbonate by the mass of aggregate.

3.2.6 Microstructure analysis

Scanning electron microscopy (SEM) and energy-dispersive spectroscopy (EDS) were conducted to characterise the deposition of calcium carbonate on the surfaces of the crushed aggregate. A sample of the crushed aggregate that was treated by three-soil lift strategy was retained after completing the UCS tests for conducting micro-scale investigations. The fractions were oven-dried; carbon coated and then examined using an SEM (JEOL 7001F FEGSEM,) with a beam intensity of 15 kV.

3.3 Results and discussion

3.3.1 Multi-soil lift strategy

Figure 3.4 shows the relationship between the amount of calcium carbonate precipitation in three regions (top, middle and bottom) of the bio-cemented crushed aggregate and the UCS with the number of soil lifts. The bio-cemented crushed aggregate showed a variation in the amount and distribution of calcium carbonate deposition, though the total number of biochemical treatment cycles was similar in each column. Calcium carbonate precipitation was visible as a white deposition on the surfaces of the crushed aggregate (see Figure 3.5(a)). Figure 3.5(a) reveals that calcium carbonate had deposited under the influence of gravity on the crushed aggregate particles. SEM was also used to further investigate the white deposition, where Figure 3.6(a) shows that calcium carbonate was deposited as white crystals on the surface of the aggregate particles. In addition, Figure 3.6(b) indicates that the chemical constituents of the precipitated crystals are mainly composed of calcium, carbon, and oxygen, and this validates the fact that the crystals are $CaCO_3$ polymorphs. It can therefore be inferred that MICP method can be successfully applied using surface percolation to improving coarse materials without needing bumping technique as it is usually used in the case of fine and medium sand. A similar trend was reported by Van der Star et al. (2011), in which gravel deposits were stabilised using bio-cementation process with the aim of installing pipes via horizontal directional drilling. It can be clearly seen from Figure 3.4 that calcium carbonate precipitation was more abundant within the lower portions of the multi-crushed aggregate lift columns than the upper portions. This is reasonable as the total number of treatments was less in the upper portions than in the lower portions of the multi-soil lift columns. The amount of calcium carbonate precipitation in the bottom portions was around 2× and 3× greater than the top portions in case of three-soil lift and four-soil lift samples, respectively. It is also worth noting that differences of calcium carbonate precipitation between top and bottom portion

increased with increasing crushed aggregates lift numbers. This is because the bottom portion received more biochemical treatment cycles than the top portion as shown in Figure 3.3.
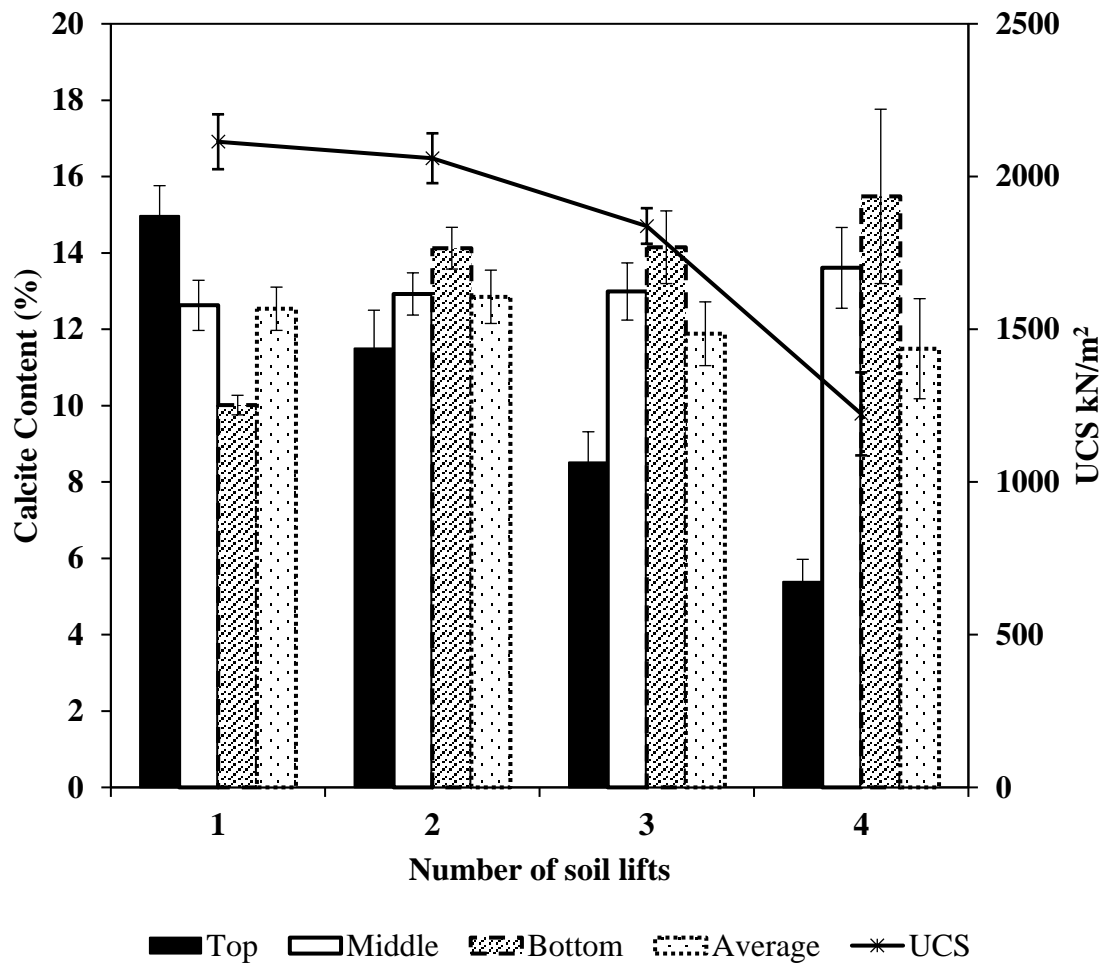
Figure 3.4. Effect of number of soil lifts on the amount of calcium carbonate precipitation at the upper, middle and lower sections of the bio-cemented crushed aggregate and its overall unconfined compressive strength (UCS).
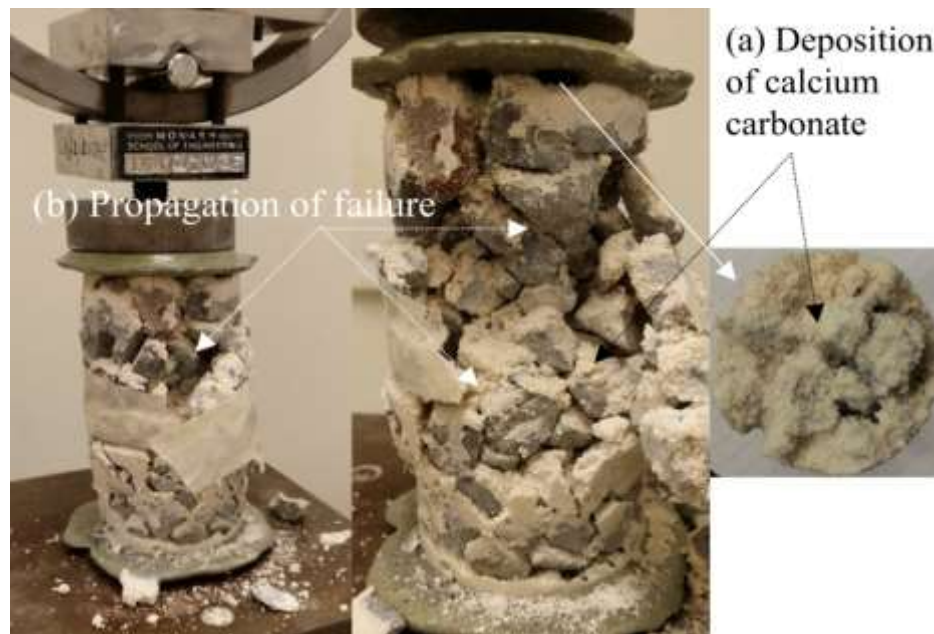
Figure 3.5. MICP-treated crushed aggregate using treatment of three-soil lifts, where (a) deposition of calcium carbonate on the surface of the crushed aggregate (b) propagation of failure in the interface between the lifts.

The amount of calcium carbonate precipitation in the bottom portions was around 2× and 3× greater than the top portions in case of three-soil lift and four-soil lift samples, respectively. It is also worth noting that differences of calcium carbonate precipitation between top and bottom portion increased with increasing crushed aggregates lift numbers. This is because the bottom portion received more biochemical treatment cycles than the top portion as shown in Figure 3.3. In contrast, the one-soil lift column differed from the multi-soil lift samples because more calcium carbonate was deposited in the upper portion than in the lower portion. Utilising a one-soil lift column having a high calcium chloride concentration can lead to increase ionic charges, and might tend to stimulate adsorption and flocculation of the bacterial cells particularly in portions close to injection points (Faibish et al. 1998, Foppen and Schijven 2006, Harkes et al. 2010) because bacterial cells have a high negative zeta potential that are attracted to soil particles under physical infiltration (Dick et al. 2006). The

average calcium carbonate content in all bio-cemented crushed aggregate columns was similar with a marginal difference in amount of deposited calcium carbonate in the three-soil lift column and four-soil lift column. This may be related to lose some deposited calcium carbonate during placement and vibration of multi-soil lift. Figure 3.4 also shows the effects of multi-soil strategy on the compressive strength of crushed aggregate treated by bio-grouting. The experimental results indicate that an increased number of soil lifts negatively affects the strength of bio-cemented crushed sand samples. It is postulated here that the interaction between soil lifts resulted in the creation of regions with weak resistance, which eventually led to weaker samples. Shear failures were observed to originate from the interface between the lifts (Figure 3.5(b)), and were primarily dependent on calcium carbonate–calcium carbonate contacts (Figure 3.6) as it was reported that a thin layer of calcium carbonate resided on the surfaces of the soil particles (DeJong et al. 2010). Also, the calcium carbonate deposited was relatively weaker than the crushed aggregate particles due to the heterogeneous distribution of the calcium carbonate. Furthermore, because each lift was vibrated in place, this process might have also resulted in reduction of UCS values of multi-soil lift columns. Notwithstanding the above, it can be seen from Figure 3.4 that the strength of bio-cemented crushed aggregate was improved despite the fact that strength decreased as the number of lifts increased. The one-soil lift treatment technique achieved the highest strength (about 2100 kPa).
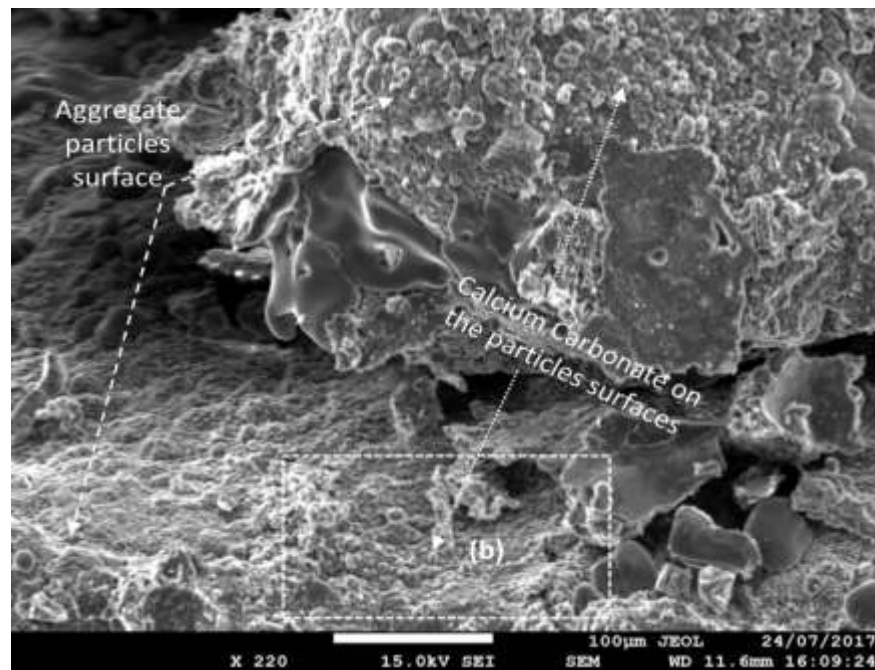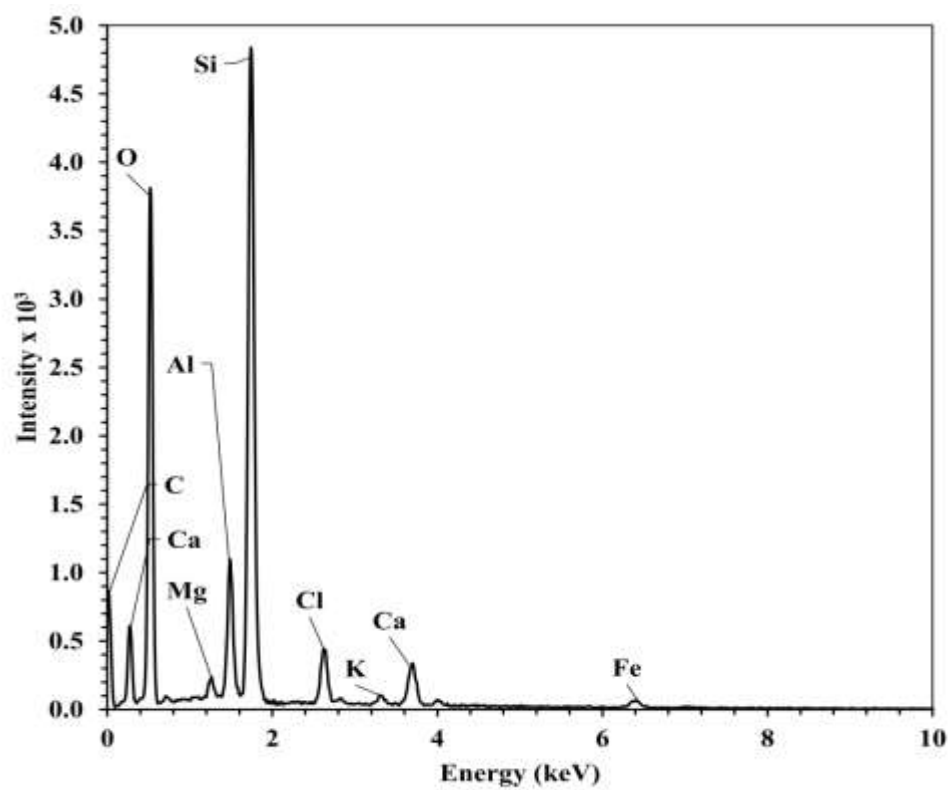
Figure 3.6. SEM and EDS for crushed aggregate treated via three-lift treatment strategy: (a) the deposition of calcium carbonate on the surface of the crushed aggregate; (b) EDS for chemical composition of the CaCO3 crystals.

3.3.2 Multi-reagent phase strategy

This strategy allowed investigation of the effect of using various reagent phase numbers on the distribution and retention of bacterial cells within crushed aggregate columns. Using a multi-phase percolation of bacterial suspension and cementation solution may provide better intermixing of bacterial cells and cementation solution, especially when using higher number of reagent phases (Cheng and Cord-Ruwisch 2012). It was postulated that bacteria adhesion to surfaces within crushed aggregate columns might be achieved as substantial amount of calcium carbonate precipitated in bio-cemented crushed aggregate columns (Figure 3.7). Similar trend was observed by Cheng and Cord- Ruwisch (2012) when bio-cementation was used to stabilise fine sand. When the bio-cemented columns were taken out of the moulds, it was noticed that increasing number of reagent phases resulted in an increase of amount of calcium carbonate precipitation near to the percolation points (i.e. the top portion of the samples). It is clearly seen from Figure 3.7 that an increase of reagent phase number led to increasing amount of calcium carbonate at the upper parts of the bio-cemented crushed aggregate. This can be useful if bio-grouting is applied to stabilise specific locations, for example improvement of the upper part of granular piles where bulging tends to mostly occur (Hughes and Withers 1974). In addition, using high calcium chloride concentration can result in increase of ionic charges, and promotion of bacterial adhesion (Faibish et al. 1998, Foppen and Schijven 2006). Figure 3.7 further shows that the gap between upper and lower portion in terms of amount of deposited calcium carbonate seems to increase with an increase of reagent phase numbers. However, the column with four-reagent phase strategy was marginally different in distribution of calcium carbonate as there was a little difference between top, middle and bottom portions compared with other strategies. The total amount of calcium carbonate precipitation remained within the range of 12.4–13.2% in all bio-cemented crushed aggregate columns. It can also be seen from Figure 3.7 that an

increase of reagent phase numbers associated with increasing strength of bio-cemented crushed aggregate occurred up to the four-reagent phase, and then the strength slightly decreased with increasing number of reagent phases. Maximum compressive strength was achieved using four-reagent phase, which was linked with the minimum calcium carbonate precipitation (bottom portions), and was greater than the other bio-cemented crushed aggregate columns. Thus, it is important to investigate the spatial distribution of calcium carbonate precipitation within the bio-cemented granular columns in which it is directly associated to the gained strength as stressed by Martinez and DeJong (2009).
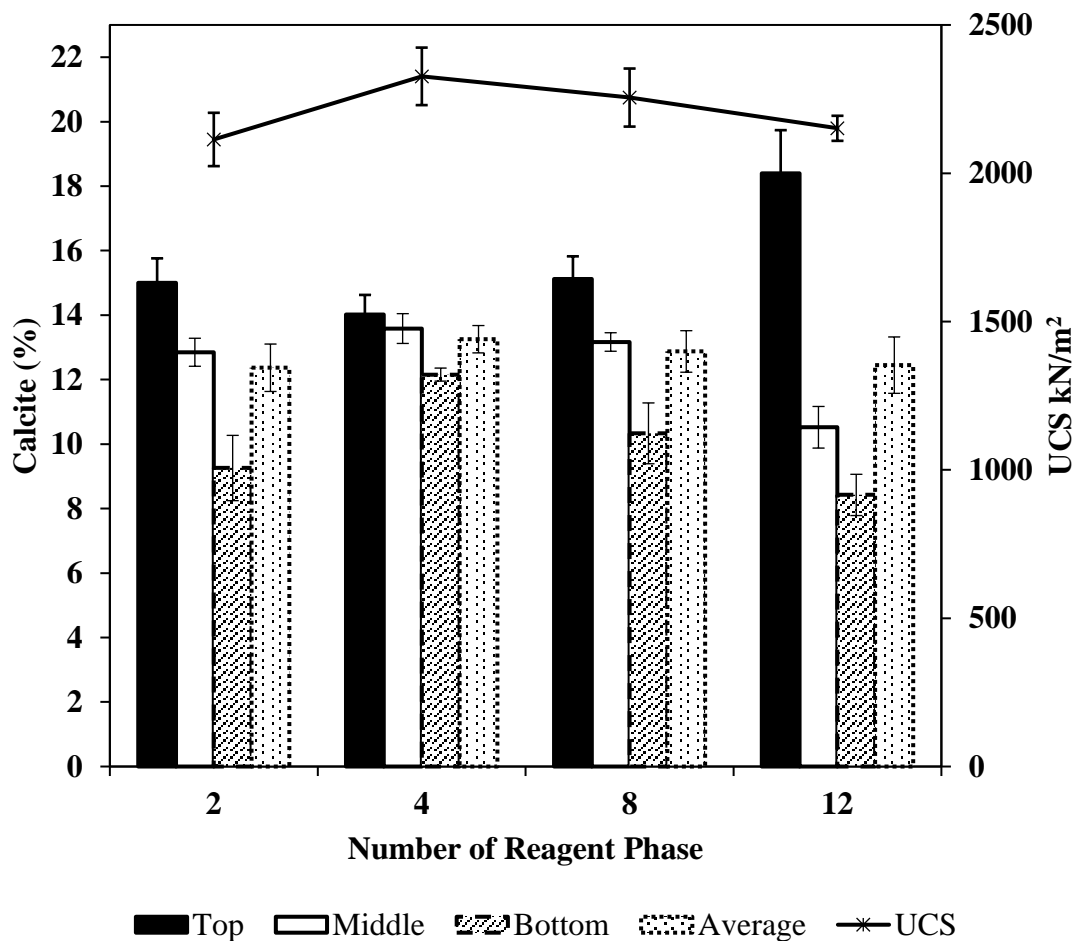
Figure 3.7. Effect of reagent phase additions on calcium carbonate concentration at the upper, middle and lower sections of the biocemented crushed aggregate and its overall unconfined compressive strength (UCS).

3.4 Conclusion

Crushed aggregate is very often used as raw material for stone column installation within weak soils. Crushed aggregate tests have shown that bio-grouting can be used to enhance their engineering properties, such as compressive strength. Bio-grouting by means of urease-producing bacteria (S. Pasteurii) was used throughout this study. It was applied using two different typical techniques, namely a multi-soil lift strategy and a multi-phase treatment strategy. A multi-soil lift strategy included an option of 1, 2, 3 and 4 crushed aggregate lifts, and it was shown that using a one-soil lift resulted in higher strength than using multi-soil lifts due to the interaction between soil lifts. However, the minimum strength achieved (around 1.2 MPa) in this study should still be sufficient to mitigate slumping of a stone column that may occur as a result of excessive radial expansion. A multi-reagent phase treatment strategy comprised various phases (2, 4, 6 and 12 phases) of bacterial suspension and cementation solution and experienced a different tendency. An increase of multi reagent phase number associated with increasing in compressive strength was observed up to four-reagent phase, and then a decrease in strength was recorded. A maximum strength of approximately 2.3 MPa was achieved using four-reagent phase strategy. The total amount of calcium carbonate precipitation was in the range of 12.4–13.2%. Furthermore, bio-grouting bounding crushed aggregate particles can be useful to gain localised bio-cementation either in the upper or lower portion by utilising a multi-reagent phase, which can be combined with a multi-soil lift strategy to gain a uniform calcium carbonate and a high strength along a granular column. The multi-soil lift strategy could also be used to treat a deep granular pile where it is impossible to strengthen the bottom lifts and heterogeneous distribution of calcium carbonate precipitation can be overcome by increasing number of treatment cycles in the top lifts. Microstructure analysis has further confirmed the precipitation of calcium carbonate on the surfaces of the crushed aggregate particles.

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Chapter 4: Factors affecting the bio-cementing process of coarse sand

Factors affecting the bio-cementing process of coarse sand

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Abstract

Microbial induced calcite precipitation (MICP) has received increased attention in recent years as a potential technique for green soil improvements. MICP mimics natural biochemical processes, but utilizes cultured urease-producing bacteria that more efficiently catalyse the hydrolysis of urea in the presence of calcium ions to induce precipitation of calcium carbonate. However, optimization of the MICP method for practical use has hindered its widespread uptake and application. This paper explores the impact of factors such as equimolar and non-equimolar cementation solution, various reaction times and reaction temperatures, which may impact on engineering design of MICP projects. The results reveal that MICP is strongly influenced by the choice of cementation solution concentration, reaction times and temperatures adopted. Higher strength and time-efficient bio-cementation of coarse sand columns were achieved over 24 hours incubation time with equimolar cementation solution (1M) at 20^oC temperature. The effect of molarity of cementation solution and temperature were also analysed and confirmed by scanning electron microscopy and energy dispersive spectroscopy.

4.1 Introduction

Microbial induced calcite precipitation (MICP) has emerged recently as an alternative method, for traditional green soil improvements. MICP can treat cohesionless soil by enhancing natural processes. This process relies on non-pathogenic, halophilic, alkalophilic and urease-producing bacteria, such as *Sporosarcina pasteurii*, and cementation solutions (urea and $CaCl_2$). Biotreatment is initiated by adding urease-producing bacteria into a soil matrix to catalyse urea hydrolysis as described by the following equations (Hammes and Verstraete 2002).

$$CO(NH_2)_2 + H_2O \xrightarrow{\text{Urease Enzyme}} CO_2 + 2NH_3 \qquad (4.1)$$

$$2NH_3 + CO_2 + H_2O \longrightarrow 2NH_4^+ + CO_3^{2-} \qquad \uparrow \text{ pH} \qquad (4.2)$$
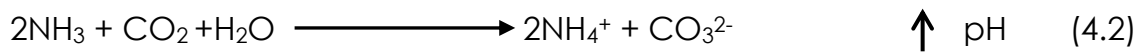
Urea hydrolysis produces an excess of dissolved carbonate ions as can be seen in Eq. 4.2. In the presence of excess supplemental calcium ions, calcium carbonate is spontaneously precipitated:

$$Ca + CO_3^2 \longrightarrow CaCO_3 \qquad \downarrow \text{ pH} \qquad (4.3)$$
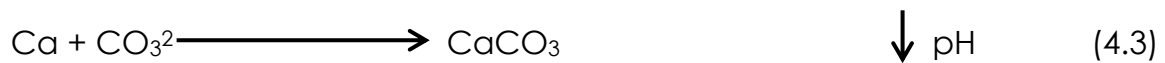
The pH with an arrow up in equation 4.2 indicates that the pH increases (pH $\approx$9.5,Stocks-Fischer et al. 1999) with urea hydrolysis, and then decreases to pH $\approx$8.3 after precipitation of sufficient calcium carbonate as presented in equation 4.3 with an arrow down. To achieve the required saturation state of the bio-reagents, four key factors governing the precipitation of calcium carbonate must be accounted for: (1) calcium concentration, (2) concentration of dissolved inorganic carbon (DIC), (3) pH and (4) availability of nucleation sites (Castanier et al. 1999, Kile et al. 2000, Hammes and Verstraete 2002).

Soluble divalent cations (e.g., calcium ion) in the presence of negatively charged bacterial cells are requisite for carbonate precipitation, and when adsorbed onto the surfaces of negatively charged bacterial surfaces, provide ideal nucleation sites crystal growth (DeJong et al. 2006). The concentration of soluble carbonate is mostly dependent on the saturation

of the DIC, the solution pH and water temperature. Furthermore, several physical and environmental parameters have an effect on the amount and efficieny of calcium carbonate precipitation in a soil matrix, including salinity, temperature, geometric compatibility of bacteria, methodology, soil gradation, treatment conditions (cementation solution (e.g., urea and calcium) concentrations, incubation time and injection rate), ammonium concentration, oxygen availability and ureolytic activity for viable bacterial cells (Rivadeneyra et al. 2004, De Muynck et al. 2010, Mortensen et al. 2011, Pepper et al. 2011, Qabany et al. 2012, Mahawish et al. 2016, 2017a and 2017b, Amin et al. 2017).

The effects of parameters such as cementation solution (urea and $CaCl_2$ concentrations), temperature, incubation time (reaction time) as well as the appropriate methodology to introduce the bacteria into the soil matrix, need to be understood to enable a controlled use of MICP and to scale up the MICP method from laboratory to field applications. Urease activity and soluble carbonate are significantly, to one another, affected by temperature, and hence the rate of calcium carbonate precipitation can be influenced (i.e., higher temperatures, higher urease activity, and better carbonate production rate) (Nemati and Voordouw 2003, Whiffin 2004). However, Cheng et al. (2017) indicated that calcium carbonate precipitating at high temperatures can be the least efficient way to retain strength improvement.

Selecting ideal cementation solution concentrations is important for $CaCO_3$ precipitation as it is considered to be a key control factor for improving the strength of biotreated soils. Nemati et al. (2005) and DeJong et al. (2009) indicated that a chemical optimization of the cementation solution can also contribute to obtain a uniform $CaCO_3$ precipitation. Nemati et al. (2005) indicated that a better conversion to calcium carbonate could be accomplished by utilising equimolar concentrations of both reactants (urea and $CaCl_2$). This latter observation is supported by the study of Whiffin (2004), who reported that the deposited $CaCO_3$ proportionally increased with an

increase in equimolar cementation solution concentrations. A significant increase in soil strength and a decrease in permeability were also reported at high cementation solution (1 M) when investigating the enzyme of urease (Yasuhara et al. 2011). However, a decrease in $CaCO_3$ precipitation was seen when using cementation solution concentrations above 1.5 M, due to inhibitory effects of high salinity on microbial activity and $CaCO_3$ precipitation (Nemati and Voordouw 2003, Nemati et al. 2005, De Muynck et al. 2010). Also, the crystals of deposited calcium carbonate can become larger as an increase of cementation solution concentrations can lead to a reduction in precipitation uniformity in the soil media (Al Qabany et al. 2012). By contrast, other studies found that the precipitation of calcium carbonate at lower cementation solution concentrations is more efficient than when higher reagent solution concentrations are used (Nemati et al. 2005, De Muynck et al. 2010, Okwadha and Li 2010, Al Qabany et al. 2011, 2012). The increased efficiency was attributed to a gradual reduction in the permeability with lower cementation solution concentrations (Al Qabany et al. 2012), and can contribute to preventing clogging near injection point by promoting more homogeneous cells and ultimately metabolic activity distribution in porous media (Phillips et al. 2013). Application of a greater number of injections of relatively low cementation solution concentrations was suggested by Al Qabany et al. (2012) to induce the same amount of calcium carbonate precipitation and at the same time attain high soil strength. On the other hand, the effect of non-equimolar (e.g., disproportionate amounts of calcium and urea) cementation solutions has also been studied. For instance, Yasuhara et al. (2011) stated that using non-equimolar cementation solutions resulted in an increase of UCS and decrease in permeability of a biotreated sand. Okwadha and Li (2010) investigated various non-equimolar cementation solutions and concluded that a higher amount of deposited calcium carbonate was associated with a higher cementation solution concentration., Although, a lot of research work has been conducted to investigate the effects of cementation solutions on fine to medium sand, there is still uncertainty about selecting the

most adequate cementation solution concentration that can be adopted for practical use. A lack of knowledge also exists concerning the proper cementation solution concentration that would be used with coarser materials and whether the bio-cementation process can be achieved in the same way as with fine and medium sands as was discussed in previous studies (Al Qabany and Soga 2013, Cheng et al. 2017), because more calcium carbonate precipitation may be required to attain a targeted strength. In this paper the effect of using different equimolar and non-equimolar (urea-$CaCl_2$) cementation solutions, incubation times (reaction times) and temperatures on the mechanical properties (strength) of bio-cemented coarse sand is examined. The effects are assessed based on the measurements of the unconfined compressive strength (UCS), the amount of precipitated calcium carbonate, scanning electron microscopy and energy dispersive spectroscopy.

4.2 Materials and Methods

4.2.1 Soil type

The sand used in this study has an average grain size of 1.6 mm, and its particle grain size distribution is shown in Figure 4.1. It is classified as poorly graded coarse sand (SP) according to the Unified Soil Classification System (ASTM 2006). Maximum and minimum dry density measurements were conducted in accordance with ASTM D4253 (2014a) and ASTM D4254 (2014b) to control the density of the column model and were respectively 1.70 g/cm^3 and 1.44 g/cm^3 . The sand properties (specific gravity (G_s), coefficient of curvature (C_c), coefficient of uniformity (C_u), median grain size (D_{50}), maximum and minimum void ratios (e_{max} and e_{min}) are summarised in Table 4.1. The sand particles were typically sub-angular in shape and were deemed to be representative of stone aggregates used in full-scale stone columns (Gniel and Bouazza 2009, 2010) since the intention was to develop eventually rigid columns suitable for improvement of soft and weak soils.
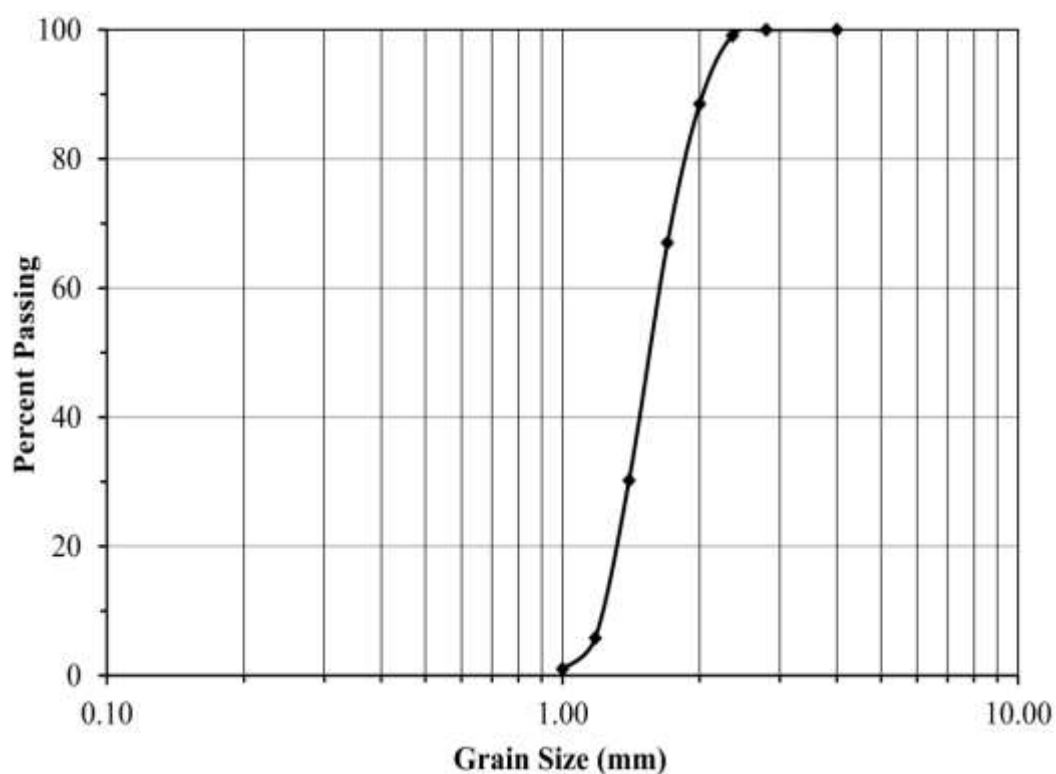
Figure 4.1. Sand particle size distribution

Table 4.1: Sand characteristics

Soil Type	Classification	G_s	C_c	C_u	D_{50} (mm)	e_{max}	e_{min}
Sand Grade 8/16	SP	2.64	0.97	1.35	1.60	0.84	0.55

4.2.2 Microorganism and culture medium

Sporosarcina pasteurii (American Type Culture Collection (ATCC) 11859) was used as urease-producing bacteria throughout this study. The bacterial strains from the frozen stock were cultivated on ATCC 1376 ammonium-Yeast extract (NH_4-YE) petri dishes (20 g yeast extract, 10 g of ammonium sulphate (($NH_4)_2SO_4$) and 20 g of agar in 130 mM Tris buffer in pH 9.0) (Yoon et al. 2001). One colony from the stock culture (petri dishes) was transferred to 5 mL of NH_4-YE growth medium (20 g yeast extract, 10 g ($NH_4)_2SO_4$ and 130 mM tris buffer (pH=9.0) per litre of distilled water) to grow the bacterial cells at 30°C. Individual ingredients of the recipe were separately dissolved

in distilled water bottles, autoclaved (121°C, 100 kPa for around 2 hr process) separately and then combined post sterilisation. Suspended bacterial cultures were grown in a shaking water bath (200 rpm) for 24 hrs before harvesting at a final optical density (OD_{600}) of 3.0-3.5 using a WPA CO 8000 spectrophotometer (BioChrom Ltd). Such treatment allowed achieving an optimum concentration of bacterial cells in which all readily available nutrients were considered to be consumed from the growth medium (i.e., the start of the stationary growth phase) (Cheng and Cord-Ruwisch 2012). The number of bacterial cells (Y, per ml) at OD_{600} =3.0- 3.5 was estimated to be 3.8×10^8 - 4.7×10^8 cells/ml (Eq.4) (Ramachandran et al. 2001).

Concentration of cells per mL = $8.59 \times 10^7 \times OD_{600}{}^{1.3627}$ (4.4)

An activity value of 19.38-21.45 mM/min for urease enzyme was used in this study based on the maximum optical density of the harvested bacterial cells. Urease activity was based on an electrical conductivity assay (labCHEM-CP) result, which was converted to an ureolysis rate using the following equation (Whiffin 2004);

Urea hydrolysed (mM) = Conductivity (mS) x 11.11   (R^2=0.9988)   (4.5)

The main reason for harvesting bacterial cells at the beginning of the stationary phase was to minimise further growth of bacterial cells in the granular columns during retention time (incubation time) from occurring, because no nutrient broth has been percolated onto the soil columns after the percolation of bacteria. The preliminary culture medium was transferred aseptically to inoculate 250 ml of Ammonium-YE medium, and the previous process can hence be repeated accordingly. The bacterial suspension was stored up to 14 days at 4°C until used in the experimental works.

4.2.3 Reagents Concentrations

The ureolytic process was driven using urea-$CaCl_2$ cementation solutions. The reason for choosing $CaCl_2$ as a calcium source for $CaCO_3$ precipitation was because $CaCl_2$ has been identified to be the most adequate for the production of $CaCO_3$ among the different sources of calcium that were used with cementation solutions containing urea (Achal and Pan 2014).

Table 4.2. Chemical combination concentrations used in this study.

Cementation Solution combinations	Urea (M)	$CaCl_2$ (M)
Non equimolar		
1	0.50	0.25
2	1	0.50
3	1.50	0.75
4	2.0	1.0
Equimolar		
5	0.25	0.25
6	0.5	0.5
7	0.75	0.75
8	1.5	1.5

Table 4.2 summarises different combinations of urea and $CaCl_2$ concentrations utilised in this study. Predetermined masses of $CaCl_2$ were dissolved into distilled water based on the different equimolar and non-equimolar requirements, taking into consideration the molecular weight of both dehydrated $CaCl_2$ (147.02 g/mol) and urea (60 g/mol).

4.2.4 Experimental factors

The cementation solution concentration which gave the maximum UCS was selected as the optimal cementation solution concentration. The effects of environmental factors such as incubation time (reaction time) and reaction temperatures were investigated on the specimens treated at this optimal concentration. Various incubation times were chosen to investigate the effectiveness of MICP to treat coarse sand over both short and long biochemical treatment periods. Incubation time was postulated herein to

have a significant impact on the applications of the biotreatment process. Incubation time is the amount of time that the urease enzyme requires to catalyse the hydrolysis of the urea. The adopted incubation times were 12, 24, 48, 72, and 96 hr.

The enzyme urease produced by bacteria is analogous to any other enzymatic reactions where it depends on an environmental temperature. As temperature increases from 10 $^\circ$C to 60 $^\circ$C, the urease activity increases resulting in an increase in the precipitation rate of $CaCO_3$ (Ng et al. 2012). However, it was found that most urease enzymes typically worked within an optimum temperature range of 20 $^\circ$C to 37 $^\circ$C (Mitchell and Ferris 2005, Okwadha and Li 2010) and it was completely stable at 35 $^\circ$C (Dhami et al. 2013). Thus, changing or controlling the in-situ temperatures to suit MICP in field situations may be difficult. Consequently, for MICP to be suitable for engineering applications where there are spatial and temporal variabilities in soil temperatures in the field (Barry Macaulay et al. 2013, 2014, Singh et al., 2015), requires more research effort. The effect of using different temperatures to replicate ground temperature is still limited, especially their effect on the mechanical properties of soils (compressive strength). Therefore, the current study explores the effect of various temperatures (i.e., 0, 10, 20, and 40 °C) to imitate a range of field temperatures. The incubation time applied between the biochemical treatments of the specimens that were prepared to study the effect of temperature was 24 hr.

4.2.5 Experimental setup and Methodology

The column used in the current study was made of a split mould Poly Vinyl Chloride (PVC) pipe, 220 mm high and with an internal diameter of 51 mm when assembled together (Mahawish et al. 2016). The soil column specimens have a length to diameter aspect ratio of 2:1. A wire mesh and filter paper were placed at the bottom of the column to minimise the loss of sand grains during preparation and treatment. The columns were positioned vertically with fully open top and a control valve at the bottom. Dry coarse sand was pluviated into the specimen moulds and then compacted up to

a relative density of 60% (equivalent to 1.59g/cm³) due to their sub-angular particle shape and poor grading, using a vibratory compaction method. After placement of the soil, tap water was flushed through it to expel the extra air from the pore matrix.

The test protocol adopted in this study was a four-phase percolation strategy based on the percolation method described in Mahawish et al. (2016). The amount of bacterial suspension and cementation solution in each treatment cycle was determined based on the pore volume (pore water capacity) of the sand columns. The percolation strategy consisted of the following steps:

i.   Percolation of tap water to expel air from the specimens was applied at 200% the total pore water capacity of the granular columns was performed without retention (i.e., the bottom valve was opened to allow drainage).

ii. Percolation of cementation solution at a rate of 100% the total pore water capacity of granular columns was performed without retention to promote a high ionic strength (Table 2) within the pore matrix.

iii. After the cementation solution volume was drained until no apparent dripping of the liquid from the bottom was observed, then the bottom valve was closed, and the first treatment cycle was started by percolating the bacterial suspension (BS) alternately with the cementation solution (CS) for a total of four times to achieve a four phase percolation strategy (10 mL BS + 10 mL CS + 10 mL BS + 10 mL CS) (totally 40 mL was percolated which is equivalent to 50% of the pore water capacity of the soil column).

iv. Incubation (retention time) was 24 hr (unless otherwise stated in the case of studying an effect of various incubation times) at 20 ±2°C, allowing the percolated liquids to facilitate reactions between microorganisms and reagents solutions.

v.   The second treatment cycle included the percolation of only a cementation solution (50% pore water capacity of the granular

columns) and allowed ureolysis and calcium carbonate deposition to take place for the same aforementioned retention time.

vi. Steps iii-v were considered as two biochemical treatment cycles and were repeated until reaching the required number of treatment cycles (12 biochemical treatment cycles were chosen to investigate different environmental factors impact within short testing periods).

After the completion of the bio-cementation treatments, all the specimens were flushed with tap water to remove residual chemical reagents from the pore space. UCS tests were conducted on samples cured at room temperature ($20 \pm 2°C$) over 14-d. All the tests were carried out at a constant rate of 1%/min to monitor the propagation and the observation of the shear failure in the samples. Calcium carbonate precipitation was quantified after completion of the UCS tests.

Calcium carbonate content was measured using a gravimetric acid washing (2 M hydrochloric acid, HCl) technique. For this purpose, subsamples of the bio-cemented coarse sand were dried in an oven at a temperature of 105 $°C$ and their masses were measured before and after the acid wash. The quantification of calcium carbonate was determined by rinsing dissolved calcium carbonate with HCl several times through a sieve (No. 200). The difference between two masses was taken as the mass of calcium carbonate, and the percentage of calcium carbonate was obtained from dividing the mass of calcium carbonated by the mass of sand.

4.2.6 Microstructure analysis

Scanning electron microscopy (SEM) and energy dispersive spectroscopy (EDS) were used to examine the deposition of microbially induced calcium carbonate precipitation on the surface of the coarse sand matrix. For this purpose, subsamples of specimens treated under low and high temperatures and various chemical concentrations were retained after completing the UCS tests with the aim of conducting micro-scale

investigations. The fractions were oven dried; carbon coated, and then examined using an SEM (JEOL 7001F FEGSEM,) with a beam intensity of 15kV.

4.3 Results and Discussions

4.3.1 Effects of Cementation Solution Concentrations

The effect of cementation solution concentrations was investigated by treating coarse sand samples with different equimolar and non-equimolar of cementation solution reactants (urea and $CaCl_2$). Figure 4.2 shows the variation of the unconfined compressive strength (UCS) and calcium carbonate precipitation content at three regions (top (about 30 mm from the top of the specimen), middle (about 60 mm from top of the specimen) and bottom (about 90mm from the top of the specimen)) against the concentration of equimolar cementation solution reactants.
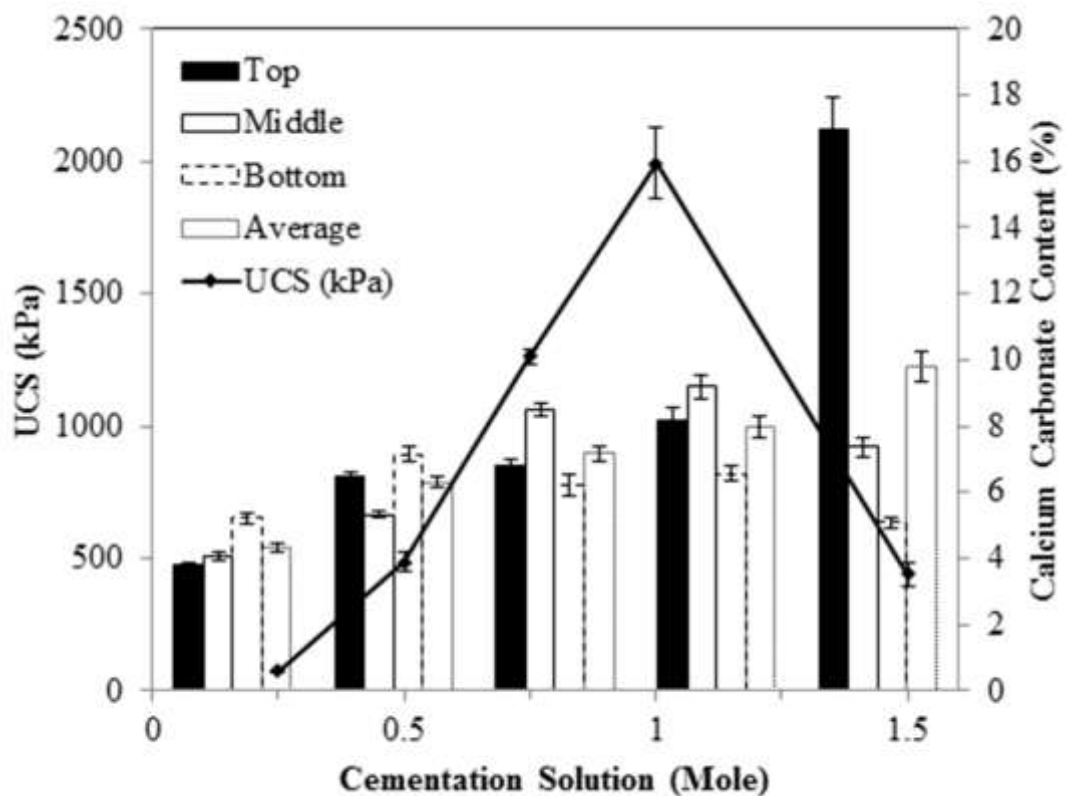
Figure 4.2. Effect of equimolar cementation solution concentration on UCS and calcium carobnate content (note: All bar columns represent calcium carbonate content at the same cementation solution concentration of 0.25, 0.50, 0.75, 1.0 and 1.50 M, respectively).

The amount of deposited calcium carbonate at the top and the middle regions of the bio-cemented coarse sand columns increased with an increase of equimolar cementation solution as a result of MICP process. Perhaps not surprizingly, more calcium carbonate precipitated at the bottom part of the column than in any of its other parts when using low cementation solution concentrations (e.g. 0.25 M). This may be related to abrading of bacterial cells from the upper ports to the bottom ports of the samples, thus resulting in higher amount of $CaCO_3$ precipitation in the bottom part. This was previously reported by Harkes et al. (2010) who indicated that utilising low cementation solution concentrations can promote movement of microbes in porous media. However, the tests exhibited a reduction in the amount of bio-mineralised materials at the bottom regions of the samples treated with higher cementation solution concentrations (0.75-1.5 M) due to the fact that most of the deposited calcium carbonate aggregated in the areas near to top and middle regions of the sand columns (Figure 4.2). Such an effect was evident in treatments with very high cementation solution concentration (1.5 M) where a greater amount of calcium carbonate precipitated in the area close to the percolation points (top portion of the column) (Figure 4.2). These observations are supported by the findings of van Loosdrecht et al. (1989), Scholl et al. (1990) and Harkes et al. (2010) who have shown that adsorption of bacteria increases with an increase in salinity of the environment. Increased ionic strength sue to soluble calcium ions might encourage bacterial cells fixation in areas close to the percolation points (Faibish et al. 1998, Foppen and Schijven 2006, Mahawish et al. 2016), leading to significant amount of precipitation in these areas. This process is further confirmed by the significant amount of calcium carbonate that precipitated at the top portions of the sample treated by 1.5M cementation solution (Figure 4.2). SEM images provided in Figure 4.3 show the amounts, characteristics and distribution of calcium carbonate deposited near the middle surfaces of the coarse sand samples treated by non-equimolar cementation solution (0.25 M $CaCl_2$ and 0.50 M urea) and equimolar

cementation solutions (0.25 M, 0.75 M and 1.50 M), as compared with the untreated sand shown in Figure 4.3a. It can be seen from Figure 4.3a that the surfaces of coarse sand sample are, as expected, smooth and have no evident precipitations. Figure 4.3c shows that calcium precipitated as a result of treatment with an equimolar cementation solution of 0.25 M as small crystals that are lightly distributed over the coarse sand grain surfaces, with more calcium carbonate deposition formed at inter-particle contacts. The amount of calcium carbonate precipitated on the surfaces of the sand grains using a 0.75 M cementation solution is significantly greater than the one used 0.25 M cementation solution and covers almost the entire sand grain' surfaces (Figure 4.3d). It is also interesting to note that the deposited calcium carbonate crystals grew bigger when using a highly concentrated cementation solution as per Figure 4.3d. In addition, utilising higher cementation solution, i.e. 1.5 M has resulted in calcium carbonate precipitation with significant larger crystals that accumulated extensively over the grain surfaces and formed dispersed connections within the pore spaces (Figure 4.3e). Therefore, it can be inferred that cementation solution concentration has a great effect on the by-product of ureolysis process and using various cementation solution concentrations results in different amount, size and distribution of calcium carbonate crystals. The SEM observations provide an evidence on the reasons of the macro-mechanical changes (i.e., UCS changes) observed in the current study as increasing equimolar cementation solution led to accumulation of more calcium carbonate precipitations on and within the coarse sand particles (Figure 4.3e), resulting in possible clogging in the pore matrix and an inhomogeneity of calcium carbonate distribution. EDS was also used to verify the components of the bio-cement material induced from using equimolar cementation solution (1.5 M) and is presented in Figure 4.3f, where the chemical compositions confirm the existence of calcium carbonate precipitation.
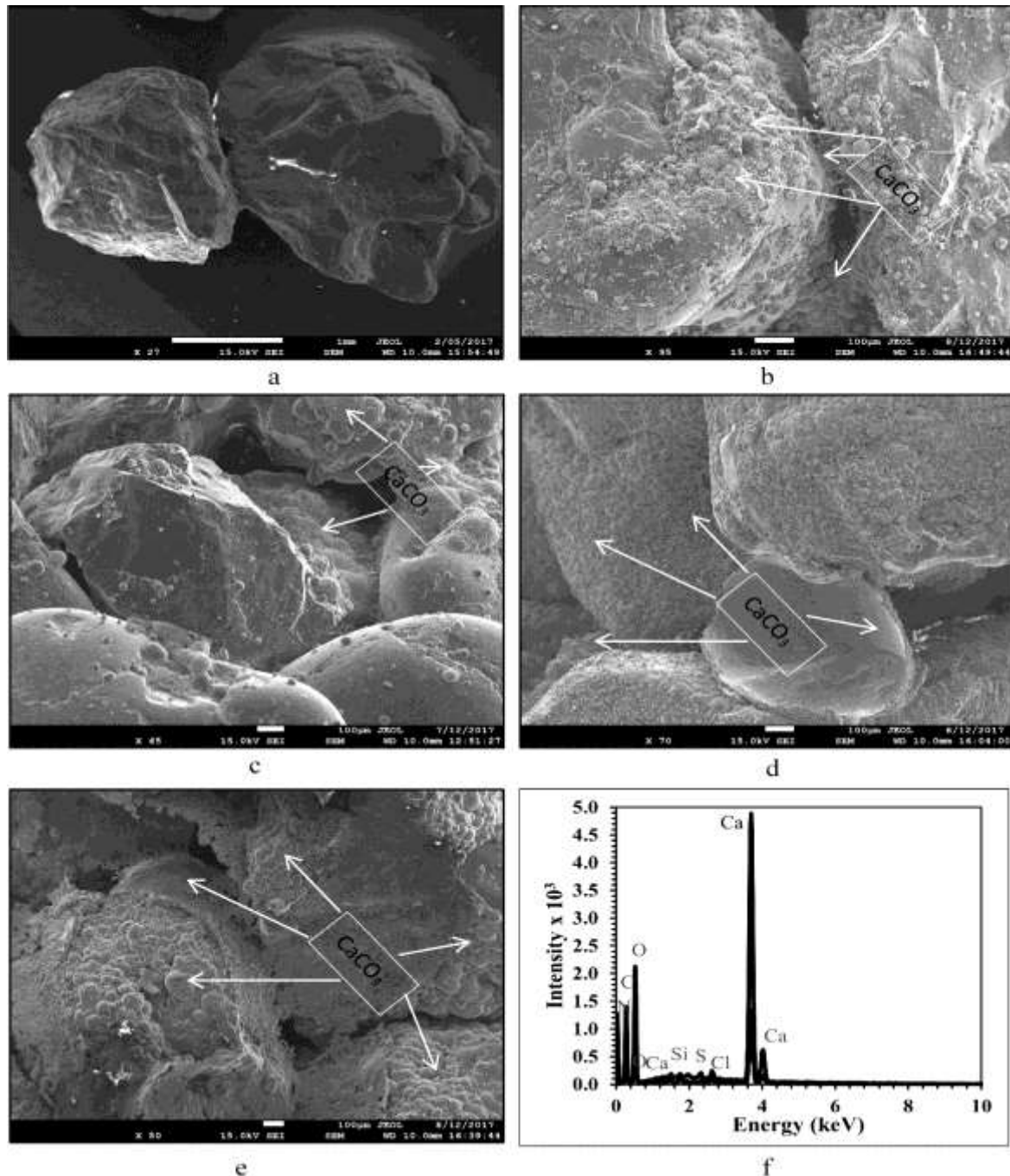
Figure 4.3. SEM images of bio-cemented coarse sand samples treated with equimolar and non-equimolar cementation solution: (a) untreated sample 0 kPa and $CaCO_3$ content =0%; (b) Non-equimolar 0.25M $CaCl_2$ and 0.50M urea, UCS=82.14 kPa and $CaCO_3$ content = 4.48%; (c) Equimolar 0.25M, UCS=73.71 kPa and $CaCO_3$ content = 4.35%; (d) Equimolar 0.75M, UCS=1262.45 kPa and $CaCO_3$ content = 7.18%; (e) Equimolar, 1.50M, UCS=439.10 kPa and $CaCO_3$ content = 9.80%; and (f) EDS of 1.5M equimolar.

It remains also plausible that bacterial cells were not affected by using a high concentration of cementation solution. Soon et al. (2014) reported that some bacteria, such as *Bacillus megaterium,* have no detectable urease activity when using high cementation solution concentrations (i.e.1.0 M). It is also worth noting that the efficiency of $CaCO_3$ precipitation with the 12-biochemical treatment cycles was determined based on the calcium used in each cementation solution concentration and was found to be about 100%, 77%, 58%, 48 and 40% for equimolar cementation concentration of 0.25 M, 0.5 M, 0.75 M, 1.0 M and 1.5 M, respectively. The efficiency reduction with increasing cementation solution concentration indicates that bacterial cells required more time to induce enough enzyme urease to catalyse the hydrolysis of highly concentrated urea solution.

An increase of equimolar cementation solution also resulted initially in a steep increase in unconfined compressive strength (UCS) before it drops at 1.5 M cementation solution (Figure 4.2). The reduction in the UCS of samples treated by higher cementation solution may be linked to the inhomogeneity of the deposited calcium carbonate within the pore matrix of the coarse sand, where there was very low calcium carbonate precipitated at the bottom of the specimen compared with other areas (top and middle). It has also been observed by Dejong et al (2010) that shear failure tended to originate from places where there was less calcium carbonate contact within the sand particles. A maximum UCS of around 2000 kPa was recorded when using 1.0 M of urea and $CaCl_2$. This substantial increase in UCS is in agreement with the study reported by Yasuhara et al. (2010). However, it is worth noting that Al-Thawadi (2008), Al Qabany and Soga (2013) and Soon et al. (2014) reported that no improvement was noticed in the strength of bio-cemented sand specimens for a high cementation solution concentrations (1.0 M). One possible explanation for this apparent contradiction with the current results may be related to particle size effects, as the previous studies focussed only on fine sand. It can also be seen that when equimolar cementation solution increased from 0.25 M to 0.5 M, the

UCS rose from around 74 kPa to around 490 kPa, approximately a 6 times increase. UCS increased from 490 kPa to 1262 kPa when the concentration of cementation solution increased from 0.5 M to 0.75 M. However, there was a substantial decrease in the UCS for the sample treated with high equimolar chemical reagent (1.5 M of urea and $CaCl_2$) although there was high average amount of calcium carbonate precipitation. This is because of the heterogeneous distribution of calcium carbonate precipitation as it mostly precipitated in the top portion of the bio-cemented coarse sand as shown in Figure4.2. Thus, the minimum cementation solution concentration that can be used with coarse sand to retain an acceptable improvement in strength is 0.50M equimolar cementation solution. Using lower cementation solution concentration may require higher number of treatments particularly with a significant pore matrix as it is the case of coarse sand column (Al Qabany et al. 2012),

Table 4.3 summarises the distribution of the amount of calcium carbonate precipitation at specific location within the coarse sand column and UCS values achieved for various non-equimolar chemical reagents (urea and $CaCl_2$). Distribution of deposited calcium carbonate was essentially uniform within the coarse sand samples treated using non-equimolar cementation solutions up to 0.75 M $CaCl_2$ and 1.5 M urea. For instance, utilising 0.5 M of urea with 0.25 M $CaCl_2$ resulted in a uniform distribution of precipitated calcium carbonate at top, middle and bottom of the bio-cemented coarse sand. This is in agreement with the findings reported by Shahrokhi-Shahraki et al. (2015). This concept is further confirmed via the SEM image shown in Figure 4.3b. However, one can notice that using a high chemical reagent concentration (2.0M of urea and 1.0M of $CaCl_2$) affected the distribution of calcium carbonate within the bio-cemented samples. The samples treated with non-equimolar solutions also exhibited a continuous increase of their UCS as the molarity increased. The maximum UCS achieved was around 1155 kPa at 2.0 M urea and 1.0 M $CaCl_2$.

From the results shown in Figure 4.2 and Table 4.3, it can be inferred that utilising a low non equimolar cementation solution (0.50 M urea and 0.25 M Ca) resulted in more and uniform distribution of $CaCO_3$ precipitation compared with using low equimolar cementation solution (0.25 M urea and 0.25M Ca). It is also interesting to note that the precipitated calcium carbonate crystals are bigger in the specimen treated by a low non equimolar than the one treated by a low equimolar cementation solution (Figure 4.3b and c). In addition, compressive strength of samples treated with low nonequimolar concentration solutions were greater than samples treated with low equimolar cementation solutions. However, at high (0.75-1.0 M) cementation solution concentration the compressive strength was lower, although the amount of $CaCO_3$ was greater in the case of non equimolar (i.e. 2.0 urea and 1.0M Ca, Table 4.3).

Table 4.3. Effect of non-equimolar and equimolar cementation solution on UCS and calcium carobnate content.

Cementation Solution combinations	Urea (M)	CaCl₂ (M)	UCS (kPa)	Calcium carbonate content (%)			
				Top	Middle	Bottom	Average
1	0.50	0.25	82.14±5.00	4.380±0.114	4.550±0.138	4.510±0.141	4.480±0.131
2	1	0.50	478.27±32.20	7.250±0.243	5.460±0.154	4.690±0.120	5.80±0.172
3	1.50	0.75	846.66±31.21	8.390±0.322	8.930±0.286	8.920±0.282	8.75±0.297
4	2.0	1.0	1154.60±53.41	17.480±0.775	12.060±0.445	8.850±0.233	12.80±0.484
5	0.25	0.25	73.71±4.00	3.792±0.107	4.034±0.129	5.214±0.175	4.35±0.140
6	0.50	0.50	486.32±37.36	6.440±0.158	5.321±0.114	7.153±0.206	6.30±0.160
7	0.75	0.75	1262.45±26.38	6.798±0.177	8.497±0.206	6.240±0.327	7.18±0.24
8	1.0	1.0	1993.00±134.21	8.190±0.357	9.180±0.329	6.570±0.231	7.98±0.31
9	1.50	1.50	439.10±45.99	16.941±0.986	7.374±0.287	5.076±0.138	9.80±0.47

The trend of having a high amount of calcium carbonate precipitation when using a high non equimolar cementation solution might be related to the production of high amount of ammonium salts because one mole of urea induces two moles of ammonium ions. A significant amount of ammonium salts may have accumulated in the coarse sand media, and washed out with the deposited calcium carbonate. This can infer that the precipitated calcium carbonate might be less efficient with high non equimolar cementation solution. Further research on this possible trend is warranted.

4.3.2 *Effect of Incubation time on bio-cemented coarse sand*

Optimisation of the incubation time (reaction time) is important to achieve better intermixing between bacteria and cementation solution. Also, it is an essential factor in the scale up of MICP to field applications. As the maximum UCS was achieved using an equimolar cementation solution of 1.0 M concentration at $20 \pm 2\ ^{\circ}C$, the effects of incubation times were investigated on the specimens treated with this concentration.

The effect of different incubation times on calcium carbonate precipitation at various locations (top, middle and bottom) and UCS (Figure 4.4) indicate that an increase of incubation time was accompanied by an increase of deposited calcium carbonate at top and middle of the column. While small differences in the average calcium carbonate precipitation occurred between samples treated under 12 hr and 24 hr incubation time, a substantial increase in deposited calcium carbonate was observed with the samples treated ≥ 48 hr incubation time. It is worth noting that the calcium carbonate efficiency increased with the increase of the incubation time and was found to be in range of 46-67%. This also supports the earlier observation indicating that bacteria required more time to digest highly concentrated cementation solution to induce calcium carbonate precipitation. It is also worth mentioning that the incubation time was not the only factor to lead to an increase in the ureolysis process; addition of

bacterial cells in every two treatments as described earlier contribute to the enhancement of the precipitation rate.
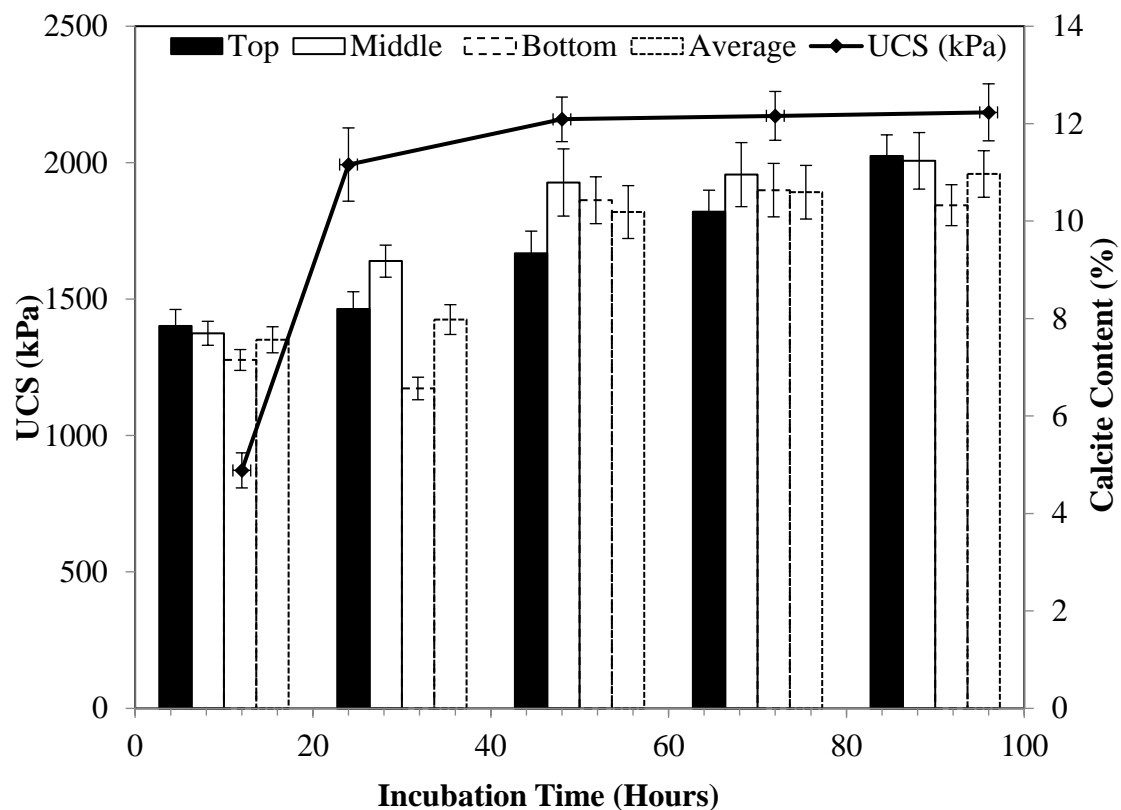
Fig 4.4. UCS and Calcium carbonate content for various incubation times (note: All bar columns represent calcium carbonate content at the same incubation time of 12 , 24, 48, 72 and 96 hrs, respectively).

It can also be seen that an increase of incubation times resulted in an increase of the UCS. The UCS of the samples subjected to 24 hr incubation time was almost 2.5 times of the samples subjected to 12 hr incubation time. This may be related to the urease-producing bacteria, which were given enough time to catalyse the hydrolysis of high urea concentration. The current observations are consistent with previous studies (Cheng and Cord-Ruwisch 2012, Zhao et al. 2014). It can also be observed from Figure 4.4 that the average amount of precipitated calcium carbonate for sand columns subjected to 12 and 24 hr incubation time is almost the same because some of the calcium salts present in the specimen subjected to 12 hr incubation time might not have been used (data not shown) in the production of

calcium carbonate. The calcium salts may still be residing in the pore space of the sand resulting in probably not a very accurate estimate of calcium carbonate content. This shortcoming is also one of the disadvantages of the acid washing technique used to quantify the amount of deposited calcium carbonate. However, UCS data confirms that 24 hr incubation time was more efficient than the 12 hr incubation time. It is also interesting to note that although a significant increase in the amount of deposited calcium carbonate (about 25%) occurred in the specimen treated for 48 hrs compared to the specimen treated for 24 hr it only resulted in a gain of 170 kPa in UCS. The above observations are all pointing to an incubation time of 24 hr being the target time to aim for to provide an optimum calcium carbonate precipitation and gain in UCS. Increasing the incubation time beyond 48 hrs only marginally increased the UCS. A maximum UCS of 2200 kPa was achieved after 96 hrs incubation, compared to an UCS of 2160 kPa reached after 48 hrs incubation. This is probably because there was a little difference in precipitated calcium carbonate between the two conditions.

4.3.3 Effect of temperature on the bio-cemented coarse sand

The variation of UCS and the amount of calcium carbonate precipitated at three positions (top, middle and bottom) of the bio-cemented coarse sand column with various treatment temperatures is shown in Figure 4.5. It can be observed that an increase in temperature increased the average bio-cementation of the coarse sand columns, probably because increased temperatures increased the activity of urease. The lowest average calcium carbonate precipitation was observed at 0°C, whereas the maximum amount of deposited calcium carbonate was achieved at 40°C. This implies that urease-producing bacterial cells might be influenced by the change in temperature, which is in agreement with the work of Ng et al. (2012) who indicated that microbial activity (urea hydrolysis) is less sensitive to variations in temperature within the range of 20-30°C, and that high urease activity tend to occur above 30°C. Also, the urease activity can proportionally increase with an increase in temperature in the case of urease-producing

bacteria (Whiffin 2004). This is also consistent with the current study as the highest calcium carbonate was achieved at temperature of 40°C. Also, it is worth noting that the efficiency of calcium carbonate precipitation increased with increasing the incubation temperature, ranging from about 36% at 0 °C temperature to about 65% at 40°C. The increase of calcium carbonate precipitation efficiency is further evidence of accelerated precipitation, which implies that the urease activity was increased with increased temperature. The UCS exhibited an increase from ≈50 kPa to ≈700 kPa, a 1300% improvement, when the temperature increased from 0°C to 10°C. The specimens treated at 20°C resulted in further increase of the UCS, 3 times greater than the UCS of the specimens treated at 10°C. However, at a higher temperature (40°C) the UCS substantially decreased indicating that the optimum temperature needed for a maximum strength improvement is near 20°C. This drop in UCS may be related to less stable calcium carbonate precipitation at elevated temperatures, such as vaterite formation (Weiss et al. 2014). The results presented herein support the previous observations made by Cheng et al. (2017) who reported that precipitated calcium carbonate crystals formed at 40°C were least efficient in obtaining strength enhancement. The microstructure investigation performed in the current study reveals that the surface of the coarse sand grains treated under the highest temperature (40°C) is coated with a thin layer of calcium carbonate precipitation (Figure 4.6a-b). It has been previously stated by Cheng et al. (2017) and reiterated herein that the calcium carbonate formed at a high temperature is well distributed over the surface of the particles with significant gaps between the particles, leading to an ineffective precipitation. This associated shortcoming could explain why there was a drop in the UCS of the sample treated under the highest temperature (40°C). Interestingly, the microstructure analysis of the specimen treated at the lowest temperature (0°C) (Figure 4.6c), shows a different shape of the deposited calcium carbonate crystals which consist of small spherical masses, scattered on the surface of the coarse sand grains. Even though, the EDS analysis confirmed that the by-product of the ureolysis

was calcium carbonate as shown in Figure 4.6d, the precipitated calcium carbonate was apparently weak to resist the compressive load resulting in a UCS value of about 44 kPa. This significant reduction in the UCS value may be related to a so-called pseudo precipitation of calcium carbonate (thin layer of calcium carbonate covering a mass layer but still not effective although its amount was 5.8%). The protocol adopted herein was based upon percolating the bacterial suspension continuously as indicated earlier, which could end up in bacteria accumulating in a spherical shape as they tend to form colonies when facing a harsh environment such as encountered at very low temperatures (0°C). Hence, the incubation temperature can also result in different amount and shape of calcium carbonate precipitation which can contribute significantly to the change in compressive strength.
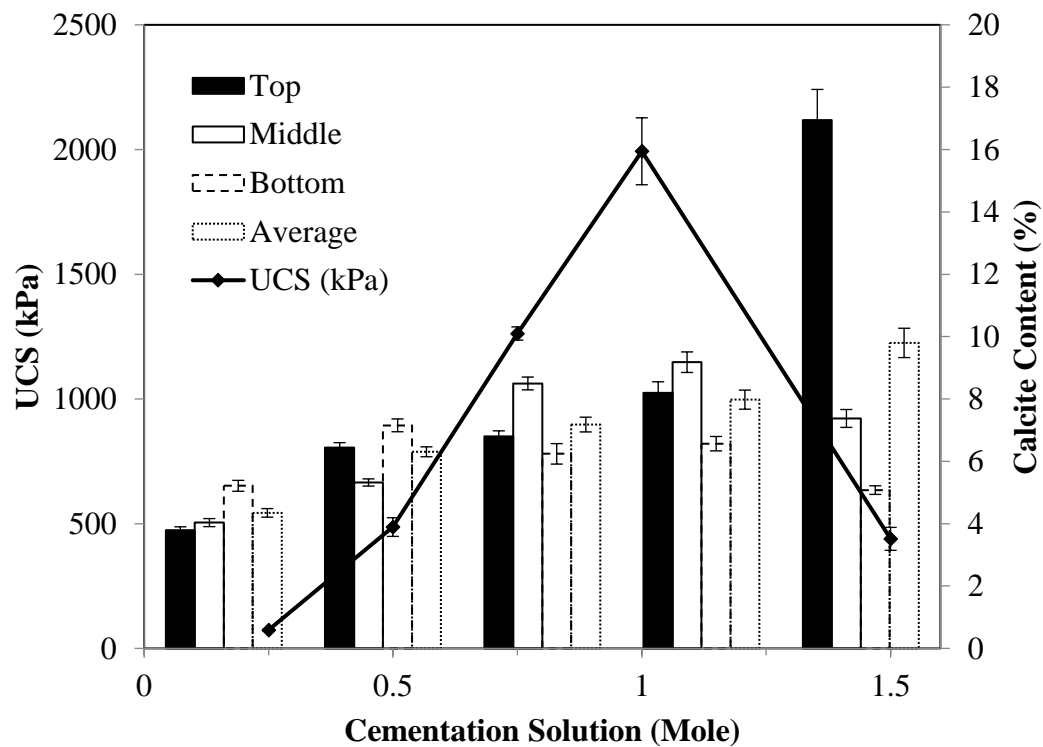
Fig 4.5.UCS and calcium carbonate content percentages of samples treated with different temperatures (note: All bar columns represent calcium carbonate content at the same incubation temperature of 0 , 10, 20 and 40 $^{\circ}$C, respectively).
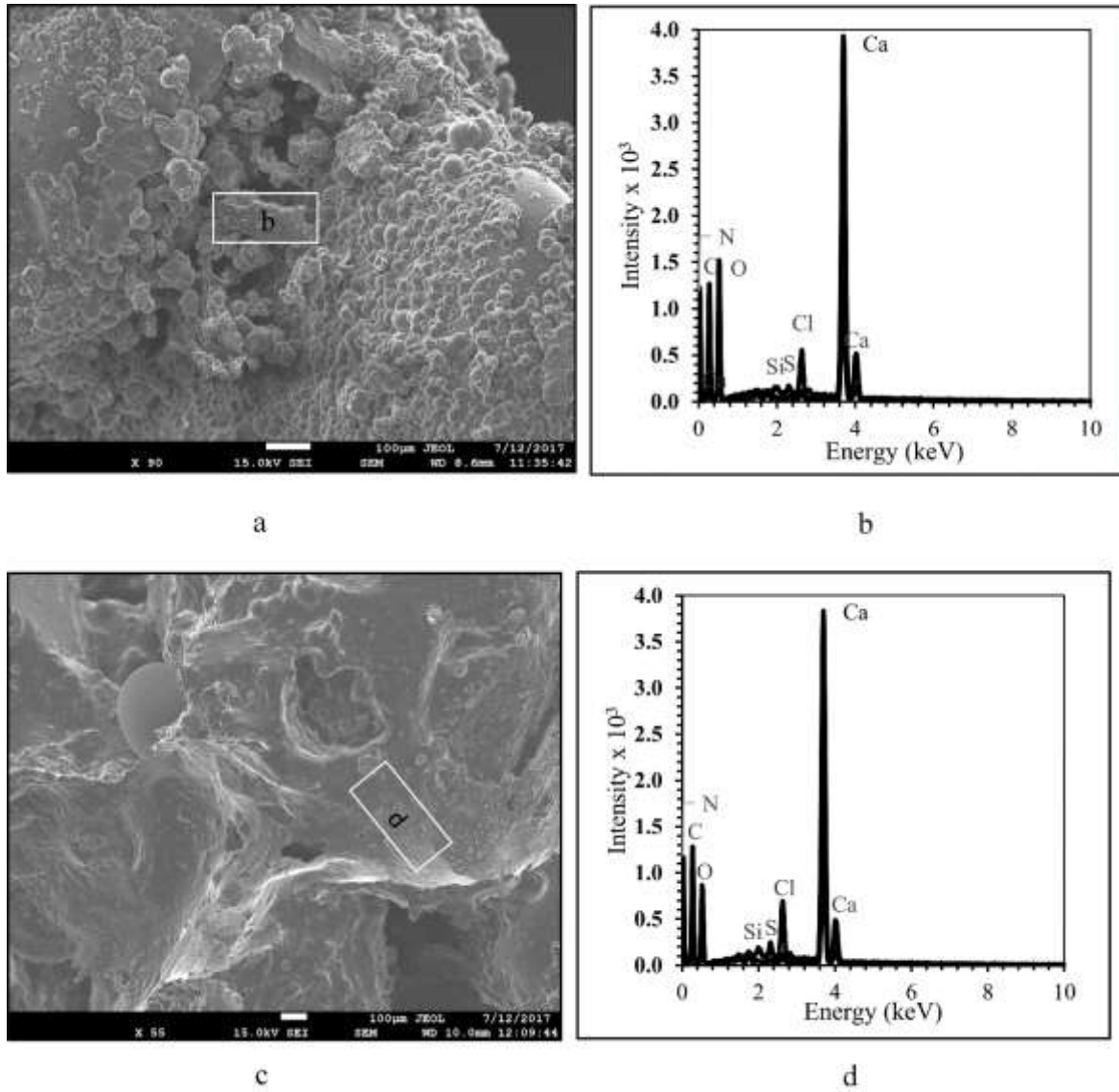
Figure 4.6. SEM images of bio-cemented coarse sand samples treated by a 1.0 M cementation solution concentration under high and low temperatures : (a) 0°C, UCS=44.32 kPa and $CaCO_3$ content =5.84%; (b) EDS of the sample treated at 0°C; (c) 40°C, UCS=1373.93 kPa and $CaCO_3$ content =10.60%; (d) EDS of the sample treated at 40°C.

4.4 Conclusions

MICP technique for treating coarser materials for possible application in geotechnical engineering was investigated. The chemical composition concentrations and environmental conditions, such as temperature and chemical reagent incubation time are important factors that need to be optimised before scaling up the MICP method for field applications. In this respect, the salient conclusions that can be drawn from this work are:

- An increase of equimolar cementation solution was associated with increased calcium carbonate precipitation and an increase in the UCS. The maximum strength (around 2 MPa) was achieved at 1 M equimolar cementation solution concentration.
- Utilizing low concentrations of non-equimolar cementation solutions resulted in greater uniformity of calcium carbonate distribution in coarse sand columns than for similar concentrations of equimolar cementation solutions.
- The content of precipitated calcium carbonate increased with an increase in the reaction temperature of MICP treated coarse sand, but the efficiency of the deposited calcium carbonate was found to be high with a moderate reaction temperature of 20°C as it resulted in a high compressive strength. However, a reasonable improvement in the UCS of coarse sand can also be obtained at a low reaction temperature (0°C).
- An increase of incubation time leads to an increase deposited calcium carbonate and UCS. This study also shows that a 48 hrs incubation time has been identified as the optimum time that can result in a high UCS. In addition, it was found that the amount of calcium carbonate precipitation reached the highest level when cementation solution concentration, incubation time and temperature were very high, i.e. 1.5 M, 96 hrs and 40°C, respectively. However, the UCS was adversely affected by high temperature and cementation solution concentration.

- SEM and EDS analysis have confirmed that having large agglomerated calcium carbonate crystals filling the gaps between coarse sand grains lead to improvement of the compressive strength.

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Chapter 5: Improvement of coarse sand engineering properties by microbially induced calcite precipitation

Improvement of coarse sand engineering properties by microbially induced calcite precipitation

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Abstract

This paper investigates the effectiveness of microbially induced calcite precipitation (MICP) method in improving the strength and stiffness of coarse sands using treatments based on a four-phase percolation technique. An increase of biochemical treatment cycles was associated with increased deposition of calcium carbonate ($CaCO_3$) and consequently an increase in compressive strength. Furthermore, the bio-cemented coarse sand retained reasonable porosity and permeability, which should allow dissipation of pore water pressure if required. The results also establish a correlation between the strength gained and stiffness of the bio-cemented coarse sand with the increase in the amount of deposited $CaCO_3$, initial relative density and dry density. Scanning electron microscopy (SEM) and electron dispersive spectroscopy (EDS) analysis indicate that the inter-structure of the bio-cemented coarse sand tends to change in morphology based upon the number of biochemical treatments used.

5.1 Introduction

Recently, biologically mediated processes have been used to improve soil properties to minimise liquefaction, erosion, and improve hydraulic and strength properties of granular soils (DeJong et al. 2010). In particular, microbially induced calcite precipitation (MICP) by urea hydrolysis has shown to be a viable ground improvement alternative (Stocks-Fischer et al. 1999, DeJong et al. 2006, 2010, Whiffin et al. 2007, Van Paassen, 2011, see also, Bang et al. 2009, DeJong et al. 2010, Chou et al. 2011, Mortensen and DeJong 2011, Yasuhara H et al. 2011, Chu et al. 2012, Montoya 2012, Rong et al. 2012, Al Qabany and Soga 2013, Cheng et al. 2013, Soon et al. 2013, O'Donnell and Kavazanjian 2015, Mahawish et al. 2016, 2017, 2018).

Ureolysis is one of the methods used for MICP treatment of soils. It is driven by urease producing bacteria, which catalyses the hydrolysis of one mole of urea to create one mole of ammonia and one mole of carbamic acid (Eq.1). The carbamate spontaneously decomposes to carbonic acid and another mole of ammonia (Eq.5.2) and further hydrolysis of ammonia produces ammonium (NH_4^+) and hydroxide ions (Eq. 5.3). The latter process will increase pH and promote the generation of bicarbonate (HCO_3^-) (Eq 5.4) and carbonate (CO_3^-) ions (Eq. 5.5) (Stocks-Fischer et al. 1999, Dick et al. 2006, Phillips et al. 2013).

$$CO\,(NH_2)_2 + H_2O \xrightarrow{\text{Urease Enzyme}} NH_2COOH + NH_3 \tag{5.1}$$

$$NH_2COOH + H_2O \longrightarrow NH_3 + H_2CO_3 \tag{5.2}$$

$$2NH_3 + 2H_2O \longrightarrow 2NH_4^+ + 2OH^- \tag{5.3}$$

$$H_2CO_3 \longrightarrow HCO_3^- + H^+ \tag{5.4}$$

$$HCO_3^- + H^+ + 2OH^- \longrightarrow CO_3^{2-} + 2H_2O \tag{5.5}$$
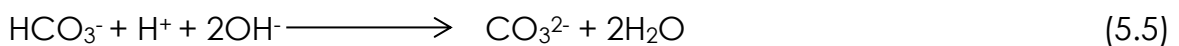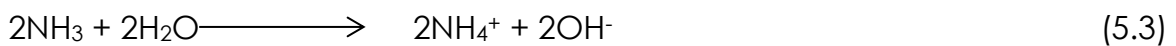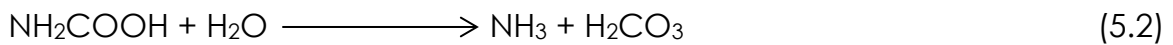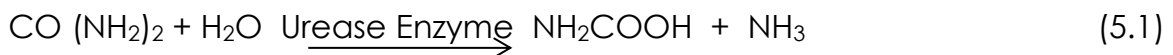
Precipitation of calcite ($CaCO_3$) will occur under saturation conditions of both dissolved carbonate and calcium ions. Since carbonate is often overproduced from metabolic activity in MICP, usually insufficient

concentrations of dissolved calcium ions exist, and thus, supplemental Ca^{2+} is needed to complete the precipitation of $CaCO_3$ (Eq. 5.6).

$$Ca^{2+} + CO_3^{2-} \longrightarrow CaCO_3 \text{ (Solid)} \qquad (5.6)$$

Another by-product of ureolysis is ammonium (as shown in Eq. 5.3), which is regarded as a contaminant. Therefore, attempts have been made to eliminate the detrimental effect of this by-product by converting it into struvite, which could also contribute to strength gains (Qian et al. 2015, 2016). Bio-cementation is mostly applied to fine to medium sand (Nemati and Voordouw 2003, Whiffin et al. 2007, Al-Thawadi 2008, DeJong et al. 2010, Phillips et al. 2013) with limited field applications (Van Paassen 2009, Van der Star et al. 2011). However, very limited studies have considered the use of the MICP technique to improve the engineering properties of coarse granular soils (Shahrokhi-Shahraki et al. 2015, Mahawish et al. 2016, 2017, 2018). Coarse cohesionless soils are classified into two groups including gravel and sand. These two groups are further divided into coarse cohesionless soils (particle size 20-60 mm for gravel, and 0.6-2.0 mm for sand), medium cohesionless soils (particle size 6-20 mm for gravel, and 0.2-0.6 mm for sand), and fine cohesionless soils (particle size 2-6 mm for gravel, and 0.06-0.2 mm for sand).

The objective of this paper is to present the outcome of a recent investigation using a bio-cementation process based on MICP to increase the stiffness and strength of very coarse sand with the global view of developing rigid coarse sand columns suitable for improvement of soft and weak soils. For this purpose, an effective biochemical treatment technique was developed to treat coarse materials. Furthermore, a correlation between the number of treatments and calcium carbonate precipitation with strength parameters was identified through an optimisation of the MICP-treated coarse material process.

5.2 Materials and Methods

Stone columns are usually composed of angular and sub-angular granular materials, such as crushed rock, having an average particle size ranging between 25 mm and 75 mm. A typical stone column diameter ranges from 0.40 m to 1.20 m, and this leads to a standard column diameter to average particle size ratio ranging between 5 to 50. The model sand columns used in the current investigation to mimic stone columns were comprised of coarse sand with an average grain size of 1.6 mm. The sand column diameter to the particle size ratio was about 30, which is within the practical range reported above. The sand particles were typically sub-angular in shape and were deemed to be representative of the stone aggregate used for full-scale stone columns (Gniel and Bouazza 2009, 2010).

A commercially available sand (Grade 8/16, supplied by Unimin Australia Pty. Ltd.) was used in the current investigation. It is classified as poorly graded coarse sand (SP) according to the Unified Soil Classification System (ASTM 2006). Its particle size distribution is presented in Figure 5.1. Maximum and minimum dry density measurements were conducted in accordance with ASTM D4253 (2014a) and ASTM D4254 (2014b) to control the density of the column model and were respectively 1.70 g/cm^3 and 1.44 g/cm^3. Sand characteristics such as specific gravity (G_s), coefficient of curvature (C_c), coefficient of uniformity (C_U), median grain size (D_{50}), maximum and minimum void ratios (e_{max} and e_{min}) are presented in Table 5.1.
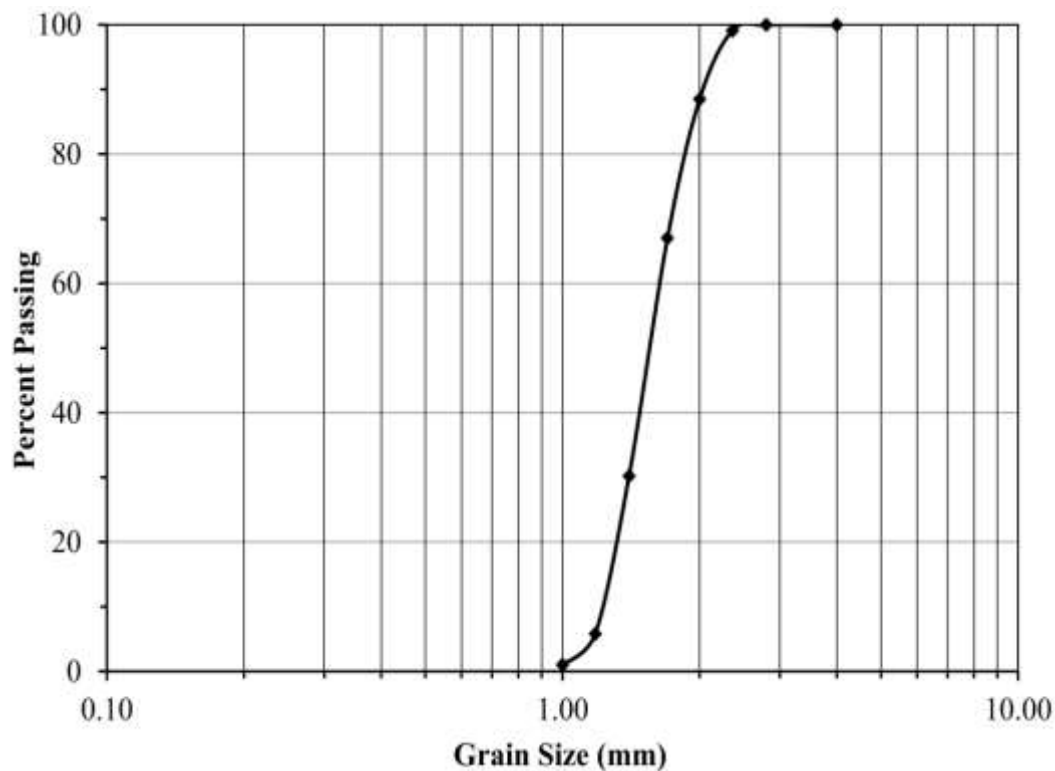
Figure 5.1. Sand particle size distribution

Table 5.1: Sand characteristics

Soil Type	Classification	G_s	C_c	C_u	D_{50} (mm)	e_{max}	e_{min}
Sand Grade 8/16	SP	2.64	0.97	1.35	1.60	0.84	0.55

5.2.1 *Specimen Preparation*

A specimen mould was made of a split poly vinyl chloride (PVC) pipe, 220 mm high and with an internal diameter of 51 mm when assembled as shown in Figure 5.2. A wire mesh and filter paper were placed at the bottom of the mould to reduce potential losses of sand grains during preparation and treatment. A sample of dry coarse sand was initially air pluviated via a single nozzle without allowing any free fall. Additionally, a vibratory compaction method was used to further compact the sample to a 60% target relative

density unless otherwise stated. A similar preparation methodology was used to prepare the columns used to investigate the effect of relative density on the strength and stiffness of bio-treated coarse sand. The additional target relative densities were 40%, 80% and 100%.
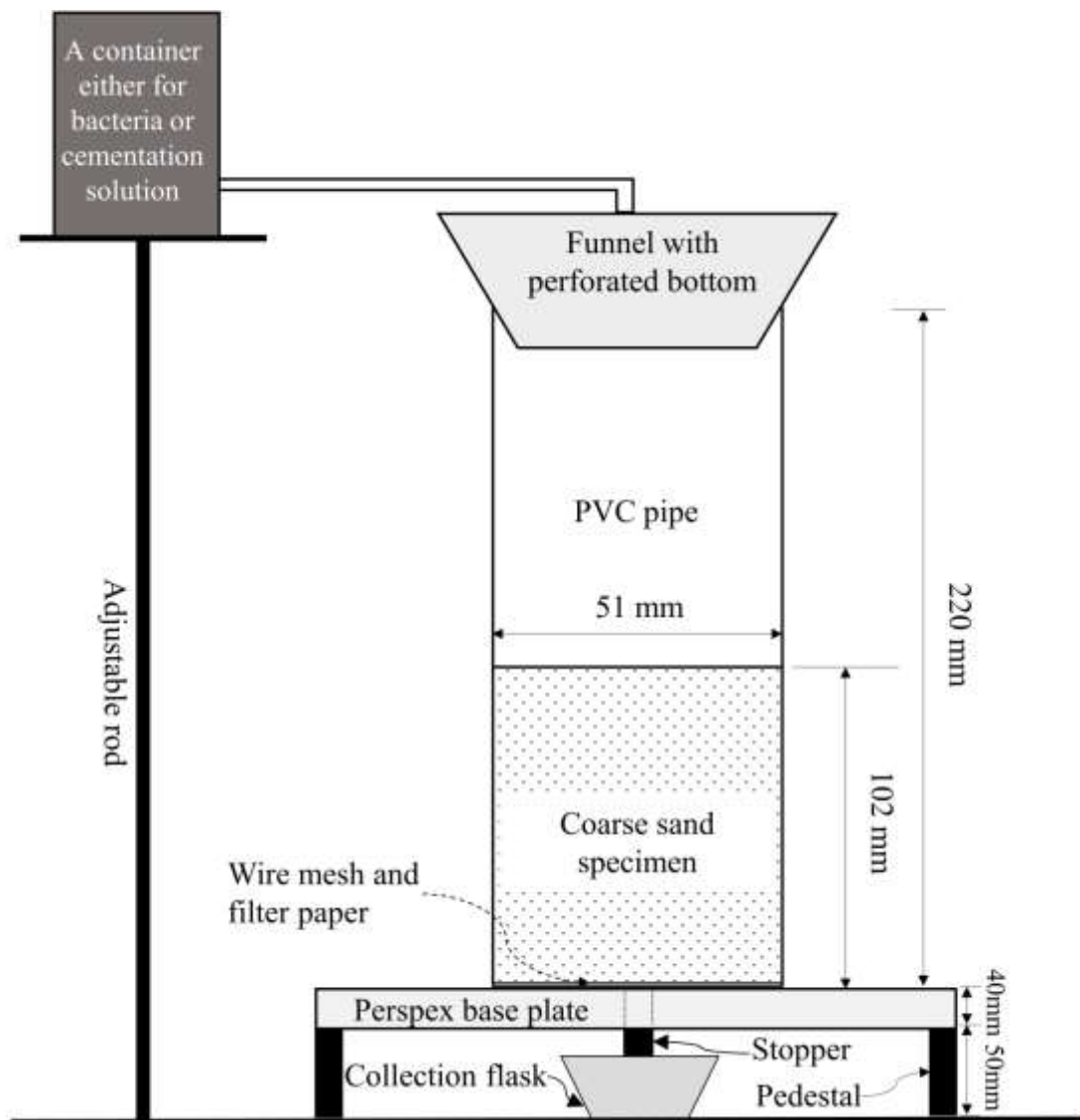
Figure 5.2. Schematic of the experimental set-up for coarse sand column.

5.2.2 Preparation of the bacterial suspension and cementation solution

The urease-producing bacterium used was *Sporosarcina pasteurii* (supplied by ATCC® 11859). One colony from the stock culture (Petri dishes containing bacterial colonies, 20 g/L yeast extract (Scharlau), 10 g/L ammonium

sulphate AR and 20 g/L agar (BP Scharlau) in 130 mM Tris buffer AR (pH 9.0) (the growth medium recipes were supplied by Labtek) was transferred to 5 mL (in a 24 ml vial) of ammonium-YE growth medium (20 g yeast extract, 10 g $(NH_4)_2SO_4$ and 130 mM tris buffer (pH=9.0) per litre of distilled water) to grow the bacterial cells at 30°C (Stocks-Fischer et al. 1999). Individual ingredients of the recipe were separately dissolved in distilled water bottles and autoclaved separately and then combined post sterilisation. Suspended bacterial cultures were grown at 30°C in a shaking water bath (200rpm) for 24 hrs before harvesting at a final optical density (OD_{600}) of 3.0-3.5 to achieve an optimum concentration of bacterial cells in which all readily available nutrients were considered to be consumed from the growth medium (i.e., the start of the stationary growth phase) (Cheng and Cord-Ruwisch 2012). The final optical density was determined using a WPA CO 8000 spectrophotometer (BioChrom Ltd) taking into account the ten times dilution factor. The concentration of bacterial suspension (cells, per ml) used was estimated to be about 3.8×10^8 - 4.7×10^8 cells/ml based on the equation 5.7 (Ramachandran et al. 2001, Okwadha and Li 2010, Shahrokhi-Shahraki et al. 2015). The referenced calibration curves for OD_{600} were used and hence the estimated bacterial cell concentrations herein are relative cell concentration, but not actual bacterial cell concentrations.

Concentration of cells per mL = $8.59 \times 10^7 \times OD_{600}{}^{1.3627}$ (5.7)

Urease activity was determined by a conductivity assay as described by Whiffin et al (2007), where a 20 mL Falcon tube containing 9 mL of urea solution with a concentration of 1.11 M was inoculated with 1 mL of bacterial suspension. Then, LabChem-CP instrument was used to record the relative electrical conductivity change over a period of 5 minutes at 20±2°C. Afterwards, the urease activty was calculated using equation (5.8) (Whiffin 2004) taking into account the dilution factor of 10 times.

Urea hydrolysed (mM) = Conductivity (mS) x 11.11   (R^2 = 0.9988) (5.8)

An activity value of 19.38-21.45 mM urea/min/mL for urease enzyme activity was used in this study based on the maximum optical density of the harvested bacterial cells. The main reason for collecting bacterial cells at the beginning of the stationary phase was to minimise the further growth of bacterial cells in the granular columns during retention time (incubation time) from occurring. The preliminary culture medium was transferred aseptically to inoculate 250 ml (in a bottle reagent boro, 1 L) of the Ammonium-YE medium, and the previous process can hence be repeated accordingly. The bacterial suspension was stored up to 14 days at 4ºC, until used in the experimental work. The cementation solution consisted of 1 M urea grade AR, $CO(NH_2)_2$ and 1 M calcium chloride dihydrate AR, $CaCl_2$ and both reagents were supplied by Labtek.

5.2.3 Methodology of fixation and distribution of bacteria in the soil

Bacterial cells themselves, whether live or dead, can function as nucleation sites for $CaCO_3$ crystals (Schultze-Lam et al. 1996) by attracting divalent cations to the wall of the bacterial cells (Greenfield 1963, Stocks-Fischer et al. 1999, Van Lith et al. 2003, Aloisi et al. 2006). Bacterial cells may flush from a soil matrix if an improper injection method is used. An effective method is required to induce an adequate $CaCO_3$ precipitation and to obtain a uniform distribution of $CaCO_3$ crystals within a granular soil matrix. Different bacterial fixation methodologies and their effectiveness in MICP-treated granular soils have been reported by several researchers (Le Metayer-Levrel et al. 1999, Whiffin et al. 2007, Harkes et al. 2010, Cheng and Cord-Ruwisch 2012, Tobler et al. 2012, Al Qabany and Soga 2013, Shahrokhi-Shahraki et al. 2015). One technique that attempted to stabilise coarse granular soils was to a mix bacterial suspension with cementation solution before they were injected into the soil matrix (Le Metayer-Levrel et al. 1999). However, this protocol caused the inhomogeneous formation of small clumps or masses of $CaCO_3$ throughout the matrix. Another approach was based on a two-phase injection strategy, where bacterial cells suspension were injected first, followed by injection of 50 mM calcium chloride solution (Whiffin et al. 2007).

This protocol seemed to have had a positive effect on the bacterial cell fixation. A maximum strength of 570 kPa in a 5 m column was obtained at approximately 1 m from the treatment point and was not associated with clogging close to the injection points. The main drawback associated with this protocol was that it necessitated the use of 0.5 M calcium concentrations, much of which was flushed after the bacterial cells fixation process. This process can compromise the application of the MICP method when it is scaled up to the field as using too much of calcium without inducing calcium carbonate precipitation can have a significant effect on the cost of the MICP process. Other strategies to note were staged injection of bacterial suspension followed by cementation solution, with or without retention time for bacterial suspension and cementation solution to intermix (Harkes et al. 2010). Retention time is necessary to facilitate a number of reactions occurring between microorganisms and reagents solutions (Al Qabany et al. 2011, Rong et al. 2012).This approach led to more homogeneous distribution of $CaCO_3$ crystals within the specimen and improved immobilisation of bacterial cells within sand grains (Tobler et al. 2012). As a modification for the protocol above (staged injection), bacterial suspension and cementation solution were sequentially percolated using multiple thinner layers (lesser volume) of the bacterial suspension and cementation solution followed by incubation time (retention time) (Cheng and Cord-Ruwisch 2012). This resulted in immobilisation of bacteria within long columns with a UCS value as high as 390 kPa. The effect of using multi-soil lifts was studied as another method of adopting MICP method where it is impossible to inject biochemical treatments deeply in the ground (such as stone column applications). It was found that a single lift treatment could lead to a very high increase in strength and stiffness of coarse sand up to 8.9 MPa and 2.3 GPa, respectively (Mahawish et al. 2016). A final methodology to note is the single-stage injection where cementation solution is injected simultaneously with injection of bacterial suspension into the sand. This was considered to be an ineffective method due to rapid clogging of the injection points which restricted injection of more fluids (Shahrokhi-Shahraki

et al. 2015). Hence, based on the strategies above, the staged approach, where the suspension of bacteria is first injected, followed by injections of cementation solutions was the strategy adopted for MICP applied to very coarse sand. However, its adaptation for use as a sand/stone column inserted within a soft clay bed requires a special attention to obtain a uniform distribution within a coarse sand matrix with retention of high compressive strength. The treatment strategy proposed herein is further discussed and justified in the next section.

5.2.4 Soil treatment procedures

A percolation method was used for the treatment of the very coarse sand specimens as it allowed a gravity-driven infiltration process. The percolation process is based on the methodology reported in Mahawish et al (2016, 2018). In summary, a percolator was fabricated by making uniform holes (1 mm diameter) in a small bottle with a diameter slightly less than the 50 mm diameter mould, allowing it to rest on the top edge of the mould. The percolator was assumed to simulate a system of irrigation tubing. External tubes containing bacterial suspension and treatment cementation solution (Urea and Calcium chloride) were fixed above the percolator to keep a constant percolation rate of the liquid into the sand of about 1 litre/hour (see Figure 5.2).

It is postulated herein that coarse materials tend to have larger pores throat diameters, which need a high amount of $CaCO_3$ to be precipitated at the interface of inter and intra-particle pores. Sufficiently high amount of precipitated $CaCO_3$ cannot be achieved by using a single flush of bacterial suspension as suggested in previous studies (Le Metayer-Levrel et al. 1999, Whiffin et al. 2007, Harkes et al. 2010, Cheng and Cord-Ruwisch 2012, Tobler et al. 2012, Al Qabany and Soga 2013, Shahrokhi-Shahraki et al. 2015). Also, a large number and volume of biochemical treatment cycles may be needed to reach the required cementation level. Thus, higher costs associated with the treatment of coarser materials can be expected which

might detract from the use of MICP in soil stabilisation applications. Hence, it is necessary to establish new protocols to use MICP in coarser materials. The protocol that has been developed throughout this study is a four-phase percolation protocol.

The strategy consists of the following steps;

vii. Percolation of tap water to expel air from the specimens (200% pore volume ((PV) determined by multiplying the total volume of the specimen with its porosity at 60% relative density unless otherwise stated) of sand columns (160 ml)). This step was carried out by closing the bottom valve initially, then percolating deionised water until ponding occurred on top of the specimen, after that the bottom valve was opened, the percolation of water was continued until reaching 2PV.

viii. Flushing of cementation solution (100% PV of sand columns (80 ml)). This process was performed to promote high ionic strength within the pore matrix as the cementation solution contained divalent cations (i.e. calcium ions), which increases the ionic charges. Urea plays a significant role in providing a suitable environment for the bacterial cells as they have a high affinity to urea. This step was applied once before the beginning of the treatment cycles.

ix. After the cementation solution volume has been drained out from each specimen, the bottom valve was closed and percolation of lesser volume of bacterial suspension (BS) and cementation solution (CS) without ammonium-YE media were alternated to achieve a four-phase percolation protocol (10 ml BS + 10 ml CS + 10 ml BS + 10ml CS). The amount of liquids percolated is equivalent to 50% of the PV of the sand columns, (40 ml).

x. Incubation (retention time) for 24 hrs at 20±2°C was adopted to allow the percolated liquids to facilitate the occurrence of reactions between microorganisms and reagents solutions. When the incubation time ended, the bottom valve was opened to discharge

the liquids waste (effluent) and prepare the pore matrix for the next treatment.

xi. This treatment step included percolation of fresh cementation solution (40 ml) only and allowed ureolysis and $CaCO_3$ deposition to take place for the same retention time. The effluent was also discharged after finishing the incubation time for the purpose mentioned in the previous step.

Steps iii, iv and v were considered as two biochemical treatment cycles and were repeated until reaching the required number of treatment cycles (i.e. 4, 8, 12, 16, 20, 24, 28 and 32 cycles). For instance, 4-biochemical treatment cycles mean that steps iii, iv and v were repeated two times.

5.2.5 Unconfined Compressive Strength (UCS) Tests

After completion of biocementation treatments, all specimens were flushed with dionised water to remove any residual chemical reagents from the pore spaces. The specimens were then dried for 14-days at 20 ± 2°C to ensure that they all had the same initial test conditions after treatment cycles ceased. These specimens had a different amount of $CaCO_3$ deposition after having undergone a different number of treatments (0, 4, 8, 12, 16, 20, 24, 28, and 32 biochemical treatments). UCS tests were also conducted on samples that were treated by cementation solution only and samples that were prepared with different relative densities (40, 60, 80 and 100%). All the tests were performed on specimens having 51 mm diameter with a selected height to diameter ratio of 2:1. An Instron 4204 50 kN constant-displacement mode UCS machine equipped with a laser extensometer was used in the current study. The axial load was applied at a constant strain rate of 1%/min.

After completion of the UCS tests, $CaCO_3$ content was measured using gravimetric acid washing (2 M HCl) technique. For this purpose, soil samples were dried in an oven at a temperature of 105°C for 24 hrs and their masses were measured before and after the acid wash. The samples were washed

several times with HCl passing through sieve No.200 (standard test sieve, ASTM D1921) and allowing the dissolved salts to be rinsed out from the samples, and were again dried in an oven at a temperature of 105°C for 24 hrs. The difference between two masses was taken as the mass of $CaCO_3$, the percentage of $CaCO_3$ was obtained by dividing the mass of $CaCO_3$ by the mass of soil.

5.2.6 Permeability Tests

Permeability tests were carried out on the untreated coarse sand, treated sand with only cementation solution, and MICP-treated coarse sand specimens using a different number of biochemical treatment cycles (0, 4, 8, 12, 16, 20, 24, 28, 32, 36 and 40 biochemical treatment cycles). The laboratory permeability measurement was conducted in accordance with ASTM D2434 using a constant head permeability test. Separate perspex columns (32 mm inner diameter x 190 mm height) were used to perform the permeability tests. The specimens for permeability tests were prepared and bio-cemented in the same way as the UCS specimens. All specimens were also flushed with DI water after finishing the biochemical treatments to remove residual chemicals from the pores of coarse sand and then saturated before commencing the permeability test by percolating water through the coarse sand to de-air residual air in the pore matrix (using a hydraulic head of about 102 cm). The average of the last three measurements was reported as permeability value. All tests were conducted at a room temperature (20±2°C) and a hydraulic head ranging between 58 and 122 cm.

5.2.7 Microstructure analysis

Scanning electron microscopy (SEM) and energy dispersive spectroscopy (EDS) were undertaken to characterise the bonding achieved by a low and a high number of biochemical treatment cycles. For this purpose, subsamples of specimens treated by 4 and 32 treatments were retained after completing the UCS tests with the aim of conducting micro-scale

investigations. The fractions were oven dried, carbon coated, and then examined using an SEM (JEOL 7001F FEGSEM,) with a beam intensity ranging between 5 to 15 kV.

5.2.8 Porosity measurement

Subsamples from untreated and MICP treated coarse sand samples were taken from the UCS tested specimens to conduct porosity tests using the gravity method (Rong et al. 2012). The gravity method is a well-known technique that uses vacuum saturation and has the advantages to be fast and easy to apply. The subsamples were firstly dried in an oven at a temperature of 60°C. When the mass of the samples became constant, their dry mass (M_{dry}) was recorded using an analytical balance (down to 1/10000 of a gram). Next, the subsamples were saturated using a vacuumed desiccator filled with distilled water, the fully saturated condition was achieved when the mass of the subsamples (denoted as M_{sat}) was stable. The stability of the subsamples mass was assessed based upon measuring the mass of the samples at different times using the same balance with a variation not exceeding 0.1%. Finally, the volume of subsamples (V_s) was determined by immersing them in de-aired water using a basket and a hanger as well as an analytical balance (down to 1/10000 of a gram) based on the methodology described in Liu and Buzzi (2014). The porosity (n) was determined using the following equation (Rong et al. 2012).

$$n = (M_{sat} - M_{dry}) / (\rho_{water} \times V_s) \qquad (5.9)$$

5.3 Results and Analyses

5.3.1 *Effect of number of biochemical treatments on the mechanical properties of coarse sand*

A correlation between the number of biochemical treatment cycles and the UCS is presented in Figure 5.3a, where it can be seen that the UCS increased with the increase of the number of biochemical treatment cycles. This increase in UCS could be related to the introduction of more variables (i.e., bacteria, urea and calcium) that led to more calcium carbonate precipitation within the pore matrix of coarse sand. The maximum UCS of about 14 MPa was associated with a high number of biochemical treatments (32-treatments). However, the coarse sand samples that were treated by the cementation solution only showed no UCS improvements (data not shown) as it could not bear any applied load, not even its own weight. This implies that the bacterial cells played a significant role in the UCS improvements of the coarse sand. The most prominent component beside calcium in the cementation solution used herein is the urea content as it is plausible that the more number of treatment cycles was applied, the more amount of urea accumulatively participated in the precipitation of calcium carbonate. This is evidenced in Figure 5.3b where the urea content is directly correlated with the induced $CaCO_3$ precipitation in three regions (top, middle and bottom) of the bio-cemented coarse sand column. It can be seen from Figure 5.3b that as the accumulated amount of urea increased with a number of treatment cycles, the average $CaCO_3$ precipitation in the coarse sand matrix increased. Therefore, it can be inferred that the bio-cemented coarse sand specimens showed a substantial compressive strength increase with an increase in the amount of urea used in the cementation solution over a given number of biochemical treatment cycles. A urea content of 4.5% in the bio-cemented coarse sand produced a compressive strength (about 1 MPa), which was linked with the amount of calcium carbonate precipitation (about 6.7%, Figure 5.3b).
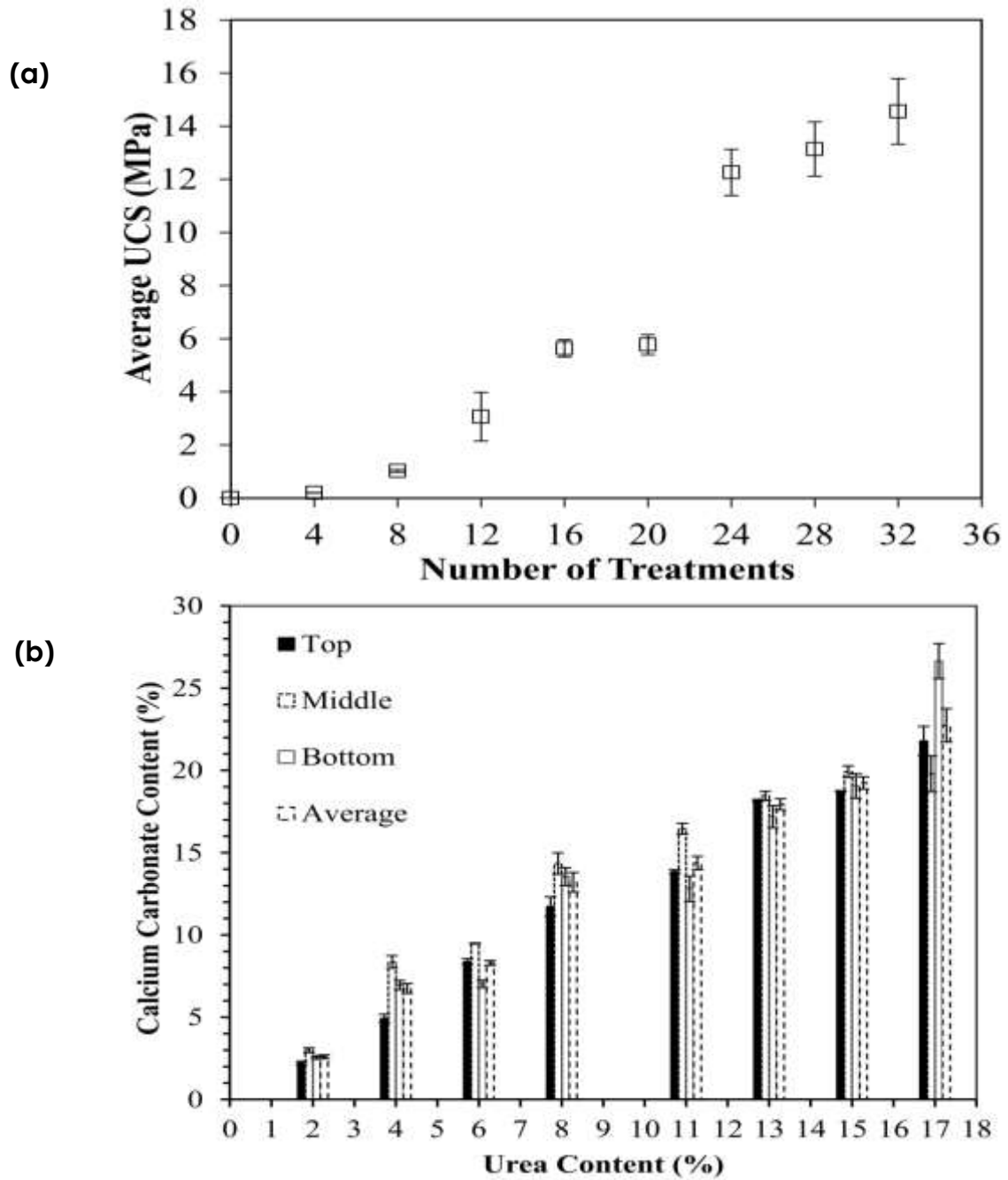
Figure 5.3. **(a)** Variation of number of treatment versus average UCS with a standard error; **(b)** Relationship of urea used with the precipitated calcium carbonate with a standard error. UCS: unconfined compressive strength.

Furthermore, a UCS of approximately 14 MPa was achieved when using 17% urea, which led to precipitation of roughly 22% of $CaCO_3$. It is also worth noting that the adopted protocol has proven to be successful in providing a higher amount of deposited $CaCO_3$ along with improving mechanical properties. The simple process used in the biochemical treatment cycles is

an advantage as a gravity driven drip system was developed. This means that there is an alternative to pumping technique as has been applied in other studies (Whiffin et al. 2007, van Paassen et al. 2010, Al Qabany and Soga 2013, Lee et al. 2013, Montoya and DeJong 2015). Additional bacterial suspension cycles based on the adopted protocol herein may therefore be considered as a critical factor leading to more calcium carbonate precipitate. Urease-producing bacteria can keep high urease activity to breakdown urea to carbonate into the pore matrix of the coarse sand (Okwadha and Li 2010) and can also work as nucleation sites for $CaCO_3$ crystals (Hammes and Verstraete 2002), leading to increased bio-calcification masses. Thus, a significant amount of carbon dioxide (CO_2) was sequestered, probably as part of the $CaCO_3$, as shown from the amount urea used. This is because one of the sources of CO_2 is the hydrolysis of urea and respiration of bacteria. Hence, urea as one of the cementation solution components can be involved in CO_2 sequestration (Okyay and Rodrigues 2015).

It has been postulated by DeJong et al. (2010), and reiterated herein, that the critical aspect in $CaCO_3$ precipitation is the distribution of $CaCO_3$ crystals within the soil pores. Point to point inter-particle contact of $CaCO_3$ precipitates is necessary to achieve strength and stiffness increase (Al-Thawadi 2008, DeJong et al. 2010). While the inter-particle contacts were not evaluated in the current investigation, it can be argued that having sufficient $CaCO_3$ precipitation might lead to a higher chance for having inter-particle contacts because $CaCO_3$ randomly precipitates within the inter-particle pores around the coarse sand particles (Figure 5.4). Figure 5.4a shows $CaCO_3$ precipitated on and between the coarse sand particles treated by 32-biochemical treatment cycles, fully supporting the argument that $CaCO_3$ provides more bridging contacts between coarse grains (Figure 5.4b). It is also interesting to note that large trigonal-rhombohedral $CaCO_3$ crystals precipitated in the coarse sand matrix (Figure 5.4b), and the chemical compositions of these crystals were also confirmed by EDS analysis

(Figure 5.4c). Figure 5.4c indicates that the chemical constituents of the precipitated crystals are mainly composed of calcium, carbon, and oxygen, and this validates the fact that the crystals are $CaCO_3$ polymorphs. The identified $CaCO_3$ crystals form an irregular morphology (about 100 microns thick), which could be created by the approximately trigonal to the rhombohedral superposition of thin layer flakes of $CaCO_3$. The deposited calcium carbonate at a higher number of biochemical treatments (32-treatment cycles) was considered as calcite based upon its morphology (Li et al. 2011). However, spherical-shape calcium carbonate crystals deposited in the coarse sand specimens resulted from using 4-biochemical treatment cycles and was observed microscopically (Figure 5.5a). Also, Van Paassen (2009) reported that spherical-shape crystals, resulting from MICP, were identified as vaterite based on X-ray diffraction (XRD) analysis. Thus, different crystal morphologies can be deposited depending on the number of biochemical treatment cycles used. EDS was also used to verify the components of the bio-cement material induced from using 4-biochemical treatment cycles and is presented in Figure 5.5b, where the chemical compositions imply the existence of calcium carbonate precipitation. The involvement of bacterial cells in the precipitation of more calcium carbonate was also observed and is shown in Figure 5.5c.

(a)

(b)

(c)
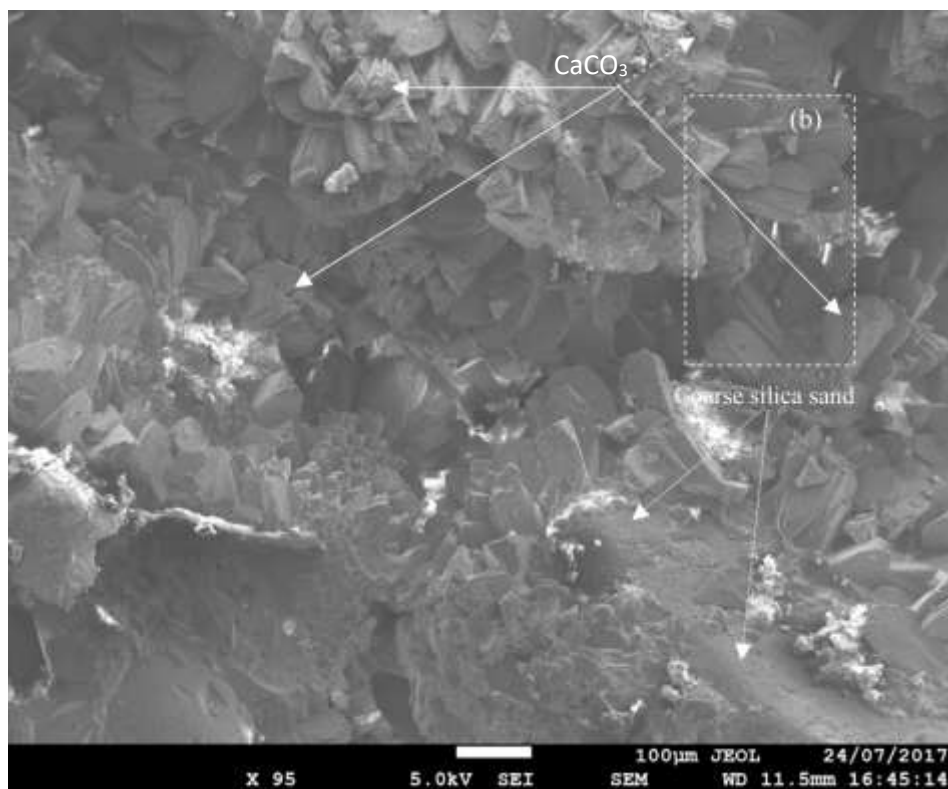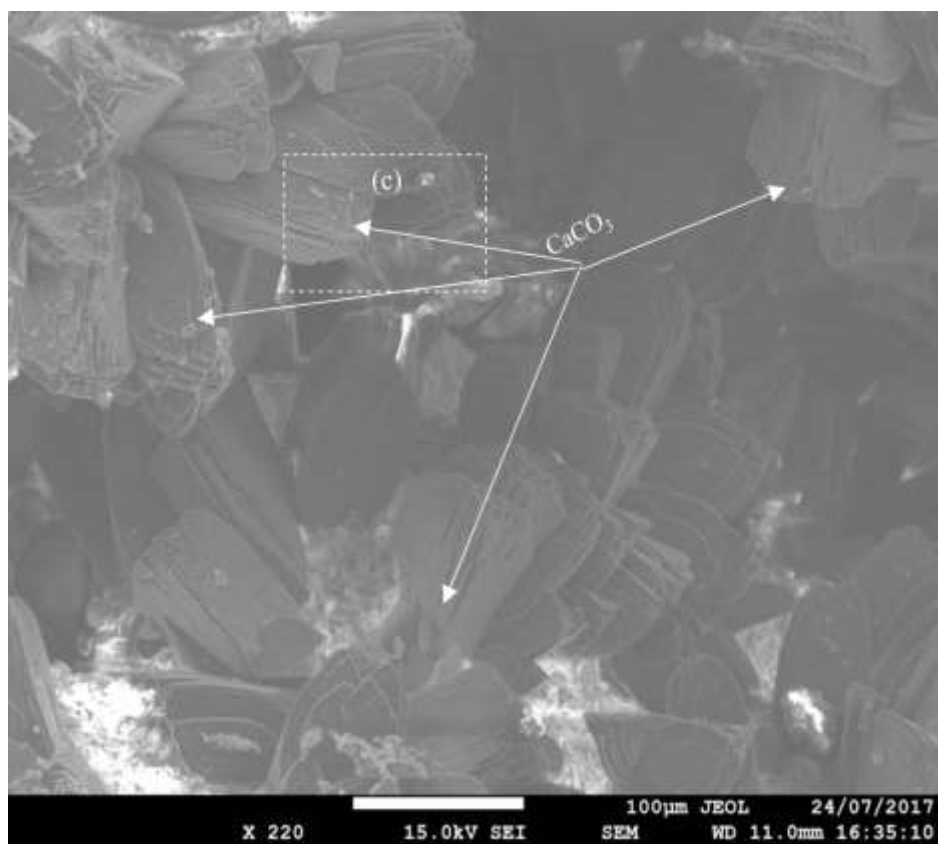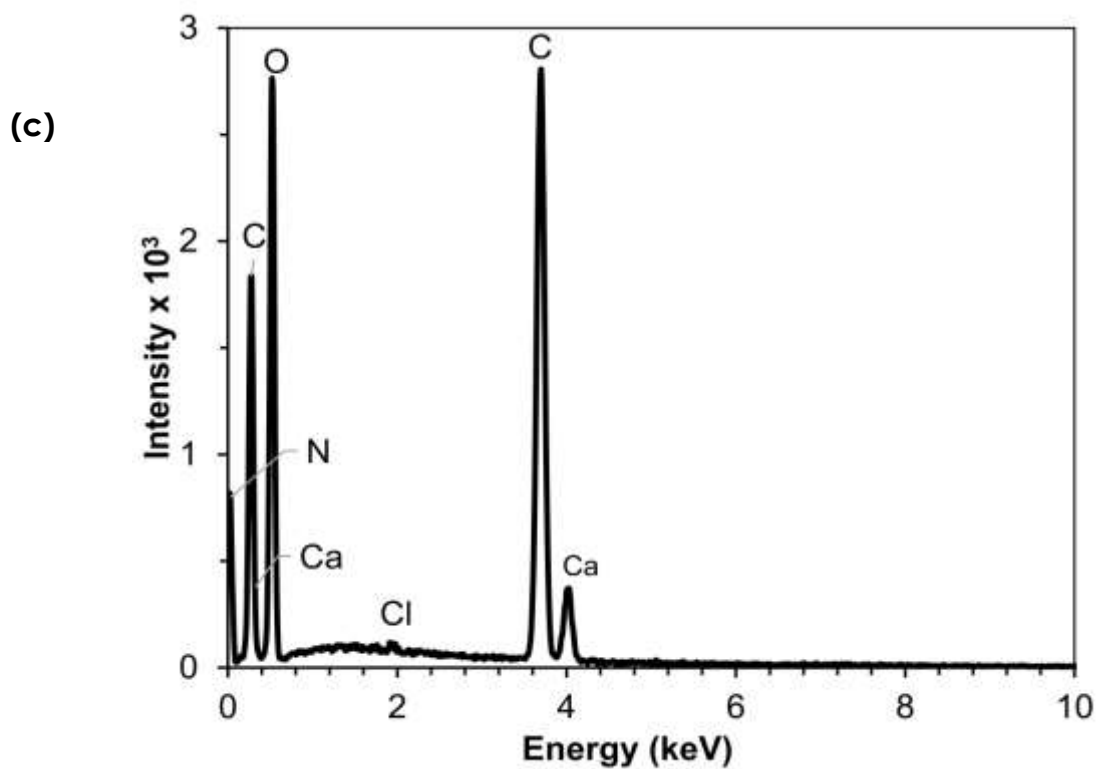
Figure 5.4. SEM and EDS for coarse sand treated via 32 biochemical treatment cycles: (a) Large CaCO3 crystals filling the voids and surrounding coarse sand particles; (b) Trigonal-rhombohedral calcium carbonate crystals; (c) EDS for chemical composition of the CaCO3 crystals. EDS: energy dispersive spectroscopy; SEM: scanning electron microscopy.

(a)
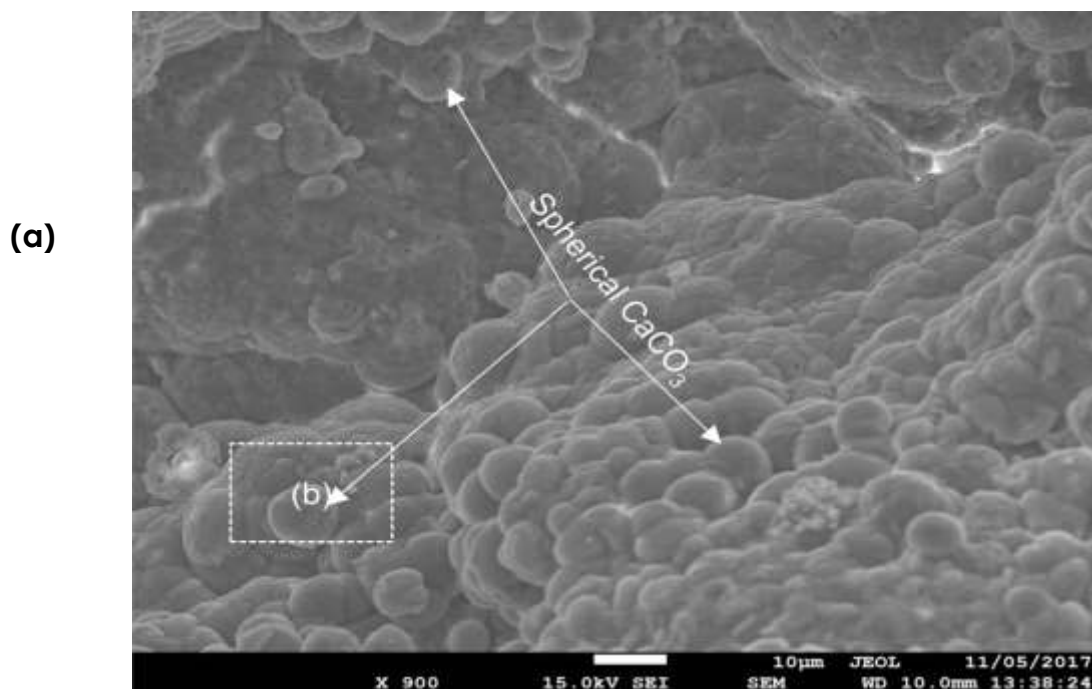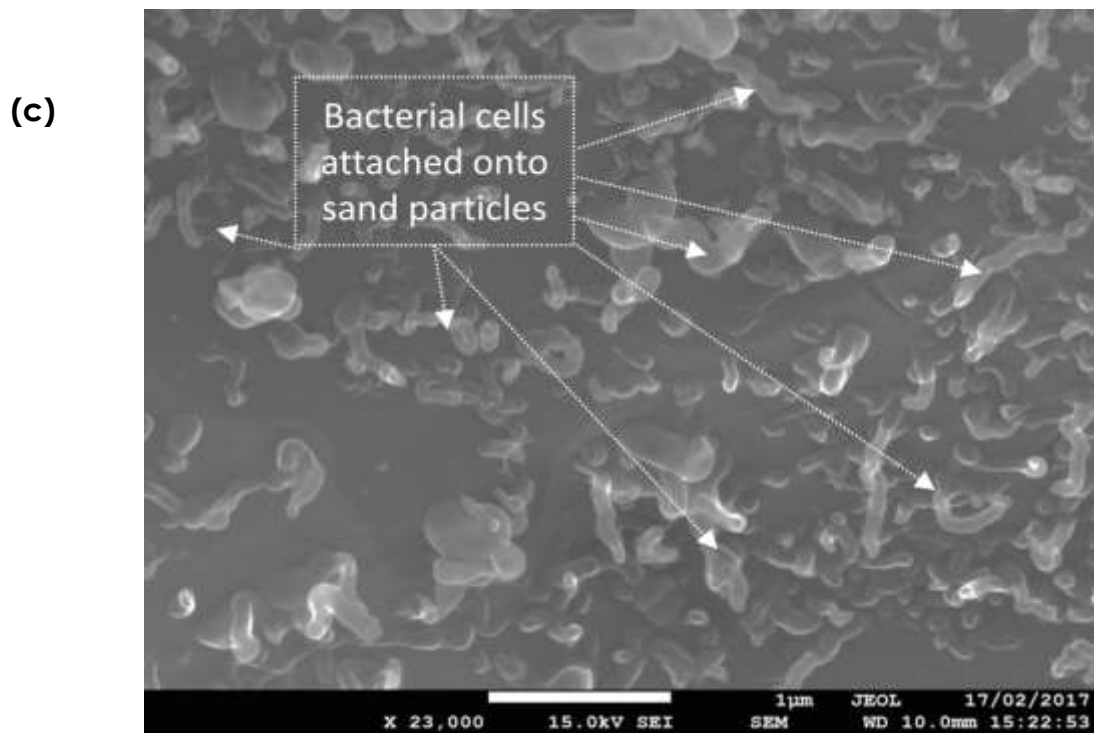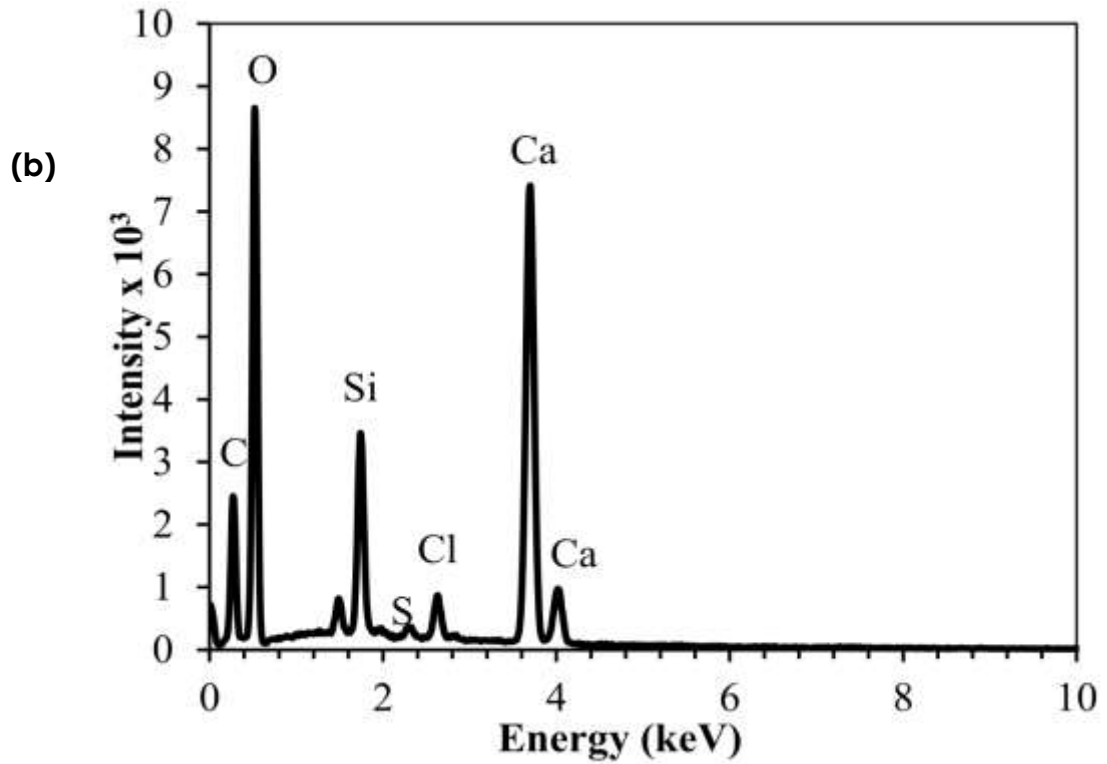
Figure 5.5. SEM analysis of calcium carbonate crystals with four biochemical treatments: (a) Spherical-shape crystals resulted from lightly bio-cementation; (b) EDS analysis for chemical composition of the $CaCO_3$ crystals; (c) Footprints of bacterial cells involvement. EDS: energy dispersive spectroscopy; SEM: scanning electron microscopy.

These bridging contacts were only correlated with the amount of deposited $CaCO_3$, because as described above, heterogeneous $CaCO_3$ precipitation led to failure propagation primarily from regions where a low bio-cementation occurred (Figure 5.6).

A direct relationship between strength and stiffness with average $CaCO_3$ precipitation is presented in Figure 5.7. Both strength and stiffness increased as the average amount of precipitated $CaCO_3$ increased. The minimum $CaCO_3$ to gain an improvement in strength and stiffness (about 200 kPa and 259 MPa, respectively) of bio-cemented coarse sand was around 2.6%. A minimum of four-biochemical treatments was required to obtain a minimum $CaCO_3$ cementation, consistent with Whiffin et al. (2007) who concluded that a minimum of around 2.8% $CaCO_3$ is required for a measurable increase in strength. Stiffness is another essential factor that has been improved as bio-cemented samples showed a nearly linear relationship with the number of biochemical treatments and an average amount of deposited $CaCO_3$ (Figure 5.7). Furthermore, the maximum strength and stiffness were also associated with the dry unit weight equated to about 19 kN/m^3 as shown in Figure 5.8 It can also be seen from Figure 5.8 that an increase in dry density of the biocemented coarse sande associated with increasing of compressive strength and stiffness. This behaviour is well known in the case of using artificially cemented soils as the strength increases with an increase of cement content and dry unit weight. The high strength and stiffness achieved in this study indicate that MICP is a promising ground improvement method, which can be used in coarse sand alternatively to other mixing processes.

Figure 5.6. Typical failure pattern of a bio-cemented coarse sand column.
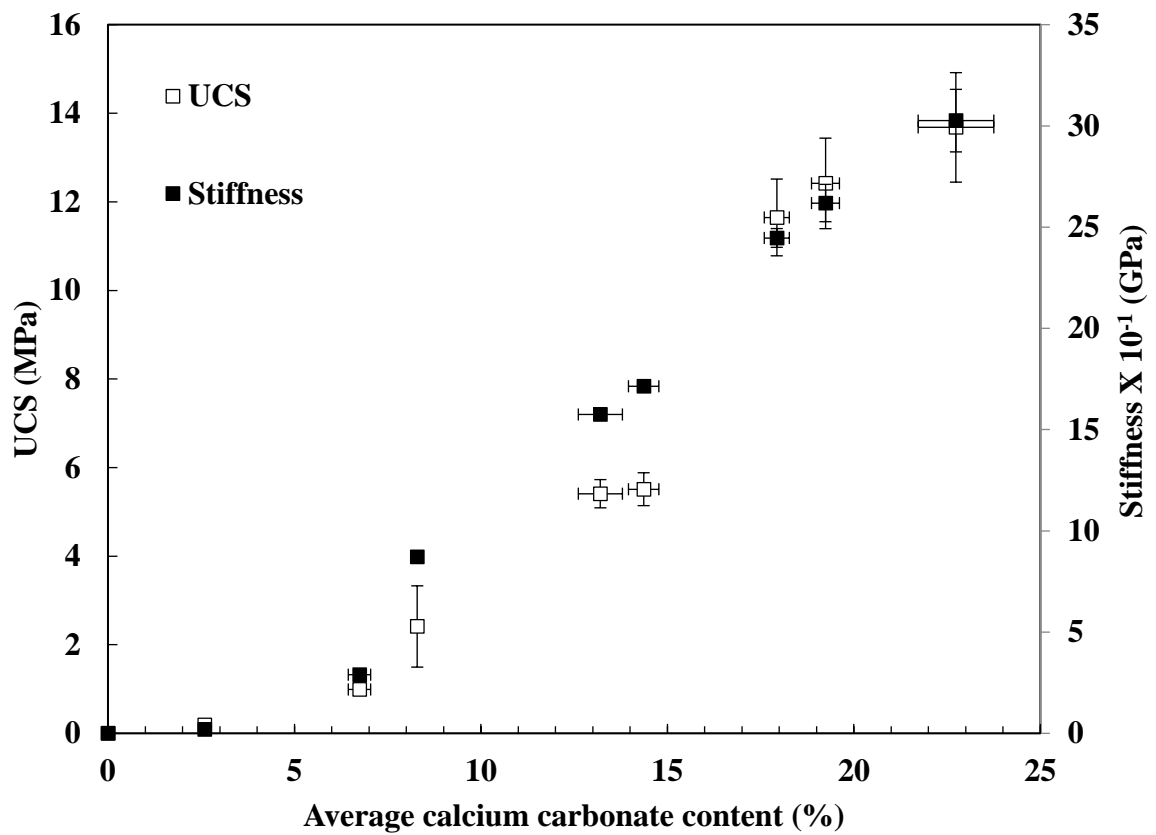
Figure 5.7. Variation of the precipitated calcium carbonate against the average UCS and stiffness. UCS: unconfined compressive strength.
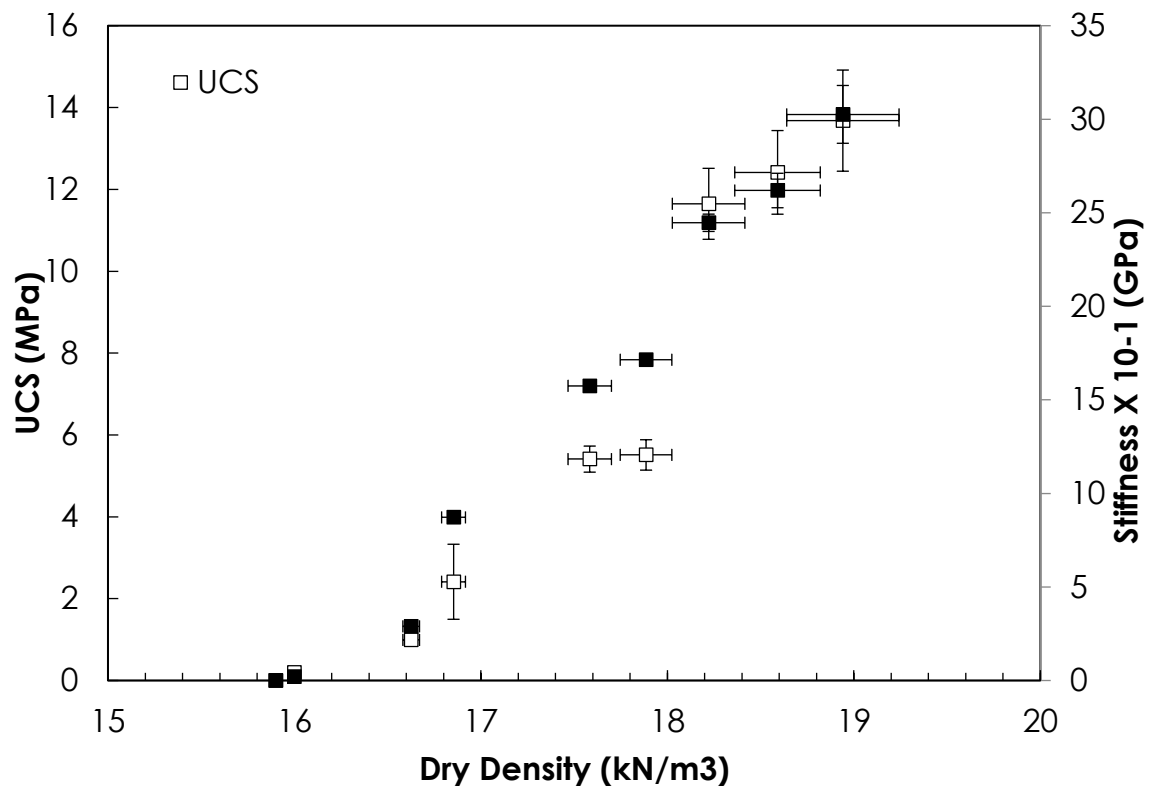
Figure 5.8. Variation of the dry density against the average UCS and stiffness. UCS: unconfined compressive strength.

It is also worth noting that the efficiency of $CaCO_3$ precipitation with the number of biochemical treatment cycles was determined based on the calcium used in cementation solution and was found to be in the range of about 47-62% as shown in Table 5.2.

Table 5.2: Calcium carbonate precipitation efficiency versus number of treatment cycles.

Number of treatment cycles	0	4	8	12	16	20	24	28	32
$CaCO_3$ precipitation efficiency (%)	0	47.72	62.11	50.92	60.80	52.92	55.06	50.62	52.35

5.3.2 Effect of initial relative density on the mechanical properties of bio-cemented coarse sand

Figure 5.9 summarises the UCS and amount of $CaCO_3$ precipitation in three locations (top, middle and bottom) with percentages of the relative density of coarse sand treated by MICP. As all specimens were similarly treated by 32-biochemical treatment cycles, there were variations in the amount of deposited $CaCO_3$. The efficiency of $CaCO_3$ precipitation was about 45% for specimens with an initial relative density of 100%, and the maximum $CaCO_3$ precipitation efficient (about 58%) was achieved with the specimen having an 80% relative density. However, there is still an acceptable relationship between the amount of $CaCO_3$ precipitation and the relative density. More $CaCO_3$ was observed at the bottom locations with specimens compacted up to 80% relative density. By contrast, 100% relative density showed that a higher $CaCO_3$ was deposited in the location near the percolation points (the upper section). This is because packing coarse sand to the maximum density resulted in the decrease of the soil pore throat.

A higher UCS and stiffness were reached when the relative density of MICP-treated coarse sand increased as the maximum UCS and Stiffness were achieved at 80% and 60% relative densities, respectively. This is in agreement with other studies (Martinez and DeJong 2009, Al Qabany and Soga 2013), as more particle contact numbers, will exhibit a high increase in UCS compared with loose state samples. However, a drop in UCS and stiffness was observed in samples packed up to 100% relative density. This may be because urease-producing bacteria could not infiltrate deeper in the soil specimen due to the reduction in the soil pore throat. This led to a heterogeneous distribution of $CaCO_3$, which was high in the region close to the percolation points. A strength of 16 MPa was achieved at 80% relative density, which was associated with higher $CaCO_3$ precipitation (about 25%).
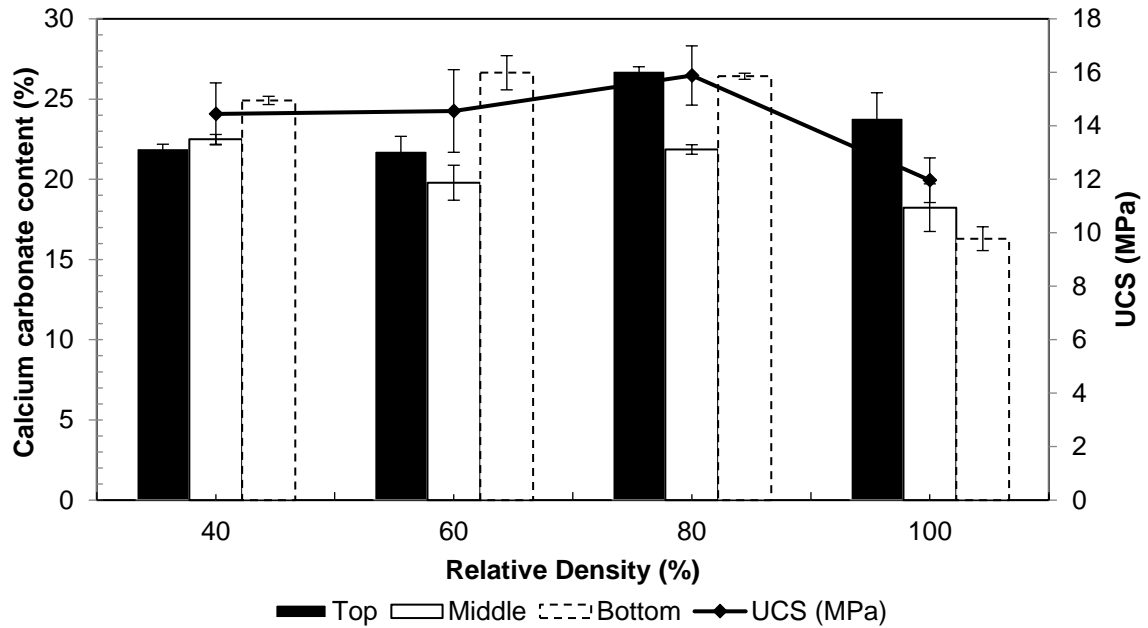
Figure 5.9. Variation of initial relative density against the calcium carbonate content and the UCS. UCS: unconfined compressive strength.

5.3.3 Effect of biochemical treatment on the Permeability and porosity of coarse sand

The measured permeability values for the MICP-coarse sand specimens including untreated specimens and specimens treated with cementation solution only are presented in Figure 5.10. The permeability of the untreated coarse sand specimen and the sample treated with only cementation solution were 2.22×10^{-3} m/s. In general, an increase of biochemical treatment cycles caused the permeability to decrease, as clearly observed where only 4-treatments resulted in a sharp (around 40%) reduction in permeability. An increase of biochemical treatment numbers can be exponentially correlated with reduction of permeability up to 24-treatments and then there is a marginal reduction in the permeability. This is because these specimens were treated without incubation time where bacterial suspension and cementation solutions were left to drain out after treatments.

The reasons for permeability reductions when using different biochemical treatment numbers may be related to pore clogging by the precipitation of

calcium carbonate, which reduces the porosity of coarse sand specimens (see SEM images, Figure 5.4a). Consequently, the coarse sand pore spaces were partially filled by the $CaCO_3$ crystals, which caused only small volume changes with the increased number of biochemical treatment cycles (Figure 5.4a and Figure 5.5a). The reduction in porosity of the bio-cemented coarse sand is presented in Figure 5.11, where it can be seen that the porosity is substantially reduced with an increase of biochemical treatment cycles. The quantified porosity of untreated and treated coarse sand is supporting the hypothesis that calcium carbonate is filled the pore spaces leading to a substantial reduction in porosity and permeability. It is also worth noting that an increase in porosity of the biocemented coarse sand led to increase in its permeablity as shown in Figure 5.12. This is also evidenced in Figure 5.4 and Figure 5.5 as discussed earlier. Even though the results indicate significant reductions in the permeability when using a different number of biochemical treatment cycles, the specimens still maintained reasonable permeability. This is an advantage as it is essential to dissipate pore water pressure in a soil matrix.
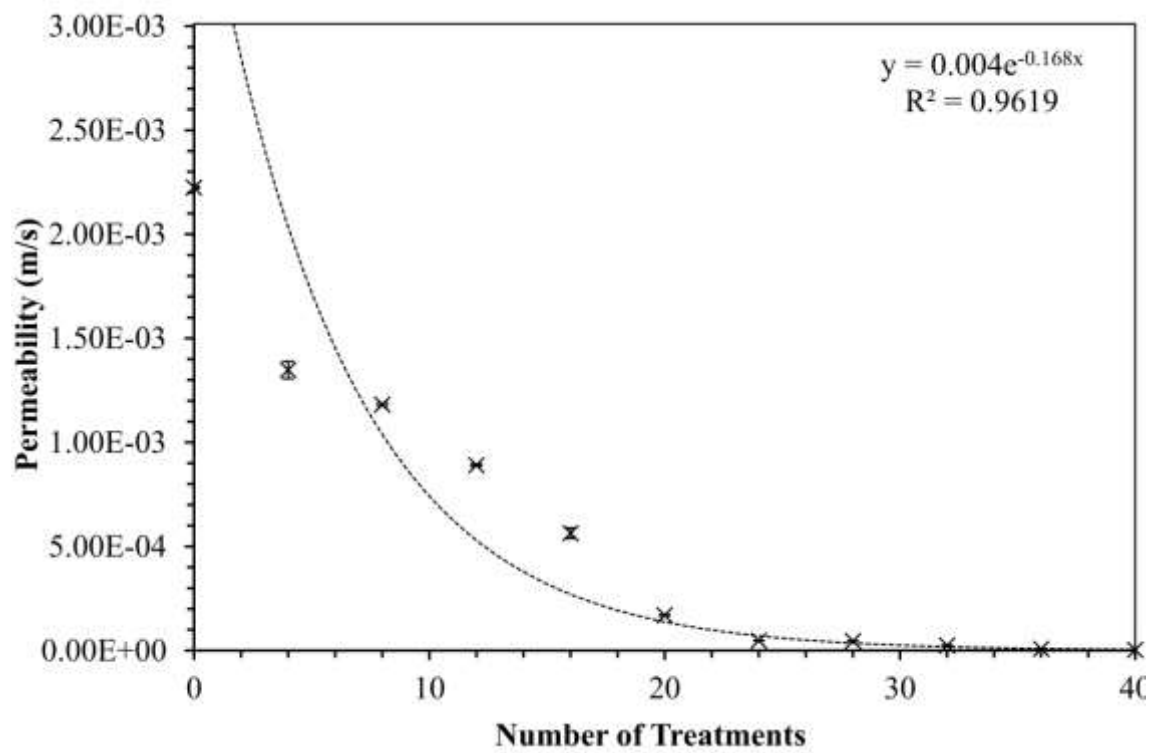
Figure 5.10. Permeability of MICP-treated coarse sand against the number of biochemical treatments. MICP: microbially induced calcite precipitation.
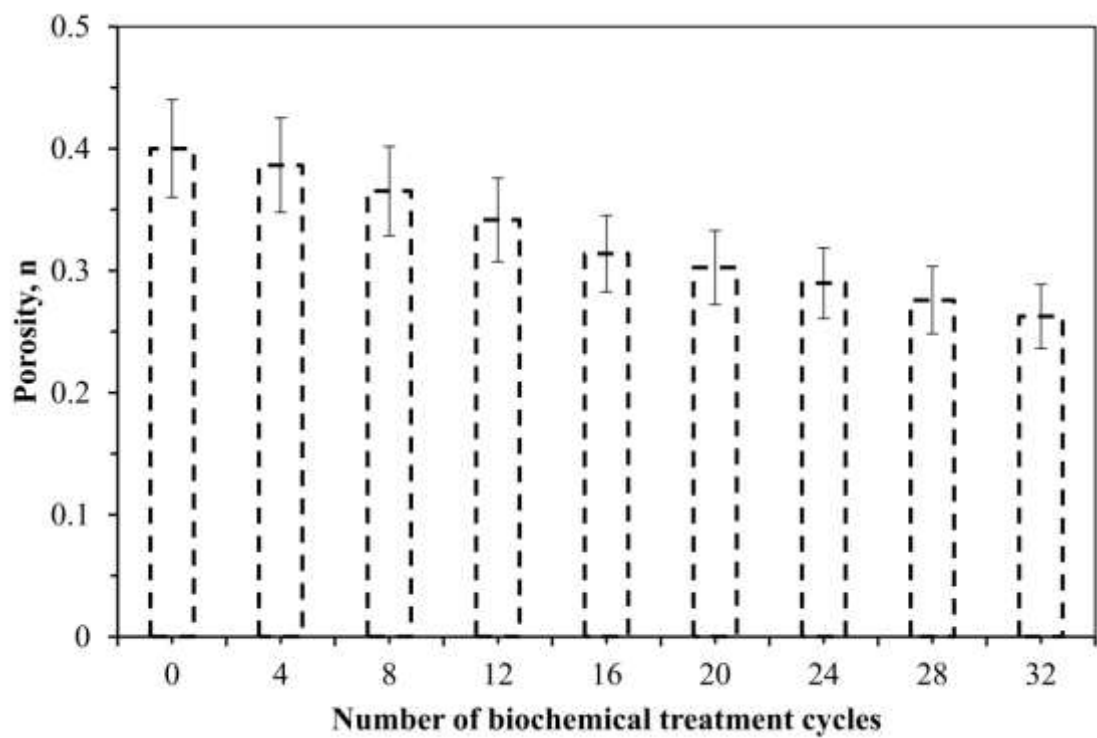
Figure 5.11. Variation of porosity against the number of biochemical treatment cycles.
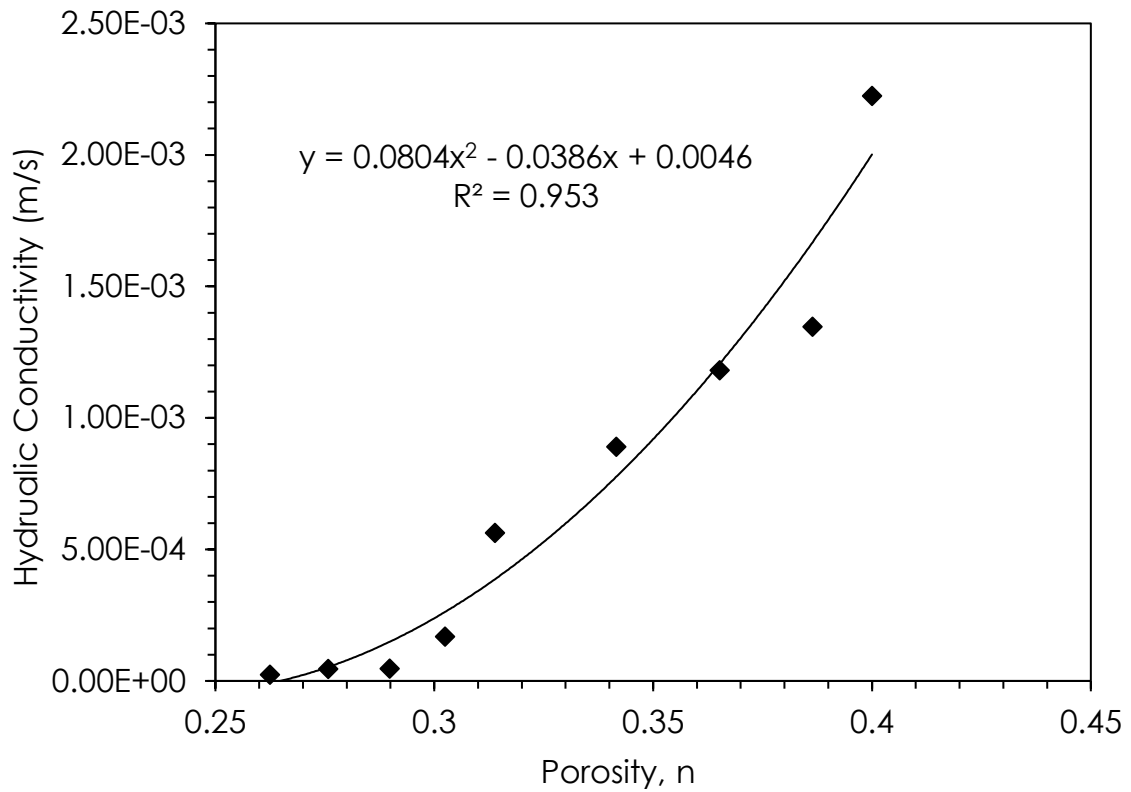
Figure 5.11. Relatioship between porosity and permeabity of the biocemented coarse sand.

5.4 Conclusions

The effects of biochemical treatments number, amount of urea use, amount of $CaCO_3$ precipitation, and initial relative density, on the engineering properties of coarse sand, as well as the optimisation of the bio-cementation process treated coarse sand have been investigated in the current study. The salient conclusions that can be drawn are:

- The amount of deposited $CaCO_3$ tended to increase with increasing number of biochemical treatment cycles and the amount of urea used.

- The amount of deposited $CaCO_3$ correlated with the increase of the UCS and stiffness of bio-cemented coarse sand, and the maximum UCS attained was about 14 MPa.

- The minimum $CaCO_3$ to obtain an improvement in strength and stiffness (about 200 kPa and 259 MPa, respectively) of bio-cemented coarse sand was around 2.6% in agreement with previous studies. A

minimum of four-biochemical treatments was required to obtain a minimum CaCO$_3$ cementation.The UCS and stiffness were increased with the increase of the initial relative density of MICP-treated coarse sand up to 80% relative density and then decreased at 100% relative density. A UCS value of 16 MPa was achieved at 80% relative density, which was associated with higher CaCO$_3$ precipitation (about 25%).

- The current results indicate substantial reductions in the porosity and permeability when using a different number of biochemical treatments. The treated specimens maintained a permeability in the range of 3.76 x 10^{-6} m/s which is desirable if pore water pressure in a soil matrix needs to be dissipated.

- Microstructure analysis has further confirmed the precipitation of calcium carbonate within and around coarse sand grains. Large trigonal- rhombohedral calcium carbonate crystals precipitated in the pore matrix of coarse sand when using a higher number of biochemical treatment cycles, whereas adopting a low number of treatments resulted in small spherical calcium carbonate crystals.

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Chapter 6: Effect of particle size distribution on the bio-cementation of coarse aggregates

Effect of particle size distribution on the bio-cementation of coarse aggregates

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Abstract

The effect of grain size distribution on the unconfined compressive strength (UCS) of bio-cemented granular columns is examined. Fine and coarse aggregates were mixed in various percentages to obtain five different grain size distributions. A four-phase percolation strategy was adopted where a bacterial suspension and a cementation solution (urea and calcium chloride) were percolated sequentially. The results show that a gap-graded particle size distribution can improve the UCS of bio-cemented coarser granular materials. A maximum UCS of approximately 575 kPa was achieved with a particle size distribution containing 75% coarse aggregate and 25% fine aggregate. Furthermore, the minimum UCS obtained has applications where mitigation of excessive bulging of stone/sand columns, and possible slumping that might occur during their installation, is needed. The finding also implies that the amount of biochemical treatments can be reduced by adding fine aggregate to coarse aggregate resulting in effective bio-cementation within the pore matrix of the coarse aggregate column as it could substantially reduce the cost associated with bio-cementation process. Scanning electron microscopy results confirm that adding fine aggregate to coarse aggregate provides more bridging contacts (connected by calcium carbonate precipitation) between coarse aggregate particles, and hence, the maximum UCS achieved was not necessarily associated with the maximum calcium carbonate precipitation.

Keywords Bio-cementation, Coarse aggregate, Fine aggregate, MICP, Strength

6.1   Introduction

Bio-cement is produced via a microbially induced calcium precipitation (MICP) method. This process mimics natural biochemical processes that produce insoluble bio-products such as calcium carbonate. The most prominent process of the MICP method relies on the urease enzyme produced by bacteria that catalyses the urea hydrolysis into ammonium and carbonate. Subsequently, in the presence of supplemental calcium ions, this can result in carbonate ions deposit as calcium carbonate crystals (bio-cement). Bio-cement process forms cementing bridges between the granular soil particles to create a solid mass. The bio-cement process is suitable for many geotechnical applications such as improving the mechanical properties of granular soils, erosion control, dust control, etc. (Le Metayer-Levrel et al. 1999, Ramachandran et al. 2001, Nemati and Voordouw 2003, Nemati et al. 2005, DeJong et al. 2006, Whiffin et al. 2007, Ivanov and Chu 2008, De Muynck et al. 2010, De Muynck et al. 2010, Al Qabany and Soga, 2014, Montoya and DeJong, 2015, Salifu et al., 2016, Mujah et al., 2016, Mahawish et al. 2016, Hamdan et al., 2017).

Although soil improvement using a bio-cement process has shown a great promise; limitations exist in applying this method to coarser materials. Coarser materials are defined herein as coarse aggregate which contains more than 50% coarse fraction retained on No.4 (4.75mm) sieve in accordance to the unified soil classification system (USCS). This type of coarse material is located with the acceptable range of coarse material (average particle size of between 25 mm to 75 mm) often used to form stone columns in the field to improve soft clay deposits (Gniel and Bouazza, 2009, 2010). Due to the fact that coarser materials require a high amount of cementitious material to gain an acceptable strength, very often they are considered not suitable for treatment by biogrout process as they need higher number of biochemical treatment cycles. Thus, making the treatment less cost-effective. The objective of the present paper is to explore the effect of particle size distribution on the efficacy of the MICP method to improve

the strength of coarser materials as the intention of this investigation is to mitigate slumping of a sand/stone column installed in soft clays that may occur during installation or excessive radial expansion. Different percentages of coarse aggregate and fine aggregate were mixed to obtain different ranges of particle size distributions (five particle sized distributions). Unconfined compression tests (UCS) were conducted on the bio-cemented soil columns, and the precipitated calcium carbonate was acid quantified after the completion of the UCS tests.

6.2 Materials and methods

6.2.1. Type of Bacteria and cementation solution

The urease producing bacteria used was *Sporosarcina pasteurii* (ATCC® 11859). One colony from the stock culture (Petri dishes containing bacterial colonies, 20 g/L yeast extract, 10 g/L ammonium sulphate and 20 g/L agar in 130 mM Tris buffer (pH 9.0) (Stocks-Fischer et al. 1999) was transferred to 5mL of ammonium-YE growth medium (20 g yeast extract, 10 g $(NH_4)_2SO_4$ and 130 mM tris buffer (pH=9.0) per litre of distilled water) to grow the bacterial cells at 30°C. Individual ingredients of the recipe were separately dissolved in distilled water bottles and autoclaved separately and then combined post sterilisation. Suspended bacterial cultures were grown in a shaking water bath (200 rpm) for 24 hrs before harvesting at a final optical density (OD_{600}) of 3.0-3.5 determined using a WPA CO 8000 spectrophotometer (BioChrom Ltd) to achieve an optimum concentration of bacterial cells in which all readily available nutrients were considered to be consumed from the growth medium (i.e., the start of the stationary growth phase) (Cheng and Cord-Ruwisch, 2012). The concentration of bacterial suspension (cells per ml) used was estimated to be 3.8×10^8 - 4.7×10^8 cells/ml based on equation 6.1 (Ramachandran et al. 2001, Okwadha and Li 2010, Shahrokhi-Shahraki et al. 2014).

Concentration of cells per mL = 8.59 X 10^7 X OD_{600} $^{1.3627}$ (6.1)

An activity value of 19.38-21.45 mM/min for urease enzyme was used in this study based on the maximum optical density of the harvested bacterial cells. Urease activity rate was determined via a solution electrical conductivity assay (labCHEM-CP) and equation (6.2) (Whiffin 2004). Equation 2 provides an indication of the urease enzyme activity of the bacteria based on electric conductivity.

Urea hydrolysed (mM) = Conductivity (mS) x 11.11 (R^2 = 0.9988) (6.2)

The main reason for harvesting bacterial cells at the beginning of the stationary phase was to minimise further growth of bacterial cells in the granular columns during retention time (incubation time) from occurring. This is because no nutrient broth has been percolated into the soil columns after the percolation of bacteria. The preliminary culture medium was transferred aseptically to inoculate 250 ml of Ammonium-YE medium, and the previous process can hence be repeated accordingly. The bacterial suspension was stored up to 14 days at 4°C until used in the experimental work.

The cementation (reagent) solution consisted of 1 M urea (CO $(NH_2)_2$) and 1 M calcium chloride ($CaCl_2$). The cementation solution has been used according to the protocol adopted in this study.

6.2.2. Granular Columns Specimen Preparation

Pakenham Blue Metal (Old Basalt) coarse aggregate (sieves 2.36 to 16 mm) and fine aggregate (sieves 0.075 to 9.5 mm) were used to prepare cylindrical samples for the bio-cementation treatments and UCS tests. Different percentages of fine aggregate (0, 25, 50, 75 and 100 %) were respectively added to various percentages of coarse aggregate (100, 75, 50, 25 and 0 %, respectively) to study the effect of different particle size distribution on the soil columns treated by MICP. All mixtures are classified as poorly graded materials according to the Unified Soil Classification System (ASTM 2006). Their particle size distribution is presented in Figure 6.1. The

mixtures were named as A, B, C, D and E, representing respectively coarse aggregate only, fine aggregate only, coarse aggregate with 25% fine aggregate, coarse aggregate with 50% fine aggregate, and coarse aggregate with 75% fine aggregate. Maximum and minimum dry density measurements were conducted in accordance to ASTM D4253 (2014a) and ASTM D4254 (2014b) to control the density of the column model. Summary of the mix properties (specific gravity (G_s), coefficient of curvature (Cc), coefficient of uniformity (C_U), the medium grain size (D_{50}), maximum and minimum void ratios, e_{max} and e_{min}) are presented in Table 6.1.

Preparation of the samples was performed by mixing coarse aggregate and fine aggregate by dry weight according to the required percentages. Both materials were blended in a dry condition, and then 5% of water was added to the mixture to wet the specimens. All the test samples were packed up to the relative density of 60% with an initial gravimetric water content of 5%. The soil columns had a length to diameter aspect ratio of 2:1. The columns were made of a split mould polyvinyl chloride (PVC) pipe, 200 mm in height and with an internal diameter of 100 mm when assembled. A wire mesh and filter paper were placed at the bottom of the column to minimise possible loss of soil mixture particles during preparation and treatment. The columns were positioned vertically with fully opened upper surface, and a drainage control valve was connected to the base.
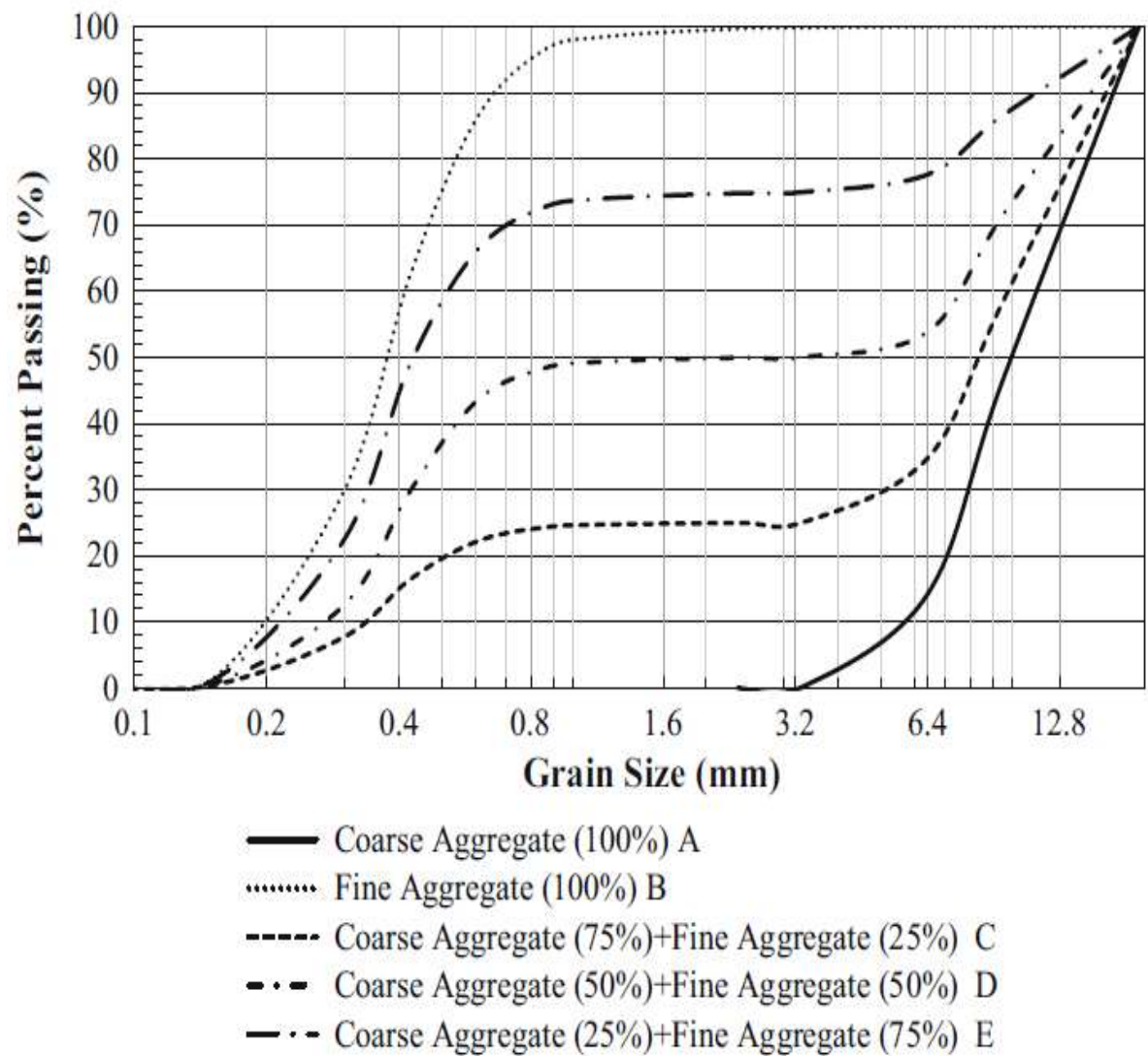
Figure 6.1 Particle size distribution of granular materials (mixtures).

Table 6.1: Properties of granular materials.

Material Type	Symbol	Class.	G_s	C_c	C_u	D_{50} (mm)	e_{max}	e_{min}
Coarse aggregate (100%)	A	GP	2.78	1.011	2.14	9.90	0.86	0.72
Fine aggregate (100%)	B	SP	2.65	1.12	2.21	0.37	0.73	0.55
Coarse Aggregate (75%) +Fine Aggregate (25%)	C	GP with Sand	2.73	6.71	28	8.10	0.41	0.28
Coarse Aggregate (50%) +Fine Aggregate (50%)	D	SP with Gravel	2.69	0.09	28.84	3.0	0.36	0.25
Coarse Aggregate (25%) +Fine Aggregate (75%)	E	SP with Gravel	2.67	0.90	2.75	0.44	0.49	0.43

6.2.3. Soil treatment procedures

A percolation method, described in Mahawish et al. (2016), was used to bio-cement the very coarse grained soil specimens. The protocol that has been adopted throughout this chapter was a four-phase percolation strategy. The amount of bacterial suspension and cementation solution in each treatment cycles was determined based on the pore volume (PV) of the soil columns. The strategy consisted of the following steps:

i. Percolation of tap water to expel air from the specimens (200% PV of granular columns). The step was performed by closing the bottom valve, percolating deionised water until ponding on top of the specimen, and then opening the bottom valve.The percolation of water was continued until reaching a volume of water equivalent to 200% PV.

ii. Percolation of cementation solution (100% PV of granular columns). This process was performed without retention time to promote high ionic strength within the pore matrix because the cementation solution contained urea and divalent cations (i.e. calcium ions), which tend to increase the ionic charges. Urea plays a significant role in providing a suitable enviroment for the bacterial cells as they have a high affinity to urea. This step was applied once before the beginning of the treatment cycles.

iii. After the cementation solution volume was drained out from each specimen, the bottom valve was closed, and the first treatment cycle was followed by percolating bacterial suspension (BS) without the ammonium-YE media alternately with cementation solution (CS) for a total of four times to achieve a four phases percolation strategy (12.5% PV (BS) + 12.5% PV (CS) + 12.5% PV (BS) + 12.5% PV (CS)). Total percolated liquids are equivalent to 50% of PV of the granular columns.

iv. Incubation (retention time) for 24 hrs at 20±2°C, allowing the percolated liquids to facilitate reactions between microorganisms and reagents solutions.

v. The second treatment cycle included the percolation of only cementation solution (50% PV of the granular columns) and allowed ureolysis and calcium carbonate deposition to take place for same aforementioned retention time.

vi. Steps iii-v were considered as two biochemical treatment cycles and were repeated until reaching the required number of treatment cycles (6 biochemical treatment cycles).

6.2.4. Unconfined Compressive Strength (UCS) Tests and Calcium Carbonate Quantification

After completion of the bio-cementation treatments, all the specimens were flushed with tap water to remove the residual chemical reagents from the

pore space. UCS tests were conducted on samples that were conditioned over 14-days at 30±2°C. The axial load was carried out at a constant strain rate of 1%/min.

After completion of the UCS tests, calcium carbonate content was measured using gravimetric acid washing (2 M HCl) technique. For this purpose, subsamples from each 20 mm increments along the bio-cemented granular columns were dried in an oven at a temperature of 105°C and their masses were measured before and after the acid wash. The quantification of calcium carbonate was determined by rinsing dissolved calcium carbonate-HCl several times through sieve No.200 and allowing the dissolved salts to be rinsed out from the samples. The difference between two masses was taken as the mass of calcium carbonate, and the percentage of calcium carbonate was obtained by dividing the mass of calcium carbonate by the mass of soil.

6.2.5. Microstructure analysis

Scanning electron microscopy (SEM) and energy dispersive spectroscopy (EDS) were conducted to characterise the location of the fine aggregate within the coarse aggregate column, and to investigate the bonding behaviour (bridging) between fine aggregate grains and fine and coarse aggregate grains,. Bio-cemented coarse aggregate with 50% fine aggregate were retained after completing the UCS tests for the purpose of conducting micro-scale investigations. The fractions were oven dried, carbon coated, and then examined using an SEM (JEOL 7001F FEGSEM,) with a beam intensity of 15kV.

6.3. Results and Discussion
6.3.1. Effect of particle size distribution on calcium carbonate precipitation of aggregate columns

Cementation uniformity is a major factor that can potentially affect the use of MICP bio-grouting process as a soil improvement technique as it can affect the targeted strength. MICP tends to produce different cementation

levels at various heights of bio-cemented soil columns (Whiffin et al. 2007, DeJong et al. 2009). The distribution of calcium carbonate precipitation along the bio-cemented granular columns for the current study is shown in Figure 6.2. It can be seen that the calcium carbonate precipitated more in the upper portion of columns B, D and E (i.e., columns containing 100%, 50% and 75% fines, respectively) than in any other parts of these columns. This is because the areas closest to the percolation point were exposed to significantly more cementation than in the other regions of the columns. In this instance, the pores available for percolation at the injection point were probably obstructed by the presence of a larger amount of fine aggregate, thus reducing the flow velocity. This increased cementation may also be related to bacterial cells being filtered by the soil matrix during the subsurface injection process resulting in a reduction of microbe concentration along the injection path (Ginn et al. 2002). Furthermore, it is plausible that utilising the four-phase percolation strategy with high calcium chloride concentration may have led to increased ionic charges, and promoted bacterial adhesion (Faibish et al. 1998, Foppen and Schijven 2006) due to bacterial cells having a high negative zeta potential being attracted to soil particles during infiltration (Dick et al. 2006). Previous studies had demonstrated that fixation of bacterial cells occurred when using staged strategy that included injection of bacterial suspension, followed by cementation solution when bio-cementation was performed on fine to medium sand (Harkes et al. 2010, Cheng and Cord-Ruwisch 2012). The present study adopted a similar strategy with some modifications about the sequence of bacterial suspension and cementation solution as well as the surface percolation process. Therefore, it is postulated that bacterial cells fixations could occur within the pore matrix of coarser materials. The column with a low percentage of fine materials (column C) showed a different tendency since a reasonably better distribution of calcium carbonate precipitation could be observed along the column, as illustrated in Figure 6.2. Quantitative analysis was conducted for each bio-cemented column and the coefficient of variation was found to be, respectively, 24%, 23%, 18%,

31% and 25% for columns A, B, C, D and E. Column C gave the best coefficient of variation for calcium carbonate distribution along its length compared to other columns confirming the trend seen in Figure 6.2. The column with purely coarse aggregate (column A) had more calcium carbonate at the lower part than in other regions. This is because it had the largest pore volume (686 cm^3) which allowed easier flow for reagent solution and bacterial cells to occur.
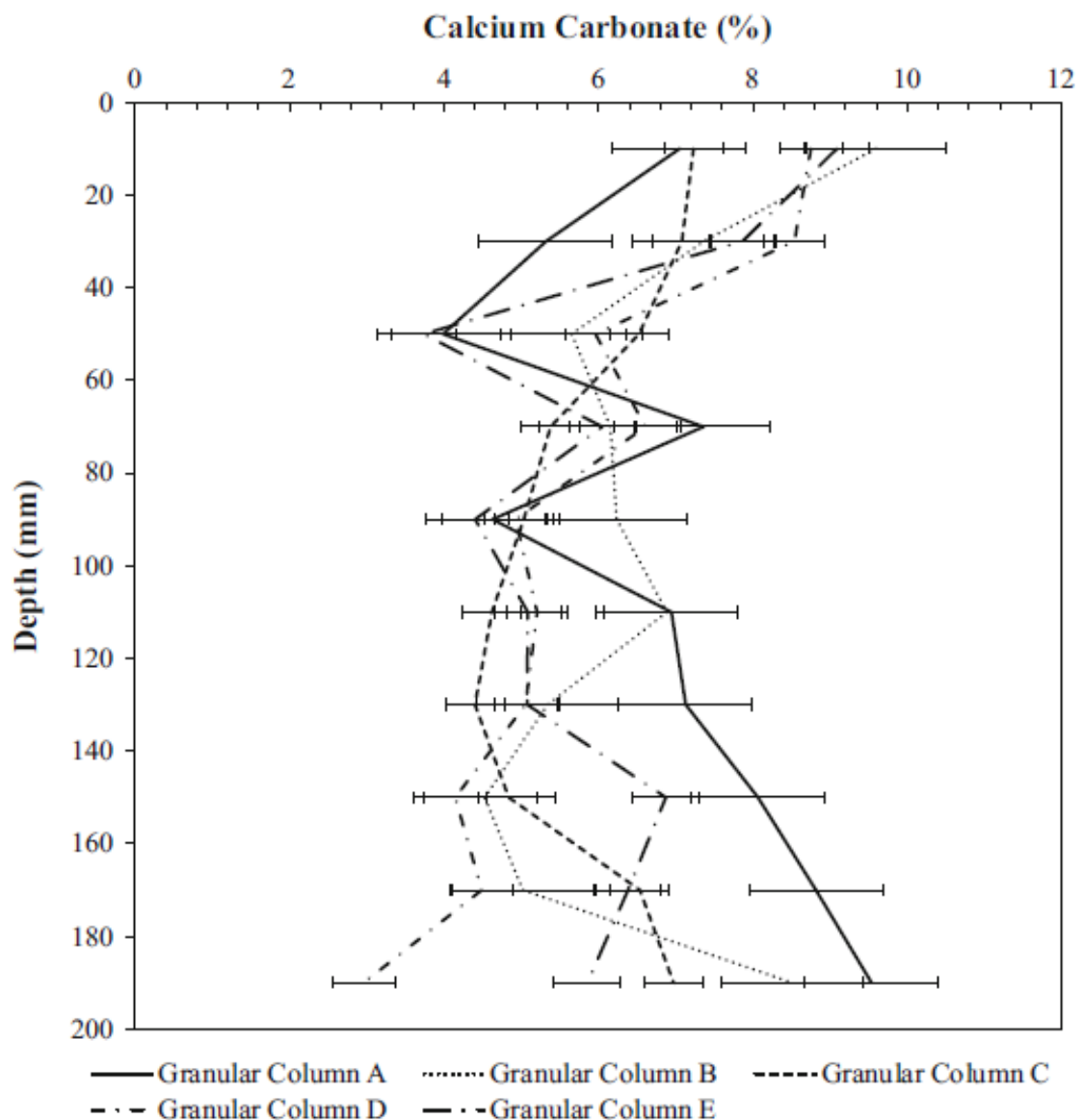
Figure 6.2 Acid quantification of an average calcium carbonate in 20 mm increments over the depth of the bio-cemented granular columns.

6.3.2. Effect of particle size distribution on strength of aggregate columns

The correlation between the UCS and the average calcium carbonate content is presented in Figure 6.3. The average precipitated calcium carbonate varied from 5.7% to 6.9%, despite the fact that the number of biochemical treatment cycles was equal (6 treatment cycles) in each column. This is because coarse aggregate columns with different fine aggregate contents have different pore volumes. In other words, the amount of biochemical treatment used in each treatment cycle (343, 302, 197, 180, and 246ml for columns A, B, C, D, and E, respectively) was determined based on the pore volume of the soil columns. This can cause a difference in the amount of deposited calcium carbonate in each column. The maximum calcium carbonate precipitation was associated with the columns containing only coarse aggregates (column A) or fine aggregates (column B). This may be again related to having the highest pore volumes (686 cm^3 and 608 cm^3) in columns A and B, respectively, than in the other soil columns. Furthermore, it is worth noting that increasing the percentage of fine aggregate to coarse aggregate associated with decreasing the amount of biochemical treatment used in each treatment cycle, and the amount of deposited calcium carbonate was consequently reduced. However, the reduction of biochemical treatment employed in each treatment cycle, while keeping a high precipitation efficiency, could be beneficial. The efficiency of calcium carbonate precipitation in each column was evaluated in relation to the amount of calcium used in the total treatment volume and found to be around 52, 58, 100, 100 and 74% for the columns A, B, C, D, and E, respectively. It is also interesting to note that the maximum efficiency was found in the columns containing either 25% or 50% fine aggregate, which could have a significant positive impact on the cost of the bio-cement process. As an example, the addition of 25% fine aggregate (column C) could reduce the cost of the biochemical treatment to around a half the cost of treating coarse aggregate alone (Column A).
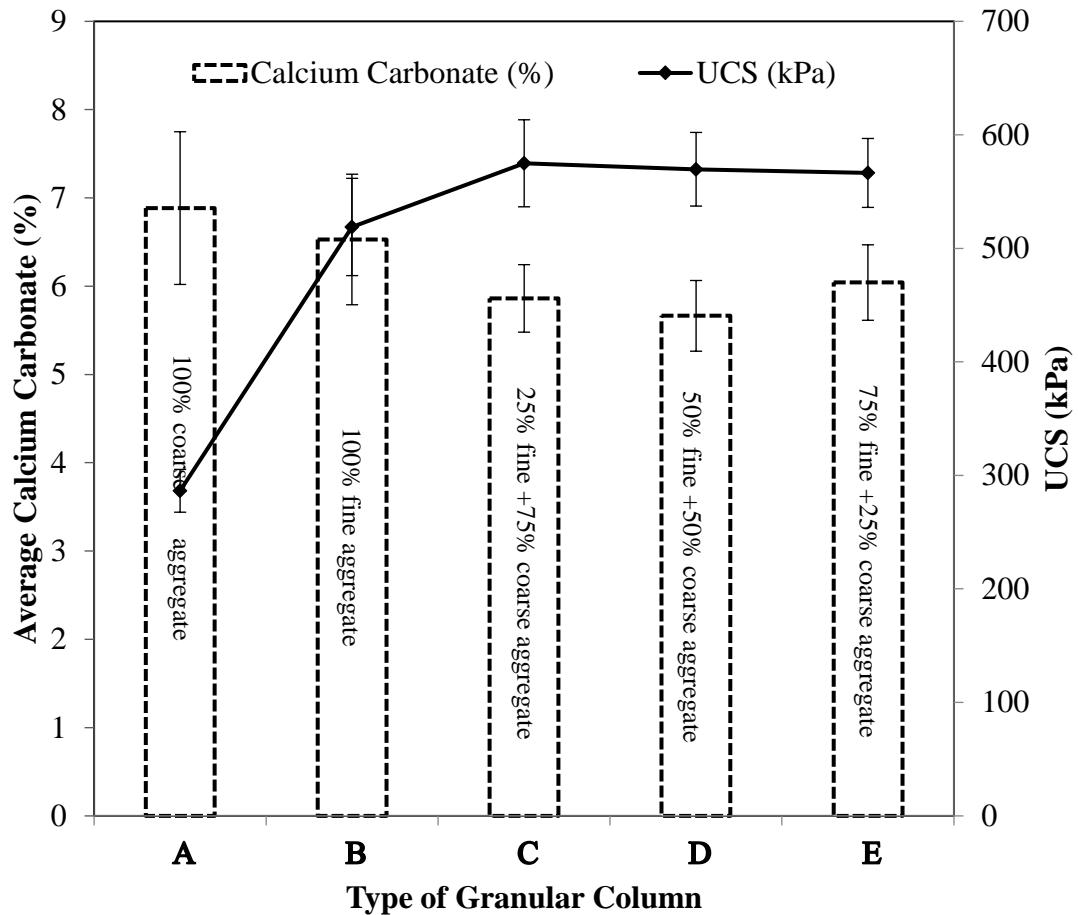
Figure 6.3. UCS and calcium carbonate content for various granular columns.

Figure 6.3. UCS and calcium carbonate content for various granular columns. Thus, the bio-cement process could become a cost-effective and efficient method to improve the strength of coarse aggregate materials by adding given amounts of fine aggregates as it is the case of column C. This process can lead to better distribution of deposited calcium carbonate.

The variation of UCS against the percentage of fine aggregate indicates that there is a substantial increase in the UCS value (around 2x) of the column containing 25, 50 and 75% fine aggregate (Column C, D and E) compared with the UCS value of the coarse aggregate column (Column A). This is because adding fine aggregate material to coarse aggregate material resulted in gap graded particle distribution. This change in particle size distribution provided improved bridging contacts (connected by calcium carbonate precipitation) between the particles and this, in turn,

had an effect on the mechanical properties (compressive strength). Bridging of sand grains by calcium carbonate crystals is evidenced in Figures 6.4a and 6.4b where it can be seen that the bio-cemented fine aggregate particles filled the pore spaces of the coarse aggregate (Figure 6.4a.) and the fine aggregate particles connected to each other and to coarse aggregate particle by precipitated calcium carbonate (Figure 6.4 a and b). The chemical composition of the precipitate is shown in Figure 6.4c and comprises primarily calcium, oxygen, and carbon which is a further evidence of the presence of calcium carbonate as a bio-product of the bio-cementation process. The presence of fine aggregates tended to partially reduce the voids size present between coarse aggregate particles, leading to smaller voids being available thus facilitating a better cementation process. This is evidenced in Figure 6.3 and Figure 6.4a as a high UCS is associated with the less deposited calcium carbonate content achieved in Columns C, D and E. It can also be seen that a marginal decrease in UCS (around 5-8 kPa) is associated with increasing fine aggregate percentages (50-75%). This reduction in the UCS may be related to the precipitation of more calcium carbonate in the upper part of the column than in the other parts. The maximum UCS attained was in the column that contained 75% of coarse aggregate and 25% fine aggregate because there was a better distribution of calcium carbonate along the column (Figure 6.2). Thus, the UCS of coarse aggregate containing different fine aggregate content can be substantially enhanced, despite the small heterogeneous distribution of calcium carbonate precipitation within the pore matrix of these columns.
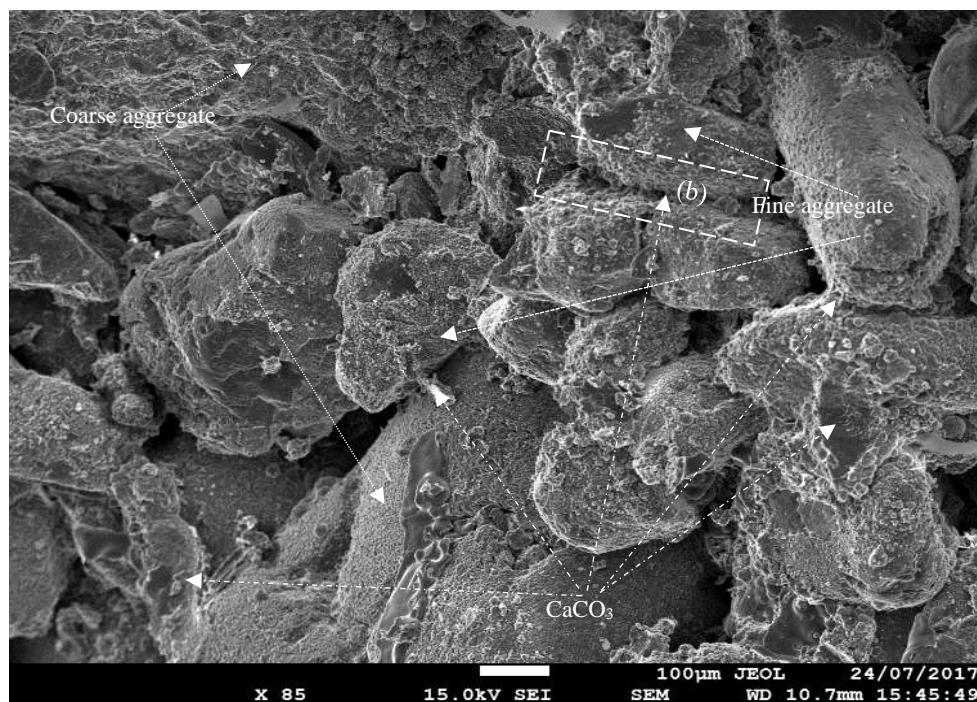
(a)
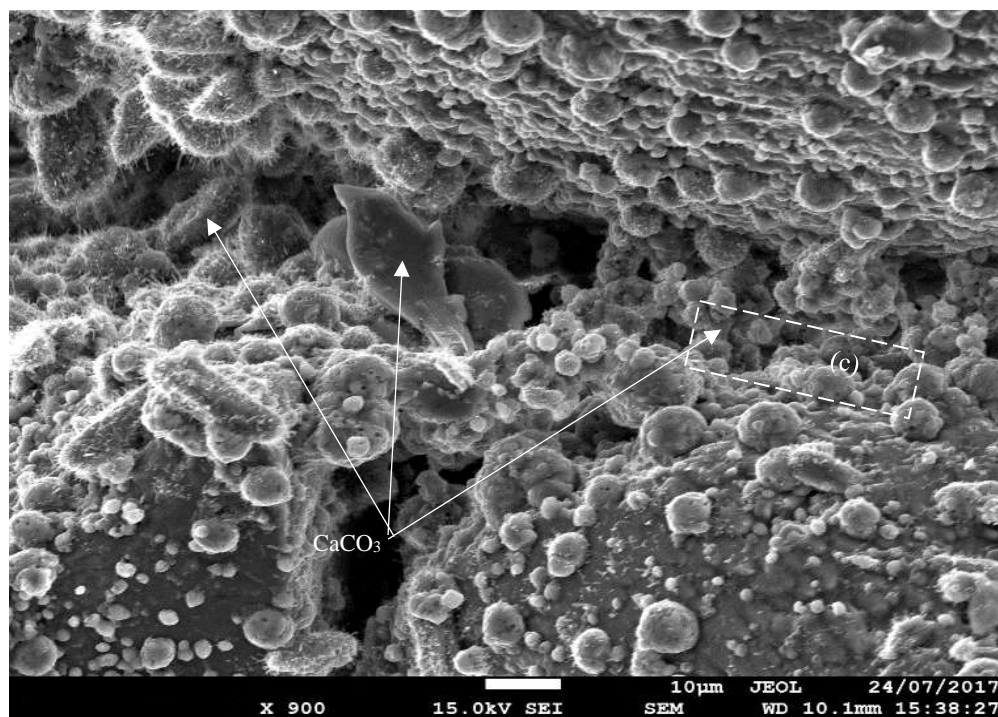
(b)
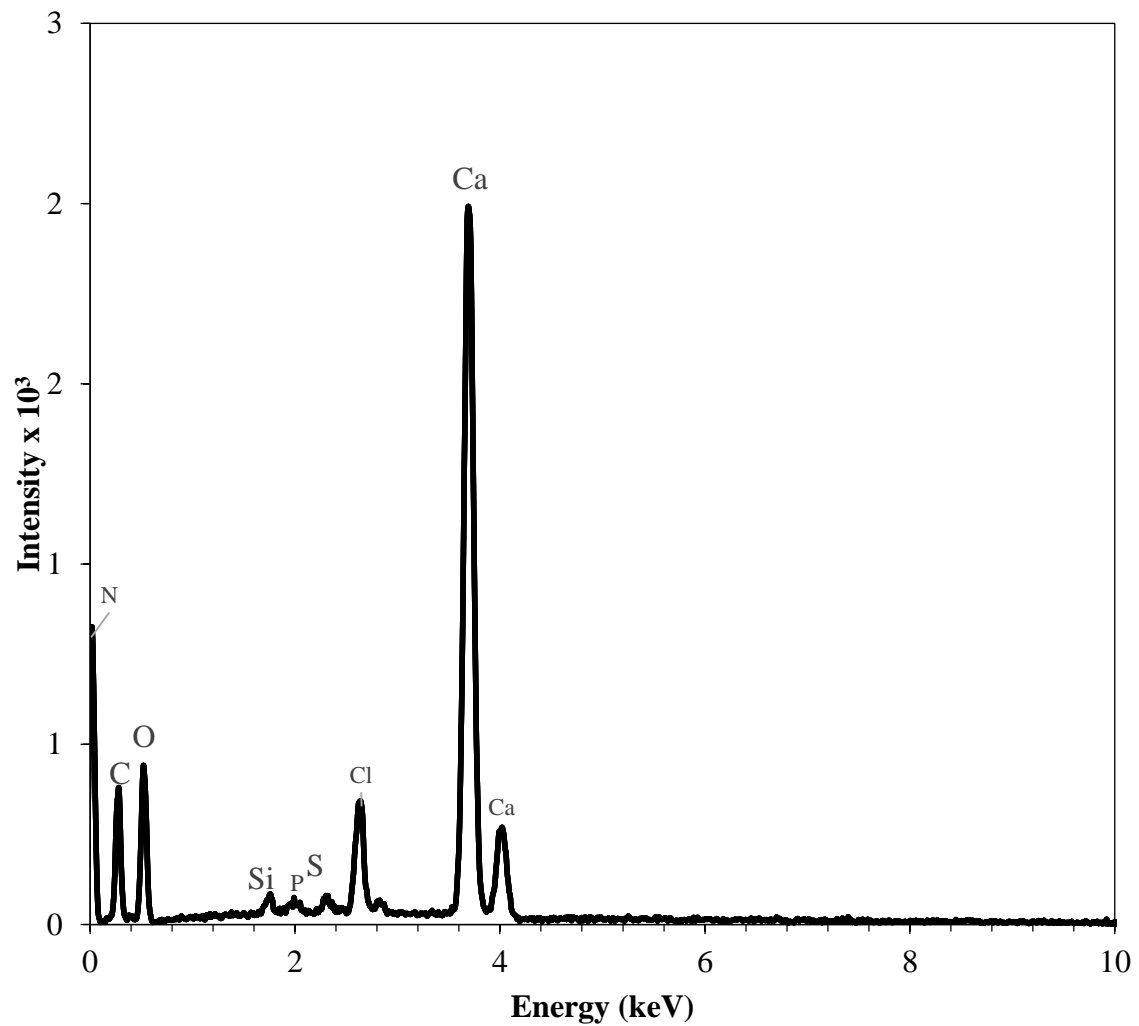
(c)

Figure 6.4 SEM and EDS analysis for column D containing 50% coarse aggregate with 50% fine aggregate treated by MICP: (a) fine aggregate filling the coarse aggregate voids; (b) fine aggregate grains bridged by spherical-shaped calcium carbonate crystals; (c) EDS analysis for chemical composition of the spherical calcium carbonate crystals

6.4 Conclusions

A series of laboratory experiments were conducted to investigate the effect of particle size distribution on the efficacy of bio-cementation of granular columns. The salient conclusions that can be drawn from this work are as follows:

- The column (column C) with the lower percentage of fine materials (25%) showed a better distribution of calcium carbonate precipitation along its length compared to the columns which contained 100%, 50% and 75% fines (columns B, D and E, respectively). The column with purely coarse aggregate (column A) had more calcium carbonate at the lower part of the column than in other regions of the column.

- Bio-cementation of merely coarse aggregates can be facilitated by the addition of fine aggregates particles. The inclusion of 25% fine aggregate particles to form a gap graded distribution was found to be sufficient to increase the unconfined compressive strength (UCS) from 0 kPa (untreated mix) to about 575 kPa (treated mix). The presence of larger fine contents (>25%) led to very limited changes in UCS of the bio-cemented soil and more non-uniform distribution of calcium carbonate precipitation. Thus, the bio-cementation process can be an effective method to improve the strength of coarse aggregate materials if their fine content is about 25%. This process might be beneficial for stone columns installed in soft clays to mitigate slumping that may occur in the column during installation or excessive radial expansion. Further works is warranted

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Chapter 7: Unconfined Compressive Strength and Visualization of the Microstructure of Coarse Sand Subjected to Different Bio-cementation Levels

Unconfined compressive strength and visualization of the microstructure of coarse sand subjected to different biocementation levels

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Abstract

Biocementation processes rely on microbial-induced calcite precipitation (MICP), which is a naturally occurring biochemical process. Bio-cement materials are a form of environmental cementitious agents used to improve the mechanical properties of granular soils by physically binding soil particles together. Efficient improvement of the macro-mechanical behaviour of coarse sand treated by various amounts of bio-cement materials requires an in-depth understanding of its microstructure. This paper examines the effect of number of bacterial suspension and cementation solution treatments on the macro-mechanical behaviour of coarse sand. Also, X-ray computed tomography (XCT), scanning electron microscopy (SEM), and energy dispersive spectroscopy (EDS) were used to investigate the changes occurring at micro levels. The results show that the compressive strength increased with an increase in bio-cement materials, and the maximum compressive strength achieved was around 14 MPa. The microscopic investigations were linked to the macro-mechanical changes, thus providing unique insight into the causation of the changes. Furthermore, several common soil properties (calcium carbonate content, dry density, void ratio, and porosity) were successfully identified using the XCT technique.

7.1 Introduction

Biocementation using microbial-induced calcite precipitation (MICP) is an environmentally benign alternative to conventional chemical grouting used to improve soils with poor geotechnical engineering properties. This process has been recently utilised in numerous geotechnical applications such as, liquefaction mitigation, dust suppression, protection of coastal areas, slope stability, subgrade reinforcement, among many other (DeJong et al. 2006, 2010, Mortensen and DeJong 2011, Montoya 2012, Weil et al. 2012, O'Donnell and Kavazanjian, 2015, Stabnikov et al. 2015, Mahawish et al. 2016, 2018a, Naeimi and Chu, 2017). The biocementation process refers to a natural biochemical reaction that takes place within a soil matrix to induce an insoluble bio-product (calcium carbonate precipitation) in the pore spaces and/or at the surfaces of the soil particles (Chu et al. 2012). This forms links/bonds between the soil particles to modify some engineering properties of the soil (DeJong et al. 2010). The calcium carbonate precipitate acts as a cementing agent to bind the soil particle together, and thus improves the strength and reduces the hydraulic conductivity of the soil. Biocementation strengthening effect on soils has been commonly assessed using the unconfined compressive strength (UCS) test as reported by a number of studies (Whiffin et al. 2007; Van Paassen et al. 2010; Harkes et al. 2010; Al Qabany and Soga 2013; Cheng et al. 2013; Nafisi and Montoya 2018; Mahawish et al. 2016, 2018a, 2018b, 2018c).

The most common biocementation processes relies on ureolytic (urea hydrolysis by non-pathogenic) bacteria, such as *Sporosarcina pasteurii* (formerly *Bacillus pasteurii*), which have been shown to be effective because of their ability to produce high amount of carbonate within a short period of time due to their high urease activity (Bang et al. 2010, Dhami et al. 2013, Wei et al. 2015). This bacterium impacts the concentration of dissolved inorganic carbon (DIC) and soil pH by catalysing the hydrolysis of urea (Wei et al. 2015). The bacterium may also provide a nucleation site for calcium carbonate precipitation as the negatively charged bacterial cell

wall adsorbs divalent cations (in this case calcium ions Ca^{2+}) to its walls (Hammes and Verstraete 2002, Thawadi 2008).

Ureolysis is initiated through a series of chemical reactions by urease-producing bacteria which catalyse the hydrolysis of urea to create ammonia (NH_3) and carbon dioxide (CO_2). Further hydrolysis of ammonia (NH_3) to ammonium (NH_4^+) and hydroxide ions (OH^-) increases the pH value around the bacterial cells. The carbon dioxide produced is readily soluble in water and forms bicarbonate (HCO_3^-), which reacts with the hydroxide ions to induce carbonate ions, and in the presence of calcium ions, calcium carbonate ($CaCO_3$) is precipitated (Stocks-Fischer et al. 1999, Dick et al. 2006, Phillips et al. 2013). The overall ureolysis process, including microbially induced formation of calcium carbonate on a soil particle, is presented by Dejong et al. (2010).

Although the bio-cementation process and its implication on the improvement of soil engineering properties have been extensively studied over the past decade at laboratory scale (Whiffin et al. 2007, Van Paassen et al. 2009, DeJong et al. 2010, Al Qabany et al. 2012, Rong et al. 2012, Cheng and Cord-Ruwisch 2014, Shahrokhi-Shahraki et al. 2014, Stabnikov et al. 2015, Mahawish et al. 2016, 2018a), uncertainty remains with regards to its overall effectiveness at field scale due to localised inhomogeneity of deposited calcium carbonate. Calcium carbonate precipitation is usually quantified based upon small sub-samples of bio-cemented soil that are taken from locations which may not necessarily represent the entire bio-cemented soil sample. Inhomogeneous distribution of biocementation can lead to erroneous conclusions (Terzis and Laloui 2017).

Linking the macroscopic mechanical behaviour of a bio-cemented material with a calibrated microscopic tool such as X-Ray computed tomography (XCT) may prove a more reliable method to evaluate changes in material engineering properties (Tagliaferri et al. 2011, Mahawish et al. 2017; Terzis and Laloui 2018). XCT is a non-destructive 3D imaging technique

capable of closely describing and analysing internal structures of soils, to a resolution of less than a micron.

This paper explores the use of laboratory-based XCT to track microstructure changes in the amount and distribution of calcium carbonate along a bio-cemented sand sample associated with its macro-mechanical behaviour. Other soil characteristics such as porosity, dry density, void ratio and pore volume that could change due to the bio-grouting process were also identified and analysed in the current study.

7.2 Materials and methods

7.2.1 Type of bacteria and cementation solution

The urease producing bacteria used was *S. pasteurii* (ATCC® 11859). One colony from the stock culture (Petri dishes containing bacterial colonies, 20 g/L yeast extract, 10 g/L ammonium sulphate and 20 g/L agar in 130 mM Tris buffer (pH 9.0), Stocks-Fischer et al. 1999) was transferred to 5 mL of ammonium-YE growth medium (20 g yeast extract, 10 g $(NH_4)_2SO_4$ and 130 mM tris buffer (pH=9.0) per litre of distilled water) to grow the bacterial cells at 30°C. Individual ingredients of the recipe were separately dissolved in distilled water bottles and autoclaved separately ((121°C, 100 kPa for around 2 hr process) and then combined post sterilization. Suspended bacterial cultures were grown at 30°C in a shaking water bath (200 rpm) for 24 hrs before harvesting at a final optical density (OD_{600}) of 3.0–3.5 to achieve an optimum concentration of bacterial cells in which all readily available nutrients were considered to be consumed from the growth medium (i.e., the start of the stationary growth phase; Cheng and Cord-Ruwish 2012). The final optical density was determined using WPA CO 8000 spectrophotometer (BioChrom Ltd) taking into account the ten time dilution factor.

The maximum optical density of the harvested bacterial cells was used to estimate an activity value of 19.38-21.45 mM/min for urease enzyme. Urease

activity was based on an electrical conductivity assay (labCHEM-CP) result, which was converted to an ureolysis rate using equation 7.2 (Whiffin 2004) based on electric conductivity:

Urea hydrolysed (mM) = Conductivity (mS) x 11.11   (R^2 = 0.9988)   (7.1)

The preliminary culture medium was transferred aseptically to inoculate 250 ml of growth medium, and the previous process can hence be repeated as required. The bacterial suspension was stored up to 14 days at 4°C until used, and at the time of use had a urease activity of 19.38 mM/min.

The cementation (reagent) solution consisted of 1 M urea (CO (NH_2)$_2$) and 1 M calcium chloride $CaCl_2$. The cementation solution has been used according to the technique adopted in this study.

7.2.2 Soil type and soil column preparation

A commercially available sand (Grade 8/16, supplied by Unimin Australia Pty. Ltd.) was used in the current investigation. It is classified as poorly graded coarse sand (SP) according to the Unified Soil Classification System (ASTM 2006). It had an average grain size of 1.6 mm (Figure 1). Maximum and minimum dry density measurements were conducted in accordance with ASTM D4253 (2014a) and ASTM D4254 (2014b) to control the density of the column model and were 1.70 g/cm^3 and 1.44 g/cm^3, respectively. Mix properties (specific gravity (Gs), coefficient of curvature (Cc), coefficient of uniformity (Cu), the medium grain size (D_{50}), maximum and minimum void ratios (e_{max} and e_{min})) are summarised in Table 1. The sand particles were typically sub-angular in shape to represent surfaces of stone aggregate commonly used in full-scale stone columns (Gniel and Bouazza 2009, 2010) since the intention was to eventually develop rigid columns suitable for improvement of soft and weak soils. The sand columns had a length to diameter aspect ratio of 2:1. A polyvinyl chloride (PVC) pipe, 220 mm high and with an internal diameter of 51 mm, unless otherwise stated in the case of specimens prepared for XCT, was cut in half length-wise to form a split-mould for the columns. This enabled demoulding without disturbance of the

treated coarse sand samples. A wire mesh and filter paper were placed at the bottom of the mould to minimise possible losses of soil particles during preparation and treatment. The sand-filled moulds were positioned vertically with the upper surface fully exposed, and a drainage control valve was connected to the base. Dry coarse sand was pluviated into the specimen moulds and then compacted, using a vibratory compaction method, to a relative density of 60% (equivalent to a dry density of 1.59g/cm^3) due to their sub-angular particle shape and poor grading. After placement of soil, tap water (200% pore volume (PV) of sand columns (160 mL)) was flushed through it to expel the extra air from the pore matrix. This step was done by closing the bottom valve, percolating deionised water until ponding on top of the specimen, then opening the bottom valve, the percolation of water was continued until reaching to 2 pore volumes (PV). After completion of the bio-cementation treatment cycles, all the samples were flushed with tap water (1 litre, this is equivalent to over 12 times the pore volume of sand column) to remove residual chemical reagents from the pore space. Zeng et. al 2018 also mentioned that using at least 10 pore volume water could cut off the reagent reactions and eliminate the effect of salts on strength. The samples were air dried for 14 days at 20±2°C, and then were taken out of the mould to prepare them for the UCS and the XCT tests. The degrees of saturation of the specimens following treatment for 0, 4, 8, 16, and 32 treatment cycles were respectively 8.67%, 9.19%, 10.97%, 13.83% and 18.45%. This indicates that the specimens had very low water content in which the negative pore water pressure had a less effect on the UCS attained.
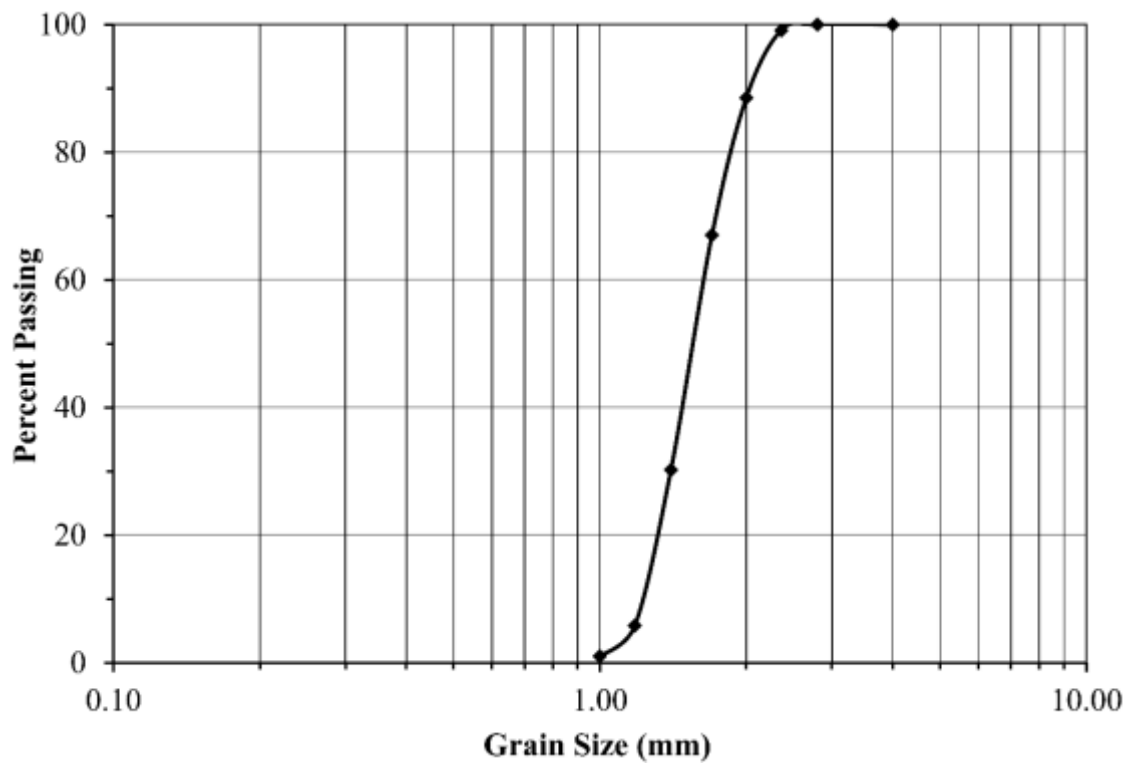
Figure 7.1. The particle size distribution of coarse sand.

Table 7.1: Properties of coarse sand.

Soil Type	Classification	Gs	Cc	Cu	D_{50} (mm)	e_{max}	e_{min}
Grade 8/16	SP	2.64	0.97	1.35	1.60	0.84	0.55

7.2.3 Soil column treatment procedures

A percolation method was used to promote biocementation of the very coarse-grained soil specimens as described in Mahawish et al. (2016, 2017b, 2018a). The protocol adopted for this study was a four-phase percolation strategy. The amount of bacterial suspension and cementation solution in each treatment cycles was determined based on the pore volume (pore water capacity) of the soil columns. The strategy consisted of the following steps:

i.   Flushing cementation solution (100% pore volume ml of sand columns) to promote high ionic strength within the pore matrix. The divalent calcium ions increase the ionic charge at the particle surfaces. Urea plays a significant role in providing a suitable environment for the bacterial cells as they have a high affinity for urea. This step was applied once before the beginning of the treatment cycles.

ii.   After the cementation solution volume has been drained out from each specimen, the bottom valve was closed. A lesser volume (12.5% PV) of bacterial suspension (BS) and cementation solution (CS) without ammonium-YE media were alternately but individually percolated to achieve a four-phase percolation protocol (12.5% PV BS + 12.5% CS + 12.5% BS + 12.5% CS). The amount of liquids percolated is equivalent to 50% of the PV of the sand columns.

iii.   Incubation (retention time) for 24 hrs at 20±2ºC, allowing the percolated liquids to facilitate the occurrence of reactions between microorganisms and reagents solutions. When incubation time ended, the bottom valve was opened to discharge the liquids waste (effluent) and prepare the pore matrix for the next treatment.

iv. This treatment step included percolation of fresh cementation solution (50% PV, ml) only and allowed ureolysis and $CaCO_3$ deposition to take place for same retention time. The effluent was also discharged after finishing the incubation time for the purpose mentioned in the previous step.

Steps iii and iv were considered as two biochemical treatment cycles and were repeated until reaching the required number of treatment cycles (i.e. 4, 8, 16, and 32 cycles).

7.2.4 Unconfined Compressive Strength (UCS) tests

Samples with different amounts of $CaCO_3$ deposition depending on the number of biochemical treatment cycles were used to conduct UCS tests. All the tests were performed on samples having a 51 mm diameter with a selected height to diameter ratio of 2:1. The axial load was carried out at a constant strain rate of 1%/min. An Instron 4204 50 kN constant-displacement mode UCS machine equipped with a laser extensometer was used in the current study.

7.2.5 Calcium carbonate quantification

Calcium carbonate ($CaCO_3$) content was measured using a conventional gravimetric acid washing technique (Choi et al., 2017). For this purpose, subsamples from the specimens that were prepared and biotreated for UCS test and XCT (within 30 mm of the top, middle and bottom of the specimen) were first oven-dried at 105°C for 24 hrs, and their masses were measured before and after the acid wash. The quantification of $CaCO_3$ was determined by rinsing with 2M HCl several times through No.200 sieves and allowing the dissolved salts to be rinsed out from the samples. The difference between dry mass before and after acid washing was taken as the mass of $CaCO_3$ removed and the % $CaCO_3$ was obtained by dividing the mass of $CaCO_3$ by the mass of soil. The calcium carbonate was quantified in the specimens that were only treated with a cementation solution (i.e. no bacterial cells), and there was no trace of calcium carbonate in these specimens.

7.2.6 Porosity measurements

Subsamples from untreated and MICP treated coarse sand samples were taken from the UCS, and the XCT tested specimens to conduct porosity tests using the gravity method (Rong et al. 2012). The gravity method uses vacuum saturation and has the advantages to be fast and easy to apply. The subsamples were firstly dried in an oven at a moderate temperature of 60°C. When the mass of the samples became constant, their dry mass (M_{dry}) was recorded using a balance with 1 mg precision . Next, the subsamples were saturated using a vacuumed desiccator filled with distilled water; the fully saturated condition was achieved when the mass of the subsamples (denoted as M_{sat}) was stable. The stability of the subsamples mass was assessed based upon measuring the mass of the samples at different times using the same balance with a variation not exceeding 0.1%. Finally, the solids volume (V_s) was determined by immersion in de-aired water based on

the methodology described in Liu and Buzzi (2014). The porosity (n) was determined using equation 7.2 (Rong et al. 2012):

$$n = (M_{sat} - M_{dry}) / (\rho_{water} \times V_s) \tag{7.2}$$

For comparative purposes, mercury intrusion porosimetry (MIP) was conducted on a coarse sand sample treated by a higher number of biochemical treatment cycles (32 biochemical treatment cycles). The MIP test was performed using a mercury autopore IV porosimeter 9520. A subsample of highly bio-cemented coarse sand (around 1 g) was outgassed in a vacuum at 1.3 Pa and at a temperature of 150°C for 24 hrs in accordance with ASTM D4404 (2004). The porosity of the sample with a highest number of biochemical treatment was 19.78 %. The MIP gived lower porosity values lower than the experimental results.

7.2.7 Scanning electron microscopy (SEM) and energy dispersive spectroscopy (EDS)

Scanning electron microscopy (SEM) and energy dispersive spectroscopy (EDS) were undertaken to characterise particle bonding achieved when using a different number of biochemical treatment cycles (0, 4, 8, 16 and 32 treatments). Subsamples were retained after completing the UCS tests; they were oven dried; carbon coated, and then examined using an SEM (JEOL 7001F FEGSEM,) with a beam intensity ranging between 5 to 15 kV.

7.2.8 X-Ray computed tomography (XCT) and image processing

XCT tests were conducted on five bio-cemented coarse sand columns (0, 4, 8, 16 and 32 biochemical treatment cycles). The coarse sand samples were packed into small tubes up to 60% relative density, each with an internal diameter of 25 mm and 50 mm height. The coarse sand columns had similar dry density (ρ=1.59 ± 0.015 g/cm³). These sand columns were similar to the ones that were prepared for the UCS tests. For verification purposes, the amount of $CaCO_3$ in the central portion of the bio-cemented coarse sand

columns was quantified using the gravimetric acid washing technique as described earlier.

A ZEISS Xradia 520 Versa (Xradia, Pleasanton, CA, USA) X-ray CT-imaging instrument was used in this study to visualise changes in the microstructure of the uncemented and bio-cemented coarse sand. To acquire the image projections, the sample-stage was incrementally rotated 360° around its vertical axis to obtain 1004 image projections (X-ray computerised tomographs) with a pixel size of 25 microns. The acquired image projections represented the central portion of the bio-cemented coarse sand column (around 25 mm height above the base) to avoid edge effects. The image size of the projections was 1024 x 1024 pixels. The values of optimized XCT scanning parameters are shown in Table 7.2.

The 3D images were processed using the commercially available image processing software Avizo (V9.1. FEI, Hillsboro, OR, USA) to visualize and analyse the microstructure of the bio-cemented coarse sand specimens. For the analysis, 2D tomographs (image projections) were firstly reconstructed to 3D volume using an X-ray microscopy (XRM) software (Cone Beam-10, Xradia, Pleasanton, CA, USA) (Al Mahbub and Haque 2016). Next, the assembled 3D images were cropped from top and bottom regions to remove the excessive noises from these regions. The central 800 slices were selected in each sample to perform statistical calculations to minimise edge effects. After successfully applying the AVIZO interactive thresholding routine to satisfactorily segment the soil phases (voids, soil particles, and calcium carbonate) of each sample, the area of each phase was determined using the Avizo tools (Segmentation/Material Statistics tool). The segmentation process was applied to selected slices of the samples and then replicated to the entire reconstructed 3D sample. Different filter combinations were examined to reduce the remaining noises, and a suitable filter (Non-local means filter) was selected for all columns. Finally, a segmentation of the voids, soil particles and $CaCO_3$ was conducted, and materials statistics were recorded. The operations of image processing were performed by:

- Loading reconstructed 3D tomographs (greyscale images) of the coarse sand column.
- Cropping the coarse sand column to the height (20 mm) to visualise regions of low or no apparent noise.
- Applying non-local means filters for grayscale enhancement to reduce noise.
- Applying auto-thresholding to evaluate greyscale intensity ranges, and then applying interactive thresholding to segment voids, coarse sand particles and deposited $CaCO_3$.
- Scrutinising and manually adjusting the interactive thresholding to identify (visual judgment) voxel parameters as representing either voids, particle or $CaCO_3$ (Hasan and AlShibli 2010).
- Performing materials statistics of the segmented 3D images to quantify V_v, V_s, ρ, η and % $CaCO_3$ and the ratio of % $CaCO_3$ to V_s of coarse sand particles. The $CaCO_3$ content was obtained by dividing the volume of segmented $CaCO_3$ by the segmented volume of soil particles.

Table 7.2: Values of XRCT scanning parameters.

Parameter	Value
Voltage (keV)	140
Power (W)	10
Exposure Time (s)	0.5
Camera binning	2
Source to Sample Distance (mm)	50
Detector to Sample Distance (mm)	90
Geometric magnification	2.8x
Objective (scintillation screen)	0.4
Pixel Size (μm)	25

7.3 Results and discussion

7.3.1 Macroscopic mechanical behaviour of bio-cemented coarse sand

The macroscopic mechanical response of the biochemically treated coarse sand, as assessed by UCS, is related in Figure 7.2 to the % $CaCO_3$ precipitated in three regions (top, middle and bottom) of the biocemented coarse sand columns. Figure 7.2 shows that $CaCO_3$ deposition increased with increasing number of biochemical treatments. For instance, <3% (of total mass) of $CaCO_3$ was deposited after 4 biochemical cycles, whereas around 22% of $CaCO_3$ was precipitated after 32 treatment cycles. The average %$CaCO_3$ deposited increased from 6.74% to 13.20% as the number of treatments increased from 8 to 16 cycles. This is acceptable as more treatment cycles mean more reactants (bacterial suspension and cementation solution). However, the coarse sand samples were also treated with the cementation solution only and showed no UCS improvement as they could not support any load even their own weight.

The pore spaces of coarse sand columns are quite large and therefore require a higher number of biochemical treatment cycles (i.e. 32 cycles), which played an essential role in the change of the mechanical behaviour of coarse sand. Additional bacterial suspension cycles, based on the adopted strategy reported herein, may, therefore, be considered a key factor to precipitate more $CaCO_3$ in the environment containing a high concentration of cementation solution (1M). This increase in % $CaCO_3$ is attributed to the higher concentration of substrates introduced into coarse sand pores due to multiple biochemical treatment cycles. It is also showed that only one flush of bacterial suspension in the coarse sand column was insufficient to have a high amount of calcium carbonate precipitation (Mahawish et al. 2018b).
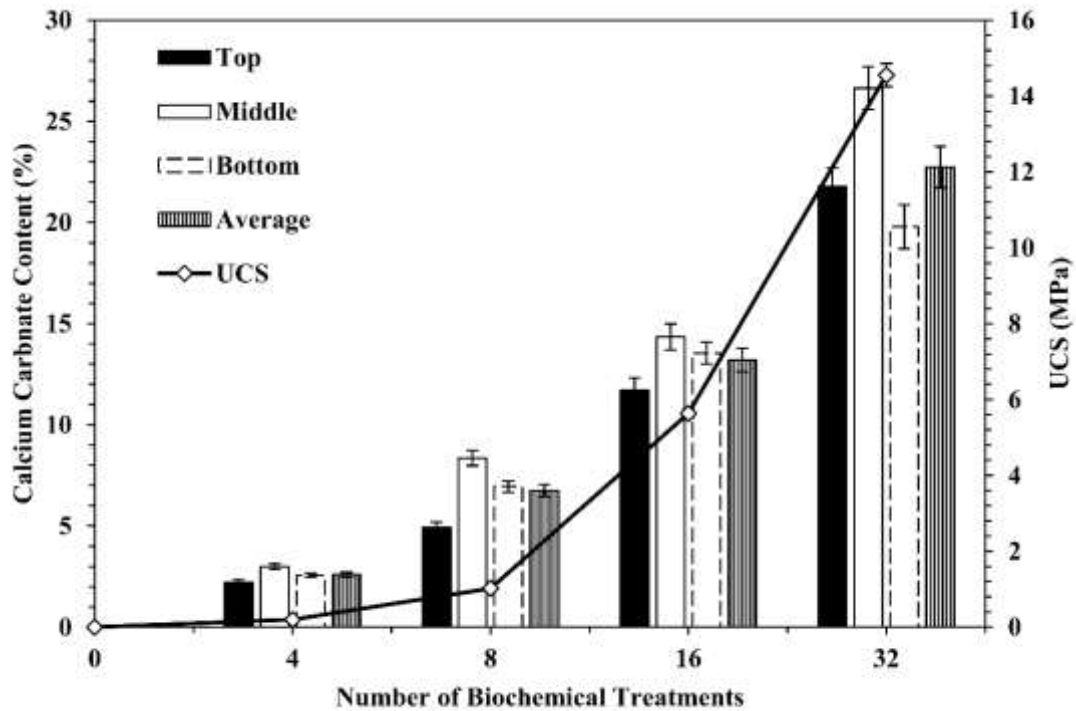
Figure 7.2. Variation of number of biochemical treatment cycles with precipitated calcium carbonate at three regions (top, middle and bottom) and UCS of bio-cemented coarse sand.

Figure 7.2 shows that as the very coarse silica sand received the biochemical treatment cycles, its UCS increased as the amount of $CaCO_3$ precipitation increased. The UCS had an approximately linear relationship with the number of biochemical treatment cycles in the range of 8 to 32 cycles. This is again related to the increase of the mass of $CaCO_3$ deposited, which provided more bridges between coarse sand particles to create a solid mass (Mahawish et al. 2017b).

An insight into the microstructural changes is provided later in the paper to support the findings related to the shear strength of treated coarse sand.

7.3.2 Microscopic investigations of coarse sand

7.3.2.1 Microstructure visualisation

Figure 7.3 shows the segmentation of the coarse sand specimens that underwent 0, 4 , 8, 16 and 32 biochemical treatment cycles (referred to as

S0, S4, S8, S16 and S32, respectively). The intensity ranges (0-65535 voxels) used in the current study allowed the investigation of the inner structure of untreated and bio-cemented specimens. This inner structure was divided into three regions (coarse sand particles, void spaces and the deposited $CaCO_3$) as shown in Figure 7.3a, b, c, d and e. Figure 7.3a shows that the untreated coarse sand specimen (S0) had significant void spaces. These void spaces were reduced after conducting 4-biochemical treatment cycles as precipitation of $CaCO_3$ occurred between coarse sand particles as shown in Figure 7.3b. It is interesting to note that only a small number of biochemical treatments could induce precipitation of effective $CaCO_3$ crystals that bridged soil particles (Figure 7.3b and Figure 7.4b). This is further confirmed by the UCS results shown earlier where a gain in strength is evident in S4 compared to S0 (Figure 7.2). Also, using the XCT technique, it was found that ≈3% $CaCO_3$ precipitated in the coarse sand column when treated with up to 4-biochemical treatments, whereas the laboratory acid washing technique indicated that the % $CaCO_3$ was around 2.9%. The error percentage between XCT and laboratory determined % $CaCO_3$ was approximately 0.1%. Hence, one can infer that XCT can be used to quantify MICP within the pore matrix of soil with a high level of accuracy.

Figure 7.3c displays the inner structure of the bio-cemented coarse sand specimen which underwent 8 biochemical treatment cycles (S8) and shows the presence of $CaCO_3$ crystals. This is further evidenced in Figure 7.4c where it can be seen that these crystals are large and bridging has occurred between the coarse sand particles. It was reported earlier that the UCS of the specimen S8 was higher than the one which underwent 4-biochemical treatment cycles (S4). This increase in UCS is attributed to the larger amount and distribution of $CaCO_3$ precipitation in S8 compared to S4 as can be seen when comparing Figure 7.3b to Figure 7.3c.

Figure 7.3d and e show a segmentation of heavily bio-improved coarse sand specimens (S16 and S32, respectively). The $CaCO_3$ crystals deposited are evident in inter-particle and intra-particles of S16 and S32. $CaCO_3$

deposits (Figure 7.3d and Figure 7.3e) were thicker than S8 for example (Figure 7.3c). The most important feature shown in Figure 7.3d and e is the distribution of $CaCO_3$ crystals, deposited within the pores and at the surfaces of the coarse sand particles, is consistent with previous observations reported by Martinez and DeJong (2009) using scanning electron microscopy (SEM) analysis. It can also be seen in Figure 7.3d and e that the distribution of $CaCO_3$ over the diameter of S16 and S32 was random (some particles were heavily cemented by $CaCO_3$). This may be evidence of microbes that were augmented within the pore matrix of the coarse sand columns as shown in Figure 7.5c. It can also be seen that the pore spaces were reduced as a result of the precipitation of large crystals (Figure 7.4e and Figure 7.5a and b). The XCT results indicated that around 12% and 26% $CaCO_3$, respectively, was present in S16 and S32. This is in agreement with the $CaCO_3$ measured by acid dissolution (about 11% and 25%, respectively). It is also worth noting that as the layer of precipitated $CaCO_3$ increased, the UCS increased. Hence, image analyses could be used as an alternative approach to the acid wash technique to quantify the amount of precipitated $CaCO_3$ and also visualise how the increase in the amount of $CaCO_3$ led to the increase in UCS via bio-cementation.

Figure 7.3. XRCT Visualisation of coarse sand samples treated with various number of biochemical treatment cycles: (a) control sample (no treatment, S0); (b) S4,4 cycles, 2.93% $CaCO_3$; (c) S8, 8 cycles, 6.84% $CaCO_3$; (d) S16, 16 cycles, 10.87% $CaCO_3$; and (e) S32, 32 cycles, 24.44% $CaCO_3$.

Further investigations of the microstructural effects of biocementation were also conducted using scanning electron microscopy (SEM) and energy dispersive spectroscopy (EDS). SEM analysis was conducted on the surface of bio-cemented coarse sand specimens, which have undergone various levels of cementation (i.e. 0, 4, 8, 16, and 32 treatment cycles). The surfaces of the control specimen (0 treatment cycle) and the post treatments specimens were compared to assess the impact of applying a different level of bio-cementation on the microscopic changes of coarse sand fabric characteristics. The untreated coarse sand consisted of large particles (Figure 7.4a) with smooth, clean and even surfaces and was comprised mostly of silica (Figure 7.6a). Figures 7.4b to 7.4e show the micrographs of coarse sand treated with a various number of biocementation cycles. Application of 4 biochemical cycles led to the appearance of a thin layer of $CaCO_3$ precipitation as shown in Figure 7.4b. Bio-cementation transited from isolated clusters of $CaCO_3$ (Figure 7.4b), when less number of cycles were used, into deposits largely covering the coarse sand grains (Figure 7.4e and Figure 7.5a and b) when a high number of biochemical cycles (32 treatments) were used. It is hypothesised herein that each biochemical cycle provided nucleation points for $CaCO_3$ precipitation dispersed randomly at the surfaces of coarse sand particles (see Figure 7.5a-c). It is also worth noting that Figure 7.3 shows that with increasing treatment cycles, carbonates were more widely distributed throughout the pore surfaces, implying that nucleation were occurring even after multiple treatment cycles. Crystal growth also occurred as the areas in which nucleation was observed (i.e. right half of 3b and the inset to 3b) showed a greater amount of carbonate in these areas after many cycles (e.g. 3d, e). The insets of Figure 7.3c, d, e show nicely this crystal growth as well. As the $CaCO_3$ nucleation points grew, they provided important bridging contacts between sand grains, thereby increasing strength and stiffness from nil (with control sample) to around 200 kPa at 4 treatment cycles (Figure 7.2). SEM micrographs (Figure 7.4) are in agreement with the outcomes from XCT

images as they verified that the deposited $CaCO_3$ was found on the surfaces of coarse sand samples.
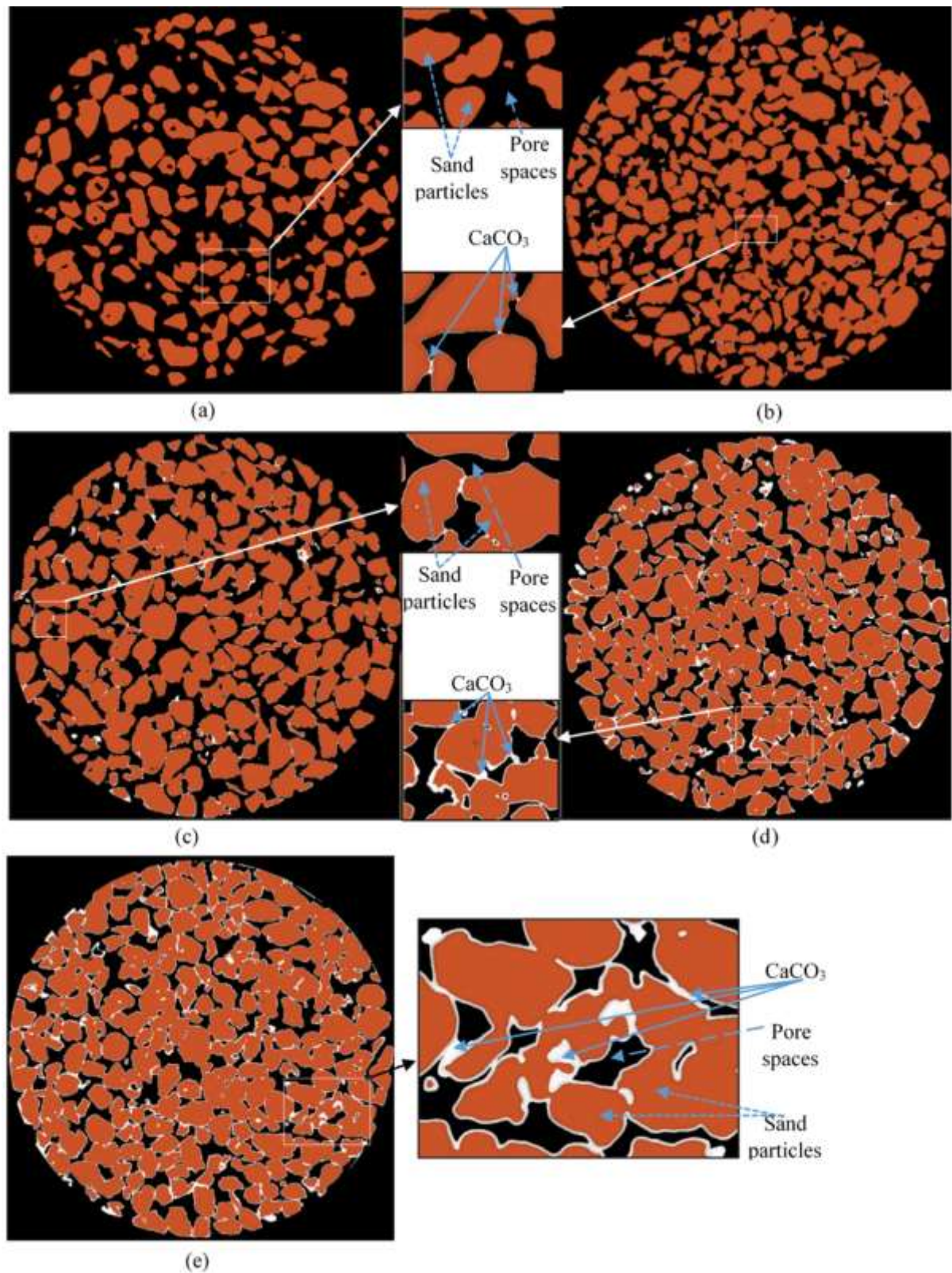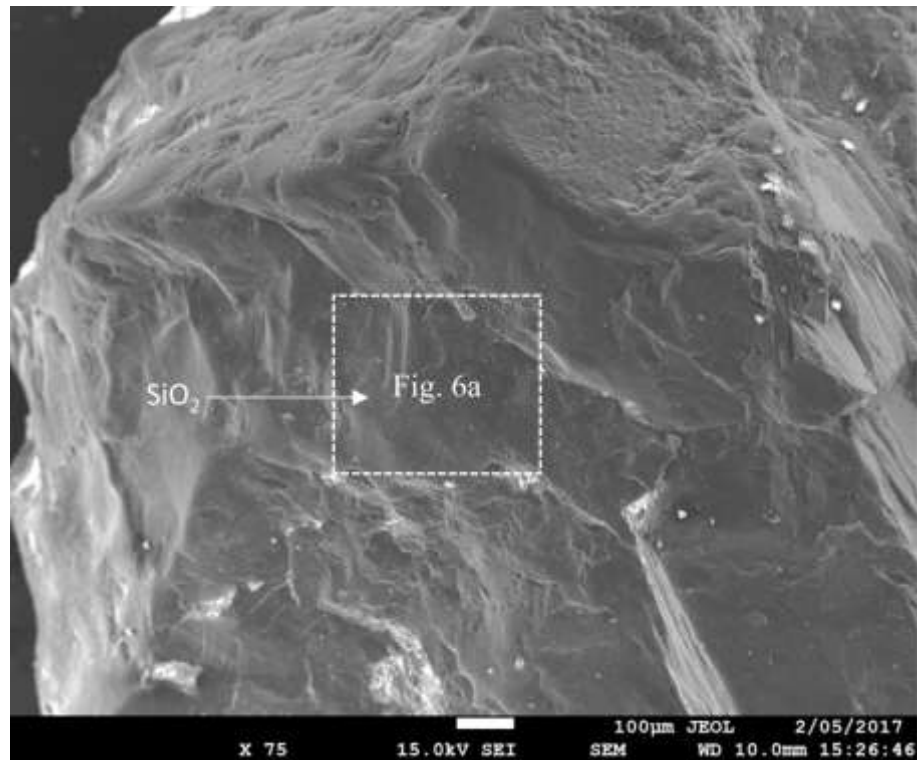
(a)

(b)
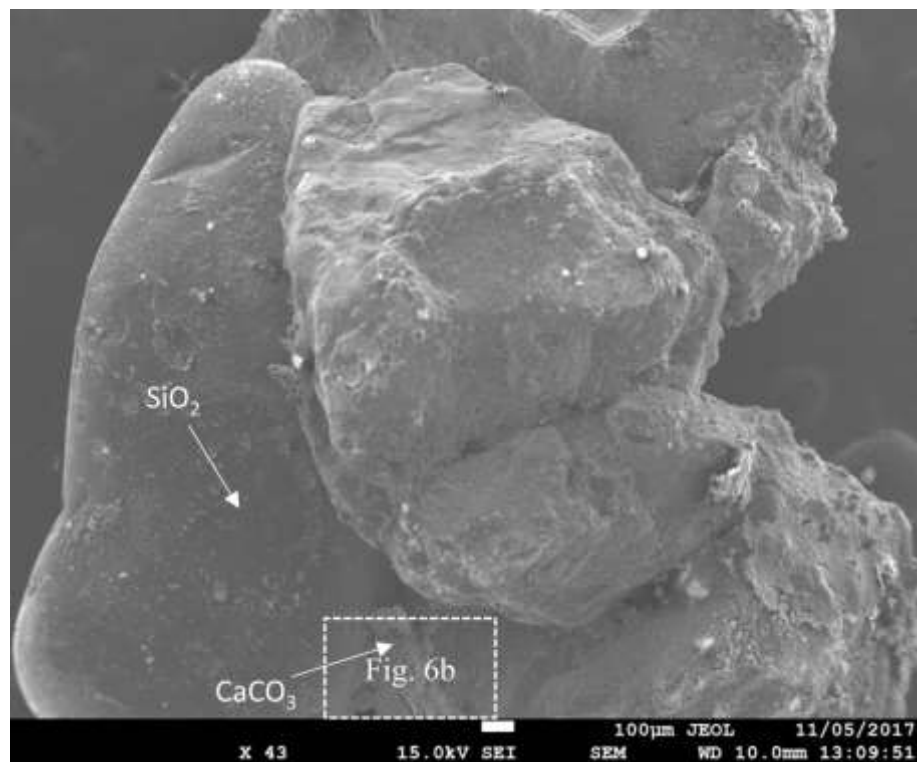
(c)
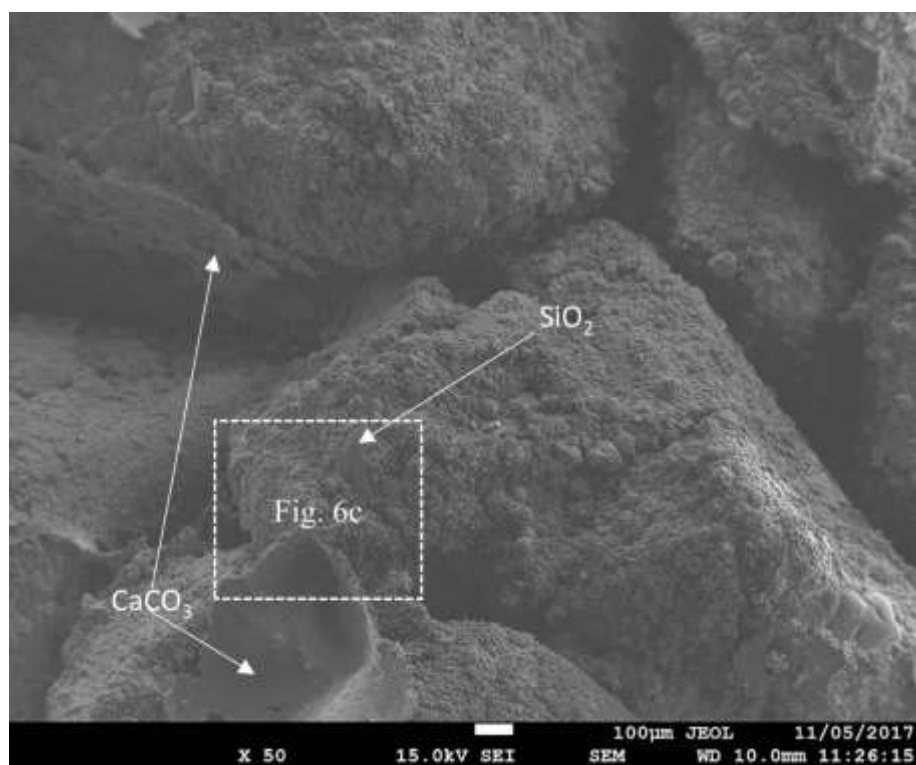
(d)
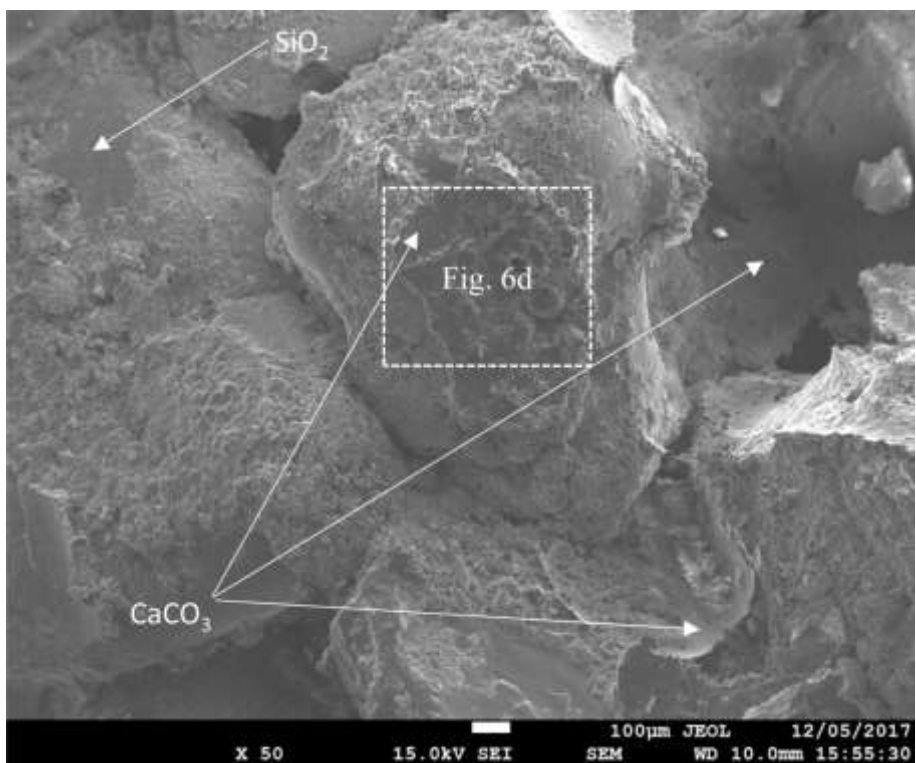
(e)
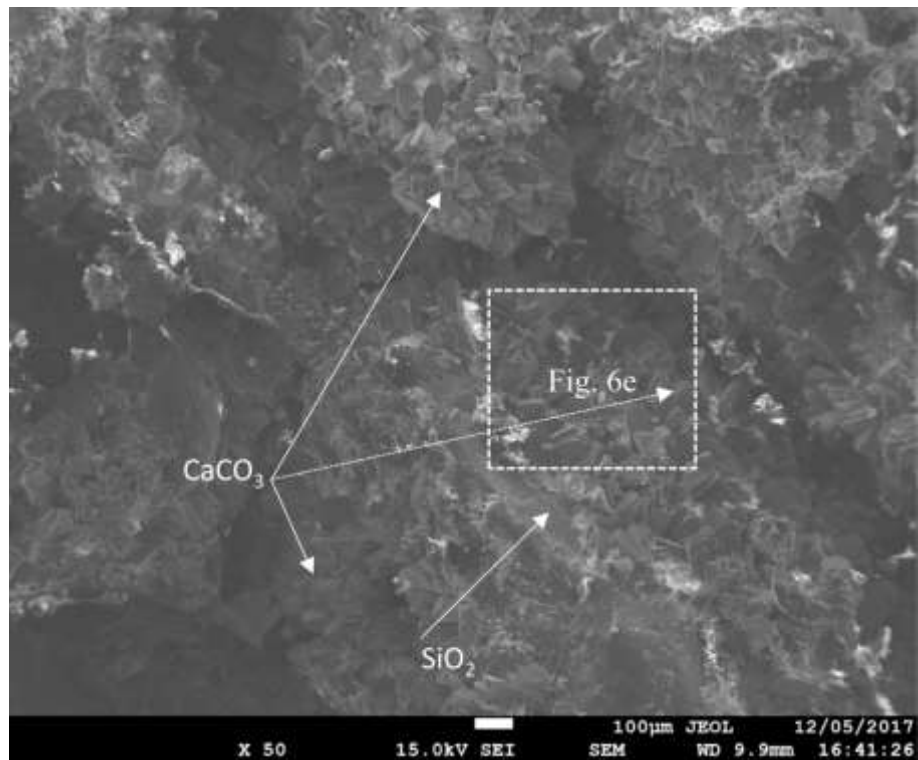
Figure 7.4. SEM of coarse sand samples treated with various number of biochemical treatment cycles: (a) control sample (no treatment cycles, S0); (b) S4, 4 treatment cycles; (c) S8, 8 treatment cycles; (d) S16, 16 treatment cycles ; and (e) S32, 32 treatment cycles.

(a)

(b)

(c)

(d)
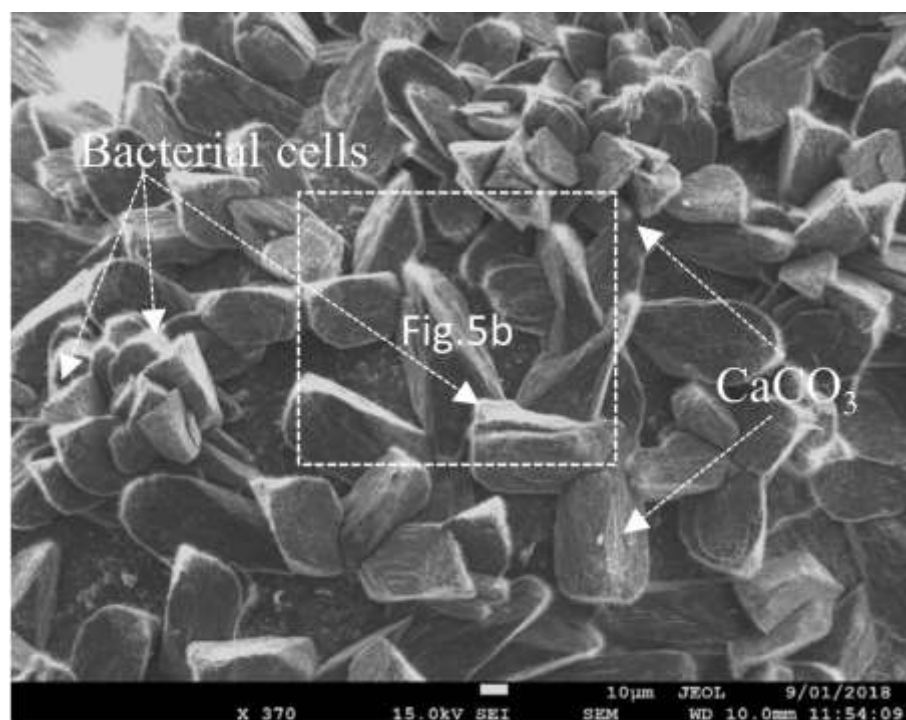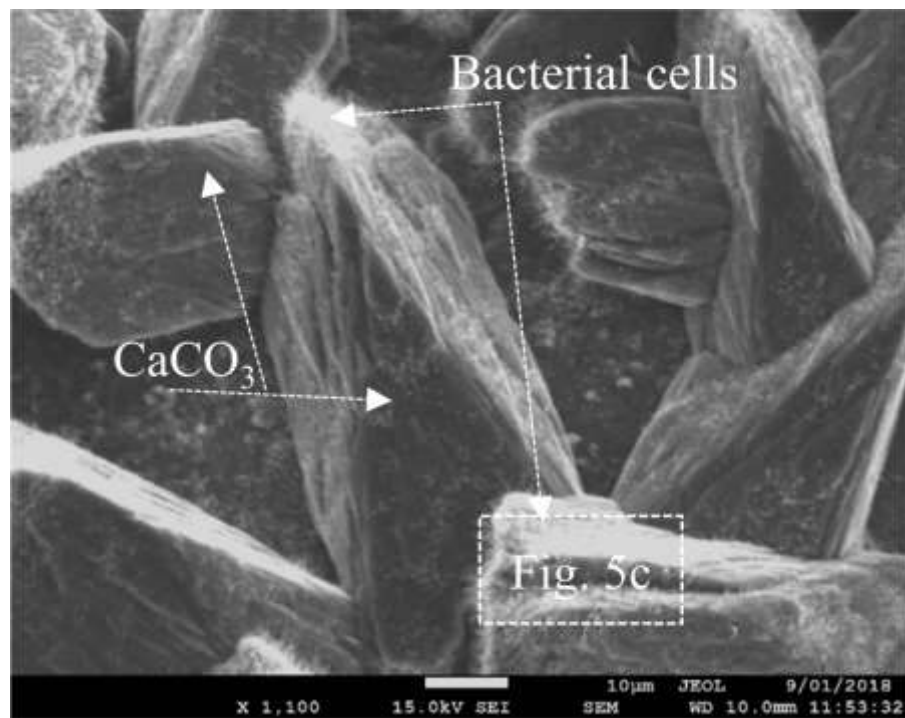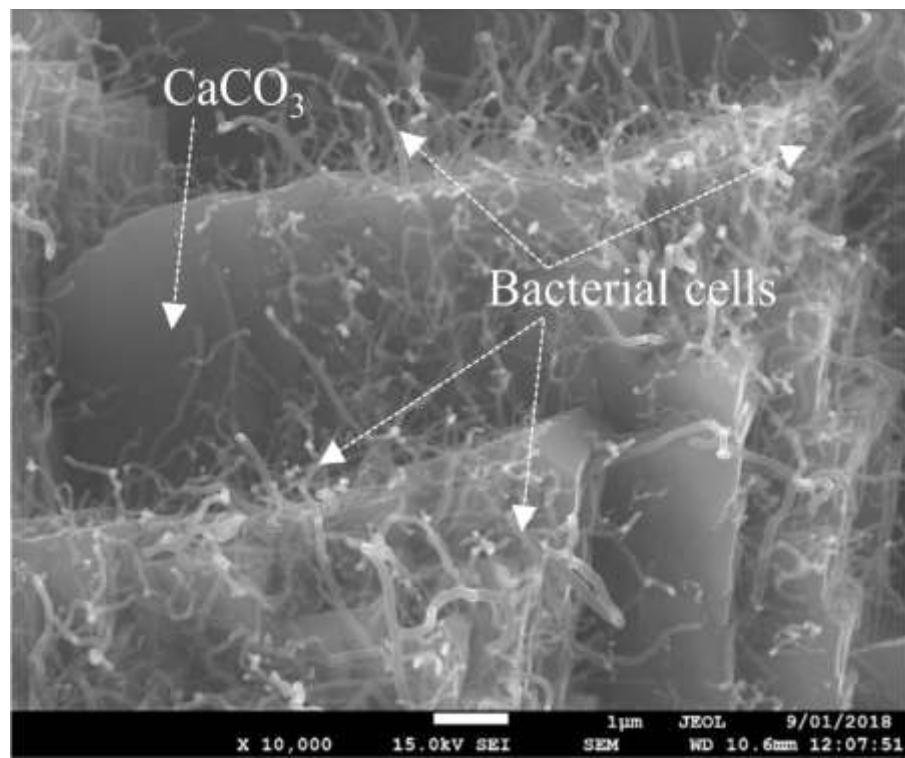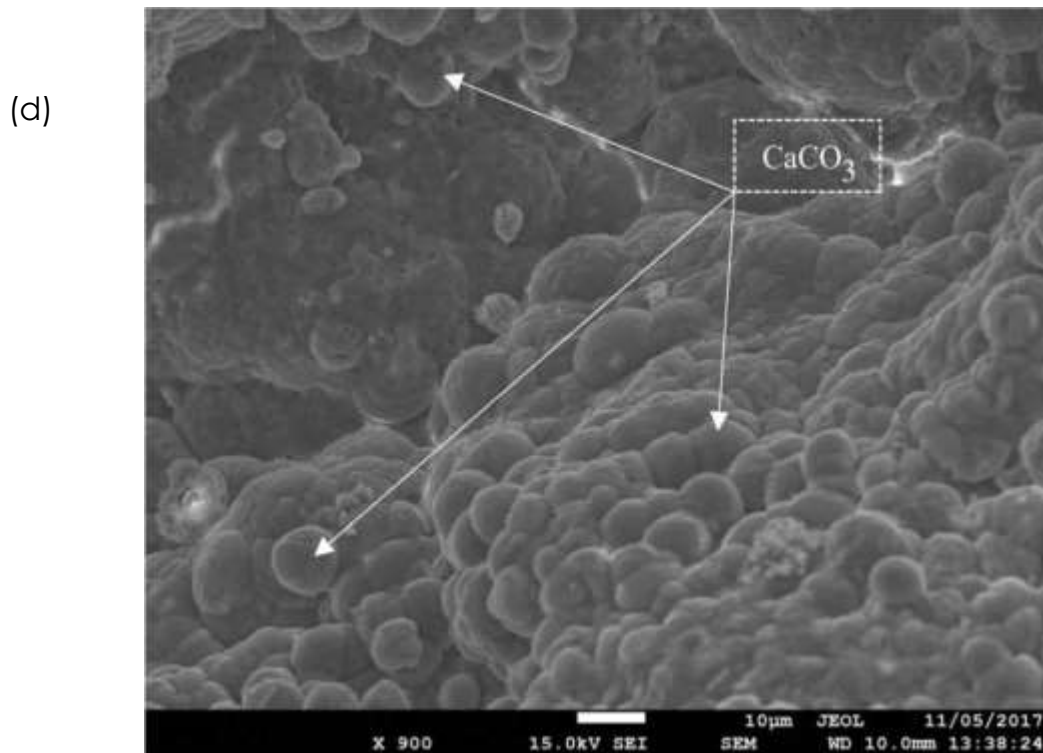
Figure 7.5. SEM for coarse sand treated via heavy and light biochemical treatments: (a) Large calcium carbonate crystals resulted from a heavy treatment (b) Trigonal-rhombohedral calcium carbonate crystals; (c) An evidence of a microbe augmentation; (d) spherical-shape crystals resulted from lightly bio-cementation.

Furthermore, the EDS data (Figure 7.6b-e) indicate that the chemical compositions comprised mainly calcium, oxygen, carbon, and silica, implying that bio-cementation is responsible for increased UCS compared to untreated coarse sand samples (Figure 7.6a). It is also worth noting that large trigonal-rhombohedral $CaCO_3$ crystals precipitated in the coarse sand matrix (Figure 7.4e and Figure 5a and b) when a high number of biochemical treatment cycles (i.e. 32 treatments) were applied. The identified $CaCO_3$ crystals form an irregular morphology (about 100 microns thick), which could be created by the superposition of thin layer flakes of $CaCO_3$ having approximately trigonal to rhombohedral forms. The deposited calcium carbonate at a higher number of biochemical treatments (32-treatment cycles) can be considered as calcite based upon

its morphology (Li et al. 2011). However, spherical-shape calcium carbonate crystals (about 10 microns thick) deposited in the coarse sand specimens resulted from using 4-biochemical treatment cycles was also observed microscopically (Figure 7.5d). Van Paassen (2009) reported that spherical-shape crystals, resulting from MICP, were identified as vaterite based on X-ray diffraction (XRD) analysis. Thus, different crystal morphologies can be deposited depending on the number of biochemical treatment cycles used and in all likelihood the form will change with time (in situ) and subsequent to any treatment during the study (e.g., drying for XRD analysis).
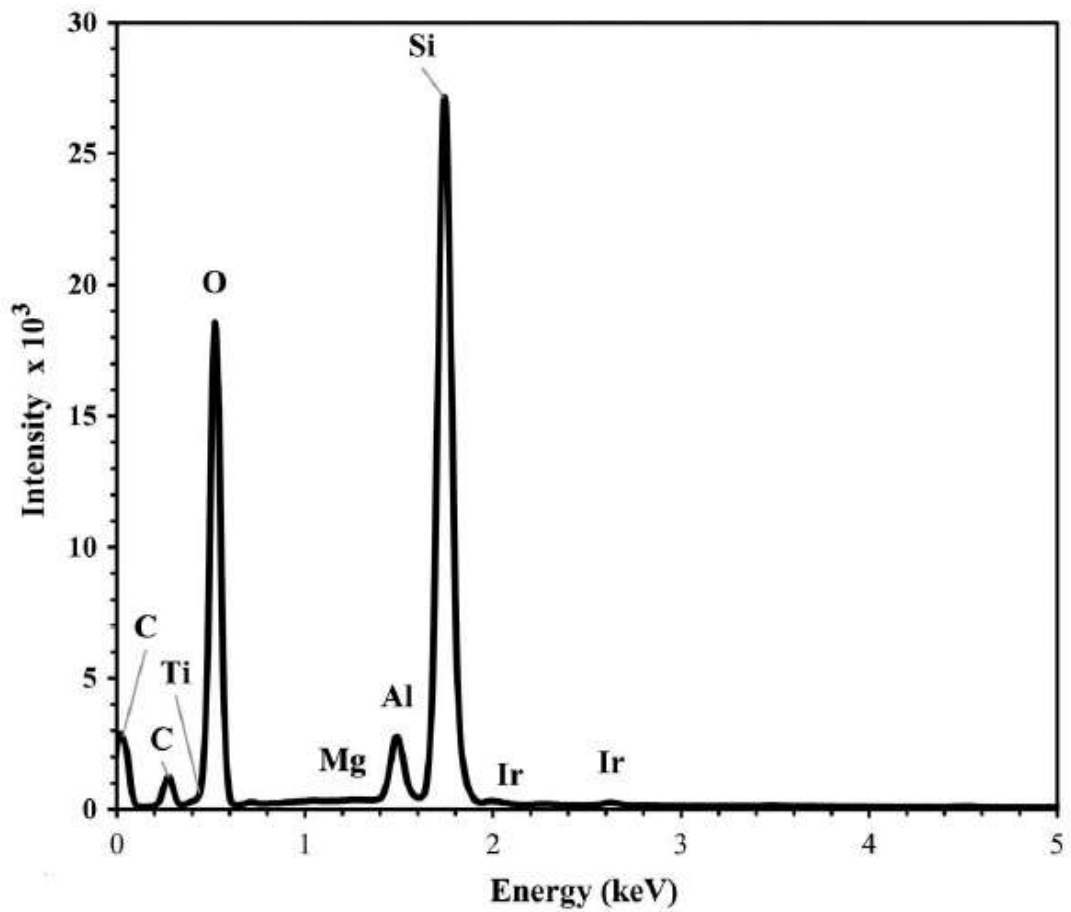
(a)
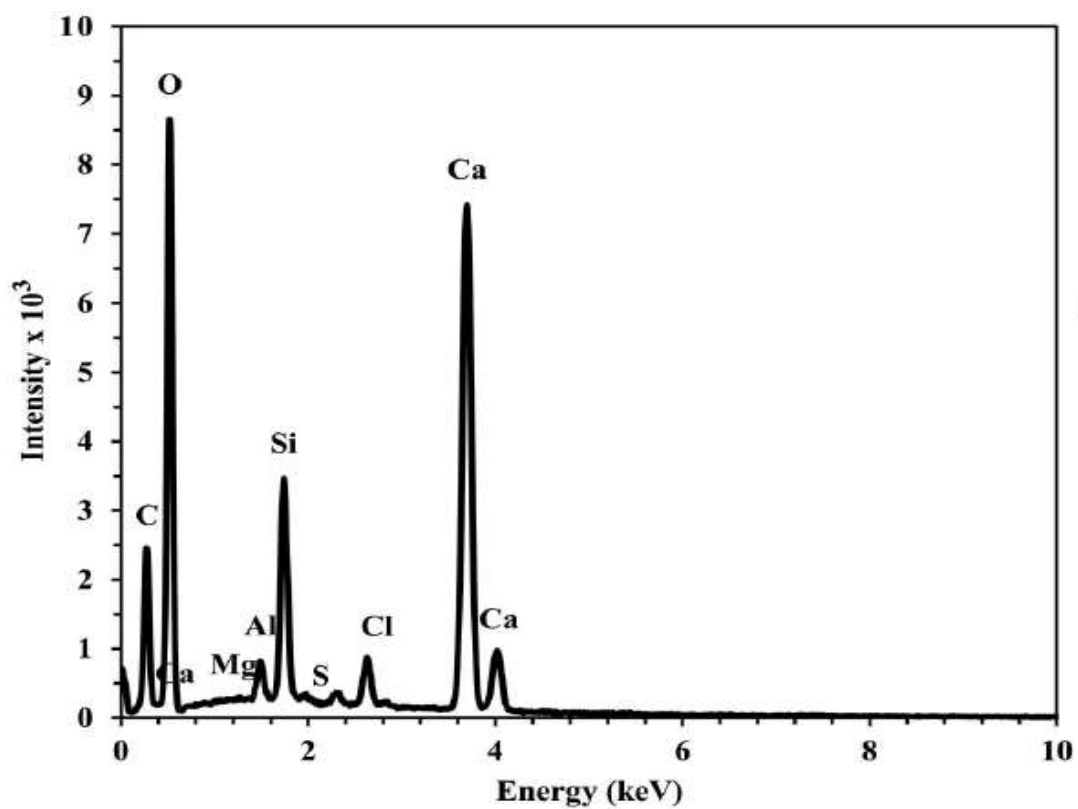
(b)
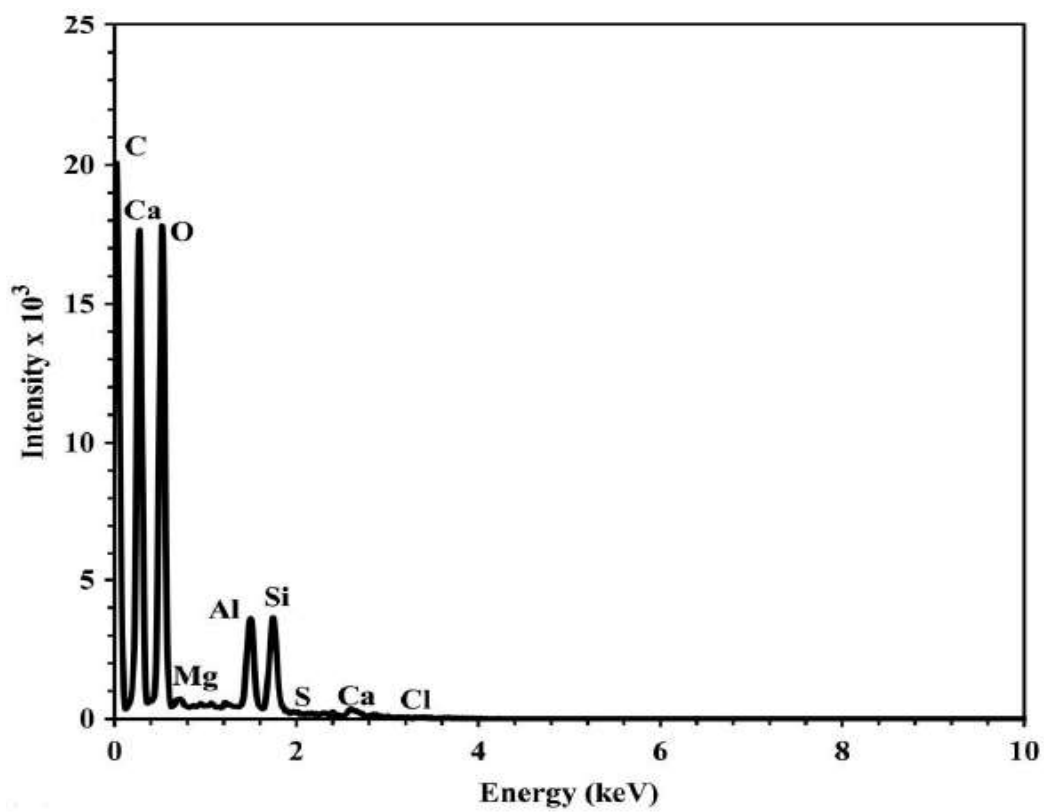
(c)
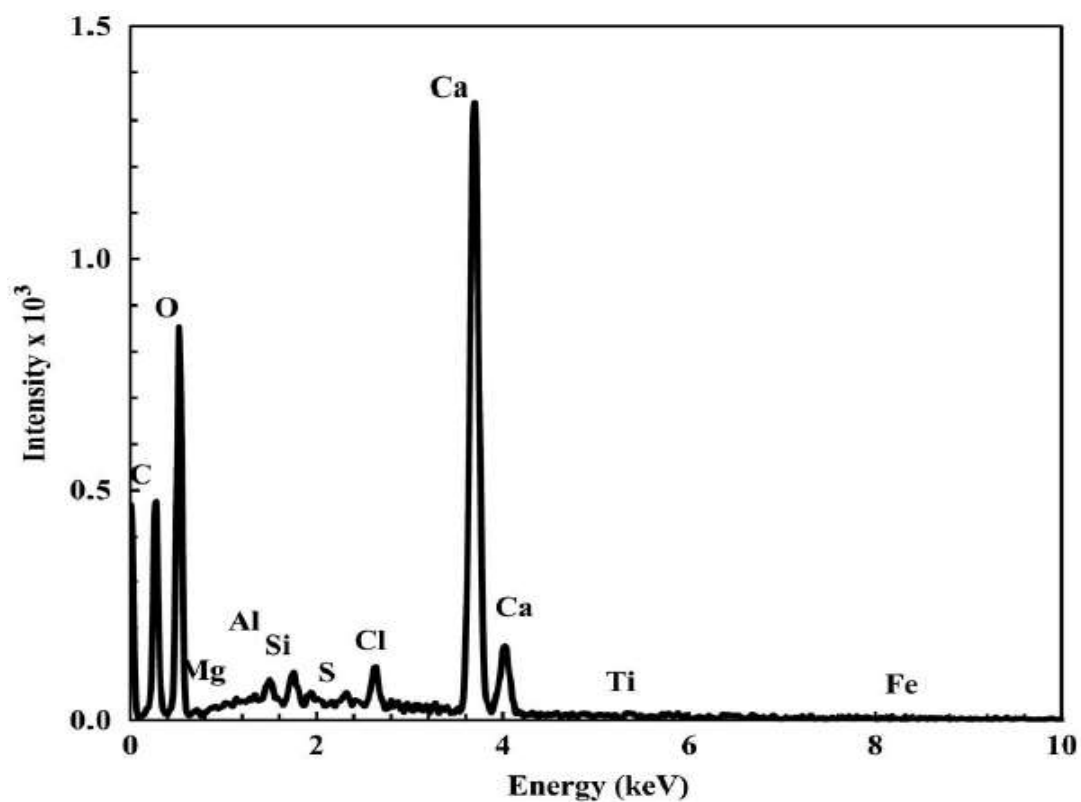
(d)
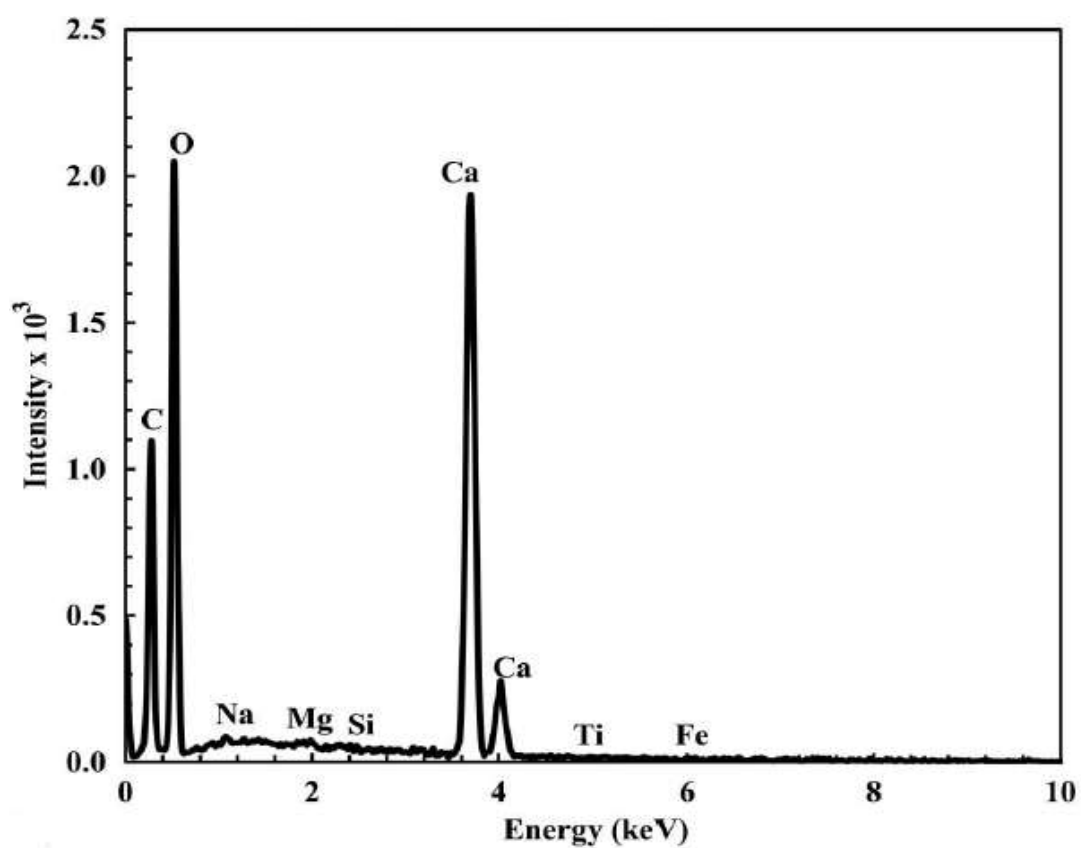
(e)

Figure 7.6. EDS results for different levels of cementation: (a) control sample (no treatment cycles); (b) 4 treatment cycles; (c) 8 treatment cycles; (d) 16 treatment cycles; and (e) 32 treatment cycles.

7.3.2.2   Evolution of the bio-cementation precipitation profile

The amount and distribution of $CaCO_3$ precipitated within the coarse sand columns derived from threshold CT-images indicate that an increase of biochemical treatment cycles is associated with an increase in the amount of biocementation (Fig 7.7). The distribution of $CaCO_3$ precipitates at the surfaces of the coarse sand column was quite uniform after a low number of biochemical treatment cycles. However, the apparent homogeneity reduced as treatment cycles increased, in particular in the sample (S32) subjected to 32 biochemical treatment cycles. This observation is consistent with laboratory data where greater heterogeneity was observed in S32 in relation to the precipitation of $CaCO_3$ (Figure 7.2). Application of more biochemical treatments such as in S32 means more $CaCO_3$ precipitation which could accumulate on and within sand particles leading to a possible clogging in the pore matrix and an inhomogeneity of $CaCO_3$ distribution (Figure 7.4e and Figure 5a and b). As stated above the average calcium carbonate content in S32 was about 26%, its minimum content was in the middle of the sample and was about 21%.

Notwithstanding this aspect, the UCS was not affected as the highest UCS achieved (about 14 MPa) was associated with the highest number of treatments (Figure 7.2). However, The UCS value could be linked to the minimum calcium carbonate present in the sample (21%). This is further evidenced in Figure 7.3e, Figure 4e and Figure 7.5a and b where a significant amount of precipitated $CaCO_3$ within and around the coarse sand particles can be observed. Furthermore, the large $CaCO_3$ deposits seem to tightly bond to, and fully cover, the coarse sand particle surfaces (Figure 7.4e).
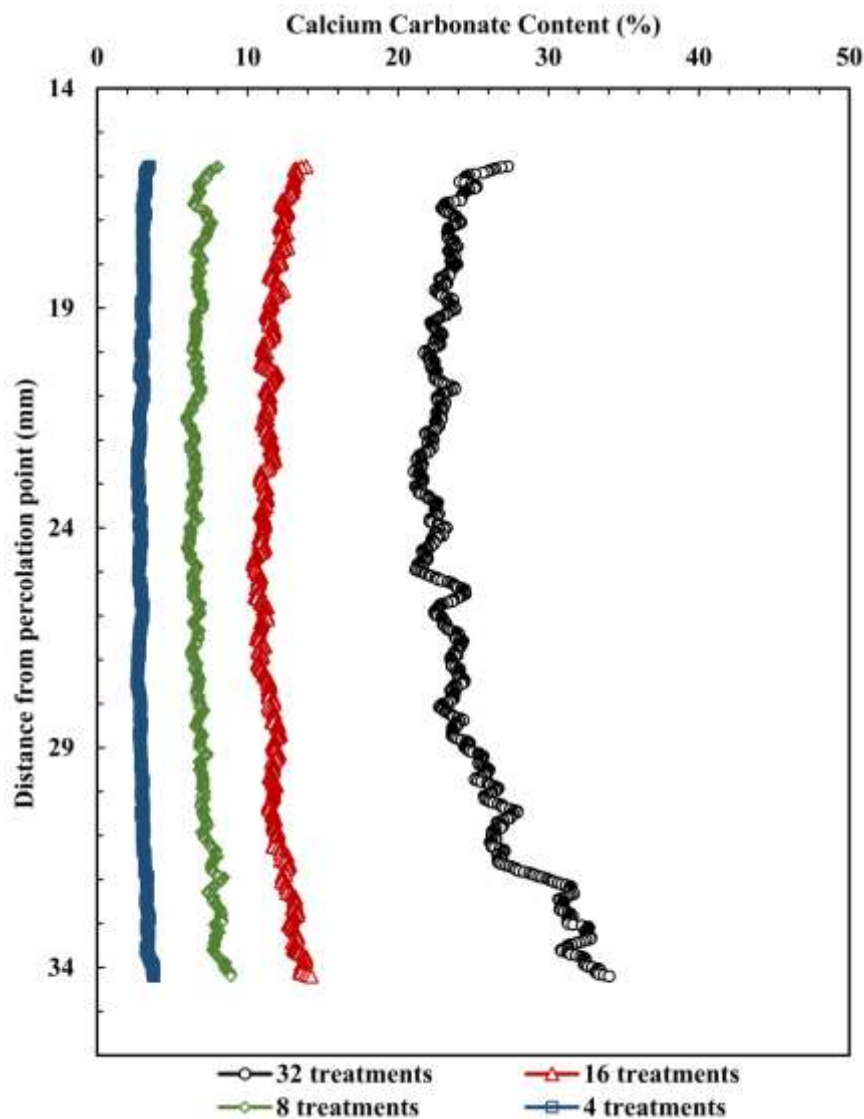
Figure 7.7. Variation of CaCO$_3$ content along the treated sand columns based on XCT analysis.

Figure 7.8 shows the relationship between CaCO$_3$ content estimated using laboratory method (acid washing technique) and XCT technique. An excellent agreement between both techniques was realised (coefficient of variation R^2=0.9986). It is worth noting that the agreement between XCT and laboratory measurements was reduced slightly at a higher number of biochemical treatment cycles that could be related to the introduction of more treatment variabilities (number of biochemical treatment cycles), resulting in greater complexity of XCT image segmentation.
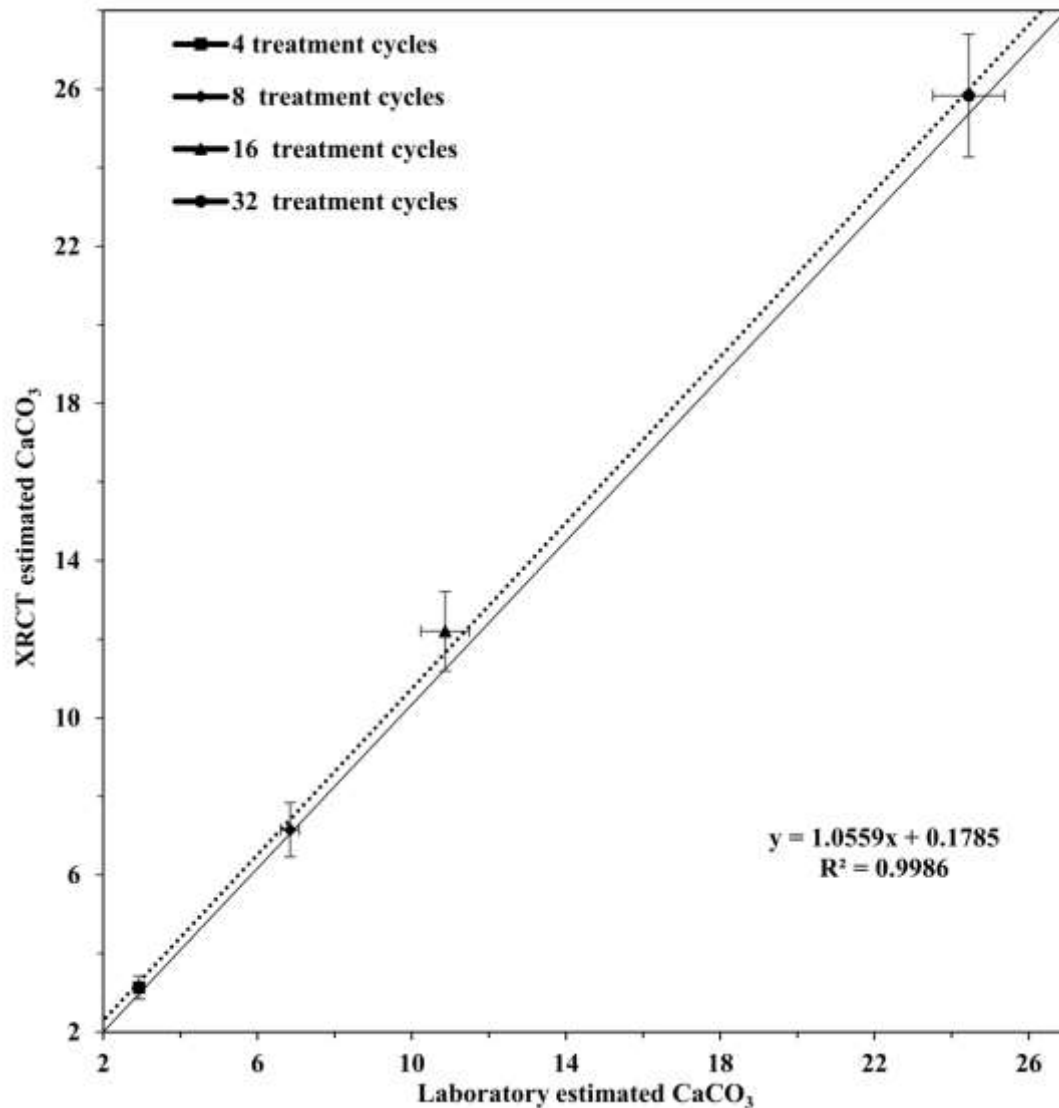
Figure 7.8 Relation between XCT and laboratory estimated CaCO$_3$ content.

7.3.2.3 Microstructural characteristics

The dry density variations along the coarse sand columns were estimated using XCT imaging as shown in Figure 7.9a. Percolating a higher number of bacterial suspension and cementation solution cycles associated with increased dry density. This increase was due to CaCO$_3$ filling the pore spaces and covering the coarse sand particles (see Figures 7.3, 7.4, and 7.5) and thus increasing the dry mass of the coarse sand. It is also interesting to note that the dry density was relatively uniform throughout the bio-cemented coarse sand column compared with the untreated column, especially when using a greater number of treatment cycles. For instance, the untreated

coarse sand sample showed a significant variation in its dry density with the depth of the column, whereas a more uniform dry density distribution was seen for example with the sample treated by 16 biochemical treatment cycles. This trend of having an even dry density distribution with samples treated by a high number of biochemical treatment cycles (i.e. 32 treatments) might be attributed to the dissimilarity of the large crystals of calcium carbonate deposited which accumulated within the larger pores of the coarse sand (see Figure 7.3e, Figure 7.4e and Figure 7.5a and 7.5b). The deposited calcium carbonate crystals could actively participate in filling the regions with the large pores resulting in more uniform dry density in the sample treated by 32 treatments (the more non-uniform the distribution of calcium carbonate, the more uniform is the dry density). This can also be further supported by exploring the effect of biocemention on the porosity and void ratio of coarse sand which are both discussed in the next paragraph. In addition, Figure 4d shows that the deposited calcium carbonate filled the large gaps between coarse sand particles leaving the sand sample that was treated by 16 treatments with small pore spaces (this point is further confirmed by exploring the effect of biochemical treatments on the void ratio of biocemented coarse sand).

The observed uniformity in density profiles might be better interpreted by exploring the XCT estimated void ratio (Figure 7.9b), which may provide insight into the pore space changes along the coarse sand columns. As there was an equal amount of coarse sand mass in each column, the only characteristic that could be changed as a result of biocementation is the pore volume associated with sand. It can be seen from Figure 7.9b that untreated coarse sand had substantial pore spaces compared with the bio-cemented samples. For the latter, the pore spaces were observed to be occupied by $CaCO_3$ particles leading to the reduction of their sizes (Figure 7.3 and 7.4), thus leading to a reduction of void ratio with the level of reduction being dependent on the number of treatment cycles. The

accompanying reduction of pore sizes can also be associated with increasing $CaCO_3$, dry density and the compressive strength of coarse sand.
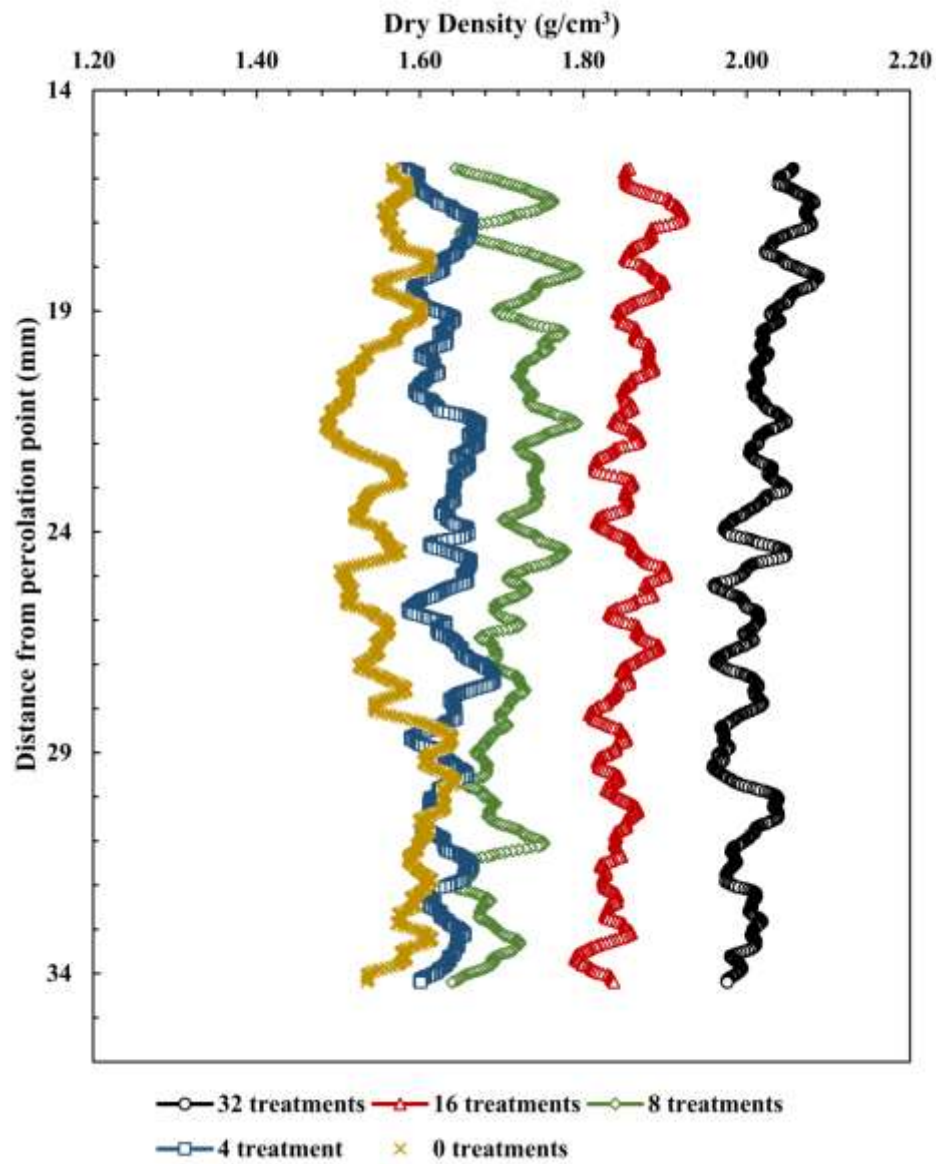
(a)
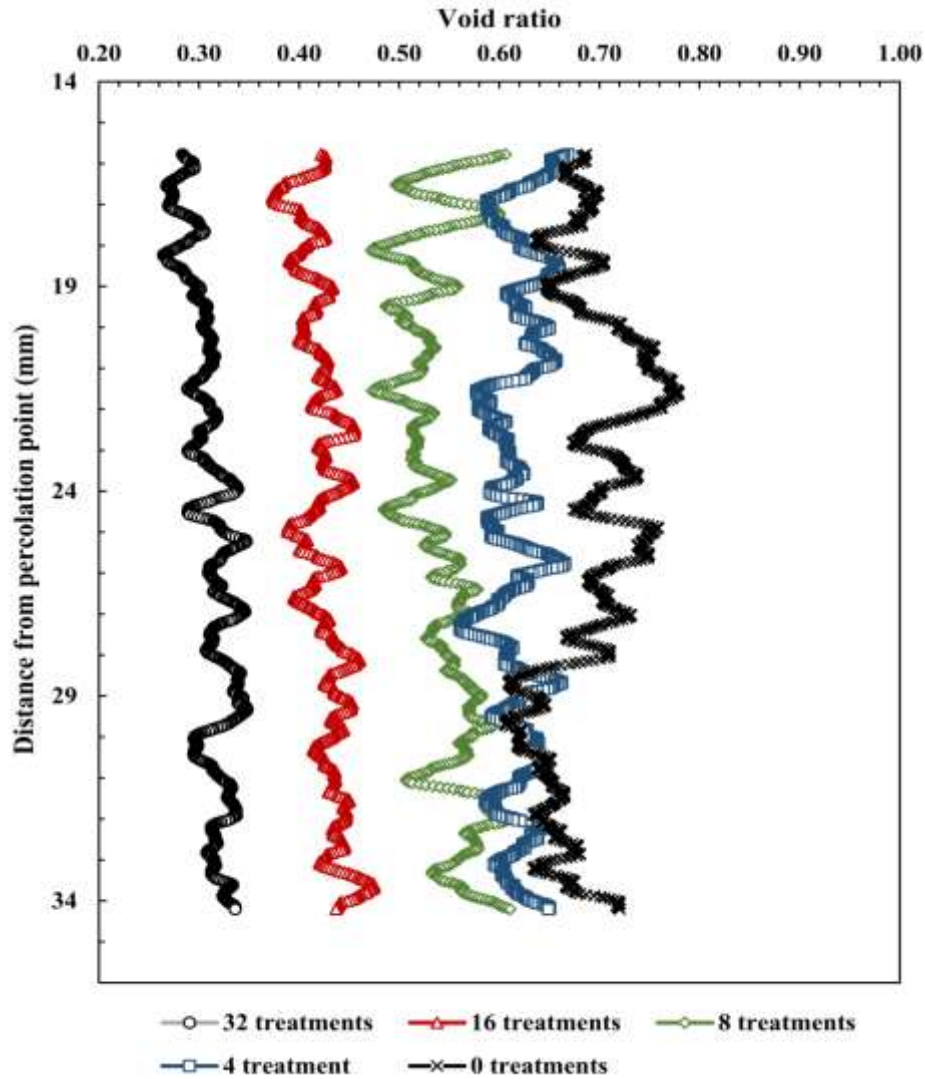
(b)

Figure 7.9 Variation of (a) dry density and (b) void ratio along the treated sand columns based on XCT analysis.

XCT estimated dry densities are compared with the laboratory estimated values as shown in Figure 7.10a. The segmentation process of XCT images was successfully verified as there is an excellent agreement between XCT estimated dry densities and the laboratory data. It is also worth mentioning that utilising a higher number of biochemical treatment cycles led to a slight difference between the two dry density estimation methods (XCT and laboratory) as indicated in Figure 7.10a. Figure 7.10b presents the relationship between XCT estimated void ratio with the laboratory estimated void ratio. There is an excellent agreement between the two estimations

reinforcing the fact that XCT can provide a very accurate estimate of the treated sand properties.

(a)

(b)
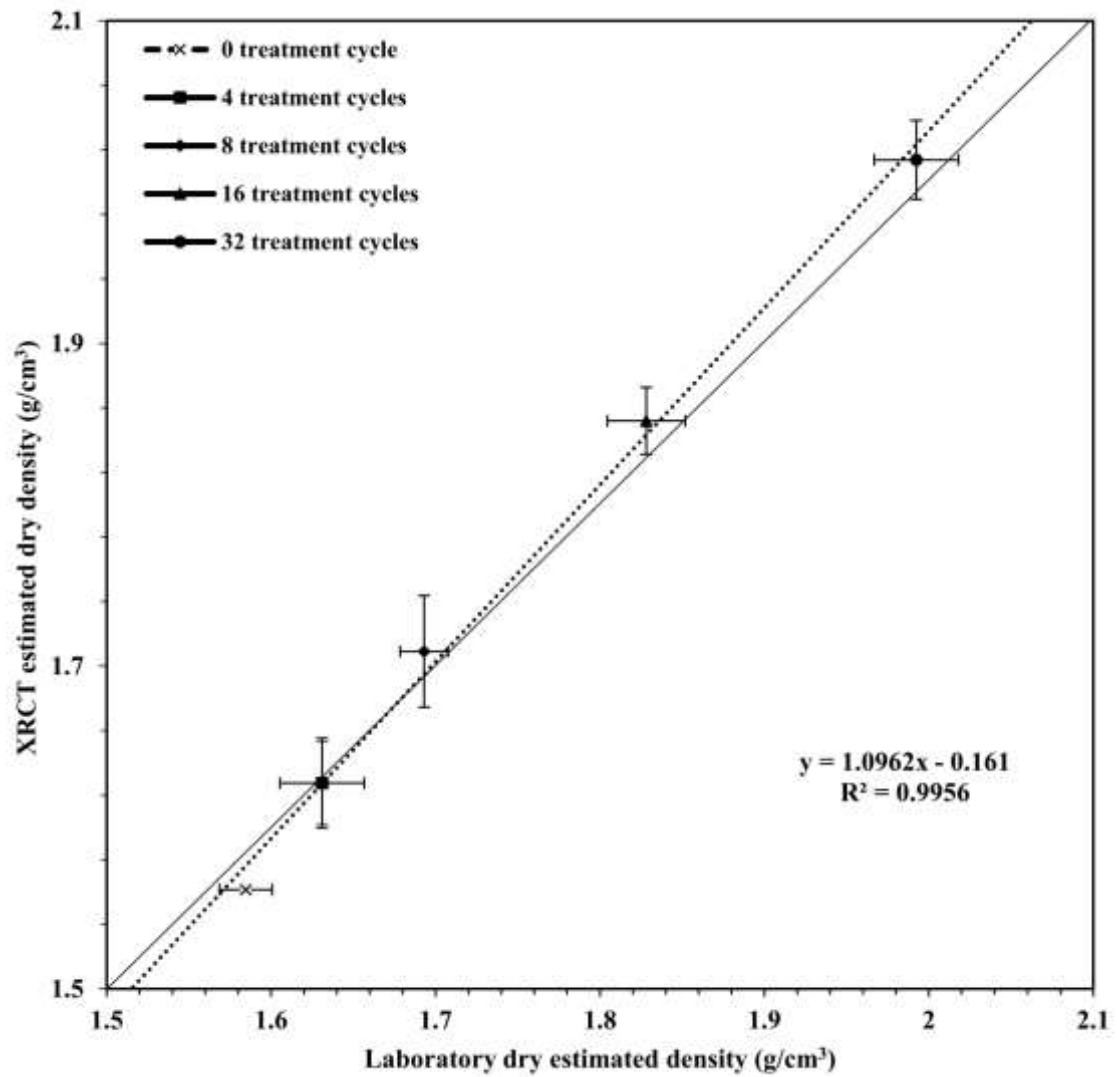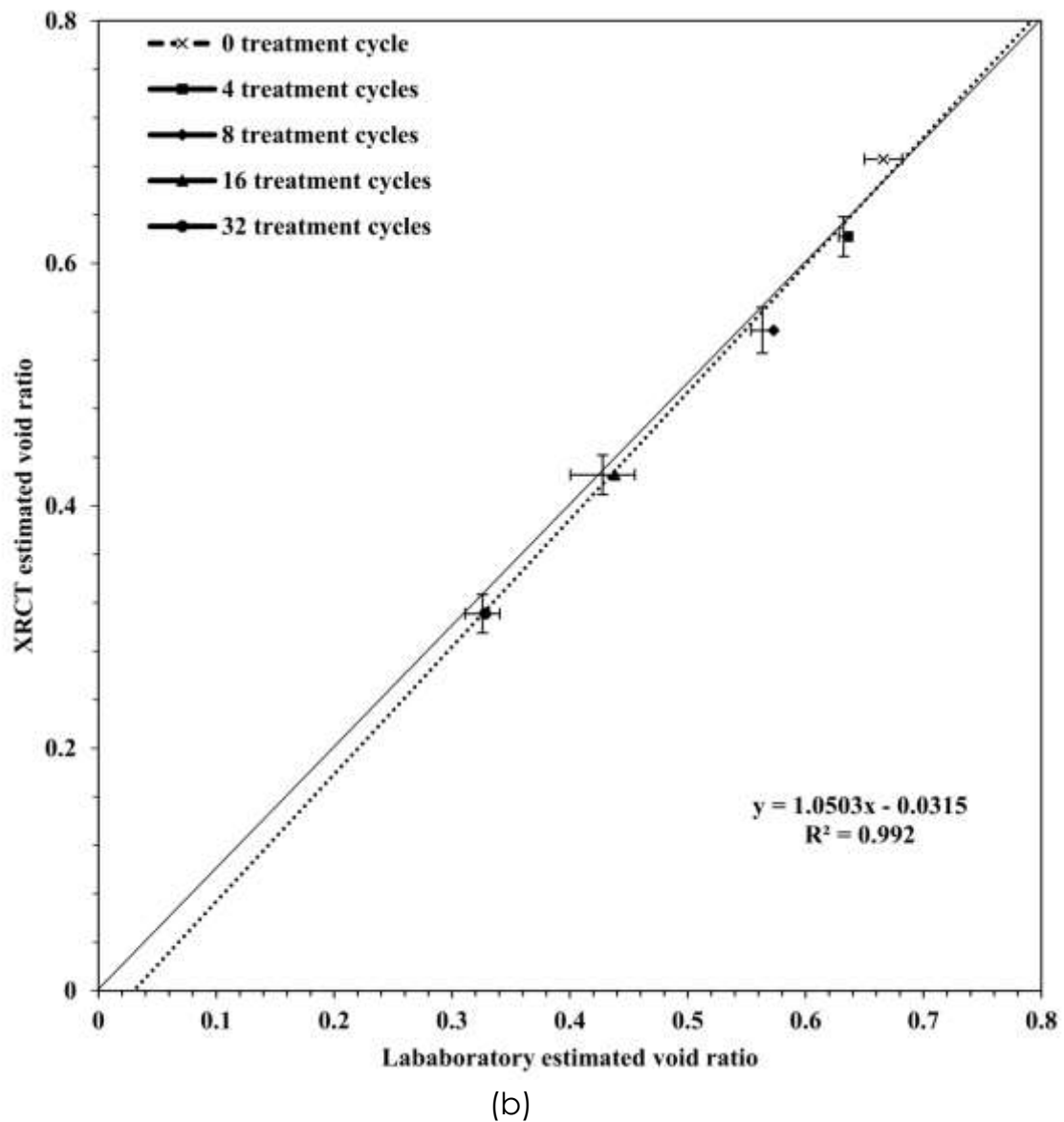
Figure 7.10 Relation between XCT and laboratory estimated (a) dry density and (b) void ratio.

Another important sand characteristic identified using XCT imaging technique is the porosity changes throughout the depth of the sand column. Figure 7.11 illustrates the variation of porosity along the coarse sand columns using a different number of biochemical treatment cycles. It can be observed that a substantial reduction in the porosity occurs as the number of treatment cycles increases. The average decrease in porosity was around 5%, 14%, 34% and 69 % for 4, 8, 16 and 32 treatment cycles, respectively, compared to untreated sand. It is important to mention that the reduction in porosity is compatible with the decrease in void ratio, as well as with the

increase in $CaCO_3$ and dry density of coarse sand as discussed earlier. The XCT estimated porosity and the laboratory estimated porosity data are presented in Figure 7.12. Once again there is an excellent agreement between the two estimations which confirms that the segmentation process of the XCT images adopted in this investigation is accurate.
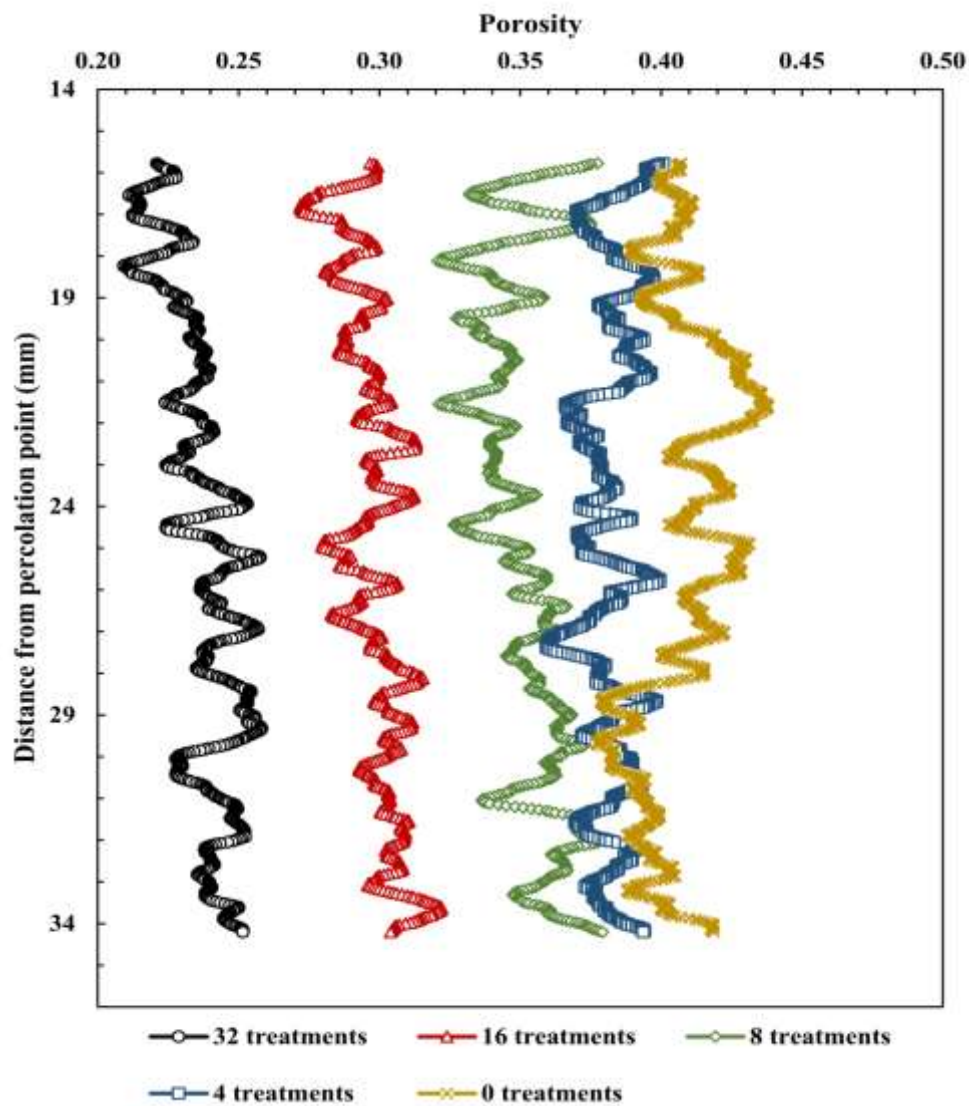
Figure 7.11 Variation of porosity along the treated sand columns based on XCT analysis.
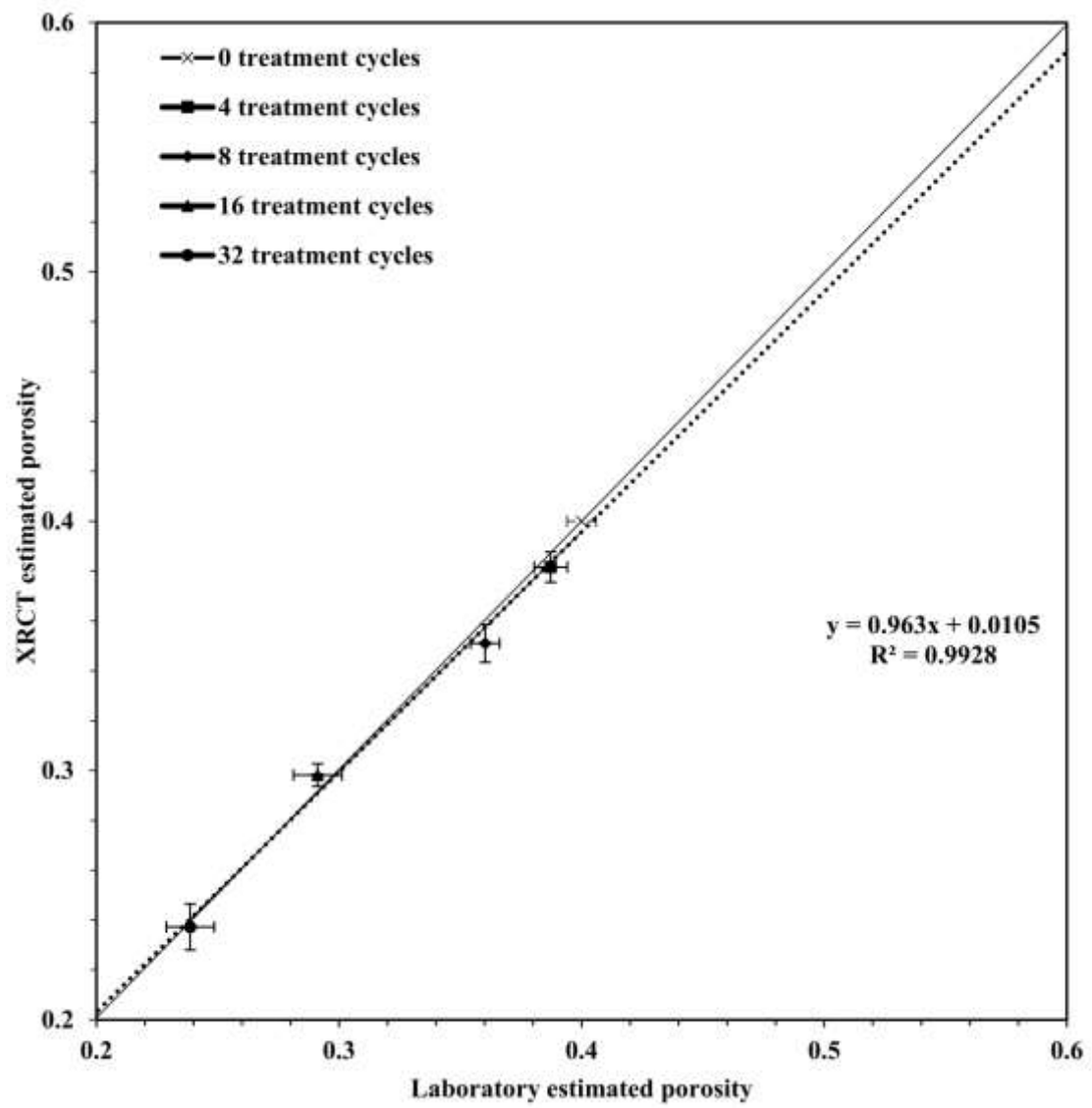
Figure 7.12 Relation between XCT and laboratory estimated porosity.

7.4 Conclusions

The macro-mechanical behaviour of coarse sand specimens treated using different levels of bio-cementation was investigated, and high-resolution XCT *in-situ* imaging of their microstructure was examined. Based on the results presented in this paper, the following salient conclusions can be drawn:

- The precipitation of $CaCO_3$ within the pore matrix of coarse sand specimens is directly related to the number of biochemical treatment cycles. More bio-treatment cycles induced greater % $CaCO_3$ precipitation.

- The increase % $CaCO_3$ resulted in bio-cementation as observed by a significant increase in strength of coarse sand columns. A maximum strength of 14 MPa was achieved for 25% $CaCO_3$ content.

- XCT imaging accurately detected the level of $CaCO_3$ precipitation achieved in coarse sand column specimens at all bio-chemical treatment cycles. Segmentation allowed visualization and quantification of significant changes that occurred at the micro-scale level during the bio-cementation reactions, supporting the findings obtained at macro-scale level. The segmentation of the XTC images adopted in the current investigation allowed an accurate estimation of % $CaCO_3$, dry density changes as well as void ratio and porosity changes, which correlated very well with laboratory-based measurements.

- XCT imaging is a viable alternative technique to quantify $CaCO_3$ content with acceptable accuracy, without the need for conventional gravimetric acid washing technique.

- SEM and EDS analysis further confirmed the changes observed in the microstructure of the bio-cemented specimens. The SEM micrographs showed that $CaCO_3$ crystal from small lumps of bonds into large $CaCO_3$ crystals. EDS confirmed that the chemical compositions of the precipitates are related to the constituents of $CaCO_3$.

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Chapter 8: Biogrouted stone columns in soft clay

The following work has been submitted to

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Biogrouted stone columns in soft clay

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Abstract

This paper discusses the results of small-scale model column tests undertaken to investigate the behaviour of un-cemented and biocemented sand columns installed in soft clay. It examines the effect of a biocementation process to reduce excessive bulging that occurs during the loading of sand columns. A 12-phase percolation biochemical treatment technique was used to obtain partial bio-cementation targeting the sand columns upper section, where bulging usually occurs. Placement of an *ex-situ* bio-cemented sand column substantially reduced the vertical strains by 43% and 56% compared with un-cemented sand column and kaolin clay, respectively. A further reduction of about 11% in the vertical strain was observed when a bio-cemented column was created *in-situ*. Bulging was reduced by 62% to 75% following *in-situ* bio-cementation and mostly occurred in the bottom section of the bio-cemented column, where bio-cementation was less evident. Other parameters such as the undrained shear strength, moisture content and the precipitated calcium carbonate along the columns were also quantified and were consistent with the above observations. Scanning electron microscopy (SEM) and energy dispersive spectroscopy (SEM) validated the existence of calcium carbonate. This study establishes a foundation for the biogrout to be used in stone columns or similar foundations in the future.

8.1 Introduction

Conventional grouped granular/stone columns are commonly used to improve soft soils beneath embankments, tank bases and other lightly or moderately loaded structures with a wide area (McCabe et al., 2009, Babu et al., 2013, Douglas and Schaefer, 2015). To extend the use of stone columns to sites with very soft soils, which typically have low undrained shear strength (e.g. < 10 kPa), stone columns encased in geosynthetics have been developed over the last two decades (Alexiew et al., 2005, Murugesan and Rajagopal, 2009, Gniel and Bouazza, 2009, 2010, Yoo and Lee, 2012, Cengiz and Guler, 2018, Alkhorshid et al., 2019) as these soils are unable to provide the confinement required to support conventional stone columns. Another alternative which has received increased attention is the possibility of grouting the stone columns by injecting cement-bentonite or cement slurries into them. The aim, in this case, is to reduce the large settlement and bulging associated with the use of traditional stone columns (Heitz et al., 2005, Liu et al., 2015, Unver and Unver, 2015) and to minimize the possible migration of fines through stone columns from natural soils during earthquakes which can be detrimental to the stability of infrastructures such as dams (Avalle et al., 2012).

Bio-grouting, based on biogeochemical processes such as microbial induced calcite precipitation (MICP), can serve as an alternative technology to cement grouting strengthening/stabilising stone columns. The enzymatic activities of microorganisms, such as *Sporosarcina pasteurii*, induce the precipitation of calcium carbonate within a soil matrix and binds granular particles together at inter-particle contacts leading to increased strength and stiffness of a given soil (Mitchell and Santamarina 2005, DeJong et al. 2006, Mortensen and DeJong 2011 Montoya 2012, Stabnikov et al. 2015, Mahawish et al., 2017, 2018a, 2019, Terzis and Laloui, 2018, 2019, Wang et al., 2019, Dejong and Kavazanjian, 2019). The applicability of this process has been shown to have a broad spectrum of applications ranging from ground improvement/stabilisation (Whiffin et al. 2007, Van der Ruyt and van der Zon

2009, DeJong et al. 2010, Harkes et al. 2010, van Paassen et al. 2010, Stabnikov et al. 2011, 2013, Weil et al. 2012, Chu et al. 2013, Ivanov et al. 2015, Hamed-Khodadi et al., 2017, Mujah et al., 2017, Mahawish et al. 2018b,c) to contamination mitigation (Cheng and Shahin; 2017, Safavizadeh et al., 2019).

The present paper examines the efficacy of a range of bio-grouting granular columns when installed in very soft soils to mitigate excessive radial column expansion during loading. Staged loading of the granular columns (un-cemented and bio-cemented) was conducted to replicate the staged construction process typically encountered in projects such as in embankments.

8.2 Materials

8.2.1 Soils

A commercially available powdered kaolin (Grade HR1F, Unimin Australia) commonly used in our laboratory experiments (Shannon, 2013; Gniel, 2009) was used in the current investigation. It was mixed with water to produce a slurry with an initial gravimetric moisture content of 115% which reduced the formation of air bubbles within the slurry and increased its workability. The slurry was prepared by mixing a predetermined amount of kaolin powder (6700g) with 7705 ml of water, for about three hours, in a large mixing bowl using an electric mixer. The homogenous mix was then left to sit for a further four hours allowing any air bubbles to rise to the surface. The testing of the slurry material in a modified oedometer cell (100 mm height and 155 mm in diameter) indicated that a vertical pressure of 120 kPa caused a vertical strain of around 37%. The resulting undrained shear strength (C_u), measured using a laboratory vane shear apparatus was about 15 kPa. The consolidated saturated unit weight of kaolin was 17.88 kN/m^3 and had a gravimetric moisture content of 51%. The properties of the consolidated soft clay are presented in Table 8.1.

Stone columns are usually composed of angular and sub-angular granular materials, such as crushed rock, having an average particle size ranging between 25 and 75 mm. A typical stone column diameter ranges from 0.40 to 1.20 m, and this leads to a standard column diameter to average particle size ratio ranging between 5 and 50. The model granular columns used in the current investigation were composed of coarse sand having an average grain size of 1.6 mm. The sand column diameter to the particle size ratio was about 30, which is within the practical range reported above. The sand particles were typically sub-angular in shape and were deemed to be representative of stone aggregates used for full-scale stone columns as reported previously by Gniel and Bouazza (2009, 2010). Commercially available sand (Grade 8/16, supplied by Unimin Australia Pty. Ltd., North Sydney, NSW, Australia) was used in the current investigation. The properties of the sand are given in Table 8.2. Sand columns were prepared to a relative density of 60%, which within the range of conventional stone columns encountered in practice (between 60% and 100%).

Table 8.1. Properties of consolidated kaolin clay.

Parameter	Unit	Value
Specific gravity	--	2.64
Plastic limit	%	29
Liquid limit	%	62
Compression index, C_c	--	0.7
Recompression index, C_r	--	0.09
Saturated unit weight	kN/m^3	17.88
Moisture content	%	51
Average undrained shear strength, C_u	kPa	15*

* tested using a vane shear apparatus.

Table 8.2. Properties of sand used in sand columns

Parameter	Unit	Value
Specific gravity, G_s	-	2.64
Coefficient of curvature, C_c	-	0.97
Coefficient of uniformity, C_u	-	1.35
Median grain size, D_{50}	mm	1.60
Maximum void ratio, e_{max}	-	0.84
Minimum void ratio, e_{min}		0.55
Minimim dry density	kN/m^3	14.4
Maximum dry density	kN/m^3	17.0
Compacted dry density	kN/m^3	16.2
Compacted saturated density	kN/m^3	20.2
Residual angle of internal friction, ϕ	°	35
Dilation, ψ	°	8 – 11
Average permeability, k	m/s	4.5×10^{-2}

8.2.2 Type of bacteria and cementation solution

The selection and preparation of the bacteria and the cementation solution followed the protocol reported in details in Mahawish et al. (2016, 2018, 2019), and it is briefly summarized herein. *Sporosarcina pasteurii*(ATCC® 11859) was grown in ammonium-YE growth medium (20 g yeast extract, 10 g $(NH_4)_2SO_4$ and 130 mM tris buffer (pH=9.0) per litre of distilled water) to at 30°C. Suspended bacterial cultures were grown in a shaking water bath (200 rpm) for twenty-four hours before harvesting at a

final optical density (OD600) of 3.0-3.5 using a WPA CO 8000 spectrophotometer (BioChrom Ltd).

Individual ingredients of the recipe were separately dissolved in distilled water bottles and autoclaved separately (121°C, 100 kPa for around two hours) and then combined post sterilisation. The urease activity was about 19.38-21.45 mM/min based on conductivity assay (labCHEM-CP). The bacterial suspension was stored up to 14 days at 4°C until used and had a urease activity of 19.38 mM/min. The cementation (reagent) solution consisted of 1 M urea ($CO(NH_2)_2$) and 1 M calcium chloride $CaCl_2$.

8.2.3 Test equipment

The design, development and construction of the column cells used in the current investigation are described in detail in Gniel and Bouazza (2009) and Gniel (2009). The column cell apparatus was required to initially act as an enlarged oedometer cell, consolidating the kaolin slurry to the consistency of very soft clay in which the model untreated and bio-treated sand columns could be installed. The cell also needed to be capable of accommodating the applied load to the column using unit-cell boundary conditions to represent a group of columns (Gniel and Bouazza, 2009). A cross-section of the cell (without the sand column) and the column cells are shown in Figure 8.1, together with the installation process of the sand column. The kaolin slurry was placed inside a stainless steel cylinder (550 mm H by 155 mm internal diameter; 6 mm wall thickness), mounted on a base plate. Both base plate and top plate were modified from triaxial cells. A piston within the cylinder loaded the slurry using air pressure. A double O-ring system was used to prevent slurry squeezing around the piston and being exposed to pressurised air. Two identical cells were used in the current investigation. The internal surface of each cell was bored and polished to reduce side resistance, and a thin layer of silicone lubricant was applied to the inner surface of the cells before filling them with the slurry. Gniel and Bouazza (2009) indicated that using the above measures, the vertical stress-strain

behaviour of the cell closely matched that of a standard oedometer, justifying the much larger height/diameter ratio, and O-ring resistance was also reduced, such that between 85% and 90% of the applied load was transferred to the kaolin. Two-directional drainage through the piston and base plate was provided to the sample during consolidation. The water level in the cell was always kept above the level of the piston to ensure that a fully saturated condition was achieved.
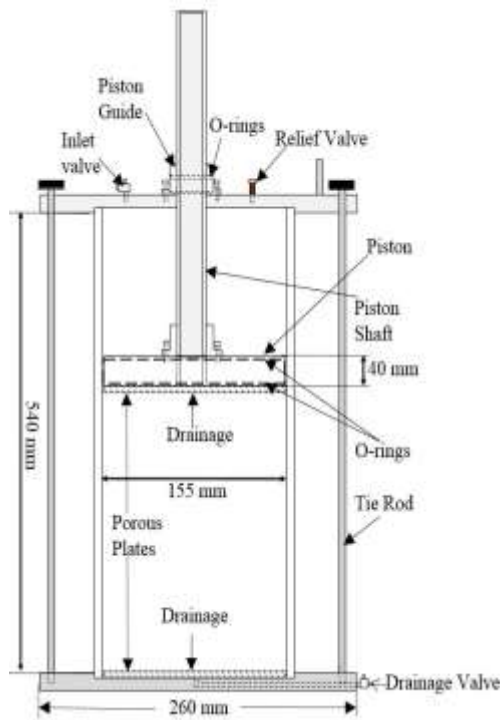
(a)   (b)

Figure 8.1. (a) Cross section of enlarged oedometer, (b) Column cells

8.2.4   Setup Instrumentation

A string potentiometer (string pot transducer) with a travel distance of about 380 mm was attached to each cell to measure the vertical displacement of the piston and settlement of the sample under given loads. Two pore water pressure transducers were mounted on the cylinder wall of both cells at distances of 100 mm and 150 mm, respectively, from the base plate. They measured excess pore water pressure during loading and excess pore pressure dissipation and also allowed observation of the impact of the

drainage path length on the consolidation rate. A 10 channel DataTaker (D600 Series 2 data logging system) and computer provided the necessary data acquisition system.

8.2.5 Cell operation

The column cells were designed to operate in two stages. The first stage consisted of applying a vertical load to the kaolin slurry by air pressurising the chamber above the piston and forcing it downwards causing consolidation of the kaolin slurry to the consistency of a soft clay ($C_u \approx 15$ kPa). The kaolin slurry took about 71 days to consolidate to the targeted soft clay consistency. Following the consolidation stage, the clay samples were unloaded, and model sand columns were installed. Based on the adopted cell geometry and material availability, a 51 mm diameter sand column was used within a 155 mm diameter cell equating a replacement ratio (ratio of sand column area to loaded footprint area), A_r, of about 11 %. Gniel and Bouazza (2009) indicated that while this replacement ratio value was at the lower end of the range typically adopted for site use, it was considered sufficient for small-scale tests given the controlled conditions within the laboratory. Thus, the adopted A_r was deemed to be adequate for assessment of column behaviour. The second stage of testing consisted of loading the clay-column samples using the piston to create the unit-cell condition. The loading was staged in increments of 50 kPa until reaching a maximum surcharge pressure of about 350 kPa (roughly corresponding to the maximum surcharge pressure that would be applied to a stone column in site). This stress range resulted in five load stages (150, 200, 250, 300 and 350 kPa), enabling suitable assessment of the relationship between applied pressure and vertical strain. The sample was allowed to undergo full consolidation under each load stage, which was assessed by monitoring both settlement and excess pore pressure dissipation.

8.2.6 Test procedure

8.2.6.1 Column construction

The kaolin slurry with an initial height of about 480 mm was consolidated in the cells under a pressure of 120 kPa, which was applied in stages starting with 50 kPa to avoid a build-up of excess pore water pressure. At completion, the kaolin clay had an undrained cohesion of 15 kPa (measured using a vane shear test, ASTM D2573) and a height of about 300 mm. This final sample height equated to a model column length/diameter ratio of about 6, within the range (3 to 20) generally accepted for stone columns (Gniel and Bouazza, 2009). Following unloading, a thin-walled aluminium tube measuring 51 mm in outside diameter was pushed slowly through the centre of the sample, located by using a guide attached to the top of the cylinder, to the base of the cell. Once the tube was retrieved, it created a cylindrical cavity of 51 mm diameter at the centre of the clay along the entire height of the clay column (Figure 8.2a). The soil within the tube was also tested for strength and moisture. A replacement technique was considered to be the most reproducible method for column installation in soft soils. *In-situ* compaction was avoided because the potential for inconsistencies in column density were considered high (Gniel and Bouazza, 2009). Model sand columns were constructed using the freezing method proposed by Sivakumar et al. (2004) and Gniel and Bouazza (2009). Two preparation methods were adopted in the current investigation, including an *in-situ* column method where coarse sand was installed in the cavity at the middle of the clay deposit and an *ex-situ* column method.

For the *in-situ* column method, a polyvinyl chloride (PVC) pipe, with an internal diameter of 51 mm, was cut in half length-wise to form a split-mould for the sand columns. This enabled demoulding without disturbance of the coarse sand samples. A wire mesh and filter paper were placed at the bottom of the mould to minimise possible losses of soil particles during preparation and treatment. The sand column was equal in length to the consolidated clay sample height. The sand-filled mould was positioned

vertically with the upper surface fully exposed, and a drainage control valve was connected to the base. A dry coarse sand sample was pluviated into the specimen moulds and then compacted, using a vibratory compaction method, to a relative density of 60% (equivalent to a dry density of 1.59g/cm^3) due to their sub-angular particle shape and poor grading. After placement of the soil, tap water (200% pore volume (PV) of sand columns (440 mL)) was flushed through it to expel any extra air from the pore matrix. This step was done by closing the bottom valve, percolating deionised water until ponding occurred on top of the specimen, then opening the bottom valve to flush the water out, the percolation of water was continued until a total of two-pore volumes (PV) had drained. Afterwards, the bottom valve was closed, and the sand was saturated with water. The column was then frozen at a temperature of -5 ^{o}C for twenty-four hours. After the column was removed from the freezer, it was allowed to thaw slightly, enabling the column to be extruded from the mould using low pressure. Once the intact frozen sand column was ejected from the mould, it was inserted in the cavity created in the centre of the consolidated clay (Figure 2b). In trials undertaken before column tests, a two hour waiting period was enforced before commencing the *in-situ* biochemical treatment cycles. Note: The same preparation process as described above was also conducted for the preparation of the untreated sand column.

The mould and preparation processes of the *ex-situ* sand columns were similar to the *in-situ* method. For this particular study, the columns were biochemically treated after completion of the preparation process. Once the biochemical treatment cycles and curing ceased, the columns were frozen to ensure that the bottom portion did not collapse during the disassembling process.

For both *in-situ* and *ex-situ* columns, a percolation treatment method was used to promote bio-cementation of the very coarse-grained soil specimens as described in Mahawish et al. (2018b). However. the protocol adopted for this study was a twelve-phase percolation strategy, where, in the first

treatment, small volumes (10 mL) of bacterial suspension (BS) and (10 ml) cementation solution (CS) without ammonium-YE media were alternately, but individually percolated through the column in the first treatment cycles, in order to achieve the twelve-phase percolation protocol (i.e., 10 ml BS + 10 ml CS + 10 ml BS + 10ml CS+ 10 ml BS + 10ml CS+ 10 ml BS + 10ml CS+ 10 ml BS + 10ml CS + 10 ml BS + 10ml CS). The second step included half pore volume cementation solution (120 ml). The other steps of biotreatment were similar the ones described in Mahawish et al. (2018b).This protocol was used to minimise bulging of the upper part of the column. The use of an alternating BS/CS solution percolation in the column has been shown to improve bacterial retention (Cheng and Cord-Ruwisch 2012). Cheng and Cord-Ruwisch (2012) stated that bacterial cells could be filtered out and accumulated near to inlet points.

The amount of bacterial suspension and cementation solution in each treatment cycles was quantified based on the pore volume (pore water capacity) of the soil columns. The total amount of percolated liquid per each cycle was equivalent to the half pore volume of the column (120 ml). After completion of the bio-cementation treatment cycles, all the samples were flushed with tap water (1 litre) to remove residual chemical reagents from the pore spaces.

For the loading stages, the cells were initially loaded by approximately 5 kPa above the clay pre-consolidation pressure to ensure intimate contact occurred between the column and surrounded clay. Afterwards, the cell was loaded in an increment of 50 kPa and the maximum cell pressure applied was about 350 kPa. The sample was allowed to undergo full consolidation under each load increment. Once the test was completed, the sample was extruded from the cylinder using the piston and was carefully bisected vertically to observe column deformation.
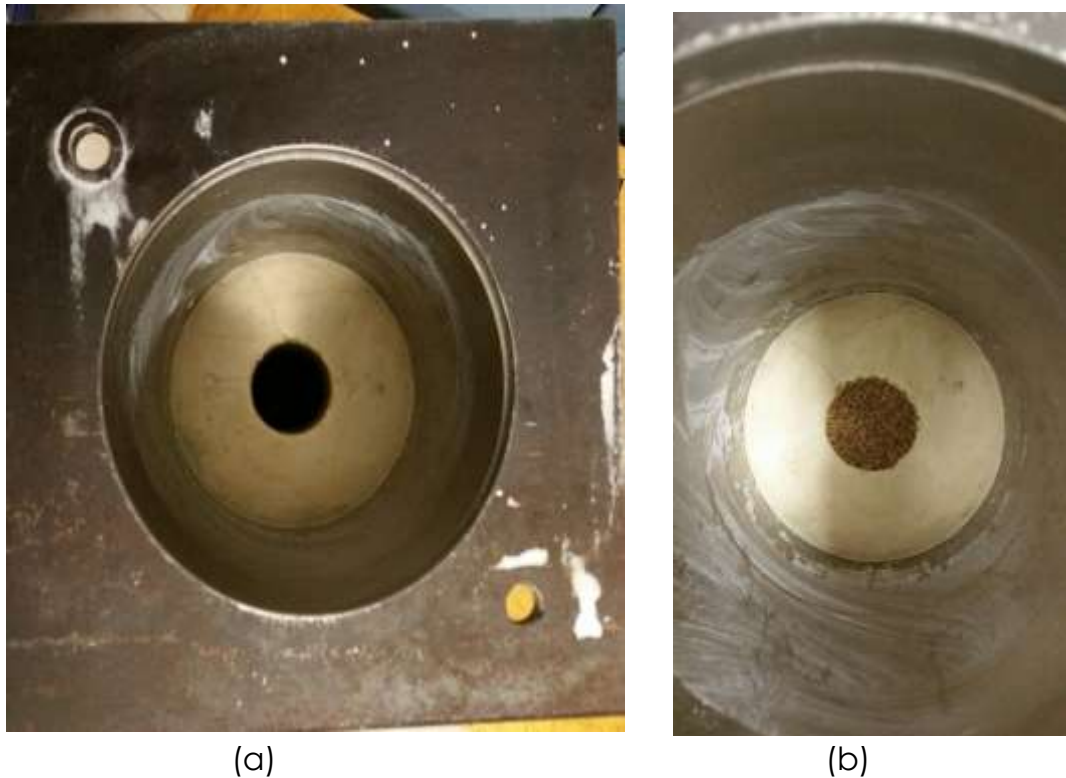
Figure 8.2. (a) Cavity created in the centre of the clay; (b) Installed sand column.

8.2.7 Calcium carbonate quantification

Calcium carbonate ($CaCO_3$) content was measured using a conventional gravimetric acid washing technique (Choi et al., 2017). For this purpose, subsamples from the specimens that were prepared and bio-treated for column test (within 30mm of the top, middle and bottom of the specimen) were first oven-dried at 105°C for twenty-four hours, and their masses were measured before and after the acid wash. The quantification of $CaCO_3$ was determined by rinsing the samples with a solution of 2M HCl several times (total solution used was 200 mL) through No.200 sieves and allowing the dissolved salts to be rinsed out from the samples. The difference between dry mass before and after acid washing was taken as the mass of $CaCO_3$ removed and the % $CaCO_3$ was obtained by dividing the mass of $CaCO_3$ by the mass of soil.

8.2.8 Scanning electron microscopy (SEM) and energy dispersive spectroscopy (EDS)

Scanning electron microscopy (SEM) and energy dispersive spectroscopy (EDS) were undertaken to characterise the calcium carbonate precipitated in the *in-situ* bio-cemented sand column. Subsamples were retained after completing the sand column test; they were oven dried; carbon coated, and then examined using an SEM (JEOL 7001F FEGSEM,) with a beam intensity ranging between 5 to 15kV.

8.3 Results and discussion of column testing

8.3.1 Vertical stress-strain behaviour

A kaolin consolidation test was initially undertaken in the column cell to provide a base reference, after which an un-cemented column test was conducted to establish its performance for comparison. Tests were then performed on either *ex-situ* bio-cemented column and *in-situ* bio-cemented column. After the bio-cemented sand was inserted within the consolidated clay, a nominal load of 125 kPa was applied incrementally to ensure intimate contact between the sand column and surrounding clay. A vertical strain resulting from the application of the nominal load was assumed to be equivalent to the strain associated with consolidation, installation disturbances and re-densification of sand columns following thawing. The vertical strain was not recorded until the first loading stage (150 kPa) following the nominal loading. The vertical stress-strain behaviour of the column is presented in Figure 8.3, where the vertical strain is plotted against the applied vertical pressure. The vertical strain was calculated using the sample height at the completion of nominal loading. Interestingly, it can be seen from Figure 8.3 that the vertical strain recorded from the test on kaolin in the column cell test paired quite well with that of the oedometer tests, indicating that the silicone lubrication worked successfully.

The inclusion of an un-cemented sand column resulted in a significant reduction in vertical strains compared to clay alone as shown in Figure 8.3. This reduction varied according to the range of applied stresses, and it ranged between about 33% for 150 kPa vertical stress to about 16% at around 350 kPa. The average strain reduction across the range of applied vertical stresses was approximately 23% a result which is typical for a low replacement ratio of A_r =11% according to Gniel and Bouazza (2009). The *ex-situ* partially bio-cemented columnar inclusion led to an even more substantial reduction in the vertical strain as can be observed in Figure 8.3. The averaged vertical strain reduction across the range of applied stresses was about 43% and 56%, respectively, compared with the un-cemented sand column inclusion and clay alone, respectively. A further reduction in the vertical strain of about 11% was observed for the *in-situ* bio-cemented sand column compared to the *ex-situ* column. This may be attributed to the interaction between the bio-cemented sand column and the surrounding clay as less ingress of clay into the pore spaces of the in-situ treated column (Figure 8.4a) was observed than in the ex-situ treated column (Figure 8.4b).

In the *in-situ* treatment, the overall reduction in vertical strain across the range of applied stresses was about 60% compared to clay alone and about 48 % compared to the un-cemented coarse sand column for just a low replacement ratio of 11% as the bio-cemented sand columns acted as stiff columns.
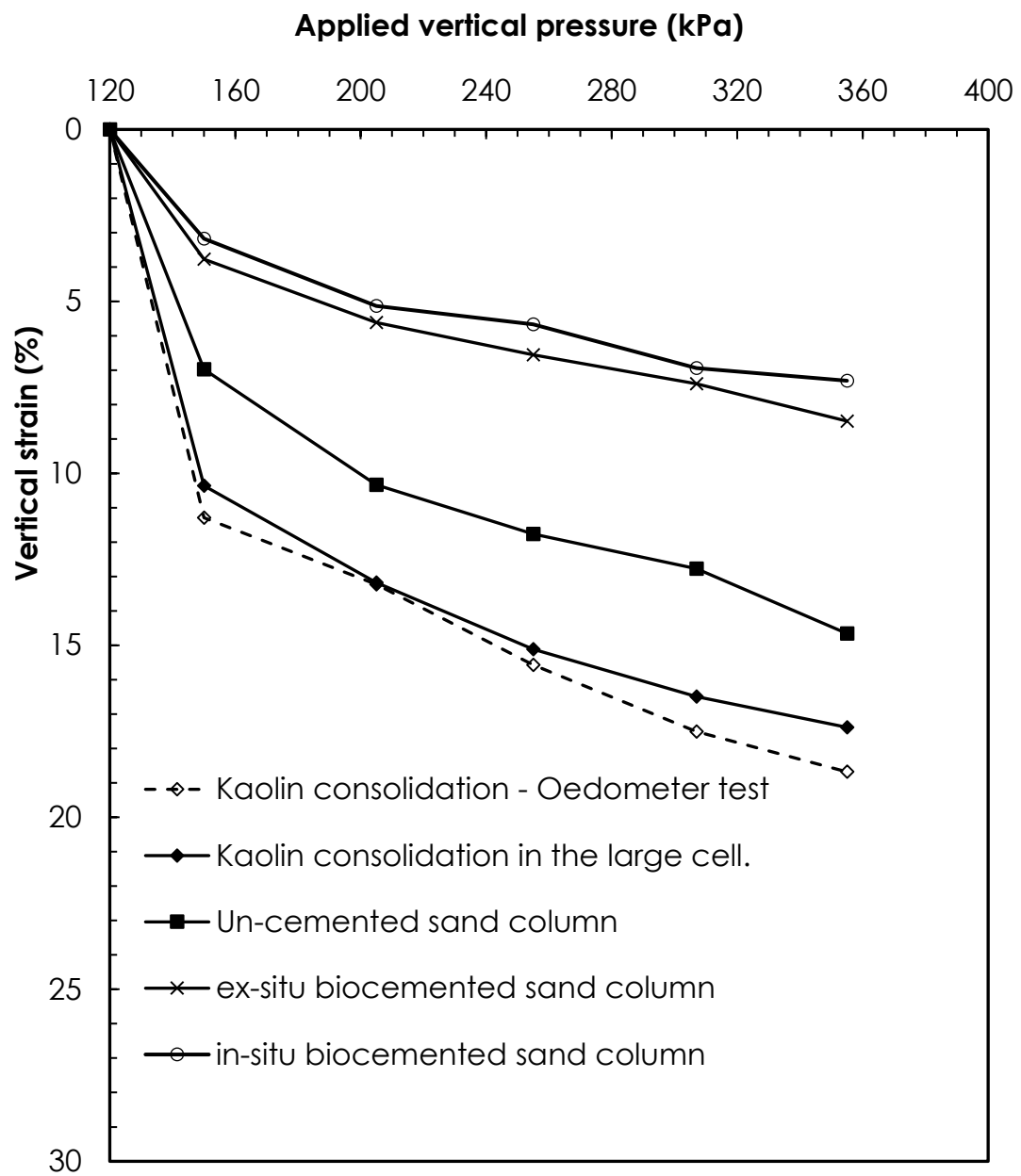
Figure 8.3. Variation of vertical strain against applied vertical stress.

Figure 8.4. Clay ingress into the column: a- *in-situ* bio-cemented sand column; b- *ex-situ* bio-cemented sand column.

8.3.2 Column radial expansion

The lateral expansion of the columns were measured to an accuracy of about 0.5 mm, given the coarse grain size of the sand. These measurements were performed after extruding the samples and carefully bisecting them to measure column deformation. Figure 8.5 shows the lateral expansion of the treated and un-treated sand columns. Bulging of the un-cemented column was observed to occur along the column to a vertical length corresponding to about four diameters of the column ($\square_c$) which is consistent with previous observations reported in the literature (Hughes and Whiters, 1974, Black et al. 2007). The maximum bulging (~21% radial strain) was recorded at the top of the column, whereas the average radial strains along the column length where most of the bulging occurred (i.e., 4 x $\square_c$) was about 11%.
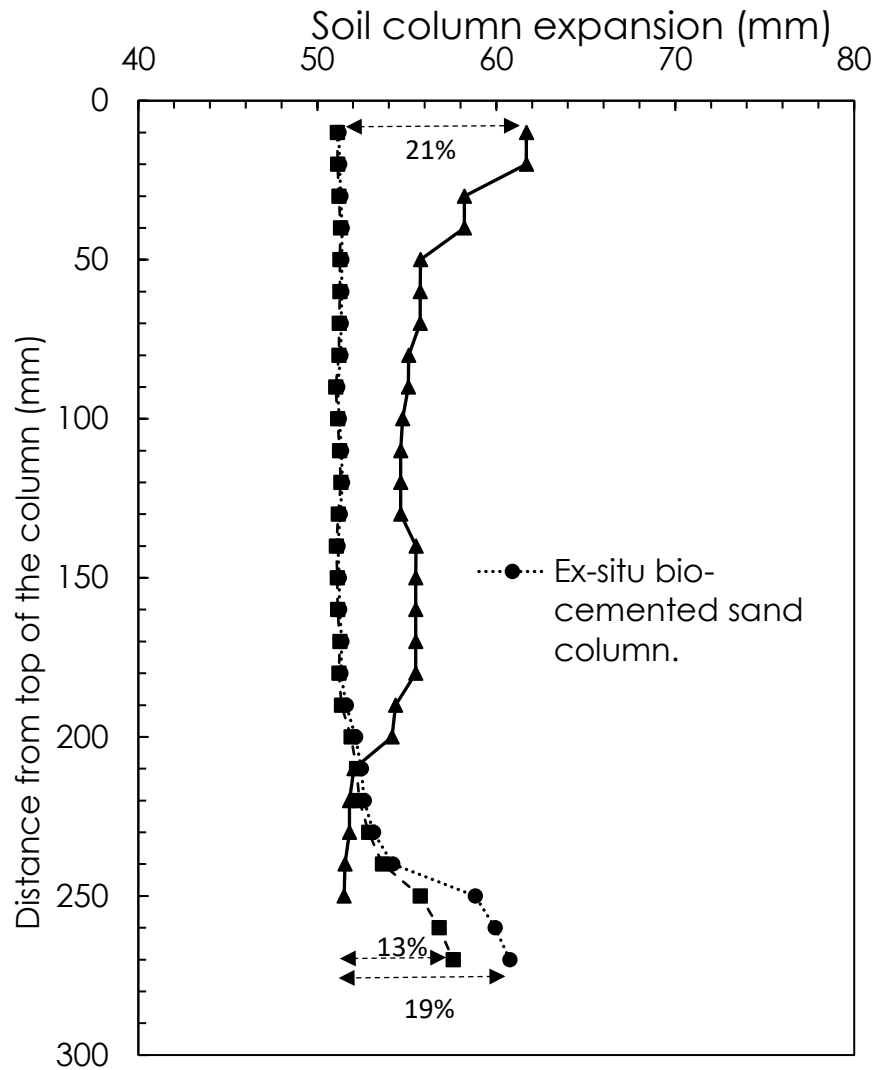
Figure 8.5. Radial expansion of sand columns

In contrast, the bulging along both the *ex-situ* and *in-situ* bio-cemented columns was dramatically reduced as the radial strain recorded was only 0.5% which is an indication that there was minimal variation in lateral stress or stiffness within the clay material. Figure 8.6 provides further evidence that the distribution of calcium carbonate played an essential role in reducing bulging along the length of the sand column. It can be seen that calcium carbonate was observed to be concentrated in the upper two-thirds of the *ex-situ* bio-cemented column, whereas it was found along most of the length (93%) of the *in-situ* bio-cemented column, albeit generally at lower concentrations in the upper profile. Less than 0.5% calcium carbonate was observed at a depth of 220 mm in the *ex-situ* column, whereas about 6%

was seen at the same depth in the *in-situ* column. It can be seen from Figures 8.7a and 8.7c that precipitation of calcium carbonate bridged the sand particles of the *in-situ* column. This may be an indication why the *in-situ* column behaved better than the *ex-situ* column. The chemical composition of the bio-cemented materials were confirmed by EDS analysis (Figures 8.7b and d), which was conducted on a random specimen from the *in-situ* treated column. Figures 8.7b and d indicate that the chemical constituents of the precipitated crystals are mainly composed of calcium, carbon, and oxygen. This further validates that these chemical compositions indicate the presence $CaCO_3$ in the sample.

More importantly, it can be inferred from Figure 8.6 that MICP treatment will reduce the bulging of the stone columns. The difference between the precipitation profiles of the *in-situ* and *ex-situ* bio-cemented column is due to the column preparation process adopted in the current investigation. In the case of the *in-situ* treated column, it can be postulated that some of the biogenic calcium carbonates which precipitated within the pore spaces of the upper column sections might have moved to the lower section during treatment flushes, resulting in better distribution with depth. In the case of the *ex-situ* treated column, precipitates might have been washed out during the treatment process, and/or during soaking and freezing after treatment. It is also worth noting that the efficiency of MICP for the *in-situ* and the *ex-situ* bio-cemented columns were determined based on the calcium used in cementation solution and was found to be 45.60% and 32.55%, respectively.
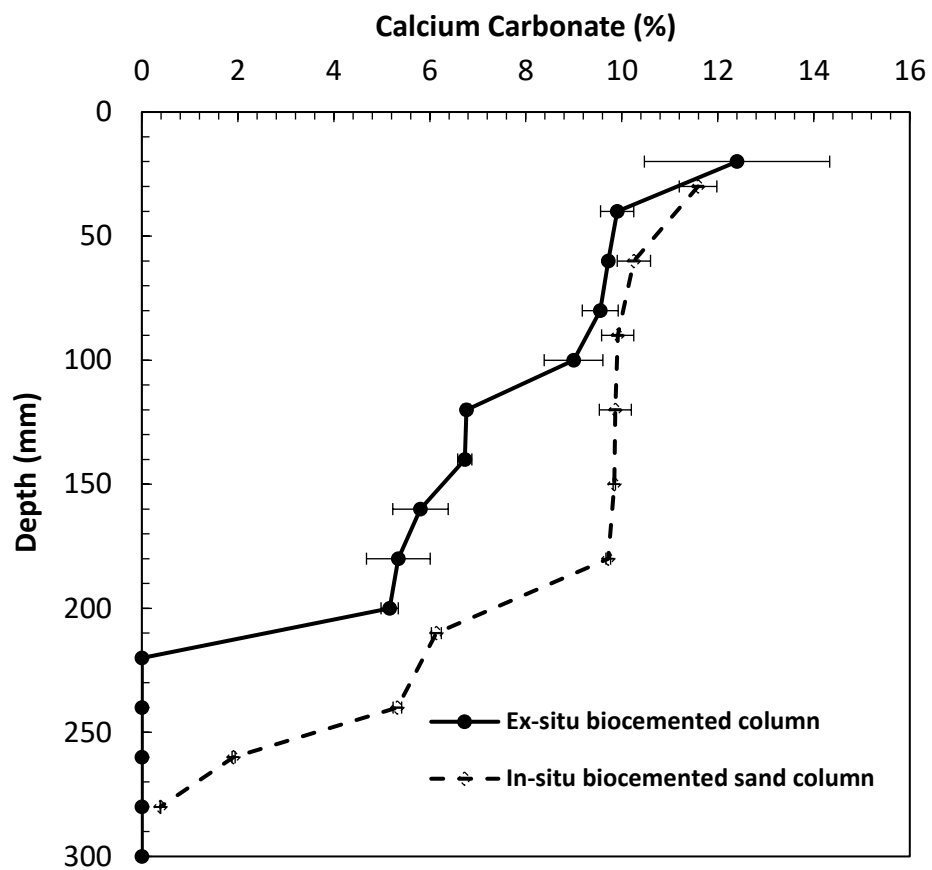
Figure 8.6. Distribution of precipitated calcium carbonate with depth of treated columns.

(b)

(c)
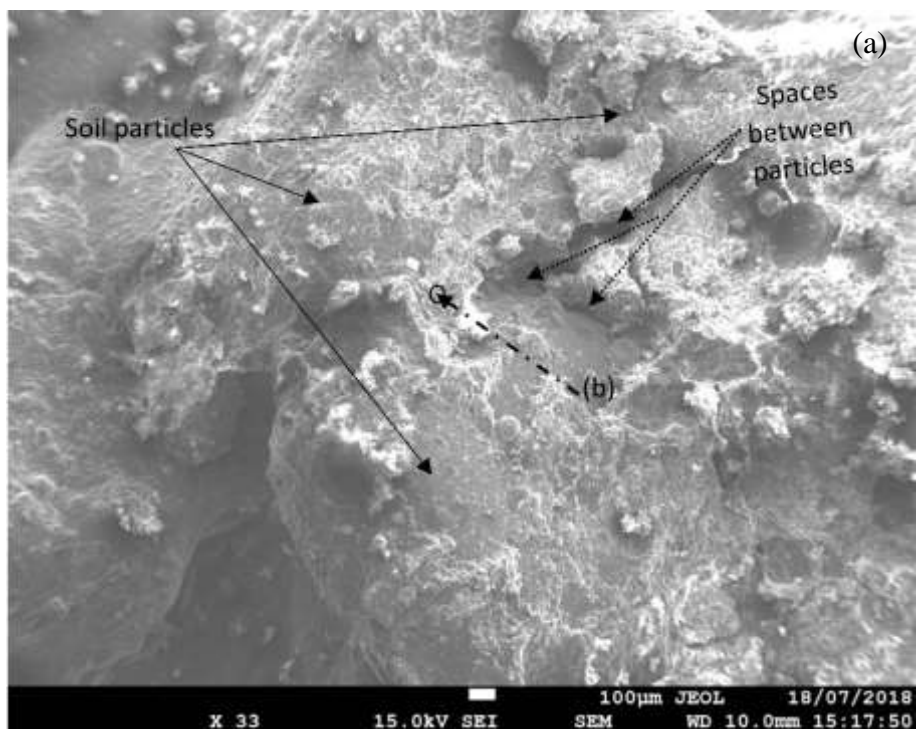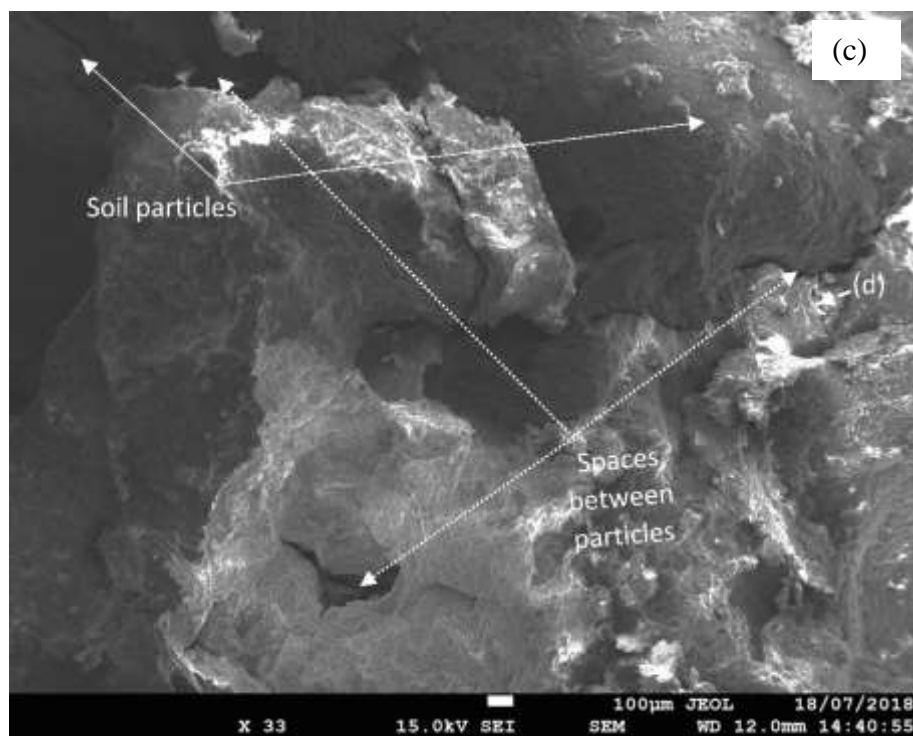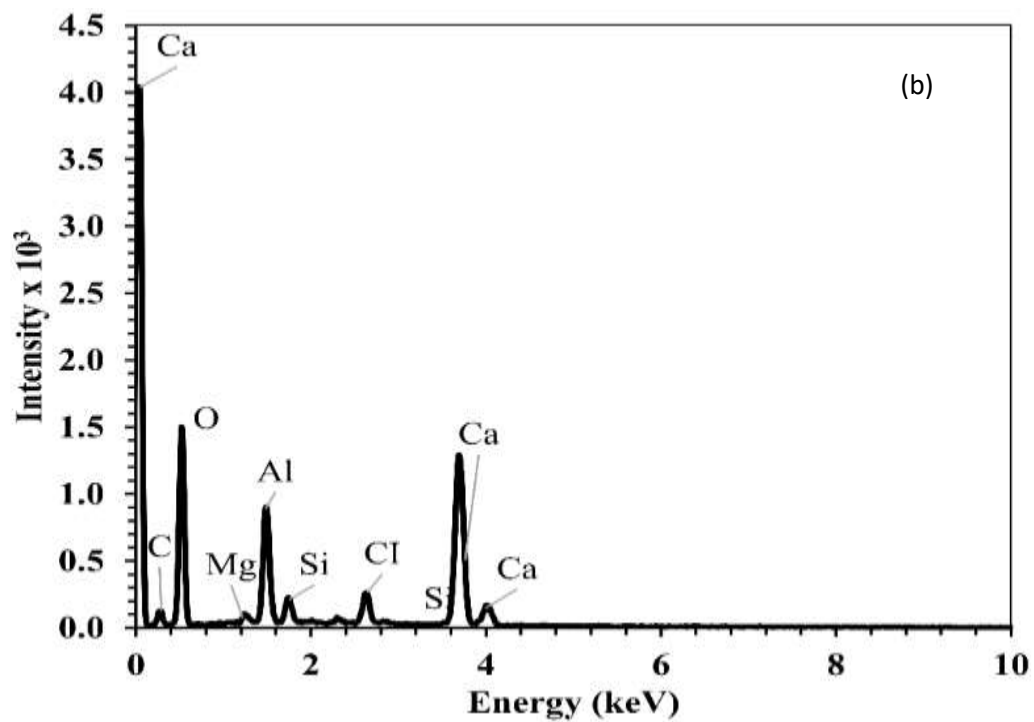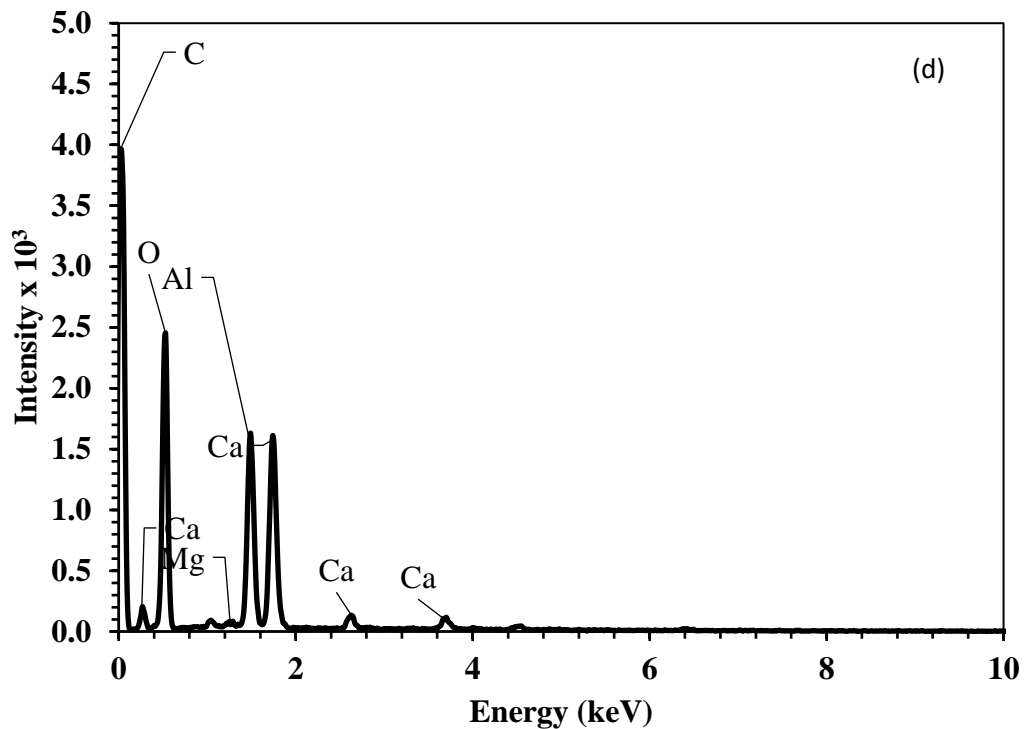
Figure 8.7. (a) and (c) SEM showing typical precipitated calcium carbonate; (b) and (d) EDS for chemical composition of the $CaCO_3$ crystals between sand particles.

It is also interesting to note that the maximum radial strain occurred at the lower portion of both treated columns, it was about 19% in the *ex-situ* bio-cemented column and 13% in the *in-situ* bio-cemented column, respectively. This observation is an indication that bulging occurred in the length of the column where treatment was not as efficient as at the top, as shown in Figure 5. Radial bulging at the base of the treated columns tended to increase in magnitude with decreased calcium carbonate content. The most substantial lateral deformations occurred along the length of the column where no calcium carbonate precipitation was recorded.

8.3.3 Variation of gravimetric moisture content and undrained shear strength

The gravimetric moisture content measurements of the kaolin were conducted on samples that were extruded before the installation of the column and at the end of the column tests. The specimens were collected from 25, 50, 75, 100, 125, 150, 175, 200, 225, 250 and 270 mm depths, respectively. Figure 8.8 illustrates the variation of the initial and final gravimetric moisture contents along the depth of the un-treated, *ex-situ* bio-cemented and *in-situ* bio-cemented columns. The initial distribution of the gravimetric moisture contents was similar for all cases, confirming that the soil columns have undergone a similar initial degree of consolidation, and therefore the repeatability of the tests. The final average gravimetric moisture content of these samples was about 51%. Furthermore, the uniformity of this distribution along the columns indicated that a homogenous bed of consolidated clay had been achieved which is an essential component to emulate a natural deposit. The distributions of the gravimetric moisture contents after the column tests were completed indicate that a noticeable reduction in moisture content occurred for all tests at test completion. For instance, the presence of columns reduced the average moisture content by about 11%.

Similar to the gravimetric moisture content measurements, clay specimens were taken at various depths of the columns pre and post-loading for vane shear testing. Figure 8.9 shows that the undrained shear strength was about 15 kPa along the column, confirming the consistency of the test bed and its repeatability. The undrained shear strengths of the clay samples differed after loading. For the case of the un-treated sand column, the maximum undrained shear strength was observed to occur in the top part of the column and decreased further down in the column. This is associated with the section that had a significant radial expansion and reduced gravimetric moisture content (see Figure 8.5 and Figure 8.8), i.e., where the bulging of the column occurred. However, the bio-cemented columns showed a

different pattern. The maximum undrained shear strength occurred in the lower part of the column where there was less bio-cemented material (see Figure 8.6) and coinciding with a higher lateral column expansion (see Figure 8.5). In this particular case, the radial bulging was transferred to the clay layer surrounding the less treated part of the column which gained strength due to the densification process induced by the bulging process.
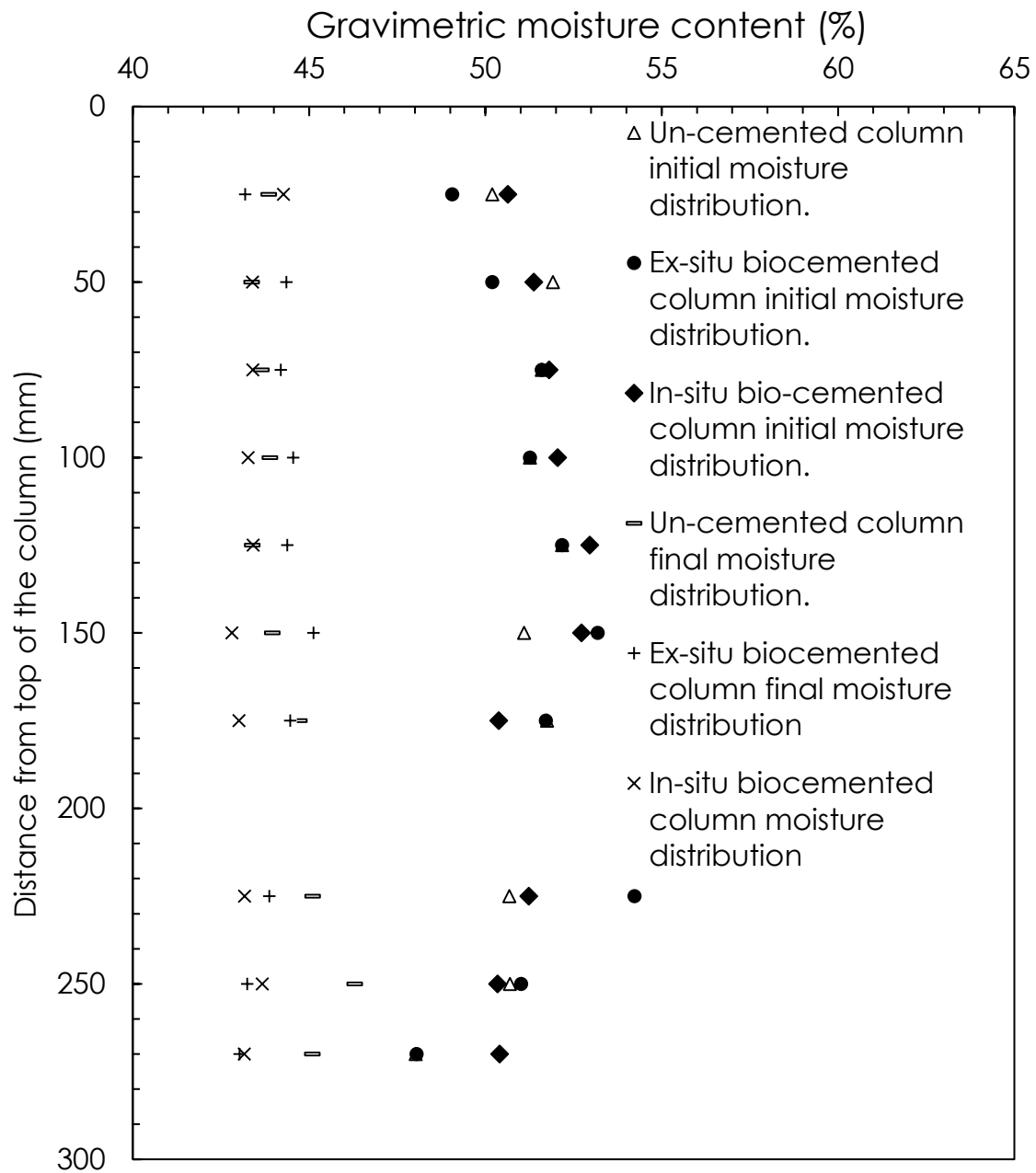
Figure 8.8. Variation of gravimetric moisture content with depth before and after loading.
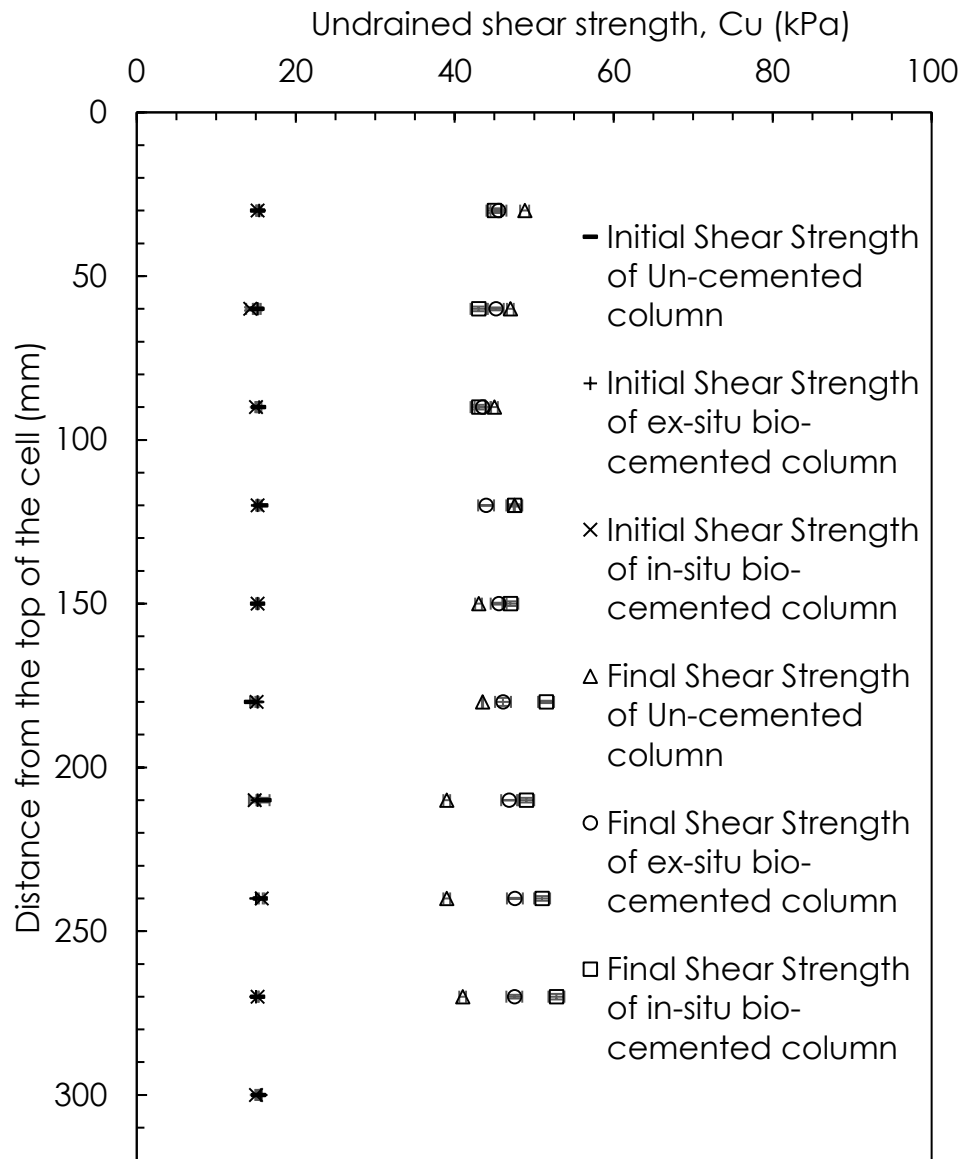
Figure 8.9. Variation of undrained shear strength against depth of columns before and after loading.

8.4 Conclusions

This research was aimed at investigating the effect of bio-cementation on stone column behaviour using laboratory scale model testing. Results were presented for un-cemented sand, *ex-situ* and *in-situ* bio-cemented sand columns. The salient conclusions that can be drawn from this work are:

1. The reduction of vertical strain for un-cemented column compared with the consolidated kaolin clay on its own varied with the range of applied vertical stresses, with an average decrease of about 22%. However, the *ex-situ* partially bio-cemented sand columnar inclusion within the clay bed showed a substantial reduction in the vertical strain as the average vertical strain reduction across the range of applied stresses was about 43% and 56% compared with the un-cemented sand column and the kaolin clay, respectively. A further reduction of about 11% in the vertical strain was observed for the *in situ* bio-cemented sand column.

2. The reduction of the bulging provided by the *ex-situ* and the *in situ* bio-cemented columns, equated about 62% and 75%, respectively compared with the un-cemented column. It was also found that the bulging occurred at the lower section of the bio-cemented column, where there was less precipitation of bio-cement materials as it was observed that the bio-cement materials were found to precipitate mostly within the upper two-thirds of the bio-cemented column. The undrained shear strength variations along the columns support the above observations.

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Chapter 9: Summary and suggestions for further research

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9.1 Summary of outcomes

The main objective of this thesis was to investigate the effectiveness of the microbial induced calcite precipitation (MICP) method to enhance the engineering properties of coarser granular soils for possible use in granular columns installed in soft soil. Experimental studies related to various methodologies and parameters that may have a potential effect on the scale-up of the MICP method have been investigated to achieve an optimised strategy in the application of MICP within granular columns. X-ray computed tomography (XCT) and scanning electron microscopy (SEM) were used to evaluate the microstructure changes of the bio-cemented coarse materials and to give a proper explanation in relation to the macro-mechanical changes. The final phase was the application of the MICP technique, where an optimised method was used with a sand column installed within soft clay.

This thesis consists of nine major chapters, of which seven are dedicated to the presentation of the major findings of the thesis, while the other two chapters provide the introduction and conclusions. Besides, each chapter has been subdivided into inter-related sections to meet the specific sub-objectives to achieve the main objective of this thesis. The major outcomes drawn from each phase are presented in the following Sub-sections.

9.1.1 *An optimisation of MICP treated coarse granular columns.*

Application of MICP to sand/stone columns has not been optimised to be used in *in-situ* yet. Therefore, various conditions and numerous parameters (such as methodologies, number and duration of biochemical treatments, amount of deposited calcium carbonate, initial relative density, dry density, cementation solution concentrations (equimolar and non-equimolar), various reaction times, reaction temperatures and gradation of soil) are investigated before harnessing the use of bio-grouting technique in coarse granular soil columns. These parameters are summarised in the following sub-sections.

9.1.1.1 *Methodology*

MICP method faces many challenges in geotechnical application, especially when it is considered as a potential enhancement to engineering properties of deeper soils when stone columns/sand piles or rammed columns are used. Although bacterial suspension and cementation solution used in the MICP method have a low viscosity, the main practical difficulty resides on how to inject these solutions to the places where improvement is needed. Bio-grouting was investigated to mitigate slumping of granular columns that may occur during excessive radial expansion. However, this sub-section aimed to present an evaluation of MICP to enhance engineering properties (compressive strength) of crushed coarse aggregate and coarse sand columns and to quantify the amount of calcium carbonate precipitation Another objective of this sub-section was to show the feasibility of treating the coarse materials using a gravity-driven percolation bio-grouting process combined with different treatments strategies. Two typical strategies to apply bio-grouting were investigated; the first protocol was based on using a multi-reagent phase strategy and the second was a multi-soil lifts strategy to treat crushed aggregate column materials. Both approaches were expected to provide an insight into the distribution of calcium carbonate precipitation and its effect on unconfined compressive strength (UCS) values of coarse materials. Based on the experimental results, the following salient conclusions can be drawn:

➢ Various soil-lift strategy (1, 2, 3, 4, and 5 soil lifts) were undertaken to test its applicability to MICP- treated coarse sand columns. Single coarse sand lift column gave more homogenous calcium carbonate distribution than multiple soil lifts columns.

➢ The main weaknesses in the multiple coarse sand lifts columns were associated with the interface between the lifts.

- Strength and stiffness were dependent on the overall mass of the bio-cemented materials and the maximum strength was associated with single coarse sand lift column.

- The study revealed that an increase of soil-lift number has a negative influence on the mechanical properties of the bio-cemented coarse sand. However, the minimum strength and stiffness achieved in this study are enough to mitigate the slumping of a soil column that may occur during excessive radial expansion during loading and improve rammed columns capacity.

- A multi-soil lift strategy included an option of 1, 2, 3 and 4 crushed aggregate lifts, and it was also shown that using a one-soil lift resulted in higher strength than using multi-soil lifts due to the interaction between soil lifts.

- A multi-reagent phase treatment strategy comprised various phases (2, 4, 6 and 12 phases) of bacterial suspension and cementation solution and experienced a different tendency. An increase of multi reagent phase number associated with increases in compressive strength was observed up to a four-reagent phase, and then a decrease in strength was recorded.

- The maximum strength of the crushed aggregate of approximately 2.3 MPa was achieved using four-reagent phase strategy.

- The total amount of calcium carbonate precipitation was in the range of 12.4–13.2%. Furthermore, bio-grouting bounding crushed aggregate particles can be useful to gain localised bio-cementation either in the upper or lower portion by utilising a multi-reagent phase, which can be combined with a multi-soil lift strategy to obtain a uniform calcium carbonate and a high strength along a granular column. The multi-soil lift strategy could also be used to treat a deep granular pile where it is impossible to strengthen the bottom lifts and heterogeneous distribution of calcium carbonate precipitation can be overcome by increasing number of treatment cycles in the top lifts.

➤ Microstructure analysis has further confirmed the precipitation of calcium carbonate on the surfaces of the crushed aggregate particles.

9.1.1.2 Factors affecting bio-grouting of coarse material

The effects of parameters such as cementation solution (urea and $CaCl_2$ concentrations), temperature, incubation time (reaction time) as well as number and duration of biochemical treatments, amount of deposited calcium carbonate, initial relative density, dry density, and gradation of soil need to be understood to enable a controlled use of MICP and to scale up the MICP method from bench-scale to large-scale applications. These factors were investigated using the optimised methodology (one soil lift and four-phase treatment strategy) that can be found in chapters 2 and 3.

Even though a lot of research work has been conducted to investigate the effects of cementation solutions on fine to medium sand, there is still uncertainty about selecting the most adequate cementation solution concentration that can be adopted for practical use. A lack of knowledge also exists concerning the proper cementation solution concentration that can be used with coarser materials and whether the bio-cementation process can be achieved in the same way as with fine and medium sands as discussed in previous studies (Al Qabany and Soga 2013, DeJong et al. 2010), because more calcium carbonate precipitation may be required to attain a targeted strength. This cementation solution (equimolar and non-equimolar (urea-$CaCl_2$)) factor among other factors such as incubation time and the temperature are presented in chapter 4. The effects of biochemical treatments number, amount of urea use, amount of $CaCO_3$ precipitation, and initial relative density, as well as dry density on the engineering properties of coarse sand, were investigated in chapter 5. A series of laboratory experiments were also conducted to investigate the effect of particle size distribution on the efficacy of bio-cementation of granular columns and are presented in chapter 6. The effects were assessed based on the measurements of the unconfined compressive strength (UCS),

the amount of precipitated calcium carbonate, scanning electron microscopy and energy dispersive spectroscopy. The salient conclusions that can be drawn from these works are:

➢ An increase of equimolar cementation solution was associated with increased calcium carbonate precipitation and an increase in the UCS. The maximum strength (around 2 MPa) was achieved at 1 M equimolar cementation solution concentration.

➢ Utilising low concentrations of non-equimolar cementation solutions resulted in greater uniformity of calcium carbonate distribution in coarse sand columns than for similar concentrations of equimolar cementation solutions.

➢ The content of precipitated calcium carbonate increased with an increase of the reaction temperature of MICP treated coarse sand, but the efficiency of the deposited calcium carbonate was found to be high with a moderate reaction temperature of 20°C as it resulted in high compressive strength. However, a consistent improvement in the UCS of coarse sand can also be obtained at a low reaction temperature (0°C).

➢ An increase of incubation time leads to an increase in deposited calcium carbonate and UCS. This study also shows that a 48 hrsr incubation time has been identified as the optimum time that can result in a high UCS. In addition, it was found that the amount of calcium carbonate precipitation reached the highest level when cementation solution concentration, incubation time and temperature were very high, i.e. 1.5 M, 96 hrs and 40°C, respectively. However, the UCS was adversely affected by high temperature and cementation solution concentration.

➢ SEM and EDS analysis have confirmed that having large agglomerated calcium carbonate crystals filling the gaps between coarse sand grains lead to improvement of the compressive strength.

➢ The amount of deposited $CaCO_3$ tended to increase with an increasing number of biochemical treatment cycles and the amount of urea used.

➢ The amount of deposited $CaCO_3$ correlated with the increase of the UCS and stiffness of bio-cemented coarse sand, and the maximum UCS attained was about 14 MPa.

➢ The minimum $CaCO_3$ to obtain an improvement in strength and stiffness (about 200 kPa and 259 MPa, respectively) of bio-cemented coarse sand was around 2.6% in agreement with previous studies. A minimum of four-biochemical treatments was required to obtain minimum $CaCO_3$ cementation. The UCS and stiffness were increased with the increase of the initial relative density of MICP-treated coarse sand up to 80% relative density and then decreased at 100% relative density. A UCS value of 16-MPa was achieved at 80% relative density, which was associated with higher $CaCO_3$ precipitation (about 25%).

➢ The current results indicate substantial reductions in the porosity and permeability when using a different number of biochemical treatments. The treated specimens maintained permeability in the range of 3.76×10^{-6} m/s, which is desirable if the pore water pressure in a soil matrix needs to be dissipated.

➢ Microstructure analysis has further confirmed the precipitation of calcium carbonate within and around coarse sand grains. Large trigonal- rhombohedral calcium carbonate crystals precipitated in the pore matrix of coarse sand when using a higher number of biochemical treatment cycles, whereas adopting a low number of treatments resulted in small spherical calcium carbonate crystals.

➢ The column (column C) with the lower percentage of fine materials (25%) showed a better distribution of calcium carbonate precipitation along its length compared to the columns which contained 100%, 50% and 75% fines (columns B, D and E, respectively). The column with purely coarse aggregate (column A) had more calcium carbonate at the lower part of the column than in other regions of the column.

➢ Bio-cementation of merely coarse aggregates can be facilitated by the addition of fine aggregates particles. The inclusion of 25% fine aggregate particles to form a gap graded distribution was found to be sufficient to increase the unconfined compressive strength (UCS) from 0 kPa (untreated mix) to about 575 kPa (treated mix). The presence of more extensive fine contents (>25%) led to minimal changes in UCS of the bio-cemented soil and more non-uniform distribution of calcium carbonate precipitation. Thus, the bio-cementation process can be an effective method to improve the strength of coarse aggregate materials if their fine content is about 25%. This process can be beneficial for stone columns installed in soft clays to mitigate slumping that may occur in the column during installation or excessive radial expansion.

9.1.2 *Microstructure evolution of the bio-cemented granular columns*

Linking the macroscopic mechanical behaviour of a bio-cemented material with a calibrated microscopic tool such as X-Ray computed tomography (XCT) and SEM has been proven a more reliable method to evaluate changes in material engineering properties as shown in chapter 7. This phase of this thesis explored the use of laboratory-based XCT to track microstructure changes in the amount and distribution of calcium carbonate along a bio-cemented coarse sand sample associated with its macro-mechanical behaviour. Other soil characteristics such as porosity, dry density, void ratio and pore volume that could change due to the bio-grouting process were also identified and analysed in the current study. Based on the results presented in chapter 7, the following salient conclusions can be drawn:

• The precipitation of $CaCO_3$ within the pore matrix of coarse sand specimens is directly related to the number of biochemical treatment cycles. More bio-treatment cycles induced greater % $CaCO_3$ precipitation.

- The increase % $CaCO_3$ resulted in bio-cementation as observed by a significant increase in the strength of coarse sand columns. The maximum strength of 14 MPa was achieved for 25% $CaCO_3$ content.

- XCT imaging accurately detected the level of $CaCO_3$ precipitation achieved in coarse sand column specimens at all bio-chemical treatment cycles. Segmentation allowed visualisation and quantification of significant changes that occurred at the micro-scale level during the bio-cementation reactions, supporting the findings obtained at the macro-scale level. The segmentation of the XTC images adopted in the current investigation allowed an accurate estimation of % $CaCO_3$, dry density changes as well as void ratio and porosity changes, which correlated very well with laboratory-based measurements.

- XCT imaging is a viable alternative technique to quantify $CaCO_3$ content with acceptable accuracy, without the need for conventional gravimetric acid washing technique.

- SEM and EDS analysis further confirmed the changes observed in the microstructure of the bio-cemented specimens. The SEM micrographs showed that $CaCO_3$ crystal from small lumps of bonds into large $CaCO_3$ crystals. EDS confirmed that the chemical compositions of the precipitates are related to the constituents of $CaCO_3$.

9.1.3 *Application of bio-cemented coarse granular columns*

The final phase of this study was to investigate column group using a bench model testing which is a common and relatively a cost-effective method of investigating engineering behaviour of sand/stone columns. The main objectives of this phase was to investigate column group behaviour of three different types of sand columns that were installed to improve the engineering behaviour of soft clay including, un-cemented sand column, ex-situ bio-cemented sand column (i.e. was treated before inserting it into

soft soil) and in-situ bio-cemented sand column (i.e. was treated while it was within soft soil). A multi-phase treatment strategy that was illustrated in phase one was used to treat the coarse granular columns, and the suitable factors such as cementation solution concentration, temperature, several biochemical treatments and incubation time that were observed in phase one to give a better result have been taken into account. The study was also aimed to use a staged loading of sand columns model to replicate the staged construction commonly used in stone column projects such as an embankment. Scanning electron microscopy (SEM) and energy dispersive spectroscopy (EDS) were also harnessed to observe the existence of the bio-cement material. Based on these results, the following conclusions are drawn:

> The reduction of vertical strain for un-cemented column compared with the consolidated kaolin clay on its own varied with the range of applied vertical stresses, with an average decrease of about 22%. However, the ex-situ partially bio-cemented sand columnar inclusion within the clay bed showed a substantial reduction in the vertical strain as the average vertical strain reduction across the range of applied stresses was about 43% and 56% compared with the un-cemented sand column and the kaolin clay, respectively. A further reduction of about 11% in the vertical strain was observed for the in situ bio-cemented sand column.

> The reduction of the bulging provided by the ex-situ and the in situ bio-cemented columns, equated about 62% and 75%, respectively compared with the un-cemented column. It was also found that the bulging occurred at the lower section of the bio-cemented column, where there was less precipitation of bio-cement materials as it was observed that the bio-cement materials were found to precipitate at two-third of the bio-cemented column. The undrained shear strength variations along the columns support the above observations.

6.2 Suggestion for further research

Further investigations are suggested as they may provide a greater understanding of the bio-grouting process of coarser materials. This is because several aspects of the bio-cemented coarse materials could not be adequately investigated due to time constraints. Most aspects related to bio-grouting process and its application onto sand/stone columns have been not thoroughly investigated in this thesis and thus require further research. Suggestion for further investigations are summarised below:

- Bio-grouting to improve coarse materials using two typical strategies to apply bio-grouting were proposed; the first protocol was based on using a multi-reagent phase strategy and the second on a multi-soil lifts strategy to treat crushed aggregate column materials. However, combining the two methods to resolve the problem of heterogeneity of soil improvement, which generally exists in engineering application, has not been investigated. Thus, this requires further work to optimise these strategies so they can be used in the field.

- MICP treated soil is mostly developed in laboratory-based tests, where environmental conditions are controlled thoroughly. However, it is necessary to establish a microbial growth model under different real soil environments to simulate the formation of contact among soil particles. As it is very hard to quantify and control bacterial cells growth accurately and their enzyme activity, further consideration needs to focus on the establishment of microorganisms and soil particle models. This may provide an insight concerning the attachment of bacterial cells onto surfaces of soil particles.

- Another by-product of MICP treatment is ammonium (NH_4^+), which is regarded as a contaminant. Though this was out of the scopt of the current study, attempts have been made to eliminate the detrimental effect of this by-product by converting it into struvite, which could also contribute to strength gains (Qian et al. 2015, 2016). A reclamation of unfavourable bio-product (ammonium chloride) using the struvite

precipitation process can be an effective method to have a clean environment after calcite precipitation. Extensive research is warranted in this field.

➢ Although this study has shown that there are potential advantages and application of bio-grouting process, upscaling of the process is a challenge which requires extensive field investigation to evaluate the long-term durability of compressive strength induced by the process. A large-scale based on the procedure presented in this thesis is highly recommended.

➢ Small-scale testing was undertaken in the current study to simulate group column behaviour of bio-cemented coarse granular column installed in soft soil. However, isolated columns behaviour has not been investigated and requires further investigation before any scale-up. Several cases also need to be investigated, such as using column comprised of crushed aggregate, different cementation levels, full column cementation versus partial cementation, various shear strength of weak soil, and use of optic fibre to monitor lateral deformation.

➢ To investigate practical methods of bio-cemented column construction, it is suggested to investigate a medium-scale column model, where the aggregates will consist of coarse rock gravel or coarser-sized crushed rock as commonly used in a full-scale column material. A numerical model has not been used to simulate the coarse sand column – weak soil behaviour due to the complexity of the current work and time constraints. However, it is recommended to look into the possibility of numerically simulating the interaction between coarse sand materials with surrounding soft soils as well as calcite precipitation.

6.3 Reference

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