

**School of Civil and Mechanical Engineering**

**Stabilisation of Geomaterials using Microbially Induced Calcium  
Carbonate Precipitation**

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**This thesis is submitted for the Degree of  
Doctor of Philosophy  
of  
Curtin University**

**July, 2018**

# Author's Declaration

To the best of my knowledge and belief this thesis contains no material previously published by any other person except where due acknowledgement has been made.

This thesis contains no material which has been accepted for the award of any other degree or diploma in any university.

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[Hannah Porter]

Date: 6/08/2018

# Authorship Declaration: Co Authored Publications

This thesis contains the following work that has been published and / or prepared for publication:

## Journal Papers

- PORTER, H., DHAMI, N.K. & MUKHERJEE, A. 2018. Sustainable road bases with microbial precipitation. *Proceedings of the Institution of Civil Engineers – Construction Materials*, 171, 95 – 108
- PORTER, H., DHAMI, N.K. & MUKHERJEE, A. 2017. Synergistic chemical and microbial cementation for stabilization of aggregates. *Cement and Concrete Composites*, 83, 160-170.
- PORTER, H., BLAKE, J., DHAMI, N.K. & MUKHERJEE, A. 2018. Rammed earth blocks with improved multifunctional performance. *Cement and Concrete Composites*, 92, 36-46
- PORTER, H., DHAMI, N.K., MUKHERJEE, A. AND TULADHAR, R. Can engineered biocement be sustainable?, (Currently submitted to journal for publication), 2018.

## Conference Papers:

The following paper was submitted to the Sustainable Construction Materials and Technologies, (SCMT4) Conference, Las Vegas, 2016 and received the title of an ‘award winning paper’.

- PORTER, H., DHAMI, N.K., & MUKHERJEE, A. 2016. Sustainable Road Bases with Microbial Carbonate Precipitation. *Fourth International Conference on Sustainable Construction Materials and Technologies*. Las Vegas, USA: SCMT4

# Abstract

The production of Portland cement worldwide is estimated to contribute 6% of carbon dioxide from all man-made emissions. Although Portland cement has proven to be a durable and reliable construction material, the development of a cementing material that does not produce carbon dioxide during the manufacturing phase is required to ensure the sustainability of the global cement industry.

The Australian landscape has a large number of naturally cemented structures, which provide inspiration for sustainable engineering materials. Structures such as corals, beach rocks and stromatolites are cemented through the process of Microbically Induced Calcium Carbonate Precipitation, (MICP). During the process of MICP, the metabolic process of microbes aids in the precipitation of calcium carbonate crystals, which naturally 'cement' soil particles together. The process of MICP in nature occurs under ambient conditions with minimal embodied energy.

Recently MICP has gained much attention for its applicability to both geotechnical applications and structural building materials. To date, the majority of investigations have focused on the use of MICP in sand, (for grouting applications) or as a crack sealant and surface treatment for reinforced concrete structures. However, MICP has the potential to improve the sustainability of many other geotechnical structures, such as stabilised road base layers, or rammed earth structures. The overall theme of this thesis has been to establish the efficacy of MICP in stabilising geomaterials for the construction industry. This thesis is presented in a publication-based format comprising of four journal papers and one conference paper.

Chapter 3 of the thesis reports on the potential for MICP as a replacement or augmentation to Portland cement in cement stabilised or plain sand and road base materials. The results demonstrate that MICP may be used on its own, or in conjunction with other chemical binders, (such as Portland cement). When used as a supplementing material in stabilised road base structures, MICP will decrease the overall requirement for cement thereby increasing the sustainability of the material. Chapter 4 further investigates the synergistic relationship between MICP and other chemical binders, (such as geopolymer), and further defines the mechanism of MICP through a microstructural analysis.

Chapter 5 reports on the multifunctional performance properties of rammed earth materials using MICP as a surface treatment on both cement stabilised, and plain rammed earth blocks. The results of chapter 5 demonstrate that the surface treatment of rammed earth blocks with MICP is a worthwhile solution, not just to decrease erosion and water absorption, but also to sustainably increase strength.

Chapter 6 of the thesis reports on the sustainability of the engineered MICP process. A cradle-to-gate LCA was used as a tool to compare the sustainability of different MICP metabolic pathways with that of the traditional carbonation process. The results of this analysis demonstrate that the sustainability of different metabolic pathways to MICP is varied. Calcium carbonate produced using carbonic anhydrase producing bacteria proved to be the most environmentally sustainable, followed by methanogens, (methane oxidation) and then cyanobacteria, (photosynthesis). The least sustainable pathways to MICP were via ureolytic and denitrifying bacteria. The substitution of laboratory grade input materials, with a lesser purified, commercial equivalent results in significant improvements to the sustainability of all MICP pathways. Treatment of the gaseous waste produced by urea hydrolysis, denitrification or ammonification pathways is also required for the process to be truly sustainable.

Chapter 7 provides key conclusions from the research project and reveals the directions for future research.

# Acknowledgements

The multidisciplinary nature of a project that combines engineering and microbiology means that many people, from different backgrounds, and with different skillsets, have contributed to this project. I am thankful for the time, energy and generosity of the team at Curtin University, and for the collaborators further afield at James Cook University.

Prof. Abhijit Mukherjee, thank you for your supervision over the past 3 years. Your enthusiasm, excitement and ability to generate new ideas has inspired me. You have shaped the way that I approach research, and I am very fortunate to have had the opportunity to learn from, and work with you.

Dr. Navdeep Dhimi, thank you for introducing me to the exciting world of microbiology! Thank you for your time, assistance and patience in the laboratory. I have benefited greatly from working with such a competent microbiologist, and have loved developing my skills beyond the world of civil engineering.

Dr. Rabin Tuladhar, thank you for your generosity in allowing me to utilise JCU facilities and software for the life cycle assessment, completed as part of this project.

Dr. Megan Walske, thank you for sharing with me your expertise in the field of geotechnical engineering. Thank you also for the many encouraging conversations whilst I was in the midst of 'PhD land'.

I am very grateful to my fellow post-graduates within Prof. Abhijit's team at Curtin University. Thank you for the many exciting team discussions! Without a doubt, you have contributed much to my own personal and academic development.

On a personal note, I would like to thank my parents, for their continued support and encouragement to continue. Thank you Dad for being willing to read this thesis, and thank you Mum for the many encouraging phone calls over the past 3 years.

And last but not least, to my husband Ben. Thank you for being here with me on this journey, and thank you for the many delicious dinners you have cooked for me along the way!

# Dedication

For Ben.

Thank you for your unwavering support, kindness and encouragement.

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# Glossary of Terms

DIC Digital Image Correlation

EDS Energy Dispersive X-Ray Spectroscopy

LCA Life Cycle Analysis

MICP Microbially Induced Calcium Carbonate Precipitation

OPC Ordinary Portland Cement

RE Rammed Earth

REB Rammed Earth Block

SEM Scanning Electron Microscope

UCS Unconfined Compressive Strength

# Chapter 1 Introduction

## 1.1 Motivation

The production of Portland cement (OPC) worldwide is estimated to contribute 6% of carbon dioxide from all man-made emissions, (Imbabi et al., 2012), and is estimated to be the second most consumed resource in the world, (after water), (Flower and Sanjayan, 2007). The production of one tonne of OPC produces an equal amount of carbon dioxide to the atmosphere. Approximately 50% of the carbon dioxide produced by the cement industry occurs in the manufacturing phase, due to the emissions produced during the calcination of limestone while 30% occurs due to the burning of fuels to create heat for the calcination process, (CIF, 2013).



Although OPC has proven to be a durable and reliable construction material, sustainability research undertaken by (Imbabi et al., 2012) indicates that the development of a cementing material that does not produce carbon dioxide during the manufacturing phase, would ensure the sustainability of the global cement industry. OPC is used for two principal applications: to produce concrete, or to stabilise granular materials such as soil or mining backfill. In Australia, the ready mix and precast concrete industry consumes the highest proportion of OPC; however, other bulk works, such as soil and road base stabilisation, and mining backfill consumes the second largest proportion, (CIF, 2013)

The Australian landscape has a large number of naturally cemented structures, such as corals beach rocks and stromatolites (Figure 1.1), which are formed through the process of Microbial Induced Calcium Carbonate precipitation, (MICP). The process of MICP uses microbes indigenous to the soil, to naturally ‘cement’ materials together by producing calcium carbonate at ambient conditions. Recently MICP has gained much attention for its applicability to both geotechnical applications, (Dejong et al., 2013b) and structural building materials, (Achal and Mukherjee, 2015). MICP in nature has a low embodied energy, is reversible, recyclable and self – healing, and as such, provides a way to sustainable cementation, (Mukherjee, 2010). Dejong et al. (2013b) envisaged that harnessing the biological processes that occur in natural formations would represent the next transformative practise for geotechnical engineering.



**Figure 1-1** Naturally cemented materials, (a), (b) Corals, (Nelly Bay, Magnetic Island, Queensland), and (c) Beach rock, (Lucky Bay, Esperance, Western Australia)

## 1.2 Thesis outline

This thesis is presented in a publication-based format comprising of four journal papers, and one conference paper, (Chapter 3 to Chapter 6). The overall theme of this work has been to establish the efficacy of MICP in stabilising geomaterials for the construction industry. Chapter 7 provides key conclusions of the research, as well as recommendations for future work. The overall structure of the thesis is shown in Figure 1-2. A brief summary of each chapter, and the relationship to the overall thesis objectives is discussed below.



**Figure 1-2** Structure of the thesis

**Chapter 1: Introduction** details the motivation of the project and the overall thesis outline.

**Chapter 2: Literature Review** discusses the background and significance of the work. Identifies current gaps in literature and defines the scope of the project and research objectives.

**Chapter 3: Sustainable road bases with microbial precipitation (Paper 1 and 2)** reports the performance of MICP as a stabilisation technique within road base and sand materials, and as an augmentation technique for previously cement stabilised materials. Unconfined Compressive Strength (UCS) testing was used to characterise the bulk strength and stiffness of sand columns. Digital Image Correlation (DIC) was undertaken concurrently with UCS testing to help correlate the deformation and strain build up throughout the test. The behaviour of samples at the micro scale was also investigated using Scanning Electron Microscope, (SEM) and nano-indentation testing. The results of chapter 3 have been previously presented at the Sustainable Construction Materials and Technologies (SCMT4) conference in Las Vegas, (2016) and subsequently published as a journal paper in the Proceedings of the Institution of Civil Engineers – Construction Materials, (Porter et al., 2016, Porter et al., 2018b).

**Chapter 4: Synergistic chemical and microbial cementation for stabilisation of aggregates, (Paper 3)** further explores the use of MICP combined with traditional, chemical binders. A combination of chemical and microbial binders was attempted. The performance of the stabilised systems was

characterised in terms of compressive strength, elastic modulus and water absorption. The chemical and microbial cementation mechanisms were revealed to work synergistically. A microstructural investigation was undertaken to reveal the synergistic nature of the two binding systems. The manuscript of chapter 4 has been published in the Journal of Cement and Concrete Composites, (Porter et al., 2017a).

**Chapter 5: Rammed earth blocks with improved multifunctional performance (Paper 4)** reports the performance of MICP surface treatment, and the incorporation of crumb rubber on the engineering properties of rammed earth blocks. Surface application of MICP was found to increase the strength of rammed earth blocks by 25% and reduce the permeability and erosion of the blocks by 24% and 62% respectively. The addition of crumb rubber was found to improve the thermal resistance of blocks, however it also reduced the overall strength. The results of this research demonstrate that significant improvement of overall performance of rammed earth blocks can be achieved using MICP and crumb rubber. However, the overall performance requirements are specific to the engineering application and synergistic and antagonistic interactions must be considered to obtain an optimal performance. The manuscript of chapter 5 has been published in the Journal of Cement and Concrete Composites, (Porter et al., 2018a).

**Chapter 6: Is Biocement Sustainable? (Paper 5)** Although the natural process of MICP is undoubtedly sustainable, its engineered emulation deviates substantially from the natural process. To date, researches have used purified and concentrated reactants to induce MICP through the ureolytic pathway. This paper uses Life Cycle Assessment to evaluate the sustainability of the engineered MICP process. The results demonstrate that different metabolic pathways of MICP have considerably varying sustainability performance. The sustainability of man-made MICP process can be improved by using alternative biotic pathways and industrial grade nutrients. The manuscript of chapter 6 has currently been submitted to a journal for publication.

**Chapter 7: Conclusions** provides key conclusions from the research project and reveal the directions for future research.

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## Chapter 2

## Literature Review

### 2.1 Biological process

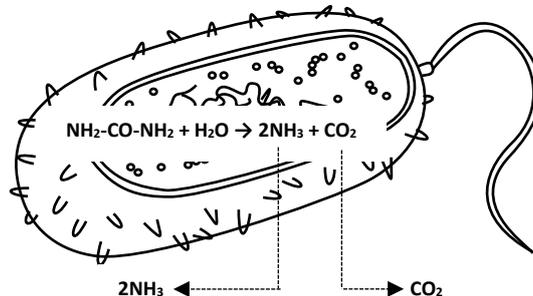
In nature, the process of MICP occurs when indigenous microbes metabolize available nutrients, and in the presence of  $\text{Ca}^{2+}$  aid in the precipitation of calcium carbonate. The process of MICP in nature can occur through heterotrophic pathways; where bacteria utilise organic compounds, such as urea, for energy and cellular material, or through autotrophic pathways where bacteria obtain energy from the sun, and reduce atmospheric carbon dioxide for energy and cellular material. Heterotrophic pathways to MICP include urea hydrolysis, (van Paassen, 2011, DeJong, 2010, DeJong et al., 2013b), denitrification, (Hamdan et al., 2017, Van Paassen et al., 2010), ammonification, (Rodriguez-Navarro et al., 2003, González-Muñoz et al., 2010, Chekroun et al., 2004), methane oxidation, (Ganendra et al., 2014, Whittenbury et al., 1970) and MICP induced through the carbonic anhydrase enzyme, (Kaur et al., 2016, Dhami et al., 2016b). Autotrophic pathways to MICP occur via photosynthesis, (Zhu and Dittrich, 2016, Dittrich et al., 2003).

The metabolic pathway which requires the lowest amount of free energy, and has the fastest reaction rate is MICP via urea hydrolysis, (DeJong, 2010). To date, the majority of experimental investigations using MICP for engineering applications have utilised the ureolytic pathway, and the microbial strain *Bacillus Pasteurii* (DeJong et al., 2014, Montoya and DeJong, 2015, Mortensen, 2011, Al Qabany and Soga, 2013, Bernardi et al., 2014, Harkes et al., 2008, Harkes et al., 2010, Whiffin et al., 2007, Dhami et al., 2016a, Cheng et al., 2013).

The process of microbial urea hydrolysis is illustrated in Figure 2-1. During urea hydrolysis the bacteria metabolize urea to produce ammonia, and carbon dioxide within the cell. These chemicals diffuse through the cell wall into the surrounding pore space between grains. The ammonia reacts with pore water to produce ammonium and hydroxyl ions, which cause a corresponding increase in the pH of the pore water solution. In the presence of an increased pH the carbon dioxide then equilibrates to form bicarbonate. Bicarbonate reacts with the hydroxyl ions, and an available calcium source to precipitate calcium carbonate, (Dejong et al., 2013b).

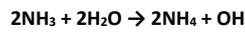
In natural systems the calcium carbonate crystals from MICP generally take the form of anhydrous polymorphs; calcite, aragonite or vaterite. Calcite is the most stable crystal form and has a distinct rhombohedral shape, while aragonite and vaterite are less stable forms that may transform into calcite over time, (Al-Thawadi, 2008, Tai and Chen, 1998, Dhami et al., 2013b). An amorphous, pre cursor phase, (ACC) has also been reported during the mineralisation process, (Hammes et al., 2003, Chen et al., 2009). Typical polymorphs of calcium carbonate are shown in Figure 2-2.

Bacteria metabolise urea and produce ammonia (NH<sub>3</sub>) and carbon dioxide, (CO<sub>2</sub>) within the cell

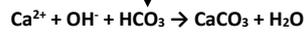


These chemicals diffuse through the cell wall and into the surrounding pore spaces

The ammonia (NH<sub>3</sub>) reacts with water (H<sub>2</sub>O) to produce ammonium (NH<sub>4</sub>) and hydroxyl ions (OH<sup>-</sup>), which increase the pH of the pore water

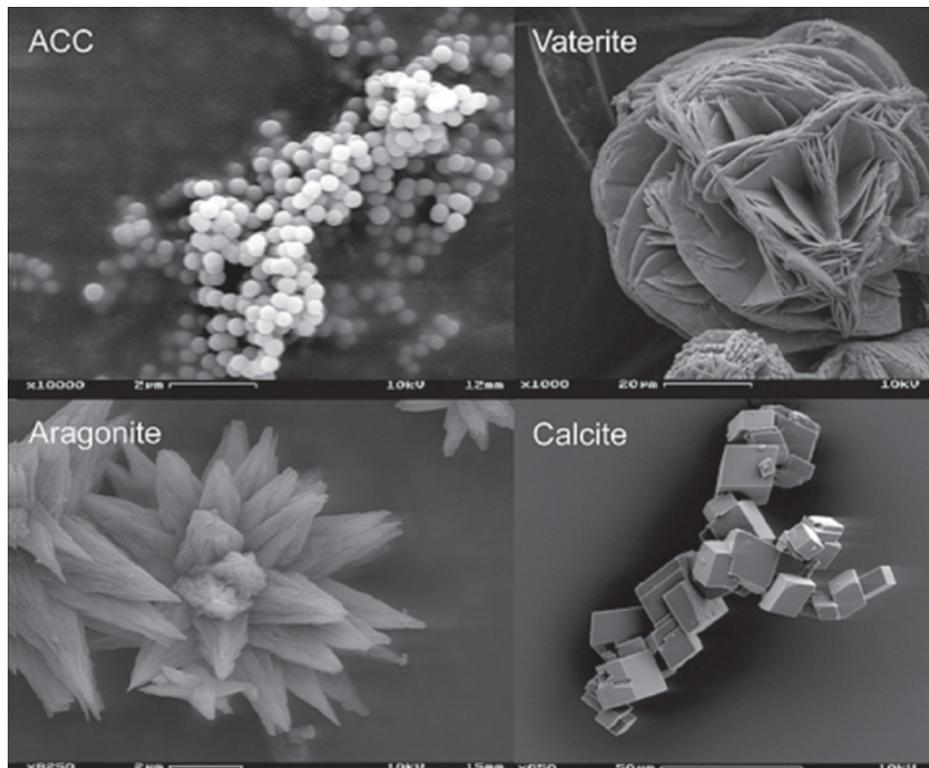


The carbon dioxide (CO<sub>2</sub>) equilibrates to form bicarbonate (HCO<sub>3</sub><sup>-</sup>)



In the presence of an introduced calcium source, the bicarbonate (HCO<sub>3</sub><sup>-</sup>) then reacts with the hydroxyl ions (OH<sup>-</sup>) to precipitate calcium carbonate (CaCO<sub>3</sub>). The precipitation nucleates at the cell wall.

**Figure 2-1** MICP via urea hydrolysis

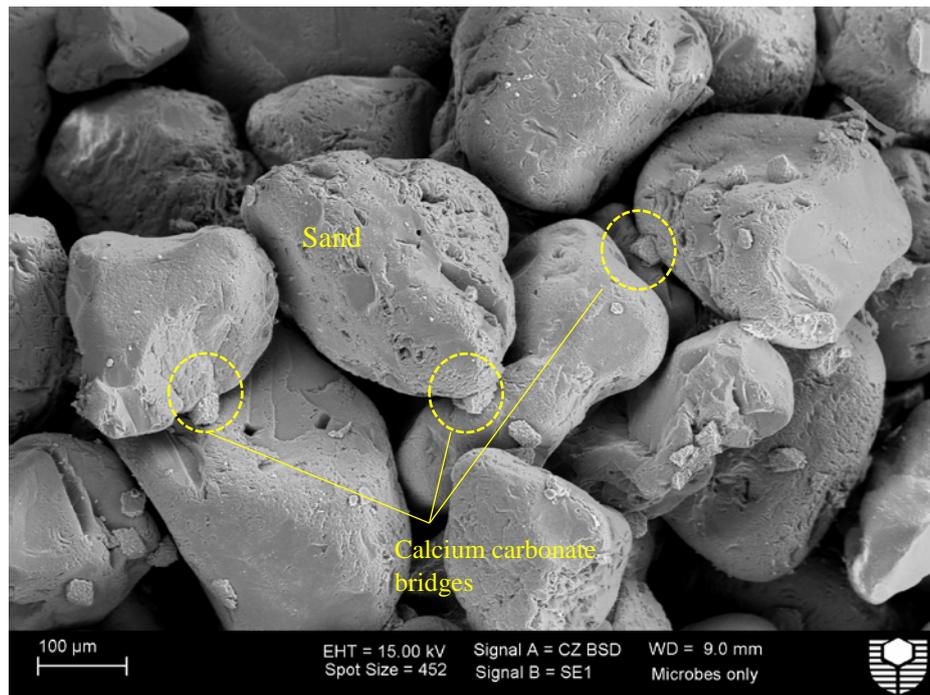


**Figure 2-2** Polymorphs of CaCO<sub>3</sub> <http://www.ruhr-uni-bochum.de/sediment/forschung.html>, Niedermayr (2017)

## 2.2 Current engineering applications for MICP

When MICP occurs within a granular soil matrix, the resulting calcium carbonate crystals attach to the granular particles and act as bridges between particles (Figure 2-3), increasing the stiffness and compressive strength of the material, (Ferris et al., 1996, Le Métayer-Levrel et al., 1999, Whiffin., 2007) and reducing its permeability, (Van Paassen et al., 2010). It has been observed that while the treatment works for medium grade materials, treatment of a finer, clayey material will result in rapid clogging at the injection point, (Le Métayer-Levrel et al., 1999, DeJong, 2010) and as such, the majority of geotechnical investigations to date have been conducted on sand. Within the geotechnical field MICP has demonstrated potential for stabilisation of slopes, (DeJong, 2010, Harkes et al., 2010), resistance to liquefaction, (Burbank et al., 2011) and dust suppression, (Chu J, 2012). When applied as a surface treatment to reinforced concrete structures, MICP has been found to increase resistance to freeze – thaw cycles, sulphate attack and drying shrinkage, (Ramachandran et al., 2001) as well as preventing moisture ingress, and subsequent corrosion of steel reinforcement, (Dhami et al., 2012a). MICP also shows promise for self-healing of reinforced concrete structures. (Sangadji, 2015) compared MICP as a crack sealing technique with traditional grout and epoxy sealants by pumping the repair agents through pre cracked concrete samples with a porous core. Leakage and three-point bending testing of the repaired samples showed that MICP crack sealing was as effective as a traditional epoxy or grout sealant.

Geotechnical field trials using MICP have been conducted in the Netherlands and USA. (Van Paassen, 2010) undertook a large scale (100 m<sup>3</sup>) experiment to stabilise sand. UCS testing on extracted sand cores showed a strength of between 0.7 and 12.4 MPa while the calcium carbonate content varied between 12.6% and 27.3%. (Burbank et al., 2011) undertook dike reinforcement using bio cementation on the shore of the Snake River, USA. 1% (by mass) of calcium carbonate was reported at the ground surface while 1.8 – 2.4% calcium carbonate was reported at a depth of < 90 cm below the ground surface.

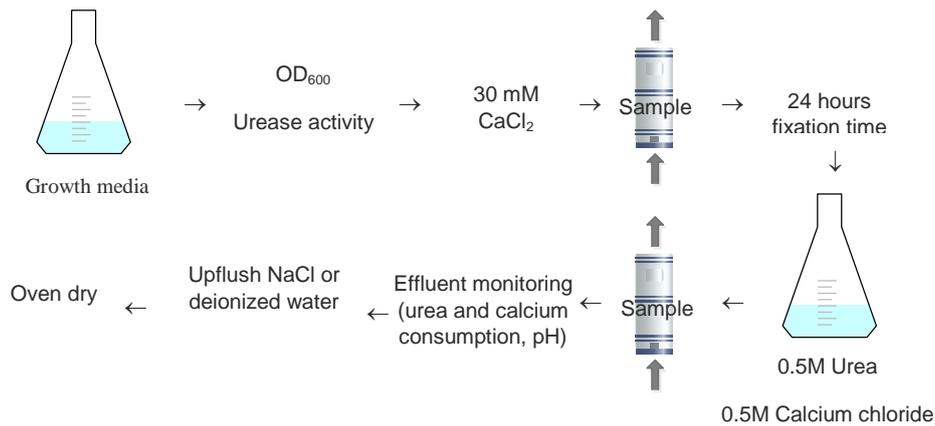


**Figure 2-3** Early stages of biocementation, calcium carbonate forming ‘bridges’ between sand grains

A typical experimental methodology for the stabilisation of sand using MICP is shown in Figure 2-4. Laboratory experiments to date have been column – based and MICP has been conducted using the ureolytic pathway, (Harkes et al., 2010, Rong and Qian, 2012, Whiffin., 2007, Al Qabany and Soga, 2013, Cheng et al., 2013). An ureolytic strain of bacteria, (such as *Sporosarcina pasteurii* or *Bacillus cereus*) is cultivated in a nutrient broth or yeast extract growth media. The optical density and urease activity is monitored during growth. Upon harvesting the bacteria the addition of a small amount of calcium chloride, (30 mM to 50 mM) to the solution has been found to enhance bacterial attachment to the sand grain walls, . The bacterial solution is then mixed with the sand prior to compaction or injected through the compacted sand sample and retained within the sample for a period of 24 hours to enhance bacterial attachment. A cementation media, containing equal molar concentrations of calcium chloride and urea (0.5 to 1.0M) is then injected into the inlet valve. After the predetermined amount of cementation media has been pumped into the specimen the outlet valve is closed and the nutrient solution is retained to allow the MICP reaction to occur. The injections are conducted either via up flushing, (Montoya and DeJong, 2015, Harkes et al., 2010) or down flushing, (Harkes et al., 2010, Whiffin., 2007) and are repeated cyclically. An increase of the pH of the effluent solution will indicate the commencement of the MICP reaction whilst the degree of MICP can be estimated through measuring the effluent for urea and calcium consumption, (Dhami et al., 2016c). After the MICP treatment phase is complete the sample is flushed with deionized water or sodium chloride solution and oven dried.

Several studies have reported the blockage of granular pore throats at the inlet during the MICP process, and subsequent uneven distribution of calcium carbonate through the specimens, (Rong et al., 2012,

Whiffin., 2007). The optimal pressure to avoid blockages has been investigated. Whiffin., 2007 has observed that a flow rate of 350 ml/h is required to avoid clogging in a 5 m long column. Other studies have adopted a two-phase injection system to prevent blockages, such that bacteria solution is first injected, followed by an injection of calcium chloride, (as a fixation agent), and then injection of the cementation media, (Harkes et al., 2010).



**Figure 2-4** Typical experimental methodology for stabilisation of sand using MICP

### 2.3 Sustainable road bases with MICP

One area in which the usefulness of biocementation has not yet been investigated is as a replacement for OPC in stabilised road base materials. The Australian road network is nearly a million kilometres long, of which 83% is classified as rural or remote roads, (DoIRD, 2015). The size, and remote nature of the Australian road network, introduces significant challenges in terms of monitoring and maintenance works. The incorporation of a binder material, (such as cement) into a granular pavement layer, is a practise commonly adopted to improve the strength and durability of a granular pavement structure, (Austroads, 2007, Austroads, 2012).

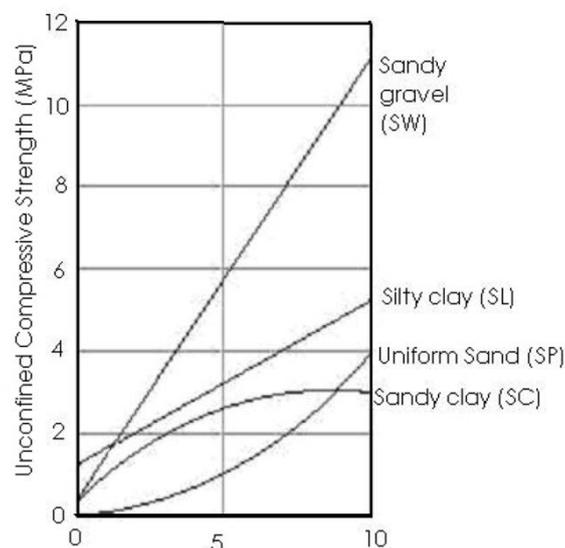
Road base materials with an added cement binder can be classified as modified, lightly bound or heavily bound, (refer Table 1- 1) (AustStab, 2012). Modified materials are granular materials to which a low proportion of cement has been added, such that the 7 day UCS is less than 1.0 MPa. The objective of modification is to improve the plasticity, modulus and durability of the material, without increasing the tensile strength. During the design process modified layers are considered to behave in the same way as unbound layers, anisotropic, and with no tensile strain development throughout the layer, (Austroads, 2012). Lightly bound pavement layers, however, are considered isotropic for design purposes and have a 7 day UCS of between 1 and 2 MPa, (AustStab, 2012). The objective of lightly bound materials is to improve the stiffness and tensile strength of a pavement layer, while limiting the potential for cracking,

(AustStab, 2012). Heavily bound layers have a 7 day UCS of greater than 2 MPa and are typically used in high traffic scenarios to reduce the overall thickness of the pavement. According to current design guidelines, (Austroads, 2012), heavily bound layers should be located lower down in the pavement structure, with sufficient cover to prevent the occurrence of reflective cracking, and subsequent moisture ingress and deterioration of the pavement structure.

The UCS of cement stabilised materials is also dependant on the type of granular material used, (Figure 2-5). In general, cement stabilised sands will demonstrate lower strengths, (1 MPa for 5% cement) while the use of a material with a wider particle size distribution, (such as a sandy gravel or silty clay) will exhibit significantly higher strengths, (AustStab, 2012).

**Table 1- 1** Typical properties of modified, lightly-bound and heavily –bound materials, (AustStab, 2012)

Material type	Layer thickness (mm)	Design strength (MPa at 7 days)
Modified	Applicable for any thickness	UCS < 1.0
Lightly bound	≤ 250 mm	UCS 1 – 2
Heavily bound	≤ 250 mm	UCS ≥ 2



**Figure 2-5** Strength of various soils stabilised with general purpose cement, (7 days curing at 25°C and constant moisture content), (AustStab, 2012)

Although cement has proven to be a durable and reliable stabilisation technique in Australia, the continued use of cement, over such a vast road network is unsustainable into the future, (Imbabi et al., 2012). Typical energy consumption rates for road materials have been published by (Austroads, 2007).

The production of a cement or lime binder for use in stabilised base layers is estimated to consume approximately 5,000 MJ/tonne. The actual placement and construction of the stabilised road base is estimated to consume only 50 MJ/tonne. Evidently, a sustainable binder for road base material with dramatically lower embodied energy and carbon emissions must be developed. A biologically based system, which mimics the process of stabilisation inherent in nature, would eliminate the need for a manufactured binder, thereby significantly decreasing the energy consumption and environmental impacts of stabilised pavement layers.

## 2.4 MICP combined with other chemical binders

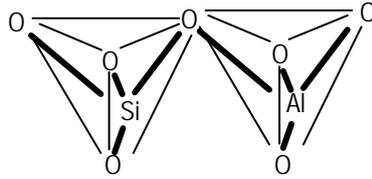
Recent research into the use of biotechnology in Europe has shown that despite unparalleled progress in bio-based research and investment, the take up of bio-based products to the commercial stage has been slow, (Dupont-Inglis and Borg, 2018). Several strategies have been proposed to promote the industrial development of bio technology, including the development of a skilled work force, as well as improved public perception and awareness of bio-based technology and products, (Dupont-Inglis and Borg, 2018).

Biological cementing systems are new for the construction industry in Australia and it is unlikely that the construction industry will solely depend on MICP for soil stabilisation. As such, it may be prudent to first introduce MICP as an augmentation or rehabilitation technique to systems stabilised using traditional, (OPC) or developing binders, (such as geopolymers). To date, geo polymer materials have developed independently of MICP, and a combined stabilisation system using MICP and geopolymers or MICP and OPC has seldom been attempted.

### 2.4.1 Geopolymerisation

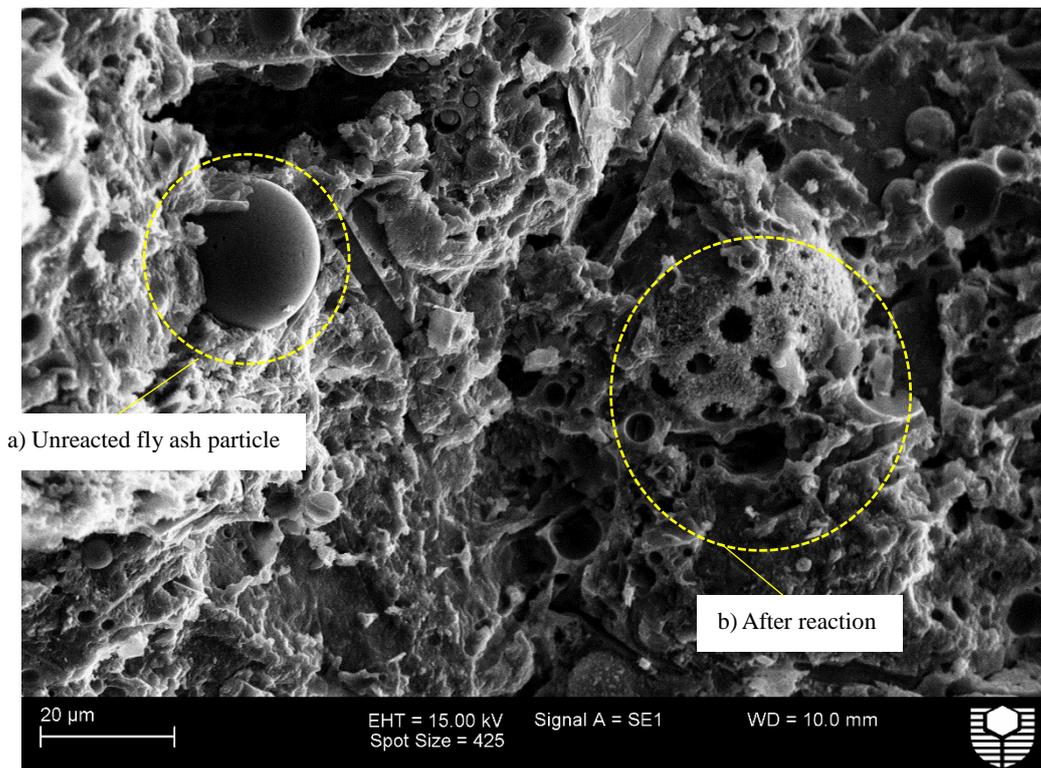
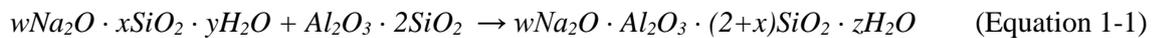
Geopolymer is a method of chemical binding, which has significantly lower energy requirements and produces less carbon emissions than OPC. (Davidovits, 2013) reports that the embodied energy of OPC is approximately 4700 MJ/tonne whilst the embodied energy of an activated fly ash or metakaolin geopolymer is 1965 MJ/tonne or 2715 MJ/tonne. Similarly while the carbon dioxide emissions of OPC are between 0.9 to 1 tonne CO<sub>2</sub> / tonne cement, the carbon dioxide emissions for fly ash and metakaolin geopolymers are much lower, (0.208 tonnes CO<sub>2</sub> and 0.308 tonnes CO<sub>2</sub> respectively).

The geo polymerisation process involves the chemical reaction of alumina-silicate oxides, (such as metakaolin or fly ash), with alkali silicates, to produce a three dimensional polymer comprising of silicon, oxygen and aluminium bonds, (Davidovits, 1994),( Figure 2-6).



**Figure 2-6** Silicon-oxygen-aluminium bonds in a geopolymer, (Davidovits, 1991)

The geopolymerisation reaction occurs over two stages. In the first stage the alumina-silicate materials are dissolved in a highly alkaline solution and form reactive silica and aluminium colloids. In the second stage of the reaction the silica and aluminium colloids form repeating polymer chains through the process of polycondensation, (Rowles and O'Connor, 2009), (refer Equation 1-1). When geopolymerisation takes place within a granular soil matrix the resulting polymer will bind granular particles together, resulting in stabilisation, (Figure 2-7).

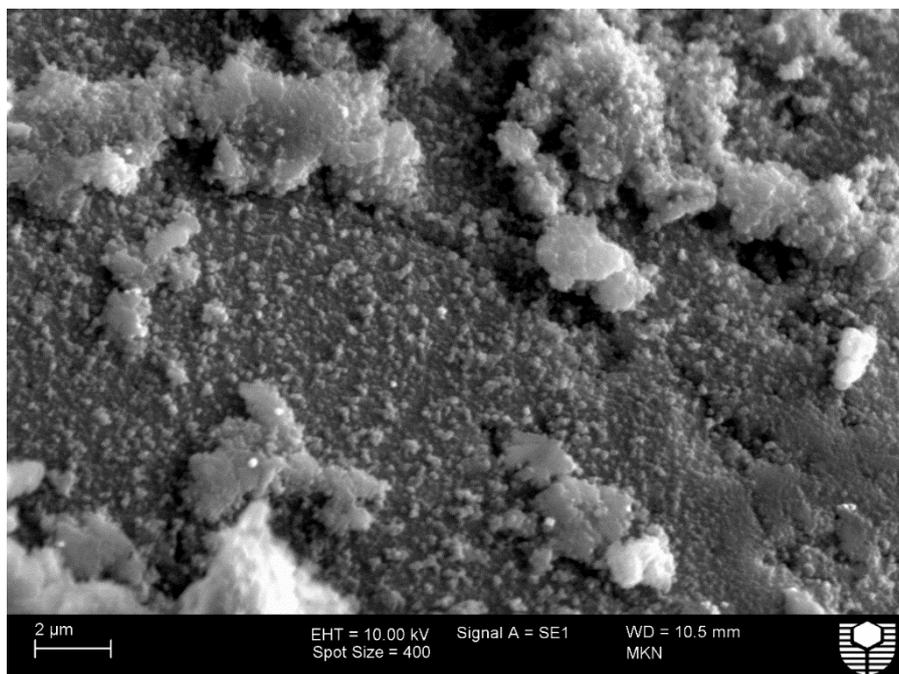


**Figure 2-7** SEM of fly ash geopolymer within a sandy clay soil matrix showing (a) flyash particle before geopolymerisation reaction, (b) after geopolymerisation reaction

Significant research has been undertaken into the optimal conditions for the geo polymerisation reaction. Some of the factors that have been found to influence the engineering properties of a geo polymer material include the source material, the type and concentration of the alkaline activator, the liquid to solid ratio of the activator to source material and the curing conditions.

The source material for geo polymerisation can be calcined material, (such as metakaolin or fly ash) or a non-calcined material, (such as kaolinite, stilbite or red mud). Investigations undertaken by (Xu and Van Deventer, 2000) have revealed that calcined materials have a greater reactivity during the geo polymerisation process, and are therefore more suited to the shorter timeframes required for curing in the construction industry.

The use of a combined sodium silicate/sodium hydroxide solution for activation of the geopolymer has been found to increase both the strength, and the environmental sustainability of the resulting geopolymer, (Komnitsas and Zaharaki, 2007, Turner and Collins, 2013). Experiments conducted by (Liew et al., 2012) found that a sodium silicate: sodium hydroxide ratio of 0.2 provided the best compressive strength properties for metakaolin while investigations by (Abdulkareem, 2015) found that a sodium silicate: sodium hydroxide ratio of 1.0 or 2.25 provided the best compressive strength properties for fly ash. The volume and molarity of the alkaline solution required for fly ash or metakaolin activation also varies. (Liew et al., 2012) investigated the impact of the alkaline solution on the strength of metakaolin geopolymer pastes and reports an optimal liquid: solid ratio of 0.8 and an optimal concentration of 8M. In contrast, (Cristelo et al., 2011) reports an optimal liquid: solid ratio of 0.375 when activating flyash geopolymer using a 12.5M activator solution. The differences in required activator solution for fly ash or metakaolin geopolymers are largely attributed to the difference in particle shape, (Liew et al., 2012). SEM imaging of the two source materials reveals that while fly ash particles have a distinct spherical appearance, (Figure 2-7), metakaolin particles are smaller and flakier in nature, (Figure 2-8).



**Figure 2-8** Metakaolin particles on a sand grain

Although geo-polymers are energy efficient there are a number of challenges when applying geopolymers for engineering applications. While OPC will gain the majority of its strength within the first 28 days, a 90 day period is required when curing geopolymers at ambient conditions, (Cristelo et al., 2011, Villa et al., 2010). Geopolymers are often cured at elevated temperatures, (60°C to 90°C), to enhance the rate of curing, (Liew et al., 2012). While it is possible to use elevated temperatures for curing in the laboratory, these curing conditions may not be practicable for bulk applications.

There is limited work to date on the usefulness of MICP when combined with other chemical binders, (such as OPC or geopolymer). In the case of geopolymers, augmentation with MICP has the potential to improve early strength conditions, while maintaining a sustainable cementing strategy.

## 2.5 MICP to improve rammed earth materials

Rammed earth (RE) is an ancient construction technique that has a significantly lower embodied energy than many modern construction materials, (such as OPC or fired bricks), (Dobson, 2015). Rammed earth structures are constructed from a mixture of sand, clay, silt and gravel, which is compacted progressively in layers. Today, rammed earth is a construction technique that is commonly adopted within developing countries, (Pacheco-Torgal and Jalali, 2012, Dahmen, 2015). While rammed earth is a sustainable, and low cost building material, it also exhibits variable strength and poor resistance to erosion, (Walker, 2002, Ciancio and Boulter, 2012, Venkatarama and Kumar, 2011, Silva et al., 2013, Bui et al., 2009). The compressive strength of rammed earth materials is impacted by the particle size distribution of the soil, the density after compaction and the water absorption. (Beckett and Ciancio, 2016) conducted strength testing on core samples of rammed earth with a dry density of between 1800 and 1910 kg/m<sup>3</sup> and a water content, (at mixing) between 15.2% and 12%. The UCS of the samples ranged between 5 MPa (for a dry density of 1800 kg/m<sup>3</sup>) and 7MPa (for a dry density of 1910 kg / m<sup>3</sup>). Clearly the level of compaction during construction has a significant impact on the overall strength of rammed earth structures. Moisture absorption over the life of the rammed earth structure also has an impact on the strength. A high proportion of fines, (such as clay) promotes the absorption of water leading to softening and a subsequent loss of strength, (Venkatarama and Kumar, 2011, Silva et al., 2013).

One methodology to improve the strength and erosion resistance of rammed earth materials is the incorporation of a stabilising binder, (such as OPC or hydrated lime). (Ciancio and Boulter, 2012) conducted UCS testing on rammed earth cores stabilised with between 0% and 15% cement, (by mass). Both the compressive strength and the Young's Modulus increased with increasing cement content, up to 10% cement. The UCS of samples stabilised with 15% cement was lower. This was attributed to an inadequate amount of water for full hydration. An average UCS of 10.8 MPa was achieved for a rammed earth core stabilised with 10% cement, while an average UCS of 1.0 MPa was attained for

rammed earth cores with no cement. The incorporation of cement into a rammed earth mix has also been found to improve erosion resistance. During accelerated erosion testing unstabilised rammed earth samples disintegrate within a 60 minute time interval, however samples stabilised with 10% cement (by mass) demonstrate no observable erosion over the same time period, (Ciancio and Boulter, 2012, Ciancio et al., 2013). Longer term observations over a 20 year time period show that under natural weathering conditions stabilised rammed earth walls erode to an average depth of 2 mm, while rammed earth walls with no stabilisation erode to an average depth of 6 mm, (Bui et al., 2009).

While the incorporation of OPC does improve the strength and durability of rammed earth it also increases the embodied energy. Recent research reports that a 2% increase in cement content, (by mass) results in an increase in embodied energy of 125 MJ/m<sup>3</sup>, (Venkatarama Reddy and Prasanna Kumar, 2010). To date, there is limited information available regarding the use of MICP to improve the performance of rammed earth materials. However, MICP has the potential to improve both the erosion and water resistance of rammed earth materials, without impacting on sustainability.

## 2.6 Sustainability of MICP

The process of MICP in nature deviates substantially from the man-made process adopted for construction applications. MICP in nature occurs at ambient temperatures using carbon and calcium sources at micromolar concentration. As a result, MICP in nature occurs over a geological timeframe. When applied to construction scenarios it becomes necessary to enrich the bacteria and nutrients to allow MICP to occur within a timeframe appropriate to the construction industry, (Van Paassen et al., 2010, Whiffin et al., 2007, Van Paassen, 2010, DeJong et al., 2014, Dhami et al., 2016a). While there is no doubt that MICP is sustainable in nature, the concentrated, and purified chemicals typically used for man-made MICP are likely to have a significant impact on the sustainability of the product. To date, MICP via urea hydrolysis has been the most commonly adopted pathway for MICP applied to construction applications, (DeJong, 2010). Alternative heterotrophic pathways to MICP, (such as denitrification, ammonification, methane oxidation), or autotrophic pathways, (such as photosynthesis), may be more sustainable options due to the differing levels of nutrient and waste products. A comprehensive assessment of the alternative pathways to MICP is required to determine the sustainability of MICP as a construction technique, and help provide the direction for future research.

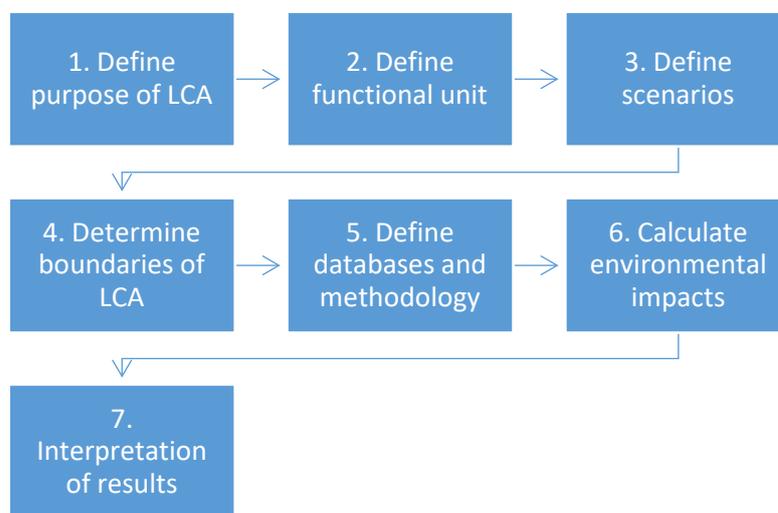
### 2.6.1 Life cycle analysis

Life Cycle Analysis (LCA) offers a technique for the systematic and objective assessment of the sustainability of alternative pathways to MICP, (Vieira et al., 2016, Buyle et al., 2013, Cabeza et al., 2014, Guinée et al., 2011). During a LCA the key impacts to the environment through each stage of a products life are evaluated. Current international standards for life cycle assessments include ISO14040

Environmental Management-lifecycle assessment, principles and frameworks, (ISO, 2006a) and ISO 14044 Environmental Management-lifecycle assessment, requirements and guidelines, (ISO, 2006b).

LCAs have gained popularity for use as an evaluation tool in the construction industry since the 1980s, (Guinée et al., 2011). Within the construction sector LCAs have been used to evaluate the sustainability of individual building elements, (Feiz et al., 2015, Vieira et al., 2016) as well as to compare the sustainability of entire buildings, or road pavements, (Mroueh et al., 2001, Chowdhury et al., 2010, Cabeza et al., 2014, Takano et al., 2014).

The general methodology for conducting an LCA in accordance with ISO guidelines is shown in Figure 2-9.



**Figure 2-9** LCA Methodology, (ISO, 2006a, ISO, 2006b)

There are several databases and methodologies available for the calculation of environmental impacts. A summary of some databases and LCA methodologies which are commonly used within the construction sector is provided in Table 2-1 and Table 2-2.

**Table 2-1** Common LCA databases, (Prè Consultants, 2016, Vieira et al., 2016, AusLCI, 2017)

Material Database	Developer	Country
GaBi	PE International	Germany
Ecoinvent	Swiss Centre for Life Cycle Inventories	Switzerland
Ecoinvent (with adaptation to Australasian context)	Australian Life Cycle Assessment Society	Australia
IBO	Austrian Institute for Healthy and Ecological Building	Austria
CFP	Japan Association for Industry	Japan
Synergia	Finnish Environmental Institute	Finland

**Table 2-2** Common LCA methodologies to calculate environmental impacts, (Prè Consultants, 2016, Vieira et al., 2016, AusLCI, 2017)

<b>Methodology</b>	<b>Developer</b>	<b>Country</b>
AUSLCI V3.0	Australian Life Cycle Assessment Society	Australia
Cumulative Energy Demand	Prè Consultants	Netherlands
Eco-indicator 99	Prè Consultants	Netherlands
CML 2002	Institute of Environmental Sciences, Leiden University	Netherlands
EDIP97-EDIP2003	Technical University of Denmark	Denmark
EPS 2000	Swedish Environmental Research Institute (IVL)	Sweden
Impact 2002+	Swiss Federal Institute of Technology Lausanne (EPFL)	Switzerland
Lime	National Institute of Advanced Industrial Science and Technology (AIST)	Japan
LUCAS	International Reference Centre for the Life Cycle of Products, Processes and Services (CIRAIG)	Canada
ReCiPe	Radboud University Nijmegen (RUN)	Netherlands
TRACI	US Environmental Protection Agency	United States
MEEuP	Van Holsteijn en Kemna (VHK)	Netherlands

While LCA is a powerful evaluation tool, there are some limitations inherent to the process. A construction project or product may generate different LCA results depending on the material database used for the analysis, (Buyle et al., 2013). A comparison of building greenhouse gas emissions using the commonly adopted databases shown in Table 2-1 has shown that the greenhouse gas emissions can vary by 25% due to differences in the material databases, Takano et al. (2014). The difference is attributed to the difference in geographical locations, with the electricity and technical material production processes contributing to significant variations in environmental impacts. Other issues inherent to the LCA process include the difference in assessment methodologies, (Cradle to Gate, Cradle to Grave, Cradle to Cradle), (Vieira et al., 2016), non-availability of data for recycled materials, (Cabeza et al., 2014) and an inability to accurately reflect local environmental impacts, (Buyle et al., 2013) (Cabeza et al., 2014).

Due to the weaknesses inherent to the LCA process it is essential to clearly define the input properties of all materials, databases and methodologies adopted. For the purposes of the analysis conducted in this research project the Ecoinvent version 2.2 material database has been used, with adaptations to an Australasian context where available. The AUSLCI V3.0 and Cumulative Energy Demand V2.01 methodology has been used to calculate environmental impacts

LCA offers a technique to objectively compare the sustainability of different MICP pathways, and verify the sustainability of man-made MICP when applied for construction applications, however, there is little data available on industrial scale projects with biocement to conduct a full lifecycle analysis. An initial embodied energy analysis on biocement demonstrates that in the case of MICP by urea hydrolysis, the urea and calcium chloride sources are the key contributors to embodied energy, (Suer et al., 2009). This varies from OPC where the key contributor to energy usage and fossil fuels is the burning of limestone during the calcination process. The production of OPC is estimated to consume approximately 6.21 MJ/kg, (Frischknecht R., 2005), while urea consumes 30.54 MJ/kg (Nemecek, 2007) and calcium chloride 11.76 MJ/kg, (Hischier, 2004b). Field scale experiments demonstrate that 0.6 kg of urea and 1.1 kg of calcium chloride is required to produce 1 kg of calcium carbonate, for biocement, through ureolytic hydrolysis, (Van Paassen, 2010, van Paassen, 2009).

## 2.7 Significance

The following gaps have been identified during the literature review:

- To date, the majority of investigations have focused on the use of MICP in sand, (for grouting applications), or as a crack sealer and surface treatment for reinforced concrete structures. However, MICP has the potential to improve the sustainability of many other geotechnical structures, such as use of MICP as a replacement, or augmentation to Portland cement in cement stabilised roads.
- The introduction of MICP into Australian construction practices will require concerted effort from all stakeholders. The introduction of MICP as an augmentation over conventional methods may provide a stepping stone to adopt MICP as a construction technique in Australia. Augmentation with MICP has the potential to improve both the engineering properties and sustainability outcomes of OPC and geopolymer binders.
- Rammed earth structures are a low cost and sustainable construction technique. However to be widely adopted improvements must be made in terms of reliability and durability. Progress has been made in this area through the incorporation of chemical binders into the mix, (such as OPC), however this technique also increases the embodied energy and impacts on the sustainability of the material. Like cement, MICP has the potential to improve the strength and durability of rammed earth materials, without significantly impacting on sustainability. Limited information is currently available regarding the performance of MICP when combined with rammed earth structures.

- The process of MICP in nature deviates substantially from the man-made process adopted for construction applications. While it is recognised that MICP is a sustainable process in nature, limited research has been undertaken on how the man-made emulation of MICP alters the sustainability. A comprehensive assessment of the alternative pathways to MICP is required to determine the sustainability of MICP as a construction technique, and help provide the direction for future research. LCA provides a tool for the objective assessment of competing pathways to MICP.

## 2.8 Scope of work

Key objectives of the research project are as follows:

- To investigate the potential of MICP as an environmentally sustainable replacement to Portland cement for stabilised road base materials.
- To investigate the potential of MICP to augment the performance of chemically stabilised, (cement or geopolymer) materials.
- To investigate the potential of MICP as a technique to improve the engineering performance of rammed earth materials.
- To objectively compare the sustainability of alternative pathways to engineered MICP using a the tools available within a life cycle assessment.

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**Statement of Authorship**

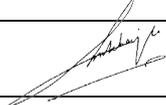
Title of Paper	Sustainable road bases with microbial precipitation
Publication Status	<input checked="" type="checkbox"/> Published <input type="checkbox"/> Accepted for Publication <input type="checkbox"/> Submitted for Publication <input type="checkbox"/> Unpublished and unsubmitted work written in manuscript style
Publication Details	<ul style="list-style-type: none"> <li>• PORTER, H., DHAMI, N.K. &amp; MUKHERJEE, A.2018. Sustainable road bases with microbial precipitation. <i>Proceedings of the Institution of Civil Engineers – Construction Materials</i>, 171, 95 – 108</li> <li>• PORTER, H., DHAMI, N.K., &amp; MUKHERJEE, A. 2016. Sustainable Road Bases with Microbial Carbonate Precipitation. <i>Fourth International Conference on Sustainable Construction Materials and Technologies</i>. Las Vegas, USA: SCMT4</li> </ul>

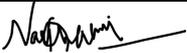
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# Sustainable road bases with microbial precipitation

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## Abstract

The Australian road network is nearly a million kilometres long of which 83% can be classified as rural roads. The size and remote nature of Australia's road network poses unique challenges in terms of asset monitoring and maintenance costs. Cement stabilisation is a practice commonly used to improve durability and reduce inspection and repair costs. However, due to the sheer length of the road network a huge quantity of cement is consumed in stabilisation work making the practice unsustainable. This paper explores a sustainable alternative through augmentation of traditional cement based stabilisation with microbial carbonate precipitation. The calcium carbonate crystals formed within the samples have been investigated by scanning electron microscopy and X-ray diffraction. Bulk mechanical properties have been characterised through Unconfined Compressive Strength (UCS) testing and Digital image correlation (DIC) technique has been utilised during UCS testing to extract more refined information such as local deformation and strain build-up. Mechanical properties at the microscopic scale have been characterised through nanoindentation testing. The results show that microbial precipitation is able to significantly increase the strength of a cement stabilised and road base materials and can considerably improve the technological, economic and social sustainability of Australian road bases.

## Keywords

Granular materials, Roads & highways, Sustainability

## List of notation

DIC	Digital Image Correlation
E	Modulus
EDS	Energy Dispersive X-Ray Spectroscopy
SEM	Scanning Electron Microscopy
UCS	Unconfined Compressive Strength

## 1. Introduction

Australia is a continent with sparse population density. Connecting remote locations with road network has resulted in the country having the highest road length per population. The Australian road network comprises of 873,573 km of roads of which 83% can be classified as rural roads (DoIRD, 2015). Such a vast and remote road network creates a formidable problem in terms of monitoring deterioration in pavement structures as well as the identification and prioritization of maintenance and rehabilitation works. In the past 10 years, (2005-2015) Australia has spent \$21 billion on road and bridge maintenance works (DoIRD, 2015). Understandably, a durable road base system that would minimise the need for frequent inspection and maintenance has been opted for. Cement stabilisation is a practice commonly used in the Australian road industry for ensuring adequate strength and stiffness of a granular road base material. According to Austroads, (2006) it is common for the cost of the binder material (cement) in stabilisation works to be approximately 40% or greater of the total cost of the works. Typical energy consumption rates for cement stabilised materials have been estimated to be approximately 5,000 mJ/tonne while the actual placement and construction of the stabilised road base is estimated to consume only 50mJ/tonne (Austroads, 2007). Although the present road base has proved to be very functional, mandate on developing sustainable technologies necessitates drastically limiting its energy consumption and emission. Evidently, a sustainable binder for road base material with dramatically lower embodied energy and emission must be developed.

The Australian landscape has a large number of sustainable natural formations such as ant hills, corals and stromatolites. These structures also bind granular materials but using negligible embodied energy. Recent research has revealed the role of microbes in cementing the natural formations such as stalagmites, stromatolites and corals (Chen et al., 2012). The process of natural cementation has been emulated in laboratory protocols known as Microbially Induced Calcium Carbonate Precipitation (MICP) (Stocks-Fischer et al., 1999). MICP is a form of bio mineralisation where the metabolic products of microbes result in precipitation of calcium carbonate in the presence of an ionic calcium and carbon source, (Castanier et al., 1999). During urea hydrolysis the bacteria within the soil metabolize urea and produce ammonia (NH<sub>3</sub>), and Carbon Dioxide (CO<sub>2</sub>) within the cell (3-1). These chemicals diffuse through the bacterial cell wall into the surrounding pore space between soil grains. The ammonia (NH<sub>3</sub>) reacts with water (H<sub>2</sub>O) to produce ammonium (NH<sub>4</sub>) and hydroxyl ions (OH<sup>-</sup>) (3-2) resulting in a corresponding increase in the pH of the solution while the carbon dioxide (CO<sub>2</sub>) equilibrates to form bicarbonate (HCO<sub>3</sub><sup>-</sup>) (3-3). In the presence of introduced calcium (attracted to the bacterial cell walls) the bicarbonate (HCO<sub>3</sub><sup>-</sup>) then reacts with the hydroxyl ions (OH<sup>-</sup>) to precipitate calcium carbonate (CaCO<sub>3</sub>) (3-4).





The most remarkable feature of MICP is that unlike traditional cement, the bio-cement is produced at ambient conditions with water as the solvent. Thus, it obviates the consumption of energy in cement manufacturing (Achal et al., 2015). (Mitchell and Santamarina, 2005) report that a kg of soil has nearly  $10^{12}$  microbes and many of them can be classified as calcifying. Thus, it is possible to harness the calcifying bacteria present in the soil of road bases for cementation action. MICP is an emerging technology for various geotechnical applications (DeJong et al., 2014). It has also proved to be a very attractive measure to improve the engineering properties of all building materials (Achal and Mukherjee, 2015). During the process of MICP, bacteria within the soil digest nutrients and precipitate calcium carbonate. There are several different biological processes that can cause calcium carbonate precipitation to occur, however, the process that occurs the fastest, and requires the least free energy, is precipitation via urea hydrolysis (DeJong, 2010).

Laboratory experiments have demonstrated the potential of MICP in improvement of soil strength (Le Métayer-Levrel et al., 1999), (Whiffin.,2007), (Ferris et al., 1996), reduction of permeability (Harkes et al., 2010), stabilisation of slopes (DeJong, 2010) (Harkes et al., 2010), improved resistance to liquefaction (Burbank et al., 2011) and also suppression of dust, (Chu J, 2012). MICP forms “cohesive bridges” between grains of sand through the deposition of calcium carbonate crystals increasing the stiffness of the material and reducing permeability, (van Paassen et al., 2010). MICP was found to resist the deterioration of concrete in freeze thaw, sulphate attack and drying shrinkage (Ramachandran et al., 2001). In the case of reinforced concrete MICP retards moisture and chloride ion diffusion and the corrosion of steel (Dhami et al., 2012a). Several progresses have been made in unravelling the biochemical processes (Castanier et al., 1999, Ercole et al., 2012, Bains et al., 2015), improving the bacterial strains (Bergdale et al., 2012, Achal et al., 2009b), use of industry by-products (Achal et al., 2009a, Dhami et al., 2012b), and process optimisation (Okyay and Rodrigues, 2015, Dhami et al., 2016b). After the success of laboratory scale experiments in-situ soil strengthening at the field (bio-grouting) has been attempted where the biological fluid is injected in the soil. It has been observed that although the technique works well for surface treatments, sands and mixed-in-place applications, in the case of fine grained materials the injection well clogs rapidly, (Le Métayer-Levrel et al., 1999). The optimum pressure to avoid clogging has been investigated and it was observed that a flow rate of 350 ml/hr could avoid clogging of a 5m long tube, (Whiffin., 2007). (van Paassen et al., 2010) reports that in large scale experiments (treatment volume of up to  $100 \text{ m}^3$ ), use of bio-grouting is technically feasible

under conditions common to what is found in practice (van Paassen, 2009). Large scale laboratory tests on dike reinforcement using biocementation have already been carried out. (Burbank et al., 2011) reported formation of around 1% CaCO<sub>3</sub> in near surface and 1.8- 2.4 % calcite below 90 cm after stimulating indigenous ureolytic microbes in soils on the shore of the Snake river, USA.

Although MICP has shown a lot of promise the field trials must be implemented in a phased manner. Thus, it is advisable to apply MICP in Australian road bases in addition to cement stabilisers. Some challenges in the compatibility between cement stabilised soil and MICP are envisaged. The MICP process may impose restrictions on control of moisture in soil to achieve optimum consolidation, on the other hand, it may be affected by the high pH of cement. This paper reports performance of MICP as a stabilising technique on its own, and as an augmentation to cement stabilised road base materials. It is noted that pre- stabilisation with a cement binder increases carbonate precipitation of carbonates at intergranular bridges and thus, a small quantity of carbonate crystals improves the strength considerably. This was evident through the combined results of strength testing, scanning electron microscopy and nanoindentation. UCS results showed an improvement in strength in the combined MICP / cement system greater than the strength increase obtained through MICP or cement stabilisation alone, while micrographs of the granular structure showed cement evenly coating the soil grains and carbonate crystals precipitating at bridges between particles. Nano indentation testing conducted on the samples revealed that MICP treatment on cement stabilised samples increased the stiffness of cementitious bridges between particles and at the sand / bridge interface to a value greater than that obtained for MICP only (SM) or cement only (SC) treated samples. Through digital image correlation the deformation and strain build up at the time of performing unconfined compression test has been monitored. It is discovered that DIC is able to pinpoint the local strain build up at early stages of loading. These locations ultimately cause cracking and collapse. Evidently, DIC has the potential to be a convenient technique for early identification of deterioration in the road surfaces and a useful road asset management tool.

## 2. Experiment and methods

In this investigation, both standard methods of testing cement stabilised samples and the modified ones with MICP have been followed. In case of standard samples maximum compaction is achieved by maintaining an optimum moisture content and standard compaction technique on a sample of standard size. In case of MICP stabilisation the addition of cementation fluid violates the optimum moisture content conditions. Preparation of standard (100 mm diameter) MICP treated samples is also difficult under laboratory conditions. Thus, samples of three sizes have been prepared (Table 3-1). SC100 are the standard samples in terms of size, moisture content and compaction. SC65 are the intermediate scale samples while SC30 samples are smallest where neither the optimum moisture content nor the

compaction has been maintained. These samples are amenable to MICP. However, MICP has been tried hitherto in sand only and not in road base materials. Therefore, both sand and road base materials have been investigated for MICP. The samples have been tested for unconfined compression strength with digital image correlation. Their microstructure has been studied by SEM and EDS and their mechanical performance at the micro scale has been investigated using nanoindentation.

**Table 3-1** Details of samples

<b>Identifier</b>	<b>Description</b>	<b>Diameter (mm)</b>	<b>Height (mm)</b>	<b>Repeats</b>
SC100	Sand + 7% cement	100	200	3
SC65	Sand + 7% cement	65	130	2
SC	Sand + 7% cement	30	60	3
SM	Sand + MICP	30	60	3
SCM	Sand + 7% cement + MICP	30	60	3
R	Road base	30	60	3
RM	Road base + MICP	30	60	3
RC	Road base + 5% cement	30	60	3
RCM	Road base + 5% cement + MICP	30	60	3

## 2.1 Materials

Manufactured sand sourced from Cook Industrial Minerals, Perth, Western Australia was used as the granular component of samples SC100, SC65, SC, SM and SCM. The sand can be classified as a medium graded sand in accordance with ISO 14688, (ISO, 2002). Roadbase material was provided by the Department of Transport and Main Roads, West Australia and was used for samples R, RM, RC and RCM. Road base material was sourced from Graham Pit, located approximately 5 km from the township of Narrogin. Commercially available general purpose grey cement sourced from Swan Cement, Perth, complying with (AS3972, 2010) and standard tap water have been utilised.

Two ureolytic strains of bacteria were used for MICP treated samples. In the case of sand samples the bacterial strain *Bacillus cereus* (ATCC 11778) was used. The bacteria culture was grown in a nutrient broth growth media as detailed in Table 3-2. The bacteria was grown at a temperature of 37°C at 200rpm for 24 - 36 hours. The optical density of the bacterial solution at harvesting varied between  $5 \times 10^7$  -  $8 \times 10^8$  cfu/ml.

In the case of road base samples *Sporosarcina pasteurii* (ATCC 11859) was used for MICP treatment. The bacteria were inoculated in an Ammonium-Yeast extract media as described by Bernardi et al. (2014) and detailed in Table 3-3. The bacteria was grown at a temperature of 37°C at 200rpm for 24 - 36 hours till  $1 \times 10^9$  cfu/ml.

After compaction the microbially treated samples were cured for 20 days in an incubator at a temperature of 37°C. Each day 1 pore volume of cementation solution was added to the top of samples and allowed to filter through to the outlet at the bottom of the sample via gravity. The cementation solution was autoclaved, prior to adding the urea component. After autoclaving the solution was stabilised to a pH of between 8.0 and 8.5. The cementation solution for MICP treated sand and road base samples is detailed in Table 3-2 and Table 3-3.

**Table 3-2** Materials for MICP treatment on sand samples

Description	Materials	Concentration
Microbial Growth Media	<i>Bacillus Cereus</i> (ATCC 11778) isolate	$5 \times 10^7$ - $8 \times 10^8$ cfu/ml
	Bacto nutrient broth	13g/L
	Nickel sulphate	1mM
	Urea	.05M
	Tris buffer (pH 8.4)	0.15M
Cementation Solution	Urea	0.5M
	Calcium chloride	0.5M
	Bacto nutrient broth	3g/L

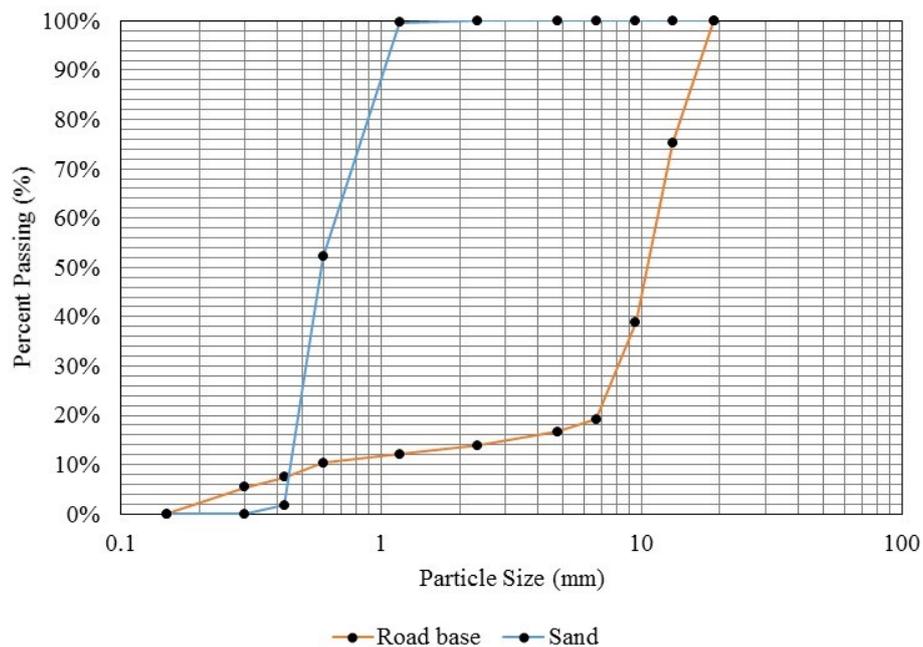
**Table 3-3** Materials for MICP treatment on road base samples

Description	Materials	Concentration
Microbial Growth Media	<i>Sporosarcina pasteurii</i> (ATCC 11859) isolate	$1 \times 10^9$ cfu/ml.
	Yeast extract	20 g/L
	Ammonium sulphate	10 g/L
	Nickel chloride	2 $\mu$ M

	Tris buffer (pH 9)	0.15M
Cementation Solution	Urea	0.5M
	Calcium chloride	0.5M
	Yeast extract	0.5 g/L

### 2.1.1 Particle size distribution

To characterise the particle size distribution of the two mixes a sieve analysis was conducted in accordance with the requirements of Australian Standards, (AS1289.3.6.1, 2009). The particle size distribution for the manufactured sand and for the road base mix is shown in Figure 3-1. It is noted that sand had a much narrower distribution of grain size in comparison to the road base that contained a considerable amount of both large aggregates and fines.



**Figure 3-1** Particle size distribution

### 2.2 Sample preparation

The standard SC100 samples were prepared in three steps; finding out the maximum dry density; preparing the samples through compaction and curing. Cement was added at a proportion of 7% (by mass) of sand. Maximum dry density of the cement-sand mixtures was obtained by finding the optimum moisture content according to (AS1289.5.2.1, 2003). An optimum moisture content of 10% by mass was found to achieve a maximum dry density of 1.6 g/cm<sup>3</sup> in the samples. Specimens were prepared

by pouring the cement-sand mixture containing 10% water in a compacting cylinder and applying 25 blows per layer. The compaction was performed in five (5) even layers; and each layer was scarified to allow for good bonding between them. The samples were demoulded after 1 days and cured for 7 days in a humidity cabinet in accordance with standard practice for a cement stabilised material.

SM, SCM, SC and SC65 samples were prepared as follows. Where required cement was added at a proportion of 7% (by mass) of sand. Water was added at a proportion of 10% (by mass) to SC and SC65 while bacterial solution was added in lieu of water at a proportion of 10% (by mass) to SCM and SM samples the samples were compacted to a maximum dry density of  $1.6 \text{ g/cm}^3$ . Following compaction cementation solution was added daily to MICP treated samples while water was added to control samples as described in Section 2.1.

RC and RCM samples were prepared as follows. Cement was added at a proportion of 5% (by mass) of roadbase material. Water was added at a proportion of 12% to achieve a maximum dry density of  $2 \text{ g/cm}^3$ . The bacterial solution was added in lieu of water at a proportion of 12% (by mass) to RCM samples. For non-cement stabilised samples (R and RM) water or bacterial solution was added at a proportion of 11% to achieve a maximum dry density of  $2 \text{ g/cm}^3$ . Following compaction cementation solution was added daily to MICP treated samples while water was added to control samples as described in Section 2.1.

### 2.3 Compressive strength testing

After the end of the curing period the specimens were tested for unconfined compression. UCS testing was performed on a universal testing machine (Shimadzu AGS - X) in accordance with (AS5101.4, 2008). The specimens were placed vertically on the bottom platen. A digital camera was mounted in front of the specimen and snapshots were taken at regular interval while compressing the sample at a rate of 1 mm/minute. Open source Digital Image Correlation Matlab software (NCORR) was used to trace 2D displacement and strains throughout the deformation process.

### 2.4 SEM and EDS

After UCS testing SEM and EDS analysis was undertaken on small portions of the samples. Samples were mounted on aluminium stubs and coated with a 10 nm thick carbon coating. SEM images and EDS were taken with a Zeiss Neon 40EsB dual beam FESEM/FIB-SEM.

### 2.5 Nanoindentation testing

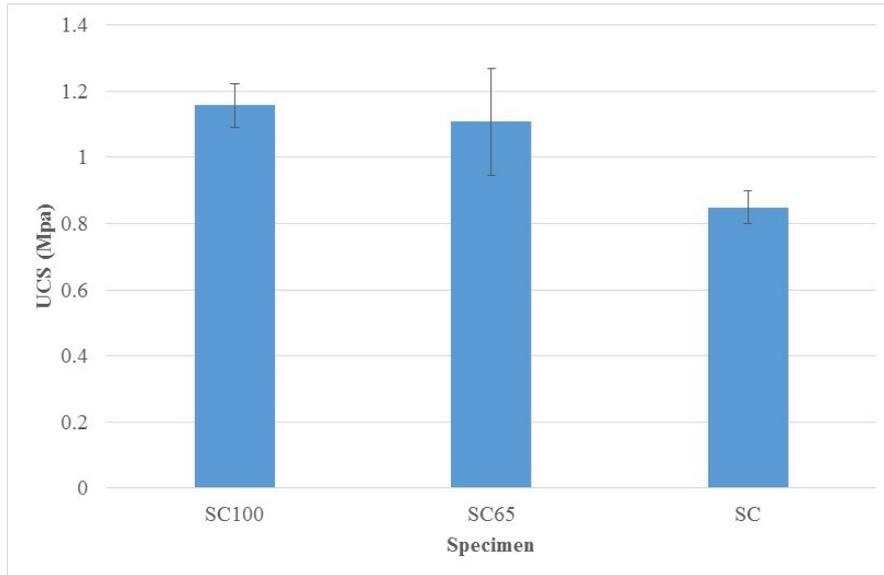
Nanoindentation testing was conducted on samples SM, SC and SCM in order to gain a deeper understanding of the mechanical properties of the samples at the microscopic level. Nanoindentation was undertaken using a G200 Agilent Technologies Nano indenter.

Samples were mounted in an epoxy resin and allowed to cure for a period of 24 hours. After curing the samples were ground back and polished using a Buehler EcoMet grinder polisher. Locations for nanoindentation were manually selected using the 40X optical microscope fitted to the nano indenter. Selected locations for nanoindentation were on the sand grain, on the bridge between grains, on the sand grain /bridge interface and on the epoxy resin. A minimum of 100 indentations was undertaken on each location type for each sample. Samples were indented to a depth of 1000nm using a Berkovich diamond tip at a loading rate of 0.5 mN/s. The indenter was controlled with Testworks 4 (version 4.10), (MTS System Corporation) using an allowable drift rate of 0.3 and a surface approach distance of 2000nm. A Poisson's ratio of 0.3 was assumed for the purpose of calculating the modulus and hardness. Modulus and hardness calculations were performed based on the load / indentation curves utilizing NanoTest Platform Four V40.08 software, (Micro Materials Ltd).

### 3. Results and discussion

#### 3.1 Scale effect and impact of MICP treatment on compaction

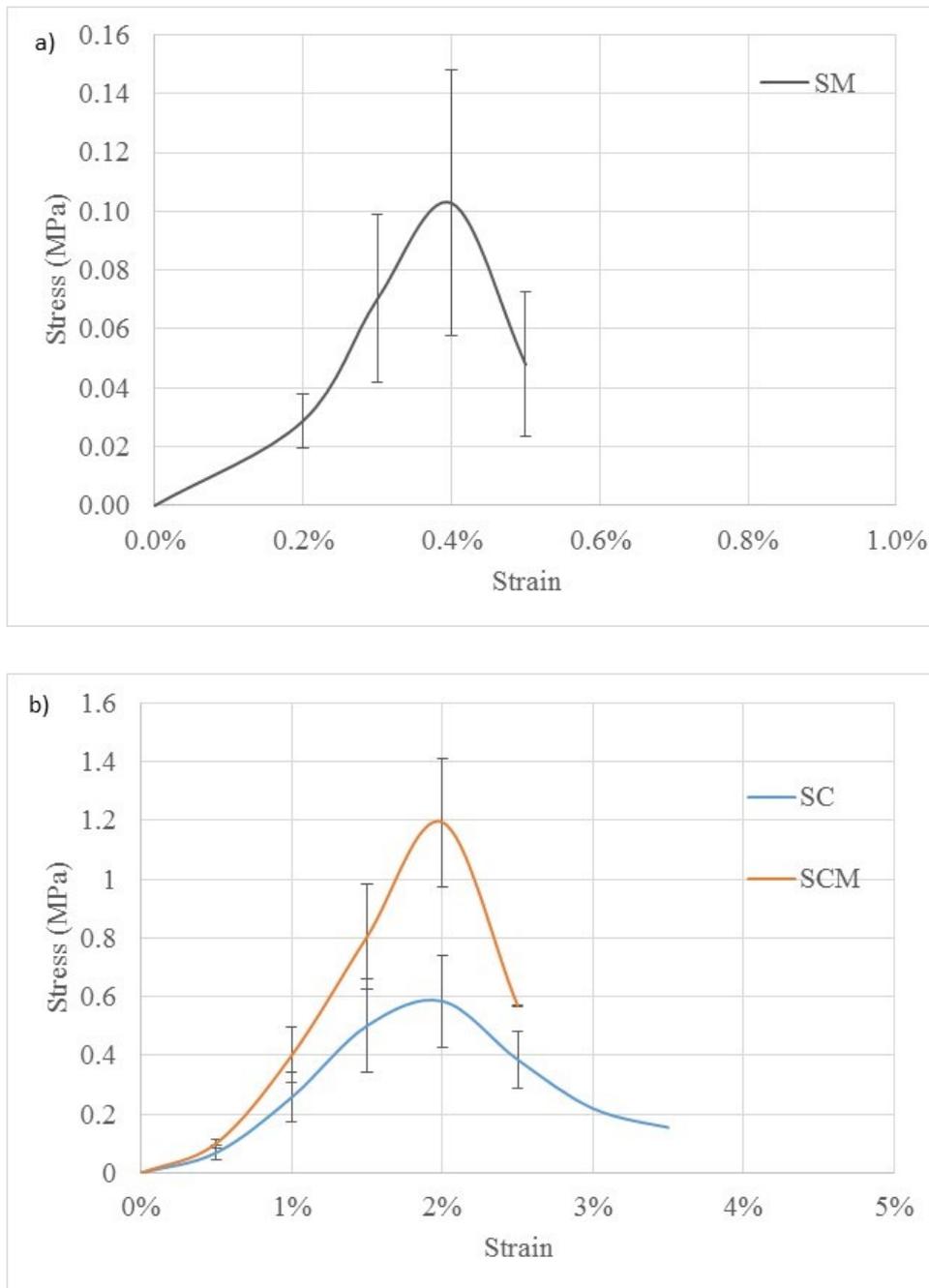
The results of the control cylinders of three length scales are shown in Figure 3-2. They indicate an increase in strength with increasing cylinder size. Although smaller scale usually increases strength a reverse trend is noticed here due to the difference in compaction. SC100 was fully compacted at optimum moisture content. However, in SC65 the addition of fluid during the curing process changed the moisture conditions. It is observed that there is a marginal reduction of compressive strength due to lack of compaction. In case of SC the optimum moisture content too could not be maintained. In fact, in this case the specimens were supersaturated. As a result, the strength has decreased by nearly 25%. It is known that soil expands in volume due to the buoyancy effect in supersaturated condition. Thus, the void space in soil is increased resulting in reduction in strength. This result also indicates that it may be wise to devise MICP techniques with frugal water content where optimum moisture conditions can be maintained. The results of SC samples are used later in determining the effect of MICP on UCS.



**Figure 3-2** Unconfined compressive strength for samples of varying dimensions

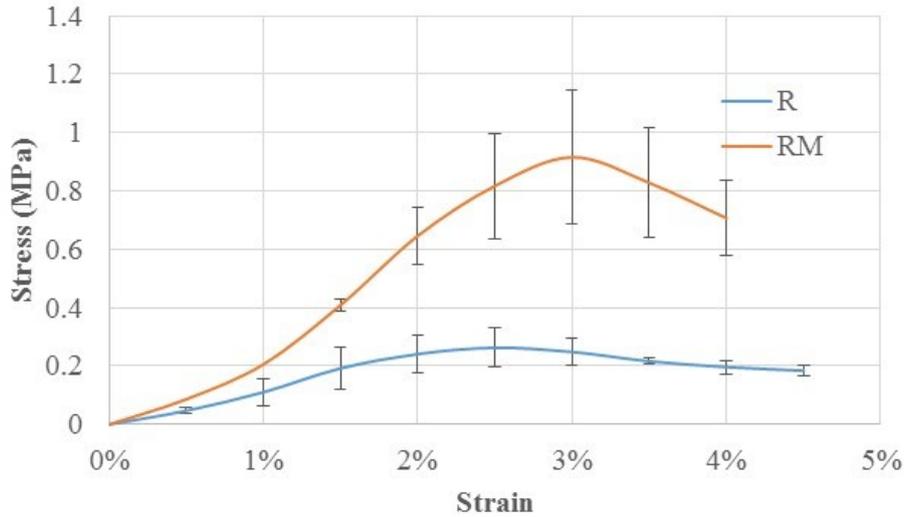
### 3.2 Compressive strength

Next the effect of MICP on sand and road base samples is reported. The stress-strain curves for sand stabilised with MICP only is shown in Figure 3-3 (a) while the stress-strain curves for cement stabilised sand with and without MICP treatment are shown in Figure 3-3(b). The range of values is indicated in the error bars. In the case of combined cement and MICP stabilised samples (SCM) a significant improvement in strength (in the order of 50%) was observed due to MICP, far greater than the strength improvement achieved in samples treated using MICP only, (SM). The underlying reason for such dramatic improvement of strength will be discussed later through microstructural analysis and nanoindentation testing. The strain at the maximum load remained similar (2%). All except one SC and SCM samples collapsed at around 3% strain.



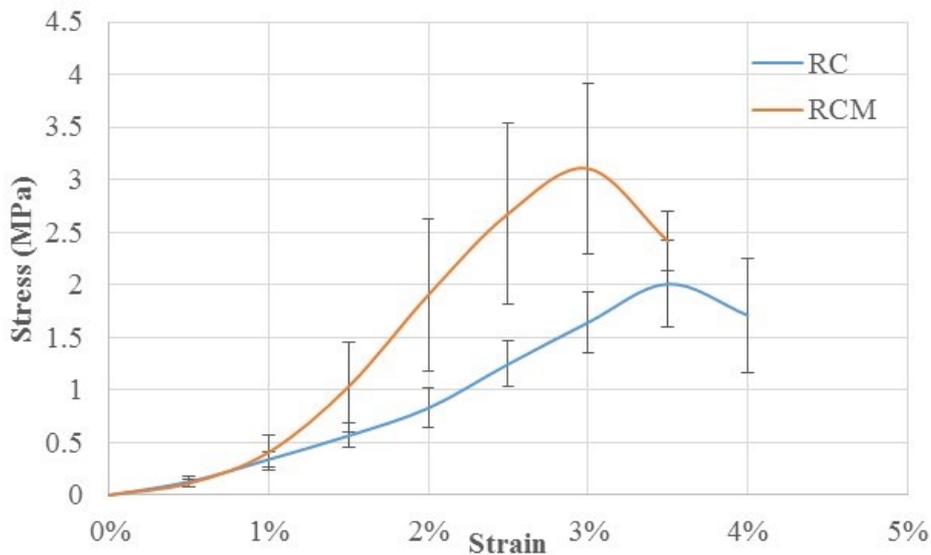
**Figure 3-3** Stress – strain curves for stabilised sand samples a) SM and b) SC and SCM

It may be recalled that the road base samples had a wider grain size distribution than sand with the presence of both finer and coarser particles. Significant improvement in strength were also recorded in road base samples. In this investigation, the performance of MICP on road bases is reported for the first time. Road base materials, ostensibly due to the presence of clay, do aggregate without a binder. Therefore, their load capacities without binders have been tested (Figure 3-4). They had an average strength of 0.3 MPa at 2.5% strain (R). When they were treated with MICP (RM) there was a dramatic (threefold) increase in strength. The load capacity reached 0.9 MPa at 3% strain. However, the unloading portion of the curve was shorter. Clearly, MICP can bind road base materials well.



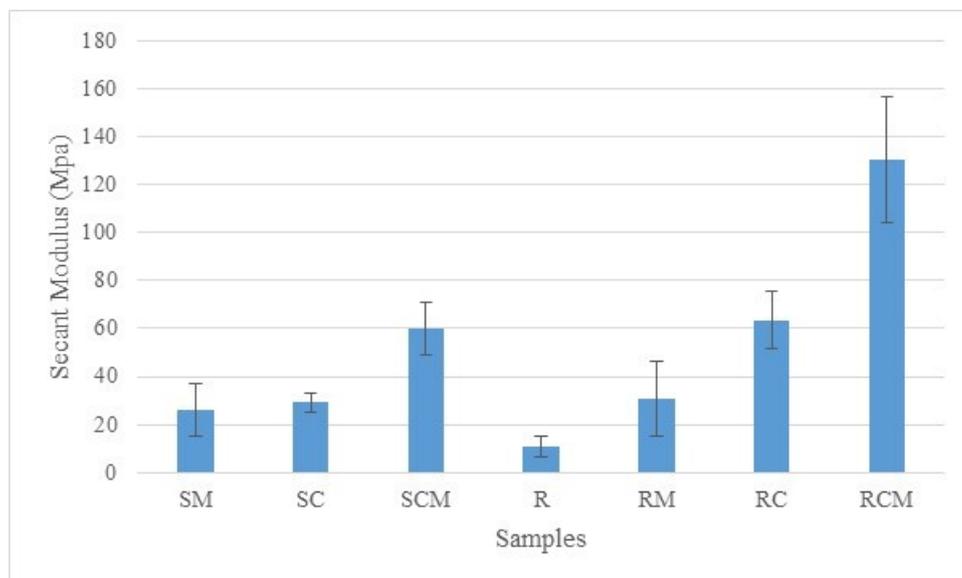
**Figure 3-4** Stress-strain curves for road base samples R, RM

It may be recalled that in case of sand, there was a dramatic increase in strength when cement and MICP was used simultaneously (Figure 3-3). We report the performance of road base materials here (Figure 3-5). It is noted that the cemented road base material had a load capacity of around 2MPa at a strain of 3.5%. Clearly, the stabilised road base material has a much higher load capacity than sand alone. This is due to the wider grain size distribution of the material in comparison to that of sand, which leads to better packing and compaction. The more compacted material may pose a challenge for MICP as the pores act as channels of flow of cementation fluid. Figure 3-5 shows that the RCM samples had a load capacity of 3.6 MPa, which is 80% improvement. However, the strain at maximum load is reduced to 3%. There is a reduction in the ultimate strain from 4% to 3.5%. Thus, MICP increases the strength and effective stiffness of cemented road bases.



**Figure 3-5** Stress – strain curves for road base samples RC, RM

Secant moduli for sand and road base samples with and without MICP have been calculated from the stress-strain graphs by joining the origin with the point of maximum stress and measuring the slope. The secant moduli are shown in Figure 3-6. It may be noted that a column of loose sand would have no stiffness. When it is stabilised it gathers stiffness. Thus, the secant modulus is a measure of the degree of cementation achieved. A comparison between SC and SCM shows that the secant modulus has increased at least two fold due to MICP. This is a huge increase considering a small percentage of carbonate deposition is expected from MICP. The road base samples (R) had a small stiffness (10 MPa). When they were stabilised with MICP the modulus of the samples (RM) went up three times (30 MPa). When they were stabilised with cement (RC), the modulus was a little above 60 MPa. However, when they were further treated with MICP (RCM), the modulus rose nearly two fold again to above 120 MPa. Thus, MICP stabilises the road base material very effectively and it also reinforces cement stabilised road base materials to a great extent.

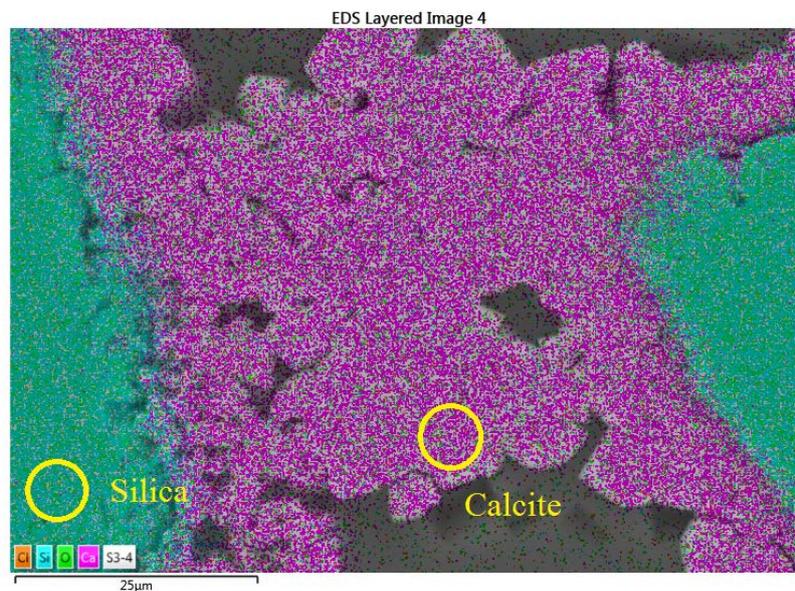


**Figure 3-6** Secant moduli for sand and road base samples

Evidently, MICP can offer a huge benefit in cutting down cement consumption in road bases, and thus improve both economic and environmental sustainability. As mentioned previously, the cost of the binder material in stabilisation works can be equivalent or greater than 40% of the total cost of the stabilisation works (Austroads, 2006), while the embodied energy in the cement binder (5,000 MJ/tonne) is overwhelmingly the largest component in the stabilised road base works (Austroads, 2007). The use of MICP has a great potential to turn around the sustainability challenges that Australian road industry is facing. However, these are early results and the authors are aware several breakthroughs are imperative for successful field implementation of the technology.

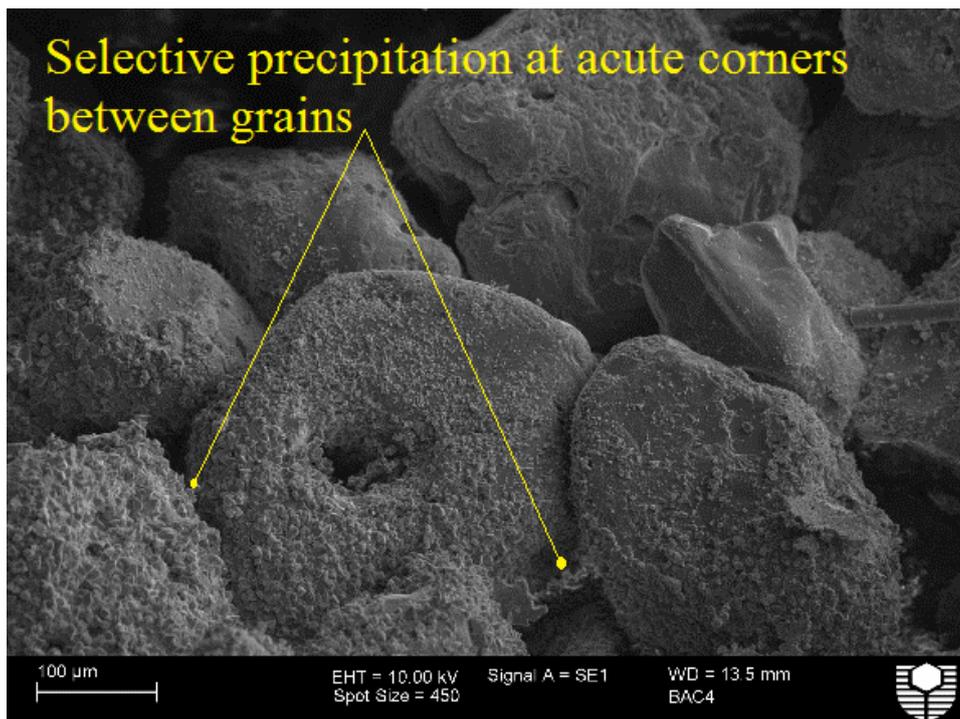
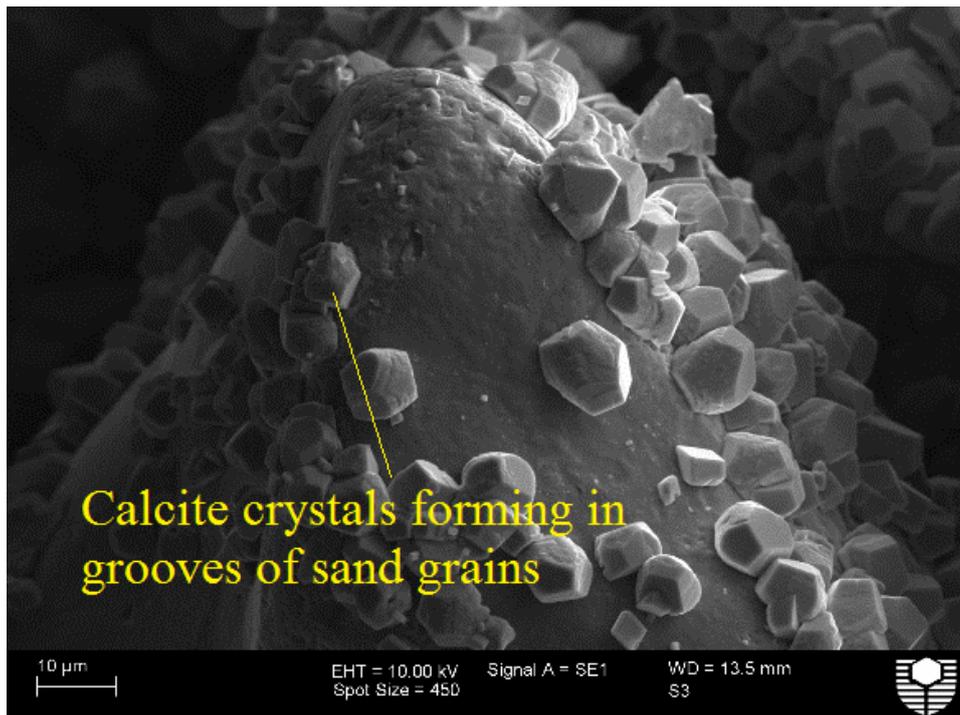
### 3.3 Microstructural investigation

A microstructural analysis was undertaken to reveal the cause of the dramatic increase of UCS and modulus due to MICP. Scanning electron microscopy (SEM) coupled with energy dispersive X-ray spectroscopy (EDS) to possibly identify the sand grains, the hydrated cement and the calcium carbonate particles. Figure 3-7 illustrates a typical micrograph with EDS of SM, (sand stabilised with MICP). The sand is identified by the abundance of silicon and oxygen in the EDS while the calcium carbonate crystals are identifiable through the presence of calcium. Clearly, the calcium carbonate deposit is bridging two sand particles.

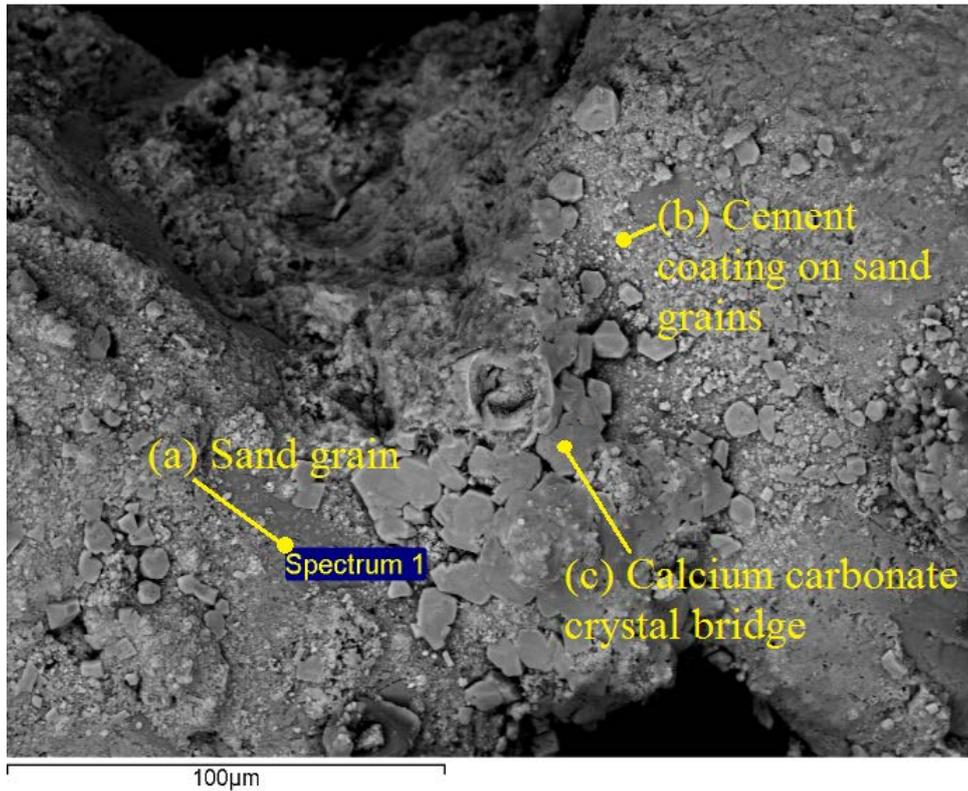


**Figure 3-7** Typical micrograph with EDS of calcium carbonate crystals attached to sand particle

SEM images revealed that carbonate deposits take place at two locations: 1) at the grooves on the sand grains (Figure 3-8 (a)); and 2) at the acute corners created by the contacting grains of sand (Figure 3-8 (b)). This occurs because bacteria can securely attach themselves in those corners and are not easily dislodged. Their metabolic activity in those critical areas results in nucleation and growth of calcium carbonate crystals. MICP can therefore reinforce a material by preventing inter-granular slip or rotation, and building bridges between sand grains. Thus, it creates a topologically optimal configuration of cementation. Cement, on the other hand, coats the grains more evenly and thus only a fraction of it is effective in binding the particles. In the case of cement stabilised sand with MICP the cement was observed coating the sand grains whilst rhombohedral crystals were observed reinforcing the bridges between grains (Figure 3-9).

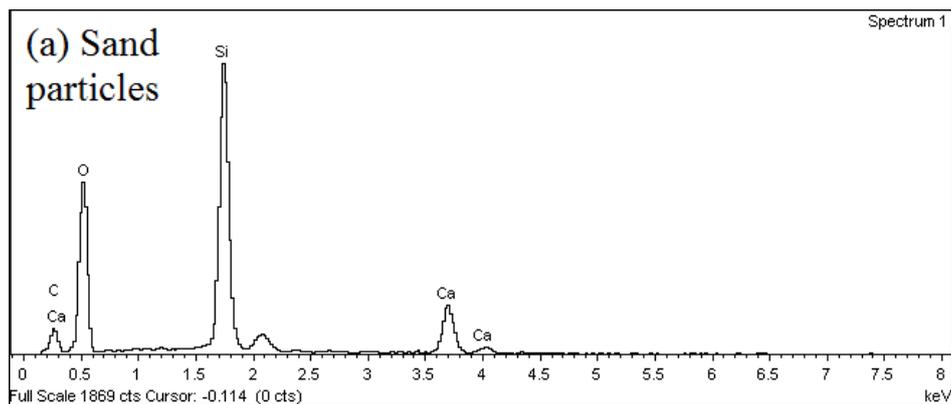


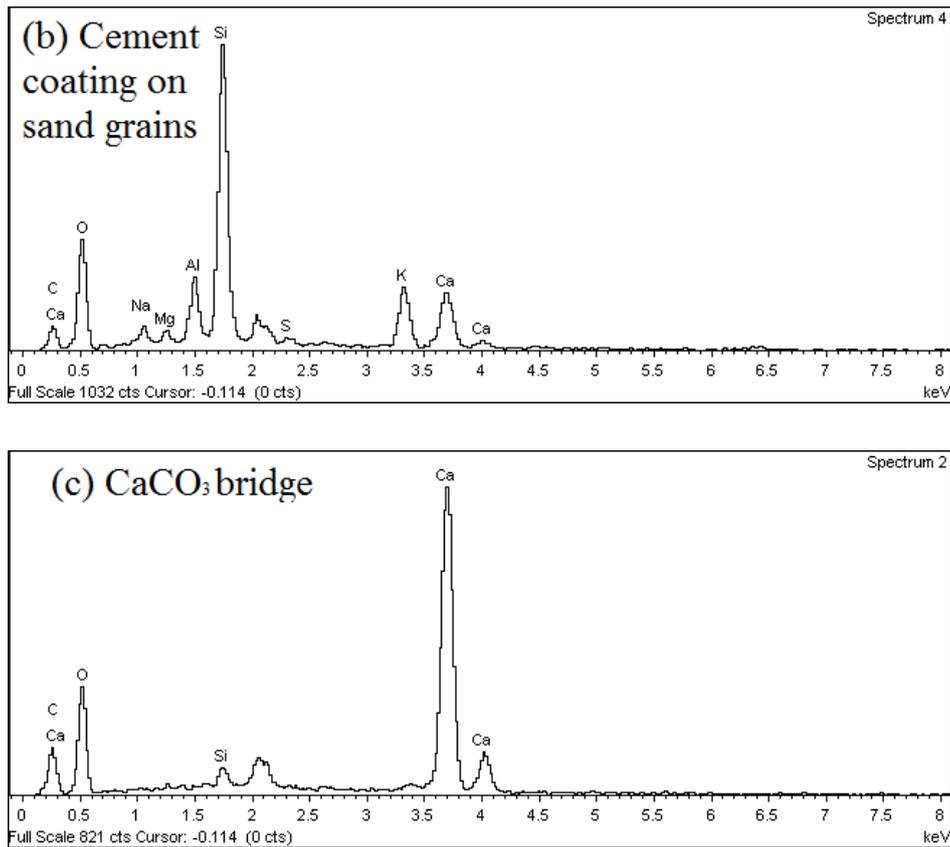
**Figure 3-8** (a) Crystal formation from rough areas of sand grains and (b) carbonate bridging at acute corners between grains



**Figure 3-9** SEM micrograph of cement stabilised + MICP treated sand

Figure 3-10 (a) shows a typical spectrum on the sand grain, with peaks of silica and oxygen. Figure 3-10 (b) shows EDS results of the cement particles coating the sand grains. Silica is indicative of the sand grain while potassium, aluminium, sodium and calcium are typical peaks observed in cement. Figure 3-10 (c) shows EDS results taken at the bridge between particles. Peaks of calcium and oxygen are indicative of calcium carbonate precipitation.

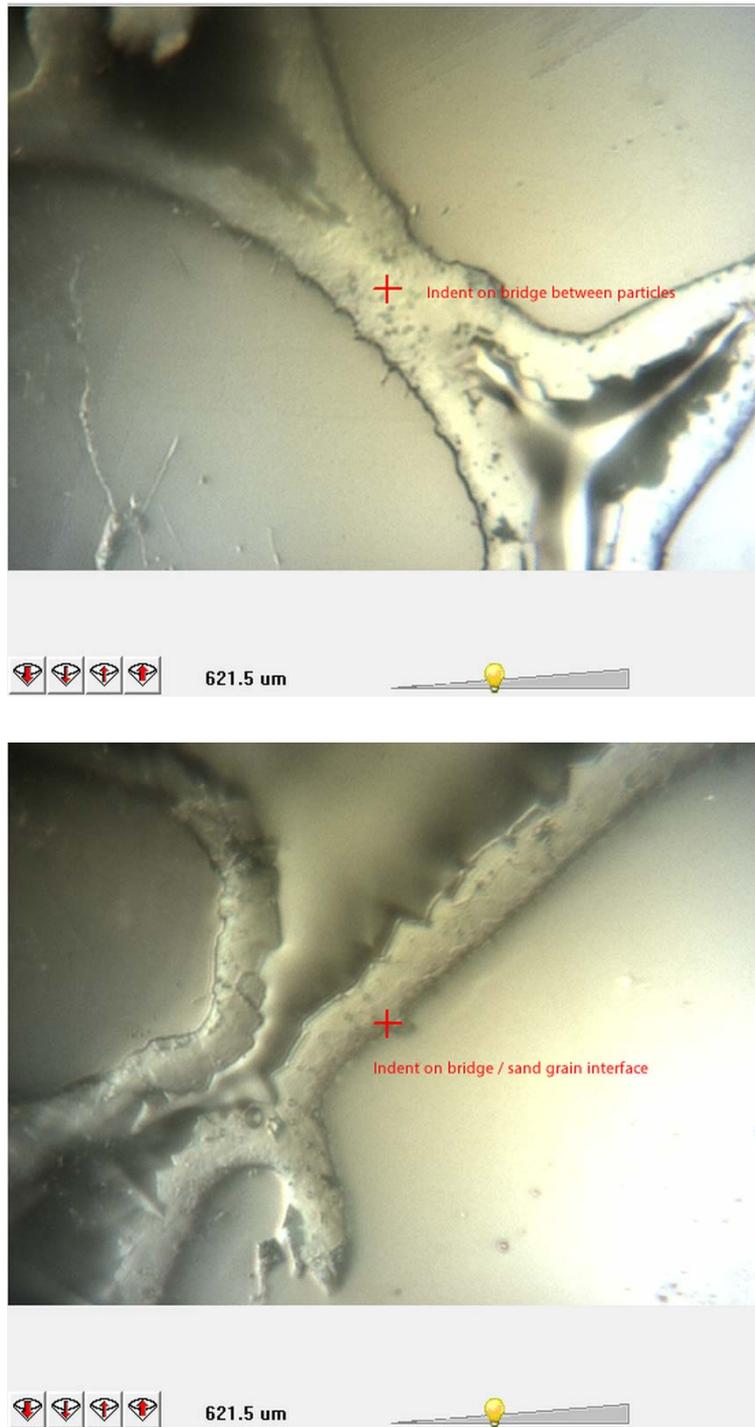




**Figure 3-10** EDS of cement stabilised + MICP treated sand at locations (a), (b) and (c)

### 3.4 Nanoindentation testing

To estimate the relative bond strengths between the binder and the sand, nanoindentation testing was performed on samples SC, SM and SCM. Nanoindentation allows evaluation of mechanical properties at a microscopic scale. Thus it is possible to evaluate the components individually as well as the interfacial transition zone. Of special interest in this investigation are the mechanical properties of cement bonding, MICP bonding and a combined MICP-cement bonding system. Previous studies have demonstrated the microscopic mechanical properties of calcium carbonate crystals with differing morphologies, (Dhami et al., 2016a), (Ren et al., 2013), (Muller et al., 2014), (Merkel et al., 2009). However, information regarding the mechanical performance of crystals precipitated within a granular soil structure is hitherto unavailable. Indentations were undertaken on the sand grain, on the bridges between particles, on the grain-bridge interface. An example of nanoindentation locations on the bridge and bridge interface is shown in Figure 3-11.



**Figure 3-11** Typical location of indents (Sample SM) (a) on bridge, (b) on interface

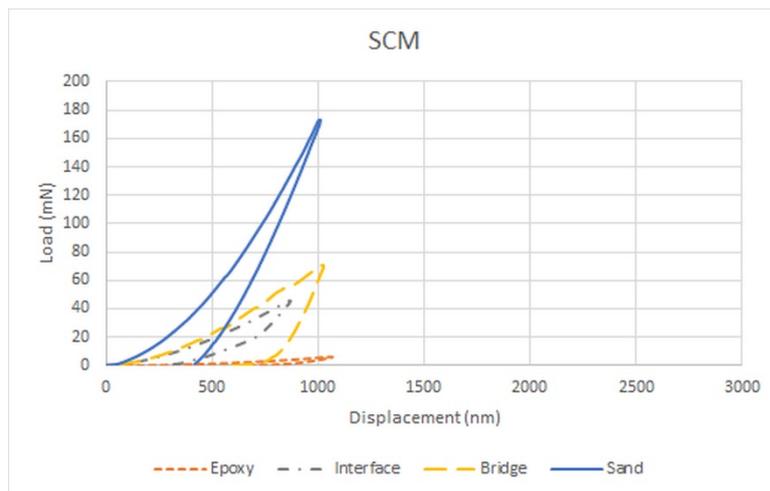
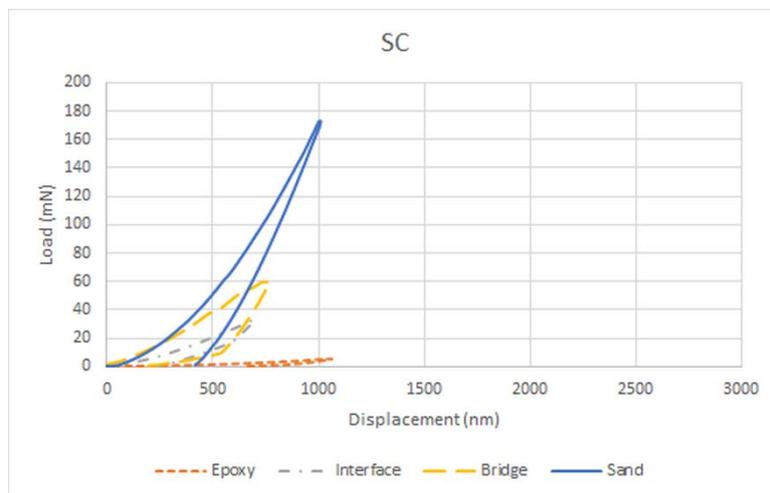
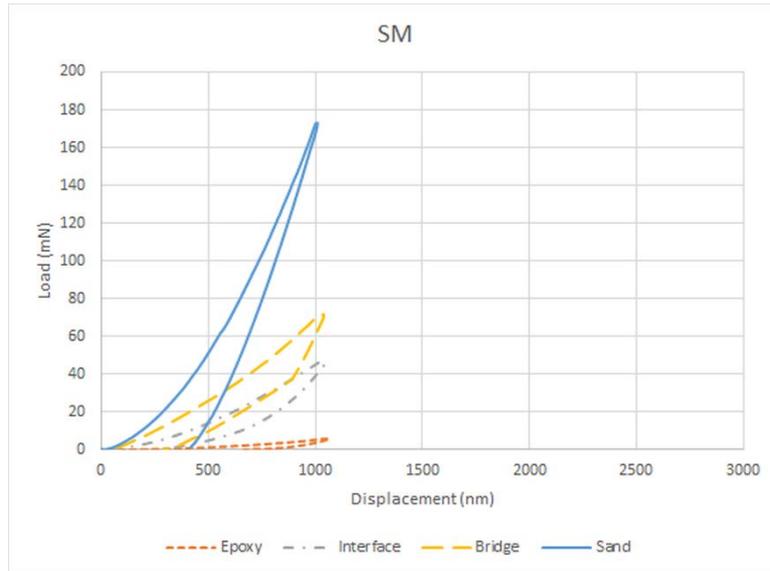
Typical load-indentation curves for the samples are shown in Figure 3-12. It can be seen that the components: sand, binder and the holding epoxy have very distinct load-indentation responses. Therefore, it is simple to correlate the response with the location of indentation. Sand is the hardest component followed by the cement and the epoxy has an order of magnitude lower stiffness. Thus, the epoxy is unlikely to influence the results. The interface has a stiffness comparable to, but always lesser than that of the cement. A summary of the average modulus and hardness obtained from all the

indentations is shown in Table 3-4. The average modulus and hardness results for precipitated calcium carbonate crystals in sample SM are similar to previously published results, (Ren et al., 2013), (Dhami et al., 2016a). Dhami et al., 2016a reports a modulus of  $64.50 \pm 2.7$  and a hardness of  $3.92 \pm 0.43$  for pure calcium carbonate crystals precipitated using *Sporosarcina pasteurii* (ATCC 11859). This proves that the mechanical property of the carbonate crystals in deposited on roadbase materials is similar to that obtained in the flask.

In the case of samples treated only with MICP (SM) or with cement (SC) the modulus results for the interlocking bridge structures are similar, (77.07 and 74.32 GPa respectively). However, when the cementitious systems are combined (SCM) the modulus of the bridge structures increases by approximately 9% to 84.58 MPa.

The modulus results obtained for the interface between bridge and sand grain were consistently lower than the modulus obtained on the bridge itself. In the case of the cement stabilised samples (SC) the average modulus was 35% lower than that obtained on the cement bridges. In the case of MICP stabilised samples (SM) the average modulus at the interface was 29% lower than that obtained on the bridges between grains, while for the combined MICP / cement system the average modulus at the interface was 24% lower than that obtained on the bridges.

Unconfined compressive strength samples revealed that when MICP treatment is performed on cementitiously stabilised samples the increase in strength due to MICP is significantly higher than that obtained when MICP treatment is applied to unstabilised samples. SEM imaging of these samples revealed that in a combined system cement would evenly coat the sand grains while MICP crystals are likely to precipitate at bridges between grains, providing a higher degree of reinforcement at bridges and resulting in a higher overall compressive strength. The results obtained from nanoindentation testing demonstrate that in the case of cement stabilised specimens treated with MICP the stiffness of the bridges between particles and bridge-grain interface is increased due to the precipitation of calcium carbonate at these locations.



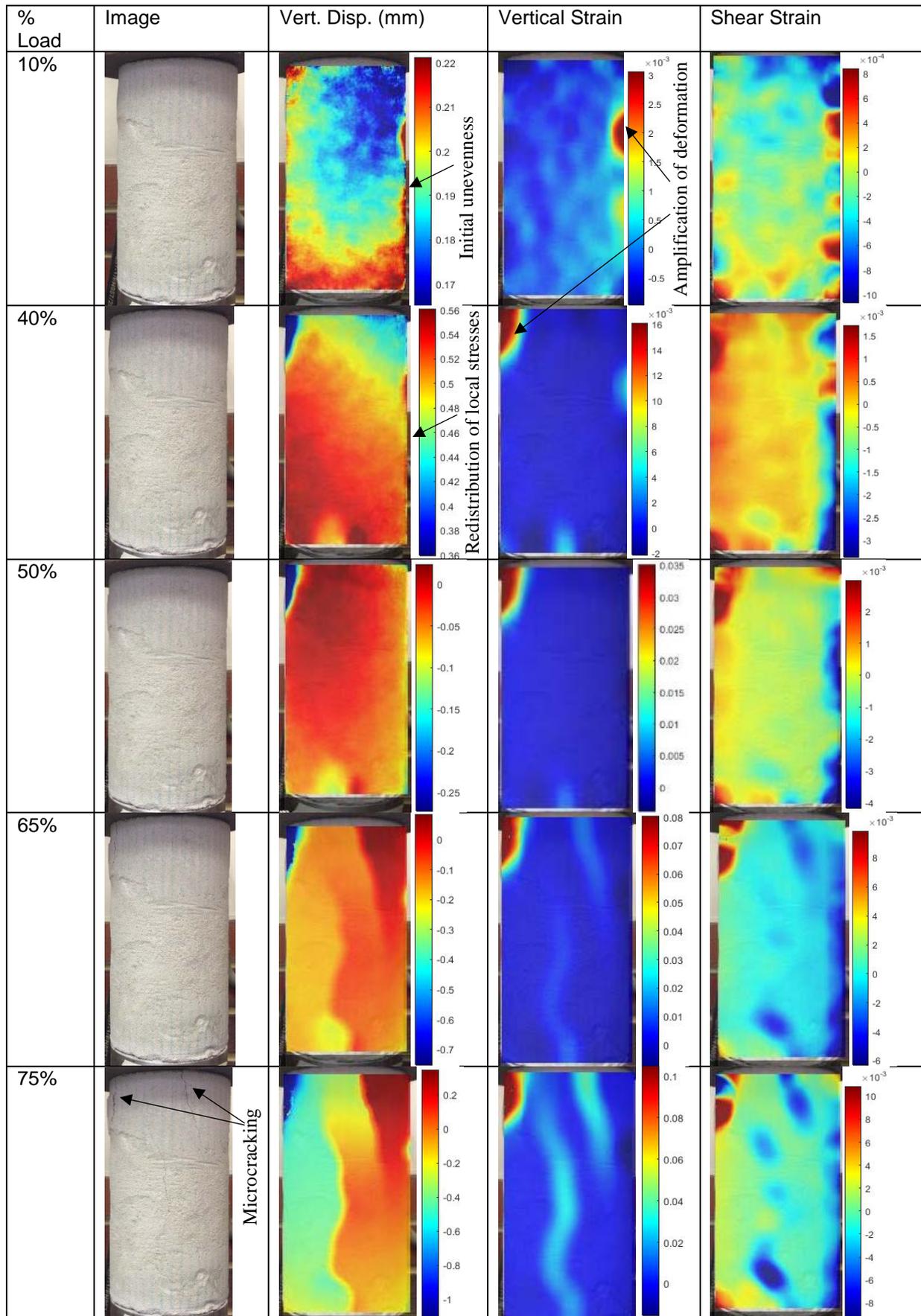
**Figure 3-12** Typical load-indentation curves for (a) SM, (b) SC and (c) SCM

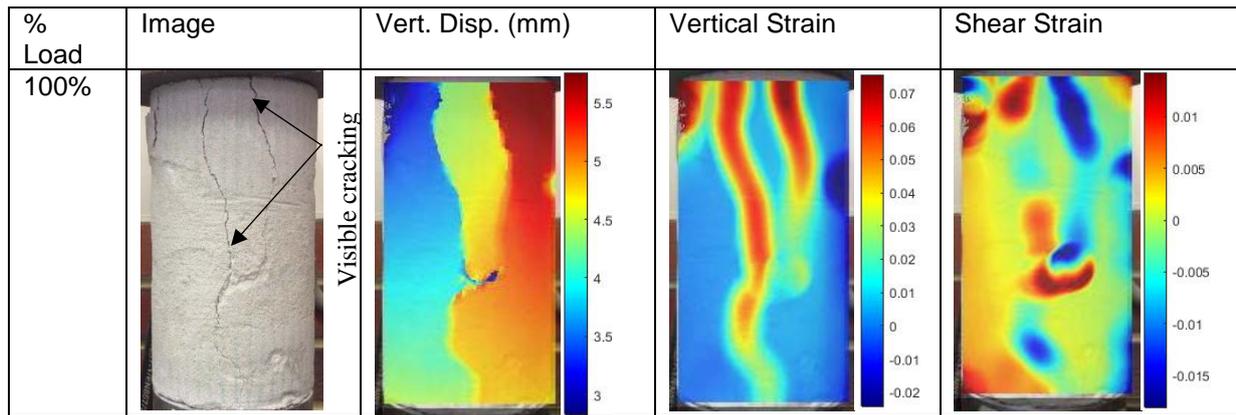
**Table 3-4** Mechanical properties obtained from nano indentation testing

<b>Sample</b>	<b>Location</b>	<b>Modulus (GPa)</b>	<b>Std. Error</b>	<b>Hardness (GPa)</b>	<b>Std. Error</b>
SM	Sand grain	118.64	1.00	16.79	0.18
	Epoxy	4.25	0.07	0.34	0.008
	Bridge (CaCO <sub>3</sub> )	77.07	4.17	7.67	0.77
	Bridge-grain interface	54.72	2.49	4.93	0.45
SC	Bridge (Cement)	74.32	2.27	3.53	0.36
	Bridge-grain interface	35.44	1.18	5.00	0.08
SCM	Bridge (CaCO <sub>3</sub> + Cement)	84.58	7.61	9.39	1.15
	Bridge-grain interface	64.25	2.74	5.66	0.43

### 3.5 Digital image correlation

To have a deeper understanding of the failure modes of the stabilised samples, digital image correlation (DIC) analysis was performed. Open source Matlab software, NCORR, was used for the DIC analysis. Photographs were taken during UCS testing for the purposes of the DIC analysis. Figure 3-13 shows the DIC results for a standard specimen stabilised with 3% cement. Three parameters; vertical displacement, vertical strain and shear strain have been shown at varying load steps throughout the UCS test. The results of the DIC analysis effectively show the variation of strain and deformation in the sample at a much more detailed level than has typically been available through the use of strain gauges. At 10% of the total load no damage to the specimen is visible however the displacement graph shows local stresses down the side of the specimen which are amplified in the strain graphs. At 50% of the total load these local stresses have disappeared, likely due to the localised redistribution of grains. At 40% of the total load (prior to any visible cracking) the origin of this cracking is observable in both the vertical strain and vertical displacement graphs. At 65% of the total load, the propagation of the cracking is visible in the vertical displacement and vertical strain plots before the cracks can be visibly observed in the specimen. At 100% of the total load, both the vertical displacement and vertical strain graphs accurately map the crack locations. The results of this experiment indicate the potential of DIC to identify the location of cracking in a stabilised material before it becomes visible. The use of DIC in the field may have significant benefits for asset management, allowing the identification of areas of failure and early remedial actions prior to substantial damage of an asset.





**Figure 3-13** Digital image correlation results at varying loads

#### 4. Conclusion

The investigations undertaken in this paper seek to determine the strength improvements that can be obtained by combining cement stabilisation with MICP. Key conclusions made from this investigation are as follows:

- It is important to consider the scale effect of samples for comparing the performance of MICP and conventional stabilisation.
- Saturation of soil due to MICP affects its compaction and UCS. An MICP technique that allows reduction of water to optimum moisture content level would be extremely beneficial.
- MICP can be successfully used to improve the strength of cement stabilised sand and road base materials as well as a plain, compacted road base. In the case of plain road base materials, treatment with MICP resulted in a 70% increase in unconfined compressive strength, providing values consistent with that required for a modified road base material. In the case of cement stabilised road base and sand materials MICP treatment resulted in an increase of between 33 and 50%. In cement stabilised samples MICP improves the strength by reinforcing the bridging structure between grains, and the bridge/ sand grain interface. These results demonstrate that MICP may be used as a supplementing material in stabilised soil structures, reducing the overall requirements for cement, thereby increasing the sustainability of such materials. MICP may also be useful for rehabilitation works on cement stabilised pavement structures.
- DIC can be successfully used for observing the degradation process of stabilised materials. The use of both displacement and strain maps from DIC can provide an indication of where cracking is going to occur and may provide a useful asset management tool for road surfaces.

## Acknowledgements

The authors would like to acknowledge the Department of Transport and Main Roads, West Australia for the provision of the road base material utilized as part of this investigation.

The authors acknowledge the use of Curtin University's Microscopy & Microanalysis Facility, whose instrumentation has been partially funded by the University, State and Commonwealth Governments.

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**Statement of Authorship**

Title of Paper	Synergistic chemical and microbial cementation for stabilisation of aggregates
Publication Status	<input checked="" type="checkbox"/> Published <input type="checkbox"/> Accepted for Publication <input type="checkbox"/> Submitted for Publication <input type="checkbox"/> Unpublished and unsubmitted work written in manuscript style
Publication Details	<ul style="list-style-type: none"> <li>PORTER, H., DHAMI, N.K. &amp; MUKHERJEE, A. 2017. Synergistic chemical and microbial cementation for stabilization of aggregates. <i>Cement and Concrete Composites</i>, 83, 160-170.</li> </ul>

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Certification:	This paper reports on original research I conducted during the period of my Higher Degree by Research candidature and is not subject to any obligations or contractual agreements with a third party that would constrain its inclusion in this thesis. I am the primary author of this paper.		
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# **Synergistic chemical and microbial cementation for stabilisation of aggregates**

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## **Abstract**

The use of Portland cement as a stabiliser of granular materials such as sand, is comparable to that in concrete. Although a relatively small percentage of cement is used for a stabilised soil system, the consumption of cement in Australian road bases and mining backfills is only second to the concrete industry. Conventionally, Portland cement is used as a binder for these applications. This paper explores low embodied energy alternative binders such as geopolymers and microbial cementation. A combination of the chemical and microbial binders has been attempted. The performance of the stabilised systems in terms of compressive strength, elastic modulus and water absorption was determined. It is noted that the chemical and the microbial cementation work synergistically. A microstructural investigation has been performed to reveal the synergistic nature of the two binding systems. The new technology is able to significantly reduce the embodied energy and emission of stabilised granular materials.

## **Key Words**

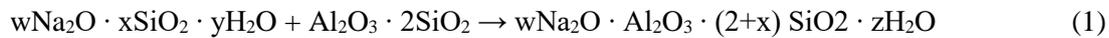
Road bases, Maintenance, Cement stabilisation, Geopolymer, Bacteria, Microbial calcite, Sustainability

## **1. Introduction**

The construction industry is heavily reliant on ordinary Portland cement (OPC) that produces roughly the same amount of greenhouse gases as its own weight (Imbabi et al., 2012). Worldwide, nearly 3.6 billion tonnes of OPC are produced which account for approximately 6% of man-made greenhouse gases. To achieve sustainability in construction it is imperative to drastically reduce the consumption of OPC. OPC is used for two principal applications: to produce concrete or to stabilise granular

materials such as soil or mining backfill. In Australia, the ready mix and precast concrete industry consumes the highest proportion of cement; however, other bulk works, such as soil stabilisation and mining backfill consume the second largest proportion. So far, the attention of researchers has been focussed towards developing sustainable concrete. Sustainable stabilisation of soil can hugely improve the sustainability score of construction industries. Two potential alternative and low energy routes are: 1) chemical binding, where OPC is replaced by a chemical alternative such as geopolymer, (Davidovits 1994, 1991, 2013); and 2) microbial, where microbes are used for in-situ cementation through deposition of calcium carbonate (DeJong 2010, DeJong et al. 2013). These two technologies were developed independent of each other and possibly, due to perceived challenge of controlling the two processes, a combination of the two is seldom attempted.

The geopolymer binding system is a chemical alternative to OPC with water, which comes with a considerably lower embodied energy (Davidovits, 1994, Davidovits, 2013). Geopolymerisation involves the chemical reaction of aluminium silicate oxides, (such as metakaolin or fly ash), with alkali silicates, to produce a polymer comprising of silicon- oxygen-aluminium bonds, (Davidovits, 1994). The reaction occurs over two stages. In the first stage, the alumina-silicate solution is dissolved using an alkaline activator solution; and in the second stage, silica and aluminium colloids form repeating chains through the process of polycondensation (Equation 1) (Rowles and O'Connor, 2009). The resulting inorganic polymer material can cement sand grains together resulting in stabilisation.



Although geopolymers are energy-efficient there are a number of challenges for its application in the bulk material stabilisation. Geopolymers are often cured at temperatures between 60°C and 90°C (Liew et al., 2012) that may not be practicable for bulk application. When cured at ambient temperatures, geopolymers typically take a considerably longer time for strength development (Cristelo et al., 2011, Liew et al., 2012, Villa et al., 2010). A 90 day curing timeframe has been found to be required when curing geopolymers at ambient conditions in order to achieve the same strength as an equivalent cement mixture at 28 days (Cristelo et al., 2011, Villa et al., 2010).

The microbial cementation utilises bacteria for the formation of carbonates and binding through microbially-induced calcium carbonate precipitation (MICP) process (Dhami et al., 2013b). Most bacteria induce or produce calcium carbonates under suitable conditions (Castanier et al., 2000, Dhami et al., 2013c, Dhami et al., 2016b). This biogenic technology is natural, environmental-friendly, novel, compatible with most building materials as well as durable (De Muynck et al., 2010). The production of carbonates through microbes occurs via a number of routes, however, MICP through the ureolytic pathway has been found to be most convenient route due to its simplicity. DeJong (2010) calculated the Gibbs free energy (kJ / mol) for MICP through urea hydrolysis, denitrification, iron reduction and

sulphate reduction and found that the change in free energy for urea hydrolysis was significantly lower than other metabolic pathways, (DeJong, 2010). During the process of urea hydrolysis, the bacteria within the soil metabolize urea, produce ammonia (NH<sub>3</sub>) and Carbon Dioxide (CO<sub>2</sub>) (Equation 2). These chemicals diffuse through the bacterial cell wall into the surrounding pore space between soil grains. The ammonia (NH<sub>3</sub>) reacts with water (H<sub>2</sub>O) to produce ammonium (NH<sub>4</sub><sup>+</sup>) and hydroxyl ions (OH<sup>-</sup>) (Equation 3) resulting in a corresponding increase of the pH of the solution while the carbon dioxide (CO<sub>2</sub>) equilibrates to form bicarbonate (HCO<sub>3</sub><sup>-</sup>) (Equation 4). In the presence of calcium, the bicarbonate (HCO<sub>3</sub><sup>-</sup>) then reacts with the hydroxyl ions (OH<sup>-</sup>) to precipitate calcium carbonate (CaCO<sub>3</sub>) (Equation 5).



The precipitated calcium carbonate binds the granular materials, by forming bridges in between the grains (De Muynck et al., 2010, Abdulkareem, 2015, Kaur et al., 2016). Unlike traditional cement, the formation of bio-cement occurs at ambient temperatures, thereby dramatically reducing the consumption of energy involved in the production of OPC (CIF, 2013). Prior studies have demonstrated the potential of MICP with regard to improvement of stiffness and strength of granular materials (Le Métayer-Levrel et al., 1999, Whiffin et al., 2007, Ferris et al., 1996) and reduction of permeability (Harkes et al., 2010, Van Paassen et al., 2010). The potential of MICP has also been investigated for slope stabilisation (DeJong, 2010, Harkes et al., 2010), improved resistance to liquefaction (Burbank et al., 2011) and suppression of dust (Chu J, 2012). Previous studies have demonstrated the potential of MICP for the reduction of porosity and permeability as well as strength improvement of sand columns (Dhami et al., 2016c, Dhami et al., 2013a). Large scale field tests have also been reported through biogrouting of microbes within soil (Van Paassen et al., 2010, Burbank et al., 2011, Dejong et al., 2013a, Dhami et al., 2012b). In the case of concrete structures, MICP has been reported to improve the strength and reduce the permeability and porosity as well as to enhance corrosion resistance (Ramachandran et al., 2001, Dhami et al., 2013b, Bang et al., 2010, Ramakrishnan et al., 1998, De Muynck et al., 2008). However, in bulk applications achieving uniform deposition of calcium carbonate is a challenge (Whiffin., 2007). Prior experiments indicated the issues of surface clogging and non-uniform calcite distribution in MICP leading to poor mechanical performance (Harkes., 2010, Whiffin., 2007, Rong et al., 2012). Thus, the construction industry is unlikely to solely depend on MICP for soil stabilisation. Moreover, the construction industry is guided by codes and practices that must be obeyed in formulating

the specifications for construction projects. Biological systems are rather new for this community and it would require a concerted effort from all stakeholders to introduce MICP into construction practice. On the other hand, the construction industry is aware of its responsibility to dramatically improve its sustainability performance.

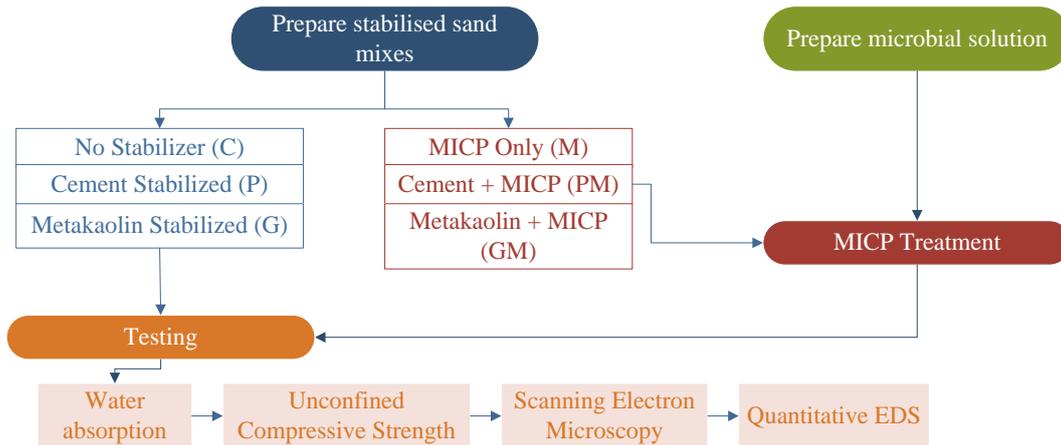
It is prudent to introduce MICP as an augmentation over conventional stabilisation methods. However, simultaneous control of the chemical and the microbial process has its own challenges. The efficacy of any biogenic process including MICP is significantly dependent upon the metabolic activity and performance of the biological agent (Harkes et al., 2010). The MICP process, on one hand, may impose restrictions on control of moisture in soil to achieve optimum compaction; on the other hand, it may be affected by the high pH of cement or the alkaline activator. The accomplishment of potential outcome can be expected through effective ureolytic activity and carbonate precipitation in a cementitious environment (Dejong et al., 2013a, De Muynck et al., 2010).

This paper explores the stabilisation of granular materials to achieve a strength of around 1MPa. Chemical cementation with conventional OPC and geopolymer and microbial cementation with MICP and combinations thereof have been performed. At first, samples have been stabilised chemically by OPC or geopolymer or microbially by MICP. The samples stabilised by OPC or geopolymer are further treated by MICP to investigate the effect of their combination and the potential of MICP to be used as a retrofitting solution for a stabilised granular material. It is noticed that the samples that received a combined chemical and microbial treatment performed better than those treated either chemically or microbially alone. To analyse the underlying reasons for differences in performance of the combined treatment methods, Microstructural analysis using Scanning Electron Microscopy (SEM) and Energy Dispersive X-Ray Spectroscopy (EDS) was executed.

## 2. Materials and methods

### 2.1 Summary of work plan

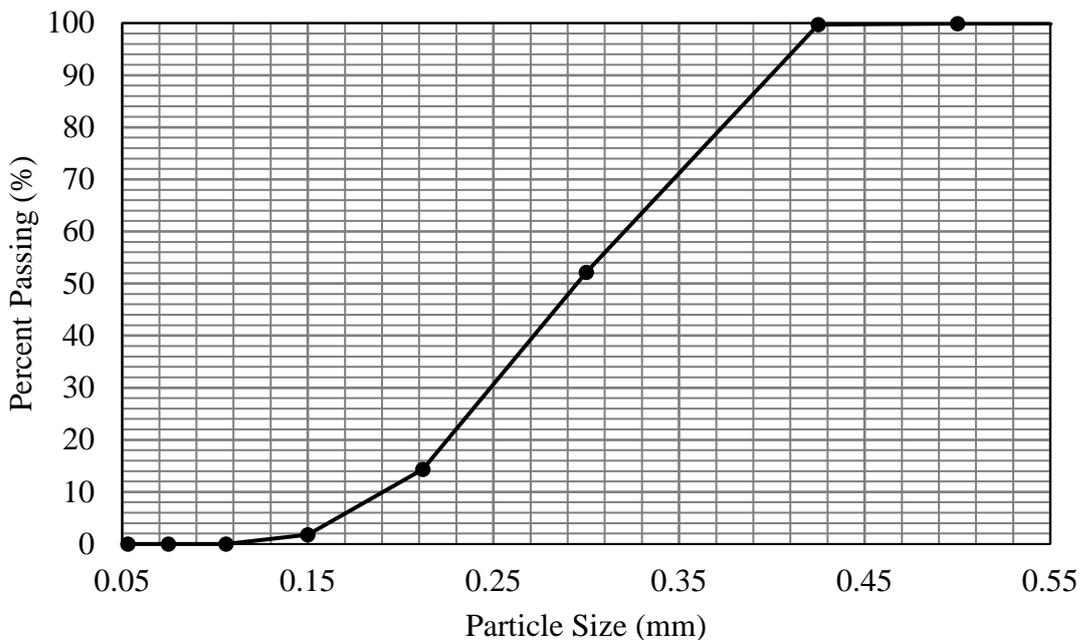
Chemically stabilised sand columns with both OPC and geopolymer were prepared at optimum moisture conditions and maximum density. Microbially stabilised sand columns were prepared separately. After a 3 day curing period of the chemically stabilised samples, MICP was undertaken on those specimens. The samples were tested for resistance to water absorption and unconfined compressive strength. The microstructure of the samples was characterized using Scanning Electron Microscopy (SEM) and quantitative energy dispersive X-ray spectroscopy (EDS). A schematic of the experimental process is shown in Figure 4-1.



**Figure 4-1** Experimental schematic for current study

## 2.2 Materials

Stabilisation was performed on manufactured sand sourced from Cook Industrial Minerals, Western Australia. The size of the sand mix varied between 75 and 425  $\mu\text{m}$ , (Figure 4-2). Standard, OPC stabilised specimens were prepared using a general purpose grey cement sourced from Swan Cement, Perth (AS3972, 2010). The chemical composition of the OPC is shown in Table 4-1. Geopolymer stabilised specimens were prepared using Metakaolin sourced from Amtrade, Australia. The chemical composition of the metakaolin as provided by Amtrade, is shown in Table 4-2. Prior to adding it to the sand, the metakaolin was activated using an alkaline solution comprising 10M sodium hydroxide and sodium silicate solution (Liew et al., 2012). The alkaline solution was prepared at a NaSi/NaOH ratio of 2.5:1 (Abdulkareem, 2015).



**Figure 4-2** Particle size distribution of manufactured sand

**Table 4-1** Chemical composition of OPC

Compound	Proportion (%)
Portland cement	< 97%
Blast furnace slag	0 – 5%
Gypsum (CaSO <sub>4</sub> 2H <sub>2</sub> O)	3 – 8%
Limestone (CaCO <sub>3</sub> )	0 – 5%
Chromium (VI) (Cr <sup>6+</sup> )	< 20 ppm
Quartz (SiO <sub>2</sub> )	0 – 25%

**Table 4-2** Chemical composition of metakaolin

Compound	Proportion (%)
SiO <sub>2</sub>	52-55
Al <sub>2</sub> O <sub>3</sub>	41-44
Fe <sub>2</sub> O <sub>3</sub>	<1.90
TiO <sub>2</sub>	<3.0
SO <sub>4</sub>	<0.05
P <sub>2</sub> O <sub>5</sub>	<0.2
CaO	<0.2
MgO	<0.1
Na <sub>2</sub> O	<0.05
K <sub>2</sub> O	<0.75

**Table 4-3** Materials for MICP treatment

Description	Components	Concentration
Bacterial Solution	<i>Sporosarcina pasteurii</i> (ATCC 11859) isolate	-
	Yeast extract	20g/L
	Ammonium sulphate	10g/L
	Tris base (pH 9)	0.15M
	Nickel chloride	2 μM

	Calcium chloride	5mM
Cementation Solution	Urea	0.5M
	Calcium chloride	0.5M
	Yeast extract	0.5g/L

### 2.3 Sample preparation

Samples were first prepared using two types of stabilisation, OPC or geopolymer. Loose sand, OPC stabilised and geopolymer stabilised samples were then subjected to microbial treatment. MICP treatment was undertaken after stabilisation to investigate the effectiveness of MICP as a retrofitting technique of stabilised soil systems. Stabilised systems were prepared under optimum moisture conditions to achieve maximum density. An optimum moisture content of approximately 10% by mass was found during proctor density testing.

For the OPC stabilised samples, cement, sand and water were hand-mixed and compacted into a 50 mm diameter by 100 mm high cylinder in three layers. Each layer was scarified prior to adding another layer to ensure good bonding between layers. OPC was added at a proportion of 5% by mass of sand. Metakaolin samples were also prepared with a metakaolin proportion of 5% (by mass) of sand. The metakaolin powder and alkaline activator were first combined at a ratio of 1:1. Water was added to the mix to achieve the optimum moisture conditions. After mixing, the metakaolin/sand mix was compacted into the cylindrical mold in even layers. Both the OPC stabilised and metakaolin stabilised samples were cured at ambient conditions for 3 days prior to MICP treatment.

Ureolytic bacterial isolate used in this study for MICP treatment was *Sporosarcina pasteurii* (ATCC 11859). The culture was grown in an Ammonium-Yeast extract media described in Bernardi et al. (2014). The bacteria were inoculated in the media and incubated at 37°C at 200rpm for around 24 - 36 hours till the optical density reached 2 which was monitored using a spectrophotometer at a wavelength of 600 nm. The grown bacterial solution was harvested and 30 mM of calcium chloride solution was added to it in accordance with Harkes et al. (2010) for fixation of the cells to the sand grains.

To begin the MICP treatment, 1 pore volume of bacterial solution was up flushed through the specimen and retained for a period of 24 hours. The bacterial solution was refreshed twice during the curing period, on day 5 and day 10. The cementation solution was up-flushed through the specimen once per day and retained for a period of 24 hours. MICP treatment phase was carried out for 15 days. Cementation effluent was collected at regular intervals and monitored for consumption of urea and calcium along with record of pH as proposed by Dhimi et al. (2016c) to investigate the efficacy of MICP.

## 2.4 Analytical methods for examination of the effluent

pH, urea hydrolysis and soluble  $\text{Ca}^{2+}$  content in the effluent were estimated at regular intervals. The urease activity of the bacterial solution was measured using the conductivity method as defined by Harkes et al. (2010). 1 ml of bacterial solution was added to 9 ml of 1M urea. The relative conductivity change (mS/min) was then recorded over a 5 minute interval. The dilution factor was then applied to the relative change to obtain the undiluted value. The specific urease activity was defined as the ratio of urease activity (mS/min) and the  $\text{OD}_{600}$  value. The soluble  $\text{Ca}^{2+}$  content in the effluent was determined by modified EDTA titration method of Stocks-Fischer et al. (1999). For an estimation of soluble  $\text{Ca}^{2+}$ , the effluent was centrifuged to recover the supernatant. Briefly, to 5 ml of the effluent, 0.5 ml sodium hydroxide (5N) was added followed by addition of a few drops of hydroxyl naphthol blue indicator. The mixture was finally titrated against 0.25M EDTA. The end point was noted from pink to blue which can be easily observed and visualized. The  $\text{Ca}^{2+}$  standard to compare with was prepared using 10-100mM  $\text{CaCl}_2$  solution.

A colorimetric method of analysis was used to determine the amount of urea present in the effluent (Knorst et al., 1997). 2 ml of effluent was mixed with 2 ml of 24% Trichloroacetic acid (TCA). The mixture was filtered through whatman paper and 1 ml of filtrate was added to 1 ml of 1.6% Dimethylbenzaldehyde (DMAB). The mixture was incubated for 10 mins at 37°C and the optical density was measured at 425 nm against a blank comprising of 1 ml diluting reagent and 1 ml of DMAB.

## 2.5 Mechanical performance investigation

### 2.5.1 Unconfined compressive strength (UCS)

UCS testing was conducted in accordance with AS 5101.4 (AS5101.4, 2008). A universal testing machine (Shimadzu AGS – X) was used for the tests. The specimens were mounted on the bottom plate of the testing machine and the top plate was lowered until it just touched the top of the specimen. The specimen was compressed at a constant displacement of 1 mm/min until failure. Force (N) and displacement (mm) were recorded electronically during the test.

### 2.5.2 Water absorption

Resistance to water absorption was determined as per AS5101.5 (2008). At the end of the curing period, (day 15), the specimens were oven-dried for a period of 72 hours. The dry mass of the specimens was recorded and the samples were immersed in 1 cm of water. The change in mass of the specimen was recorded over a 24 hour period at intervals of 10 minutes, 20 minutes, 30 minutes, 40 minutes, 50 minutes, 60 minutes, 2 hours and 24 hours.

## 2.6 Microstructural analysis

A microstructural analysis was undertaken on a portion of the samples using SEM and EDS. For SEM images small portions of the samples were mounted on aluminium stubs. After mounting, a 10 nm carbon coating was applied on the samples to ensure conductivity through the sample. A Zeiss Neon 40 EsB dual beam FESEM/FIB-SEM was used for SEM imaging at 10 kV and WD = 10 -11 mm.

For quantitative EDS small portions of the samples were coated with a 10 nm carbon coating and mounted in an epoxy resin. After mounting the surface of the sample was polished. Quantitative EDS mapping of the elements was done at points across the sample using a W-filament Zeiss EVO 40XVP.

## 3. Results and discussions

### 3.1 Unconfined compressive strength

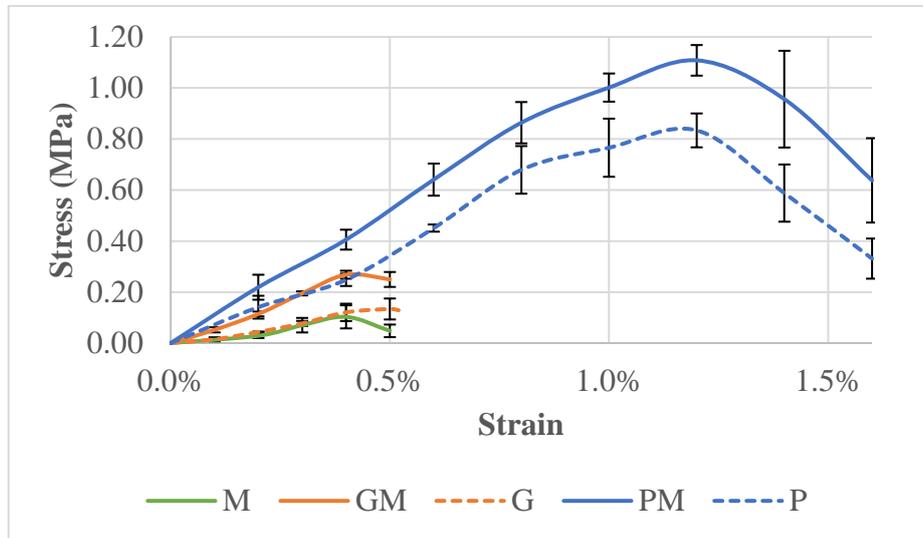
After MICP treatment, all specimens were tested for Unconfined Compressive Strength (UCS). The mode of failure, stress-strain curves, secant modulus as well as the ultimate strength were recorded. The average stress-strain curves of all specimens are plotted in Figure 4-3.

Reported strengths for sand stabilised using MICP treatment vary widely depending on the degree of stabilisation. Van Paassen (2010) undertook UCS testing on cores extracted from a large scale experiment at which plain sand was treated using MICP; Van Paassen (2010) recorded variable UCS values between 0.7 and 12.4 MPa. Whiffin et al. (2007) found confined compressive strength in the range of 0.5 MPa for 5 m long columns. In the present case, the column was lightly stabilised and a strength of around 168 kPa was noted. There is no report hitherto of a combination of MICP on columns chemically bound with geopolymer or OPC. The aim of this investigation is to determine the effect of a relatively light MICP on chemically stabilised samples.

Figure 4-3 presents the stress-strain graphs of both OPC and geopolymer stabilised materials with and without MICP. The geopolymer stabilised system is at the lower range of strength and the OPC is at the higher range of strength. The plain geopolymer stabilised system reached a maximum strength in the same range of MICP. It may be noted that in this study the geopolymer was cured in ambient conditions. When the geopolymer samples were further stabilised with MICP the maximum strength increased by more than 100%. Clearly, a combination of geopolymer and MICP works synergistically.

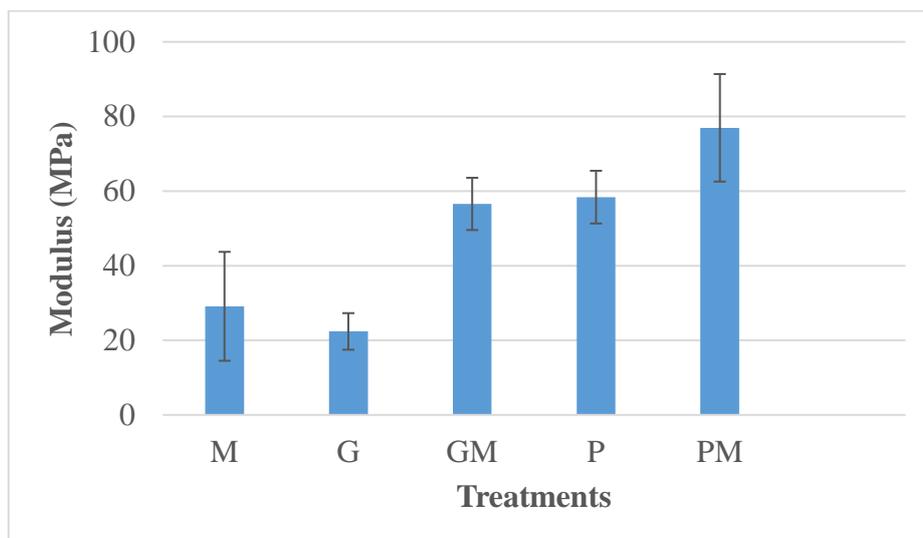
The OPC stabilised samples, on the other hand, had a far higher strength (almost four times of geopolymer). They also withstood more than three times the strain. To examine the efficacy of MICP in such a case, the OPC samples were further stabilised with MICP. The UCS of the combination was 22% higher than OPC alone. Evidently, MICP also works synergistically with OPC. An interesting point is that the UCS of the PM samples is higher than the combined strength of M and P separately.

Thus, the whole is larger than its parts. Ostensibly, MICP reinforces the stabilisation achieved through OPC in a very efficient manner. Thus, MICP is effective in reinforcing a wide range of stabilised samples. A microstructural investigation of this phenomenon is included later in the discussion.



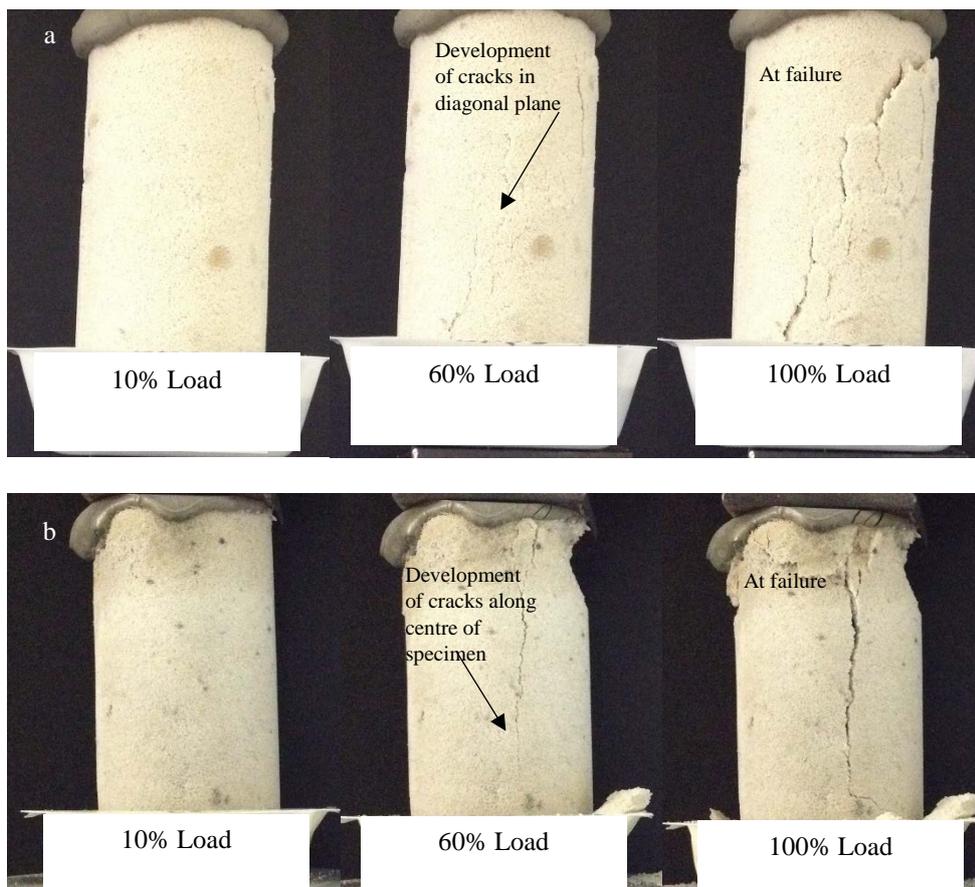
**Figure 4-3** Average stress – strain curves for control and MICP treated specimens after 15 days of MICP treatment

The secant moduli of the columns were calculated from the slope of the line obtained by joining the point of maximum stress to the origin. A higher modulus value indicates a higher degree of cementation. The secant moduli varied between 20 and 80 MPa. The M and G samples had a similar secant modulus. Remarkable improvement in stiffness was seen in case of all MICP stabilised samples. The average secant modulus of GM (56 MPa) was nearly double of that of G. In case of PM the secant modulus went up from 58 MPa to 77 MPa. The experiment illustrates that MICP works synergistically with chemical stabilisation and it can substantially increase the modulus of elasticity of the resulting material.

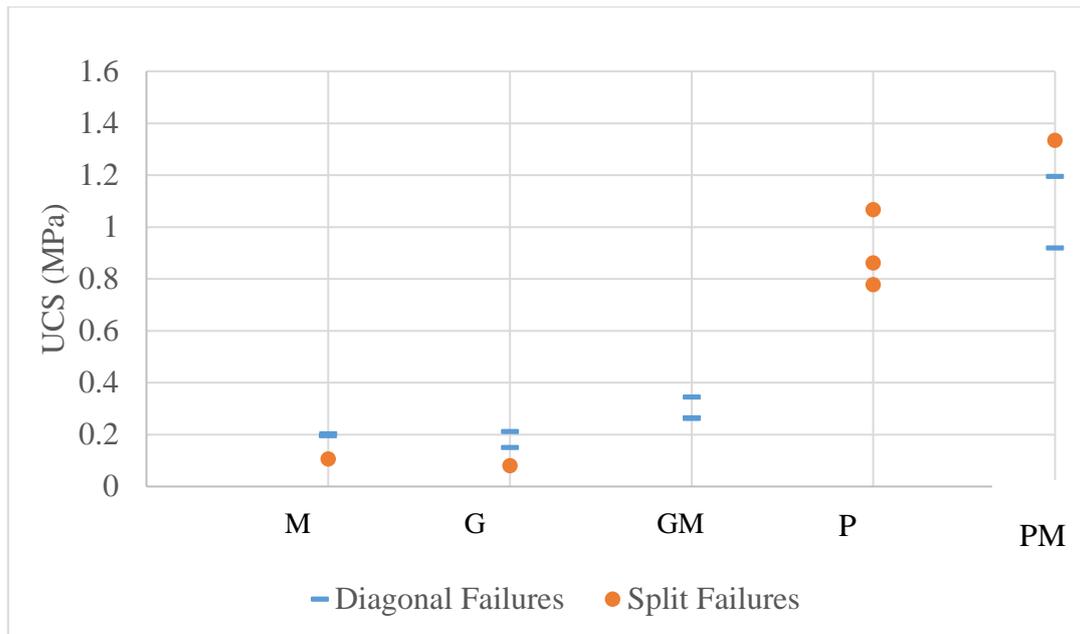


**Figure 4-4** Average secant modulus results for control and MICP treated specimens after 15 days of MICP treatment

The mode of failure of the samples was noted to assess a possible correlation between the failure modes and the stress-strain behaviour. Two principal modes of failure were noted: 1) diagonal cracking, and 2) vertical splitting (Figure 4-5). In failure mode 1, diagonal cracks developed creating a slip plane of failure. In failure mode 2, a vertical crack developed through the middle of the specimen ultimately splitting the specimen in two halves. Both failure modes occur at ultimate strength and are indicative of the actual UCS of the material. The failure mode and corresponding UCS for all specimens are shown in Figure 4-6. No real pattern was found relating the failure mode to the unconfined compressive strength results or sample type.



**Figure 4-5** Failure modes a) diagonal failure, b) split failure



**Figure 4-6** Failure modes and corresponding UCS for control and MICP treated specimens

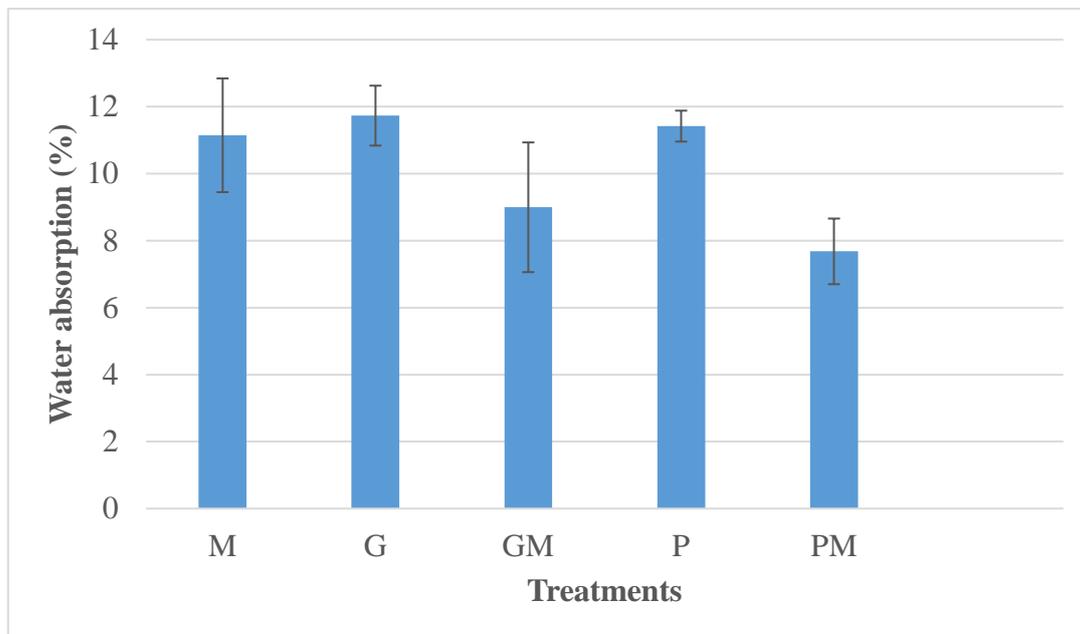
The curing of geopolymers at high temperatures is not always practicable on site and for this reason ambient curing conditions were adopted for the geopolymer. Conventionally at ambient temperatures, geopolymers are known to have a significantly longer curing period. Cristelo et al. (2011) undertook investigations with regard to strength properties of a fly ash mixture cured at ambient conditions and found that on average it took the fly ash mixtures 90 days to achieve the same strength as the equivalent OPC mixtures at 28 days. From our study, the augmentation of MICP has helped in minimising the initial curing time for geopolymers aiding in achieving higher strengths at early ages. This may therefore provide a sustainable alternative to traditional OPC modification techniques.

MICP in our study has dramatically increased the initial modulus and the UCS of OPC and geopolymer stabilised systems indicating the potential for MICP to be used as a retrofitting technique (such as in pavement rehabilitation works) or as a replacement of OPC in traditional OPC stabilised soil systems. The resistance to water absorption of MICP-treated stabilised soil systems is reported on in the following section.

### 3.2 Resistance to water absorption

One of the major causes of deterioration in stabilised soil systems is damage due to moisture ingress (Venkatarama Reddy, 2005). Moisture ingress can cause erosion within the stabilised material or cracking due to differential deformation of underlying layers. A decrease in porosity of the stabilised soil will aid in impeding moisture ingress, thereby increasing the design life of stabilised soil structures. In addition, resistance to water absorption is an indicator of the pore volume and their connectivity obtained through different stabilisation techniques. Limited data is available on the water absorption or

permeability properties of MICP treated stabilised materials. Ferris et al. (1996) found permeability reductions between 15 to 20% for plain sand material treated using MICP while Dhami et al. (2013a) demonstrated a 30-40% reduction in water absorption in MICP treated soil–OPC blocks compared to the control sets. In order to investigate the effect of stabilisation and MICP in different samples, water absorption tests were conducted at the end of 15 days (Figure 4-7). Significantly lower water absorption was recorded in all MICP treated samples compared to the control samples. The control sand specimen C could not be tested as it collapsed upon demolding. In case of GM, 23% reduction in water absorption was recorded compared to G. The PM columns had 30% less water absorption than P columns. The reduction in water absorption is attributed to the formation of calcite bridges and filling within intergranular spaces of the material which impedes the flow of water (De Muynck et al., 2010). The present investigation supports this observation. However, long term durability of the material need to be tested through further investigation. To have a better understanding of the microstructure a micrographic study was executed.



**Figure 4-7** Water absorption of stabilised samples

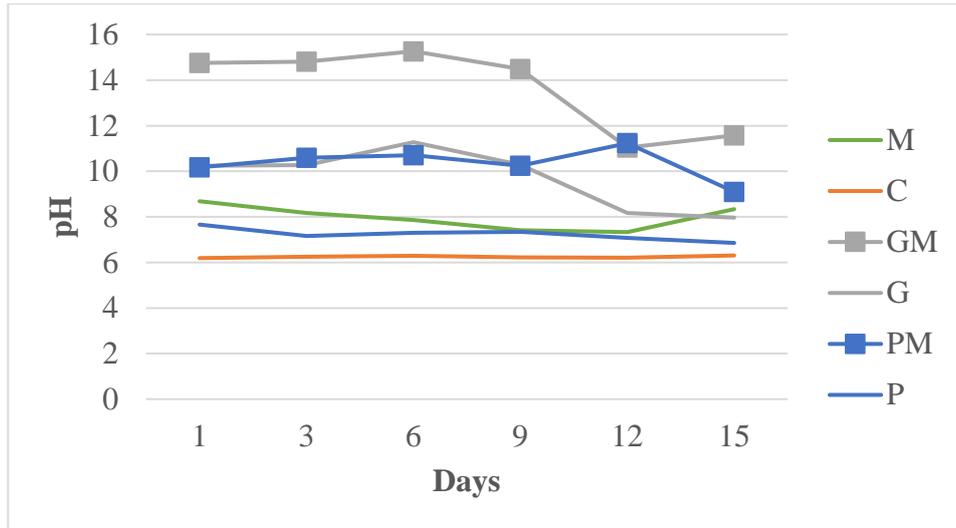
### 3.3 Analysis of effluents

At the time of MICP treatment, it is important to measure the pH. An initial increase in pH indicates the occurrence of MICP as demonstrated in previous work (Dhami, 2016). However, in the case of a combination of chemical activation and biological stabiliser the pH is influenced by both. Therefore, other measures such as consumption of urea and calcium is also included. This section reports the chemical analysis of the effluent. Moreover, a measure for MICP in the columns is obtained by analysing the effluents to estimate the residual calcium. The difference between the inflow and outflow of calcium leads to a measure of calcium remaining in the column. Similarly, the amount of hydrolysed urea reveals the efficacy of the MICP process and provides an indirect estimation of carbonate deposit.

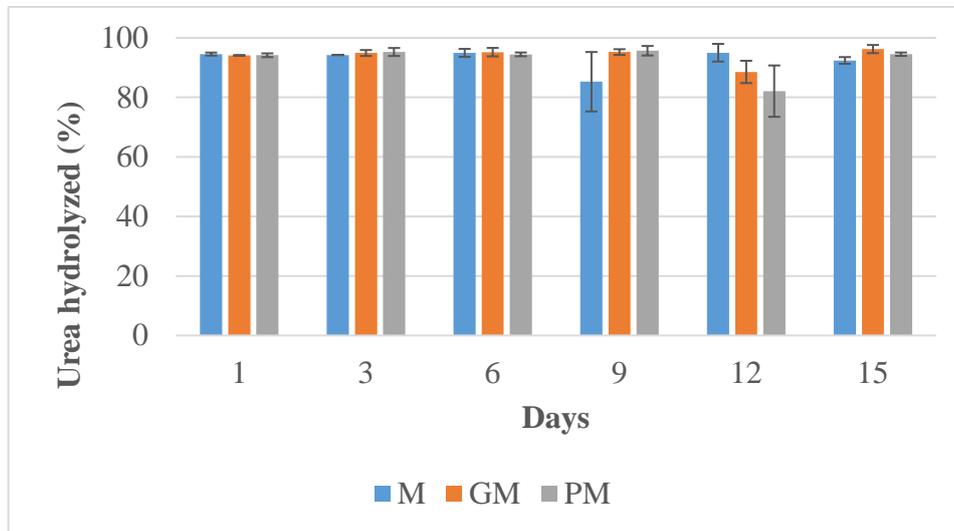
The effluent was analysed for pH, urea and calcium content throughout the MICP curing period. Only pH was monitored for the sample without MICP (Figure 4-8). The cementation solution added to the specimens had an initial pH of  $6 \pm 0.2$ . After passing through the columns there is no appreciable change in the control samples while an increase in pH was recorded in all other samples. In case of MICP-only treated sand columns (M), a significant increase in pH was noticed at each time indicating successful urea hydrolysis. In geopolymer-stabilised MICP sand column (GM), OPC-stabilised MICP sand column (PM), untreated geopolymer-stabilised sand column (G) and untreated OPC-stabilised sand column (P), the increase in pH was noticed in all samples due to the alkaline nature of the chemical stabilisers. A slightly higher pH was recorded in the case of MICP augmented sets. The noteworthy increase in only MICP sand columns confirmed the successful hydrolysis of urea within the first 24 hours. The pH of the effluent from M ranged between  $7.20 \pm 0.2$  and  $8.60 \pm 0.3$ . The pH of the effluent from GM samples was the highest, with an average value of  $13 \pm 0.2$  and that of the PM samples was in between with an average value of  $10 \pm 0.23$ . With time, the pH of all MICP samples converged within a range of 8 and 11, while that without MICP was lower between 7 and 8. Clearly, MICP occurs in the pH range of chemical stabilisers.

To quantify the MICP with regard to consumption of provided nutrients, the consumption of urea and calcium was estimated. Both urea and calcium consumption results showed that over a 24 hour retention period, more than 90% of the cementation solution was utilised in the MICP reaction in all the MICP treated samples. Approximately equal volumes of urea and calcium were utilised. Effective carbonate formation requires equal amounts of urea hydrolysis and calcium availability to precipitate calcium carbonate. This experiment demonstrates that a retention period of 24 hours is adequate for the MICP reaction under optimal bacterial cell numbers (Okwadha and Li, 2010). This result also showed that the bacteria were active throughout the entire period of the test and MICP was achieved consistently. Above 98% consumption of urea and calcium in the stabilised systems indicated the survival and high metabolic activity of bacterial cells in presence of the chemical stabilisers. Based on the utilization of calcium chloride the approximate weights of calcium carbonate have been estimated for each specimen. Approximately  $0.045 \text{ g CaCO}_3/\text{g}$  of sand is present in each MICP-treated sample. This is close to the  $.05 \text{ g cement/g sand}$  and  $.05 \text{ g metakaolin/g sand}$  present in the chemically stabilised samples. These results once again confirm that the chemical and biological stabilisers work synergistically in sand stabilisation.

(a)



(b)



(c)

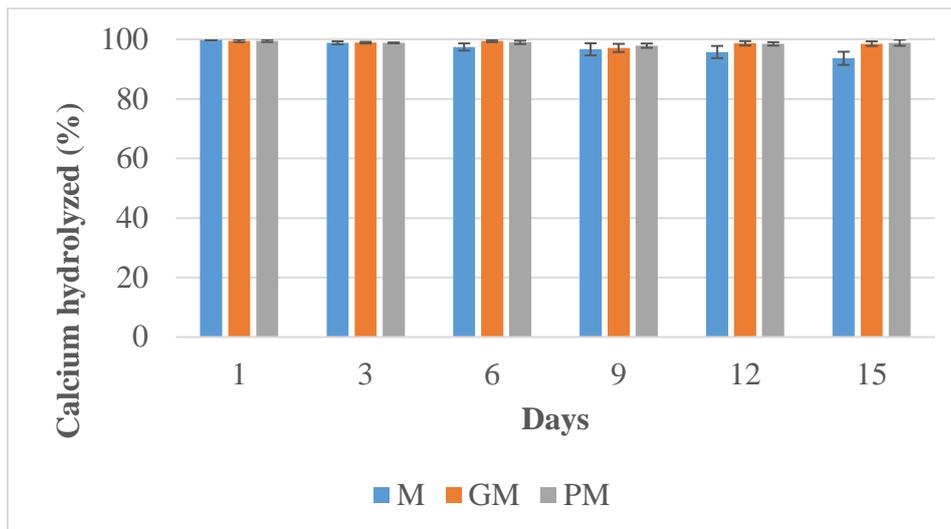
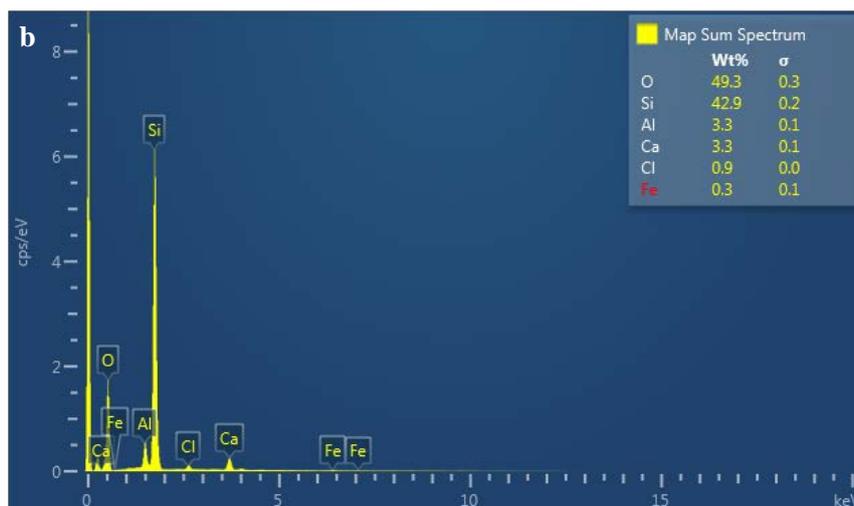
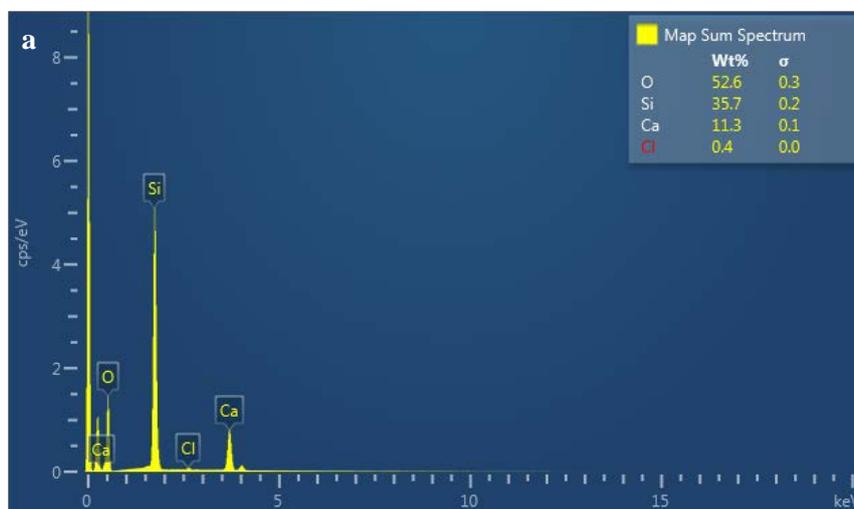
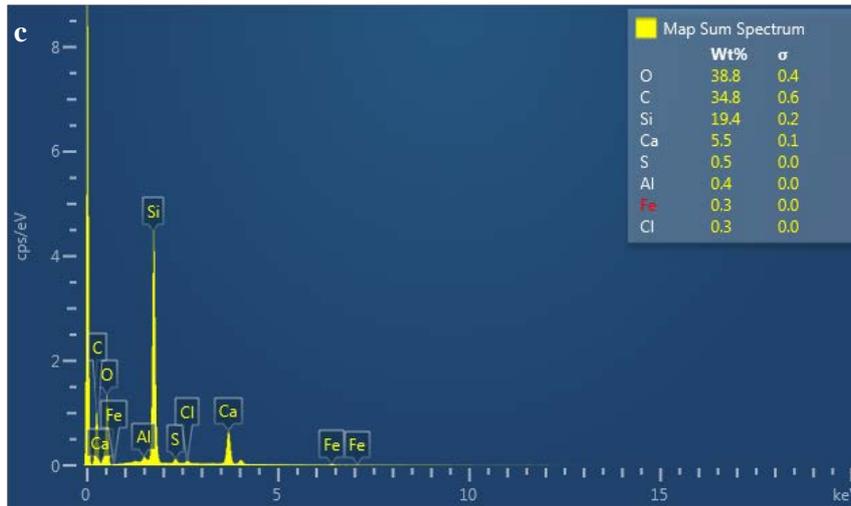


Figure 4-8 Estimation of (a) pH, (b) Urea and (c) Calcium consumption in sand columns

### 3.4 Micrographic analysis

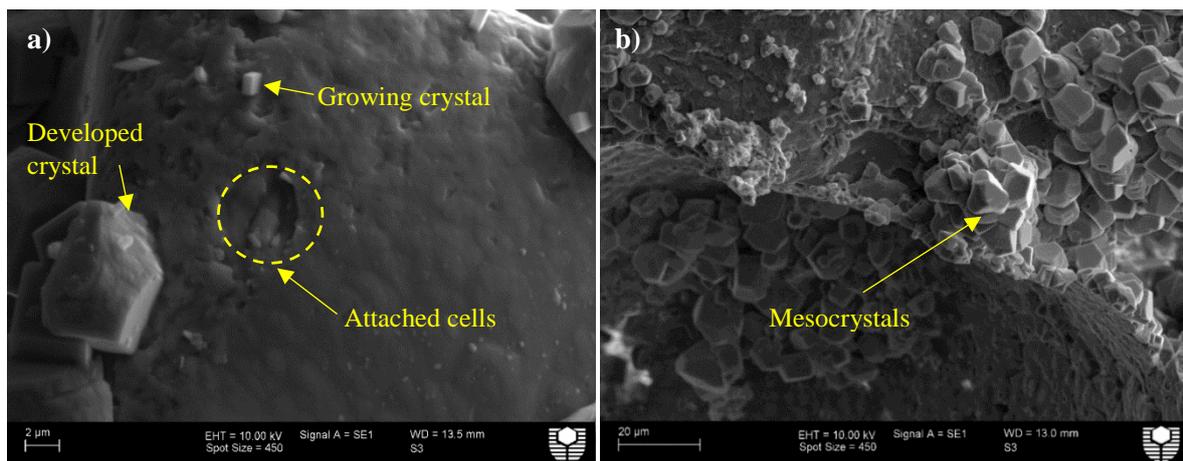
SEM was undertaken along with quantitative EDS in order to characterize the microstructure of stabilised specimens. EDS showed the abundance of silicon and oxygen that are present in the sand particles in all specimens (Figure 4-9). Apart from silicon and oxygen the major element present in the MICP sample M was calcium (Figure 4-9 a). Trace amounts of chloride were also detected. This is likely the residual calcium chloride that remained in the sample. It is clear that the majority of calcium has been converted from chloride to its carbonate form. In case of GM samples, comparable peaks of aluminium and calcium were observed (Figure 4-9 b). In these cases, the aluminium source was metakaolin while calcium was from MICP. In case of sample PM a large peak of calcium was noticed (Figure 4-9 c). The source of this calcium is the reaction product of both the OPC hydration process as well as MICP. Trace iron, aluminium and sulphur were also present and are constituents of cement.





**Figure 4-9** Energy dispersive X-ray spectrum of samples (a) M, (b) GM and (c) PM

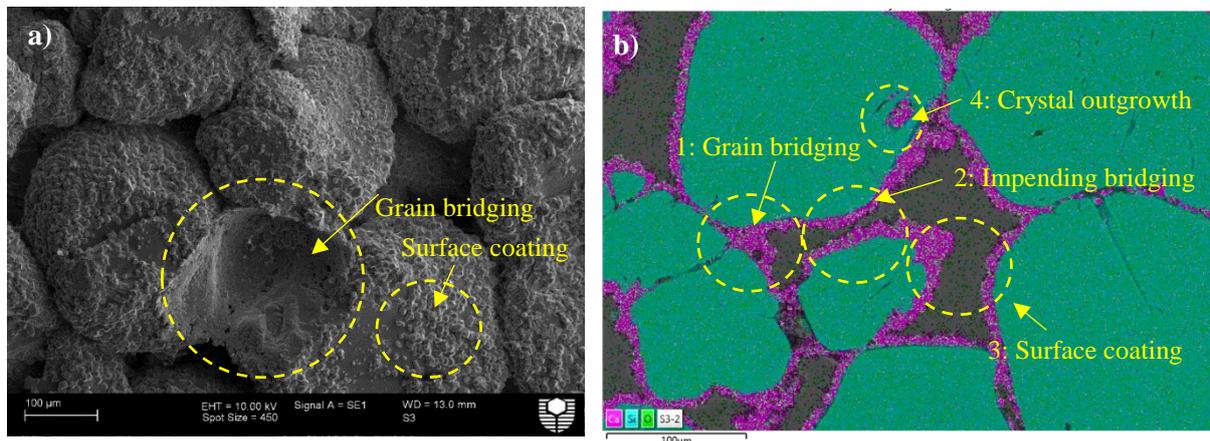
Crystal deposits from different sand columns were characterised through SEM and mapped with EDS. Figure 4-10 presents a sand grain with crystal outgrowth. It can be seen in the highlighted area that the bacterial cells have attached inside the grooves of the grain. Understandably, they control the microenvironment of the groove by producing urease. As a result, the mineralization of calcium nucleates from the grooves and gradually the crystals grow out of the grooves in rhombohedral shapes. Thus, the calcite crystals are secured in the grooves of the sand grains. Clearly, the surface morphology of the sand particles plays a vital role in the cementation process. The crystals of size 10 – 50 μm were observed in specimen M (Figure 4-10 a) and gradually they coalesce to grow mesocrystals (Figure 4-10 b). A similar structure was observed by (Terzis et al., 2016). When the mesocrystals growing from different particles join together, they create inter-granular bridges that cement the grains.



**Figure 4-10** (a) Crystals start forming from grooves in a sand grain (b) Rhombohedral crystals in Sample M

Figure 4-11(a) presents a micrograph of the M sample after UCS testing. The crater at the centre is created because a grain has slipped out at the time of testing. Thus, an interfacial failure at the grain

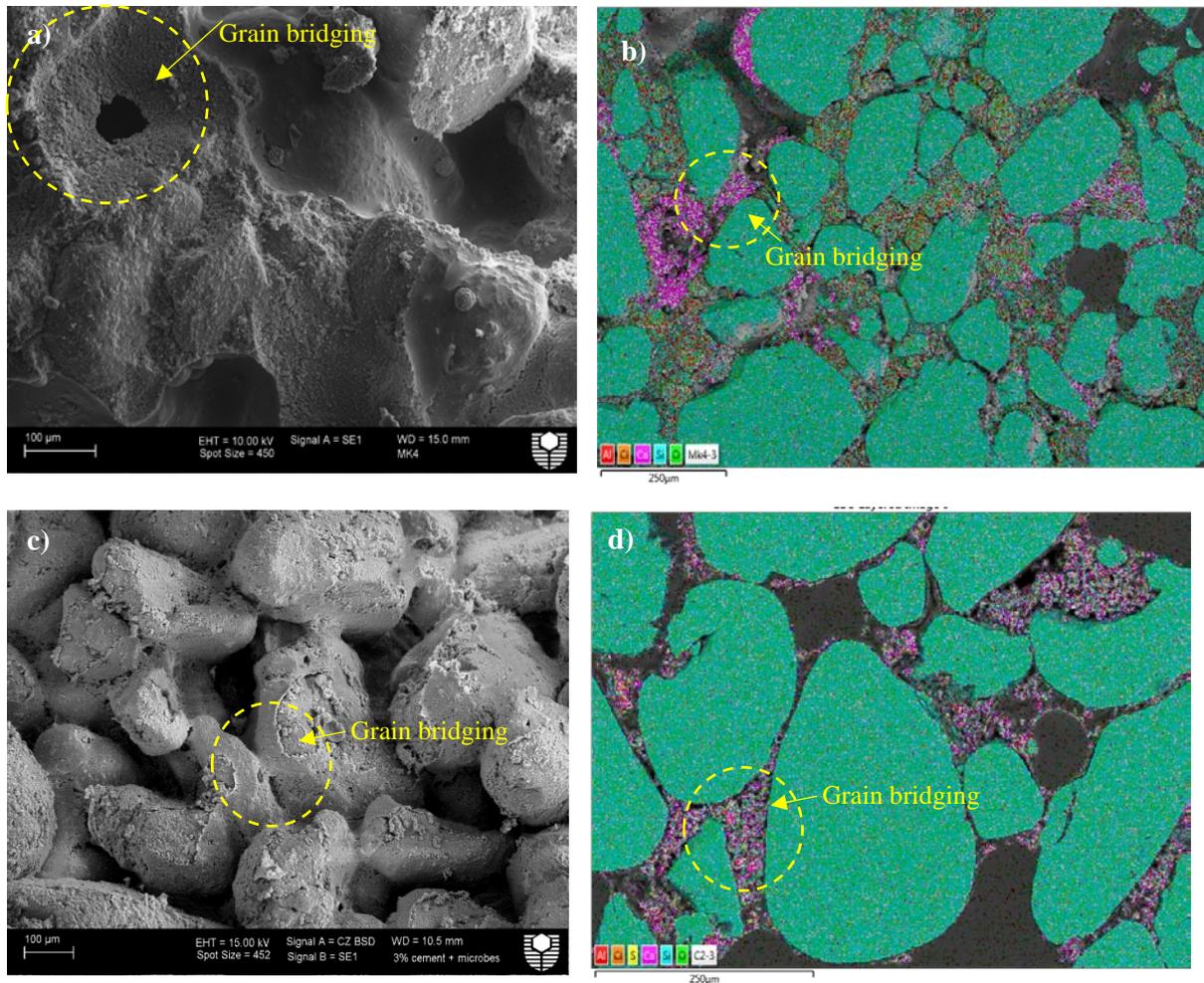
interface has occurred. The circled portion of the crystals is responsible for grain bridging. Crystal outgrowth is abundantly visible on the surface of the grains. However, at this stage, the crystals have not formed bridges between the grains. Thus, although crystals may form overall on the surface of the grains a fraction of them cements grains through bridging; the rest acts as a coating of the surface of the grain. As the MICP progresses a higher fraction of the crystals would form the bridges, increasing the degree of cementation. To further estimate the proportion of coating and bridging crystals, a quantitative EDS of the sample was performed (Figure 4-11b). In this case the sand particle is characterized by the cyan colour indicating silica and the purple in the periphery of the grains is calcium. Clearly, in the areas where the particles are in contact or in close proximity, grain bridging has occurred. The progressive formation of surface coating leads to the bridging is observed in Figure 4-11 b (location 2). In Figure 4-11 b (location 3), the coating is still too thin in comparison the distance of separation between the grains to form a bridge. Thus, these crystals just coat the surfaces. In Figure 4-11b (location 4), a crystal outgrowth from the groove of the grain is discernible.



**Figure 4-11** SEM and EDS Mapping of Sample M

In the case of the PM and GM samples the SEM images are drastically different. The abundant crystal outgrowth as seen in M is conspicuously missing (Figure 4-12). Instead the calcium, (indicated in purple) is concentrated at the inter-granular bridges (Figure 4-12 b, d). It may be recalled that in the case of M samples the crystal growth was noticed both on the surface of sand grains and also at the grain interfaces. In case of PM and GM samples, the chemical stabilisation preceded the MICP. From the images in Figure 4-12 a and c, it is clear that the sand grains were already coated with the chemical stabiliser in these cases. Therefore, the grooves on the surface of the grains were filled up and most of the bacteria did not settle on the surfaces of the grains instead securing themselves in the acute corners of the grain interfaces. Thus, nucleation sites for MICP were predominantly at the grain interfaces. As a result, MICP reinforces the bridges formed by the chemical stabilisers. Selective bacterial precipitation at the granular contact points has considerably improved the cementation, which seems to have resulted in considerably higher strength and stiffness of the material as witnessed in the UCS

results. The synergy between the chemical and the biological binders is conclusively demonstrated in this experiment.



**Figure 4-12** SEM and EDS mapping images of a-b) GM and c-d) PM specimens

The micrographic study has revealed that MICP on sand grains does both 1) coat the grains and 2) bridges them. While calcium carbonate deposited in the coating zone does not add to the strength, carbonate that is deposited in the bridging area certainly does. This section reports the fractions of coating and bridging carbonates observed in different samples. The EDS images were subjected to image analysis to estimate the proportions of coating and bridging MICP. The results are reported in Table 4-4. In case of sample M, the majority of calcium carbonate is deposited in the coating zone (64%) while only 36% is in the bridging zone (Table 4-4). In case of chemically stabilised samples PM and GM, 87-93% of the carbonates are deposited in the bridging zone. As a result, there is a significant increase in both strength and stiffness of the sand columns. Evidently, prior chemical stabilisation of the grains leads to selective deposition of the biological stabiliser at locations where they are most effective. Thus, there is a synergistic relationship between the chemical and biological stabilisers.

**Table 4-4** Distribution pattern of microbial carbonate binders inside granular materials

Sample	% Coating grains	% Bridging grains
M	64%	36%
GM	13%	87%
PM	7%	93%

#### 4. Conclusions

The present paper identifies a huge consumption of OPC in the class of construction materials that achieves about 1 MPa compressive strength using stabilised granular materials. The consumption of cement in this class of materials is only second to concrete. Therefore, this application has a significant share of the production of greenhouse gases and there is a need for low embodied energy alternatives construction materials in order to minimise the usage of OPC based systems. Two alternative cementation techniques were assessed and have been demonstrated: chemical and microbial. Both conventional stabilisation with OPC and a more recent lower energy alternative, geopolymer, have demonstrated chemical stabilisation. Microbial stabilisation through MICP has also been demonstrated. More importantly, a combination of the chemical and microbial techniques is also explored. The investigation reveals a synergistic relationship between the chemical and microbial cementation. It is noticed that if sand columns are stabilised by the chemical technique followed by the microbial one as a result there is a considerable increase in the compressive strength and stiffness of the columns. The water absorption also reduces to a great extent. The underlying mechanism for the superior performance is revealed through both chemical and microstructural analysis.

The chemical analysis of the effluent of MICP revealed that the bacteria were unaffected by the chemically-stabilised environment and they could consume both the urea and calcium more or less at the same rate in both OPC and geopolymer-stabilised samples as in case of MICP alone. Thus, the present microbial strain was able to withstand the relatively high pH of OPC and geopolymer. Thus, the rate of MICP in the chemically-stabilised samples was not significantly different from that in their absence.

The underlying mechanism of significantly superior performance of MICP in combination of OPC and geopolymer was revealed by the microstructural analysis. It is noted that bacteria secure themselves in the grooves of sand grains as well as in the inter-granular spaces and nucleate the growth of calcium carbonate crystals. As a result, a fraction of carbonates just coats the grains and do not form the interfacial bridges that leads to cementation. When a prior chemical stabilisation is performed before MICP, the grains get coated with the chemical stabiliser. Thus, a selective deposition of carbonates in

the bridging zone takes place resulting in further reinforcement of the bridges. This leads to significantly lower permeability and higher strength and stiffness of the stabilised material. Thus, the chemical and the microbial stabilisers work synergistically. Moreover, the microbial stabilisation can be applied to heal chemically stabilised material or augment their strength.

Through an optimal combination of the chemical and microbial stabilisation a material system can be synthesized that can hugely cut down the embodied energy of stabilised granular materials while offering the flexibility of future repair and maintenance. Through a combination of mechanical, chemical and microstructural testing this paper was able to open up new pathways of investigating stabilisation mechanisms.

## Acknowledgements

The authors acknowledge the use of Curtin University's Microscopy & Microanalysis Facility, whose instrumentation has been partially funded by the University, State and Commonwealth Governments.

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**Statement of Authorship**

Title of Paper	Rammed earth blocks with improved multifunctional performance
Publication Status	<input checked="" type="checkbox"/> Published <input type="checkbox"/> Accepted for Publication <input type="checkbox"/> Submitted for Publication <input type="checkbox"/> Unpublished and unsubmitted work written in manuscript style
Publication Details	<ul style="list-style-type: none"> <li>PORTER, H., BLAKE, J., DHAMI, N.K. &amp; MUKHERJEE, A. 2018. Rammed earth blocks with improved multifunctional performance. <i>Cement and Concrete Composites</i>, 92, 36-46</li> </ul>

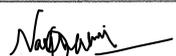
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Certification:	This paper reports on original research I conducted during the period of my Higher Degree by Research candidature and is not subject to any obligations or contractual agreements with a third party that would constrain its inclusion in this thesis. I am the primary author of this paper.		
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Contribution to the Paper:	Performed experiments as part of fourth year undergraduate thesis.		
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# Rammed earth blocks with improved multifunctional performance

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## Abstract

Rammed earth is a traditional construction technology that has proven to be sustainable. This paper explores further improvement of its multifunctional performance by increasing the strength, reducing moisture permeation and increasing the thermal resistance. Surface application of microbial cementation was found to increase the strength by 25%. The water permeability and erosion of the blocks also reduced by 24% and 62% respectively, due to surface application of microbial cementation. The thermal test showed that addition of crumb rubber resulted in a temperature difference of around 30oC even after 6 hours. However, the addition of crumb rubber also reduced the strength. This research demonstrates that significant improvement of overall performance of rammed earth materials can be achieved through various treatments. However, the overall performance requirements are specific to the engineering application and synergistic and antagonistic interactions must be considered to obtain an optimal performance.

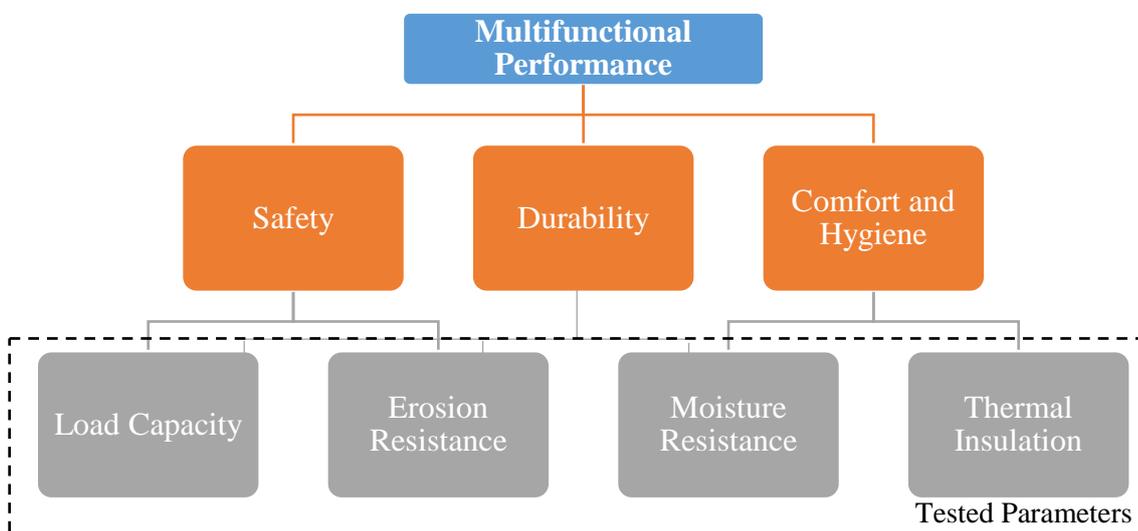
## Key words

Rammed earth, Bio cementation, Sustainability, Crumb Rubber, Microbial calcite, Stabilisation, Bacteria

## 1. Introduction

Modern construction industry is confronting the dichotomy of rapidly developing infrastructure such as buildings, roads and bridges, while adhering to the norms of sustainability (Achal et al., 2016). Construction and maintenance of buildings is estimated to consume approximately 20-40% of the total energy use in developed countries (Perez - Lombard et al., 2007). 3.6 billion tonnes per annum of Portland Cement is produced, contributing approximately 6% of all anthropogenic greenhouse gases

(Imbabi et al., 2012). Technologies that dramatically reduce or possibly eliminate Portland cement must be explored in order to improve the sustainability quotient of civil infrastructure. Rammed earth (RE) buildings have been built worldwide over a few millennia with miniscule consumption of energy in comparison to modern technologies (Dobson, 2015). RE buildings utilise earth consisting of varying proportions of sand, gravel, silt and clay which is compacted between temporary formwork in progressive layers to form walls. RE has negligible embodied energy in comparison to the fired clay bricks or concrete. However, to gain mainstream acceptance, its performance in terms of safety, durability, comfort and hygiene must be commensurate with the expectations of the modern world. Thus, it is imperative to improve the properties such as load capacity, moisture resistance, and thermal resistance and explore their interrelationships, (Figure 5-1).



**Figure 5-1** Key performance parameters for rammed earth building materials

The original mix design of RE incorporating sand, gravel, silt and clay, has a wide variation of load capacities and poor resistance to erosion. To control these limitations, a stabiliser, often Portland cement, is added to the mix. Understandably, the dosage of the stabiliser must be kept to a minimum to reduce its negative effect on the embodied energy of RE. For a rammed earth mix stabilised with 10% cement, Ciancio and Boulter (2012) achieved a compressive strength ranging between 5.5 and 13.3 MPa. The strength increased with increasing dosage of the stabiliser; however, it also depended on the particle size distribution of the soil. The moisture absorption of RE increases with increasing clay content and reduces with the dosage of the stabiliser, (Venkatarama and Kumar, 2011). The absorbed moisture in turn, affects the compressive strength of RE. Venkatarama and Kumar (2011) found an inverse linear relationship between the moisture content and compressive strength. The fine soil types

within the mix, especially the clay particles, absorb water that leads to softening and consequently a loss of strength, (Silva et al., 2013).

Erosion of RE walls is another concern. Bui et al. (2009) investigated the performance of unstabilised and lime stabilised rammed earth walls built in France in 1985 and exposed to 20 years of natural weathering. The level of erosion, (determined using stereo-photogrammetry) showed that the lime stabilised systems had eroded to an average depth of 2 mm while the unstabilised systems had eroded to an average depth of 6.4 mm. Laboratory tests with a water jet for a 60 minute period on rammed earth blocks (REB) stabilised with 10% cement, (by mass of total solid content) found negligible erosion below measurement limits, (Ciancio and Boulter, 2012), however unstabilised RE blocks could not sustain the jet and collapsed within an hour, (Ciancio et al., 2013).

For an improved lifecycle performance of RE buildings, it is essential to control energy consumption in order to maintain thermal comfort. The thermal resistance of RE has been a concern, (Correia da Silva et al., 2015, Dobson, 2015). It has been demonstrated that the thermal conductivity is dependent on both the density and the particle size distribution, (Faria et al., 2012). Often adequate thermal insulation requires a larger wall thickness than what would be required for adequate strength (Sola and Ros, 2015). A double wall configuration is not practicable. Another method is addition of an insulating material in the mix. Incorporation of granulated cork as insulation has shown to considerably increase the thermal resistance but it also reduces the compressive strength, (Correia da Silva et al., 2015). Thus, a trade-off between the strength and insulation must be reached. Evidently, in the case of RE, the performance parameters and their control techniques are intimately interconnected and they must be studied in conjunction with one another.

This paper reports a multifunctional study for improvement of sustainability, load capacity, moisture resistance and thermal insulation of REBs with the aim of improving the overall safety, durability and hygiene of RE materials. Techniques that are likely to have synergistic rather than antagonistic effect on these properties have been chosen. A combination of a cement stabiliser, a heat insulating particulate admixture and a biocementation surface treatment has been reported. Biocementation or microbially induced calcium carbonate precipitation (MICP) is emerging as a sustainable alternative to conventional cement, (Achal et al., 2015). MICP via urea hydrolysis occurs when ureolytic bacteria digest urea and in the presence of an introduced carbon source precipitate calcium carbonate, (2013b). MICP has been found to reduce the permeability of granular materials (Harkes et al., 2010, Van Paassen et al., 2010), enabling MICP to be used as a form of surface treatment, particularly on concrete structures (Ramachandran et al., 2001, Dhami et al., 2013b, Bang et al., 2010, Ramakrishnan et al., 1998, De Muynck et al., 2008). Dhami and Mukherjee (2015) conducted MICP surface treatment on earth blocks stabilised with 6% cement and found a 40% reduction in moisture absorption. Bernardi et al. (2014)

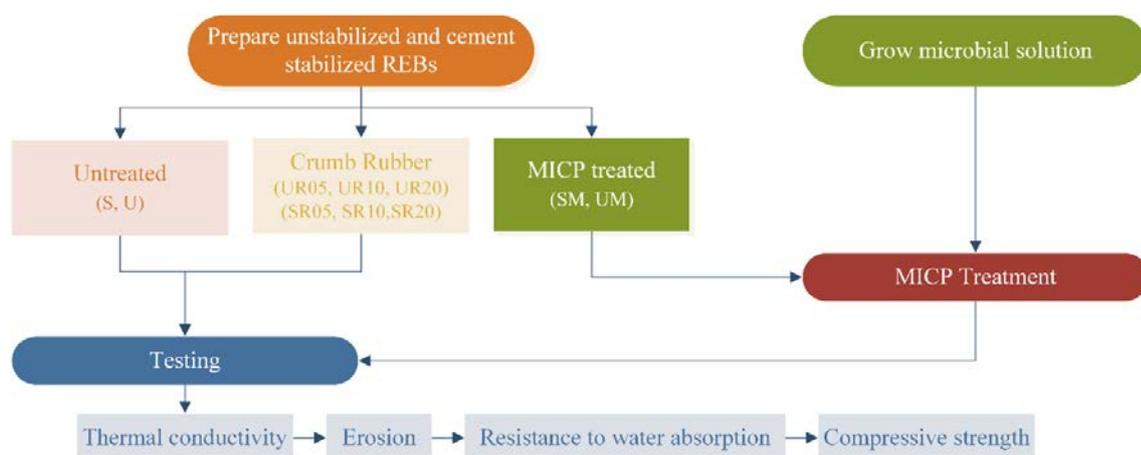
used MICP to create bio-bricks out of a moderately graded masonry sands. This paper reports the results of MICP surface treatment on REBs.

In the past, crumb rubber, a recycled industry by-product, has been used for improvement of thermal insulation of concrete, (Sukontasukkul, 2009) and bricks, (Turgut and Yesilata, 2008). While up to 10% reduction in thermal conductivity was achieved there was also a significant reduction in compressive strength and density (Turgut and Yesilata, 2008). In this investigation we study the thermal performance of REBs with varying dosages of crumb rubber and its repercussions on other properties such as compressive strength and moisture diffusion. Both unstabilised and stabilised rammed earth systems have been investigated. The samples were evaluated for compressive strength, erosion resistance, resistance to water absorption and thermal conductivity. This study demonstrates that a significant improvement in the performance of rammed earth can be achieved through innovative use of techniques to control different performance parameters, but their synergistic and antagonistic interactions must be considered to obtain an optimal performance.

## 2. Materials and methods

### 2.1 Summary of work plan

A schematic of the experimental process is shown in Figure 5-2. Rammed earth samples were prepared maintaining optimum moisture conditions to achieve maximum density. Both unstabilised and samples stabilised using cement have been prepared. Varying proportions of crumbed rubber (CR) was incorporated into the mix. After a 2 day setting period MICP surface treatment was undertaken on selected specimens for a period of 24 days. The remaining samples were demoulded after 2 days and cured for a further 26 days. The samples have been tested for resistance to water absorption, erosion, thermal conductivity and compressive strength.



**Figure 5-2** Experimental schematic for current study

## 2.2 Materials

The rammed earth mix comprised of 25 mm and 10 mm aggregate, sand, clay, rubber and cement. The aggregate material was sourced from Boral, Australia. Manufactured sand was sourced from ROCLA quarry products and an unreactive kaolin clay was sourced from Sibelco, Australia. A 30-mesh recycled crumb rubber manufactured in Somerton, Victoria was sourced from Tyrecycle, Pty Ltd, (Figure 5-3 and Table 5-1). Cement stabilised specimens were prepared using a general purpose grey cement sourced from Swan Cement, Perth.



**Figure 5-3** Recycled crumb rubber

**Table 5-1** Crumb rubber properties, (Tyrecycle, 2015)

<b>Product</b>	30- Mesh
<b>Moisture Content (%)</b>	0.5%
<b>Bulk Density (kg / m<sup>3</sup>)</b>	290
<b>Particle Size Analysis</b>	
<b>(% retained on sieve)</b>	
1.180 mm	0%
0.710 mm	2%
0.600 mm	23%
0.425 mm	36%
0.250 mm	27%
0.150 mm	11%
Pan	1%

## 2.2.1 Particle size distribution

To characterise the particle size distribution of the mix a sieve analysis was conducted in accordance with the requirements of AS1289.3.6.1 (Standards Australia, 2009b). A hydrometer test was used to characterise the proportion of the mix below 75  $\mu\text{m}$  in accordance with AS1289.3.6.3 (Standards Australia, 2003). The particle size distribution for the rammed earth mix is shown in Figure 5-4.

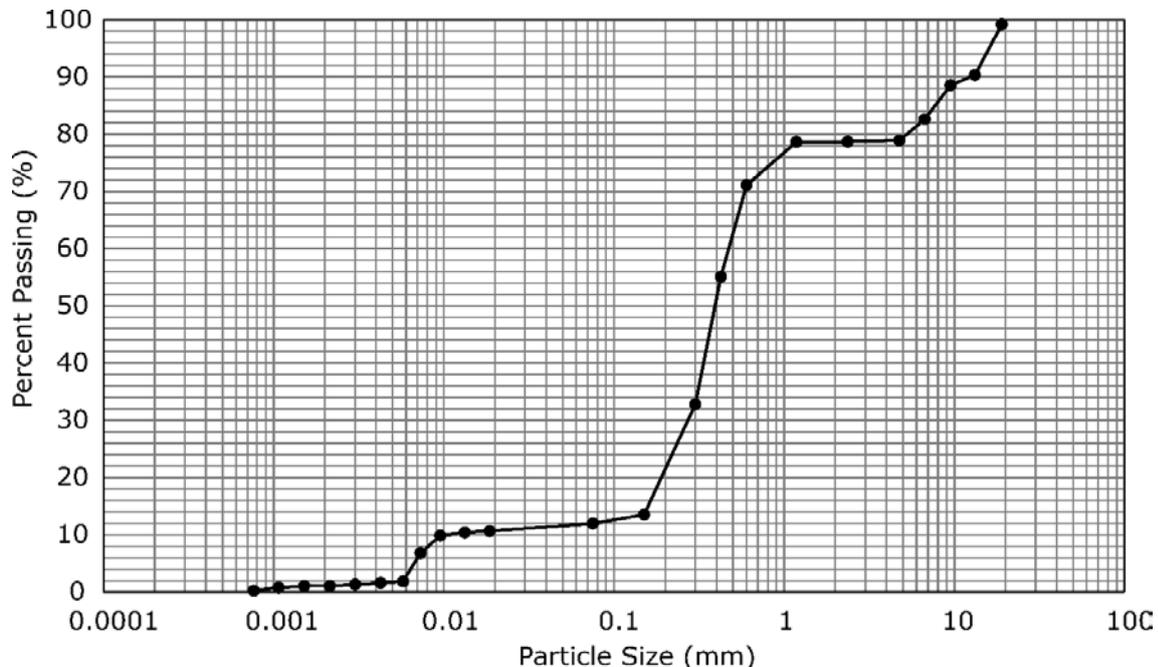


Figure 5-4 Particle size distribution for rammed earth mix

## 2.2.2 Atterberg limits

Atterberg limits, including the Liquid Limit (LL), Plastic Limit (PL) and Plasticity Index (PI) were determined in accordance with AS1289.3.1.1, (Standards Australia, 2009a) and AS1289.3.9.1, (Standards Australia, 2015). Atterberg limits for the rammed earth mix are shown in Table 5-2. All values fall within acceptable limits for a rammed earth application as defined by Walker (2002).

Table 5-2 Atterberg limits for rammed earth applications

Atterberg Values			Acceptable Limits, Walker (2002)	
LL (%)	PL (%)	PI (%)	LL (%)	PI (%)
13.4	9.3	4.1	< (35 – 45)	< (15 – 30)

## 2.3 MICP treatment method

### 2.3.1 Bacteria and growth conditions

*Sporosarcina pasteurii* (ATCC 11859), a ureolytic strain of bacteria, was used in this study. The bacteria were inoculated in an Ammonium – Yeast extract media as described by Bernardi et al. (2014). The bacteria was grown at a temperature of 37°C at 200rpm for 24 - 36 hours until the optical density (OD<sub>600</sub>) reached 2.

### 2.3.2 Cementation solution

The cementation solution used for the MICP reaction comprised of a 0.5M urea and 0.5M calcium chloride.

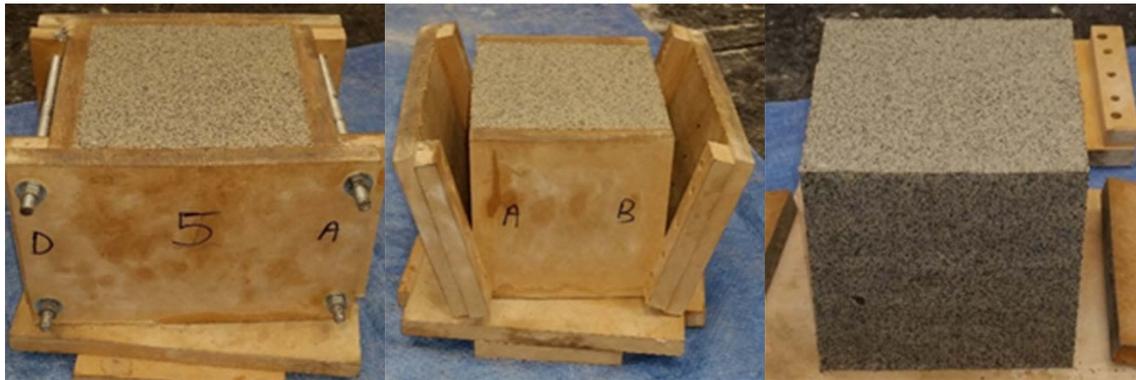
### 2.3.3 MICP

After harvesting, 30 mM of calcium chloride solution was added to the bacterial solution to aid with fixation, (2010). After demolding the blocks, 17 mL of the bacterial solution was then sprayed on each face of the rammed earth blocks and allowed to fix for 24 hours.

After the 24 hour fixation period the cementation solution 8 mL was sprayed onto each face of the rammed earth blocks, twice a day, for a period of 24 days.

### 2.3.4 Sample preparation

Both unstabilised and cement stabilised samples were prepared as part of this investigation. Prior to mixing the optimum moisture content for each mix was determined using the modified Proctor density test. The optimum moisture content varied depending on the proportion of cement and crumb rubber, an optimum moisture content of between 6.20% and 7.60% was found to achieve maximum compaction. The dry components of the mix were hand mixed with water and compacted using a 2.38 kg flat based rammer in 150 × 150 × 150 mm cubes in 5 layers of 3 cm height. Each layer was scarified prior to addition of another layer to ensure good bonding between layers. Upon completion of compaction, a straightedge was used on both the bottom and top surfaces of the specimen to ensure a flat surface was achieved. For stabilised samples, cement was added at a proportion of 6% by mass of dry constituents. Crumb rubber was added at proportions of 5, 10 and 20%. All samples were cured for 2 days prior to demolding,(Figure 5-5). Following demolding the stabilised samples were wrapped in damp hessian for 7 days to allow the cement to hydrate, before curing in ambient conditions for 19 days. The unstabilised samples were left to cure at ambient conditions for a period of 26 days after demolding. A summary of the test specimens is shown in Table 5-3.



**Figure 5-5** Demolding a stabilised rammed earth specimen with crumb rubber

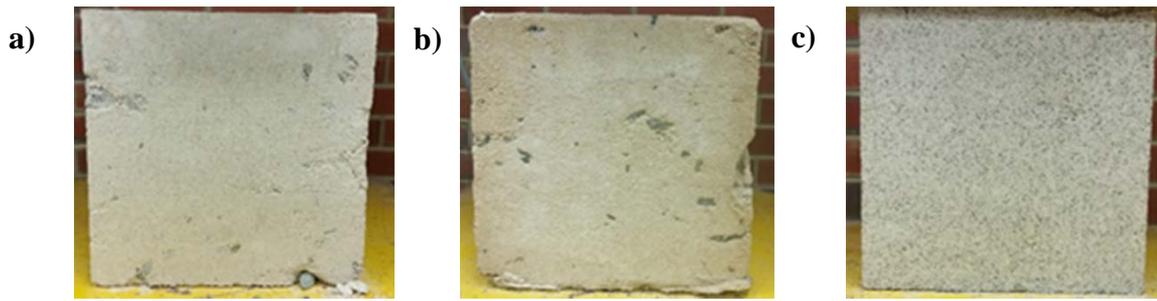
**Table 5-3** Test matrix

Label	Repeats	Cement %	Rubber %	25 mm Gravel	10 mm Gravel	Sand	Clay
U	6	0	0	10	10	65	15
UM	6	0	0	10	10	65	15
UR05	3	0	5	10	10	65	15
UR10	3	0	10	10	10	65	15
UR20	6	0	20	10	10	65	15
S	6	6	0	10	10	65	15
SM	6	6	0	10	10	65	15
SR05	3	6	5	10	10	65	15
SR10	3	6	10	10	10	65	15
SR20	6	6	20	10	10	65	15

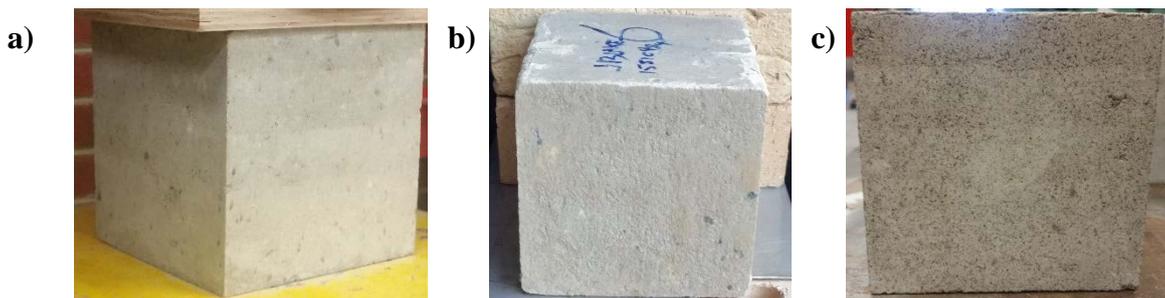
S = stabilised with cement, U = unstabilised, M = MICP treated and R = crumb rubber

Rammed earth blocks are often used for their aesthetic appeal and as such it is worthwhile to note how the addition of MICP or crumb rubber impacts on the appearance of the blocks. Figure 5-6 shows the samples of different classes of unstabilised rammed earth blocks. MICP treatment on rammed earth blocks resulted in some damage / erosion to the corners of the blocks. White precipitation was observable on the surface of the blocks due to MICP. In the case of crumb rubber a speckled appearance was observable on the face of the blocks.

Figure 5-7 shows the samples of different classes of stabilised rammed earth blocks. MICP treatment on stabilised systems did not result in damage or erosion to the blocks. Similar to unstabilised systems MICP treatment did result in a white precipitate viewable over the surface of the block while the addition of crumb rubber resulted in a speckled appearance.



**Figure 5-6** Unstabilised samples, (a) Sample U, (b) Sample UM and (c) Sample UR20



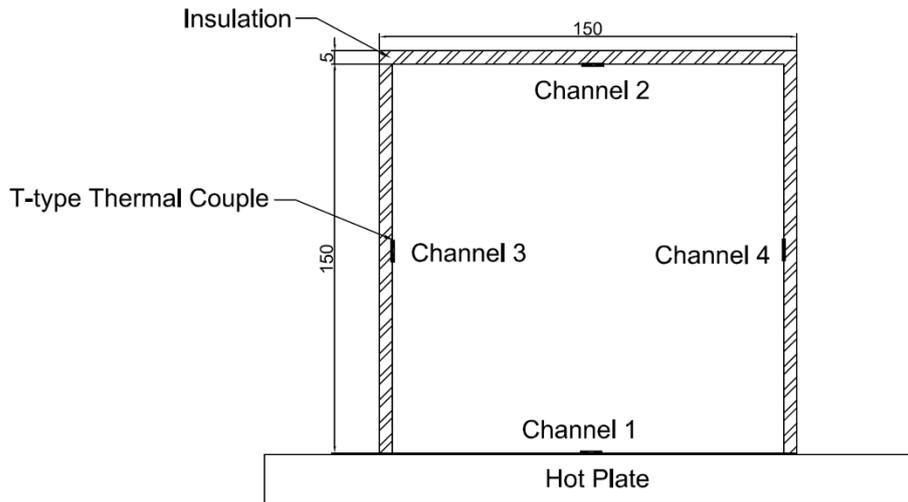
**Figure 5-7** Stabilised samples, (a) Sample S, (b) Sample SM and (c) Sample SR20

## 2.4 Compressive strength testing

Compressive strength testing was conducted in accordance with Middleton (1992). A universal testing machine (300kN Shimadzu and MCC8 Compression machine) was used for testing. The specimens were mounted on the bottom platen and the top platen was lowered until it touched the top of the specimen. The specimen was compressed at a constant loading rate of 0.15 MPa / second until failure. Force (N) and displacement (mm) were recorded electronically during the test.

## 2.5 Thermal conductivity testing

The thermal conductivity of the samples was determined using a method based on (Laaroussi et al., 2013). A schematic of the setup is provided in Figure 5-8. Four T-type thermocouples were connected to the bottom, two sides and top of the rammed earth block. Glass wool insulation was then placed around the block to prevent heat loss and the block was heated on a hot plate at a constant temperature of 150°C. The thermocouples were connected to a data logger and the temperature on the bottom, sides and top of the block was measured over time. Through these measurements the thermal conductivity of the rammed earth blocks was estimated.

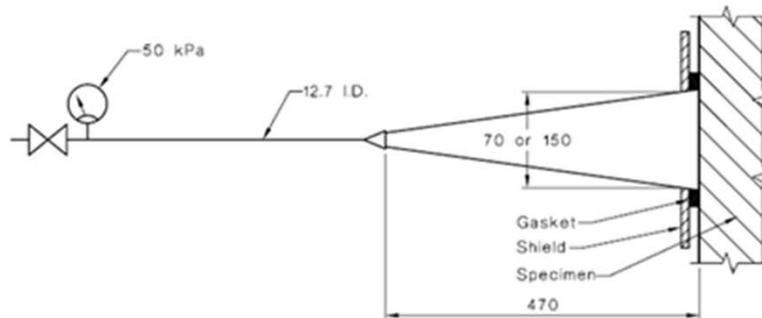


**Figure 5-8** Experimental set up for thermal conductivity testing

## 2.6 Accelerated erosion testing

Accelerated erosion testing was undertaken on the blocks based on the testing methodology outlined by (Walker, 2002). The erosion set up outlined by (Walker, 2002) is shown in Figure 5-9 a. The erosion set up in operation is shown in Figure 5-9 b. A jet of water was sprayed onto the surface of a specimen at a constant pressure and a distance of 470 mm. The erosion depth was recorded at frequent intervals through a 60 minute period. The maximum erosion rate was then determined for the specimen.

**a)**



b)



**a. Schematic b. Experimental setup**  
**Figure 5-9** Accelerated erosion test

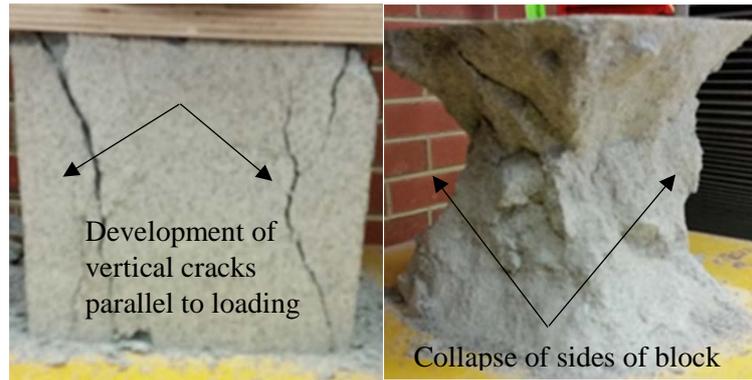
## 2.7 Resistance to water absorption

Resistance to water absorption was determined as per (Walker, 2002). At the end of the curing period the specimens were oven dried for a period of 48 hours at a temperature of 105°C. The dry mass of the specimens was recorded and the samples were immersed in water for 24 hours. After a 24 hour period the blocks were removed from the water and the saturated mass was recorded.

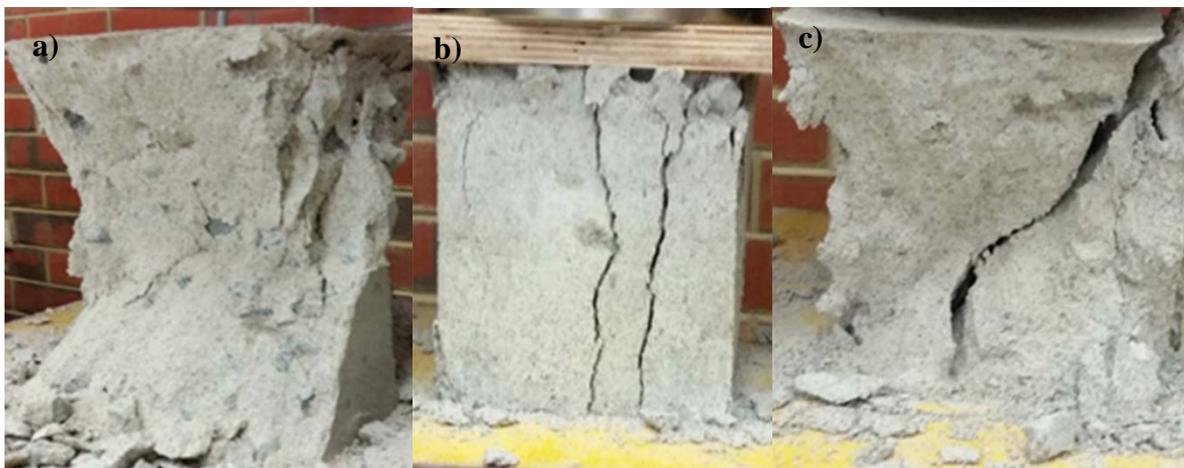
## 3. Results and discussion

### 3.1 Compressive strength

All samples were tested for compressive strength. Unstabilised REBs all failed in a similar manner. Progressive vertical cracks developed parallel to the load, spreading evenly around the block and leading to the eventual collapse of the sides of the cube, Figure 5-10. Stabilised REBs failed in three different ways. The most common mode of failure was an hour glass failure pattern, similar to that observed in unstabilised REBs, Figure 5-11 (a). In the case of samples with 20% crumb rubber (SR20) two additional modes of failure were observed. In the first case vertical cracks developed through the specimen, splitting the block in half Figure 5-11 (b), in the second case a shear crack developed through the block, splitting the block in the diagonal direction, (Figure 5-11 c). Surface treatment with MICP appeared to have limited impact on the failure mode of the REBs.



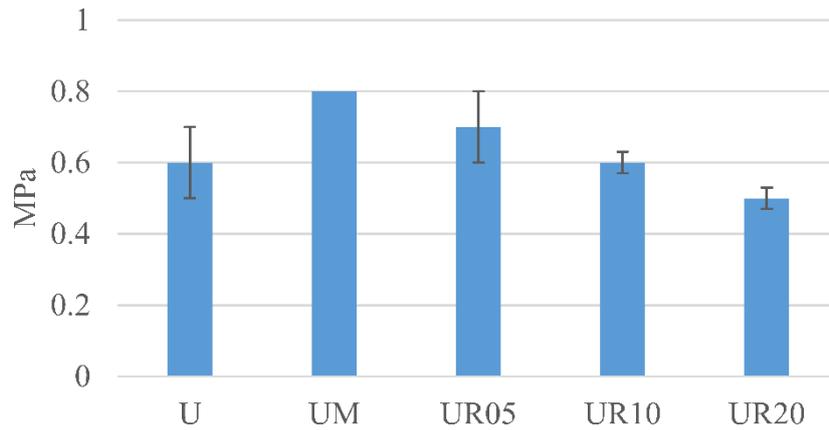
**Figure 5-10** Progressive failure of unstabilised REBs



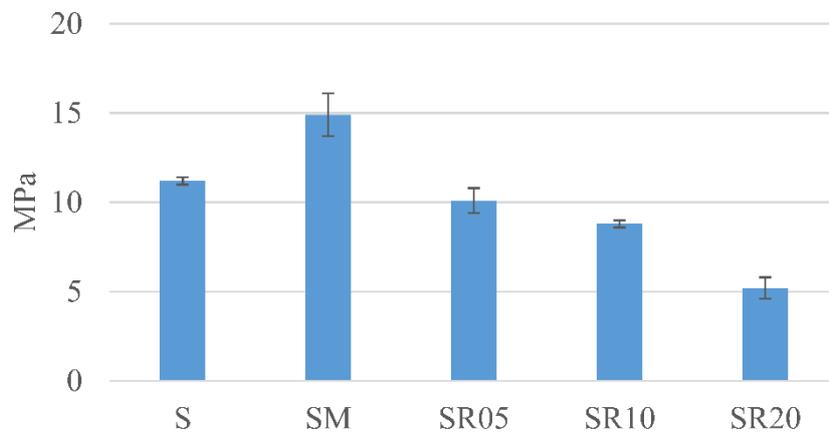
**Figure 5-11** Failure modes for stabilised blocks

The average compressive strength and standard deviation for unstabilised and cement stabilised REBs is plotted in Figure 5-12 and Figure 5-13. As expected, samples stabilised with cement achieved higher compressive strengths than unstabilised samples. Cement stabilised samples achieved compressive strengths of between 5.2 and 14.9 MPa whilst unstabilised samples achieved compressive strengths of between 0.5 and 0.8 MPa. In the case of cement stabilised samples an increasing proportion of crumb rubber was found to decrease the overall compressive strength of the sample. This is due to the addition of a softer material into the cement stabilised matrix which reduces the bulk properties of the mix. In the case of unstabilised rammed earth samples the addition of crumb rubber did not have a significant impact on the compressive strength. A small proportion, (5.0%) crumbed rubber was found to increase the compressive strength.

It is noted that the addition of a MICP surface treatment on the rammed earth blocks, (aimed at reducing the water absorption and erosion) also increased the overall compressive strength of the specimens. In the case of cement stabilised samples the addition of MICP increased the compressive strength by 24% whilst in the case of unstabilised samples the addition of MICP increased the strength by 25%.

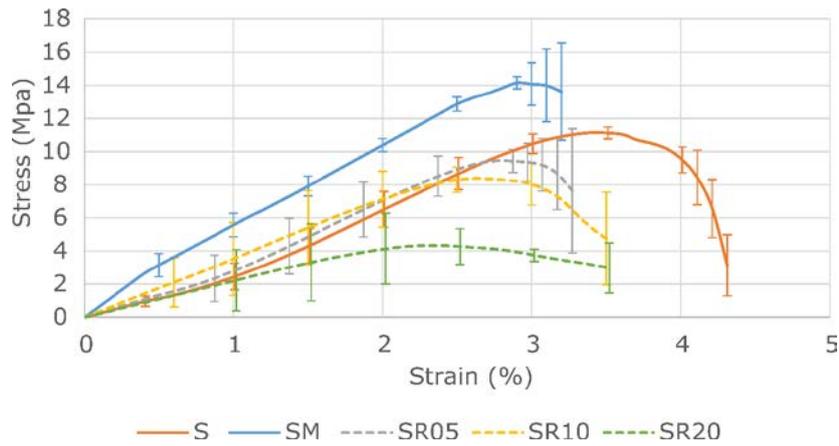


**Figure 5-12** Compressive strength of unstabilised REBs

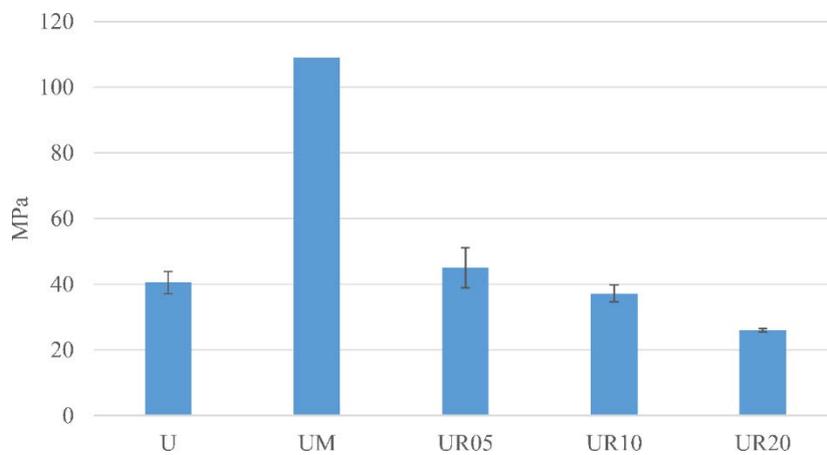


**Figure 5-13** Compressive strength of stabilised REBs

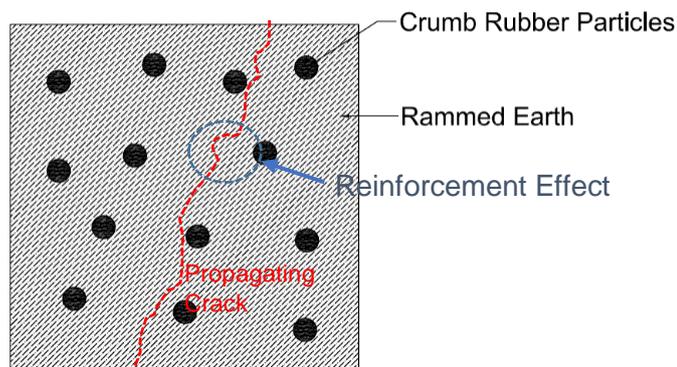
Stress-strain curves for unstabilised REBs are shown in Figure 5-14. The stress – strain curves for REBs can be used to determine the ultimate load, as well as the stiffness of the material. The stiffness of unstabilised REBs was further characterized through calculation of the secant modulus, (Figure 5-15). Like compressive strength, the modulus of unstabilised REBs was found to increase due to surface treatment with MICP. An improvement of 62% in modulus was found on unstabilised REBs due to MICP. The addition of a high proportion of crumb rubber (20%) resulted in a corresponding 35% decrease in the modulus of unstabilised REBs as compared to plain samples (U). On the other hand, the addition of a small proportion of crumb rubber (5%) resulted in an increase in modulus of 10%. A small proportion of crumb rubber particles in an unstabilised samples will not reduce the overall bulk properties of the mix but instead is likely to introduce a reinforcement effect, as illustrated in Figure 5-16. Propagating cracks are forced to travel around crumb rubber particles, thereby slowing down the rate of cracking and increasing the withstanding load before failure. Increasing the crumb rubber beyond 5% impacts the overall bulk properties of the unstabilised soil mix, again causing a softening effect and reducing the overall strength.



**Figure 5-14** Stress-strain curves for unstabilised REBs



**Figure 5-15** Modulus of unstabilised REBs



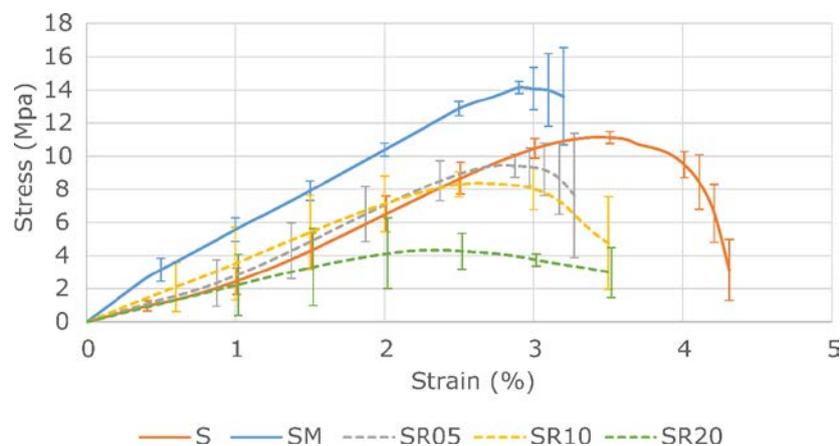
**Figure 5-16** Reinforcement effect of crumb rubber particles in unstabilised REBs

The stress-strain curves for the stabilised samples are presented in Figure 5-17. The secant modulus of the REBs is shown in Figure 5-18. Comparing Figure 5-14 with Figure 5-17, it is noted that stabilisation has resulted in a quantum jump (~10 times) in strength of the REBs. These results concur with the

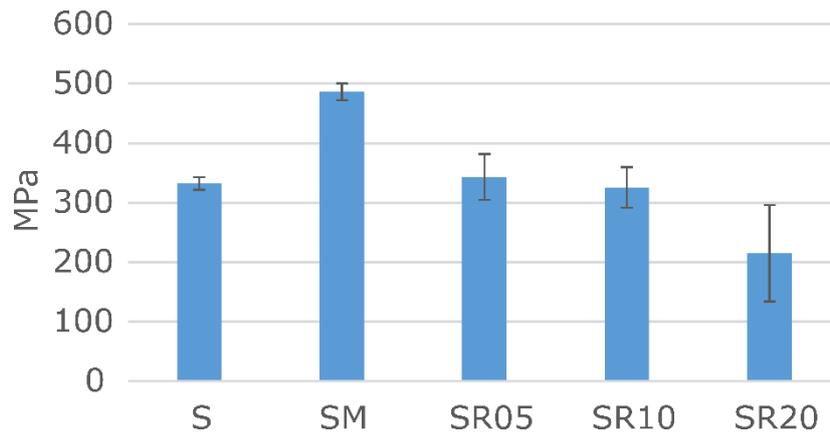
design values for rammed earth construction published by (Walker, 2002). For unstabilised rammed earth walls a dry compressive strength of between 0.4 and 0.6 MPa is recommended while for cement stabilised rammed earth walls a dry compressive strength of up to 15 MPa is recommended (Walker, 2002).

The results presented in this paper indicate that the dosage of the stabiliser can be optimised in REBs to obtain the desired strength. It is possible to use crumb rubber in combination of the stabiliser to obtain an optimal combination of strength and thermal property. The secant moduli followed a similar trend.

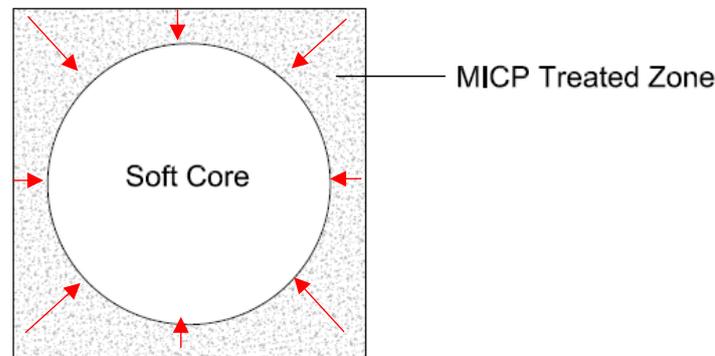
Like the case of unstabilised REBs, MICP has markedly improved the strength of stabilised REBs. A significant increase in secant modulus was observed in both unstabilised and stabilised samples surface treated using MICP. The secant modulus for cement stabilised samples increased by 31% due to MICP. These results demonstrate the potential of MICP to increase the overall compressive strength and stiffness of rammed earth blocks, using just a surface treatment methodology. The reasons for the increase in the strength and the modulus due to MICP surface treatment are envisaged as 1) confinement and 2) moisture resistance. The secant modulus of the REB has increased considerably due to MICP. The outer layer of the block, where MICP is performed is stiffer than the core, where the MICP treatment is unlikely to have penetrated. Thus the surface layer acts as a confinement to the core of the block (Figure 5-19). It is also reported that the strength of REBs is very sensitive to moisture, (Reddy and Kumar, 2011), (Reddy et al., 2017). Due to the MICP surface treatment, the moisture ingress in the block would reduce considerably, thus increasing the strength and durability of the REB. Evidently, use of MICP on rammed earth blocks is a worthwhile solution, not just to decrease erosion and water absorption (discussed later), but also to improve the strength of the REB. Thus, it is possible to replace some cement by MICP surface treatment. A judicious combination of cement and MICP surface treatment will allow tailoring of the elastic modulus of the REB to the desired level.



**Figure 5-17** Stress-strain curves for cement stabilised REBs



**Figure 5-18** Modulus of cement stabilised REBs

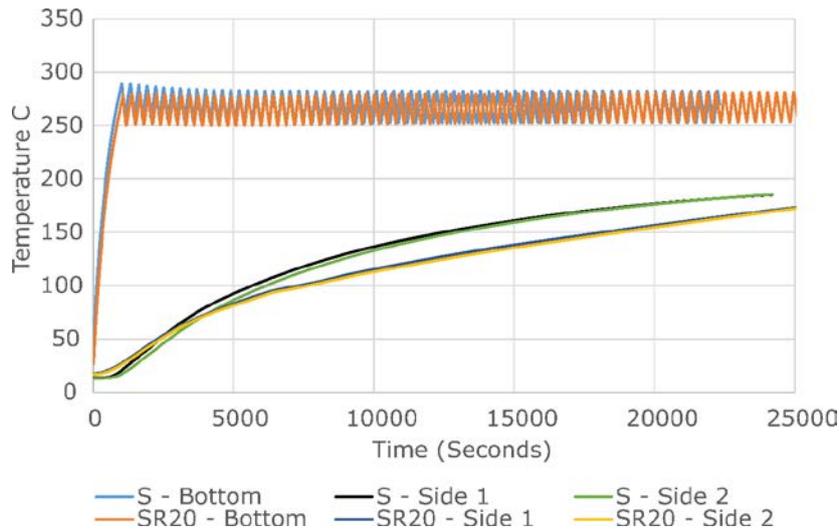


**Figure 5-19** Confinement effect of MICP surface treatment on REBs

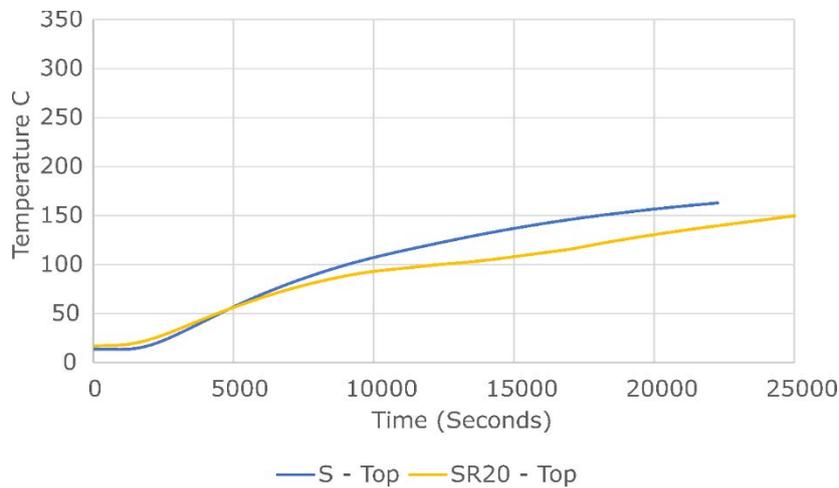
### 3.2 Thermal resistance

Both a standard cement stabilised sample and a sample incorporating 20% crumb rubber were tested for thermal conductivity using the setup discussed in Section 2.6. Heat was applied from the bottom of the block while temperature was measured at the top and the sides (Figure 5-20 and Figure 5-21). The bottom thermocouple recorded rapid rise in temperature stabilising at 275°C. The rate of temperature rise on the bottom was very close in the two samples indicating that there was a good level of control when applying heat to the sample. In the case of cement stabilised specimen S the temperature increased consistently on both sides of the specimen at a rate of 0.43°C per hour. In the case of specimen with crumb rubber (SR20) the temperature increased consistently on both sides of the specimen at a constant rate of 0.33°C per hour, 23% slower than the specimen without crumb rubber.

The rate of thermal increase on the top of specimens with and without crumb rubber is shown in Figure 5-21. In the case of cement stabilised specimen (S) the temperature increased at a rate of 0.38°C per hour. In the case of cement stabilised specimen with crumb rubber (SR20) the temperature increased at a rate of 0.30°C per hour, 20% slower than the specimen without crumb rubber.



**Figure 5-20** Thermal measurements for bottom and sides of REBs



**Figure 5-21** Thermal measurements for top of REBs

To estimate the performance of crumb rubber, the specific heat capacity defined as the amount of heat required (J) to increase 1 kg of a material by 1 degree (K) is calculated. The specific heat capacities of common building materials are shown in Table 5-4 along with the present results. A higher specific heat capacity indicates a material with better insulation properties. The specific heat capacity for cement stabilised REB (sample S) was calculated as 1,321 J/kg °K. This is consistent with previously published results for rammed earth walls (AIRAH, 2000). In the case of REBs with crumb rubber (SR20) the specific heat capacity increased by 27% to 1,832 J/kg°K.

**Table 5-4** Specific heat capacity of common building materials

Material	Density (kg / m <sup>3</sup> )	Specific heat capacity (J/kg.K)
Softwood (Ashby, 2013)	510	1,700
Rammed Earth Walls (AIRAH, 2000)	1,540	1,260

Concrete (ASHRAE, 1997)	2,240	1,000
Brick (Ashby, 2013)	1,800	800
Adobe (Ashby, 2013)	1,850	710
Sample S	2,138	1,321
Sample SR20	2,064	1,832

The addition of crumb rubber in rammed earth blocks has been found to significantly delay the rate of temperature increase of the material as a whole when exposed to a heat source. When stabilised with a high proportion of crumb rubber (20%) the REB increased in temperature at a rate 20% slower than specimens without crumb rubber. A corresponding increase in the specific heat capacity of the REB was noted. Clearly the incorporation of crumb rubber into a RE mix can help to improve the insulation properties of the material, however the addition of a high proportion of crumb rubber has also been found to decrease the strength of the blocks. When designing a RE mix with crumb rubber consideration should be given to optimise a mix that provides both desired strength and thermal insulation.

### 3.3 Erosion resistance

Accelerated erosion testing was undertaken on samples S, SM, SR20, U, UM and UR20. Time to full penetration, maximum erosion rate and pitting depth at various time intervals are shown in Table 5-5. Samples stabilised with 6% cement did not experience any measurable erosion over a 60 minute time period. The appearance of cement stabilised REBs at the end of erosion testing is shown in Figure 5-22.

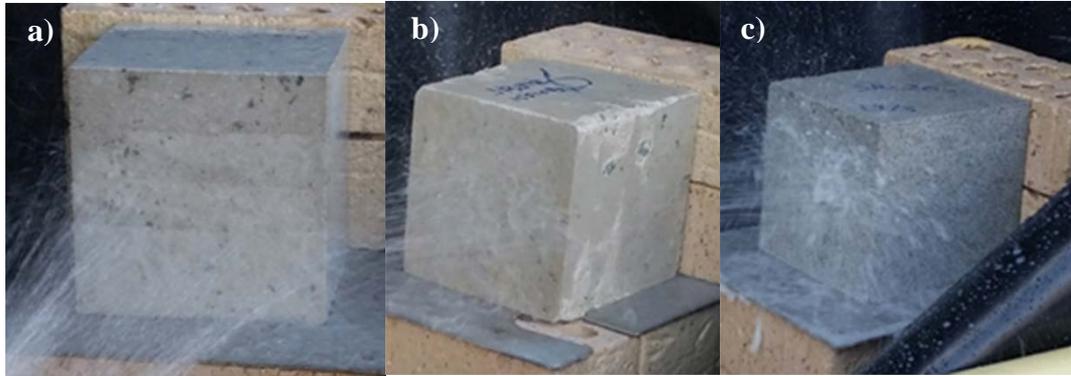
These results are consistent with the findings of (Ciancio and Boulter, 2012) who recorded no measurable erosion over a 60 minute time period on rammed earth samples stabilised with 10% cement. The addition of small proportions of cement as a stabilising agent greatly reduces the erosion of REBs and should improve their durability.

In comparison, the unstabilised blocks demonstrated rapid erosion and none withstood the 60 minute time interval. The maximum erosion rate was recorded on sample U, which eroded at a rate of 18.8 mm until full penetration at 8 minutes. Figure 5-23 shows erosion on sample U at intervals of 0 minutes, 2 minutes, 4 minutes and at failure. Surface treatment with MICP extended the time of collapse from 8 minutes to over 21 minutes. Sample UM eroded at a rate of 7.1 mm until full penetration at 21.1 minutes. Interestingly, the incorporation of 20% crumb rubber also reduced the erosion rate in unstabilised specimens. Sample UR20 eroded at a similar rate to the MICP treated specimen. Ostensibly, crumb rubber considerably impedes the pathway of the water jet.

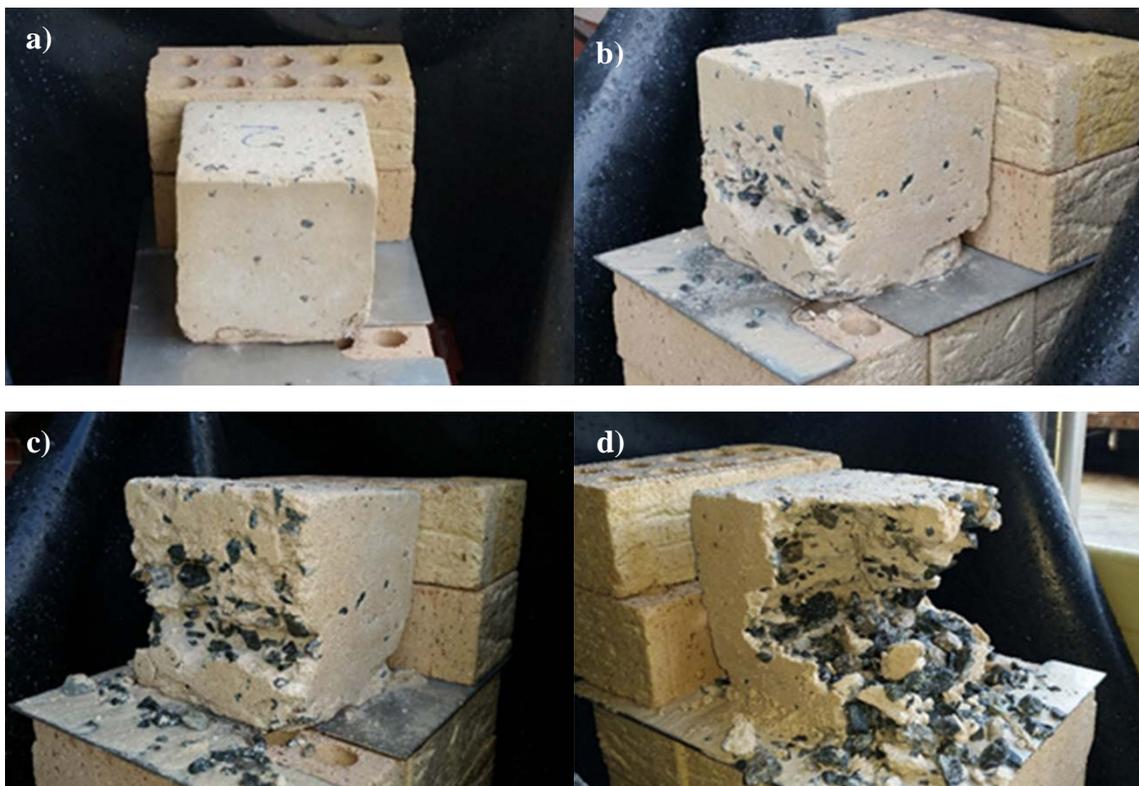
**Table 5-5** Accelerated erosion test results

Sample	Penetration Depth (mm) at Time (minutes)								Time of Full Penetration (minutes)	Maximum Erosion Rate (mm/minute)
	2	4	6	8	10	15	20	60		
S	0	0	0	0	0	0	0	0	0	0

SM	0	0	0	0	0	0	0	0	0	0
SR20	0	0	0	0	0	0	0	0	0	0
U	65	101	116	150	150	150	150	150	8.0	18.8
UM	16	29	36	48	71	125	144	150	21.1	7.1
UR20	25	38	52	65	74	104	150	150	19.9	7.5



**Figure 5-22** Sample a) S, b) SM and c) SR20 after 60 minutes exposure to water jet

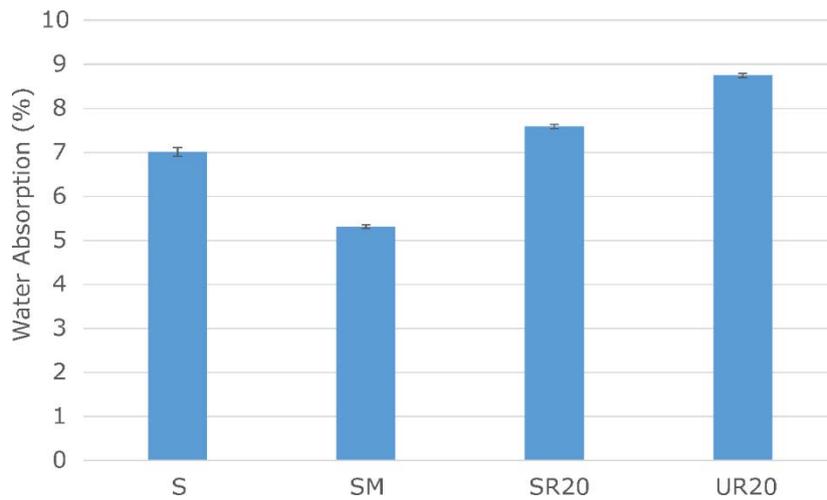


**Figure 5-23** Accelerated erosion testing on sample U: a) at 0 minutes, b) at 2 minutes, c) at 4 minutes and d) at collapse

### 3.4 Water absorption

Water absorption testing was undertaken on samples S, SM, SR20 and U and UR20, the results of which are shown in Figure 5-24. Unstabilised rammed earth sample U disintegrated during testing and no value was obtained for this sample. This is due to the low level of cohesion present in unstabilised blocks and is consistent with previous results (2014). Inclusion of 20% crumbed rubber into unstabilised

rammed earth specimens improved the resistance to water absorption and allowed the specimen to remain intact during saturation. As anticipated stabilised rammed earth specimens provided a higher resistance to water absorption than unstabilised samples, these samples are therefore likely to be more durable. Sample S, (stabilised with 6% cement) achieved a 20% lower water absorption than unstabilised sample UR20. The provision of crumb rubber in stabilised samples did not impede water absorption, however surface treatment with MICP dramatically reduced the water absorption of stabilised blocks, achieving a 24% lower water absorption than untreated samples.



**Figure 5-24** Water absorption for samples S, SM, SR20 and UR20

### 3.5 Synergistic and Antagonistic Effects

The synergistic and antagonistic effects of the REB treatment measures explored in this paper are summarised in Table 5-6. The addition of a chemical stabiliser, (such as cement) causes significant increases in both strength and erosion resistance, and some minor improvement to water resistance. In contrast, the use of MICP as a surface treatment will provide significant improvements to strength and water resistance, but have a lower impact on erosion resistance.

If a greater thermal resistance is required, crumb rubber can be added to the mix, however the addition of high proportions (> 5%) of crumb rubber will cause decreases in strength and erosion resistance. Using the results reported in this paper, the dosage of chemical stabiliser, MICP treatment and crumb rubber can be optimised according to the design requirements of the mix.

**Table 5-6** Summary of impact of REB treatment measures

REB treatment	Function	Strength	Water resistance	Thermal resistance	Erosion resistance
Stabiliser	Strength	↑	↑	-	↑
MICP	Water resistance	↑	↑	-	↑
Crumb rubber	Thermal resistance	↓	↓	↑	↑

#### 4. Conclusions

The investigations undertaken in this paper were aimed at improving the multifunctional performance properties of rammed earth blocks in areas of both structural performance, (strength and erosion) and functional performance (thermal comfort and moisture resistance). Cement stabilised and unstabilised rammed earth blocks were evaluated. A crumb rubber additive was used to improve the thermal resistance of rammed earth blocks while MICP surface treatment was used to improve resistance to water absorption and erosion.

A carbonate production, ureaolytic strain of bacteria *Sporosarcina pasteurii* (ATCC 11859), was used for the MICP surface treatment. Unstabilised and stabilised rammed earth blocks were prepared using a mix of 25 mm and 10 mm aggregates, manufactured sand and kaolin clay. 6% cement was added to the blocks for the purpose of cement stabilisation while 5%, 10% and 20% crumb rubber was added to decrease the thermal conductivity and improve insulation properties.

Compressive strength testing was conducted on the rammed earth blocks. In the case of cement stabilised samples the addition of crumb rubber resulted in a corresponding decrease in both strength and modulus. This is attributed to the crumb rubber influencing the bulk properties of the rammed earth block. In the case of unstabilised samples a small proportion (< 5%) of crumbed rubber could be incorporated into the mix without influencing the strength and stiffness of the block, however an increase above 10% resulted in a corresponding decrease in the overall compressive strength and stiffness.

MICP surface treatment was found to dramatically improve both the structural and functional properties of the rammed earth blocks. The overall compressive strength of both unstabilised and stabilised systems increased dramatically. The use of MICP surface treatment on stabilised rammed blocks increased the average compressive strength by 24% while the use of MICP surface treatment on unstabilised rammed earth blocks increased the compressive strength by 25%. This is attributed to the confinement affect, where biocementation, (due to MICP) occurred in the outer layers of the rammed earth blocks, creating a stiffer material surrounding a softer core and slowing the propagation of cracks and fractures through the block.

Accelerated erosion testing was undertaken on rammed earth blocks over a 60 minute time interval. Cement stabilised blocks exhibited minimal observable erosion over the recorded timeframe. In contrast, unstabilised rammed earth blocks eroded rapidly, with full penetration occurring at 8 minutes into the test. Both surface treatment with MICP and incorporation of 20% crumb rubber into the mix were found to impede the erosion of unstabilised samples. In the case of MICP surface treatment the erosion rate of the unstabilised blocks decreased by 62%. The incorporation of 20% crumb rubber decreased the erosion rate by 60%.

Stabilised and unstabilised rammed earth blocks were tested for resistance to water absorption. As expected, cement stabilisation decreased the water absorption of rammed earth blocks. Samples stabilised with 6% cement achieved a 20% lower water absorption than unstabilised samples. Surface treatment with MICP was found to further decrease the water absorption of stabilised blocks. MICP treated samples achieved a 24% lower water absorption than untreated samples.

Thermal conductivity testing was undertaken on stabilised rammed earth blocks to determine the impact of crumb rubber on insulation properties. In the case of samples with 20% crumb rubber the temperature in the blocks increased at a rate 20% slower than specimens without crumb rubber. As the inclusion of crumb rubber into a rammed earth mix can decrease the strength and stiffness of the blocks the mix must be optimised depending on the requirements of the design to provide both adequate thermal insulation and sufficient strength.

The surface treatment of rammed earth blocks with MICP is a worthwhile solution, not just to decrease erosion and water absorption, but also to sustainably increase the cementation of the specimen and reduce the requirement for stabilisation using chemical binders, (such as cement). MICP surface treatment may be used on its own or in conjunction with cement binders to augment the strength properties of a rammed earth material. It is known that manufacturing 1 kg of cement produces approximately 0.9 kg of carbon dioxide to the atmosphere, (Imbabi et al., 2012). MICP is a sustainable cementing approach which has minimal embodied energy, (CIF, 2013). The use of MICP for rammed earth applications could therefore significantly reduce the proportion of cement consumed within the building industry dramatically increasing the overall sustainability of building materials.

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**Statement of Authorship**

Title of Paper	Can engineered biocement be sustainable?
Publication Status	<input type="checkbox"/> Published <input type="checkbox"/> Accepted for Publication <input checked="" type="checkbox"/> Submitted for Publication <input type="checkbox"/> Unpublished and unsubmitted work written in manuscript style
Publication Details	<ul style="list-style-type: none"> <li>PORTER, H., DHAMI, N.K. &amp; MUKHERJEE, A. 2018. Can engineered biocement be sustainable?, <i>Currently submitted for publication</i></li> </ul>

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Contribution to the Paper:	Conducted literature review, analysis and wrote the manuscript.		
Certification:	This paper reports on original research I conducted during the period of my Higher Degree by Research candidature and is not subject to any obligations or contractual agreements with a third party that would constrain its inclusion in this thesis. I am the primary author of this paper.		
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By signing the Statement of Authorship, each author certifies that the candidate's stated contribution to the publication is accurate, (as detailed above) and permission is granted for the candidate in including the publication in the thesis.

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# Can Engineered Biocement be Sustainable?

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## Abstract

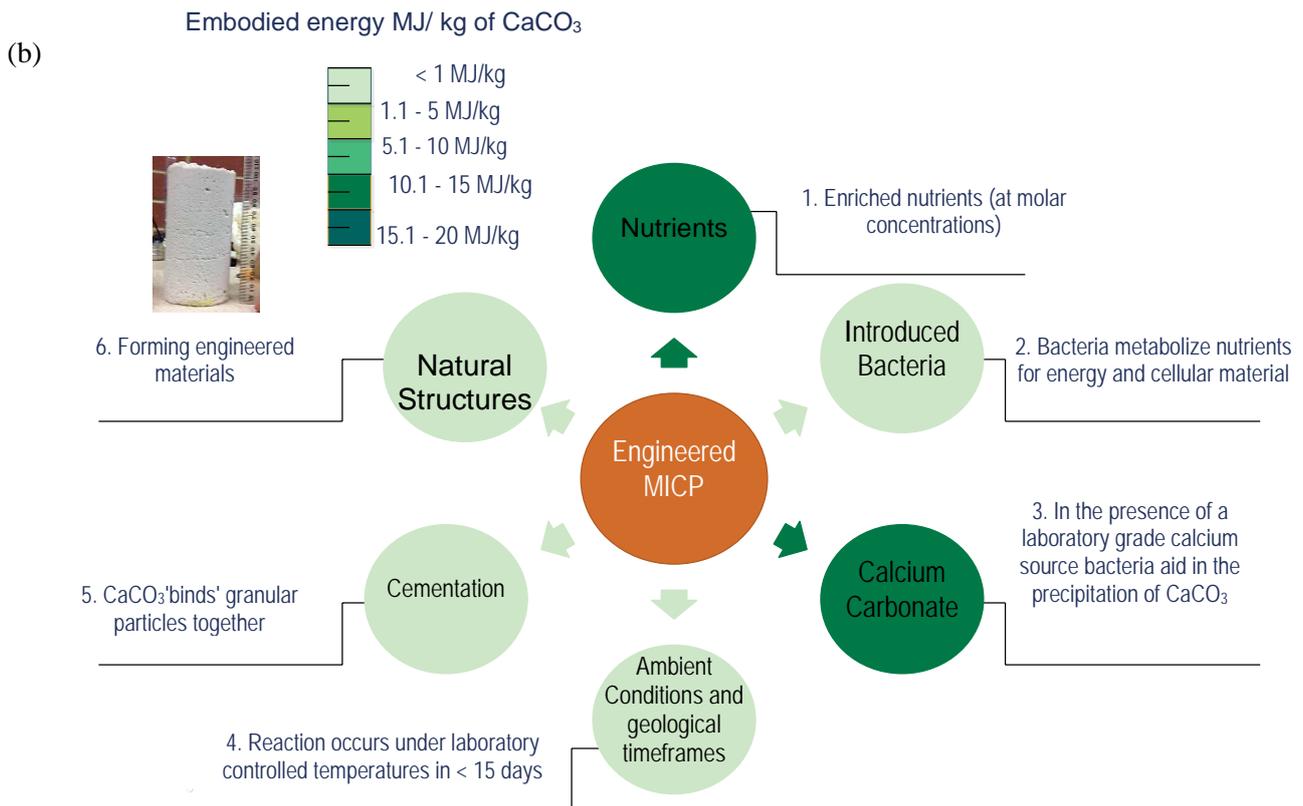
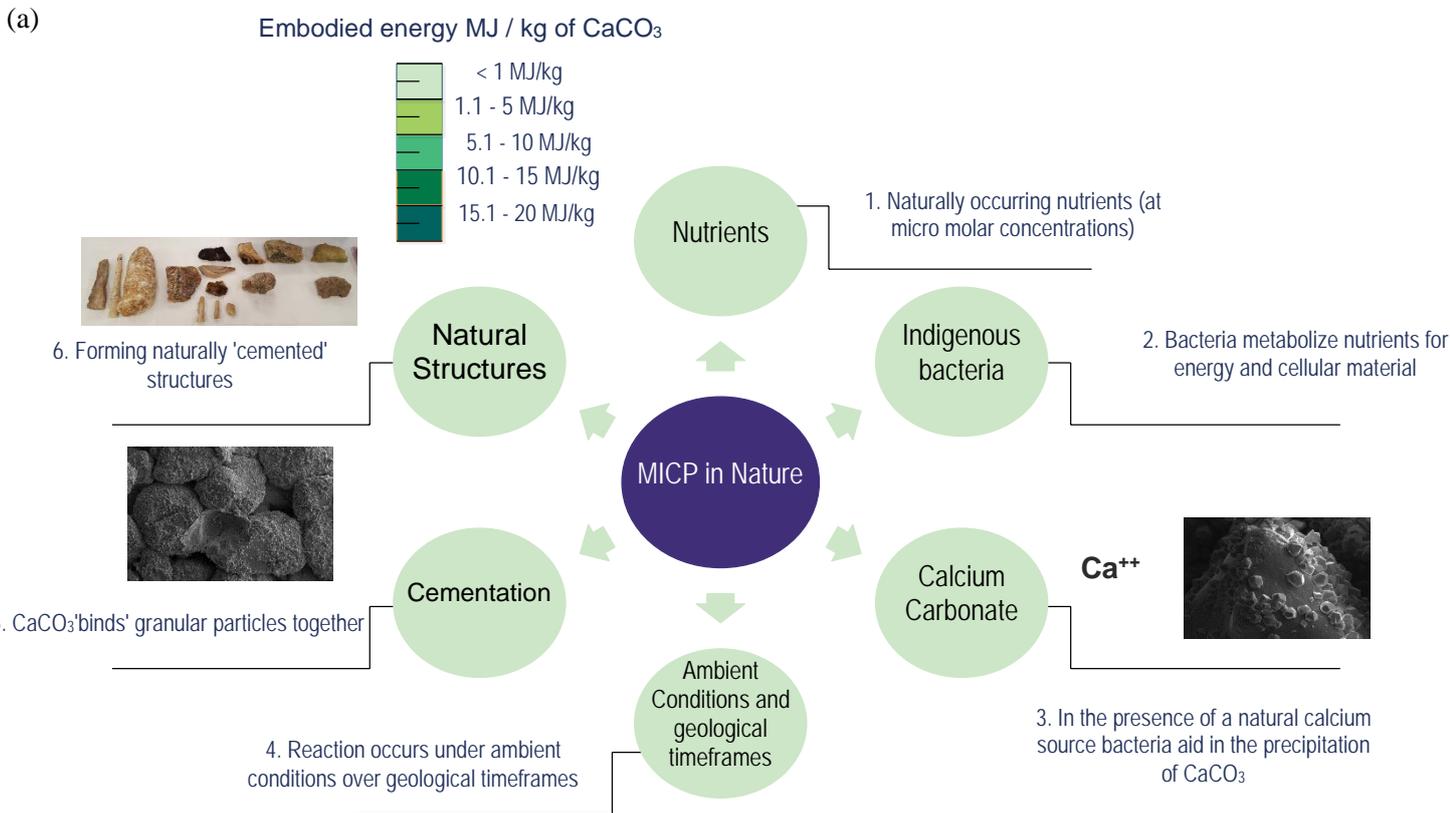
Microbially Induced Calcium Carbonate Precipitation (MICP) is a natural biocementation that takes place in corals, stromatolites and beach rocks. In recent years, researchers have explored emulation of this process as a sustainable alternative of engineered cements. Although the natural process is undoubtedly sustainable, its engineered emulation deviates substantially from the natural process. In this paper, we investigate the environmental and economic performance of the engineered biocementation process vis-à-vis present manufacturing of calcium carbonate. Our results show that different metabolic pathways of MICP have considerably varying environmental impact. We observe that nature performs MICP sustainably at ambient conditions and geological time scales utilizing naturally occurring sources of carbon and calcium at micromoles concentrations. Due to the mandate on duration of construction projects, highly purified reactants in a high concentration are used in the engineered process. This has a negative environmental impact. We conclude that the sustainability of engineered MICP is directly impacted by the metabolic pathway of bacteria as well as the purity of the input chemicals. A few biotic processes are superior to the present industrial process for manufacturing calcium carbonate if ingredients of laboratory grade purity are replaced by industrial grade products. A bigger dividend can be obtained by introducing industry by-products as nutrients. The results of this study help to direct future research for developing sustainable biocement for the construction industry.

## 1. Introduction

Microbially Induced Calcium Carbonate Precipitation (MICP) is a form of mineralisation that is responsible for major carbonate formations in nature such as corals, stromatolites and beach rocks, (Mitchell and Santamarina, 2005). Like industrial cement, the grains of sand can be bound together through MICP. Thus, MICP is biocementation that occurs at ambient conditions with no additional source of energy and with water as the solvent. The construction industry, on the other hand, is heavily reliant on ordinary Portland cement (OPC) that produces roughly the same amount of greenhouse gases as its own weight, (Imbabi et al., 2012). Worldwide, nearly 3.6 billion tonnes of OPC is produced, which accounts for approximately 6% of anthropological greenhouse gases. Researchers are exploring emulation of the natural cementation process as a means of achieving sustainability in construction. DeJong et al. (2013b) conclude that harnessing the biological processes that occur in natural formations is the next transformative practise for geotechnical engineering. Biocementation is envisaged to be sustainable due to several factors such as a low embodied energy, reversibility and recyclability and self-healing, (Mukherjee, 2010). However, there has been little attempt to objectively examine the sustainability of engineered biocementation. Biocementation has been happening in nature for millions of years and there is no doubt that the process is sustainable. However, it occurs over geological timeframes utilizing naturally available reactants often at micromoles concentrations, (Figure 6- 1 (a)). When emulating biocementation for construction applications, it becomes necessary to enrich both the bacteria and the nutrient media to allow the process to fit it into the speed, reliability and performance mandates of the construction industry (Van Paassen et al., 2010, Whiffin., 2007, Van Paassen, 2010, DeJong et al., 2014, Dhimi et al., 2016a) (Figure 6- 1 (b)). Investigators have typically used purified laboratory grade chemicals, at a much higher concentration than that occurs in nature, (Van Paassen et al., 2010, Whiffin., 2007, Van Paassen, 2010, DeJong et al., 2014, Dhimi et al., 2016a). The purification process of the chemicals is likely to consume considerable energy. Thus, it is important to evaluate the input media to ascertain the environmental impact of biocementation. Clearly, biocementation can be performed in a number of ways and a methodology to evaluate the processes to identify the best among several alternatives is essential. Lifecycle analysis has emerged as a great tool for evaluating sustainability (Guinée et al., 2011), particularly in the areas of sustainable housing technologies, building assessments (Buyle et al., 2013, Takano et al., 2014, Cabeza et al., 2014, Iribarren et al., 2015) and commonly used construction materials such as cement (Feiz et al., 2015, Vieira et al., 2016, Gäbel et al., 2004) However, there are few studies on industrial scale projects with biocement to conduct a full scale lifecycle analysis. An initial embodied energy analysis on biocement demonstrates that in the case of bio cementation by urea hydrolysis the manufacturing processes for urea and calcium chloride are the key contributors to embodied energy, while for ordinary Portland cement, the key contributor to energy usage is the burning of limestone during the calcination process, (Suer et al., 2009). The production of ordinary Portland cement is estimated to consume approximately 6.21 MJ/kg (Frischknecht R., 2005) of energy while urea consumes 30.54 MJ/kg (Nemecek, 2007) and calcium

chloride 11.76 MJ/kg (Hischier, 2004b). Field scale experiments demonstrate that 0.6 kg urea and 1.1 kg calcium chloride is required to produce 1 kg of calcium carbonate, for biocement through the ureolytic pathway (van Paassen, 2009, Van Paassen, 2010).

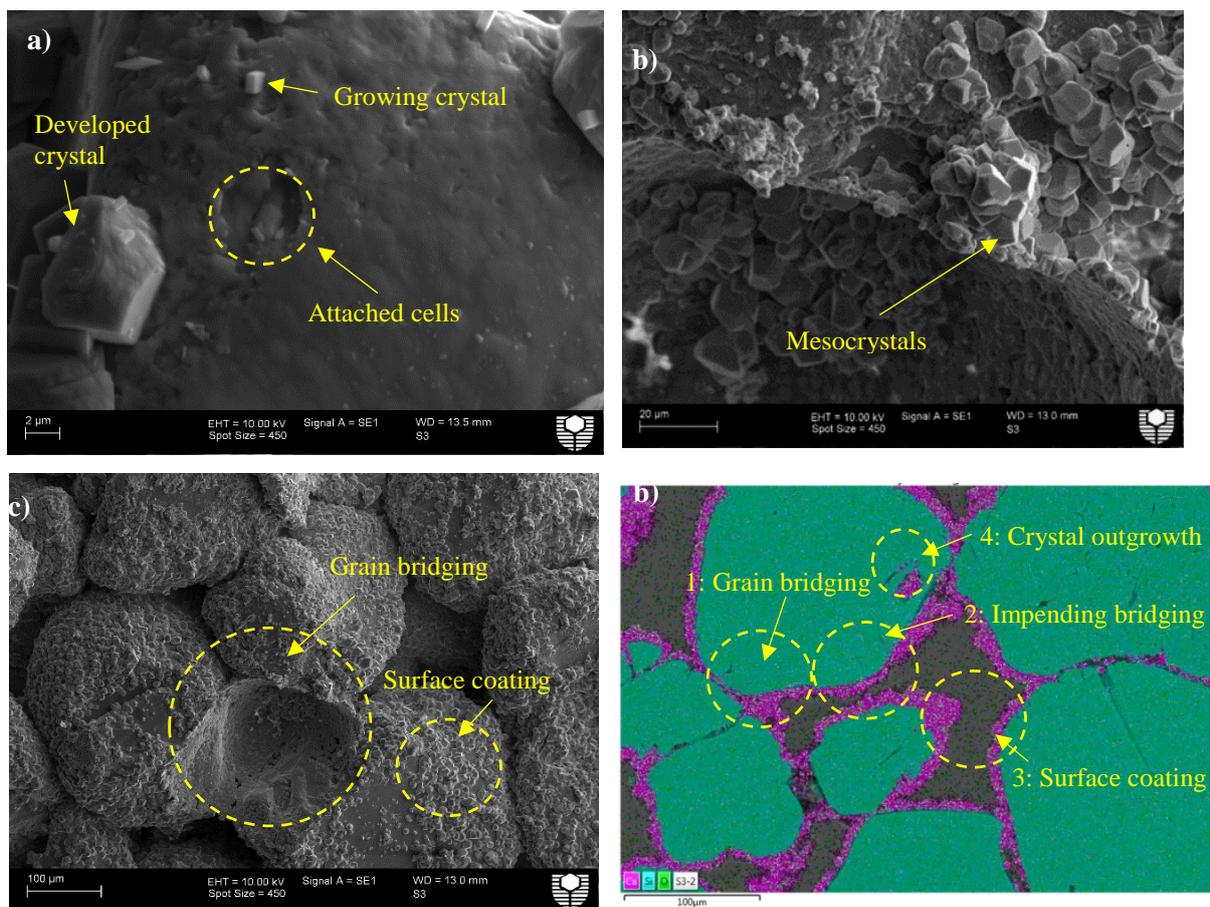
Early indications on the likely pathways to sustainable options based on already available extensive laboratory experiments would be valuable to chart the research directions towards sustainable biocement technologies. This paper employs the lifecycle analysis methodologies on the available data to identify the developments that are likely to have the deepest impact on sustainability of biocement technology.



**Figure 6- 1** Comparison of cementation techniques, (a) MICP in nature occurs utilizing indigenous bacteria and naturally occurring nutrients, at micromolar concentrations. The bacteria metabolize nutrients for energy and cellular material, and in the presence of a natural calcium source, aide in the precipitation of calcium carbonate. This reaction occurs at ambient temperatures over long timeframes. The precipitated calcium carbonate. 'cements' granular particles together resulting in the formation of naturally 'cemented structures'. When applied to construction scenarios (b) both the bacteria and cells are enriched to reduce the timeframe for cementation to suite construction applications, (< 15 days).

## 1.1 Process of Biocementation

In biocementation, the bacteria nucleate the conversion of a water soluble source of calcium such as calcium chloride into an insoluble one such as calcium carbonate. The bacterial cells secure themselves in the grooves of the substrate and act as the nucleation site for the growth of the calcium carbonate crystals (Figure 6- 2 a). Thus, the crystals grip the grains at several places and coat it, and gradually grow into mesocrystals (Figure 6- 2 b). When the mesocrystals from different sand grains join together they get cemented together (Figure 6- 2 c). Through quantitative scans of the substrate the extent of cementing of the grains can be established (Figure 6- 2 d).



**Figure 6- 2** Mechanism of biocementation. a) Bacterial cells secure themselves within the grooves of sand grains and forms the nucleation sites for the calcium carbonate crystals. b) The crystals grow to form mesocrystals. c) The crystals coat the surface and continue to grow. When the crystals bridge the neighbouring sand grains, cementations is formed. d) Quantitative scans reveal various stages of biocementation, (Porter et al., 2017b).

During MICP, naturally occurring microbes act as a biocatalyst for the precipitation of calcium carbonate,(Dejong et al., 2013b) (Figure 6- 2). MICP can occur via autotrophic or heterotrophic pathways (Table 6- 1) although presently, MICP via urea hydrolysis is the most investigated route for construction applications, due to its simplicity, (DeJong, 2010). In the case of heterotrophes, mineralization takes place as a by-product of the metabolic activity of the bacteria. In these cases,

heterotrophic bacteria utilize organic compounds, such as urea, for energy and cellular material, (Dhami et al., 2013d, DeJong, 2010). The metabolic activity of the bacteria causes a rise in the pH of the surrounding pore water, resulting in supersaturated conditions, and allowing for the precipitation of carbonates, when in the presence of an inorganic calcium source. In the cases of autotrophs, bacteria obtain energy from the sun, and reduce the atmospheric carbon, (in the form of carbon dioxide), for energy and cellular material, (Mitchell and Santamarina, 2005). Heterotrophic pathways include urea hydrolysis, denitrification, ammonification and methane reduction, while autotrophic pathways include photosynthesis and MICP through carbonic anhydrase producing bacteria. In both heterotrophic and autotrophic pathways the rate of MICP is controlled by the metabolic activity of the bacteria, the availability of calcium, the dissolved inorganic carbon source, and the pH of the surrounding environment, (DeJong et al., 2013b, Dhami et al., 2016a). Due to differing levels of nutrients and waste products produced during the reaction, natural pathways to MICP are likely to have varying impacts on the environment. Their efficacy in terms of sustainability is an important question to answer, particularly when developing MICP as a construction technique for industrial applications.

**Table 6- 1** Microbial pathways to precipitation of calcium carbonate

Metabolic Type	Pathway	Bacteria Type	Chemical Reactions
Heterotrophic	Urea hydrolysis (Van Paassen, 2010, van Paassen, 2009, van Paassen, 2011, DeJong, 2010, DeJong et al., 2013b)	Ureolytic bacteria ( <i>Bacillus pasteurii</i> )	a) $Urea + H_2O \rightarrow 2NH_3 + CO_2$ b) $NH_3 + H_2O \rightarrow NH_4^+ + OH^-$ c) $OH^- + CO_2 \rightarrow HCO_3^-$ d) $Ca^{2+} + HCO_3^- \rightarrow CaCO_3 + H_2O$
	Denitrification (Hamdan et al., 2017, Van Paassen et al., 2010)	Denitrifying bacteria ( <i>Pseudomonas denitrificans</i> )	a) $NO_3^- + 1.25 CH_2O \rightarrow 0.5N_2 + 1.25CO_2 + 0.75H_2O + OH^-$ b) $Ca^{2+} + CO_2 + 2OH^- \rightarrow CaCO_3 + H_2O$
	Ammonification (Rodriguez-Navarro et al., 2003, González-Muñoz et al., 2010, Chekroun et al., 2004)	Myxobacteria ( <i>Myxococcus xanthus</i> )	a) $Amino\ acid + O_2 \rightarrow NH_3 + CO_2 + H_2O$ b) $NH_3 + H_2O \rightarrow NH_4^+ + OH^-$ c) $OH^- + CO_2 \rightarrow HCO_3^-$ d) $Ca^{2+} + HCO_3^- \rightarrow CaCO_3 + H_2O$
	Methane oxidation (Ganendra et al., 2014, Whittenbury et al., 1970)	Methanogens ( <i>Methylocystis parvus</i> )	a) $Methane + SO_4^{2-} \rightarrow HS^- + HCO_3^- + H_2O$ b) $Ca^{2+} + HCO_3^- \rightarrow CaCO_3 + H_2O$ c) $H^+ + HS^- \rightarrow H_2S$
Autotrophic	Carbonic Anhydrase (Kaur et al., 2016, Dhami et al., 2016b)	( <i>Bacillus Megaterium</i> )	$Ca^{2+} + 2HCO_3^- \rightarrow CaCO_3 + HCO_3^- + H^+ \rightarrow CaCO_3 + CO_2 + H_2O$
	Photosynthesis (Zhu and Dittrich, 2016, Dittrich et al., 2003)	Cyanobacteria ( <i>Synechococcus</i> )	$CO_2 + H_2O + 2HCO_3 + Ca^{2+} \rightarrow CH_2O + CaCO_3 + O_2 + H_2O$

## 1.2 Cost of Biocementation

The economic cost of biocement is an important factor. De Muynck et al. (2010) conducted an economic assessment of biocement as a surface treatment for building materials. The urea/calcium chloride cementation media was found to be the highest contributor to costs, responsible for approximately 47% of the overall cost of the treatment. The cost of application and added value of the product accounted for 41% of the overall cost while the bacterial growth media accounted for approximately 12% of the total cost. The overall cost of biocement as a surface treatment was 23 – 28 €/sqm, (34 – 42 AUD).

One way of controlling the cost and embodied energy of biocement is to use unprocessed natural materials or industry by-products. Previous studies have demonstrated the potential of corn steep liquor or lactose mother liquor as an alternative, growth medium for the ureolytic bacterial strain *Sporosarcina pastuerii*, (Achal et al., 2010, Achal et al., 2009). Studies have also demonstrated the potential of using acetic acid and limestone as an alternative calcium source for ureolytic bacteria, (Choi et al., 2017). However, acetic acid typically has a higher environmental carbon footprint, (1.556 kg CO<sub>2</sub>/kg) and embodied energy (53.35 MJ / kg) when compared to the laboratory grade calcium chloride, (0.854 kg CO<sub>2</sub>/kg and 10.96 MJ/kg) making this option unlikely to have a lower overall environmental footprint than the traditional alternatives, (Hischier, 2004a, Althaus, 2007). Eggshells as an alternative calcium source has also been explored, (Choi et al., 2017, Choi et al., 2016). Whilst the study demonstrated that laboratory grade calcium chloride can be replaced with eggshells, the overall sustainability of this method cannot be assessed as the resources required to collect, and reuse eggshell waste is unknown.

## 1.3 Methods

From the above discussion it is clear that a comprehensive assessment of alternative processes is essential to evaluate the sustainability of biocementation and direct future research in the area. However, a full lifecycle analysis is infeasible due to lack of industrial scale data. This paper applies the lifecycle analysis methodologies on the available experimental results to compare the environmental and economic costs of different routes to biocementation to facilitate an informed future research with a view to sustainability. An assessment has been conducted on calcium carbonate precipitated through different MICP metabolic pathways as compared to an equal mass of manufactured calcium carbonate.

The functional unit (FU) for the assessment was defined as 1 kg of precipitated calcium carbonate. The scope of the assessment for microbially precipitated calcium carbonate included the extraction and processing of raw materials, as well as the environmental impact of any by-products produced during the MICP (Table 1). It may be remembered that the scale of allocation of MICP is relatively small. Therefore, certain factors could not be estimated. Transportation of raw materials to the plant, and the energy required to operate the fermenter were excluded from the analysis. The analysis was conducted

using, SimaPro 8.0 software,. Ecoinvent V2.2 database, (Frischknecht R., 2005), was used for material inputs, with adaptations to an Australasian context where available. The carbon footprint and eutrophication potential were calculated using AUSLCI Version 3.0 while the embodied energy for each scenario was calculated using the Cumulative Energy Demand 2.01 methodology. This is a high – level assessment for the purpose of comparing major impacts generated by different metabolic pathways, based on laboratory scale data for the purpose of directing future research. A full life cycle analysis, including other impact factors, (such as water and land use, mineral and fossil fuel depletion etc.) is outside of the scope of this study and would be conducted at a later date based on more detailed, field scale data.

The following assumptions have been made during the preparation of this analysis:

- All the reaction efficiencies are 100% and all of the provided calcium source is converted in to calcium carbonate.
- The effect of the metabolic rate of different pathways of MICP has not been considered.
- Waste products generated by the MICP process have been included in the analysis. However, treatment and recycling of these products, is not considered.
- Production of laboratory grade calcium carbonate through the carbonation process were based on the cradle-to-gate assessment conducted by Mattila et al. (2014)
- Costs (AUD) for laboratory grade materials were based on published rates from suppliers(Chemsupply, 2017, MERCK, 2017, Southern-Biological, 2017), whilst costs for commercial grade chemicals, (in bulk) were based on data published by ICIS, (ICIS, 2017).

Supplementary data to this paper has also been submitted for publication and is included in Appendix A of this thesis.

## 2. Results

The overall environmental impact of calcium carbonate produced through MICP using laboratory grade chemicals is shown in Table 6- 2. The environmental impacts of 1 kg of calcium carbonate produced using carbonic anhydrase producing bacteria, methanogens, cyanobacteria, denitrifying bacteria, ureolytic bacteria and myxobacteria is compared to the environmental impact of 1 kg of calcium carbonate produced using the traditional carbonation process.

With the exception of MICP through denitrification, calcium carbonate produced through MICP has a lower carbon footprint than an equal quantity of calcium carbonate produced through the traditional

carbonation process. This is due to the fact that MICP occurs under ambient conditions, and does not have the energy requirements of the calcination process. Autotrophic pathways to MICP, (carbonic anhydrase bacteria and photosynthesis) utilized carbon dioxide from the atmosphere, reducing the overall carbon footprint by 0.44 kg / kg CaCO<sub>3</sub>. Calcium carbonate produced using the carbonic anhydrase enzyme had the lowest carbon footprint, (0.681 kg CO<sub>2</sub> / kg CaCO<sub>3</sub>), 70% lower than the carbon footprint of the traditional carbonation process. A breakdown of the key components that contribute to the carbon footprint for each metabolic pathway is shown in Figure 6- 3.

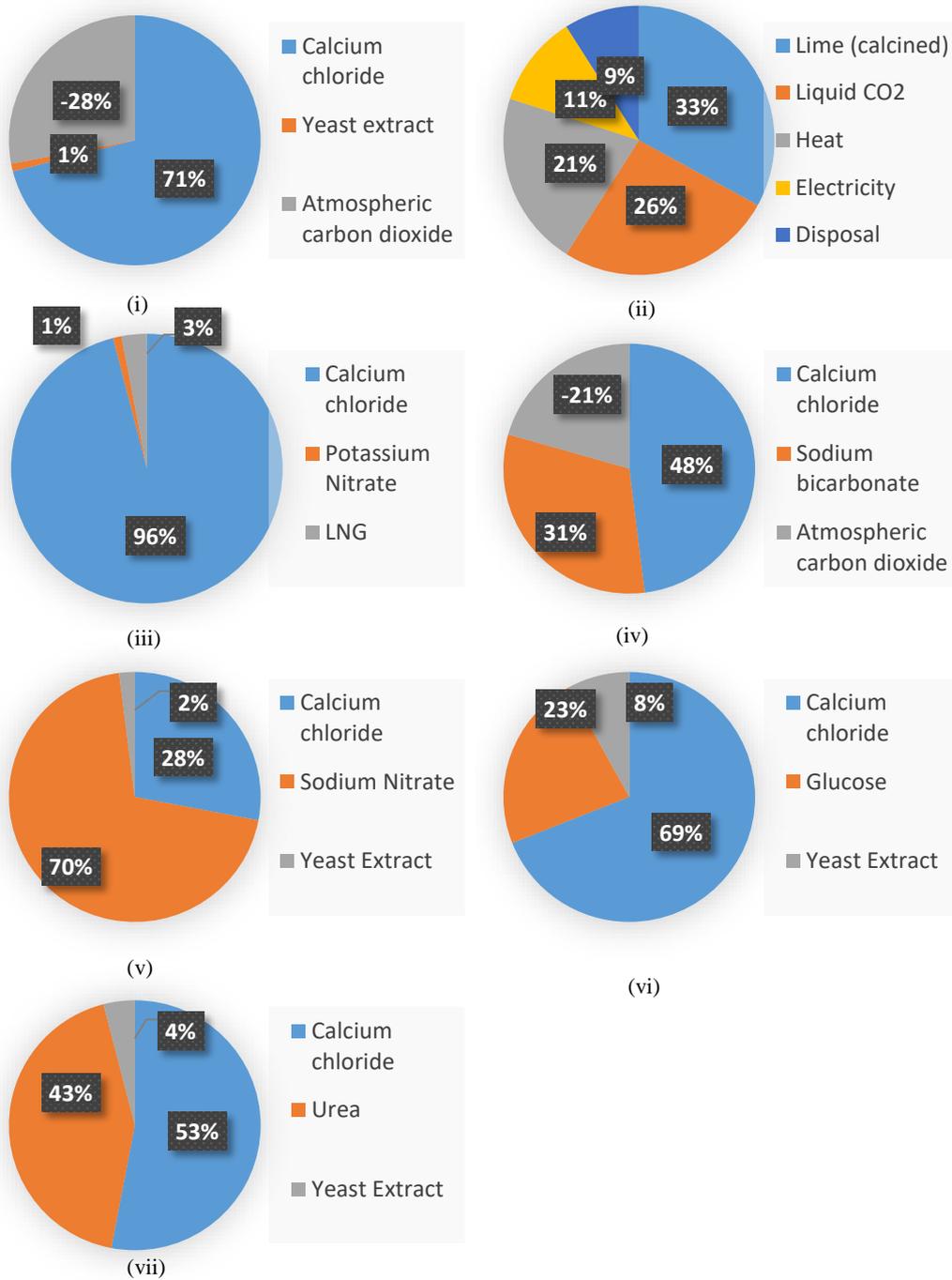
The eutrophication potential for the production of 1 kg of CaCO<sub>3</sub> using laboratory grade chemicals is also shown in Table 6- 2. MICP via ureolytic bacteria has the highest eutrophication potential, (0.24 kg SO<sub>4</sub> / kg CaCO<sub>3</sub>), followed by myxobacteria, (0.065 kg SO<sub>4</sub> /kg CaCO<sub>3</sub>) and denitrifying bacteria, (0.02 kg SO<sub>4</sub> / kg CaCO<sub>3</sub>). All three scenarios have a higher eutrophication potential than the production of 1 kg of CaCO<sub>3</sub> through the carbonation process. In the case of urea hydrolysis and denitrification the eutrophication potential can be attributed to the ammonium gas produced during the reaction, (Figure 6- 4). In the case of denitrifying bacteria the eutrophication potential is largely due to the nitrogen gas produced during the MICP, (Figure 6- 4). The eutrophication potential of all other MICP pathways is minimal. These results demonstrate that the gaseous by-products of MICP undertaken by the more commonly investigated routes (ureolytic bacteria, denitrification and ammonification), have the potential to cause significant environmental damage. If MICP is to become a truly sustainable construction methodology consideration must be given into treating or reusing the by-products of MICP in a way that does not significantly increase other environmental factors such as carbon footprint, embodied energy etc. One example of the reuse of ammonia produced by the ureolytic pathway would be as an ammonia fertilizer product for plants, (Dejong et al., 2013b).

MICP undertaken using laboratory grade chemicals has a significantly higher embodied energy than the traditional carbonation process, (Table 6- 2). MICP via ureolytic bacteria had the highest embodied energy, (28.4 MJ) whilst MICP using carbonic anhydrase producing bacteria had the lowest embodied energy (12.9 MJ). The embodied energy requirements for MICP pathways is predominantly due to the laboratory grade calcium chloride, which contributes between 44 and 98% of the total embodied energy, (Figure 6- 5).

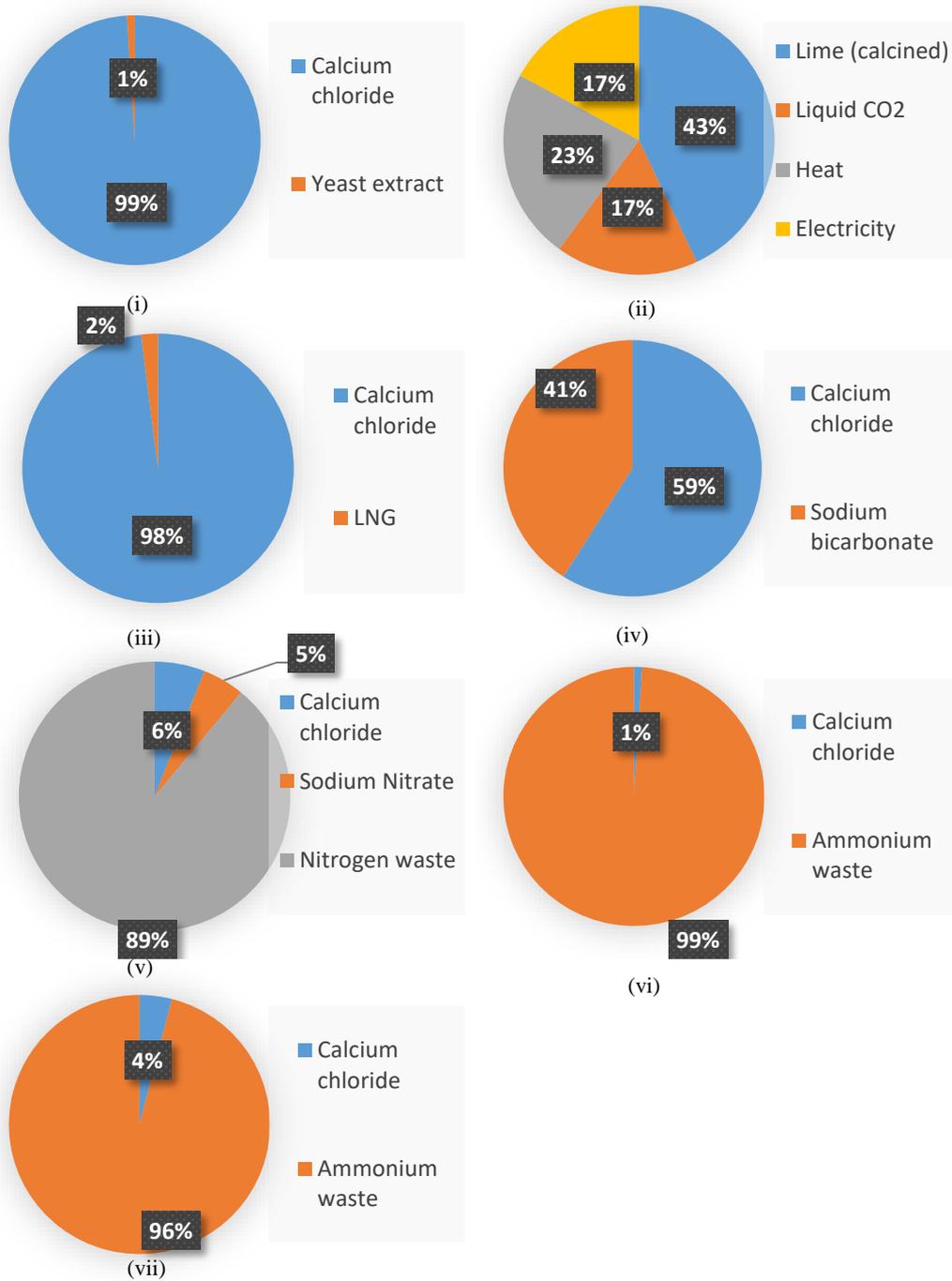
The unit costs for MICP using laboratory grade chemicals generally exceed that of calcium carbonate produced through the carbonation process. The key contributors to the overall costs are shown in Figure 6- 6. The provision of calcium chloride is a key cost across all MICP routes, other significant costs include the cost of urea (for urea hydrolysis) and sodium nitrate, (for denitrification). The most expensive route was MICP via ammonification or methane oxidation, (\$138 /kg CaCO<sub>3</sub>), while the most economic route was MICP via cyanobacteria, (\$62/kg CaCO<sub>3</sub>).

**Table 6- 2** Carbon footprint (CO<sub>2</sub>/FU), eutrophication potential, (SO<sub>4</sub>/FU), embodied energy (MJ), cost, (AUD) and overall environmental impact rating for the production of 1 kg of CaCO<sub>3</sub> through (i) carbonic anhydrase producing bacteria, (ii) the traditional carbonation process, (iii) methanogens, (iv) cyanobacteria, (v) denitrifying bacteria, (vi) ureolytic bacteria and (vii) myxobacteria using laboratory grade inputs.

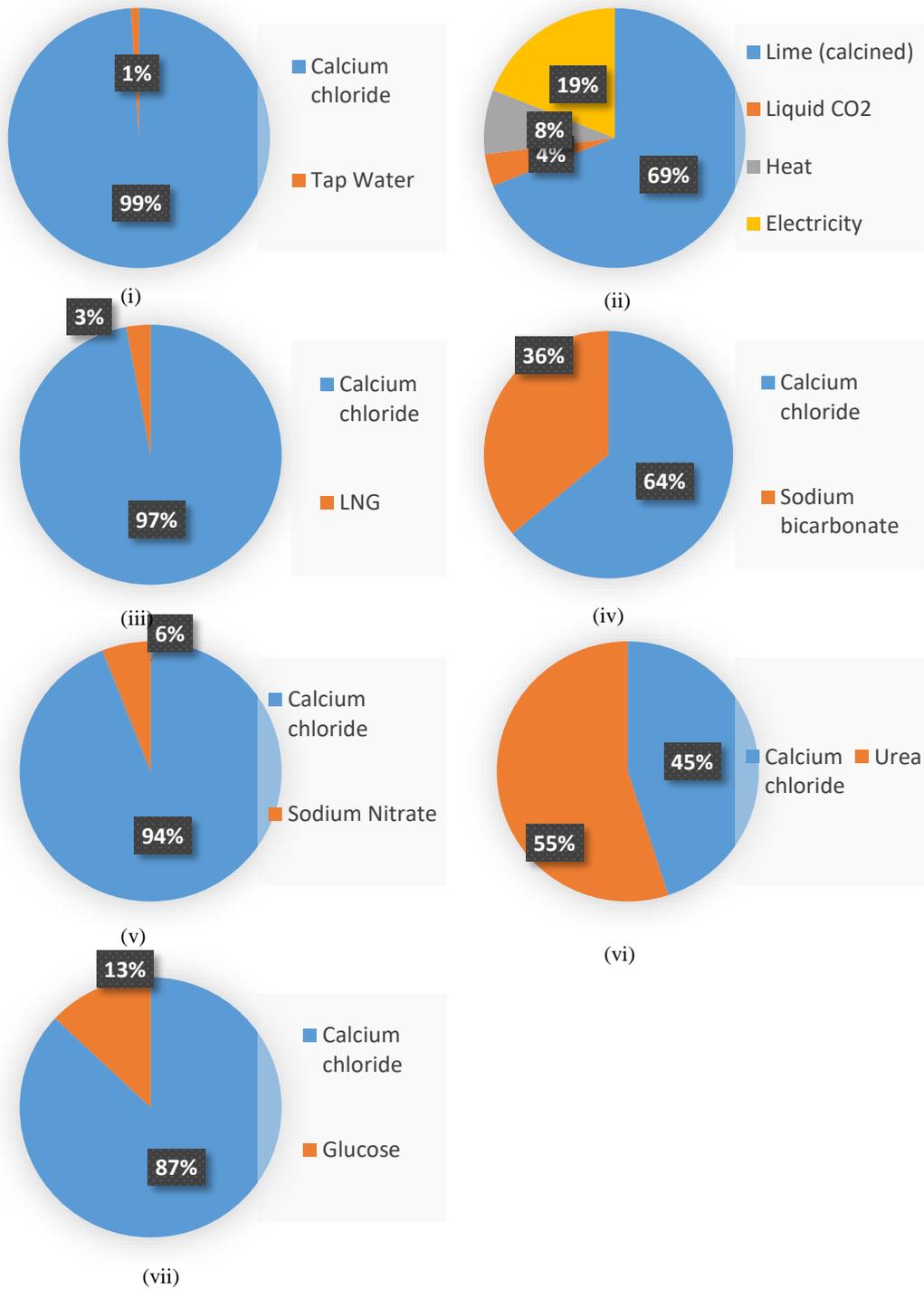
<b>Pathway</b>	<b>Carbon Footprint (CO<sub>2</sub> / FU)</b>	<b>Eutrophication Potential (SO<sub>4</sub> / FU)</b>	<b>Embodied Energy (MJ)</b>	<b>Cost (AUD / FU)</b>	<b>Overall Ranking</b>
(i) Carbonic anhydrase producing bacteria	0.671	1.77E-3	12.9	62	<b>1</b>
(ii) Carbonate	2.37	7.5E-4	7.2	61	<b>2</b>
(iii) Methanogens	1.15	1.79E-3	13.3	71	<b>3</b>
(iv) Cyanobacteria	1.38	2.97E-3	20.1	99	<b>4</b>
(v) Denitrifying bacteria	3.91	2.67E-2	13.6	102	<b>5</b>
(vi) Ureolytic bacteria	2.06	2.4E-1	28.4	107	<b>6</b>
(vii) Myxobacteria	2.34	6.5E-2	23.4	138	<b>7</b>



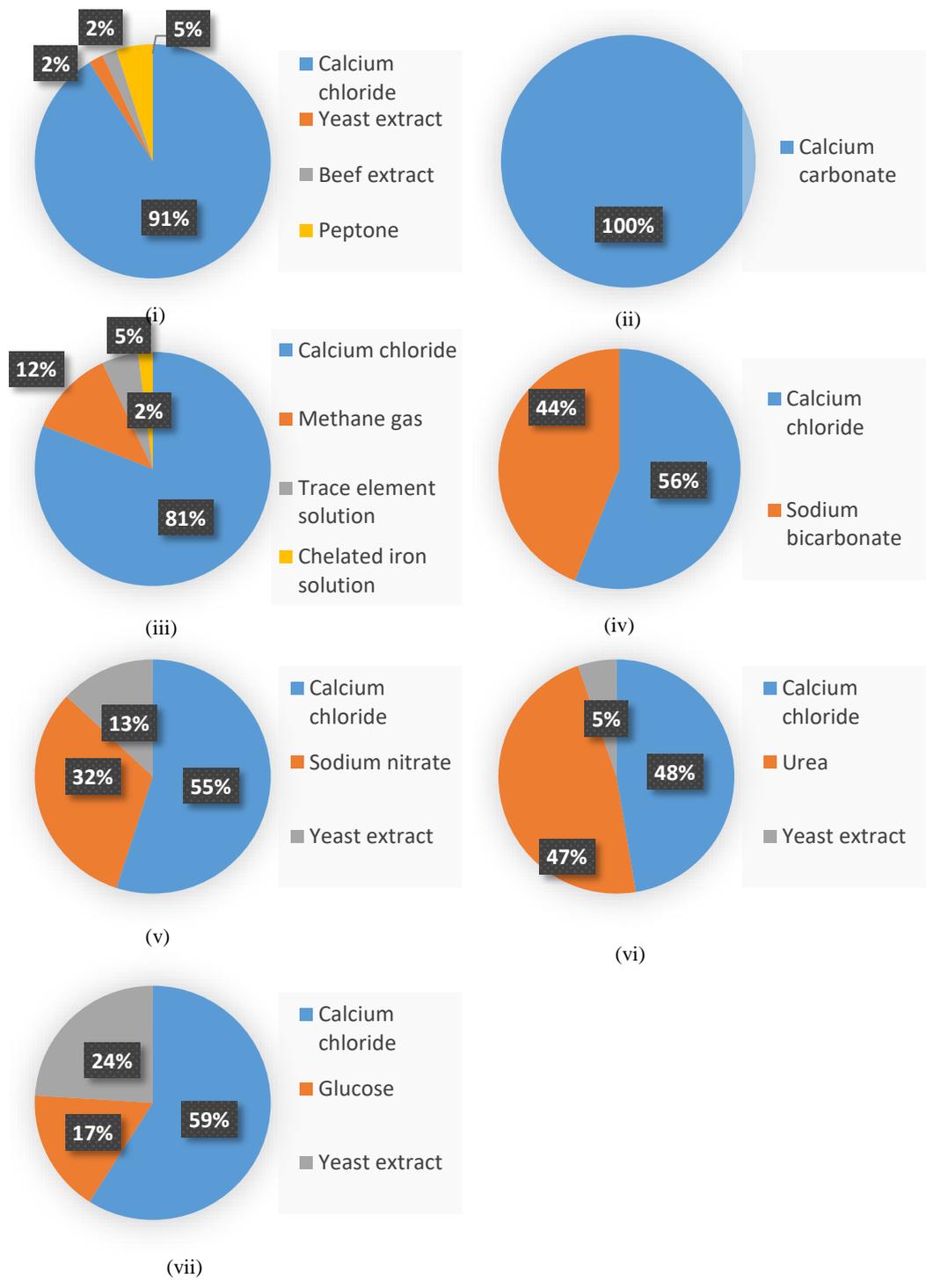
**Figure 6- 3** Breakdown of carbon footprint (CO<sub>2</sub>) for the production of 1 kg of CaCO<sub>3</sub> through (i) carbonic anhydrase producing bacteria, (ii) the traditional carbonation process, (iii) methanogens, (iv) cyanobacteria, (v) denitrifying bacteria, (vi) ureolytic bacteria and (vii) myxobacteria using laboratory grade inputs



**Figure 6- 4** Breakdown of eutrophication potential ( $SO_4$ ) for the production of 1 kg of  $CaCO_3$  through (i) carbonic anhydrase producing bacteria, (ii) the traditional carbonation process, (iii) methanogens, (iv) cyanobacteria, (v) denitrifying bacteria, (vi) ureolytic bacteria and (vii) myxobacteria using laboratory grade inputs



**Figure 6- 5** Breakdown of embodied energy (MJ) for the production of 1 kg of CaCO<sub>3</sub> through (i) carbonic anhydrase producing bacteria, (ii) the traditional carbonation process, (iii) methanogens, (iv) cyanobacteria, (v) denitrifying bacteria, (vi) ureolytic bacteria and (vii) myxobacteria using laboratory grade inputs



**Figure 6- 6** Breakdown of cost (AUD) for the production of 1 kg of CaCO<sub>3</sub> through (i) carbonic anhydrase producing bacteria, (ii) the traditional carbonation process, (iii) methanogens, (iv) cyanobacteria, (v) denitrifying bacteria, (vi) ureolytic bacteria and (vii) myxobacteria using laboratory grade inputs

The overall environmental impact of calcium carbonate produced through MICP using commercial grade chemicals is shown in Table 6- 3. The environmental impacts of 1 kg of calcium carbonate produced using carbonic anhydrase producing bacteria, methanogens, cyanobacteria, denitrifying bacteria, ureolytic bacteria and myxobacteria is compared to the environmental impact of 1 kg of calcium carbonate produced using the traditional carbonation process.

For the laboratory grade scenarios it was assumed that the calcium source ( $\text{CaCl}_2$ ) was produced using the traditional Solvay process (Hischier, 2004a). However, for the commercial grade scenarios it was assumed that the calcium source was less pure, and calcium chloride produced through the hypochlorination of allyl chloride (Hischier, 2005) was adopted for the analysis. In the case of denitrification, sodium nitrate was also replaced with a nitrate fertilizer (Nemecek, 2007).

The substitution of a commercial grade calcium chloride dramatically decreased the carbon footprint across all MICP routes. A decrease of between 18% and 49.62% was recorded across all MICP pathways. Key contributors to the carbon footprint are also shown for each metabolic pathway in Figure 6- 7. A key component for all processes is the provided calcium chloride. Clearly future research should investigate alternatives to laboratory grade calcium sources to further enhance the sustainability of carbonates produced through MICP. In the case of MICP via urea hydrolysis, or denitrification the provided organic carbon source (urea, or nitrate) results in a greater contribution to the carbon footprint than the provided calcium chloride. In the case of MICP via cyanobacteria or myxobacteria, the provided bicarbonate or glucose also results in a significant contribution to the overall carbon footprint.

A significant decrease in embodied energy, (between 43 and 95%) was achieved in all pathways, with the exception of denitrification. This decrease is largely attributed to the replacement of the laboratory grade calcium chloride with a commercial grade product. MICP using carbonic anhydrase bacteria required the least embodied energy, (0.619 MJ), 91% less than calcium carbonate produced through the traditional carbonation process. Key contributors to the eutrophication potential for each metabolic pathway are shown in Figure 6- 8.

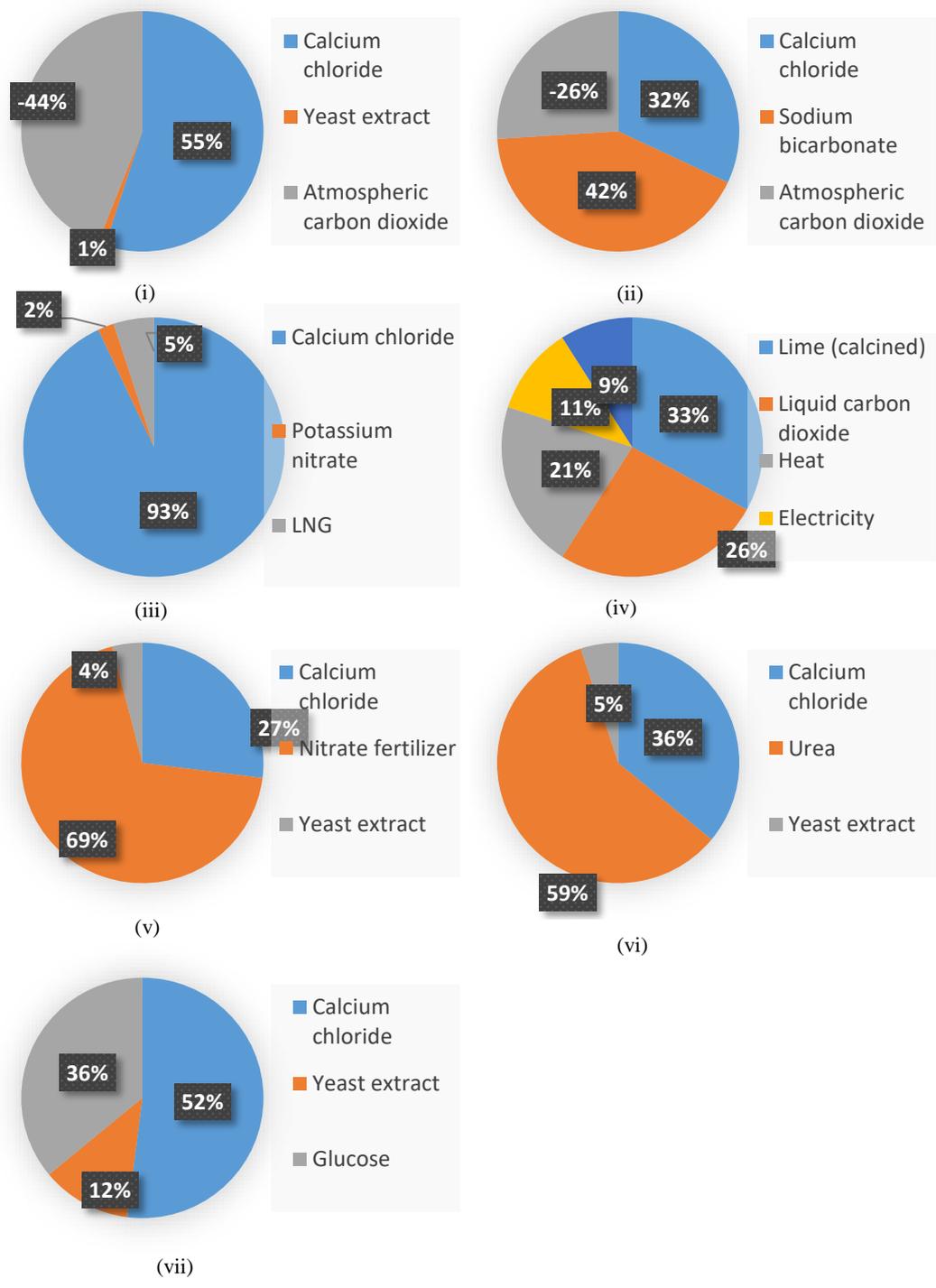
While the cost for MICP produced carbonates is still higher than calcium carbonate produced through the traditional carbonation process, (\$0.4 / kg  $\text{CaCO}_3$ ) it may be recalled that the MICP processes do not enjoy the economies of scale that the abiotic process has. However, the use of commercial grade inputs does increase the viability of carbonates produced using the MICP process, bringing the costs into the same range as calcium carbonate produced through carbonation. Key contributors to the cost for each metabolic pathway are shown in Figure 6- 9.

The metabolic pathways to MICP were ranked according to the overall environmental impact. When using laboratory grade input materials, MICP through carbonic anhydrase producing bacteria,

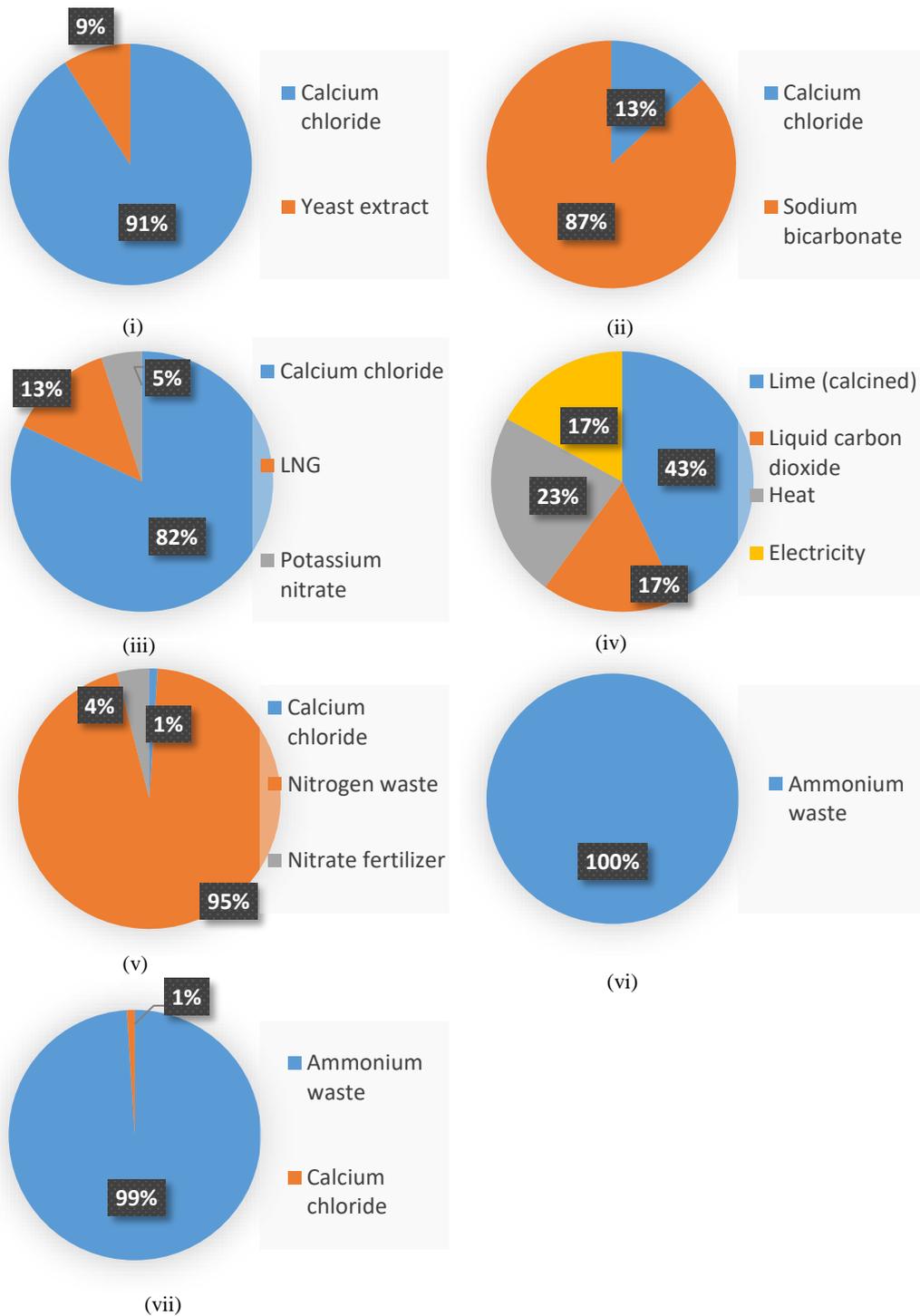
cyanobacteria or methanogens all had a lower overall environmental impact when compared to the production of an equal amount of calcium carbonate using the traditional carbonation process. When commercial grade materials were used for MICP, the overall environmental impact and cost for carbonates produced during the MICP process decreased remarkably. To date, the majority of MICP investigations have utilised laboratory grade products and as such, MICP may not be techno-economically competitive when compared to other construction materials such as Portland cement. Going forward, research in the area of MICP must prioritize low cost commercial alternatives, or waste products as the calcium and organic carbon source for the MICP reaction.

**Table 6- 3** Carbon footprint (CO<sub>2</sub>/FU), eutrophication potential, (SO<sub>4</sub>/FU), embodied energy (MJ), cost, (AUD) and overall environmental impact rating for the production of 1 kg of CaCO<sub>3</sub> through (i) carbonic anhydrase producing bacteria, (ii) the traditional carbonation process, (iii) methanogens, (iv) cyanobacteria, (v) denitrifying bacteria, (vi) ureolytic bacteria and (vii) myxobacteria using commercial grade inputs.

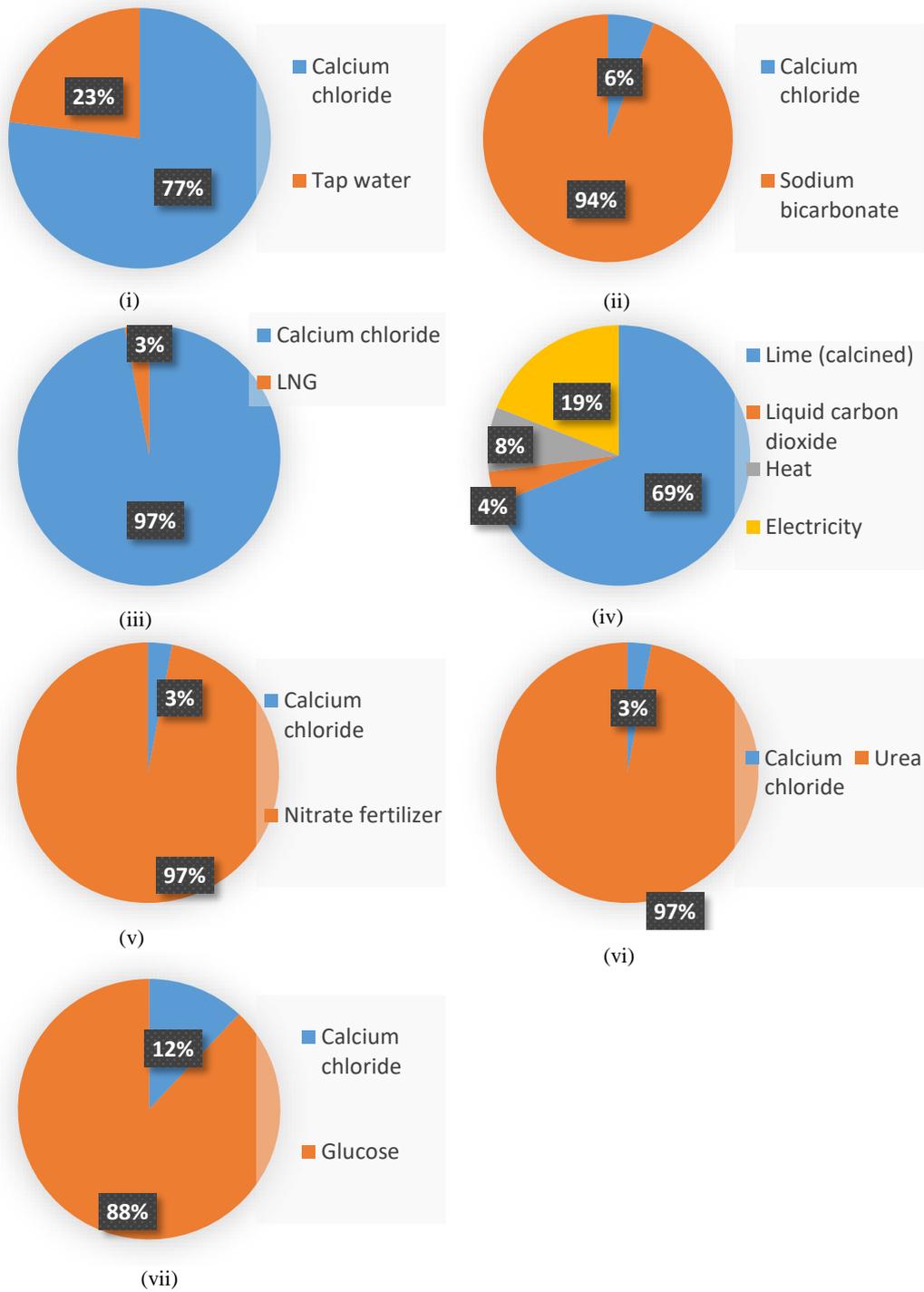
Pathway	Carbon Footprint (CO <sub>2</sub> / FU)	Eutrophication Potential (SO <sub>4</sub> / FU)	Embodied Energy (MJ)	Cost (AUD / FU)	Overall Ranking
(i) Carbonic anhydrase producing bacteria	0.555	2.09E-4	0.619	3.6	1
(ii) Cyanobacteria	0.815	1.51E-4	7.86	2.7	2
(iii) Methanogens	0.589	2.28E-4	1.01	12.9	3
(iv) Carbonation	2.37	7.35E-4	7.2	0.4	4
(v) Denitrifying bacteria	1.97	1.32E-3	13.6	7.2	5
(vi) Ureolytic bacteria	1.51	2.4E-1	16.1	3.1	6
(vii) Myxobacteria	1.54	6.27E-2	5.76	11.2	7



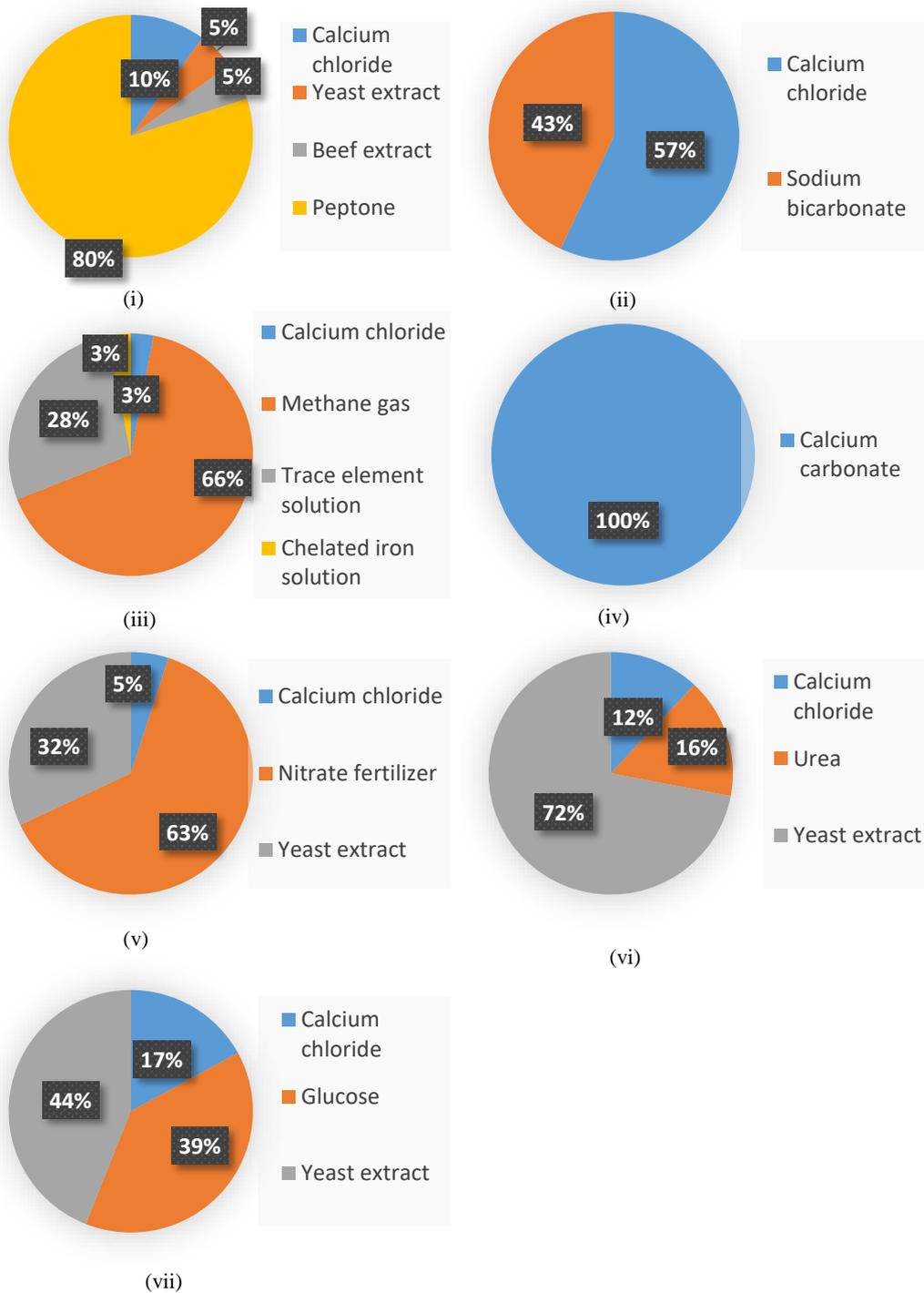
**Figure 6- 7** Breakdown of carbon footprint (CO<sub>2</sub>) for the production of 1 kg of CaCO<sub>3</sub> through (i) carbonic anhydrase producing bacteria, (ii) the traditional carbonation process, (iii) methanogens, (iv) cyanobacteria, (v) denitrifying bacteria, (vi) ureolytic bacteria and (vii) myxobacteria using commercial grade inputs



**Figure 6- 8** Breakdown of eutrophication potential (SO<sub>4</sub>) for the production of 1 kg of CaCO<sub>3</sub> through (i) carbonic anhydrase producing bacteria, (ii) the traditional carbonation process, (iii) methanogens, (iv) cyanobacteria, (v) denitrifying bacteria, (vi) ureolytic bacteria and (vii) myxobacteria using commercial grade inputs



**Figure 6- 9** Breakdown of embodied energy (MJ) for the production of 1 kg of CaCO<sub>3</sub> through (i) carbonic anhydrase producing bacteria, (ii) the traditional carbonation process, (iii) methanogens, (iv) cyanobacteria, (v) denitrifying bacteria, (vi) ureolytic bacteria and (vii) myxobacteria using commercial grade inputs

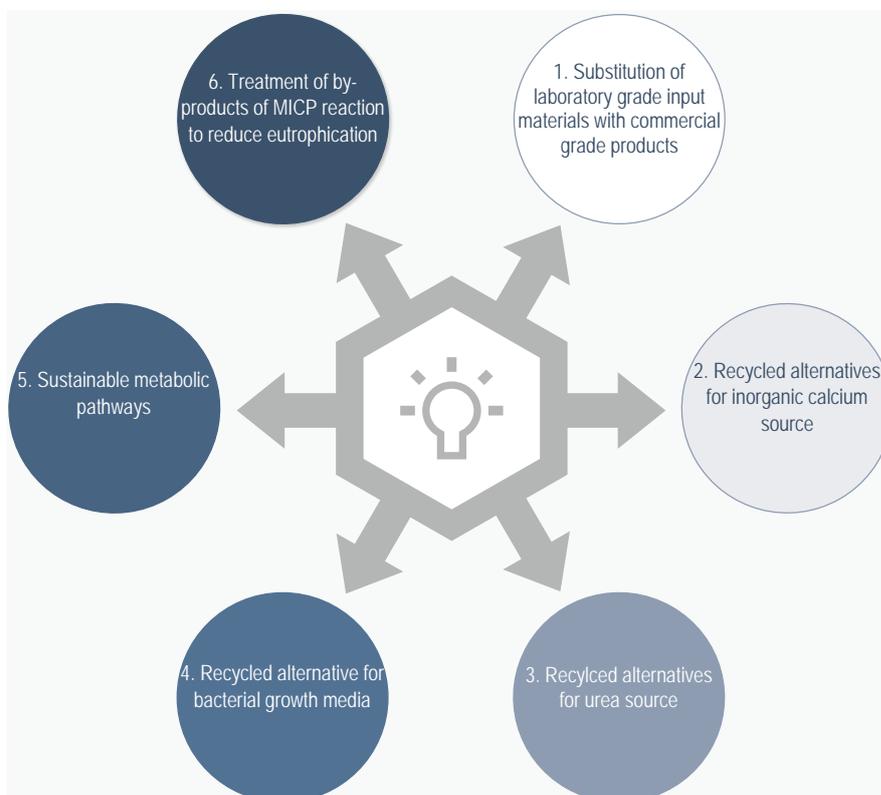


**Figure 6- 10** Breakdown of cost (AUD) for the production of 1 kg of CaCO<sub>3</sub> through (i) carbonic anhydrase producing bacteria, (ii) the traditional carbonation process, (iii) methanogens, (iv) cyanobacteria, (v) denitrifying bacteria, (vi) ureolytic bacteria and (vii) myxobacteria using commercial grade inputs

### 3. Conclusions

Nature has performed MICP for millions of years. As engineered MICP follows the same metabolic pathways, it is claimed that they are sustainable. This paper notes that engineered MICP deviates substantially from the natural process to suit the timeframe of construction projects. Thus, sustainability of the process must be examined. The present paper compares the performance of different metabolic pathways of MICP with that of the traditional carbonation process. Heterotrophic and autotrophic pathways for MICP have been investigated. A comparison of laboratory grade and commercial grade chemical inputs has also been conducted. The performance of each pathway in terms of carbon footprint, eutrophication potential, embodied energy and cost has been estimated.

A ranking of metabolic pathways to MICP has been undertaken. We note that metabolic pathways differ significantly in their performance. Calcium carbonate produced using carbonic anhydrase producing bacteria proved to be the most environmentally sustainable, followed by methanogens, (methane oxidation) and then cyanobacteria, (photosynthesis). The least sustainable pathways were MICP via ureolytic bacteria and denitrifying bacteria. In the case of MICP via ureolysis the poor overall sustainability is attributed to high carbon footprint and embodied energy of the supplied urea, as well as the eutrophication potential of the ammonium waste produced during the MICP reaction. In the case of MICP via denitrification the poor overall sustainability can be predominantly attributed to the carbon footprint and embodied energy of the required nitrate source.



**Figure 6- 11** Directions for future research

Directions for future research for industrialisation of MICP are shown in Figure 6- 11. A key component identified in the carbon footprint, embodied energy and cost for all MICP metabolic routes was the calcium source (calcium chloride). The substitution of laboratory grade calcium chloride with a commercial equivalent resulted in decreases in the carbon footprint between 26.7 and 82%. Clearly alternatives to laboratory grade calcium sources should be investigated and included in future experiments to further enhance the sustainability of carbonates produced through the MICP process.

In the case of MICP via urea hydrolysis, denitrification or ammonification the provided organic carbon source, (urea, nitrate or glucose) results in an equal contribution to the carbon footprint, embodied energy and cost as the provided calcium source. The gaseous waste produced by these pathways, (ammonium or nitrogen) also results in high eutrophication potential, which makes carbonates produced through these pathways less sustainable than the traditional carbonation method. For carbonates produced through urea hydrolysis, denitrification or ammonification to be truly sustainable, alternatives to the urea, nitrate and glucose sources should be investigated. By-products of the MICP reaction must also be treated in a way that does not significantly increase the impact in other areas, (carbon footprint, embodied energy , etc.).

A preliminary, high level cost analysis of carbonates produced through MICP revealed that as long as laboratory grade calcium and carbon sources are used, the cost of raw materials is prohibitive, when compared to carbonates produced through other, more traditional methods, such as carbonation, or when compared to commonly used construction binders, such as Portland Cement. In order for MICP to be viable economically, laboratory grade chemical inputs, typically adopted in MICP must be replaced with commercial grade alternatives.

#### 4. Acknowledgements

The authors would like to acknowledge the contribution of an Australian Government Research Training Program Scholarship in supporting this research.

The authors would also like to acknowledge the contribution of James Cook University, who provided access to the license for SIMAPRO software and databases.

#### 5. Author contributions

H.P. designed and performed analysis, analysed data, prepared figures and helped write the manuscript. N.D. provided key advice on the MICP microbial pathways and helped revise the manuscript. A.M. provided key advice on the analysis and direction of the investigation and helped revise the manuscript. R.T. provided key advice on LCA methodologies and assumptions and helped revise the manuscript.

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## Chapter 7            Conclusions

The production of Portland cement (OPC) worldwide is estimated to contribute 6% of all man-made carbon dioxide emissions, (Imbabi et al., 2012), and is estimated to be the second most consumed resource in the world, (after water), (Flower and Sanjayan, 2007). Imitating the natural process of cementation, through MICP, paves the way to sustainable and recyclable construction materials for both geotechnical and structural applications.

To date, the majority of investigations have focused on the use of MICP in sand (for grouting applications), or as a crack sealer and surface treatment for reinforced concrete structures. The main focus of this PhD was to investigate the use of MICP for other construction applications, and to characterise the sustainability of man-made MICP, used for construction purposes as to the natural process. The key objectives of the research project were as follows:

- To investigate the potential of MICP as a sustainable replacement to Portland cement for stabilised road base materials.
- To investigate the potential of MICP to augment the performance of chemically stabilised, (cement or geopolymer) materials.
- To investigate the potential of MICP as a technique to improve the engineering performance of rammed earth materials.
- To objectively compare the sustainability of alternative pathways to engineered MICP using a Life Cycle Assessment.

These objectives have been achieved, with key conclusions from the research project detailed in Chapters 3 to Chapter 6 and summarised below.

**Chapter 3** reports on the potential for MICP as a replacement or augmentation to Portland cement in cement stabilised or unstabilised sand and road base materials. The results demonstrate that in the case of plain road base materials, treatment with MICP results in a 70% increase in the compressive strength, providing UCS values consistent with that required for a modified road base material. In the case of cement stabilised road base and sand materials, MICP treatment resulted in a strength increase of between 33 and 50%. These results demonstrate that MICP may be used as a supplementing material in stabilised soil structures, reducing the overall requirement for cement and thereby increasing the sustainability of the material. Digital Image Correlation was used to monitor the degradation process of cement-stabilised samples during compressive strength testing. The use of both displacement and

strain maps from DIC can provide an indication of where cracking is likely to occur, and may provide a useful asset – management tool for road surfaces.

**Chapter 4** reports on the synergistic relationship between chemical and microbial cementation. Sand columns were first stabilised using OPC or geopolymer binders, followed by microbial cementation using MICP. The results demonstrated a considerable increase in the compressive strength and stiffness of the samples, and a corresponding decrease in the water absorption. During the microstructural analysis it was noted that on a plain system, (with no chemical stabilisation) bacteria secure themselves in the grooves of sand grains as well as in inter granular spaces and nucleate from these points, forming both a coating over the sand grains and bridges between particles. When a prior chemical stabilisation is performed the sand grains are coated with the chemical stabilizer and the bacteria are more likely to deposit in the bridging zone, resulting in reinforcement of the bridges. This leads to significantly lower permeability and higher strength and stiffness of the stabilised material. The introduction of MICP into Australian construction practices will require concerted effort from all stakeholders. The introduction of MICP as an augmentation over conventional cementation methods may provide a stepping stone to adopt MICP as a construction technique in Australia. Through an optimal combination of chemical and microbial stabilisation a low embodied energy material can be synthesised that offers the flexibility of future repair and maintenance works using further treatment by MICP.

**Chapter 5** reports on the multifunctional performance properties of rammed earth blocks using MICP to improve the strength, erosion and moisture resistance and crumb rubber to improve the thermal resistance. Surface treatment with MICP was found to improve both the strength and moisture resistance of plain and cement stabilised rammed earth blocks. Accelerated erosion resistance testing was undertaken over a 60 minute time interval. Cement stabilised blocks exhibited minimal erosion, while unstabilised blocks eroded rapidly. Surface treatment with MICP was found to impede the erosion of unstabilised blocks and the erosion rate decreased by 62%. The incorporation of crumb rubber into a rammed earth mix was found to decrease the thermal conductivity, but also decreased the strength of the blocks. As such, the incorporation of crumb rubber must be optimised depending on the requirements of the design to provide adequate thermal insulation and sufficient strength.

The surface treatment of rammed earth blocks with MICP is a worthwhile solution, not just to decrease erosion and water absorption, but also to sustainably increase strength. MICP surface treatment may be used on its own or in conjunction with cement binders. The use of MICP for rammed earth applications could therefore significantly reduce the proportion of cement consumed within the building industry, dramatically increasing the overall sustainability of building materials.

**Chapter 6** reports on the sustainability of the engineered MICP process. While the natural process of MICP is undoubtedly sustainable, when MICP is applied to construction works it becomes necessary

to enrich both the bacteria, and reactants. This has an impact on the overall sustainability of the MICP process. A cradle-to-gate LCA was used as a tool to compare the sustainability of different metabolic pathways with that of the traditional carbonation process. Heterotrophic and autotrophic pathways to MICP have been investigated and a comparison of laboratory grade and commercial grade chemical inputs has also been undertaken. The results of this analysis demonstrate that the sustainability of different metabolic pathways to MICP is varied. Calcium carbonate produced using carbonic anhydrase producing bacteria proved to be the most environmentally sustainable, followed by methanogens (methane oxidation) and then cyanobacteria, (photosynthesis). The least sustainable pathways to MICP were via ureolytic and denitrifying bacteria.

A key component identified in the carbon footprint, embodied energy and cost for all MICP metabolic routes was the calcium chloride. Substitution of laboratory grade calcium chloride with a lesser purified, commercial equivalent results in significant reductions in both the carbon footprint and cost. For carbonates produced through urea hydrolysis, denitrification or ammonification to be truly sustainable and economical, alternative to the carbon source, (urea, nitrate or glucose) should also be investigated. The gaseous waste produced by these pathways, (ammonia or nitrogen) also results in a high eutrophication potential and by-products should be treated in a way that does not significantly influence the sustainability of the overall process.

## Recommendations for future research

The following directions for future research are recommended.

- The saturation of soil due to the MICP process has an impact on soil compaction, and subsequent compressive strength. An MICP technique that allows for the reduction of moisture to the optimum moisture content for compaction would be extremely beneficial.
- The majority of work conducted to date on MICP for engineering applications has used the ureolytic pathway. However, the life cycle analysis conducted as part of this research project identified the ureolytic pathway as the least sustainable route to MICP. Further investigation is required into the potential application of alternate metabolic pathways (such as MICP via carbonic anhydrase producing bacteria) to engineered applications.
- The sustainability of engineered MICP is limited through the use of highly purified and concentrated reactants. In order for MICP to be a sustainable, and economically viable construction technique laboratory grade chemical inputs, typically adopted for the MICP reaction, must be replaced. Further research should be conducted into the use of industrial grade waste products as chemical inputs to the MICP process.

- Investigation should be conducted into viable treatment measures for waste products produced during the MICP reaction, to reduce the impact of eutrophication.

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## Appendices

Appendix A: Supplementary data to Chapter 6: Can Engineered Biocement be Sustainable?

# Can Engineered Biocement be Sustainable?

## Supplementary Data

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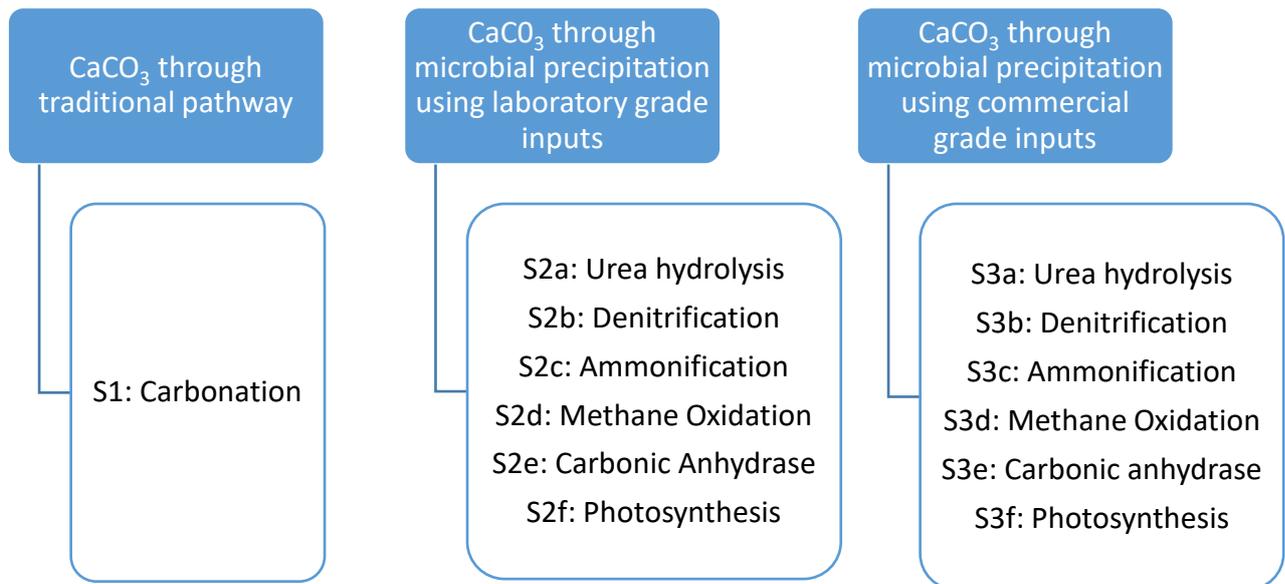
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## Methods

The purpose of the LCA was to evaluate the environmental and cost implications of calcium carbonate precipitated through MICP as compared to an equal mass of calcium carbonate precipitated using the commonly used carbonation technique. The purpose of this paper is not to conduct a full life cycle analysis, but rather to compare the environmental impacts of different biological routes to MICP. The aim of this paper is to inform future research, and enable the most sustainable and economical biological pathways to MICP to be progressed from laboratory to field applications.

The functional unit (FU) for the assessment was defined as 1 kg of precipitated calcium carbonate. The scenarios included in the analysis are shown in Figure A-1. The scope of the assessment for microbially precipitated calcium carbonate included the extraction and processing of raw materials as well as the environmental impact of any by-products produced during the MICP reaction, (Figure A-2 (b)). Transport of raw materials to the plant, and the energy required to operate the fermenter were excluded from the analysis.

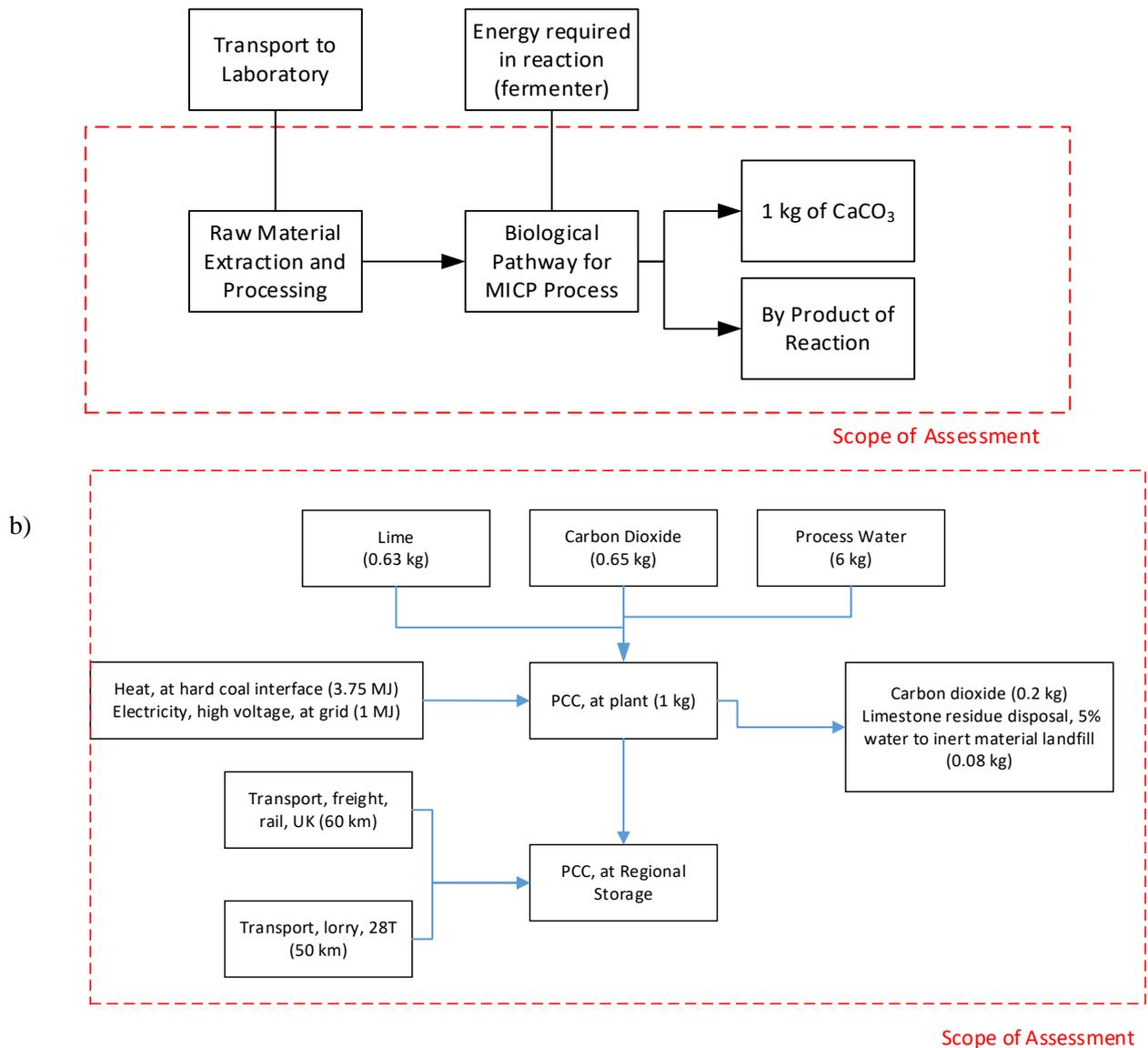


**Figure A- 1** Scenarios included in analysis

The following assumptions have been made during the preparation of this environmental assessment:

- For the purpose of this lifecycle assessment the metabolic rate of different pathways to MICP has not been considered.
- It has been assumed that the MICP reactions are 100% efficient and all of the provided calcium source is converted into calcium carbonate.
- Waste products generated by the MICP process have been included in the analysis, however the treatment of these waste products, is outside the scope of this analysis.
- The assessment has been based on ecoinvent, V2.2 database, (Frischknecht R., 2005) with adaptations to an Australasian context where available. It is possible that the use of alternative databases may vary the results obtained in this paper. Investigation on alternative database sources is beyond the scope of this investigation.

Input for the production of laboratory grade calcium carbonate through the carbonation process were based on the cradle to gate assessment conducted by Mattila et al. (2014). The scope of assessment included the extraction and transport of raw materials to the plant, energy requirements for the calcination process and waste produced during the reaction. Inputs assumed for the LCA of traditionally produced calcium carbonate are shown in Figure A-2 (b).



**Figure A- 2** Boundaries of Environmental Assessment (a) Biologically precipitated calcium carbonate, (b) Laboratory grade calcium carbonate, (Mattila et al., 2014)

The assessment was conducted using SimaPro 8.0 software. The ecoinvent V2.2 database, (Frischknecht R., 2005), was used for material inputs, with adaptations to an Australasian context where available,. The carbon footprint and eutrophication potential were calculated using AUSLCI Version 3.0 while the embodied energy for each scenario was calculated using the Cumulative Energy Demand 2.01 methodology.

A summary of the required inputs and waste products for each microbial pathway is provided in Table A-1. Chemical inputs required for the MICP reaction pathways were based on published experimental results as shown in Table A-1.

**Table A- 1** Summary of inputs / outputs for production of 1 kg of CaCO<sub>3</sub> through microbial route

Pathway	Inputs / Waste	Source
Urea hydrolysis	- 0.6 kg urea	van Paassen (2009), van Paassen (2010)
	- 1.1 kg calcium chloride	
	- 0.1 kg yeast extract	
	- 15 kg water	
	- 0.72 kg ammonium waste	
Denitrification	- 0.68 kg sodium nitrate	Hamdan et al. (2017) van Paassen et al. (2010)
	- 1.1 kg calcium chloride	
	- 0.1 kg yeast extract	
	- 0.00135 kg potassium phosphate	
	- 0.0012 kg magnesium sulphate	
	- 25 kg water	
- 0.056 kg nitrogen waste		
Ammonification	- 1.6 kg calcium acetate	Rodriguez-Navarro et al. (2003) González-Muñoz et al. (2010) Chekroun et al. (2004)
	- 0.256 kg yeast extract	
	- 0.640 kg glucose	
	- 20 kg water	
	- 0.18 kg ammonia waste	
Methane Oxidation	- 1.1 kg calcium chloride	Ganendra et al. (2014) Whittenbury et al. (1970)
	- 0.005 kg magnesium sulphate	
	- 0.005 kg potassium nitrate	
	- 6.8E-5 kg potassium phosphate	
	- 1.79E-4 kg sodium phosphate	
	- 0.00016 kg methanol	
	- 0.160 kg methane gas	
	- 0.01 kg chelated iron solution	
	- .0025 kg trace element solution	
	- 20 kg water	
- 0.34 kg hydrogen sulphide waste		
Carbonic anhydrase producing bacteria	- 1.1 kg calcium chloride	Kaur et al. (2016) Dhami et al. (2014)
	- 0.88 kg carbon dioxide	
	- 0.025 kg sodium chloride	
	- 0.0075 kg yeast extract	
	- 0.0075 kg beef extract	
	- 0.025 kg peptone	
	- 1.67E-6 kg zinc sulphate	
	- 25 kg water	
	- 0.44 kg carbon dioxide waste	
Photosynthesis	- 1.1 kg calcium chloride	Zhu and Dittrich (2016) Dittrich et al. (2003)
	- 0.88 kg carbon dioxide	
	- 1.68 kg sodium bicarbonate	
	- 25 kg water	
	- 0.44 kg carbon dioxide waste	

In the case of laboratory grade scenarios calcium chloride was produced through the commonly used Solvay process, (Hischier, 2004). In the case of commercial grade sources, laboratory grade calcium chloride was substituted with calcium chloride produced through the hypochlorination of allyl chloride, a co-product of the production of epichlorohydrin (Hischier, 2005). Laboratory grade sodium nitrate,

(used for MICP via denitrification) was also replaced with a commercial nitrate fertilizer equivalent,(Nemecek, 2007).

A high level cost assessment was conducted on all raw materials for each scenario. Unit rates for laboratory grade chemicals were sourced from laboratory chemical suppliers in Australia whilst rates for commercial grade chemicals were sourced from ICIS bulk chemical reports, (ICIS, 2017). Unit rates for laboratory and commercial grade chemicals are detailed in Table A-2 and Table A-3.

**Table A- 2** Unit Rates for Laboratory Grade Chemicals

<b>Product</b>	<b>Unit Rate (AUD) / kg</b>	<b>Source</b>
Beef extract	204.4	Southern-Biological (2017b)
Calcium carbonate	60.8	Chemsupply(Chemsupply, 2017)
Calcium chloride	50.6	Chemsupply(Chemsupply, 2017)
Chelated iron solution	151	Sigma-Aldrich (2017)
Glucose	37	Chemsupply(Chemsupply, 2017)
Magnesium sulphate	43	Chemsupply(Chemsupply, 2017)
Methane gas	49.5	Commonwealth-of-Australia (2016)
Methanol	11.75	Chemsupply(Chemsupply, 2017)
Peptone	115.5	Southern-Biological (2017c)
Potassium nitrate	55.6	Chemsupply(Chemsupply, 2017)
Sodium bicarbonate	26	Chemsupply(Chemsupply, 2017)
Sodium chloride	19.67	Chemsupply(Chemsupply, 2017)
Sodium nitrate	48.6	Chemsupply(Chemsupply, 2017)
Sodium phosphate	67	Chemsupply(Chemsupply, 2017)
Trace element solution	1362.4	MPBio (2017)
Urea	64	Chemsupply(Chemsupply, 2017)
Water	0.0027	ABS (2013)
Yeast Extract	129.8	Southern-Biological (2017a)
Zinc sulphate	23	Chemsupply(Chemsupply, 2017)

**Table A- 3** Unit Rates for Commercial Grade Chemical Replacements

<b>Product</b>	<b>Unit Rate (AUD) / kg</b>	<b>Source</b>
Beef extract	22.4	eBioChem (2017)
Calcium acetate	1.4	PulisiChem (2017)
Calcium chloride	0.34	ICIS (2017)
Chelated iron solution	39	Bunnings (2017a)
Glucose	0.64	Melbourne-Food-Depot (2017)
Magnesium sulfate	0.54	ICIS (2017)
Methanol	2.98	ICIS (2017)
Sodium bicarbonate	0.68	ICIS (2017)
Sodium chloride	0.284	Bunnings (2017b)
Sodium nitrate	6.6	Amazon (2017)
Sodium phosphate	2.38	ICIS (2017)
Urea	0.81	ICIS (2017)
Yeast extract	22.4	eBioChem (2017)
Zinc sulphate	0.92	ICIS (2017)

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