Assessing Enzyme-Based Soil Stabilisation for Unpaved Road Construction

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

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Declaration

I certify that except where due acknowledgement has been made, the work is that of the author alone. This work has not been submitted previously, in whole or in part, to qualify for any other academic award. The content of the thesis is the result of work which has been carried out since the official commencement date of the approved research program. Any editorial work, paid or unpaid, carried out by a third party is acknowledged and, ethics procedures and guidelines have been followed. I acknowledge the support I have received for my research through the provision of an Australian Government Research Training Program Scholarship.

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List of Abbreviation

AADT	*Annual Average Daily Traffic*
ADT	*Average Daily Traffic*
AMR	*Application Mass Ratio*
ARRB	*Australian Road Research Board*
CAH	*Calcium Alumina Hydrate*
CASH	*Calcium Alumina Silicate Hydrate*
CBR	*California Bearing Ratio*
CDF	*Cumulative Damage Factor*
CEC	*Cationic Exchange Capacity*
CH	*Fat Clay*
CL	*Lean Clay*
CSH	*Calcium Silica Hydrate*
CT	*Computed Tomography*
DCP	*Dynamic Cone Penetration*
DESA	*Design Equivalent Standard Axle*
DMR	*Dilution Mass Ratio*
EDS	*Energy Dispersive Spectroscopy*
FA	*Fly Ash*
GGBS/GGBFS	*Ground Granulated Blast Furnace Slag*
LL	*Liquid Limit*
MDD	*Maximum Dry Density*

MH	*High Plastic Silt*
MICP	*Microbial Induced Calcium Precipitation*
OMC	*Optimum Moisture Content*
OPC	*Ordinary Portland Cement*
PF	*Porosity Factor*
PI	*Plasticity Index*
PL	*Plastic Limit*
PSD	*Particle Size Distribution*
RHA	*Rice Husk Ash*
RQ	*Research Question*
SADT	*Standard Axle Dual Tyre*
SAI	*Soil Asphalt Interaction*
SC	*Clayey Sand*
SEM	*Scanning Electron Microscope*
TGA	*Thermogravimetric Analysis*
UCS	*Unconfined Compressive Strength*
XRD	*X-Ray Diffraction*
XRF	*X-Ray Fluorescence*

Abstract

Unpaved or unsealed roads make up the majority of the global road network. Yet, it is often not designed to appropriate standards which affect its structural integrity and compromise the life span of these pavements. The repercussions of this improper designing can incur a significant financial burden on the road governing agencies for its repair. While stabilisation of pavement soil layers using traditional additives such as cement and lime are recommended and extensively used in the current practices to mitigate this issue, these additives cause significant environmental concerns, especially during the additive production. This research investigates the effective use of enzymes as an eco-friendly, non-traditional alternative to cement-based stabilisers for problematic soils. The primary objective of this research is to understand the efficacy of enzyme-based additives in the design and construction of the unsealed pavements. This work is a combination of experimental, numerical and field trial works conducted to understand the fundamentals and application efficiency relating to enzyme-based soil stabilisation. The methodology of this study facilitates understanding of the influential parameters and stabilisation mechanism of enzymes, its optimisation, effects of combining with secondary additives, and the durability and performance of the treated pavements.

The comprehensive laboratory testing program followed within the study includes the evaluation of mechanical behaviour of the stabilised soil using physio-chemical, microstructural and pore structural techniques. The study highlights the importance of understanding the fundamentals of the stabilisation mechanism to facilitate effective enzyme-based stabilisation. Firstly, tests were conducted to identify suitable parameters and conditions for enzyme stabilisation, which includes soil type, sample preparation,

drying, curing, and testing processes. Secondly, mechanical tests were conducted to determine optimal contents for effective stabilisation. Lastly, chemical and imaging techniques were utilised to identify the stabilisation mechanism of the additive. The combination of the enzyme with fly ash (secondary additive) is also investigated in this study using a similar testing approach as a means to further improve the mechanical and durability benefits of enzyme-based soil as well as a means to improve the sustainability of the treatment by promoting higher fly ash utilisation rates in construction practices. As highlighted in the study, the combination of the two additives could not only offer a cost-effective road stabilisation method but also assist in waste mitigation for countries such as Australia, which face issues regarding the disposal of coal ash. The detailed testing conducted within the study provides optimal values of these combined additives as well as offers insight into their mechanism.

The findings from the laboratory tests for enzyme-based stabilisation were applied in trial road constructions which showed sound performance of the treatment. Mechanistic analysis of pavement is also conducted within the study to understand the immediate effect of the treatment on pavement design. However, the long-term assessment of the enzyme stabilised pavement from the trial road construction highlight the importance of combining enzymes with secondary additives. Durability assessment of the treatment is further explored experimentally by using novel experimental techniques, which include a recompaction test as well as a modified wetting and drying tests. Additionally, three-dimensional finite element analyses were conducted to further evaluate the efficiency of the stabilisation under pavement operational loads.

The findings from this thesis show that the use of enzymatic fly ash is a sustainable treatment method which offers substantial benefits in altering the mechanical behaviour of weak fine-grained subgrade soils. This additive can be used in the road construction industry instead of calcium-based approaches to effectively stabilise unsealed pavement. The results and findings from this study have been published in a number of international journals.

Chapter One.

Introduction

1.1. Preface

Road network is a major component of a nation's infrastructure as it impacts the economic growth of a country and its societal services. The Australian road network is one such network, a $280bn asset which covers more than 817,000 km of roads (Roads Australia 2020). It is understood that a significant proportion of the taxpayer's money is allocated to the repair and maintenance works of this vast road network. Seeing the need for the investment in an efficient road system across the nation, the Australian Government doubled its Roads to Recovery program budget from $350m to $750m in the 2015 – 16 financial year (Road Traffic Technology 2015). Under this program, the Australian Government aims to provide $6.2bn between 2013 to 2024 with an ongoing commitment of $500m each year following (DoIRDC 2020). Figures allude to the fact that over 50% of the Australian road network, around 466,874 km is unpaved. The commonness unpaved roads are not just limited to Australia, with over 2.2 million km of roads in the US are also unpaved. Brazil, the world's fourth-biggest road network with approximately 1.6m km only records to have only around 10% of its total roads as paved. Table 1 provides an overview of the global road network ranked based on the length of the network. The information provided within the table is based on information ranging from data obtained between 2011 – 2016. As seen from Table 1, in most countries, unpaved roads (interchangeably also known as unsealed roads) make a considerable amount of the nation's total road network.

Table 1.1. Top 10 largest global road network (CIA 2020)

Rank	Country	Road Network (km)	Unpaved Roads (%)
1	United States	6,586,610	34%
2	China	4,960,600	12.5
3	India	4,699,024	40 – 50%
4	Brazil	2,000,000	87.7
5	Russia	1,283,387	27.7
6	Japan	1,218,772	18.5
7	France	1,053,215	*
8	Canada	1,042,300	60.2
9	Australia	873,573	53.4
10	South Africa	750,000	78.9

** No accurate information reported*

1.2. Problem Definition

Unsealed roads generally consist of two layers, a subgrade layer on which a wearing course layer is laid on (Fig 1.1). Although many unsealed roads around the world provide an efficient, smooth and safe route of transport for its users, there are reportedly many that does not do so. While improvements to road geometry and safety measures are often sought after by various governing agencies, sometimes there is a lack of emphasis given to understanding the basic material properties for the construction of these pavements. This could often lead to the designing of these pavements to inappropriate standards which can force unforeseen repairs, maintenance, and the costs associated with it.

Figure 1.1. Unsealed road at Eagle Point, Victoria

Many current unsealed pavement construction methods are uneconomical and inefficient, with the use of natural resources. The washing out of these pavements from extreme weather conditions can result in loss of material and shape, which can ultimately compromise the integrity of the structure. Loss of material is evident in the form of loss of fines, often seen in the form of dust clouds when travelling on these types of roads (Fig 1.2). Loss of shape in the form of corrugations and rutting is also common on these pavements (Fig 1.3). The rise of these problems can jeopardise the integrity of the infrastructure quite heavily by causing vehicular damage and increasing safety issues.

Figure 1.2. Dust formation (loss of fines) on an unsealed road (ABC News, 2011)

Figure 1.3. Corrugations on an unsealed road (Practical Motoring, 2017)

Improper designing of these pavements could significantly affect the cost associated with pavement repair and maintenance. It is reported that in the fiscal year of 2018 – 19, Regional Roads Victoria (RRV) invested a record $941 million to repair and upgrade regional roads, of which, $333 million was for road maintenance alone (RRV 2020). The maintenance works included rebuilding and resurfacing of more than 1500 km of road.

Furthermore, RRV plan on investing another $425 million over the fiscal year of 2019 – 20, with much more maintenance works planned, with $100 million of additional support from the Victorian Government with the Fixing Country Roads program. Although these figures include general maintenance cost associated with all types of roads, they do highlight the importance of proper unsealed pavement designing in controlling the costs associated with pavement maintenance.

Generally, there are three ways to address the issues that plague unsealed pavements. The first method involves sealing the road, which could be seen as the most effective method of mitigating the issue. However, the practicality of this method is often subjected to the usage level of the pavement. For example, it is not viable to seal a road which sees low volume or seasonal traffic. Australian Road Research Board (ARRB) reports that in many cases, especially with rural councils, sealing of the road is not considered on roads with an annual average daily traffic (AADT) of 150 vehicles but only on roads with AADT of greater than 500. However, ARRB recommends that sealing of pavements is justified if the process is grounded in economic analysis, considering the costs and benefits of the expected outcome in terms of agency costs, vehicle operating costs and road user costs, and other costs including safety. The second method of mitigating unsealed pavement issues is by limiting the traffic flow. However, this is a highly impractical option as it defeats the purpose of the infrastructure. Lastly, and most commonly used technique, is introducing ground improvement techniques such as soil stabilisation.

1.3. Scope and Objectives

Stabilisation of these unpaved roads could stand as a viable countermeasure to address some of the pavement issues. Soil stabilisation is one of the oldest and the most common form of ground improvement techniques used to alter the physical properties of soils used as pavement layers, which in turn could help improve its the bearing capacity. Stabilisation of soil can be categorised into two; mechanical and chemical stabilisation. Mechanical stabilisation refers to the physical alteration of the soil matrix by external forces, whereas chemical based refers to the incorporation of additives into the soil, which helps alter the properties of the soil. Numerous research is available to date, which investigates various forms of these additives. Enzymes are one such additive reported in the literature, which has shown a mixed level of effectiveness. This research work investigates the stabilisation mechanisms of commercially available enzymes to understand its potential value for the road construction industry. This research hopes to shed light on the stabiliser's efficacy on its own as well as when combined with additives. Enzymes as soil stabilisers have been successfully used in road construction in several countries for the past 30 years. However, research has shown that the successful application of these enzymes is case specific, emphasising that enzyme performance is dependent on subgrade soil type, condition and the type of enzyme used as the stabiliser. A universal standard or a tool for road engineers to assess the performance of stabilised unsealed pavements using well-established enzymes is not available to date. Objectives of this study will aim to identify the optimised mix proportion of enzyme dilution as well as the application rate to see effective strength improvement based on the stabilisation mechanism.

The research will also provide insight into the sustainability aspect of pavement design. The study will also look into the stabilisation of pavements using fly ash, a finer form of coal ash, which contributes to one-fifth of the Australian waste stream. Despite proving its effectiveness as a cement substitute, only around 40% of the nation's coal ash waste material is recycled or utilised in the industry. In this study, efforts will be made to maximise understanding of the effectiveness of combining fly ash along with lime (a common admixture used in practice) as well as combining it with enzymes to maximise ground improvement. The study will also aim at understanding the durability effect based on time as well as environmental conditions. All the aims of this thesis combine well to provide the reader with the knowledge on soil stabilisation with the enzyme, fly ash and lime admixture, which could inherently be used by practising engineers. The specific objectives of the research are:

- Understanding and evaluating the efficacy of enzymes as a stabiliser on fine-grained subgrades based on its mechanism.
- Assessing the efficacy of enzyme-based additives when combined with other additives.
- Quantification of time-dependent strength on fine grained soil stabilised with enzyme-based additives and determining the performance pavements incorporating these materials when subjected to operational loads.
- Contribution to knowledge in designing of unsealed pavements stabilised with enzyme-based additives.

1.4. Overview of Investigation/Thesis Outline

The objectives identified in the research work have been covered across various chapters of the thesis evolving from Chapter 1, which provides background, problem definition, and the significance of the research. The chapter contains the general overview of the topic providing the importance and benefits of conducting this investigation.

Chapter 2 provides a comprehensive review of the literature on the current stabilisation practices conducted around the world. The chapter primarily focuses on chemical based stabilisation, briefly covering traditional stabilisation methods which rely on the utilisation of calcium-based additives such as cement and lime followed by the in-depth analysis on non-traditional methods primarily focusing on the use of enzymes for pavement stabilisation. Review on the use of other non-traditional additives has also been conducted in this research as the scope of the research also includes combining enzyme-based additives to other non-traditional soil stabilisers. The review of the literature highlights the origin of the concept of enzyme-based soil stabilisers, summarises case studies of its use, highlighting their efficacy, proposed mechanism, and the factors that affect their efficacy. The literature review also covers the understanding of fly ash-based soil stabilisation, as its combined use with enzyme falls within the scope of the current study. The comprehensive literature review will help identify the gap in the knowledge regarding this sort of additives in pavement stabilisation. The detailed investigation of the research gaps will help to deliver the key objectives of the research reported in Section 1.3 of this chapter.

Chapter 3 details the physical and chemical properties of the materials used in this study.

Four main materials were utilised in this research, which included the soil, the commercial enzyme, fly ash as well as lime. The materials' appearance, chemical composition and images (visual and microlevel) are also presented in this chapter. Summary of the research methodology has also been presented in the chapter.

Chapter 4 deals with the optimisation of enzyme-based soil stabilisation. This chapter investigates the stabilisation effects of the commercial enzyme-based additive, which is being applied to construct unsealed roads worldwide. The main aim of this work is to identify the optimised mix proportions of the additive by unveiling its mechanism of stabilisation for a fine-grained field soil which is common in Victoria, Australia. A series of experiments were conducted under a 4-stage test program that includes macro scale mechanical tests to micro scale imaging tests to unveil stabilisation effects and mechanism of stabilisation. The identified mechanism has facilitated five-fold enhancement in the efficiency of enzyme-based soil stabilisation compared to the strength of non-stabilised soil. The findings from this section will substantially benefit the road construction industry by not only replacing traditional construction methods with economical/reliable approaches but also eliminating site specific tests required in the current practice of enzyme-based soil stabilisation. The chapter also presents the application of enzyme on a trial road construction discussing the construction process in the field as well as its monitoring.

Chapter 5 investigates the effect of combining enzymatic stabilisers with additives such as lime and fly ash. The chapter takes the form of its preceding chapter and presents a 4-stage analysis to learns its efficacy based on its mechanism as well as finding the optimised values. The mechanistic analysis was also conducted in stage 5 based on these optimised values. This analysis shows the benefits of combining these additives on an economic point of view as well as based on sustainability aspects.

Chapter 6 investigates durability and performance of the tested additives. Durability is assessed firstly considering time and recompaction to assess how long the effects of the stabiliser last within the soil by presenting a novel recompaction method. The chapter also presents a novel method to identify the durability based on the wetting and drying of samples. Lastly, performance is assessed using finite element simulations to compare the effect of treatment on unsealed pavements.

Chapter 7 concludes the investigation conducted presenting all the findings, contribution to the field of knowledge as well as recommendations, followed by the references used in this research.

1.5. Rationale and the Significance of Research

This research contributes to the understanding of the efficacy of novel additives for pavement stabilisation. Understanding the performance and durability effects due to these additives are critical in designing more efficient pavements. The primary objective of this research is to understand the efficacy of enzyme-based additives in the design of the unsealed pavements. Unsealed roads were selected for this investigation due to its prevalence in Australia, as well as other countries across the world. The significance of the findings reported in this manuscript generally points to a better understanding of the additives at hand. In chapter 4, experiments were conducted to investigate the efficacy of enzyme-based stabiliser to understand its stabilisation mechanism and to uncover optimised levels for usage in pavement applications. The results of the experiments were used to identify the soil stabilisation mechanism, which was then used to propose the optimised mix proportions for this additive. The chapter provides insight into the optimum additive amount required to stabilise road pavements based on this stabilisation mechanism. The chapter also provides the application process of the additive for pavement stabilisation based on a case study which also offers further support for current practices. The outcomes of this study will be useful in field applications to improve the fine-grained soil strength using this enzyme additive (& similar kinds) to enhance the resilience of infrastructure.

The findings from Chapter 5 reveal the optimised combination of enzymatic fly ash and lime contents to support effect pavement stabilisation. The conclusions of the chapter highlight the sustainability of the combined additive, as well as its immediate impact in pavement design through the incorporation of CIRCLY (mechanistic design) model. The chapter highlights the effect of time on soil stabilisation which could potentially help the industry gain sound knowledge about the long-term impact of this form of stabilisation. The outcomes reported in this chapter have the potential to benefit the road construction industry by providing effective use of this waste material in engineering construction with the support of secondary additives. This research could significantly assist in enhanced fly ash waste mitigation as well as the development of alternative stabilisation practises in the road construction industry.

Findings from Chapter 6 provide further understanding on the durability aspect of the stabilisation form investigated in this research. The durability is tested using a novel wetting and drying process to provide insight into the efficacy of the additive. The performance of a pavement stabilised with the additives tested in the research is also assessed using finite element modelling to uncover the effect of the additive on the pavement stress and strain. The findings of the chapter will contribute to the knowledge in the efficacy of combining these forms of additives for stabilised pavement design in terms of durability.

The findings from this research will substantially benefit the road construction industry by not only replacing traditional construction methods with economic, reliable, and sustainable approaches but also provide insight to practising engineers regarding the use of novel additives such as enzyme-based soil stabilisation.

Chapter Two.

Literature Review

2.1. Overview

Good quality roads are significant components of infrastructure as they impact the economic growth of a country and its societal services. As highlighted in Chapter 1, a significant proportion of Australian (around 57%) as well as other nations' roads is unsealed. While many unsealed roads around the world provide an efficient, smooth and safe route of transport for its users, many others are often designed to improper standards. The inefficient use of natural resources in the construction of these roads often causes an economic burden on the road governing agencies as well as cause environmental harm to adjacent lands, aquifers, and watercourses due to winds and washouts. These pavements are also liable to have a short life span with a constant need for repair and maintenance works due to its vulnerability to shape and material loss. As noted in Chapter 1, out of the three possible methods of mitigating the issue, ground improvement techniques have been sought after in the industry as the most practical and effective process.

Ingles & Metcalf (1972) suggests that there are three options which can be undertaken on a site material prior to commencement of construction work. They include:

- Accepting the site material as it is.
- Replace the natural material.

- Altering the properties of the material.

The third option is considered as an optimal definition for soil stabilisation. This altering of properties can be achieved by chemical, thermal, mechanical or by other means based on the soil "response spectrum". Soil response spectrum simply refers to the types of soil that show a positive effect on the stabilisers. There are various types of soil stabilisation methods as summarised in Fig 2.1.

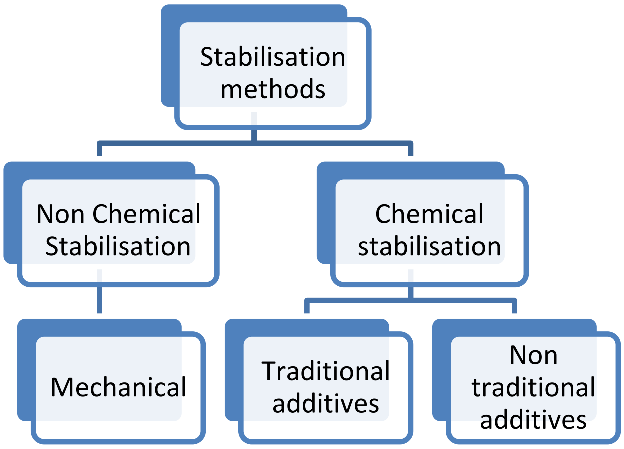


Figure 2.1. Classification of soil stabilisation

2.1.1. Mechanical Stabilisation

Mechanical stabilisation method is a non-chemical stabilisation technique that aims at the densification of soil by expelling the air from the soil voids without much change in the water content (Little & Nair 2009). This is the oldest stabilisation technique to date and continues to be the most commonly used. It was utilised during the Neolithic age between 10,200 BC to somewhere around 4500 to 2000 BC with the use of stone rammers to compact the soil. Soil compaction is defined as the reduction of soil volume as a result of applied load, vibration, or pressure, which leads to a decrease in soil bulk density and soil porosity. Mechanical stabilisation process utilises this compaction technique without the addition of any chemicals. In this process, dynamic energy is used to make the soil strata more compacted by squeezing out air and thus pushing soil particles closer together. This method, however, is more suited for the cohesionless soils where compaction energy can cause particle rearrangement and particle interlocking.

Mechanical stabilisation is based on the principles of coarse mixture strength, gradation, and properties of the soil. Altering physio/chemical properties will be more effective than densification in fine grained soils due to bonding interference with particle rearrangement and particle locking. Compaction can be conducted in the field using various equipment such as smooth wheeled rollers, pneumatic tyre rollers, sheepsfoot roller and grid rollers (Wesley 2009). However, the use of mechanical based soil stabilisation on saturated soils can result in shoving and rutting. Chemical stabilisation, the type of soil stabilisation where additives are incorporated to alter the chemical and physical properties of soil, is often accompanied by mechanical stabilisation to add to the bearing capacity of the soil.

2.1.2. Chemical Stabilisation

Soil stabilisation with chemical additives has been a field with growing research interest. Chemical stabilisation of soil is conducted in cases where adequate strength is to be attained within a short period and where more often mechanical stabilisation alone has been rendered ineffective. Chemical stabilisation has also been utilised in roadway construction for over 2000 years since the incorporation of lime in roadway construction by the Romans. History teaches us that the Roman builders used various materials to construct their roads employing multiple layers for durability and flatness. For example, shallow trenches of up to 1 m depth were excavated, with the bottom section of the road made of levelled earth and mortar or sand topped with small stones. Foundation layers of crushed rocks or gravel cemented with lime mortar were placed over the bottom section with the surface layer constructed using neatly arranged blocks made from gravel, pebbles, iron ore or hardened volcanic lava (Andrews 2018). The use of chemical stabiliser stem from this innovative design of mixing materials at hand to alter the properties of the soil. Chemical stabilisation can be categorised into two main streams; using traditional chemical additives such as cement and lime, or using non-traditional chemical additives.

2.1.3. Chapter Layout

This chapter aims at providing an in-depth review of the current state of the art in soil stabilisation emphasising on chemical stabilisation with non-traditional additives. Section 2.2 discusses the chemical stabilisation conducted by calcium-based additives. The section presents cement and lime-based additives, which are commonly used in the industry, its mechanisms, and case studies which report its efficacy as a soil additive. The use of fly ash has also been reported in this section. As highlighted in Section 2.2, this additive has also been subjected to numerous studies as a potential replacement for cement due to its pozzolanic capabilities. Section 2.3 discusses the characterisation of non-traditional additives. A detailed review of enzymatic soil stabiliser is presented in Section 2.4, highlighting its concept, hypothesised mechanisms from the literature, case studies on its use as well as parameters that affect this form of stabilisation. Section 2.5 presents the research gap and questions highlighted from the comprehensive literature review conducted.

2.2. Traditional Chemical Stabilisers

2.2.1. Cement and Lime Stabilisation

Stabilisation mechanisms of traditional calcium-based additives such as lime and cement have been widely investigated and well understood. Cement-based stabilisation is by far the most common type of soil stabilisation because of the amount of knowledge gathered from years of usage. Cement stabilisation is widely used in all types of soil except for ones with organic matter. Soil organic content has been reported as being detrimental to soil stabilisation (Tastan et al. 2011). However, a major benefit of these additives is that it can be added to very wet soil, especially in sandy soils without much compaction (Ingles and Metcalf 1972). The effects of some influential factors (i.e., water content, cement content, curing time, and compaction energy) on the microstructure and engineering characteristics of cement-stabilised soils have been extensively researched (Terashi 1979, 1980, Tatsuoka 1983, Kamon 1992, Nagaraj 1997, Yin and Lai 1998, Consoli et al. 2001, Kasama 2000, Miura et al. 2001, Kampala and Horpibulsuk 2013, Horpibulsuk et al. 2003, 2004a, 2004b, 2005, 2006, 2010a, 2010b, 2011, Suebsuk et al. 2010, 2011). In stabilisation with Portland cement, some calcium from newly formed cementing compounds Calcium – Silicate – Hydrate (C – S – H) and Calcium – Aluminate – Hydrate (C – A – H) has been reported to modify clay particles while some more Calcium is formed as a result of cement hydration. The hydrates have been reported to further stabilise flocculated clay particles through cementation (Little et al. 2000). Lime stabilised soil has also been reported as being effective in decreasing plasticity, increasing shear strength and changing the cohesion of clayey soil to a granular material (Prusinski and Bhattacharja 1997). The pozzolanic reactions between silica/alumina and calcium could also account for the strength increase in lime stabilised soil (Parsons and Milburn 2003). Lime (non-hydrated) needs more water when added to the soil, which releases heat and causes a rise in pH levels. The higher pH levels increase the solubility of alumina and silica, which results in dramatic changes to the soil lattice (Scholen 1992). Scholen (1992) recommends the use of traditional additives for the treatment of aggregate surfaces, base courses and subgrades.

Investigations have been conducted in terms of the individual applications of either cement or lime as a chemical treatment (Chen and Lin 2009, Horpibulsuk et al. 2010b, Bahmani et al. 2016, Bell 1996, Negawo et al. 2019) as well as the mixture of both over the last few decades (Azadegan et al., 2012, 2013, Jauberthie et al., 2010). The studies report enhanced soil mechanical properties as well as stability from the treatment that they allow for various application of these additives in road subgrades and other pavement layers.

Horpibulsuk et al. (2010b) investigated the improvement of strength in silty soil by the inclusion of cement based on three parameters which included water content, curing time and cement content. The study reported positive efficacy of cement-based stabilisation by improving the soil structure from the increase in the inter-cluster cement bonding and reduction in the pore space. The study goes on further to explain that there are three zones within the soil-cement fabric which affect strength development from this treatment: active, inert and deterioration. Significant pore reduction occurs in the active zones with the increase in cement content which consequently leads to an increase in soil strength. In contrast, insignificant changes occur in pore size distribution in the inert zone, which results in slight variations in strength. However, in the deterioration zone, water is not adequate for hydration which consequently causes a reduction in soil strength as it does not support the production of the cementitious products with increasing cement content. The study also reports that in the active zone, the maximum strength of the soil is attained at 1.2 times the optimum moisture content (OMC). However, the pore volume at this water content is higher than that at optimum, which suggests that both the fabric, as well as cementitious bonding, contribute to the strength development in cement stabilised soil. Chen and Lin (2009) report strength improvements as well as the reduction in swelling properties of soft soil treated with cement and incinerated sewage sludge ash (ISSA) admixture. The decrease in plasticity and the change in soil type from CL (lean clay) to CH (fat clay) indicates the improvements in the basic property of the soil. The improvement in the strength of the soil can be credited to the releasing of Ca^{2+} from the admixture. This calcium ions combine with SiO_2, Al_2O_3, Fe_2O_3, and the pozzolans from the ISSA to produce CSH and calcium hydroxide, which enhances the bonding forces among the soil particles. Bahmani et al. (2016) mainly investigate the effect of the size and content of nano-silica on strength development of cement-treated soil. Findings of the Bahmani et al. (2016) investigation includes the effectiveness of the smaller silica particles (15 nm) on initiating chemical reaction at early stages while the larger particles (80 nm) support further chemical reactions after the 14 days which lead to increase in strength during all curing stages. However, it was also noted in the study that the lower loading on the particles resulted in higher strengths. Changes in the hydration rate alluded to the higher formation rate of C-S-H, which was further supported by an increase in negative charge as shown by the Zeta potential and decrease in cationic exchange capacity (CEC) results.

The positive efficacy of lime in soil stabilisation has allowed its use also in engineering works for a very long time. Bell (1996) investigates the effect of adding lime into three of the most frequently occurring minerals in clay deposits, namely, kaolinite, montmorillonite and quartz as well as till and laminated clay. The overall outcome of the study reveals that the additive efficacy varies depending on the clay mineral, type and curing duration as well as the method and the quality of the construction. The study reports the existence of a fixation point where the calcium ions combine with or are being adsorbed by the clay minerals. The addition of lime towards this fixation point contributes to improving the workability of the soil, such as improving the plasticity. However, lime added over this fixation point helps in the cementation process depending on the development of reaction products. It was also noted that the most significant increase in the plastic limit (PL) was recorded in montmorillonitic clays. At the same time, kaolinitic soils exhibit this to a lesser extent and quartz exhibit insignificant change in plastic limits. Results also reveal an increase in the liquid limits (LL) of both kaolinitic and quartz minerals with the increase in lime content. In contrast, montmorillonitic clay exhibits a reduction in the liquid limit. The changes in the plasticity of the soil allude to the reduction in the susceptibility of volume change in expansive soil. Tests on the treated soils show that initial strength gain is more rapid in montmorillonitic clay compared to kaolinitic clays. However, with time, kaolinitic soils record higher strength compared to montmorillonite. It is also seen that lime stabilised quartz attains the highest strength compared to lime stabilised montmorillonite or kaolinite. Lime treated till and laminated clay also exhibited significant improvements in its engineering properties as well as strength tests. Optimum strength gains have been reported at 4 - 6% lime content with increase in strength noted with increasing curing time and temperature. However, general notable strength increase occurs within seven days of treatment. Negawo et al. (2019) also report similar findings from a study which investigates the efficacy of using lime to stabilise highly expansive clay soils from Highlands of Ethiopia to be used as the road subgrades. Test results show significant improvement to the soil properties such as the reduction in plasticity index (PI) and swelling potential of the soil. Increase in the Unconfined compressive strength (UCS), as well as California Bearing Ratio (CBR) of the treated soil, also highlight the efficacy of the treatment method. The study reports sufficient strength gain with 7% lime content based on the dry unit weight of the soil with a mild increase of resistance attained by adding 2% more (9%) suggesting that further addition does not contribute to additional benefits within the 7-day curing period.

An investigation by Azadegan et al. (2012) attempts to understand the effect of grain size as well as quantity of stabilising agents on the compaction and mechanical properties of a lime/cement treated soil under unconfined compressive test conditions. The tests show a linear relationship between dry density and the natural logarithm of consumed compaction energy based on the coefficients such as soil type, moisture content and the amount of the soil stabiliser used. The test results show an increase in OMC and a decrease in maximum dry density (MDD) as the size of the granular segments in treated soil decreased from 19mm to 9 mm. Unconfined compressive tests on the samples have illustrated results that higher cement content leads to a higher compressive strength and elasticity modulus. It also shows that the lime/cement stabilised gravel shows superior characteristics compared to stabilised sand.

Other notable works conducted on soil stabilisation with either cement/lime or the combination of both are summarised in Table 2.1. The table summarises the research timeframe, the type of work conducted (lab/field), the description and significant findings of the work. As seen from the section, cement and lime can be used to stabilise soil subgrades effectively. The efficacy of these form of additives can be credited to the nature of the chemical reactions that take place in the soil and the stabiliser admixture.

2.2.1.1. Mechanism

Calcium based stabilisers make use of the technique called the pozzolanic stabilisation to alter the bearing capacity of the natural soil. With the addition of pozzolans, the cationic exchange of the soil changes and affects the fabric of the soil due to the newly formed cementitious compounds based on the following Equation 2.1 and Equation 2.2 (Ismaiel, 2013):

$$Ca^{++} + 2(OH) + SiO_2 \rightarrow CSH\ (calcium\ silicate\ hydrate) \qquad \textbf{\textit{(Eq. 2.1)}}$$

(Silica) (Gel)

$$Ca^{++} + 2(OH) + Al_2O_3 \rightarrow CAH\ (calcium\ aluminium\ hydrate) \qquad \textbf{\textit{(Eq. 2.2)}}$$

(Alumina) (Fibrous)

Lime, Lime Kiln Dust (LKD) or Class C Fly Ash when mixed with water helps the ionisation of quicklime producing Ca cations which exchanges with the clay mineral (Na and K) in the lattice. The strong ionization energy of Ca ions tightens the lattice releasing water and breaking down clay clods. The increase in pH releases free alumina and silica, which reacts irreversibly with calcium ions to form calcium aluminium silicates with a net negative charge. These Calcium Aluminium Silicates with negative charges attract with the ionized water molecules in a row to form a network of hydration bonds in channels and cavities throughout aggregate mass cementing the particles together.

2.2.1.2. Case Studies

Table 2.1 summarised significant findings in various literature covering traditional calcium-based soil stabilisers, lime and cement. It covers a broad range of findings in terms of soil type, test type (i.e. lab tests or field test), the additive used and the key findings.

From Table 2.1, it can be seen that the effectiveness of these calcium-based additives is quite significant in terms of the strength of the treated soil. However, one of the issues that arise from these forms of stabilisation is its inefficacy when the soil stabilised contains significant amounts of sulphates which inadvertently causes heaves which could compromise the structural integrity of the pavement (Rollings et al. 1999). Hunter (1988) reports anecdotal evidence of this phenomena in the case of two streets in Las Vegas, Nevada. These pavements, which were stabilised with lime, caused significant heave which reportedly cost over USD 2.7m, two years post-stabilisation. Chemical analysis of the pavement shows the formation of series solution between thaumasite, a rare calcium-silicate-hydroxide-sulphate-carbonate-hydrate and ettringite, a calcium-aluminium-hydroxide-sulphate-hydrate mineral as the main constituent that cause volumetric expansion. It was noted that during the investigation, there was an increase in expansion of up to 0.1% per day to a maximum of 12% volume change (double that of untreated soil). Another major drawback of these calcium-based additives is its contribution to global carbon emission. The cement industry is the third-largest energy-consuming industry as well as the second largest contributor to global CO_2 emissions, with a total of 7% added to the global CO_2 count (WBCSD 2018).

A method followed to decrease environmental impacts from the production of calcium-based additives for soil stabilisation is the use of existing calcium-based additives derived from waste such as Fly ash for soil stabilisation.

Table 2.1. Cement and/or lime stabilised cases from the literature

Ref#	Description	Soil type	Additive	Test type	Findings
1	Case study of the failure of a pavement in Georgia which developed traverse bumps within months of construction.	Silty and clayey sand to sandy clays	**Cement**	Lab/Field	*Sulphate attack on the cement-based soil was ruled as the cause of pavement failure. Laboratory investigation shows the formation of ettringite. Calcium and alumina required to form ettringite were from Portland cement and the clay minerals in the soil, whereas the sulphur was from well water. Two types of sulphate attacks can occur in soils; type II sulphate attack occurs predominantly in cement stabilised soil rather than lime stabilised even at relatively low sulphate exposure. Type I sulphate attack is not a realistic threat for cement stabilised soils. The authors suggest looking at alternatives to cement based stabilisation with soils containing sulphates.*

Ref#	Description	Soil type	Additive	Test type	Findings
2	Evaluation of admixture properties such as compaction, strength and diffraction	Residual granite soil (kaolinitic clay)	Rice husk ash (RHA) and **cement**	Lab	*Considerable reduction in the plasticity of soil observed with treatment. Introduction on RHA reduces the amount of cement required to achieve a given UCS as compared to cement stabilised soil. CBR strength also reports being increased to a maximum of 60% at the combination of 4% cement and 5% RHA. Generally, 6-7% cement to 15% - 20% RHA is optimum to improve soil properties. Resistance to immersion also reported in the study.*
3	Long term stability characteristics of bentonite soil treated with 4% lime-based on wetting-drying and	Bentonite clay	**Lime**	Lab	*Significant reduction in plasticity index reported along with an increase in OMC and a decrease in MDD. Addition of lime shows a short-term positive effect on soil such as a reduction in the swelling potential of the soil, increased UCS and coefficient of permeability.*

Ref#	Description	Soil type	Additive	Test type	Findings
	freezing-thawing cycles as well as swelling and strength behaviours				*The authors report varied behaviour to the samples first submitted to wetting and drying, where the volumetric changes in the hydrated samples seem to stabilise within fewer cycles compared to the untreated samples. In contrast, in the dried samples, it progressively increases during cycles and renders the treatment ineffective due to the interruption of the clay-lime reactions during drying.*
4	Durability tests, including 3D volumetric swell tests, and UCS, conducted on soil treated with varying lime dosage.	Three Fat clays with varying Montmorillonite content with PI values close to 35 or above	**Lime**	Lab	*Untreated soil exhibited large amounts of volumetric strain and survived lesser wetting drying cycles compared to the treated samples.* *Montmorillonite content of the soil should also be considered along with the PI of the soil while determining lime content*

Ref#	Description	Soil type	Additive	Test type	Findings
5	Evaluation of tensile and compression strength of natural and treated soil.	Typical clay soil	Coir and **lime**	Lab	*Both indirect tensile strength (ITS) and UCS tests are sensitive to lime and coir fibre stabilisation with ITS more sensitive than the other. 1% of fibre content seems to be optimum to provide sufficient strength gain. Improvement in frictional bonding also observed with the treatment of the admixture. The fibres effectively held the cylindrical samples together resisting further development of cracks and prevented the complete failure of samples.*
6	Strength and durability evaluation of treated soil for the use in the construction of low volume traffic roads.	Lateritic soil	Arecanut coir and **cement**	Lab	*Medium improvement in soil properties observed with optimum content of 0.6% fibre to the soil. Combination of 3% cement to the optimum fibre content resulted in significant CBR and UCS improvements. Increase of fatigue life also observed with increase in coir dosage.*

Ref#	Description	Soil type	Additive	Test type	Findings
7	Investigating the effect of lime on soil fabric and strength.	Montmorillonite and quartz dominant clay soil	**Lime**	Lab	*Cementation of soil particles within the voids of the flocculated fabric in the soil reduces the compressibility while increasing the strength of the soil. The mechanism of marginal strength gain at lower lime content (up to 4%) is from the changes in the soil fabric, which shows an increase in permeability. In contrast, the drastic strength gain as seen from higher lime content (around 6%) is due to the cementation of soil particles which also reduces the permeability of the specimen. Analytical analysis confirms this hypothesis.*

[1] *Rollings et al. 1999*

[2] *Basha et al. 2005*

[3] *Khattab et al. 2007*

[4] *Pedarla et al. 2010*

[5] *Anggraini et al. 2015*

[6] *Lekha et al. 2015*

[7] *Jha and Sivapullaiah 2015*

2.2.2. Fly Ash Stabilisation

The increase in the global population has demanded a considerable amount of energy and resources, which inherently has increased the amount of waste generated globally. With the increased cases of many landfills around the world reaching near capacity, the call for proper waste management systems through recycling and reusing has been at an all-time high. The increase in the population has also increased the volume of construction work which in turn produces a considerable amount of waste materials as well as contributes to the rise in the emission of greenhouse gases. The cement industry, contributing to 7% of the global CO_2 emission, is the second largest contributor for these emissions as well as the third-largest energy-consuming sector in the world. To counter for these environmental and sustainability impacts, there is a call to decrease relying on cement-based additives as construction materials where other alternatives could be used. In the current construction industry, enhanced attention is being devoted to recycling/reusing waste materials to support engineering works. The use of fly ash has also attracted significant attention in the recent past due to its vast availability and benefits in ground improvements. Fly ash is a waste product attained from the burning of coal in thermal power plants during energy production. Although it is currently being used in various applications of construction, the utilisation rate is unable to match the rate of generation of coal ash that it contributes to around one-fifth of the entire Australian waste stream. Out of 12 million tonnes of coal ash produced per annum, only 44% is recovered, of which only half is used for beneficial purposes (Millington 2019).

The use of this chemical as a soil stabilising agent has been actively promoted as its disposal possesses a high degree of environmental pollution. However, fly ash cannot be solely used in the soil stabilisation application due to its non-cementing characteristics. In these cases, cement and lime activators might be used as traditional activators (Arora and Aydelik, 2005). Yilmaz (2015) tests the influence of fibres, fly ash, and a mixture of both into soil samples which reports that the sample that contains fly ash shows superior strength and characteristics to that of fibres alone. Samples with the fibre alone showed a decrease in the UCS of the mixture. However, when fly ash is combined with fibres, a significant increase in strength of the soil can be observed depending on the fibre type, length and dosage. Similar findings can be found in other researches with regards to the addition of fibres (Lekha et al. 2015, Anggraini et al. 2015). Anggraini et al. (2015), in their investigation, ultimately concludes that the combination of coir fibre and lime (as opposed to fly ash) resulted in the strengthening of the treated soil. Lekha et al. (2015) report that based on the soil type, coir dosage and curing length, superior improvements can be recorded in soils.

Fly ash, depending on the type of the coal burned, contain pozzolans that can instigate chemical reactions which could potentially increase the strength of existing soils. Fly ash is a by-product of powerplants that source their energy from coal and often cause considerable financial and environmental liabilities for the energy companies for its disposal. The utilisation of Fly ash holds the key to the reduction of waste disposal issues caused by fly ash as well as decrease the need to produce calcium-based additives for soil stabilisation. Application of fly ash in engineering highway embankments dates back to 1950 in England followed by other trial embankments which led to the acceptance of fly ash fill and roadway projects across European nations (Christopher et al. 2006). Reports show that the geotechnical property improvements by fly ash alone are not adequate for use in pavement and foundation design (Sharma et al. 2012). The use of fly ash has been reported as being useful in the literature (Kolias et al. 2005, Azadegan et al. 2013, Jha and Sivapullaiah 2015). Kolias et al. (2005) report the formation of a significant amount of tobermorite in fly ash – cement and clay admixture, which leads to a denser, more stable structure of the samples. The study also highlights the improvement in strength (compressive, tensile and flexural), modulus of elasticity and CBR which are considerably enhanced with the further addition of cement which provides better setting and hardening as well as an increase in early and final strength of the stabilised material. The study shows technical benefits of pavement structures incorporating subgrade improved with fly ash and cement analysed for construction traffic and operating traffic. Chen et al. (2009) investigated the influence of SO_3 content on cement – fly ash stabilised crush stoned which showed improvements of up to 120% in UCS of the material with 7.2% SO_3 when compared to the admixture with 1.8% SO_3. Brooks (2009) investigated the potential benefit of the admixture fly ash and rice husk ash (RHA) which are both waste materials. The admixture is reported to have increased UCS and CBR significantly with fly ash and rice husk content of up to 25% and 12% respectively. Sharma et al. (2012) report improvement in plasticity index (PI) and compaction limits of the soil, as well as reports optimum UCS and CBR strength improvement of samples treated using 20% fly ash and 8.5% lime. The pozzolanic reactions taking place in the fly ash and soil admixture generates long term strength gain and improve the geotechnical properties of the soil (Sharma et al. 2012). From the literature, it can be seen that many other combinations along with the fly ash such as fibres and Ground Granulated Blast Furnace Slag (GGBS) have also been trialled showing adequate strength gain (Yilmaz 2015, Sharma and Shivapullaiah 2016).

2.2.2.1. Mechanism

The efficacy of fly ash-based soil stabilisation relies on the pozzolanic reactions that occur within the additive and the soil. As highlighted previously, to date, numerous literature is available on the proposed mechanism of this additive. Kolias et al. (2005) credit the improvement in soil strength due to the formation of tobermorite, which results in a denser and stable sample structure from the cement and fly ash admixture. Sharma et al. (2012) report that the pozzolanic reaction overpowers the CEC as the stabilisation mechanism in fly ash soil interactions. In this pozzolanic reactions, calcium from lime and fly ash reacts with soluble alumina and silica from the admixture, in the presence of water to produce stable CSH and CAH, which in turn generates long term strength gain and improve the geotechnical properties of the soil (Sharma et al. 2012). The reaction observed as occurring is similar to the one seen in the cement stabilised soils.

2.2.2.2. Case Studies

Table 2.2 reports significant findings in various literature covering non-traditional calcium-based soil stabiliser fly ash. The summarised work includes the soil type, test type (i.e. lab tests or field test), the additive used and the key findings.

Table 2.2. Fly ash stabilised cases from the literature

Ref#	Description	Soil type	Additive	Test type	*Findings*
1	Assessing the usefulness of fly ash to improve the load-bearing capacity as well as the property of soil.	Three soil types	**Fly ash**	Lab	*Reduction of dry density and increase of void ratio observed with increase in fly ash content. Nonlinear increase in shear strength observed with increase in fly ash. A maximum value of cohesion can be increased in non-highly plastic soil. Increase in CBR also observed. Reduction of swelling properties observed with fly ash treatment could be due to the non-expansive nature of fly ash.*
2	Investigating the effect of fly ash on various properties of soil.	Soft organic soil	**Two types of fly ash**	Lab	*On both tested type of fly ash, moisture content decreases, and dry density increases gradually. Improvements in other geotechnical properties also noted along with the increase in strength*

Ref#	Description	Soil type	Additive	Test type	*Findings*
					properties, especially with increased curing time. Type I fly ash produce higher strength gain of the two types and is preferable for stabilising organic soil.
3	Evaluation of the effect of fly ash on fine sand compaction.	Building sand	**Class F fly ash** and cement binder	Lab	*Ordinary Portland Cement (OPC) has a profound influence on the dry density despite constituting of only 3% of the sample. Reduction in MDD and increase in OMC observed. Based on the results, fly ash sandy soil mixtures are suitable for sustainable embankment construction.*
4	Comparative study of lime stabilised and fly ash stabilised kaolin.	Soft clay-rich soil	Alkali activated high calcium **fly ash**	Lab	*New compounds formed which includes, thenardite as well as a silicate consisting of chains combined with calcium within 1 to 28 days. Compared to lime treated kaolin, the formation of*

Ref#	Description	Soil type	Additive	Test type	***Findings***
					the calcium-silicon chain phases in the alkali-activated soil is beneficial for long term stability.
5	Assessing the effect of combining MSWIFA with pre-treated cement stabilised soil.	Soft soil	**Municipal solid waste incineration fly ash (MSWIFA)** and cement	Lab	*UCS and internal friction angle of treated soil increase with OPC and pre-treated MSWIFA (PFA). 10% PFA treated soil is an ideal replacement for 5% OPC. Incorporation of PFA accelerates the formation of hydration products and performs as a cleaner form of foundation reinforcement.*

[1]*Prabakar et al. 2004*

[2]*Nath et al. 2017*

[3]*Mahvash et al. 2017*

[4]*Coudert et al. 2019*

[5]*Liang et al. 2020*

2.2.2.3. Influencing Parameters for Fly Ash-Based Soil Stabilisation

It can be seen from the previous sections that fly ash-based stabilisers have the potential to be considered as a sustainable and efficient form of pavement stabilisation, especially from an environmental aspect considering the fact that this form of stabilisation can help to mitigate the current coal ash disposal issue faced by many countries around the world. However, further studies are required to understand the fundamental mechanisms as well as the changes in both engineering and mechanical properties for it to be used in current engineering problems. Analysis of the literature review shows that these stabilisers induce some improvement in soil properties with results in some cases being inconclusive on certain soil conditions. Based on the comprehensive literature review that has been conducted, it can be identified that the following parameters are critical in determining the effectiveness of the fly ash stabilisation process.

Soil Type

It is crucial to understand the response spectrum of a stabiliser. Response spectrum refers to the type of soil in which the additive has shown positive efficacy. As mentioned earlier, the pozzolanic reaction mechanism taking place in fly ash stabilised soils are more dominant that the cationic exchange mechanism (Sharma et al. 2012). For this reason, the soil being treated should have soluble alumina and silica to produce stable CAH and CSH. It should be noted that majority of the cited literature has incorporated the use of soft clay-rich soils as the chosen material to be treated due to its weak load-bearing capacity which makes it unsuitable for pavement construction

(Prabakar et al. 2004, Nath et al. 2017, Coudert et al. 2019, Liang et al., 2020). Mahvesh et al. (2017) tested the efficacy of the additive when combined with a cement binder on building sand in contrast to soft soils tested in other literature. Chen et al. (2009) have also reported the positive efficacy on crushed stones treated with a combination of the fly ash and cement while Consoli et al. (2018) reported the effectiveness of stabilising reclaimed asphalt pavement (RAP) with fly ash, lime and a sodium chloride catalyst.

Secondary Additives and/or Activators

Sharma et al. (2012) highlighted that fly ash alone treated samples could not be adequate for the use in pavement or foundation design. Fly ash depending on the type of coal burned could be of two different classes. Type F fly ash is pozzolanic with little to no cementing value alone and is a by-product of burning anthracite or bituminous coal. In contrast, Type C fly ash has self-cementing properties as well as pozzolanic properties and is derived from the burning of lignite or sub-bituminous coal. The growing interest in alkaline-based activation dates back to 1950s. In this process, the activator reacts with fly ash to produce paste capable of setting and hardening. Cement and lime have been quite regularly used as an activator throughout the literature (Arora and Aydelik 2004, Chen et al. 2009, Sharma et al. 2012, Coudert et al. 2019, Liang et al. 2020). Combination of fly ash with other secondary additives has also gained traction, especially in the context of new and environmentally friendly binders. Fibres and Ground Granulated Blast Furnace Slag (GGBFS) have also been trialled showing adequate efficacy in terms of strength gain and geotechnical properties (Yilmaz 2015, Sharma and Shivapullaiah 2016) in the literature.

Application Rates

From the literature, it can be clearly seen that there is no set quantity of additive labelled as optimum required to see sufficient strength gain in treated samples. The tested amounts of fly ash range from as low as 5% (Khan and Sarker 1993) to 50% (Mohajerani et al. 2017) varying from literature to literature. Cement and lime as low as 3% (Eujine et al. 2017a, Mahvash et al. 2017) have also been reported in the literature as producing sufficient strength gains.

Curing Time

Investigating the effect of time on the strength of the sample is crucial as it helps better understand the mechanism of stabilisation. It can be seen in the literature that time plays a vital role in the efficacy of fly ash-based soil stabilisation. Samples have been reported to show improved strength within days of treatment using this additive. Coudert et al. (2019) credit the strength gain achieved from a high calcium fly ash and an alkali activator to the formation of thenardite as well as the calcium silicate chain phases which occur from 1 to 28 days of treatment. Kolias et al. (2005) credit the strength benefits due to formation of tobermorite in fly ash stabilised soil which continues to take place up to 6 months post-treatment.

From the literature, it can be seen that the use of calcium-based additives is continued to this day due to the improvement in strengths achieved through treatment. However, the calcium-based additives commonly encouraged to be used in engineering works is of the sustainable form such as fly ash, a by-product of coal-fuelled power plants. It can also be seen from the above sections that fly ash is often cited as being effective in cases where it is used in conjunction with other additives, i.e., while the introduction of fly ash alone can be seen as effective in strengthening the bearing capacity of the soil, in most cases, they are in need of other additives to instigate reactions. While investigation on the mechanism of combining fly ash with lime and/or cement is widely understood, there is a varying list of secondary additives that has the potential to have a positive effect on soil geotechnical and mechanical properties which has not been explored and does not have a strong understanding. This research aims to cover this research gap and contribute to the knowledge in understanding the effect of combining fly ash with non-traditional additives like enzymes. (The research gap has been reported in Section 2.5 of the chapter).

It should also be noted that the classification of fly ash as being a traditional additive is often debated. Traditionally, calcium-based additives used in the industry are limited to lime and cement due to its pronounced efficiency. As highlighted above, there is also a call to diverge from the use of calcium-based additives (mainly, cement and lime) as well as explore the efficacy of other types of stabilisers. The alternatives to these calcium-based additives are commonly referred to as non-traditional stabilisers.

2.3. Non-Traditional Chemical Stabilisers

All the available literature suggests that the above forms of calcium-based additives provide adequate stabilisation properties except for when subjected to sulphate rich soils where excessive heave is observed due to the formation of the mineral Ettringite. Another issue is that these additives are mostly termed as unsustainable. Cement industry contributes to around 5% of the global carbon dioxide emissions. The cement industry is growing annually by an average of 2.5%. Although the knowledge and availability benefits of cement stabilisation are vast and proven efficient in the current society, there is a call to rely on sustainable construction practices in the future. Inappropriate design of unbound pavements using these calcium-based additives might also cause the washing out of these additives, which in turn might cause other damages to the nearby vegetation as well as water bodies.

Among one of the oldest works on understanding non-traditional chemical soil stabilisation is reported in Oldham (1977). The report documents the history of a program initiated by the U.S military to evaluate materials for use as chemical additives for soil stabilisation. The literature evaluates a wide range of materials, both in-field and in the laboratory and recommends the use of cement, lime, and asphalt to affect the strength of the material positively. However, the authors do suggest that other additives should also be considered and investigated further to alter material properties effectively. Some of these recommended additives are:

- Lignin or lignosulphonates, a waste product from paper pulp manufacture which is a relatively cheap form of additive to acquire as well as facilitates effective stabilisation and dust control measures on specific clayey and silty soils.
- Phosphoric acid and phosphorus pentoxide, a hazardous yet effective form of stabiliser on specific clay soils.
- Aniline furfural resin, a toxic yet highly affective waterproofing agent which provides permanent waterproofing properties when added to clay soils.

All these forms of non-traditional additive investigation have led to a plethora of new additives surfacing. Tingle and Santoni (2007) classified the available non-traditional additives based on their major active component and classified them, as shown in Fig 2.2.

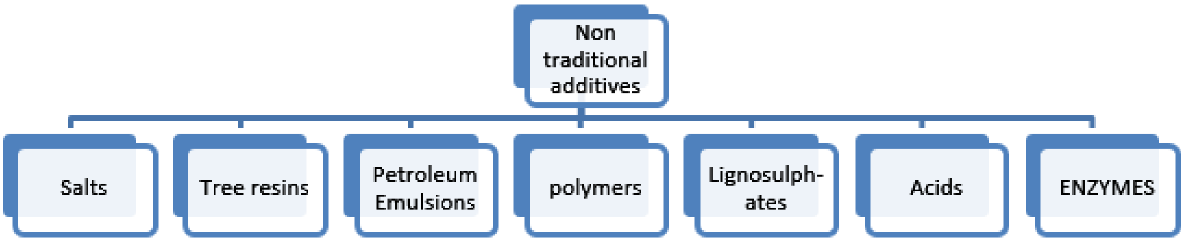


Figure 2.2. Classification of non-traditional additives (Tingle and Santoni 2007)

The classification was based on the reporting of stabilising additives identified by the U.S. Army Corps of Engineers and researchers between 1946 to 1977 (Oldham 1977). Oldham (1977) reports that these additives have potential effectiveness with varying degree of success depending on factors such as additive response spectrum, soil type and even climate and specific environmental conditions. However, a vast majority of additives tested and referred in the particular reference is no longer commercially available or has undergone formula alterations or change in trade names which renders the understanding of these additives less effective. However, the basic understanding of these additives can be identified as follows.

Salts

Salt-based soil stabilisers are generally electrolytes or crystalline salts. Tamadher et al. (2007) reported the finding on the investigation of adding chlorides including NaCl, $MgCl_2$ and $CaCl_2$ on the compaction characteristics, Atterberg limits and compressive strength of a silty soil. The increase in MDD and OMC of the treated soil with the increase in the salt concentration has been credited to the change in orientation of the clay particles under the influence of dynamic compaction. Compressive strength benefits were also reported in the study, which was due to the reduction in antiparticle repulsion and an increase in attraction resulting in better cohesion. Other studies have also been conducted to investigate the efficacy of combining salts with additives such as gypsum which highlights changes in the consistency limits and compaction characteristics with increasing additive concentration as well as substantial benefits in the CBR (Murthy et al. 2016). Oldham (1977) also noted that the commercial salts considered in the study were very particularly suited for specific climates and environmental conditions.

Resins

Resins have been subjected to investigation to understand stabilisation effects on a silty soil where a two-part epoxy system – bisphenol A/epichlorohydrin resin and a polyamide hardener at a ratio of 1:1 were combined soil to attain a significant increase in unsoaked CBR (Ajayi-Majebi et al. 1991). Depending on the admixture content, moisture content, and temperature of curing, the additive is reported in the study as being effective in producing effective strength improvement within 3 hours of mixing. Tree resins are additives which comprised of diverse emulsified by-products of the timber and paper industries. Tingle and Santoni (2003), reported effective resistance to moisture of CL soil type, although this effective moisture resistance is defined as maximum strength reduction of 50% of the dry samples as the soils disintegrate when placed in water in preparation for the wet UCS test. Anagnostopoulos (2015) reported strength development which directly depend on the water content and solid content of the soil where the moisture content adversely affects the polymerization of the resin, which in turn reduces the final strength. Despite the weakening of the polymer membrane, curing of up to 90 days shows satisfactory improvement in mechanical properties, compressive and splitting tensile strength and elastic modulus values. Combination of these polymers with cement shows significant strength improvements which could be from the consumption of large quantities of water from the cement compounds which promotes the polymerisation of epoxy resin with the hardener.

Petroleum Emulsions

Petroleum emulsions, asphalt or synthetic isoalkane fluids suspended in emulsions by a surfactant have also been reported in the literature. Abdullah and Al-Abdul Wahhab (2019) compare the efficacy of conventional emulsified asphalt (EA) with emulsified sulphur asphalt (ESA) reporting varying levels of effectiveness in mechanical properties of the soil such as improvement in indirect tensile strength and resilient modulus with ESA but decrease in the shear strength of the soils. While the study associates the decrease in the cohesion of the soil to the immiscible crystalline nature of the sulphur in the bitumen in ESA, the study highlights the importance of investigating different experimental methods to understand the effectiveness of an additive. Foamed Asphalt has also been reported in the literature as being effective in improving the strength of soil significantly, especially when coupled with as little as 2% cement with cost benefits.

Acids and Other Non-Traditional Additives

The effect of time on acid-based soil stabiliser reactions, when combined with lime, were investigated by Eisazadeh et al. (2011). Acid-based soil stabilisers are low aqueous solutions containing sulfonated molecules such as naphthalene or limestone. The findings of the literature suggest the alumina hydrate compounds are more likely to be formed with lime and acid (phosphoric acid) treatment which consequentially increase the UCS of the soil. Long term curing (up to 8 months) of the samples have also shown to make the phosphate and calcium ions less soluble in pore water. However, these reactions were mainly surface-associated reactions which cause significant changes in surface composition with time. Polymers, vinyl acetates or acrylic copolymer emulsions, lignosulphonates, organic polymers derived from lignin; and enzymes have been put to the both wet and dry UCS strength tests on varying soil types. The general efficacy of the remaining additives has also been investigated in Tingle and Santoni (2003) and compared against cement and lime additives. The key findings include that lime and cement were generally effective in stabilising clays. The acid-based additives were generally ineffective in improving the strength of clay, enzymes provided minimal effect from the treatment, and various tested commercial brands of polymers showed a varying effect on the soil. On the other hand, lignosulphonates provided excellent strength benefits from the treatment, especially in the low plastic clays and showed the best resistance to moisture.

It is evident from the literature that these non-traditional additives have the potential to serve as a sustainable alternative to calcium-based additives. However, the lack of understanding of the mechanisms of these additives deters the confident use of these additives in engineering practices. For this reason, there is a call to investigate and in-depth study on the effect of these additives on a fundamental level as well as a macroscopic level. Considering the need to utilise non-traditional additives for construction, the current study considers enzyme as a non-traditional additive which can be considered as sustainable from eco-environmental view. Enzymes have been selected within this current study as the ideal non-traditional additive due to its reported effectiveness in stabilising fine-grained soils. The manufacturers of these types of commercial additives claim their product to be a natural, biodegradable multi-enzyme product which is cost-effective and easy to utilise in pavement engineering applications (Cypher Environmental 2020). Section 2.4 provides an in-depth analysis of the concept behind these types of additives, what the available literature claims about its mechanism, detailed review of literature findings and the identified gap.

2.4. Enzyme Stabilisation

2.4.1. The Concept

The notion behind using enzymes from soil stabilisation is derived from the mound-building termites. Toronto Star published an article in 1990 introducing synthetic termite ant saliva in the Brazilian market by a former highway department employee named Anacleto Walmir Anglo. The product named ‘Dinasolo DS-328’ was being used in the state highway department on a large scale since June 1990. Anglo reported that he discovered the effects of this termite ant spit when he saw the workers use this material to fill chuckholes (Toronto Star 1990)

Pereira (2011) calls these termites ‘ecosystem engineers’ because they change the physical and chemical properties of soil. The author explains that the alteration of soil properties results from the organism’s salivary secretions and faecal excrements which improves CEC (Pereira 2011). Salivary secretions comprise of enzymes, hydro carbonates and non-digested lignocellulose whereas the faecal excretions are rich in organic matter such as organic carbon, calcium and phosphorous nutrients, potassium, magnesium, and nitrogen. Termite of the species *cornitermes cumulans*, which are known to occupy parts of South America is known to produce their mound by removing fine grained sand and adding silt to the existing soil while leaving the clay and coarse-grained sand unchanged. The top of the mounds is also discovered to have improved potassium, nitrogen and oxygen concentrations. Before their application in the construction field, *Saliva de cupim* (termite saliva) technology was utilized in the agriculture industry to improve the pH and the organic composition of the soils, which in turn assisted the crop productions.

Soil stabilisation using enzymes have been applied across the literature as a substitute for traditional calcium-based stabilisers, such as lime and cement, which are highly susceptible to heaving (Harris et al. 2006). Applications of this technology were incorporated in the engineering field due to its better clay strength, durability, resistance to weathering, abrasion and permeability characteristics. It has been recorded that the external earthen floors which use this technology increase dry resistance and comply with the Brazilian regulatory standards. Pereira (2011) suggests that the Termite Mound Soil (TMS) would be considered as an ideal solution for problematic soils provided that the soil has a significant amount of fines, a higher concentration of organic matter and a higher concentration of nutrients and/or CEC. TMS, when used in earthen floor and road constructions, are said to gain high cohesion as well as lowered permeability which helps with the durability of the road.

The cohesion and restricted permeability characteristics have led to the utilization of the technology in Brazilian structural restoration projects such as *Fazenda Jardim* farm, in 2004 which used TMS earthen walls and in the second restoration of *Igreja do Rosário* church, 2002-04 which used enzyme mortar for adobe. Both the projects have been reported to produce impressive cohesion and resistance properties. A summary of further applications of enzyme-based soil stabilisers is presented in Table 2.3 under Section 2.4.3 (Case Studies).

2.4.2. Mechanism

Soils work as electrolyte system in which they are charged particles susceptible to reaction with other materials. Soils are often classified according to the CEC. CEC is a good indicator of the holding capacity of soils. Soils hold nutrients and minerals through their ion exchanging mechanism. Clay's molecular structure provides them with a net negative charge which allows positively charged cations to cling onto it. Clay, at a molecular level, is made up sheets of silica oxide tetrahydrate and alumina hydroxide tetrahydrate. The arrangement of these sheets forms various clay minerals. For example, kaolin clays are stable layers of a positively charged silica sheet balanced by a negative charged hydrated alumina sheet. In contrast, illite and smectite, have a silica sheet on either side of the alumina sheet. The layers are held together by the shared cations as in the case of illite and smectite clays or by the attraction between the H+ ions in the alumina's hydroxyl and the O- of the silica's oxygen with the excessive negative charge holding the ionised water to the surface. Therefore, at a molecular level, clay structure is latticed made up of repeating layers of silica and alumina hydrates with charged cations attached to them, namely, potassium, sodium, and calcium and ionised water.

In most cases the cations are exchangeable. The weaker clays absorb this ionised water in between these layers facilitating the expansion and loss of density. To stabilise these clay molecules, the additive must provide strong soluble cations capable of exchanging with the cations from the clay lattice and surround the clay particles. The absence of the cation from the clay particle will result in the breakdown of clay into smaller particles which in turn helps the water to escape from the lattice forming a hardened mineral.

Enzymes, when added to the soil, requires the means to get to the reactions site. This is achieved through the pore fluid. Based on this soil chemistry, there are four main mechanisms by which enzymes could stabilise the soil:

Organic Molecular Encapsulation

Scholen (1992) proposed that when the stabiliser is added to the soil, firstly it behaves like an ionic additive by which the double layer water is reduced. The double layer water refers to the collective negative charged clay surface along with the positively charged cation that surrounds it. Firstly, when the stabiliser is added to clayey soils which have low permeability, reactions with any form of stabiliser can still be seen occurring in the clay soil mass which indicates the work of an electrokinetic phenomenon. The double layer water responds to this electrokinetic phenomenon. The point of injection of this additive would have highly concentrated negatively charged “ion cloud” which attracts the cations in the clay, facilitating the development of overall net negative charge in the silica sheets destabilising the layers subjecting the molecular bonds to collapse. This reduction in the double layer of water reduces the size of the clay particle stabilising it. Fig 2.3 presents a schematic diagram of this hypothesised mechanism illustrated by Tingle et al. (2007). Scholen (1995) also proposed that depending on the soil type, the enzyme-based additive encapsulates the organic molecule in the soil and neutralises the net charge, which would further decrease the clay particles affinity for water. Depending on the type of soil and even the type of the additive, the molecular bond collapse in the soil could be in the form of alumina sheet breaking and hydrolysing to gibbsite, while silica sheets hydrolyse and decompose to amorphous silica. Scholen (1992, 1995) differentiates the enzyme as being of two kinds. The first kind requires a high amount of clay and silt within the soil which provide organics from the humus from these fines. The second kind is a bio-enzyme, through the use of which a bacteria culture is introduced into the soil system which utilises the carbon dioxide, nitrogen, and oxygen present in the air to produce further organics which could surround the clay particle neutralising the charge of the clay. Both the type of enzymes has been reported to remain permanently active in the soil which has the potential of stabilising any further clay which could be added to the originally stabilised soil after a considerable time post-stabilisation. The author hypothesised this mechanism based on field studies conducted. When referring to the case studies which reported an increase in strength, Scholen (1992) hypothesise the strength gains in the soil to account from the combining of the enzyme to the large organic molecules within the soil. The combination forms a reactant intermediary which then breaks down the clay lattice causing covering up of the particles, which inherently reduce any further sorption of water.

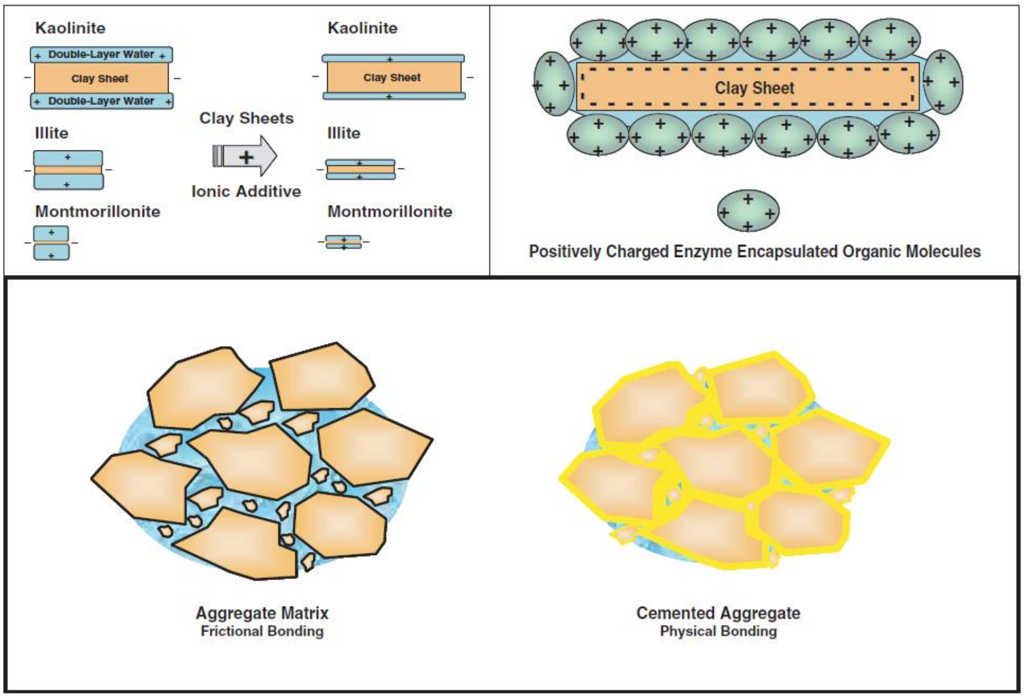


Figure 2.3. Mechanism of ionic and enzyme-based stabiliser (Tingle et al. 2007)

Aggregation of Particles

(Rauch et al. 2003) conducted investigation to identify efficacy as well as the mechanism of non-traditional three liquid chemical agents on soil stabilisation identified as ionic, enzymatic, and polymer stabilisers. The study reports the laboratory findings of these additives on three reference clay types (kaolinite, illite, and montmorillonite) and two native clays with the selected additives. The characterisation tests conducted on the enzyme revealed that the pH of the diluted enzyme was 3.26 with the conductivity measurement of 0.791 mS/m, which bears a resemblance to high purity water. The Total Organic Carbon (TOC) tests confirm the presence of a very large amount of organic carbon in the enzyme stabiliser. HPLC/MS and UV/Visible spectra tests confirm the presence of polyethylene glycol as a component of the enzyme. However, it was hypothesised that it might not be an activating ingredient. The BET nitrogen adsorption results show that for all the clay minerals stabilised with enzyme showcase a significant reduction in the surface area. The reduction in the surface area of the kaolinite soils was noted to be significantly less due to the size of the pores in kaolinite soil. As reported in the study, pores with radii less 50 Å is eliminated with the drastic reduction in pore radii from 700 Å in the untreated soil to 300 Å for the enzyme treated case which suggests the better binding of clay particles. With the exception of kaolinitic clays, microscopic imaging of other clay minerals shows a more aggregated surface of the enzyme treated clay particle. A reduction in the clay features is also noted with no change in the composition of the clay as hypothesised by Scholen (1992, 1995). The d-spacing of soil increases as the enzyme treated clay layer expands completely. Minor changes showed in XRD and surface area suggested that the stronger pull on the clay layers forces moisture out of the clay resulting in higher strength and reduction of plasticity. The decrease in the surface area indicates the binding of clay particles by aggregation. Khan and Sarker (1993) also credit the increase in the strength of the soil to this aggregation on a microscopic level on the clay surface. Rauch et al. (2003) is a vital study when it comes to enzyme-based soil stabilisation, as it proposes application rate for the additives based on two parameters, the dilution rate of the additive as well as the application rate of this diluted additive. The study also proposes a sample preparation method based on the mechanism of the additive.

Microbe Induced Calcite Precipitation

Urease, an artificially extracted enzyme from plants, have also been noted in the literature as being used to improve engineering property of soil through the process of Microbial Induced Calcium Precipitation (MICP). In this process, a highly active urease enzyme catalyses the hydrolysis of urea into ammonium and carbonate and elevate the pH. During this hydrolysis reaction, the bacterial cell with negative charge absorbs calcium ions to deposit on its surface and provide a nucleation site for crystallisation of calcium carbonate at the pore spaces which in turn help achieve desired soil mechanical properties (Cheng and Cord-Ruwisch 2014, Oliveira et al. 2017). The following Equation 2.3 can describe the process (Cheng and Cord-Ruwisch 2014).

$$CO(NH_2)_2 + 2H_2O \rightarrow 2NH_4{}^{+} + CO_3{}^{2-} \qquad \textbf{\textit{(Eq. 2.3)}}$$

Which, in the presence of dissolved calcium ions will precipitate to form crystallised calcium carbonates, as shown in Equation 2.4.

$$Ca^{2+} + CO_3{}^{2-} \rightarrow CaCO_3(s) \qquad \textbf{\textit{(Eq. 2.4)}}$$

Dejong et al. (2010) emphasise the necessity of exploring soil improvements techniques such as biological mediation of soil as an alternative to calcium-based additives which have resulted in causalities worldwide. It is believed that the alteration of soil properties through bio-mediation are dependent on the by-products of the chemical reactions occurring in the soil with the addition of these stabilisers. The authors categorise the generated by-products into inorganic precipitation, organic precipitation and gas generation via the process of biomineralization, biofilm formation and biogas generation respectively. Notable by-products of biomineralisation include magnetite, greigite, amorphous silica and calcite (Konhauser 2007). Dejong et al. (2010) highlight the importance of understanding the stabilisation process of such additives at a fundamental level to develop optimisation studies as well as to understand the possible degradation mechanisms which affect the service life of treated soil. The efficacy of the bio-mediated soil through biomineralization covers a wide spectrum of soil types, whereas the efficacy of biofilms on the soil is limited to sand and coarse gravel. This could partly be due to the soil types tested in the research available to date. The authors illustrate the process of biomineralization occurring through ureolysis from the metabolic activity of *sporosarcina pasteurrii*, with urease enzyme schematically represented, as shown in Fig 2.4. Dejong et al. (2010) utilised microscopy techniques to establish that calcite precipitation occurs at pore spaces preferentially near particle to particle contacts resulting in the densification of the soil. The examination of the image sequence with increase magnification shows the calcite coating on the surface of the particles with increased concentration of these calcites at the particle to particle contact, which in turn decreases the pore space. The authors report the effectiveness of this form of bio treated soil based on the reduction of the void ratio and densification, which in turn provides significant strength improvements. A more in-depth look into this urealytic bacteria could highlight the resemblance of this type of additive as the “bio-enzyme” referred to by Scholen (1992).

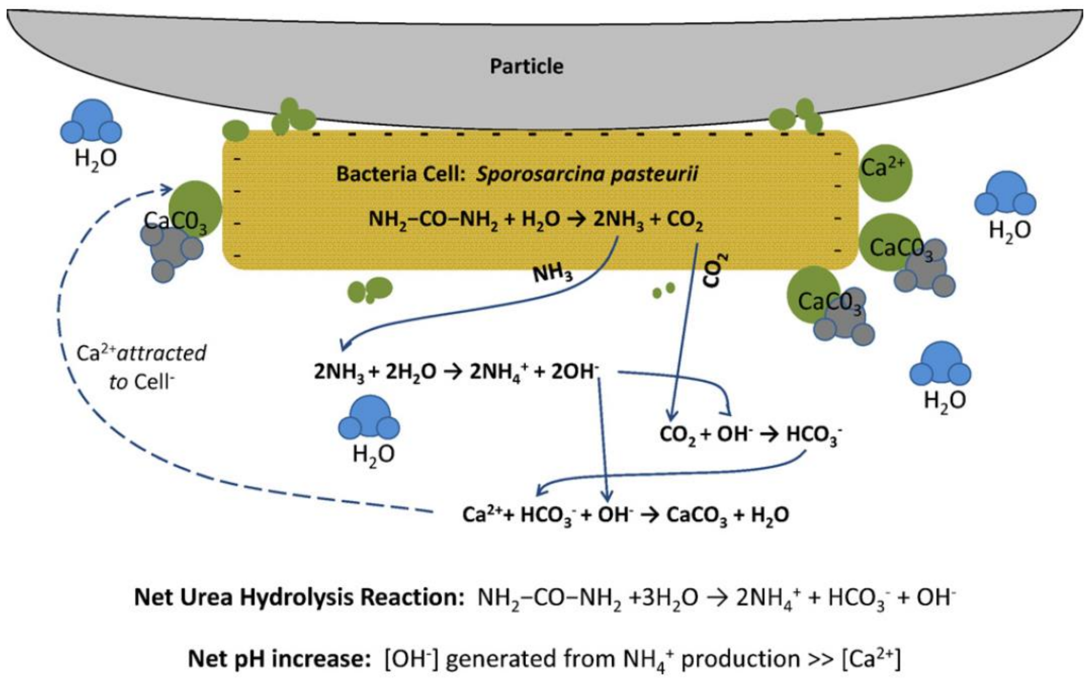


Figure 2.4. Schematic diagram of bio-mediation of soil using ureolysis (Dejong et al. 2010)

Catalytic bonding process

Tolleson et al. (2003) highlight that enzymes help stabilise the soil by employing various types of mechanisms which include a catalytic bonding process. This process refers to the decrease in the activation energy of an enzyme catalysed reactions by two of the enzyme principles. Cooper (2000) states that these principles include, increasing the rate of the chemical reactions, with the enzyme itself, not being consumed as well as not altering the chemical equilibrium between reactants and products. In this reaction, a substrate (S) is converted into a product (P) with the chemical equilibrium, which is determined by the law of the thermodynamics. The chemical equilibrium refers to the rates of forward and reverse reactions between the S and P. As highlighted by the author, the forward or the reverse reactions rates remain equal during the catalysis. The author illustrates that the energy changes occurring in the reaction, as shown in Fig 2.5, in which the S can be seen being converted to P due to P having a final energy state which is lower than the S. It should also be noted that the incorporation of the enzymes decreases the activation energy and as seen could lead to the reaction proceeding at an accelerated rate. In this catalytic stage, the enzymes bind the substrate to form an enzyme-substrate complex (ES) based on the active site. The active site refers to a specific region in the enzyme which provides a surface on which reactions can occur more readily. While bound to the appropriate specific site, the substrate is converted to the product of the reaction, which can be based on the following equation (Equation 2.5):

$$S + E \rightleftharpoons ES \rightleftharpoons E + P \qquad \textbf{\textit{(Eq. 2.5)}}$$

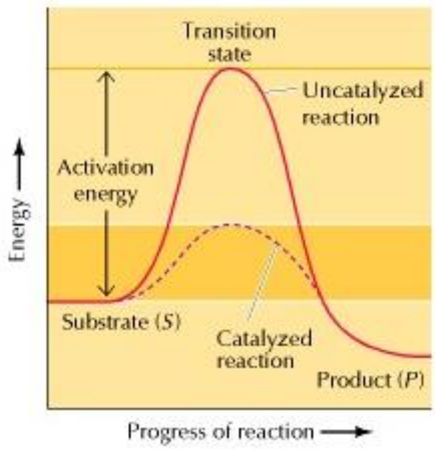


Figure 2.5. Energy diagram for reactions (Cooper 2000)

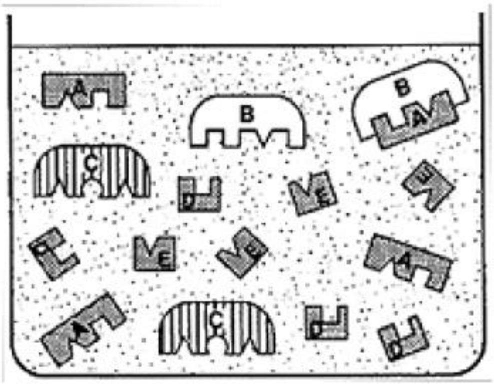


Figure 2.6. Proposed enzyme stabilisation mechanism (Tolleson et al. 2003)

The mechanism of how enzymes work in converting the substrate to the product could be of varying types depending on the active sites of the enzyme which are in the form of clefts or grooves on the surface of the enzyme. Tolleson et al. (2003) hypothesise the mechanism based on the following illustration (Fig 2.6) where the author explains the enzymes (B and C) provide a surface onto which materials in the soils (D and E) can be adsorbed onto by bringing the particles together which in turn allows better binding by producing product A.

2.4.3. Case Studies

Table 2.3 summarised findings from various literature covering enzyme-based stabilisation, including the year of application, soil type and location, application basis, the additive used and key findings. The table showcases studies which date back to field trial roads constructed in 1981 which has been recorded as the oldest use of enzyme to date. It should be noted that the segments that are left blank in the additive type refers to no additional information on the additive used is disclosed in the cited literature. The commercial name of the additive has been italicised.

Table 2.3. Enzyme stabilised cases from the literature

Ref#	***Description***	***Materials***	***Additive***	***Test Type***	***Findings***
1	Assessing the long-term effect of stabilising using enzyme-based additives based on case studies of projects from 1981 – 1990.	Silty clay (LL=20, PL=40) subgrade soil with 20% fines using	*Permazyme*	Field	*1981 - Richmond road, (Stillwater, Oklahoma) Gravel added for traction for surfacing. No maintenance work required for over ten years post-construction*
		Various types ranging from crushed rocks and aggregates with 15% - 45% fines	*Permazyme, BioCat, EMC* (commercial enzymes)	Field	*Light to little damage noted at most of the treated road section, especially compared to the untreated sections.* *Occasional scheduled blading were required at some sites whereas other sites required little to*

Ref#	*Description*	*Materials*	*Additive*	*Test Type*	*Findings*
					no maintenance.
2	Assessing the efficacy of enzymatic fly ash as a soil stabiliser	Kaolinite clay, (LL=57.26, PL=35.73)	*Fly ash, millpond sludge and enzyme*	Labs	*5% enzyme report peak results higher than lime stabilised soil*
3	Case study of enzyme treated pavement (27.2 km) in Malaysia on a plantation road constructed in 1996.	Lateritic clay soil	*Terrazyme*	Field	*No surface damage despite monsoon conditions as well as the decrease in road maintenance costs reported.*
4	Assessment of enzyme-based soil stabiliser on	Various clay and aggregate soils	*EMC squared, Permazyme,*	Field	*The trail had a soft surface that was uncomfortable to ride for longer periods and became muddy and slippery after first rain with*

Ref#	*Description*	*Materials*	*Additive*	*Test Type*	*Findings*
	universally accessible trail roads in Oregon and Arizona, USA between 1994 – 1998.		*Eco-crete*		*deep ruts forming. Overall, the enzymes did not noticeably stabilise aggregate materials. The product was also not effective in soils with 3% clay or 50% clay.*
5	Monitoring the effect of enzyme treatment on 200 mm (depth) stabilised pavements across three suburbs in Brazil	Silty and clayey soil	*Terrazyme*	Field/Lab	*Reports up to a fifteen-fold increase in CBR compared to the untreated sections along with other benefits such as elimination of washboards and ruts, dust control*
6	Understanding the effect of stabilising with enzyme-based additive based on	Clay soil (LL=30.5, PL=19.6) with 27% fines		Field/Lab	*Report reduction in plasticity and increase in CBR with time. The pavement is reported to have maintained its service four years post-construction*

Ref#	***Description***	***Materials***	***Additive***	***Test Type***	***Findings***
	economic and ecologic benefits of a pavement stabilised in Itapiuna, Ceara, Brazil				
7	A case study of four pavements constructed in the USA from late June of 2001.	Various type of soil with a high amount of fines as well as a loam mixed clay with recycled asphalt	*Terrazyme*	Field	*Report increase in CBR from 23% to 33% up to 79% six weeks post-construction as well as dust reduction of up to 75%*
8	Assessing the effect of liquid stabilisers on the engineering properties of the soil	Mesquite, kaolinite, illite and montmorillonite rich clays		Lab	*No consistent significant changes to the soil properties*

Ref#	***Description***	***Materials***	***Additive***	***Test Type***	***Findings***
9	Investigation to understand the efficacy as well as the stabilisation mechanism of liquid-based stabilisers including enzymes	Heavy silty and clayey soils stabilised		Lab	*Report reduction in surface area of all clay minerals and soils and insignificant, inconsistent improvement in soil engineering properties.* *Enzymes affected neither the OMC/MDD nor produced notable strength improvements. No consistent reduction in the expansiveness of soils is reported with the treatment.* *Minor shear strength improvements observed for two of the tested clays of which one report 1 to 3% lower swell potential.*
10	Assessing the performance of pavement subgrade stabilised with	Laterite, clay, and sandy soils	*Terrazyme*	Lab	*Soil containing large amounts of clay and silty seem to benefit the most from the treatment based on the increase in CBR (by up to 700%)*

Ref#	***Description***	***Materials***	***Additive***	***Test Type***	***Findings***
	enzymes				
11	Evaluating the performance of enzymatic stabiliser along with calcium-based stabilisers	Various clays and silty sands		Lab	*Enzyme treated soils showed modest strength gains, lowest stiffness values and showed little or inconsistent strength gain (20% - 80%) with time.* *Low strength loss also reported although the strength was low to start with on freeze-thaw tests. However, Enzyme samples did not survive the first wet-dry cycle.*
12	An Evaluation of strength change on subgrade soils stabilised with an enzyme catalyst	Sandy, silty, and clayey soils	*PZ-22X*	Lab	*Soaked CBR strength change of up to 140% with fines of up to 30% compared to negligible with higher fines. An average strength increase of 52% reported. Unsoaked strength showcased higher gain compared to soaked cases.*

Ref#	***Description***	***Materials***	***Additive***	***Test Type***	***Findings***
	solution				
13	Preliminary investigation on the effectiveness of enzyme soil stabilisers	Two soil types with a high amount of fines	Two commercial enzymes	Lab	*Report that the increase in application rate did not improve efficiency on the form of stabilisation with a minimum of 4 months required to enhance shear strength*
14	Case study reporting the use of non-traditional additives	Fine grained soil	Enzyme and urea-formaldehyde	Field	*Report that visually the treated section road as good as cement stabilised trial road. However, no monitoring was done to measure improvement due to lack of funding.*
15	An investigation into subgrade stabilisation using enzymes	Two natural Minnesota subgrade soil types with high amounts	Two commercial enzymes	Lab	*Soil I: enzyme B resilient modulus improved by 69%; enzyme A increased shear strength by 9%; enzyme B increased shear strength by 31%* *Soil II: enzyme A and enzyme B increased*

Ref#	Description	Materials	Additive	Test Type	Findings
		of fines			*resilient mod by 54% and 77%, respectively; enzyme A and B increased shear strength by 23% and 39% respectively.*
16	Understanding the efficacy of non-traditional soil stabilisers on sulphate rich soils	Four different soil types		Lab	*<1% reduction in 3d swell for 20,000ppm sulphates soil seen on enzyme stabilised soils which rendered it useless to be recommended for subgrade stabilisation for TxDOT*
17	Work conducted by the U.S army corps engineering research and development centre to compare the efficacy of non-	Fine grained soils		Lab	*Enzyme stabilisers gave low strength improvement, low to medium volume stability and low waterproofing qualities during the investigation.*

Ref#	Description	Materials	Additive	Test Type	Findings
	traditional stabilisers				
18, 19	The performance of soil stabilisers on South African unpaved roads with respect to its effects of material strength. Construction and subsequent testing of 1m × 3m × 0.15m panel in South Africa over a nine-month testing period.	Sandy to clayey soil		Field	*Enzyme increased strength when applied to sandy material with low PI over the test period. The enzyme showed significant improvement on well-graded clay and clayey sand with a significant increase in soaked strength. However, no good correlation could be made between lab and field tests.*
20	Evaluating the effect	Black clay and	*Permazyme*	Lab	*Insignificant reduction in PI, a slight*

Ref#	Description	Materials	Additive	Test Type	Findings
	of enzyme stabiliser on common subgrade soil types	reddish-brown chert stabilised	and *Earthzyme/Roadstabilizor*		*improvement in MDD and inconsistent improvement in strength. However, <50% increase in strength with increase in concentration.*
21		Soil A: Lateritic soil with 30% fines sandy silt, Soil B: Lateritic soil plus sand	*Terrazyme*	Lab	*Medium improvement in physical properties of lateritic soil observed with a certain dosage of the enzyme. However, the enzymes are ineffective for improving consistency limit.* *Significant improvement in UCS (by 450%) and CBR (by 300%) and reduction of permeability (by 42%) reported. The enzyme is not effective in soil containing cohesionless soil.*
22		Two clay soil with	Three commercial	Lab	*Increase CBR from 5% to 70% noted. However, there is an increase in swell from 5% to 350%*

Ref#	***Description***	***Materials***	***Additive***	***Test Type***	***Findings***
			enzymes		*on CH type soil. Therefore, the enzyme is highly unlikely to be a substitute for that soil type.*
23	Investigating the effect of the enzyme of the swelling characteristics of the soil	Fat clay with 95% fines	*Terrazyme*	Lab	*Lesser swell exhibited on treated soil. A decrease in swell exhibited in 30 days with no further changes reported after. The treated soil also exhibited a flocculated structure on the dry side of optimum*
24	An investigation into stabilising base course material with enzyme-based stabiliser	Natural soil with 30% fines and natural sandy gravel	*Terrazyme*	Field	*Reports effective use of the additive for the stabilisation process. The study also highlights the economic and environmental benefits of using the additive for pavement stabilisation.*
25	Comparative testing on enzyme-based soil	Soil A: fine grained, soil B:	*Permazyme*	Lab	*The study reports a significant improvement in strength between 7 and 60 days in fine grained*

Ref#	*Description*	*Materials*	*Additive*	*Test Type*	*Findings*
	stabiliser with lime-based additive	silty loam and Soil C: coarse-grained soil using			*and coarse-grained except the silty loam soil. UCS of lime stabilised soil lower than enzyme treated, which could be because the enzyme treated section was sealed during curing.*
26	Architectural works in Brazil with utilises the enzyme technology.				*Fazenda Jadim farm (1693). Restoration work in 2004 used saliva de cupim in earth walls which showcased better cohesion and stability due to water repellent properties* *Igreja do Rosário church (1728-1761). Restoration 2002-04 with enzyme mortar for adobe. Samples reported having higher cohesion, compaction and corrosion resistance.*
27		Three soils with varying levels of		Labs	*Significant increase in UCS (ranging from 150% to 200%) and CBR (ranging from 157% to*

Ref#	Description	Materials	Additive	Test Type	Findings
		fine from 12% to 40%			*673%) in all soil 1-3. It was also reported that around four weeks is required for high strengths*
28	Performance monitoring of pavements stabilised with different types of non-traditional additives	Five 100 m sections of trail road with two between 40% to 80% passing through 2 mm sieve	Two commercial enzymes	Field	*Report varying level of effectiveness. Two sections report a decrease in strength; one section seems to be unaffected by the treatment, whereas the other two sections show a positive effect of stabilisation. Comparative study shows that enzyme stabiliser performs worst, especially after factoring in the roughness of the pavement with time.*
29		Three soil types with varying level of fines using		Lab	*Report reduction in plasticity and shrinkage limits of the soil by eliminating reabsorption of water molecules. Change in compaction property also noted such as the reduction in*

Ref#	Description	Materials	Additive	Test Type	Findings
					OMC by ionizing and exchanging the water molecules on the surface of the clay platelets as well as increases the MDD by neutralizing and orderly re-arranging the clay platelets. *Increase in the compressive strength of the sample due to the increase in the inter particles bonding also reported.*
30	Follow up study to Shankar et al. 2009 with the application of the enzyme stabiliser on a 1.35 km x 3m pavement in India	Soil A: Lateritic soil with 30% fines sandy silt, Soil B: Lateritic soil plus sand	*Terrazyme*	Field/Lab	*Fatigue life increases for lateritic soil with 4% optimum dosage. A marginal increase in strength exhibited after four weeks* *The field test showed improved soil CBR values*
31	Assessing the effects	Silty soil		Lab	*Report decrease OMC by 3%, increase in MDD*

Ref#	***Description***	***Materials***	***Additive***	***Test Type***	***Findings***
	of acid, enzyme and lignosulphonate stabilised soils				*as well as 25% less compaction energy. The authors highlight that the enzymatic solution behaves similar to that of surfactants. Slightly improved UCS also reported which has been credited to the increase in MDD.*
32	Assessing the effect of the enzyme of highly common fine-grained soil	Black cotton soil, high clay content	*Terrazyme*	Lab	*Treatment increased UCS by 200% with seven days curing process reported to give significant strength increase.*
33		Montmorillonite rich clay soil with 68% fines	*Terrazyme*	Lab	*Changes in the consistency limits noted such as the increase in liquid limit within the first two weeks, which was followed by its decrease. However, the plastic limit seemed unaffected by the treatment. A twelve-fold increase in the UCS*

Ref#	***Description***	***Materials***	***Additive***	***Test Type***	***Findings***
					of the soil also noted.
34		Black cotton soil with 70% fines from Karnataka, India	*Terrazyme*	Labs	*The authors report the reduction of PI to 15% after eight weeks and an increase in stiffness. Increase in UCS from 268 kN/m^2 to 859 kN/m^2 within eight weeks also noted from the treatment. No change in MDD, however, the OMC decreases with increase in curing time. Reduction in the swell capacity from the treatment observed along with the increase in CBR with time. The samples could not, however, survive the wet-dry cycles*
35	Monitoring the effect of enzyme and sulfonated stabilised	Weathered Quartz gravel treated		Field	*Enzyme treated soil continues to show changes in terms of strength and microstructure. SEM imaging shows cornflake like grains coating the*

Ref#	***Description***	***Materials***	***Additive***	***Test Type***	***Findings***
	soil strength during a three-year testing period				*soil particles forming interparticle bonding.* *The report also shows higher in-situ DCP-CBR value after four months of curing with a minimum of two months taken to achieve max strength improvement. A slight improvement observed 31 months after construction* *However, due to the level of the immediate increase in strength of the soil, a "POOR" rating was given to the treatment.*
36		Soils obtained from five different locations ranging from clayey and silty soils		Lab	*UCS increased when the enzyme dosage and curing period increased. CBR of the treated soil higher than natural soils* *The enzyme has more effect on CL group compared to MH and CH groups*

Ref#	***Description***	***Materials***	***Additive***	***Test Type***	***Findings***
37		Two inorganic clayey soils and one sandy soil (40% fines)		Lab	*Enzyme treatment was not effective in wet conditions for one inorganic clay. However, a gradual improvement in CBR with the other treated inorganic clay. Unsoaked CBR is higher than soaked. Both soaked and unsoaked samples showed an increase in CBR in the sandy soil.*
38	Assessing the effect of combining enzyme treated adobe with fibres	Natural soil: 40% clay and 45% silt; Blended soil: 23% clay, 27% silt, 50% sand		Lab/Field	*Enzyme increased apparent density of the treated samples. The mixture of stabiliser and fibres showed higher compressive strength where the fibres limit the forming of cracks during the compressive test*
39	Effect of combining cement and enzyme for the treatment of	Clayey and silty sand with 21% fines using	Two commercial enzymes and	Field	*Enz A + cement yields higher strength compared to other treatments.*

Ref#	***Description***	***Materials***	***Additive***	***Test Type***	***Findings***
	unpaved roads		cement		
40		Red soil (71% clay and 29% silt) with sand and gravel	*Terrazyme*	Lab	*UCS peaks at seven-day curing period and decrease with time. However, the UCS is higher than untreated soil at all times*
41		Black cotton soil; Red earth	*Terrazyme*	Lab	*Black cotton: LL increased after seven days and then decreased, but PL decreases continually.* *Red earth: similar to black cotton in terms of LL. both soils showed hydrophobic nature.* *Little to no effect on compaction characteristics.* *Tremendous improvement in UCS strength and unsoaked CBR with treatment about 500%* *Air dry curing better than desiccator curing*
42		Silty Clay	*Terrazyme*	Lab	*Soaked CBR increases by 300%, unsoaked CBR*

Ref#	***Description***	***Materials***	***Additive***	***Test Type***	***Findings***
					by 500% after curing for two weeks
43		Soft soil		Lab	*LL decreases, PL increases* *Increase in additive dosage increases MDD and decreases OMC, UCS increase of up to 400%*
44	Assessing the water adsorption property of enzyme treated soil in a two-year experimental analysis	Highly plastic clay	*Earthzyme*	Lab	*Enzyme additive reduced the water retained in the pores of partially saturated soil. Treated soil also has a lower moisture content which helped with the increase in shear strength. The treated soil also absorbs the water in the capillary rise test at a slower absorption rate.*
45	Comparing the effect of enzymatic lime over lime and	Two artificial clay minerals Bentonite and Kaolinite	Lime and enzyme	Lab	*The addition of enzyme to lime treated soil increased the rate of strength gain during the first three weeks but diminished after four weeks,*

Ref#	***Description***	***Materials***	***Additive***	***Test Type***	***Findings***
	enzyme.				*albeit remaining higher than the rate of strength in either additive alone treated samples.* *The admixture is more suited for kaolinite minerals.* *The addition of enzyme decreases the quantity of lime required, which adds to the environmental friendliness of the stabiliser.* *Due to the ineffectiveness of the stabiliser on bentonite (which has the ability to mimic montmorillonite), the treatment might not be suited for montmorillonitic clays.*
46	Follow up study of ref#45	Five artificially clayey soils with varying levels of	Enzymatic lime	Lab	*Enzymatic lime imparts better and quicker stabilisation of the of soils due to the transformation of the soil into a stronger*

Ref#	***Description***	***Materials***	***Additive***	***Test Type***	***Findings***
		fines and mineral (kaolinite and Na-Bentonite)			*permanent soil matrix in half the time required by either additive alone.* *The decrease in PI suggests the reduction in swelling and shrinking of the treated soil*
47	Comparing the effects of alkali-activated ground granulated blast-furnace slag, enzyme, and OPC on the soil.	Soil (LL=42.25%, PL=18.6)		Lab	*Reduction in MDD and increase in OMC noted with the treatment of each additive. Increase in UCS and shear strength parameter also noted, especially on the cohesion of the soil.* *UCS and shear strength parameters of Alkali activated GGBS surpasses OPC treated soil with 28-day curing required to note a significant effect on the UCS.* $UCS_{enzyme\ treated} < UCS_{OPC\ treated} < UCS_{GGBS\ (alkali\ activated)}$

[1] Scholen 1992

[2] Khan and Sarker 1993

[3] Hitam et al. 1999

[4] Bergmann 2000

[5] Brazetti and murphy 2000

[6] Kensenhuis and Modi 2001

[7] Thompson et al. 2002

[8] Rauch et al. 2002

[9] Rauch et al. 2003

[10] Kuncheria et al. 2003

[11] Parsons and Milburn 2003

[12] Tolleson et al. 2003

[13] Marasteanu et al. 2005

[14] Campbell and Jones 2011

[15] Velasquez et al. 2006

[16] Harris et al. 2006

[17] Tingle et al. 2007

[18] Visser 2007

[19] Van Veelen and Visser 2007

[20] Mgangira 2009

[21] Shankar et al. 2009

[22] Yilmaz et al. 2009

[23] Naagesh and Gangadhara 2010

[24] Li et al. 2011

[25] Peng et al. 2011

[26] Pereira 2011

[27] Venkatasubramanian and dhinakaran 2011

[28] Uys et al. 2011

[29] Ali 2012

[30] Shankar et al. 2012

[31] Blanck et al. 2013

[32] Agarwal and Kaur 2014

[33] Eujine et al. 2014

[34] Lekha et al. 2014

[35] Moloisane and Visser 2014

[36] Myint and Swe 2014

[37] Thida and Swe 2014

[38] Correa et al. 2015

[39] Guthrie et al. 2015

[40] Nandini et al. 2015

[41] Ramesh and Sagar 2015

[42] Saini and Vaishnava 2015

[43] Thomas et al. 2016

[44] Chandler et al. 2017

[45] Eujine et al. 2017a

[46] Eujine et al. 2017b

[47] Thomas et al. 2018

Summarising the available literature, it can be seen that the efficacy of enzymes can be split into two: positive (strength and other benefits) as well as a negative effect. As for the positive impact of these additives, it can be seen that the addition of enzyme-based stabiliser can cause a varying range of changes in the plasticity and/or changes in compaction characteristics of soils (Kensenhuis and Modi 2001, Mgangira 2009, Ali 2012, Shankar et al. 2012, Blanck et al. 2013, Eujine et al. 2014, Lekha et al. 2014, Ramesh and Sagar 2015, Thomas et al. 2016). Kensenhuis and Modi (2001) hypothesise the change in plasticity from the effect of the enzyme additive on the double layer water of clayey soils. Clay particles are platy structures with large surface area and net negative charge. Due to the negative charge of these platy structures, positively charged cations surround the platelets in the form of a film of water. This platelet with the adsorbed swarm of cations in the form of water is called the electrical double layer. This adsorbed water gives these particles its plasticity or its ability to deform without cracking. The increase in the double layer results in the increase in the plasticity as well as a greater expulsion force of clay platelets which cause swell. This decrease in the double layer of water is hypothesised as the stabilisation mechanism of these type of additives (Scholen 1993, Rauch 2003).

As summarised in Table 2.3, literature such as Khan and Sarker (1993), Correa et al. (2015) and Guthrie et al. (2015) investigate the combination of enzyme-based additives with other available additives to explore efficacy as well as failure mechanisms. Khan and Sarker (1993) investigated the effect of enzymes the strength and stability of kaolinite and millpond sludge. However, the specifics of the adding of enzymes such as the dilution rate of the additive is not recorded. Addition of 5% (of dry weight) enzyme was reported as the optimum enzyme percentage on pure kaolinite clay to attain a sufficient UCS gain. The study also reports that higher additive levels contribute to decreasing in soil strength, with 10% additive reducing the strength of the soil to diminish to original non-treated strength values. Kaolinite soil treated with fly ash and enzyme reported continuous strength gain even at a 10% additive level. The study also notes that the fly ash and enzyme admixture treatment yield significantly greater strength than lime treated samples. However, the study also reported that the treatment with the additives on millpond sludge was ineffective.

Correa et al. (2015) investigate the effect of using “synthetic termite saliva” and *Bambusa vulgaris vittata* (bamboo particles) to stabilise adobes used as blocks for rural and urban housing. “Synthetic termite saliva” is believed to be enzyme-based additives as the text reports that this additive has been commonly used as soil stabiliser in rural roads. It is also reported that the additive bears a resemblance to the glue-like secretion produces by termites which are used to build their mounds which is the main concept of enzyme-based additives. The benefits of these additives as reported in the text include the improvement of adobe performance such as the reduction in water absorption and capillarity in the adobe and bamboo particle mixture from the enzyme additive, reduction of the adobe shrinkage, and increase in compressive strength by 90%. The catalytic effect of the enzyme in promoting ionic exchange allows the greater cohesion between the finer particles. Greater cohesion achieved in this manner facilitates more significant attraction between clay particles. The decrease in the need for water of the enzyme treated sample is evident from the reduction in suitable water content from 35% to 32% in soil treated with enzyme alone. The soil bamboo particle admixture also requires lesser water as seen from the reduction of suitable water content from 42% to 38%. Introduction of bamboo particles was reported to decrease the density of the soil as well as increase the porosity during the drying stage of the adobe due to the variation in the fibre dimension in the presence of water. However, the inclusion of enzymes increases the apparent density of the adobe. The reduction in linear shrinkage of the treated soil could be attributed to the fibre agglomeration, which could have caused the heterogeneous fibre distribution paired with the cohesive characteristics of the additive. Correa et al. (2015) also report that the addition of bamboo particles alone could increase the capillarity of the adobe due to the net of interconnected pores created by the fibres. However, the enzyme could lower this due to the agglomeration of the particles. The increase in mechanical strength of the adobes could also be credited to all the above factors with the added benefit of the introduced fibre particles interrupting the formation of crack by providing tensile resistance during compressive testing. Guthrie et al. (2015) also investigated to identify the efficacy of enzyme-based stabilisation through field experiments. The experimental analysis fielded the comparison of 6 lanes, one control, two lanes of different commercial enzymes, a lane treated with Portland cement, a lane treated with the combination of Portland cement and one of the enzymes, and lastly a lane treated with a generic liquid soap. Statistical analysis of all the tests conducted help conclude that the treatments with cement or the combination of cement and enzyme yield higher structural quality than all the other treatments evaluated. The effect of curing (ranging from 60 to 270 days) on these additives also showed an increase in the stiffness of the lane stabilised the combination of cement and one of the enzymes whereas all the other lanes decreased in stiffness. Similarly, increase in density over this curing time has also been reported in the lanes stabilised by one of the enzymes as well as the combination of that enzyme with cement, while the density of all the other lanes decreases with time. However, the analysis also reported that neither of the enzymes alone treated lanes yielded statistically significant differences in the structural quality when compared to control and soap treated lanes.

Naagesh and Gangadhara (2010) highlight the positive effect of enzyme-based stabilisation in terms of reduction of swelling capacity of the soils. Naagesh and Gangadhara (2010) report the findings of fat clay (CH) specimens mixed with varying dosages of enzyme additive which report the reduction of void ratio in the sample treated with 2% additive. The study highlights that swelling potential reduces with the increase in the enzyme content. It is also reported that the decrease in the swell pressure depends on the initial water content of the specimens. The study also reports that the reduction in swell plateaus within 30 days of curing with an insignificant reduction in swell potential to up to 120 days of the curing. Scanning Electron Microscopic (SEM) images were used to explain that this reduction in the swell potential could be attributed to the changing of the soil structure from a flocculated (seen in control sample) to a more dispersed structure upon treatment. CEC conducted on the samples does not show significant differences of treated and untreated soil with X-ray diffraction, suggesting no mineralogical changes within the samples. However, X-ray diffraction shows the reduction in the basal peaks of the Montmorillonite, Illite, and Kaolinite post-treatment.

Efficacy of the additive in terms of strength is UCS, and CBR strength is reported in numerous texts, such as Scholen (1992), Khan and Sarker (1993), Hitam et al. (1999), Brazetti and murphy (2000), Kensenhuis and Modi (2001), Thompson et al. (2002), Kuncheria et al. (2003), Parsons and Milburn (2003), Tolleson et al. (2003), Mgangira (2009), Visser (2007), Van Veelen and Visser (2007), Shankar et al. (2009), Naagesh and Gangadhara (2010), Campbell and Jones (2011), Venkatasubramanian and Dhinakaran (2011), Blanck et al. (2013), Gui et al. (2013), Eujine et al. (2014), Ramesh and Sagar 2015, Thomas et al. (2016). Increase in shear strength has also been reported in Marasteanu et al. (2005) and Velasquez et al. (2006) which show significant improvement with increasing curing time highlighting the difference in strength gains attained through two different enzymes on the same soil. The authors also emphasise the need to distinguish enzymes based on their commercial names or even more effectively, their active ingredients.

On the other hand, the negative effect of adding enzyme have also been reported in the literature as summarised in Table 2.4 below, which summarises the potential reasons of why the enzyme based additive report a negative effect. Bergmann (2000) reports a qualitative analysis on the pavements stabilised by various additives from the viewpoint of a wheelchair user as well where a qualitative evaluation was conducted highlighting parameters such as user rideability and comfort. The assessment of the enzyme treated pathway highlighted the pavement was not easy to use because of the soft surface, which was easy to scrape through with the edge of a boot. A follow-up study on another enzyme treated site with a higher amount of clay in the soil also proved ineffective despite visually appearing to look good immediately after compaction but became muddy and slippery with the first sight of rainy weather. The authors do hypothesise the effect of night-time temperature (around -3.9 ºC) as a possible reason for the ineffectiveness due to the not so ideal curing method for the pavement. It should also be noted that the study lacks in-depth laboratory analysis on the soils to determine the main mineral constituent of the soil. Moloisaine and Visser (2014) also report the analysis of field testing on enzyme-based additives on weathered gravel of a trial road in South Africa subjected to a traffic load of approximately 100 vehicles per day for three years. Significant decrease in density was noticed post-construction, which could be due to the immediate effect of the wet weather. Increased density was only observed eight months after construction due to drier weather conditions coupled with the loads induced by the traffic. The study presents electron microscopic images which show cornflake like grains coating soil particles, which results in better interparticle bonding which could be the reasoning to the slight increase in densities achieved. The impact of the additive in terms of strength which was based on DCP-CBR strength measurements show improvements four months post-construction which once again could be due to the immediate effect of the wet weather conditions to which the pavement was subjected. Due to the nature of the tests conducted as well as the lack of having no concrete evidence to prove the improvement of materials when compared to the control sections allude to the authors rating the enzyme-based additive to be poor.

The use of enzymes on stabilising soils with varying clay content has been reported to have given inconsistent results, according to Rauch et al. (2002). The study reports inconsistent and insignificant changes in the soil properties of the enzyme treated soil samples with a few cases showing effectiveness only in terms of reduction in swelling potential. The authors highlight some discrepancies in the results due to the inconsistency in sample preparation methods as well as the use of low Dilution Mass Ratio (DMR) and Application Mass Ratio (AMR) combinations of the additives. A follow-up study is conducted by Rauch et al. (2003) which aims to account for the flaws noted in the previous study. The investigation conducted follows a stricter sample preparation method which accounts for the change in the optimum moisture content of the soil due to the additive as well as the moisture loss from evaporation which occurs during the mixing stage. The improved sample preparation method showed better binding of clay particles by aggregation in enzyme treated samples. However, this aggregation of clay particles did not translate to concrete evidence in strength gain, moreover, only reporting minor improvement in shear strength of certain soils as well as the swell reduction in one of the tested soils. Harris et al. (2006) report the ineffectiveness of enzyme-based stabilisers on soils with high sulphate concentrations (around 10,000 ppm) in a two-phase study which investigates effects of soil stabilisers on three-dimensional (3-D) swell reduction and unconfined strength. It can be seen from the study that the enzyme-based additive is not considered for phase 2 of the testing as it reports an insignificant reduction in the swell potential of soil (0 to 2%). It should be noted that from the total of 12 additives, although tested enzyme-based additive did not report significant reduction in swell potential, it does not increase the swell, unlike certain additives which are subjected to testing. No other tests, including strength tests, were conducted on this additive due to its not passing phase 1, which could have hindered the better understanding of this additive type. Tingle et al. (2007) report the qualitative analyses of the hypothesised stabilisation mechanism based on reviewing of literature along with conducting laboratory tests. The authors highlight that the enzyme-based soil stabilisation mechanism could be site soil specific as it might just aid the soil to reach an end state by taking multiple pathways. The study also highlights the results of enzyme-based soil stabilisation conducted on low plastic clay soil which reports minor UCS improvement that ranges between 4% to 6% in montmorillonite rich soil as well a 3% to 6% decrease in UCS of treated kaolinite minerals. However, the authors do highlight the mixed performance results attained from the testing to come as a result of the general misunderstanding of the products in terms of the additive mix designs, and improper mixing and sample preparation. The authors also highlight the difference in the strength of two different types of clay soil fails to substantiate the hypothesised mechanism of enzyme additives in reducing the affinity for water.

Table 2.4. Summary of the ineffectiveness of enzyme stabilised cases reported in the literature

Ref#	Effect on soil	Soil type	Remarks
1	No noticeable effects of stabilisation of the aggregate materials. Deeps ruts formed with travel on the enzyme stabilised path.	Site 1: 3% clay Site 2: 50% clay	This study presents a qualitative analysis based on the visual evaluation of the disabled access trail. A few things to be noted in this study are: **Type and Percentage of fines**: Site 1: 3 % fines. Tests have also not been conducted on the type of clay mineral of the soil. **Condition of curing**: As reported in the study, Site 1 trail was subjected to snow cover of up to 1.2 m, whereas Site 2, night temperatures fell to -3.9 ℃ at night.
2	No consistent increase or decrease of PI on the enzyme treated soils.	3 Fat Clays, 1 Lean Clay and Fat Silt	**Flawed sample preparation**: decrease in swell by 10% observed in one Fat Clay. Inconsistent results with other soils due to sample preparation flaws.

	Enzymes do not significantly affect the unit weight or void ratio of enzyme treated sample. No substantial improvement in shear strength of soil.		The study did not investigate the change in OMC/MDD with the addition of stabiliser.
3	Significant reduction in surface area of the treated sample reported. Better binding and aggregation of certain soil types reported within the study.	3 New Fat Clays	Scanned imaging shows binding of clay particles by aggregation. However, **inconsistent preparation methods** (as hypothesised by the authors) could be blamed for no concrete evidence of strength gain. However, the measured change in PI is minimal, and OMC didn’t change by more than 3%. **Minor improvement in shear strength – Small reduction in swell observed in one soil.**

4	Insignificant reduction in 3D swell results for enzyme treated 20,000 ppm sulphate soil.	Soil 1: PI = 24-25 Soil 2: PI = 14-16 Soil 3: PI = 25 Soil 4: PI = 29	**sample preparation method may be at fault**: No strength tests conducted as enzyme stabilised samples didn't pass phase 1 (swell test) – enzyme only showed 1% decrease in swell.
5	A slight increase in strength reported. No noticeable reduction in affinity of water.	Low Plastic Clay	Only **4-6% increase in strength** of montmorillonite clay whereas **3-6% strength reduction** reported in treated kaolinite minerals.
6	Significant decrease in density observed eight months post-construction. A slight increase in density observed 31 months post-construction. Untreated section reported	Weathered quartz gravel with low plastic clays PI: 4-12	Additive used for the treatment of weathered quartz gravel wearing course layer Some **inconsistent improvement** observed. Significant decrease in strength observed after eight months which the authors blame the **wetter climate conditions.**

	higher strength than the enzyme treated sections of the pavement. In situ strength behaviour indicated the deterioration of strength with time, whereas soaked strength behaviour indicated improvement with time.		

[1] *Bergmann 2000*

[2] *Rauch et al. 2002*

[3] *Rauch et al. 2003*

[4] *Harris et al. 2006*

[5] *Tingle et al. 2007*

[6] *Moloisaine and Visser 2014*

2.4.4. Influencing Parameters for Enzyme-Based Soil Stabilisation

The use of enzyme in many cases can be seen as having the potential to be considered as a sustainable and efficient form of pavement stabiliser. However, from the above section, it can be seen that the effectiveness of enzyme-based additives depends on many factors. More research work is required to further understand the fundamental mechanisms as well as the changes in both engineering and mechanical properties for it to be used in current engineering problems around the world. Based on the comprehensive literature review that has been conducted, it can be identified that the following parameters play a critical role in determining the effectiveness of the stabilisation process. They are:

Soil Type

From the literature, it can be seen that the level of effectiveness in enzyme-based stabilisation with soils with higher amounts of fines reporting to have a positive influence on enzyme-based soil stabilisers. From the hypothesis of the double layer water mechanism as well as the catalytic bonding effect mechanism, it can be understood that finer soil is more likely to be affected by this phenomenon which could be the reason why the manufacturers and distributors of these form of additives suggest a minimum amount of cohesive fines in the soil. Scholen (1992), Khan and Sarker (1993), Kensenhuis and Modi (2001), Kuncheria et al. (2003), Tolleson et al. (2003), Marasteanu et al. (2005), Li et al. (2011) are a selected few of the literature available who show positive efficacy of enzymes based soil with a minimum of 20% fines. Moloisane and Visser (2014) report an insignificant improvement in the strength of enzyme stabilised weathered quartz gravel which could also highlight the importance of fines in the soil. The mineralogy of these fines are equally important in understanding the fundamental mechanisms of this form of additives as reported by Eujine et al. (2017a, b)

Dilution Mass Ratio (DMR) and Application Mass Ratio (AMR)

For a liquid stabiliser, DMR and AMR refer to two different ways of incorporating the additive into the soil. DMR refers to the ratio of the weight of the concentrated additive to the weight of water, whereas AMR is the ratio of this diluted additive to dry weight of soil. For this reason, it is imperative to understand this parameter to deduce the efficacy of this form of additive. Arguably, this could be one of the most important factors affecting stabilisation outcome. The suppliers of the additive often recommend the use of the additive at a rate of 1 L per 30 cubic meters of soil (Eko-Soil 2015). This roughly equates to 30 ml per cubic meter of the soil. Varying dosages of enzymes have been investigated in the literature. The application process highlighted in Tolleson et al. (2003) utilises a dilution rate of one unit volume of an enzyme to 1000 unit volumes of water and using this enzyme/water mix to moisten the soil to its optimum moisture content. Although this process has shown the effectiveness of the additive in the stabilisation process, no understanding of the amount of additive is not calculated, i.e., only the dilution rate of the enzyme is known, and the application rate of the enzyme is still an unknown. A similar procedure is followed by Marasteanu et al. (2005) with just the dilution factor (0.5, 1, and 1.5 cc per 5 L of water) being the variable to investigate application rates. The trend of the use of the one variable for application rate can be seen throughout the literature. Thomas et al. (2016, 2018) refer to the dosage rate in terms of the additive to the dry weight of the soil with the tested dosages including 80, 100 and 130 mL per cubic meters of soil. However, it should be noted that there is still no clear understanding of whether the enzyme should be added by the dry weight of the soil or with respect to water. Rauch et al. (2003, 2005) present a case that both AMR and DMR be considered when attempting stabilisation with the enzyme based additive. Following this methodology will provide a better understanding of the mechanism by being able to control the amount of enzyme additive in the soil.

Enzyme Type

As mentioned in the mechanism section of the chapter, it can be seen that enzyme-based soil stabilisers can also be bio-enzymes. Scholen (1992) refers to this form of the additives as the additives which introduce bacteria culture into the soil system which utilises the carbon dioxide, nitrogen, and oxygen present in the air to produce organics which could surround the clay particle neutralising the charge of the clay. However, both the type of enzymes has been hypothesised to produce similar strength benefits on the soil by following various methods of mechanism. The understanding on the mechanism of bio-enzymes have also been recently explored in detail in the literature such as DeJong et al. (2008), Cheng and Cord-Ruwisch (2014) and Oliveira et al. (2017)

Sample Preparation Method

As seen from the comprehensive literature review conducted, there is a mixed response of efficacy when it comes to enzyme stabilised soils. As highlighted in the earlier sections, this could be due to the type of tests conducted or the methodology opted to prepare samples. Therefore, it is crucial to understand sample preparation methods with improper methods leading to diminished stabilisation effects of the enzyme on the soil. Scholen (1992) highlights an example of a trial road case which did not benefit from enzyme-based stabilisation due to the oversaturation of the clayey soil during the construction and pre-traffic loading phase. Rauch et al. (2002) also highlight the importance of identifying effective and consistent sample preparation methods which could have been the reason for inconsistency and ineffectiveness of the enzyme treated soil. Harris et al. (2006) also report the ineffectiveness of the additive when submerged in water without accounting for the harshness of the form of testing. From the literature, it is seen that the enzyme treated soil do not last a single wetting cycle due to the collapsing of the soil sample when exposed to the harsh environment. Hence, it is important to identify an effective method for sample preparation and retain consistency to attain valid and repeatable results which could further gain some insight on the efficacy of these form of stabilisers.

Duration of Curing

The duration of curing could be determined based on the understanding of the stabilisation mechanism. It is important to explore this parameter as the comprehensive literature review conducted shows achievement of peak strength of enzyme stabilised soils at varying duration ranging from days to up to months. Ganapathy et al. (2017) report improvements in UCS by up to 30% seven days post-treatment at 400 ml/m^3. Continued monitoring of a trial road stabilised with the enzyme application rate of 0.005 l/m^2 on quartz gravel for up to 8 months reports a significant decrease in density with a slight increase 31 months post-construction

(Moloisane and Visser, 2014). There is limited literature available that covers the effect of time on enzyme-based soil stabilisation. Moloisaine and Visser (2014) highlight the importance of time factor in strength development of non-traditional stabilisation techniques with field tests showing that the tested soils required five months to achieve peak strength and also showing a decrease in strength after an eight-month period which the author assumes was attributed to heavy rains.

Conditions for Curing

Literature has also reported the ineffectiveness of enzyme stabilised soil caused by improper curing methods. Bergman (2000) hypothesises the harsh weather conditions to which the trial roads were subjected to daily which included a meter-deep snow cover on one such trial road as well as cold conditions on another trial road during the curing phase. Rauch et al. (2002 and 2003), Harris et al. (2006) and Tingle et al. (2007) also highlight the importance of the parameter in understanding the efficacy of enzyme-based stabilisers.

2.5. Research Gap and Questions

From the comprehensive literature review conducted and the gaps encountered in the reported knowledge, the following research questions can be identified:

1. How do enzyme-based soil stabilisers affect soil behaviour?
2. What is the response spectrum of soils that has a positive effect on enzyme-based soil stabilisation?
3. Are there notable physical and mineralogical changes induced by enzyme-based soil stabilisation?

4. How much of the additive is required to see effective strength benefits on soil?
5. What are the sample preparation methods as well as the conditions required to attain a positive effect on enzyme stabilised soil?
6. How to quantify the strength gain of soil due to enzyme-based soil stabilisation?
7. Can the efficacy of enzyme-based stabilisation be enhanced by combining with other non-traditional additives?
8. What is the time-dependent effect of enzyme-based soil stabilisation?
9. How durable is enzyme-based soil stabilisation?

This research aims to answer the above questions. Enhanced attention will be given to enzyme-based stabilisation of fine-grained soil. A series of physio-chemical and mechanical tests were conducted on composites made of varying enzyme Dilution Mass Ratio (DMR) and Application Mass Ratio (AMR) as well as fly ash contents to optimise values for each additive. The additive ratio showing peak strength was chosen to be subjected to further examination using imaging techniques to source the reason for its efficacy. At the same time, a supplementary test was conducted to gauge comparative results on how enzyme-based soil stabilisation contributes to long term strength gain in the soil where control (untreated) soil strength is compared to stabilised samples at across a three-month testing period. By investigating the mechanism of enzyme-based soil stabiliser, a better understanding on a fundamental level could be attained, which, in turn, would allow a confident approach when utilising this type of additives for unsealed pavement construction.

Chapter Three.

Material Characteristics and Methodology

The materials utilised for addressing the objectives of the research are soil, enzyme based additive and secondary additives. Fly ash and lime were selected as the secondary additives. The reasoning for its selection has been reported in the relevant sections within the latter chapters of the thesis. Section 3.1 of this chapter presents the material properties of all the additives tested in this research. Physical and chemical properties of soil, enzyme, fly ash and lime are presented within this section. The methodology adopted for research objectives is presented in section 3.2.

3.1. Material Characteristics

3.1.1. Soil

A natural fine-grained soil, obtained from a construction site in Victoria, Australia, was used for this study. The soil subjected to treatment within the study was attained from a local excavating contractor who is involved in land excavation in Melbourne. As reported in Section 2.4.4. of the thesis, the selection of fine-grained soil could facilitate higher efficacy of enzyme treatment. Another reasoning for the selection of fine-grained soil is to narrow the scope of the work. Fig 3.1 shows the pictorial depiction of the steps followed to prepare the soil for testing. After retrieving the soil from the site, the soil was stored in an open space in a bunker at RMIT Bundoora East campus, as shown in Fig 3.1a. The natural soil, being exposed to the weather conditions, was inconsistent in particles size with a mixture of many dried and saturated clumps of soil particles, including many impurities such as rocks and waste materials greater than 19 mm in diameter (Fig 3.1b). A concrete mixer was used to reduce coarse particles in the soil while breaking soil clumps, shown in Fig 3.1c. The soil was then sieved using a 2.36 mm sieve to attain consistency and to increase the reactivity of soil. The increase in reactivity, in this case, refers to the effect of surface area in affecting the rate of chemical reaction. Based on the accepted condition that increasing the surface area of the solid reactant, increases the rate of chemical reaction, sieving the soil has the potential to increase the reactivity. The soil was oven dried for 24 hours to attain a consistent moisture content distribution throughout the soil (Fig 3.1d). All the tests reported in this research study were conducted on this soil that passed the 2.36 mm sieve. The consistency limits of the tested soil have been reported in the soil characteristics summary, as shown in Table 3.1. The plasticity index was conducted based on AS1289.3.3.1 (2000). Four-point Casagrande method was utilised to attain the liquid limit of the soil (AS1289.3.1.1 2000), and plastic limit was determined using AS1289.3.1.2 (2000). The specific gravity of the soil was determined to be 2.57 using ASTM D854 (2014).

The Particle Size Distribution (PSD) of soil, shown in Fig 3.2, was conducted according to Australian Standards (AS1289.3.6.1, 2009). The distribution of the particles below 75 µ was conducted using a Mastersizer 3000 (Malvern Panalytical). Bruker D4 diffractometer was used to identify the mineralogical constituents of the samples using Cu-kα radiation at an angle scan 2θ of 15 to 75°. Results on this test identified Quartz, Muscovite and Kaolinite as the dominant minerals which make the soil, as shown in Fig 3.3. Oxide composition of the soil based on the X-ray fluorescence (XRF) results which shows the SiO_2 and Al_2O_3 dominance within the sample are shown in Fig 3.4.

Table 3.1 summarises the physical and chemical properties of this fine-grained soil. With the tested soil containing 50% materials which passes No. 200 sieve, as well as having PI of greater than 7 and above A-line, the soil used in the current research can be classified as Lean Clay (CL) based on Unified Soil Classification System (USCS) (ASTM D2487 2011). Based on these tests, the soil used in the current research can be classified as Lean Clay (CL). Based on the Liquid limit and plasticity index of the soil, the soil is low plastic inorganic clay with low compressibility as well as low swelling potential (Wagner 2013).

Figure 3.1. a. Soil stored at open space bunker at RMIT; b. Inconsistent sized dried and saturated clay clumps present in the soil; c. Concrete mixer used to breakdown large soil clumps; d. Oven drying process of soil

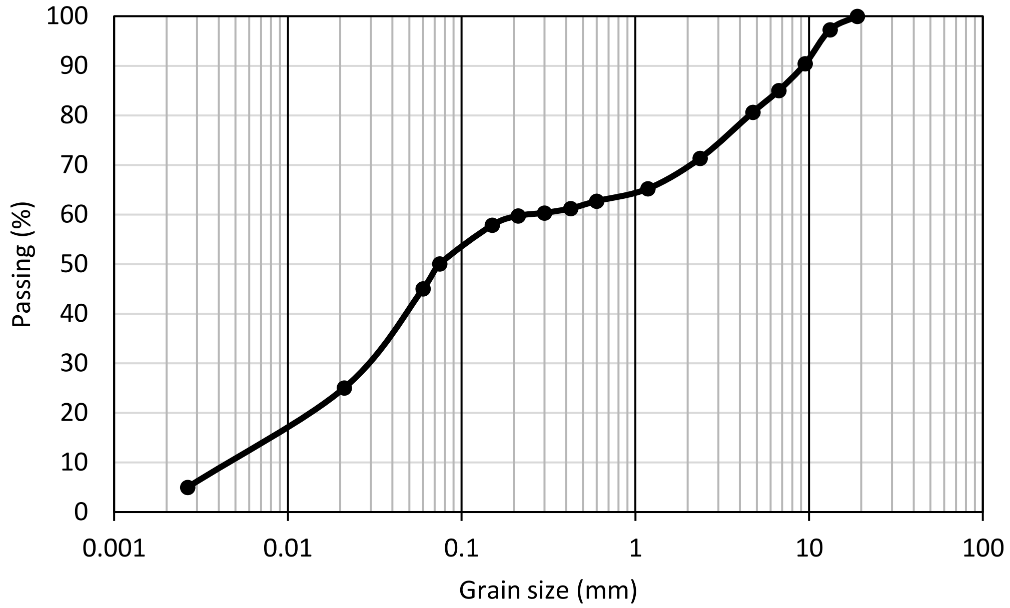


Figure 3.2. PSD of the soil

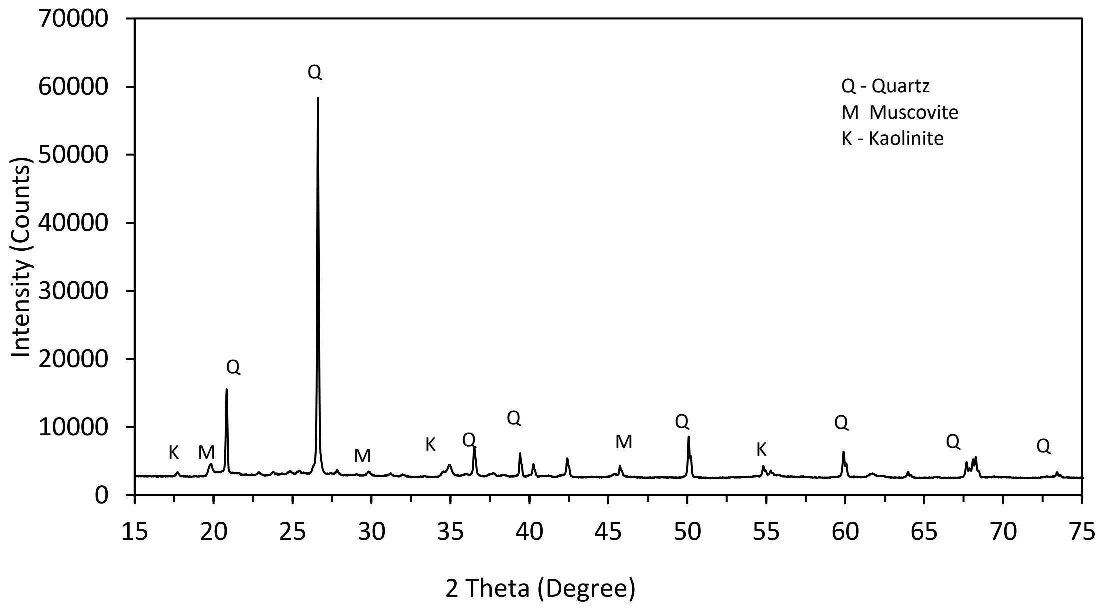


Figure 3.3. XRD of the soil

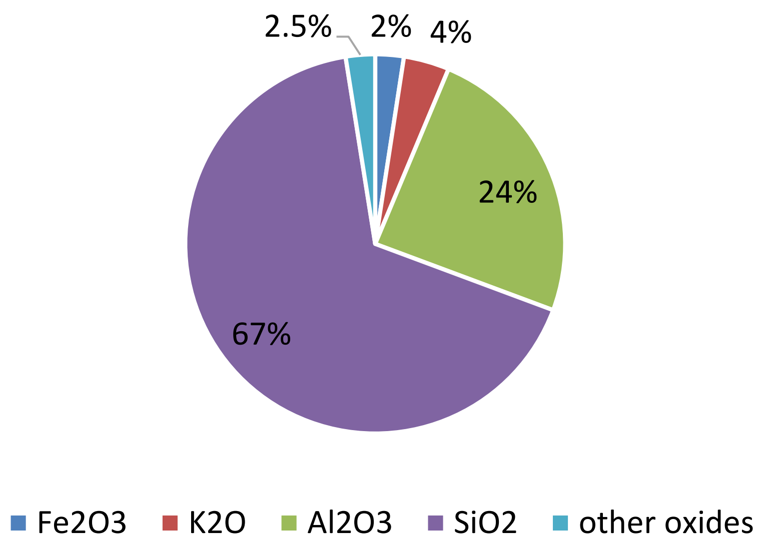


Figure 3.4. XRF of the soil

Table 3.1. Summary of the soil characteristics

Soil Property	**Value**
Specific Gravity	2.57
Liquid Limit, LL (%)	29
Plastic Limit, PL (%)	20
Plasticity Index, PI (%)	9
Maximum Dry Density# (g cm^{-3})	1.79
Optimum Moisture content# (%)	17
Unconfined Compressive Strength# (MPa)	0.21
California Bearing Ratio# (%) (*unsoaked*)	4
Maximum Dry Density* (g cm^{-3})	1.95

Optimum Moisture content* (%)	12.2
Unconfined Compressive Strength* (MPa)	0.75
California Bearing Ratio* (%) (*unsoaked*)	72
Quartz (%)	65
Muscovite (%)	27
Kaolinite (%)	8

#Standard compaction

**Modified compaction*

3.1.2. Enzyme

Eko-Soil was used as a soil stabiliser in this study. This is a non-hazardous/non-toxic and biodegradable environmentally friendly stabiliser, which is produced from water and highly purified proteins derived from plant sources. The commercial product is marketed (by the supplier) to increase the density of soil, increase the mechanical strength of the soil, and lower permeability. The pH of the pure enzyme and the pH at a Dilution Mass Ratio (DMR) of 1:500 (1 g of enzyme:500 g of water) was obtained as 4.8 and 4.42 respectively. The manufacturer revealed the general proportion of the enzyme as 20% water, 20% non-ionic surfactant and 60% ferment of base ingredients (including 30% water). The active enzymes in the additive include lipase, amylase and protease. Fig 3.5 presents the enzyme in its concentrated and diluted form. Summary of the properties of the selected enzyme has been presented in Table 3.2. Eko-Soil was used in this study as it emulates the other commercial enzymes such as Terrazyme and Permazyme, which are commonly used and cited in all the previously noted literature.

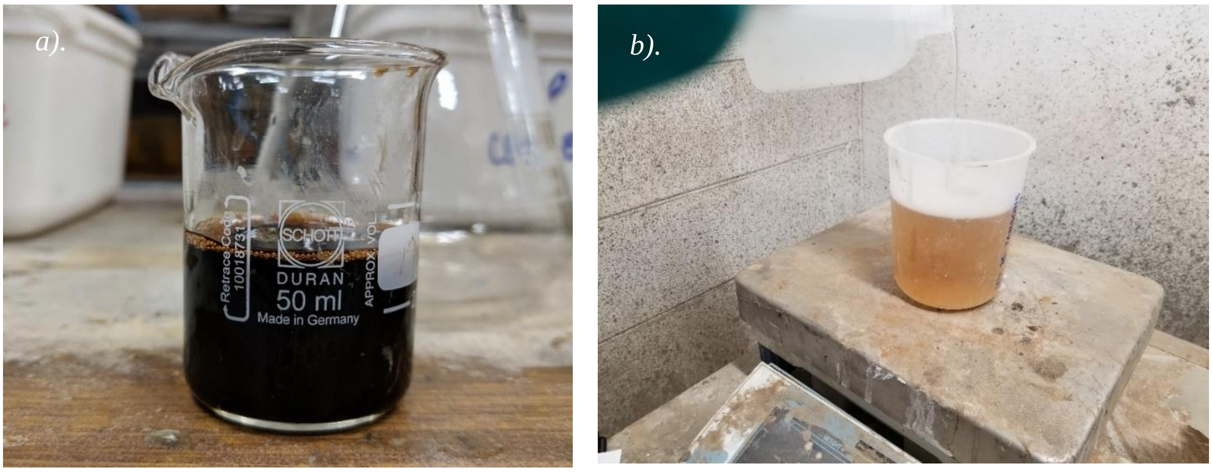


Figure 3.5. a. Enzyme (commercial form); b. Diluted enzyme prior to stabilisation

Table 3.2. Summary of the enzyme characteristics

Eko-Soil Property	**Value**
Specific Gravity	1.05
Boiling Point	100 °C
Evaporation rate	Same as water
Vapour pressure	Same as water
Appearance	Brown
Odour	Slight fermented
pH	4.4 – 4.8
Active enzymes	Amylase, Lipase, Protease

3.1.3. Fly Ash

The fly ash used in this study is a commercial product obtained from Cement Australia, the nation’s leading supplier of cementitious products and services. It is available in the form of a fine powder, light grey to fawn in appearance (Fig 3.6) with no odour, a melting point of greater than 1400 ºC, and a specific gravity of 2.35 – 2.4. XRD analysis (Fig 3.7) reveals the main mineralogical constituents can be identified as Quartz (SiO_2), Mullite ($Al_6O_{13}Si_2$), Maghemite (Y-Fe_2O_3), and Hematite (α-Fe_2O_3). X-ray fluorescence (XRF) test results showed the oxide percentages to be 42% SiO_2, 28% Al_2O_3, 15% CaO and 10% Fe_2O_3. Characterisation tests based on ASTM C618 – 05 (ASTM C618 – 05 2005) classifies the fly ash as Class F fly ash.

Figure 3.6. Fly ash used for the research

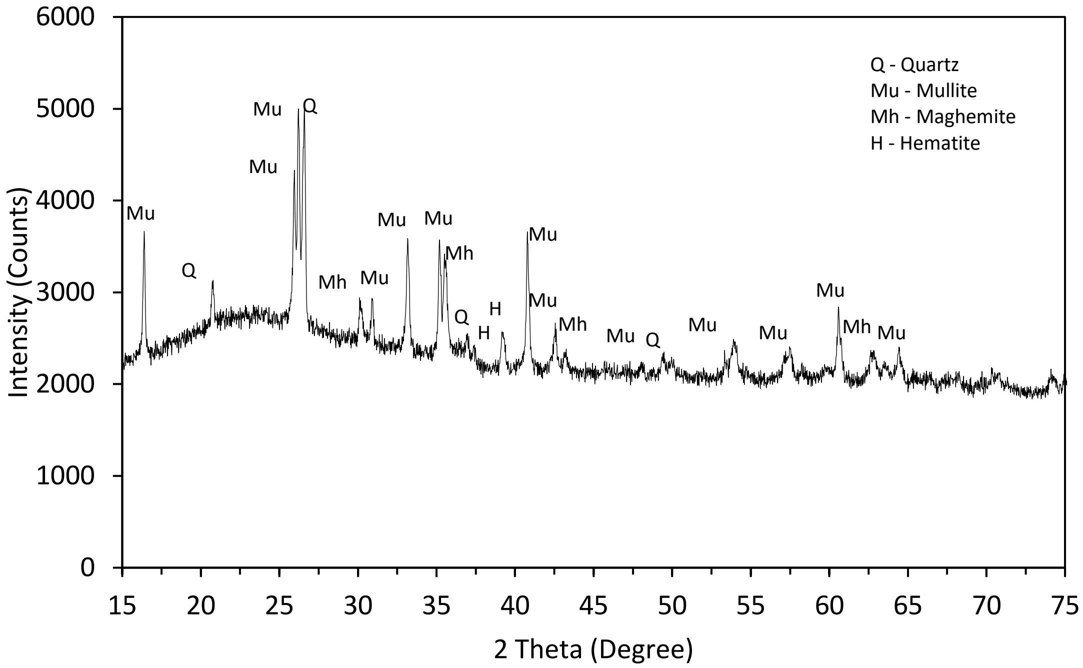


Figure 3.7. XRD of the tested fly ash

3.1.4. Lime

The lime used in this study is commercially available and was obtained from Lime Group Australia. This additive is the form of a very fine, white powder. XRF tests confirm the oxide constituents as Calcium Oxide (Cao – 72%), Aluminium Oxide (Al_2O_3 – 0.11%), Iron Oxide (Fe_2O_3 – 0.06%), Magnesium Oxide (MgO – 0.31%), and Silica (SiO_2 – 0.42%).

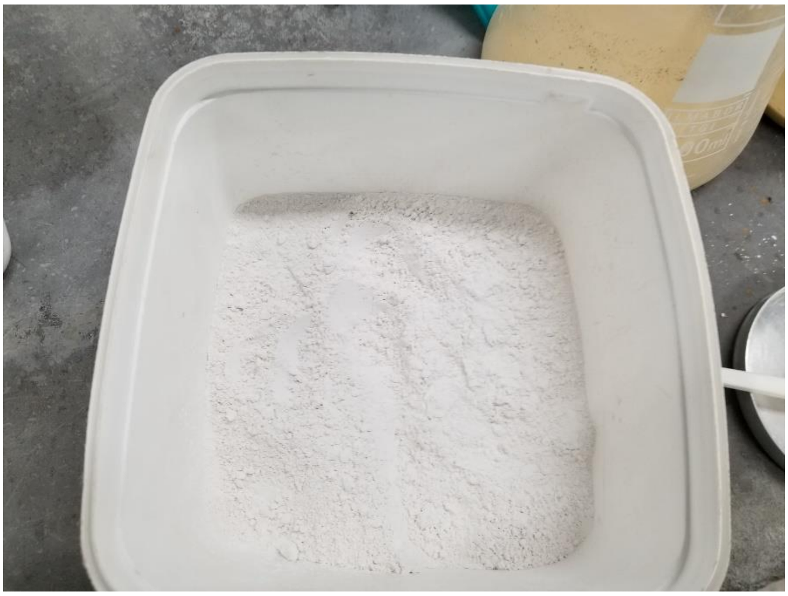


Figure 3.8 Lime used for the research

3.2. Research Methodology

The methodology followed to achieve the main objectives of the research is shown in Fig 3.9. As shown in the figure, the methodology has been divided into 5 phases; problem identification, optimisation of enzyme stabilisation, optimisation of enzyme and secondary additive stabilisation, durability and performance investigation of additive treatment, conclusion and recommendation.

Figure 3.9. Summary of research methodology

3.2.1. Phase 1: Problem Identification

The investigative problem has been presented in this phase of the research. Chapter 2 detailed the uncertainty which surrounds soil stabilisation using non-traditional stabilisers, mainly involving enzymes. Based on the comprehensive literature review conducted, materials were selected for the investigation, as reported in Section 3.1. The characterisation of the materials was based on a variety of tests ranging from chemical composition and physical characteristics at an elemental state to visual states. The expected deliverables from this phase included identification of research questions and objectives, procurement and characterisation of selected materials. The characterisation of the materials will help uncover the response spectrum of soil that has a positive effect on enzyme-based soil stabilisation (RQ 2).

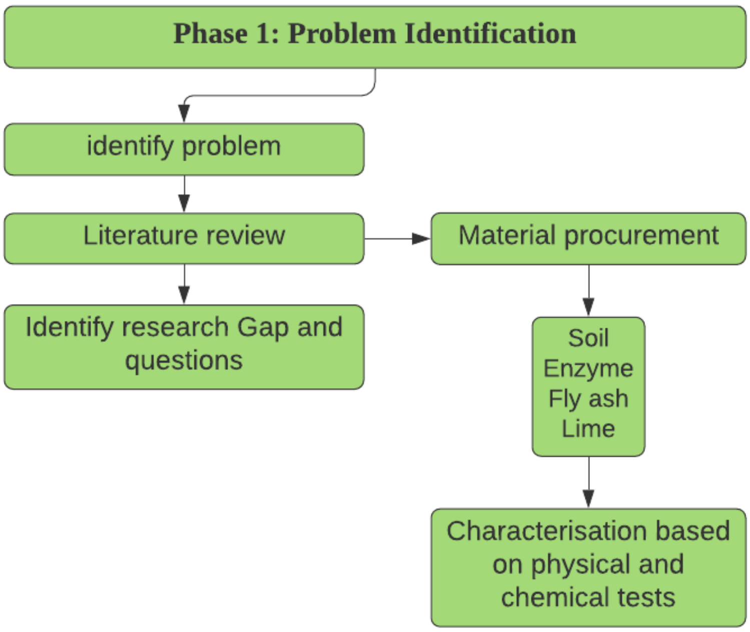


Figure 3.10. Summary of Phase 1

3.2.2. Phase 2: Optimisation of Enzyme Stabilisation

This phase of the research focussed on the selected soil and enzyme type to produce optimisation regime. Firstly, tests were conducted to identify suitable sample preparation methods and ideal curing conditions and time. The suitable conditions and the efficacy of enzyme-based stabilisation were predominantly based on the strength tests conducted (based on relevant strength testing procedures). Secondly, the phase combined the selected soil with varying levels of enzyme additive based on DMR and AMR. The tests were conducted based on two different curing conditions, soaked (CBR) and unsoaked (CBR and UCS). A detailed overview of the testing methodology is provided in the latter section of the manuscript (Section 4.2.3 and Section 4.2.4). The mechanism of the enzyme stabilisation was also explored within this phase through the use of physical and chemical tests conducted. The expected outcome of the phase include understanding on how enzymes affect the soil (RQ 1), changes in the physical properties of the stabilised soil (RQ 3), identifying the optimised levels of the enzyme additives (RQ 4), identifying suitable sample preparation methods for positive enzyme based soil stabilisation (RQ 5) as well as quantification of strength gain from the treatment (RQ 6)

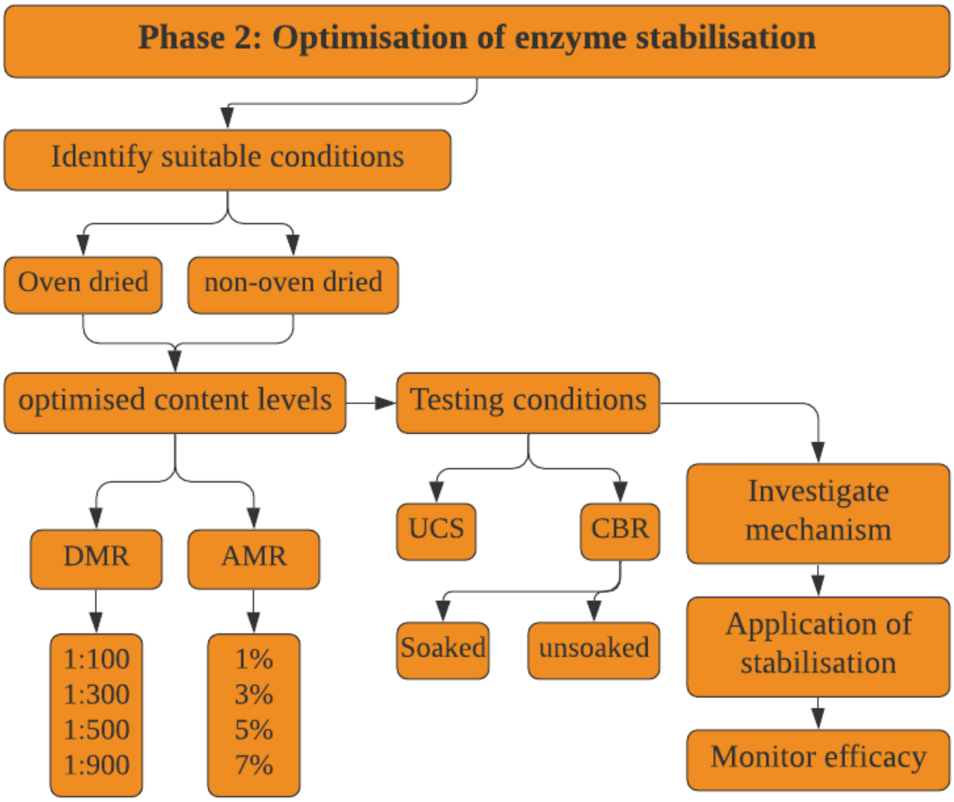


Figure 3.11. Summary of Phase 2

3.2.3. Phase 3: Optimisation of Enzyme + Secondary Additive Stabilisation

This phase mainly constitutes the effect of combining the enzyme with secondary additives selected within the scope of the research. The effect of curing time on the strength of the tested samples was also investigated within this phase. Mechanism of stabilisation (RQ 3), optimised content levels of the secondary additive (RQ 4), insight on the conditions and curing methods during sample preparation (RQ 5, 8), the effects of combining other additives with the enzyme (RQ 7) were the expected outcomes of this phase.

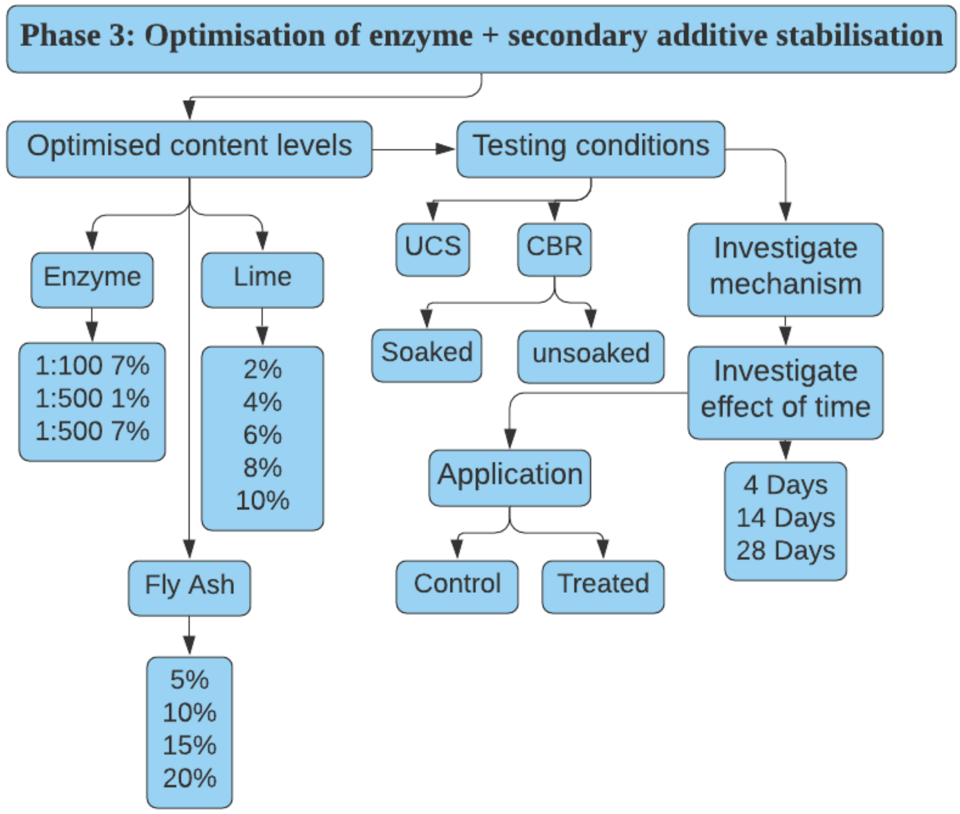


Figure 3.12. Summary of Phase 3

3.2.4. Phase 4: Durability and Performance Investigation of Additive Treatment

A series of experiments and computational analyses were conducted in this phase of the research to assess the durability as well as the performance of soils and pavement incorporating selected additives for stabilisation. The durability tests are mainly of two types, firstly the durability of the treated soil based on the effect of time and re-compaction. This form of durability testing investigates whether the properties affected by the enzyme treatment will change with time. Within this testing plan, pre-tested strength samples are removed from their mould (wherever applicable), disturbed, re-compacted ensuring no significant moisture loss, and then tested for strength with varying curing times. Secondly, the durability test is conducted using a novel wetting and drying method to assess the durability of treated samples exposed to extreme conditions. A modified wetting and drying cyclical test is performed on the selected soil type with and without the additives to measure the deterioration rate of the samples. The detailed explanation of the procedure is reported in Chapter 6. The expected outcomes of this phase include understanding on how enzymes affect soil (RQ 1), the benefits of combining enzymes with other additives (RQ 7), the effect of time on treatment (RQ 8) and lastly the long-term benefits of this form of treatment (RQ 9).

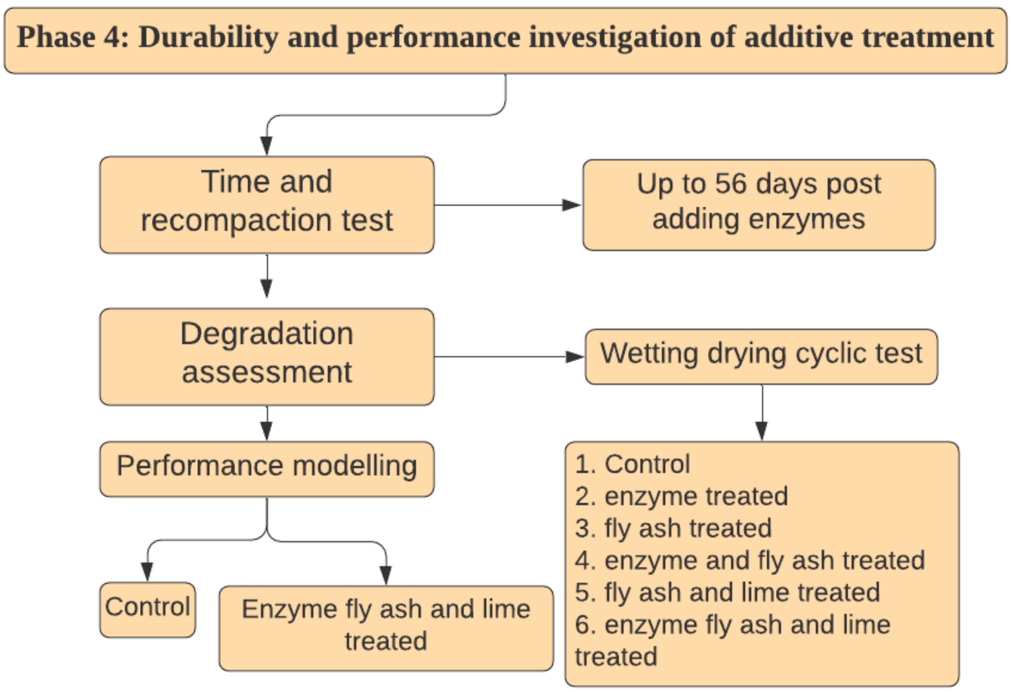


Figure 3.13. Summary of Phase 4

3.2.5. Phase 5: Conclusion and Recommendations

Phase 5 summarises the outcomes of the research while highlighting potential setbacks and recommendations for future works.

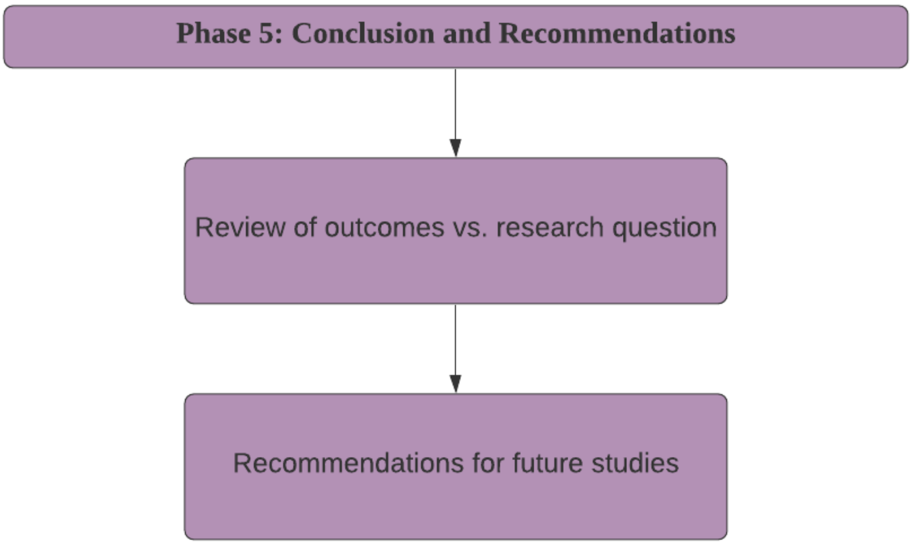


Figure 3.14. Summary of Phase 5

The thesis, within its relevant sections of the chapter, details the comprehensive methodology followed to investigate the effects of enzyme-based soil stabilisers along with other non-traditional additives and its findings. The research at hand will significantly benefit the road construction industry by not only replacing traditional construction methods with economical/reliable approaches but also provide insight on the optimum additive amounts required to stabilise road pavements based on stabilisation mechanism of these non-traditional additives.

Chapter Four.

Optimisation of Enzyme-Based Soil Stabilisation

4.1. Introduction

Soil stabilisation can be identified as one of the most effective method of ground improvement in Australia and worldwide. Various methods of soil stabilisation have been extensively tested by researchers and can be seen being rigorously used by practitioners in field applications, especially in the last four decades. These include mechanical stabilisation, which densifies the soil by expelling air from the voids without much change in the water content (Little and Nair 2009), and chemical stabilisation that incorporates additives to improve soil properties which in turn improve ground strength. Enhanced attention was devoted to chemical stabilisation methods due to their remarkable benefits in ground applications either using traditional calcium-based stabilisers such as cement and lime or using non-traditional stabilisers such as salts, acids, enzymes, lignosulfonates, petroleum emulsions, polymers and tree resins (Tingle and Santoni 2003). The efficacy of calcium-based additives has been widely explored in the past and has often proven to be effective in many of the applications. However, the limited knowledge of mechanisms of non-traditional additives restricts to devise their optimum benefits into engineering application.

Chemical stabilisation of soil has commonly been utilised mainly through calcium-based soil stabilisers due to its efficiency in providing adequate strength within a short period. The effects of some influential factors (i.e., water content, cement content, curing time, and compaction energy) on the microstructure and engineering characteristics of cement-stabilised soils have been extensively researched (Terashi 1979, 1980, Tatsuoka 1983, Kamon 1992, Nagaraj 1997, Yin and Lai 1998, Consoli et al. 2001 Kasama 2000, Miura et al. 2001, Horpibulsuk and Miura 2001, Horpibulsuk et al. 2003, 2004a, b, 2005, 2006, 2010a, b, 2011, Suebsuk et al. 2010, 2011). Moreover, the soil stabilisation using fly ash and/or lime has also been investigated (Kolias et al. 2005, Al-Hattamleh 2009, Azadegan et al. 2013, Jha and Sivapullaiah 2015). In stabilisation with Portland cement, some calcium from newly formed cementing compounds Calcium – Silicate – Hydrate (C – S – H) and Calcium – Aluminate – Hydrate (C – A – H) has been reported to modify clay particles while some more Calcium is formed as a result of cement hydration. The hydrates have been reported to further stabilise flocculated clay particles through cementation (Little et al. 2000). Lime stabilised soil has been reported to showcase decrease in plasticity, increase in shear strength and the cohesion of clayey soil to a granular material (Prusinski and Bhattacharja 1997). The pozzolanic reactions between silica/alumina and calcium could also account for the strength increase in lime stabilised soil (Parsons and Milburn 2003). Irrespective of the remarkable strength improvements from calcium-based stabilisers, research is now being focused on the other additives for the soil stabilisation mainly due to environmental impact from the production of these calcium-based additives. For example, the cement industry is the second largest industrial contributor to CO_2 emissions, with a total of 7% added to the global CO_2 count (WBCSD 2018). Another drawback of calcium based soil stabilisers include the production of expansive products such as gypsum, ettringite and thaumasite from high sulphate rich soils which could lead to cracking in soil (Hunter 1988). Such adverse influence from calcium-based stabilisers could demand the need for green, sustainable and effective non traditional stabilisers such as enzymes.

Various research work conducted since the 1990s highlighted that enzyme-based soil stabilisation could be a sustainable alternative to calcium-based stabilisers in enhancing ground performance. For example, Scholen (1992) report that enzymes were used to effectively stabilise a forest road in Oklahoma which produced significant improvement in durability in terms of a maintenance free road when compared to the adjacent non-treated sections. Other field studies that showed positive efficacy of enzymes are reported regularly and claimed various benefits such as low or maintenance free roads, a significant increase in bearing capacity (CBR, UCS and resilient modulus), dust reductions of up to 75%, and decrease in road deflections (Hitam et al. 1999, Brazetti and Murphy 2000, Campbell and Jones 2011, Li et al. 2011, Shankar et al. 2012, Guthrie et al. 2015). Laboratory findings have also confirmed these benefits as reported by various researchers (Kuncheria et al. 2003, Parsons and Milburn 2003, Shankar et al. 2009, Agarwal and Kaur 2014). However, the effectiveness of enzyme-based soil stabilisation depends on many critical factors such as soil type, construction technique, temperature and curing conditions and even the enzyme type. Hence, it is vital to gauge an insightful understanding of the enzyme-based soil stabilisation to obtain the maximum efficiency of stabilisation by controlling the critical factors.

Generally, commercial enzymes are a chemical, organic, and liquid stabiliser formed from fermented organic materials, and its stabilisation mechanism is still being debated. Tolleson et al. (2003) hypothesise that there is a catalytic bonding process from the attaching of the enzyme to the microbes present in fine soils, which form tight covalent bonds between structures that are already present in the soil, thus, decreasing the surface area and the voids in the stratum. Further literature (Marasteanu et al. 2005, Gianni and Modi 2001) suggest that CEC of the soil plays a crucial role in this stabilisation process where the enzyme cations engulf the fine particles or clay molecules and neutralise it by removing water from the weaker clay cation resulting in higher density and permanent structural change. These studies highlight the importance of having fine particles in soils for the effectiveness in enzyme-based soil stabilisation. Scholen (1995) identified that organic materials in the form of humus present in these fines are also a requirement for effective enzyme-based soil stabilisation unless a bacteria culture could be introduced in the form of enzyme additives which could potentially produce organics from the carbon dioxide, nitrogen, and oxygen present in the air. Rauch et al. (2003) demonstrated the enzyme-soil stabilisation as a possible reaction which involves the encapsulation of organic matter in the clay minerals. In this reaction, negatively charged clay minerals are neutralised with the addition of the enzymes to decrease the clays affinity for water, making it a more stable particle (Fig 4.1). Urease, an artificially extracted enzyme from plants, have also been noted in the literature as being used to improve engineering property of soil through the process of Microbial Induced Calcium Precipitation (MICP). In this process, a highly active urease enzyme catalyses the hydrolysis of urea into ammonium and carbonate and elevate the pH. During this hydrolysis reaction, the bacterial cell with negative charge absorbs calcium ions to deposit on its surface and provide a nucleation site for crystallisation of calcium carbonate at the pore spaces which in turn help achieve desired soil mechanical properties (Cheng and Cord-Ruwisch 2014, Oliveira et al. 2017). Dejong et al. (2010) utilised electron microscopic techniques to establish calcite precipitation occurs at pore spaces preferentially near particle to particle contacts resulting in the densification of the soil. However, the commercial enzyme tested within this study is not reported as having urease as an active ingredient which might suggest that MICP is not the dominant stabilisation mechanism. Although these hypotheses highlight the potential stabilisation mechanism of enzymes, there is no universal understanding of enzyme-based soil stabilisation, which can be utilised to optimise the benefits of the enzymes in field applications. This is because the reaction mechanism of the enzymes can be different depending on its chemical content and the conditions of the medium (i.e., field) as previously identified. Hence, it is crucial to identify the stabilisation mechanism, particularly for new enzymes to derive the optimum benefits prior to use in ground application.

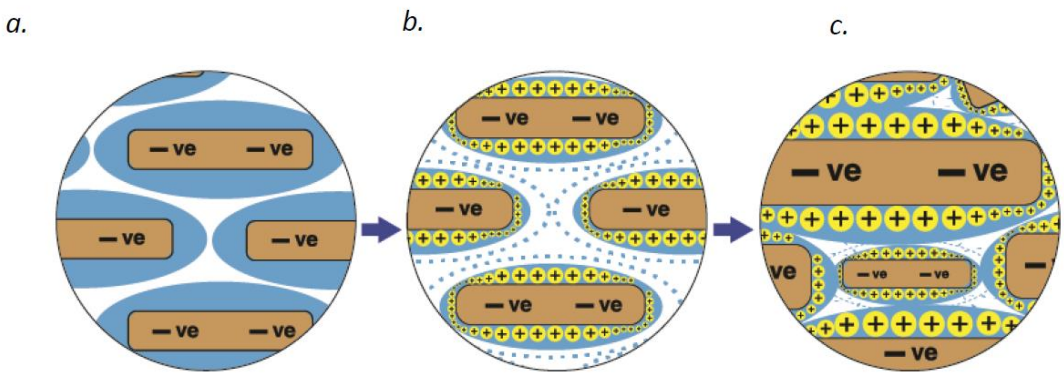


Figure 4.1. Enzyme-based soil stabilisation mechanism. a. Natural clay particle with high affinity for water; b. Organic encapsulation decreasing the double layer of water; c. Stable clay particles

This chapter investigates the optimised stabilisation mechanism of the selected novel enzyme-based additive, commercially known as Eko-Soil, which is being used to construct unpaved roads in Australia and worldwide by understanding this particular product’s working mechanism. A series of physical, chemical, and mechanical tests were conducted on enzyme-soil composites prepared using a systematically controlled 4-Stage testing program. Having investigated the effect of soil stabilisation, the mechanism of stabilisation of this enzyme was unveiled using a number of imaging tests and validated using mechanical tests. The identified mechanism was utilised to enhance the optimised strength of stabilised soil significantly.

4.2. Experimental Procedure

A series of laboratory tests were conducted under a 4-Stage test program to investigate the stabilisation mechanism and optimisation of the additive for the selected field soil. The research questions to be covered in this chapter include the following, as highlighted in Section 2.5 (Chapter 2):

- How do enzyme-based soil stabilisers affect soil behaviour?
- Are there notable physical and mineralogical changes induced by enzyme-based soil stabilisation?
- How much of the additive is required to see effective strength benefits on soil?
- What are the sample preparation methods as well as the conditions required to attain a positive effect on enzyme stabilised soil?
- How to quantify the strength gain of soil due to enzyme-based soil stabilisation?

Firstly, experiments were performed to obtain physical and chemical properties of soil used in this research work. Having characterised the soil, the effect of thermal influence on enzyme-based soil stabilisation was first explored in Stage 1 to identify a suitable oven drying temperature to change the soil from its initial saturated state to a dryer and more workable state without causing any detrimental effects to the stabilisation prior to the additive mixing and compaction. Then the effects of stabilisation were investigated in Stage 2 using mechanical tests (CBR & UCS) for soils treated at the control OMC. The observed response of soil stabilisation was explained in Stage 3 using a series of microscopic tests such as Scanning Electron Microscopy (SEM), X-ray Diffraction technique as well as Micro-CT scan and porosity analysis work. Further tests were performed in Stage 4 to optimise the enzyme-based stabilisation by facilitating the understanding of the mechanism of stabilisation. This section (Section 4.2) of the chapter presents a detailed description of the sample preparation and test procedure adopted in the research work. The application of enzyme-based additives in a trial road has been reported in Section 4.4

following Stage 4 of the experimental procedure. The detailed stabilisation application process, along with the analysis of the strength results of the treated segments of the pavement has been compared to the untreated segments based on mechanical tests conducted on the soil type as well as mechanistic analysis. Review on the state of the pavement segments two years post-construction has also been conducted to gauge the long-term effect of the additive.

4.2.1. Materials Used

A natural soil classified as Lean Clay (CL) has been tested with varying dosages and applications of Eko-Soil. The process of refining and preparing the soil for testing has been detailed in Section 3.1.1 (Chapter 3) of the thesis, along with the soil's physical and chemical properties. Section 3.1.2 (Chapter 3) details the properties of the tested enzyme.

4.2.2. Soil Preparation

Initial assessments of the soil revealed a wide variation of initial moisture in the natural soil as it was obtained from the field. This resulted in substantial variation of soil strength based on unsoaked CBR & UCS tests (standard deviation > 15%). Though soil drying could resolve this issue, there is no set standard on temperature limits to which soils can be allowed to oven-dry for specifically for the purpose of stabilisation. To control the standard deviation, all the samples prepared for this research were conducted following Australian Standards (AS1289.1.1 2001). The standard suggests air drying of soil or oven drying with temperature less than 50 ^{o}C to ensure no irreversible changes occur in soils (clause 5.3.3.2). Even though the standard highlights the temperature influence as of negligible practical significance, the effect of oven drying was investigated in the current study as the temperature influence on stabilization can be highly specific to the type of soil and additive used in the study. The results from this study can be useful to justify the temperature selection for the stabilisation process for tested materials and to identify the impact of enzyme admixture on strength behaviour independent of the adopted soil preparation technique. Thus, Stage 1 tests were conducted to simply identify the suitable drying temperature for the fine-grained soil used in this study, while understanding the strength behaviour unique to enzyme admixture influence.

4.2.3. Mix Design and Specimen Preparation

Samples were prepared in all stages of tests at various DMR and AMR. Four DMRs (1:100, 1:300, 1:500, 1:900) and AMRs (1%, 3%, 5% and 7%) were tested for strengths in the form of UCS and CBR in accordance to (AS5101.4 2008) and (AS1289.6.1.1 2014) respectively. The moisture content of the soil was determined using an OHAUS moisture analyser which helps identify universal moisture content of tested substance (Fig 4.2). The universal moisture content of a sample refers to the ratio of moisture mass to bulk soil mass (i.e., $\frac{Weight\ of\ water}{Weight\ of\ dry\ soil + weight\ of\ water}$) (OHAUS 2018). The data was used to determine the gravimetric moisture content of the soil. In contrast to the universal moisture content, gravimetric (or geotechnical) moisture content refers to the ratio of moisture mass to the dry soil mass (i.e., $\frac{Weight\ of\ water}{Weight\ of\ dry\ soil}$). The soil was prepared to OMC – AMR + 2% and allowed to reach equilibrium in a sealed container for at least 16 hours. The 2% moisture is to compensate for the unavoidable moisture loss from the sample during this preparation process. Having obtained the required mass from DMR to attain the OMC, the diluted stabiliser was added to pre-moistened soil and mixed by means of a mechanical mixer as well as by manual means to attain a high degree of homogeneity before compacting it for CBR/UCS tests. An example of the calculation is as follows:

Sample calculation:

$To\ prepare\ 4500g\ of\ wet\ soil\ to\ OMC\ of\ 14.6\%\ with\ 7\%\ AMR$

$$OHAUS\ moisture\ reading = 2.7\%^{*}$$

$$Total\ moisture\ in\ soil = \frac{2.7}{100} \times 4500 = 121.5\ g$$

$$Therefore\ dry\ soil = 4378.5\ g$$

$$OMC = 14.6\% = \frac{14.6}{100} \times 4378.5 = 639.261\ g$$

$$Therefore\ enzyme\ required = 7\%\ AMR = \frac{7}{100} \times 4378.5 = 306.5\ g$$

$$Therefore\ moisture\ to\ be\ added = 639.261 - 306.5 - 121.5 = 211.261\ g$$

*(please note that this is the universal moisture content)

This preparation method aligns well to the method reported in the literature (Rauch et al. 2003). Finally, the prepared UCS and CBR samples were sealed in aluminium foil/plastic wrap and cured under room temperature of 21 °C - 24 °C until testing. This chapter reports strength tests of both UCS and unsoaked CBR samples prepared using modified as well as standard compactor tested after a four-day curing period to allow sufficient time to achieve adequate strength improvements.

Figure 4.2. OHAUS Moisture content analyser

4.2.4. Testing

Summary of tests conducted in this study is shown in Table 4.1 – 4.2 for all the stages of the experimental program. Mechanical tests were conducted at least in duplicates or in some cases triplicates with specimens prepared at a minimum of 90% optimum density and a maximum of 2% allowance to either wet or the dry side of the OMC. Outliers (those that did not meet neither the 90% density requirement nor 2% allowable OMC requirement) were recast within these set boundaries to achieve uniform sample preparation. The samples prepared for mechanical tests were tested using a Shimadzu 50kN applying the load at a constant rate of 1 mm/min for both UCS and CBR samples as required in the standards (AS5101.4. 2008). XRD was conducted to measure the mineralogical changes, rearrangement, and spacing of atoms in crystalline materials due to the enzyme additive. Bruker D4 diffractometer was used for the analysis of the samples using Cu-kα radiation at an angle scan 2θ of 15 to

75°. Scanning electron microscope with secondary electron imaging as well as backscatter electron imaging (15 kV of energy) and Energy-dispersive X-ray spectroscopy was used to examine microstructure and elemental distribution. Specimens were hand cut to roughly 5 mm in height and carbon coated prior to scanning using FEI Quanta 200 ESEM. The micro CT scans (µCT) were used to investigate pore connectivity and pore-structure of both control and stabilised specimens. The 20 mm cubic specimens were prepared from tested UCS samples at selected DMR/AMR combination and were scanned at 20 µm resolution at 100 kV and 100 µA using a copper filter, and 1000 images recorded during a complete scanning rotation. Finally, specimen porosity was analysed using CTAN.

Table 4.1. Summary of UCS[1] tests and conditions in Stage 1

DMR & AMR	Drying Method	No of Tests
Control	Air dried	3
1:500 (1%)	Air dried	3
1:500 (1%)	20 ºC oven dried	3
1:500 (1%)	40 ºC oven dried	3
1:500 (1%)	60 ºC oven dried	3

[1]Unconfined Compressive Strength of samples prepared using modified compaction conducted in triplicates

Table 4.2. Summary of lab tests and conditions in Stage 2 – 4

Stage	Test Name	Compaction Type	DMR & AMR	No of Tests
2	CBR[1]	Modified	Control	3

		(Done in triplicates)	1:100 (1%, 3%, 5%, 7%)	12
			1:300 (1%, 3%, 5%, 7%)	12
			1:500 (1%, 3%, 5%, 7%)	12
			1:900 (1%, 3%, 5%, 7%)	12
3	SEM[2]		1:500 (0%, 7%)	2
	XRD[3]		1:500 (0%, 7%)	2
	μ-CT[4]		1:100 (0%, 7%)	4
			1:500 (7%)	2
			1:900 (7%)	2
4	Compaction[5]	Standard proctor	Control	1
			1:100 (1%, 3%, 5%, 7%)	4
			1:500 (1%, 3%, 5%, 7%)	4
			1:900 (1%, 3%, 5%, 7%)	4
	UCS[6]	Standard (Done in triplicates)	Control	3
			1:100 (1%, 7%)	6
			1:500 (1%, 7%)	6
			1:900 (1%, 7%)	6
	CBR[1]	Standard (Done in duplicates)	Control	2
			1:100 (1%, 3%, 5%, 7%)	8
			1:500 (1%, 3%, 5%, 7%)	8
			1:900 (1%, 3%, 5%, 7%)	8

[1]*California Bearing Ratio*

[2]*Scanning Emission Microscopy of treated and untreated samples*

[3]*X-Ray Diffraction of treated and untreated samples*

[4]*X-Ray Aided Micro-CT for pore distribution analysis*

[5]*Standard proctor tests on soil with DMR and AMR*

4.3. Results and Discussion

The results of these experiments are presented and discussed under four aspects of the investigation. The effect of thermal influence on enzyme-based soil stabilisation is first reported (Stage 1). Secondly, the stabilisation on the mechanical behaviour of soil at raw soil OMC is demonstrated (Stage 2). Results in Stage 3 are presented in a two-fold characterisation of the mechanism; one is to identify any new formations or chemical reactions, and the second is to explore the change in the pore structure of the soil. Visual inspection, XRD, and SEM with EDS were analysed to support the first type, whereas μ-CT scan results facilitate the second type. The results of these tests are shown and analysed in detail to conclude the mechanism of stabilisation using enzymes. Having verified the identified mechanism of stabilisation from Stage 1-3, Stage 4 tests were conducted to determine the new optimisation of enzyme stabilised soil mix.

4.3.1. Stage 1: Thermal Influence on Enzyme-Based Soil Stabilisation

Results of the tests conducted to investigate the oven drying effect of pre-enzymed soil on soil stabilisation are shown and discussed in this section (Section 4.3.1). A series of UCS tests were conducted in accordance to Australian Standards (AS5101.4 2008) using air dried soil as well as oven dried soil at 20 °C, 40 °C and 60 °C of temperature for 48 hours. The effect of soil drying is clearly visible in Fig 4.3, which shows the results from Stage 1 tests. UCS of stabilised samples dried at 60 °C decreased by 22% compared to stabilised samples prepared using air dried soil. On the other hand, the strength of stabilised samples prepared using 40 °C oven-dried temperature does show similar strength as compared to the stabilised sample at 20 °C air drying condition. These results revealed that there is no adverse effect of drying the soil at the 40 ºC in sample preparation. It appears that the soil undergoes irreversible changes at higher temperatures (> 40 ºC) affecting the mechanical behaviour of stabilised soils. Though the current scope of the study is not to investigate soil response at high temperature, results from Stage 1 assisted in selecting a suitable drying temperature during sample preparation. Hence, the soil was oven dried at 40 °C up to 48 hours for all the tests conducted in the study.

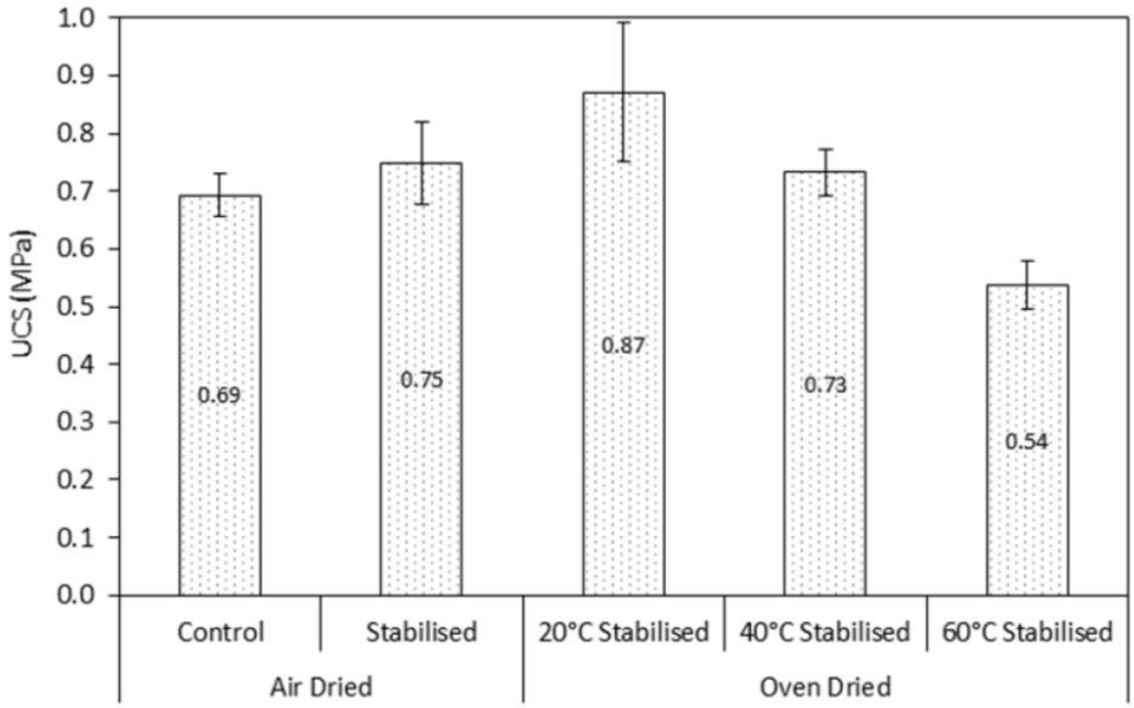


Figure 4.3. Oven drying effect on soil pre-enzyme stabilisation

4.3.2. Stage 2: Enzyme-Based Soil Stabilisation

Results of the experiments conducted to investigate the stabilisation effects of enzyme-based soil at raw OMC are summarised in Fig 4.4. It should be noted that the specimens for this stage of testing were prepared at the 1.95 g cm^{-3} and 12.2% moisture content using modified compaction effort. As seen from the figure, the additive has no positive effect on stabilisation at certain mixes when tested at raw OMC. Soil strength decreases continuously with the increase in AMR at a DMR of 1:100 for both CBR and UCS tests. One possible reason for this strength reduction at a higher DMR (i.e., highly concentrated stabiliser) could be due to the changes in soil structure by decreasing the cohesiveness of the soil, which makes the soil into a less workable and failure susceptible material. Another reason for this decrease in strength under high stabiliser concentration could be due to the increased viscous effect at high DMR. As the pure stabiliser has a high viscosity, high DMR will not facilitate the soil + water to homogenise in the medium. It can also be seen that CBR of stabilised soil at DMR 1:300 has decreased compared to the non-stabilised samples. Hence, it is clear that DMR 1:100 & 1:300 do not support enzyme-based soil stabilisation. On the other hand, soil stabilisation is effective for DMR of 1:500, which is currently being applied in the industry applications of stabilisation when using this additive. A strength increase of up to 15% can be seen for both CBR & UCS at this DMR. However, the effective AMRs based on CBR & UCS is different at DMR 1:500. This could be mainly due to the nature of the testing. Both testing methods vary in the testing format where CBR samples have confinement whereas its counterpart testing method does not. CBR is a more reliable and relatable mode of assessing the efficacy of the additive as it emulates a typical pavement characteristic. CBR tests are considered very important in pavement engineering because almost all the pavement design charts and unbound materials are characterised in terms of CBR. Out of the three pavement design procedures in Australia, both Austroads as well as CIRCLY (pavement design software) uses CBR whereas UCS is only implemented by Queensland Transport and Main Roads (QTMR) (Roads & Infrastructure Magazine 2016). CBR is also considered as being important for pavement design due to its affiliation with the constitutive properties of soil such as plasticity indices, grain size distribution, bearing capacity, modulus of subgrade reaction, resilient modulus, shear strength, density and moulding moisture content (Al Amoudi et al. 2002). Hence, UCS cannot be solely used on its own to deduce the stabilisation mechanism of the additive. However, it could complement the findings of the CBR. Another reason for the different trend in the CBR and UCS tests could also be due to non-homogeneity of the tested soil, which is highly likely to consist of non-uniform mineralogical content due to being a field soil. At lower DMR's (i.e., highly diluted enzymatic condition), CBR based strength degrades continuously with increase of AMR, in contrast to the increase in UCS up to 27% at 7% AMR. Such contradictory strength behaviour at low DMR's could be due to the inefficiency of enzyme-based stabilisation at greater dilutions. Thus, the results reveal that the stabilisation was only effective under DMR of 1:500 with AMR's of 1% & 5% for UCS and CBR based strengths, respectively when tested at raw soil OMC. The strength increase could be due to the decrease in the double layer water of the clay particle which facilitates densification and allows the particle to be more stable and aggregated as hypothesised by Scholen (1992) and illustrated in Fig 4.1.

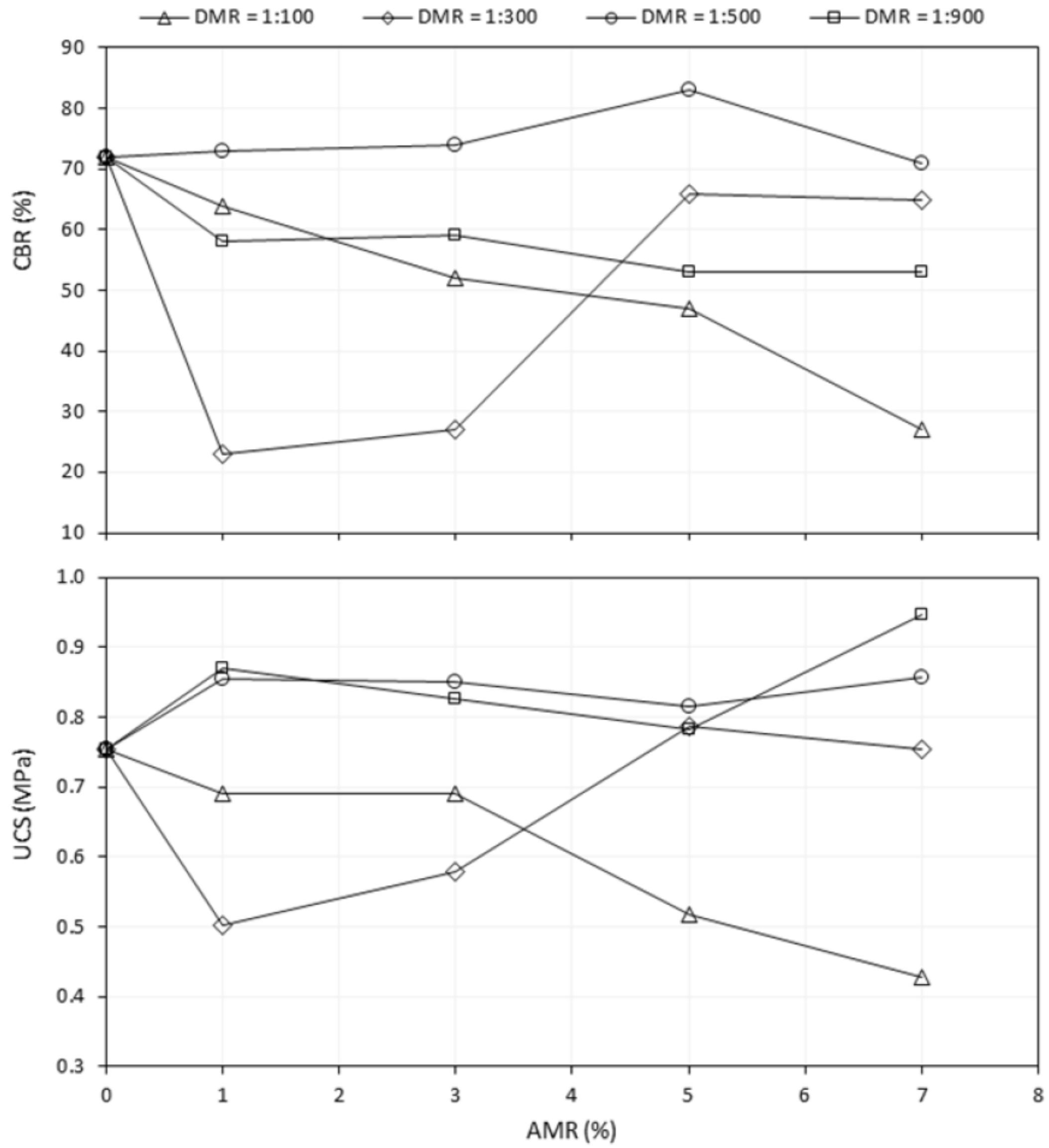


Figure 4.4. CBR and UCS based on raw soil OMC

4.3.3. Stage 3: Mechanism of Soil Stabilisation

4.3.3.1 Microstructure analysis

Detailed analysis of microstructure was conducted to identify the mechanism of stabilisation by comparing control and stabilised samples (DMR 1:500, AMR 7%)

using the enzymes. The first stage of microstructural analysis was a visual inspection of samples which showed distinct differences between the control and the stabilised sample. The sieved soil was first added with the water and enzyme-based additive. The mixture was then thoroughly hand mixed and equilibrated for 24 hours without compaction to observe the drying induced cracks on the soil surface. As seen in Fig 4.5, the control sample has a large number of wider and more distinct cracks compared to the stabilised sample. Though this inspection provides basic information regarding stabilisation efficiency, no measures of such cracks can be obtained. However, it does provide some insight into the crack formation, which could also explain the failure mechanism of this type of soil. The stabilised soil has resisted crack induced failure of the soil. This could be due to the possibility that the double layer of water might have been reduced by the cationic exchange which results in less water being absorbed and decreasing the soil's tendency to swell (Kensenhuis and Modi 2001). SEM and XRD samples were taken from both these soil batches to assess the mineralogical changes, rearrangement and spacing of atoms in crystalline materials due to the enzyme additive as well as to look at the fabric of the soil.

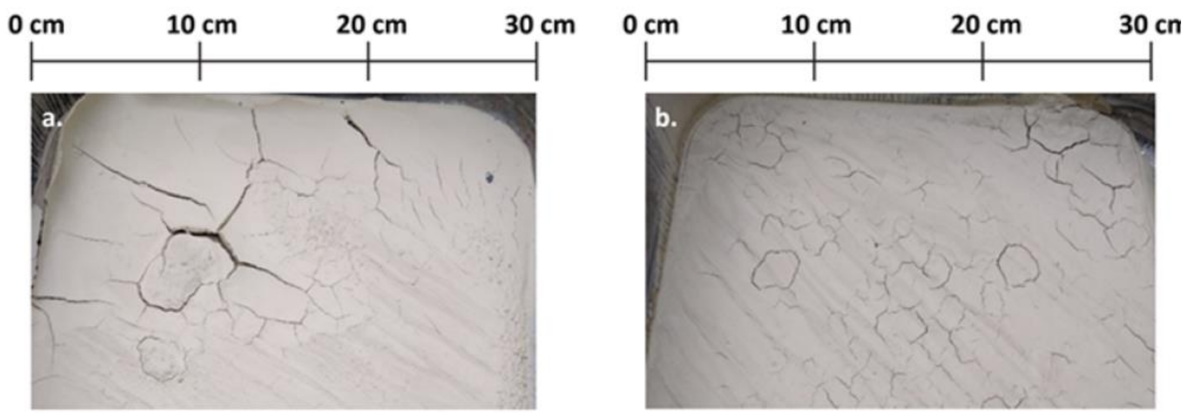


Figure 4.5. Visual inspection. a. Control sample; b. Stabilised sample

Fig 4.6 shows the results of XRD analysis conducted in the current study. The main soil minerals in the stabilised sample (i.e. Quartz, Kaolinite and Muscovite) are similar to those contained in the controlled soil sample with an insignificant difference in the basal peak or the d-scaping of the minerals. These results suggest that there are neither chemical reactions nor the presence of any new cementitious compounds during enzyme-based soil stabilisation. This aligns well with available literature that has been conducted XRD on enzymes stabilised soils. However, Naagesh and Gangadhara (2010) report a change in basal peak and d-spacing of highly plastic soils in contrast to what was observed in the current study. Such differences in observation could well be due to the difference in tested soil types. For example, Nagesh and Gangadhara (2010) have used highly expansive clayey soil for investigations on enzyme-based soil stabilisation, whereas Lean Clay was used in the current study. In order to further investigate the presence of any new compound formation, Energy Dispersive X-ray Spectroscopy (EDS) tests were also conducted in the current study.

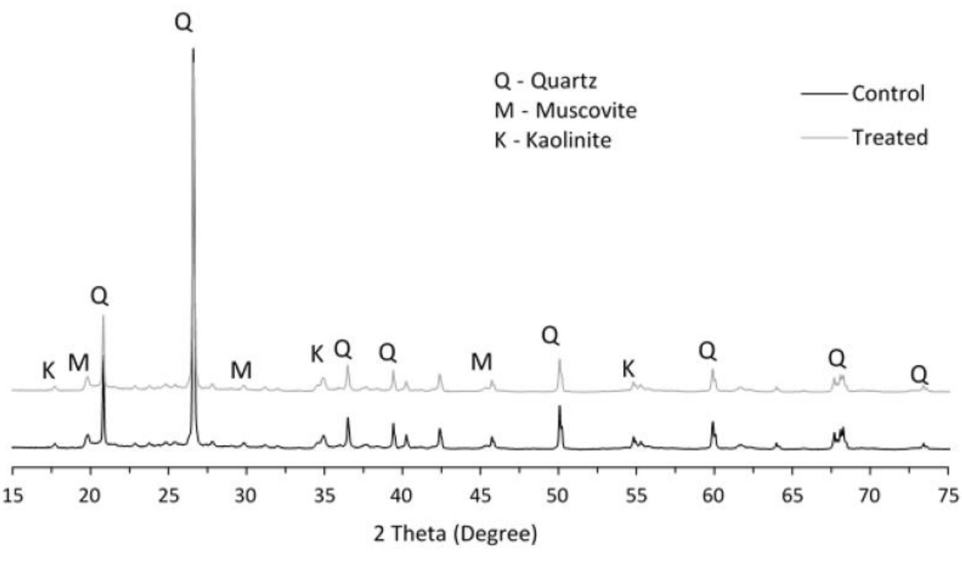


Figure 4.6. XRD results of control vs treated soil

The images from SEM analysis are shown in Fig 4.7 for the controlled and stabilised samples with 5000x magnification, which was limited mainly by the presence of oil in the additive. It can be seen that the control specimens (Fig 4.7 a – b) significantly differ from the treated specimens (Fig 4.7 c – d) in terms of surface roughness with rougher surface observed on the stabilised samples compared to that of the control samples. EDS results shown in Fig 4.8 revealed that there were no significant changes in the Al:Si ratio nor any change in compositions, verifying no chemical reaction had taken place during enzyme-based soil stabilisation. These findings support the XRD results reported in this study and align with other published research on enzyme-based stabilisation for less expansive soil (Rauch et al. 2003).

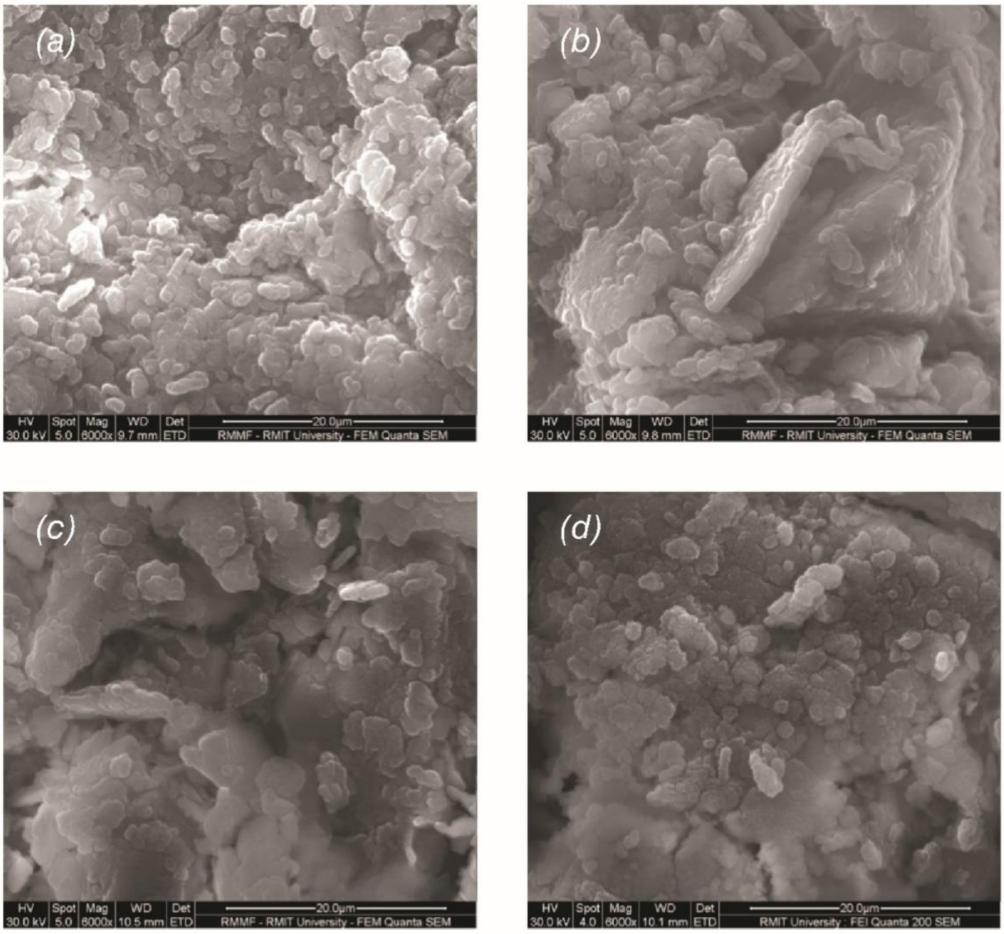


Figure 4.7. SEM Microscopic images. (a-b). Controlled sample; (c-d). Stabilised sample

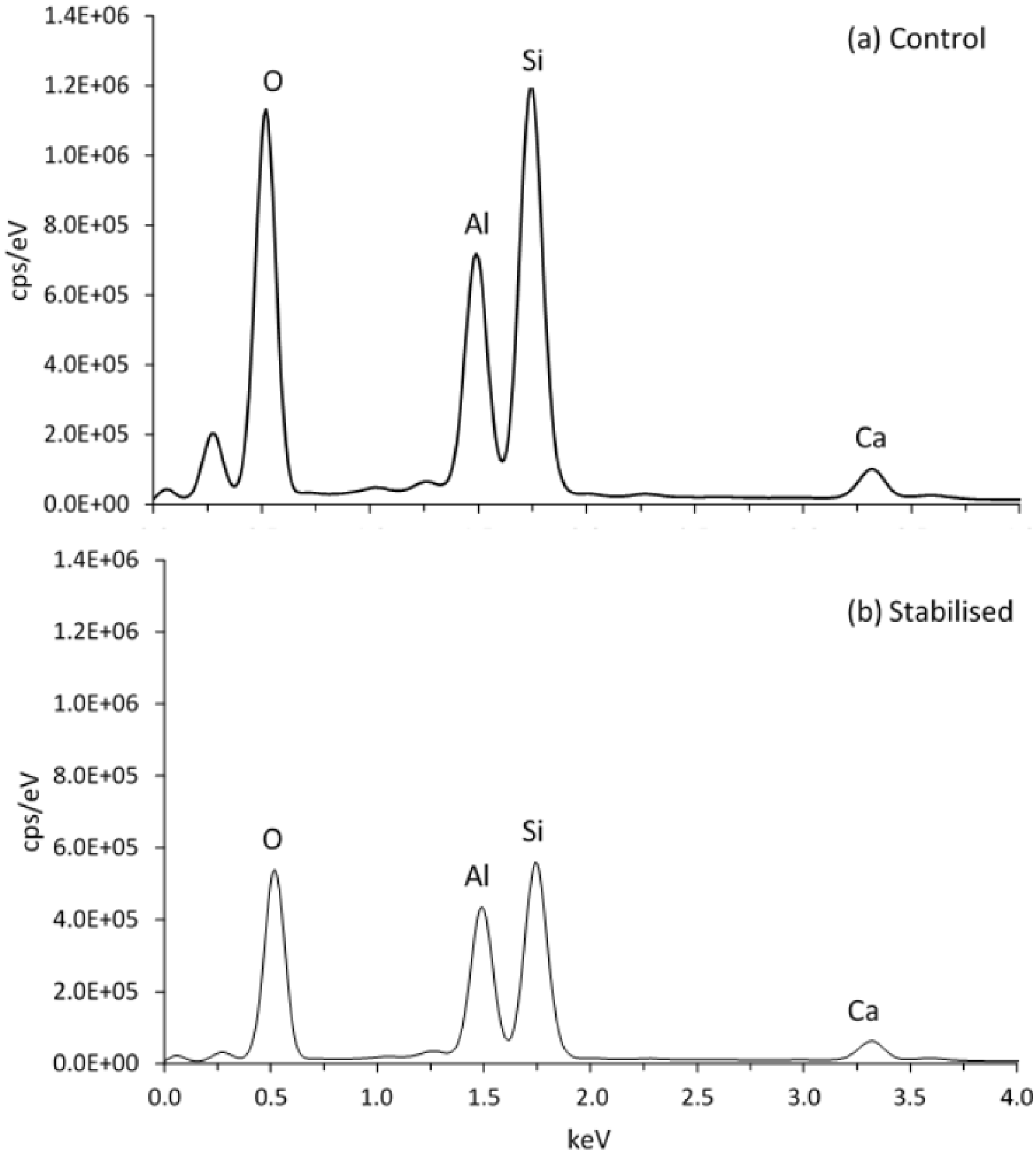


Figure 4.8. EDS results of control vs stabilised soil

4.3.3.2. Pore-structure Analysis

Micro-CT analyses were conducted to investigate the mechanism of stabilisation further using enzyme-based additives. Tests were conducted based on stabilised samples at 7% AMR at different DMRs. The selection of 7% AMR is due to the highest and lowest strengths resulted at this AMR for low and high concentrated enzyme cases, respectively, as seen in Fig 4.4. The scanning was performed on post-UCS tested samples which were cut down to 2 mm cube, dried and scanned on Bruker Skyscan Microtomography using a Cu filter with the images being reconstructed using Skyscan NRecon. X-ray aided Computer Tomography provides an accurate variation of x-ray absorption within a scanned sample by mathematically reconstructing a set of slices of a scanned object on its height using binarization (Rajczakowska et al. 2015). This binarization helped with the analysis of pore distribution as well as porosity factor determination of the scanned specimens. It should be noted herein that the visual inspection of scanning images (presented below) may not represent realistic amounts/trends of porosities, especially in stabilised samples which show reduced porosities compared to controlled samples. This is because the images obtained (shown in Fig 4.9) are a 2-D representation of the 3D sample. However, the calculated porosity factors (shown in Table 4.3) account for a general representation of porosity in the 3D sample as it considers multiple 2D images across 360°.

Results from the scanning images (Fig 4.9) showed a significant difference of voids between control and stabilised samples using the additive. Results showed a substantial difference of voids between the control and stabilised samples using the additive. It can be seen that the control sample shows a large distribution of pores with varying sizes at randomly dispersed locations across the specimen. On the other hand, samples prepared at DMR of 1:100, 1:500 and 1:900 show images with a significant decrease in pore location and distribution. However, closer inspection of the DMR

1:100 stabilised sample, seen in Fig 4.9b shows the formation of a crack across the surface of the specimen that could lead to failure of the sample during compressive loading. The pore distribution from micro CT scan is further analysed in terms of a porosity factor, as shown in Table 4.3. Porosity factor is identified in this analysis with respect to the intensity of pixels of the sample voids. i.e. the scanned image is represented by the intensity of the pixel as white or black. The part of the image that is identified as black represents the voids in the scanned sample. The porosity factor represents the total volume of this black segment in the scanned sample. As aforementioned, a clear reduction in the porosity factor is resulted due to enzyme-based soil stabilisation. Results also revealed that the strength variation observed from mechanical tests follow a similar trend that directly depends on the porosity changes based on various diluted conditions of the additive. For example, the highest porosity of the stabilised sample is at DMR 1:100 which yields the lowest UCS strength and the lowest porosity resulted for DMR 1:900 that yields the highest UCS strength as observed from Fig 4.4. Therefore, micro CT scanning results reveal that soil stabilisation using enzyme additives in this study is mainly governed by soil densification.

Table 4.3. Pore factor analysis using CTAN software

Tested Sample	**Porosity Factor (%)**
Control	3.00
1:100	0.74
1:300	0.10

1:500	0.13
1:900	0.09

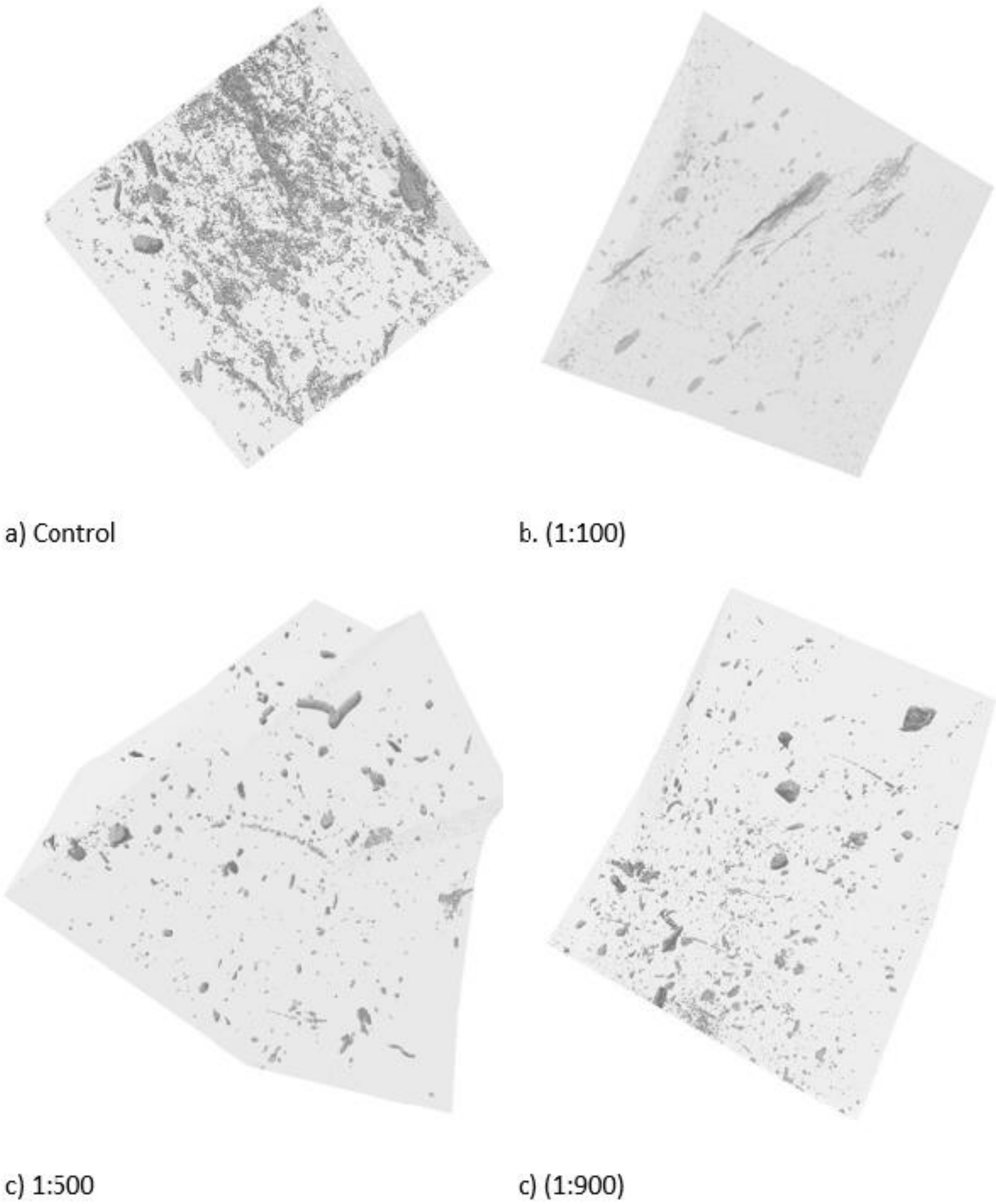


Figure 4.9. CT scans of control vs stabilised samples

4.3.4. Stage 4: Optimisation of Soil Stabilisation

4.3.4.1. Hypothesis verification

A number of standard proctor compaction tests were performed on stabilised samples to verify the densification hypothesis that was identified from the micro-imaging technology-driven analysis. Tests were conducted at four AMRs of 1%, 3%, 5% and 7% at three DMRs of 1:100, 1:500, and 1:900. In general, the results showed notable differences in OMC and MDD for stabilised soils compared to control soils, as shown in Fig 4.10 – 4.12 and Table 4.4. The OMC and MDD of the stabilised samples decreased and increased respectively at each case of enzyme treatment, as demonstrated in Table 4.4. The maximum reduction of OMC and the largest increase in MDD are at 1:500 DMR at 1% and 7% AMR of 14.1% and 1.1% respectively. Results from compaction tests can be used to explain the strengths observed in the mechanical tests which were conducted predominantly at the OMC for controlled soil reported in Fig 4.4. For instance, it can be noted that DMR 1:100 decreased the affinity of water to a consistent lower range of 15% OMC regardless of the AMR, with an increase in MDD. However, the density of the sample for DMR 1:100 is up to ~2% less than the density of the control sample at 17% moisture content, which was the OMC utilised in the mechanical tests. Hence, the reduction of strength observed from mechanical tests (Fig 4.4) for DMR 1:100 is due to the reduced density of the stabilised sample that was located at the wetter side of the OMC curve. The increase in strength at lower DMR additive can be explained as occurring due to the sample being the closest to the OMC of the treated soil. From these compaction results, it can be perceived that the change in OMC/MDD governs the efficacy of the additive and that the addition of enzymes creates a denser material with a decrease in the affinity for water. Hence, the primary mechanism of enzyme-based soil stabilisation can be verified as densification, which is the fundamental mechanism for enzyme-based soil stabilisation as identified in the literature (Scholen 1992, Rauch et al. 2003).

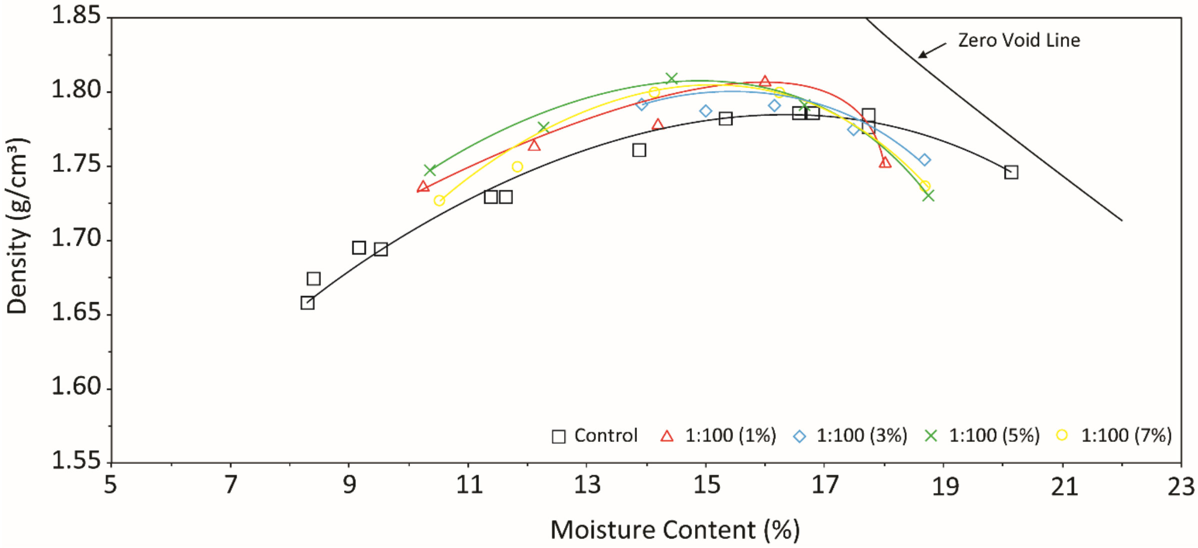


Figure 4.10. DMR 1:100 standard compaction curve

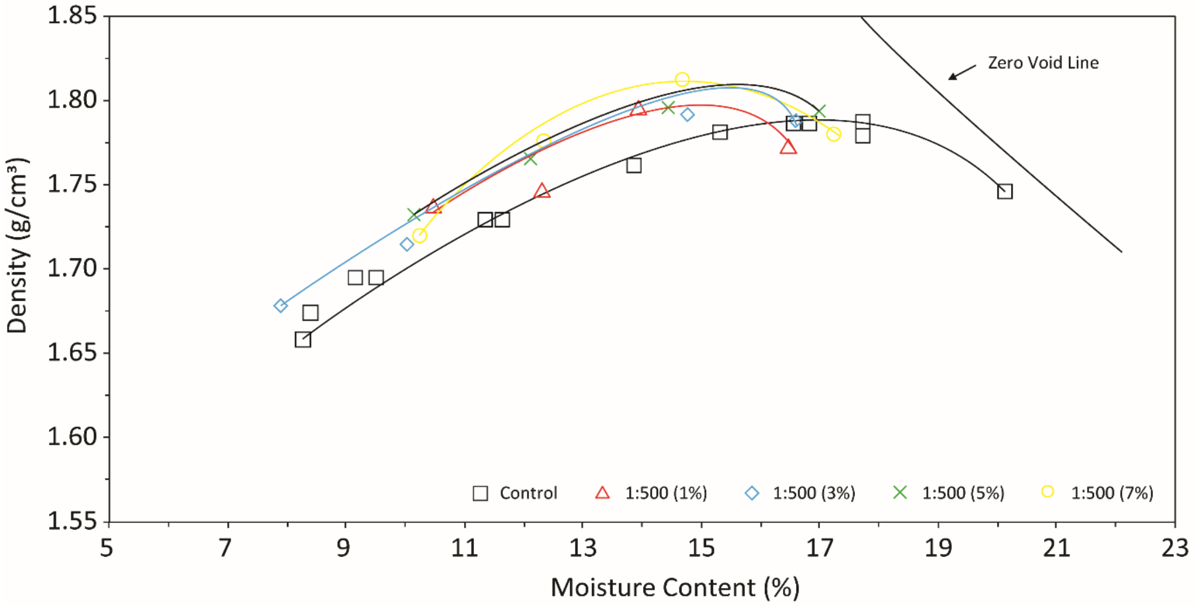


Figure 4.11. DMR 1:500 standard compaction curve

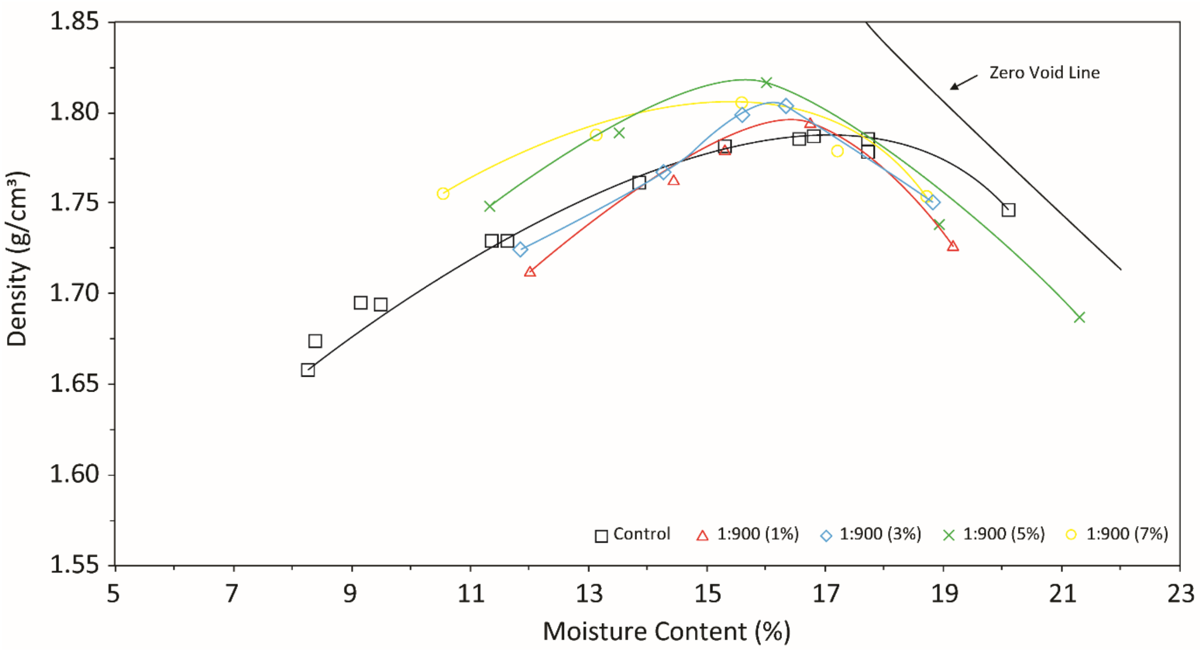


Figure 4.12. DMR 1:900 standard compaction curve

Table 4.4. Summary of OMC/MDD change

Enzyme composition		OMC, % (% change compared to controlled condition)	MDD, g/cm^3 (% change compared to controlled condition)
DMR	AMR		
0	0	17	1.79
1:100	1	15(-11.8)	1.81 (+1.1)
	3	15.4(-9.4)	1.8 (+0.6)
	5	15 (-11.8)	1.81 (+1.1)
	7	15 (-11.8)	1.81 (+1.1)
1:500	1	14.6 (-14.1)	1.8 (+0.6)
	3	15.8 (-7.1)	1.8 (+0.6)

	5	15.6 (-8.2)	1.8 (+0.6)
	7	14.6 (-14.1)	1.81 (+1.1)
1:900	1	16.8 (-1.2)	1.8 (+0.6)
	3	16.2 (-4.7)	1.8 (+0.6)
	5	15.6 (-8.2)	1.82 (+1.7)
	7	15.6 (-8.2)	1.81 (+1.1)

Having investigated and verified the mechanism of stabilisation, further mechanical tests were conducted to enhance the optimisation of enzyme-based soil stabilisation. A number of unsoaked CBR tests were conducted at three DMRs (1:100, 1:500, 1:900) and four AMRs (1%, 3%, 5%, 7%) based on the new OMC (Table 4.4) from the standard proctor compaction curves (Fig 4.10 – 4.12). It can be seen from the results (Fig 4.13) that CBR was significantly enhanced by enzyme-based soil stabilisation. For example, CBR of soil was increased up to 500%, 425% and 200% from the stabilisation based on DMRs 1:100, 1:500, and 1:900 respectively. The peak strength was observed at 1% AMR for DMR 1:500, whereas the strength gains yet to reach a plateau for DMR 1:100 & 1:900. From the results, it can also be seen that using a higher AMR at the 1:500 DMR will ensure the proper additive mixing in the soil with lesser mixing effort, i.e., the use of 7% AMR at 1:500 DMR implies that there is a higher amount of additive available to homogenise the soil in contrast to 1% AMR while maintaining the same strength gain. However, this increase in strength at each DMR/AMR was higher than what was observed from mechanical tests conducted on the basis of controlled sample OMC. i.e., marginal strength increase of 26% in CBR with 1:900 DMR at 7% AMR was observed for tests based on controlled

OMC in contrast to 200% strength increase from tests using stabilised OMC for the same AMR/DMR combination. New mechanical test results also revealed a considerable decrease in the strength variation at a given stabilised condition with a reduction of average standard deviation from 15% to 3.5% from controlled OMC to stabilised OMC tests respectively. A similar trend was also noted from the results of UCS tests conducted, as shown in Fig 4.14, i.e., an increase of strength up to 54% was observed in UCS for DMR 1:100 at 1% AMR. Hence, it can be seen from these results that the enzyme-based stabilisation derived from new compaction limits have significantly improved the efficiency of the stabilisation resulting in substantial strength gain. The stabilisation mechanism, which was identified as densification for the tested enzyme-based additive, has significantly improved the additive's efficiency. Application of enzyme-based additive has resulted in a densified structure of soil with less affinity of water as schematically presented in Fig 4.15. The increase in stabiliser efficiency is significantly larger when the stabilisation is performed at OMC_{stab} compared to the stabilisation at OMC_{raw},i.e., x_{new}>x_o, as shown in Fig 4.16. Therefore, the optimised stabilisation for the enzyme-based additive used in this study can be identified as DMR 1:500 at 1% AMR to attain the optimum strength for fine grained soils.

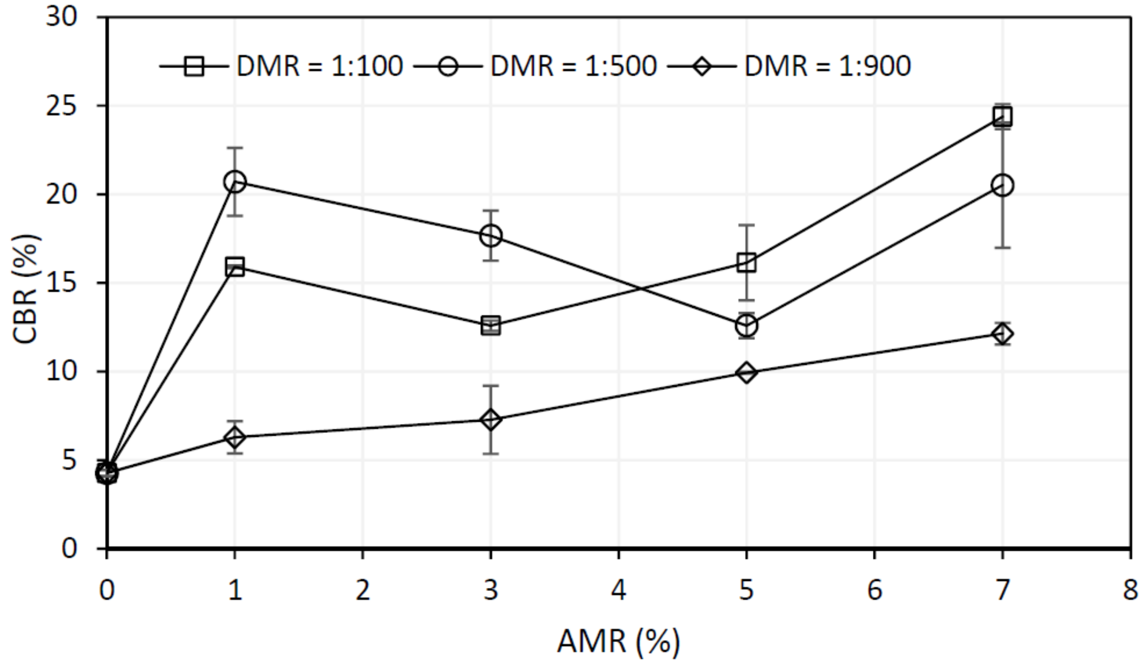


Figure 4.13. Optimised CBR

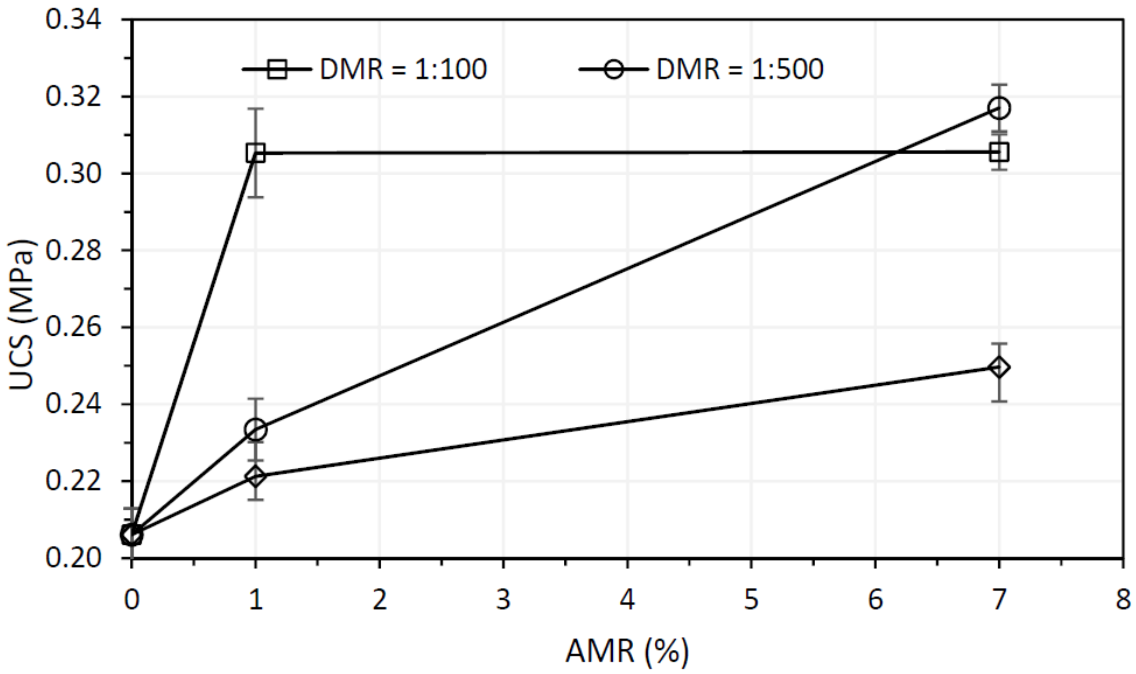


Figure 4.14. Optimised UCS

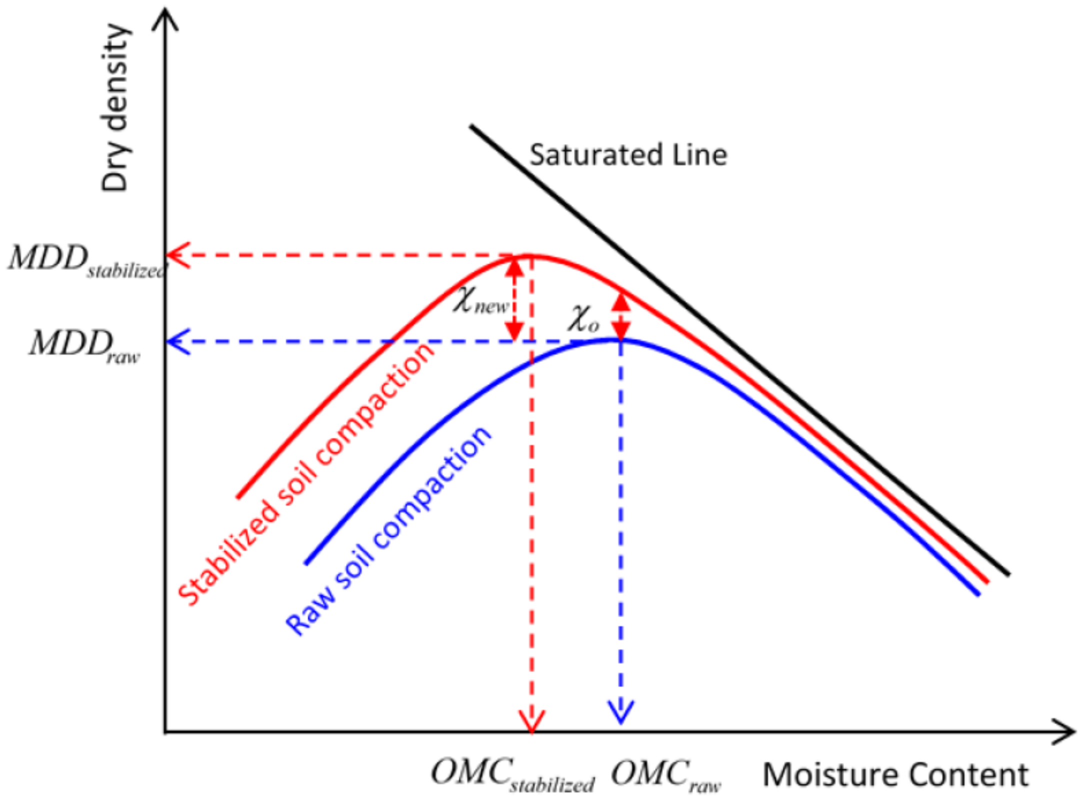


Figure 4.15. Mechanism of enzyme-based soil stabilisers unveiled through experiments

4.4. Case Study – Application of Enzymes for Soil Stabilisation

4.4.1. Trial Road Construction

Enzyme-based soil stabilisation was conducted on two segments of a trial road in Possum gully road, located 10 km south-west of Maryborough township in Victoria (Fig 4.16). The local trial section road (3 km in length) carries around 118 vehicles per day with around commercial users. The overarching objective of the project was to identify pavement/wearing course material that is readily available to the Council. Another major objective was to investigate how to improve the properties of the materials for the purposes of use as unsealed road wearing courses by using additives and/or combining with other materials. This section (Section 4.4.1) summarises the construction stages of the unsealed road construction using the developed enzyme-based stabilisation method. Prior to the construction, the trial road subbase was shaped, trimmed and compacted to at least 98% of proctor compaction by measuring the field density of compacted soil using nuclear gauge and manual dry density measurements. Imported materials from the sites having soil type S1 and S2 were added to the pavement at a depth of 150 mm at nine different segments of 300 m x 5.5 m which was selected to be stabilised as the pavement's base/wearing layer. Segments one to four consisted of imported S1 soil type, whereas segments five to nine contained S2 soil type. S1 was identified as Clayey Sand (SC) based on the particle size distribution (Fig 4.17) with a fine fraction of the soil classified as lean clay (CL). Compaction characteristics revealed that S1 had an OMC of 6.2% with a maximum dry density of 2.154 g cm^{-3} based on Australian Standards (AS1289.5.2.1 2000). S2 was identified as Clayey gravel and sand mixture (GC) (Fig 4.18) with the fine fraction classified as CL. Styles (2019) report that both the material stabilised with the additives were assessed against the ARRB criteria (ARRB 2009) which shows that both the soil types met desired characteristics such as ease of grading, compaction and traffic comfort as well as requirements for producing stable and low permeable wearing course characteristics. However, it was suggested that clay should be added to S2 soil to improve clay content and particle size range. The problem associated with S1 type soil included the high fine content mainly silty in nature with low cohesion and its high susceptibility to corrugation in its natural form. To counter this issue, segment 4 of the trial road incorporated the use of local bluestone quarry material and good quality clay. Compaction characteristics revealed that S2 had an OMC of 6.1% with a maximum dry density of 2.109 g cm^{-3} based on Australian Standards (AS1289.5.2.1 2000). The additives incorporated in each segment is as follows:

Segment 1: S1 with 3% cement and 3% foam bitumen

Segment 2: S1 with 3% polymer

Segment 3: S1 with Eko-Soil at 1 litre to 30 m^3

Segment 4: S1 with 46% class 4 FCR, 8% clay

Segment 5: S2 with Eko-Soil at 1 litre to 30 m^3

Segment 6: Crushed and screened S2

Segment 7: Crushed and screened S2 with 3% cement

Segment 8: Crushed and screened S2 with 5% clay

Segment 9: S2 with dust suppressant

In-depth analysis of treatment apart from enzyme will not be included in this chapter as it is not part of the scope of the project. However, comparative analysis of the treated segments is included in the following sections (Section 4.4.2 and Section 4.4.3). It should be noted that all imported materials (S1 and S2) were used up in the treatment with no segment prepared without treatment. It should also be noted that the soil samples, before and after adding the enzyme were collected in sealed plastic bags and delivered to the labs for conducting the mechanical testing (i.e. CBR).

Figure 4.16. Unsealed Road Treatment Trial 2016/2018

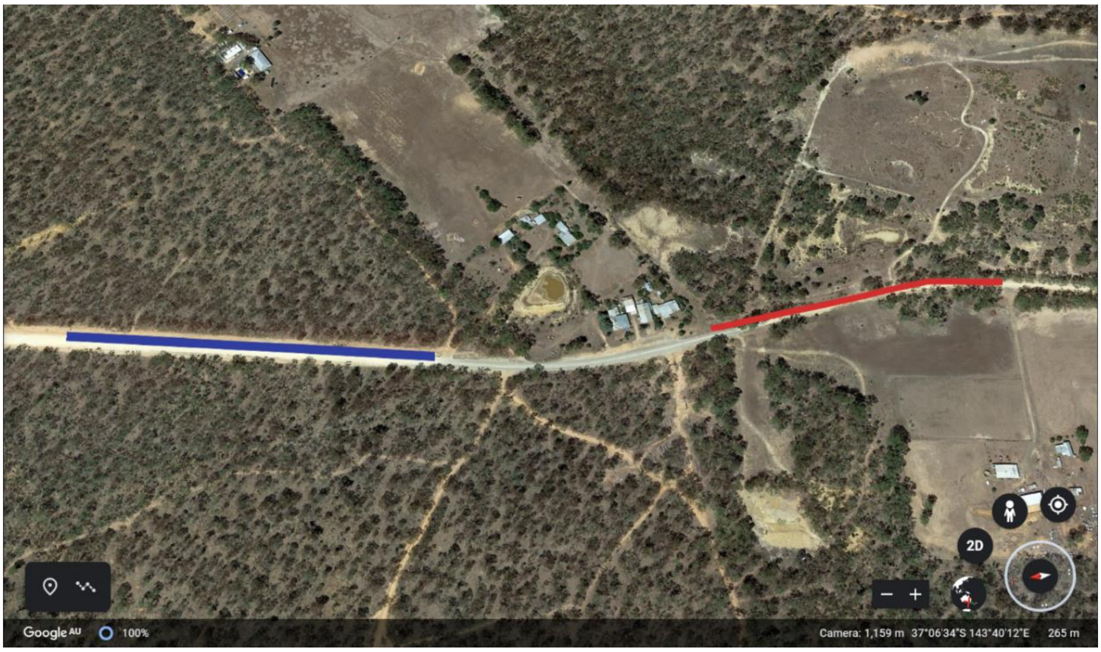


Figure 4.17. Aerial view of the enzyme treated pavement segments. (Blue = Segment 3, Red = Segment 5)

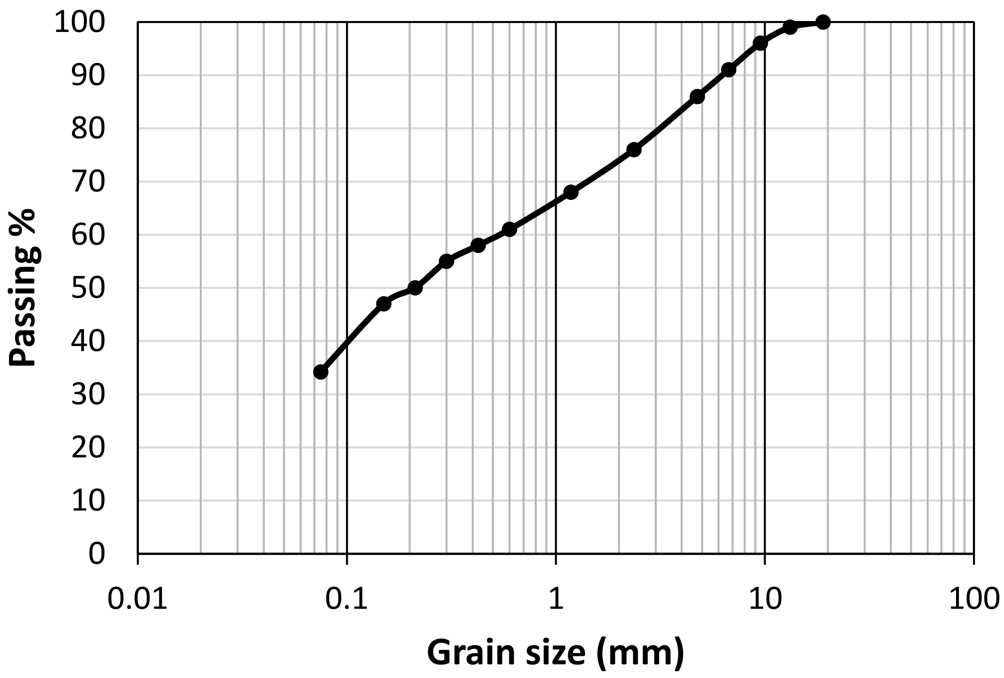


Figure 4.18. PSD of soil S1

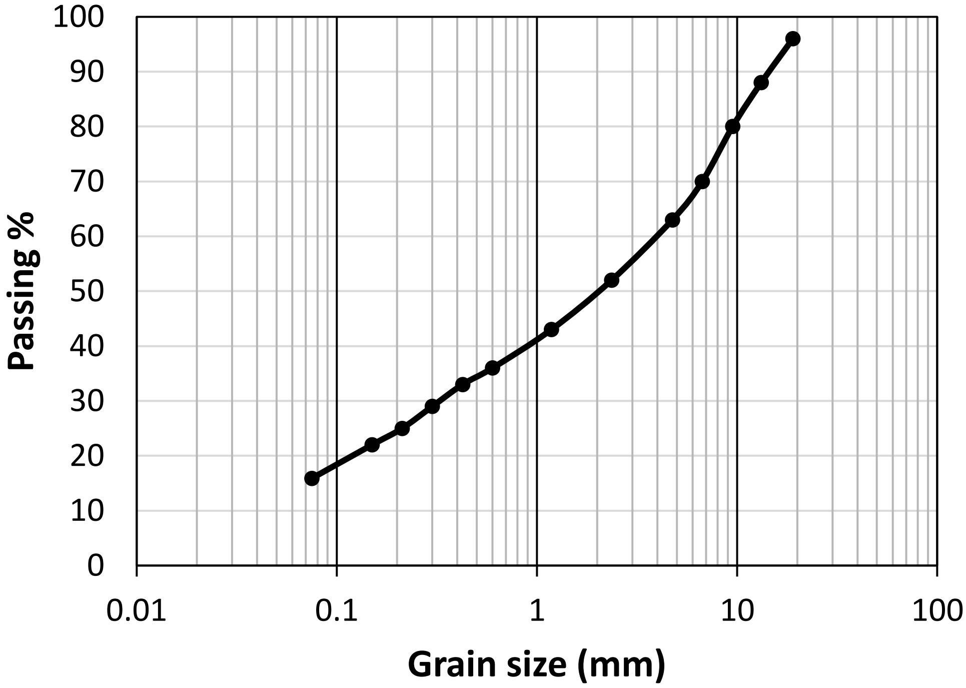


Figure 4.19. PSD of soil S2

The enzyme treatment on the trial road was performed as described below:

Construction Stage 1: Ripping

The compacted pavement was ripped to 150 mm depth with the grader, which allowed the moisture levels to be easily adjusted, increase the depth of enzyme penetration and to reduce the possibility of losing enzymes through drainage (Fig 4.20b).

Construction Stage 2: Moisture Adjustment

The moisture of the pavement was adjusted via passing the dribble bar from the watercart over the soil several times until it was deemed to be just before optimum moisture content (OMC) as determined by a squeeze test by the enzyme supplier's consultants onsite (Fig 4.20c). The controlled water content was based on the selected enzyme percentage (1% by dry weight) and the OMC of the selected soil type.

Construction Stage 3: Dilution of Enzyme and Spreading

After assessing the moisture content of the pavement's material, the pre-calculated diluted enzyme (1:500) was added into the pavement material using the same watercart. Once the enzyme was added from the watercart, it was evenly spread over the pavement for uniform bed preparation (Fig 4.20d).

Construction Stage 4: Mixing the Enzyme

Once the enzyme was sprayed into the wet soil, soil + water + enzyme were mixed thoroughly using the stabiliser (Fig 4.20e).

Construction Stage 5: Compaction, Shaping and Final Moisture Adjustment

Finally, the pavement was compacted by passing the multi-tyre and smooth drum rollers several times over the pavement. Moreover, shaping was performed by the grader until a cross fall of at least 6% was achieved (Fig 4.20f).

(a) S4 trial road segment before stabilisation

(b) Stage 1: Ripping of the trial segment with grader

(c) Stage 2: Moisture adjustment with watercart

(d) Stage 3: Soil after dispensing the enzyme

(e) Stage 4: Mixing of soil and enzyme

(f) Stage 5: Finished road segment after shaping and compaction

Figure 4.20. Construction stages of the trial road

Careful monitoring of the moisture and dry density was conducted to ascertain that the construction follows the expected construction standards. Table 4.5 summarises the pavement parameters achieved during construction. It was observed that the construction was able to achieve reasonable moisture ratios, but unable to meet 98% proctor density as specified by VicRoads Code of Practice RC 500.20 (VicRoads 2014). This could be mainly due to the use of multi-tyre roller instead of a vibrating roller.

Table 4.5. Constructed road parameters

Soil ID	**Moisture Ratio (%)**	**Density Ratio (%)**
S1	98.0	92.5
S2	96.5	89.5

CBR tests were performed as per Australian standards (AS1289.6.1.1 2014) on the basis of samples obtained from the road, before and after stabilisation. Samples were obtained at two segments of the road where imported soils S1 and S2 were utilised. Fig 4.21 summarises the test results from the lab tests. It should be noted that the testing was conducted in duplicates in which the CBR samples were compacted used modified compaction method with five layers and 55 blows and was conducted on a four-day soaked curing condition. It should also be noted that due to unavailability of proper accurate moisture content monitoring device, the compaction was based on the hand squeeze technique based on the site engineer to determine whether appropriate moisture content had been attained. For this reason, only an approximate value of OMC could be attained as opposed to the accuracy in the moisture content attained at laboratory conditions. As it can be seen, the stabilisation has been significantly effective to increase the road strength from prior stabilisation to post-stabilisation (an increase of 69 CBR and 101 CBR respectively).

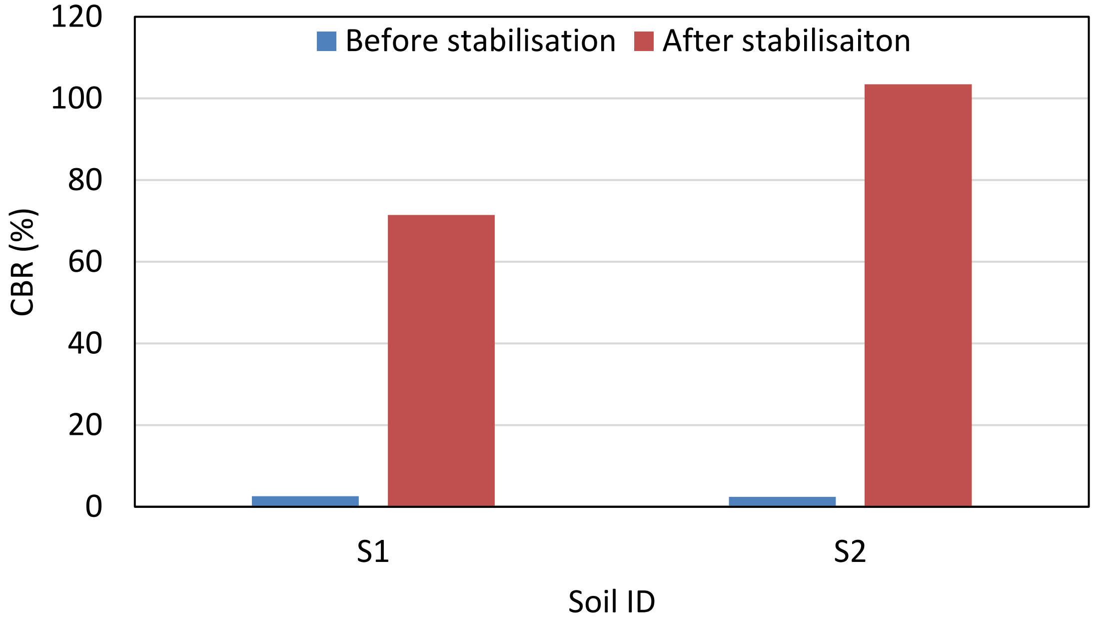


Figure 4.21. CBR results obtained from lab tests on the trial road

4.4.2. Pavement Design Analysis

4.4.2.1. Mechanistic Design CIRCLY

A mechanistic pavement design has been conducted in this study using CIRCLY to investigate the allowed traffic load, which satisfies the strain and rutting limits as specified in the Austroad standards (Austroads 2001). Results from CIRCLY analysis provide an estimate of the pavement layer depth required to sustain traffic loads. The geometry for the analysis and the input parameters are shown in Fig 4.22 and Table 4.6.

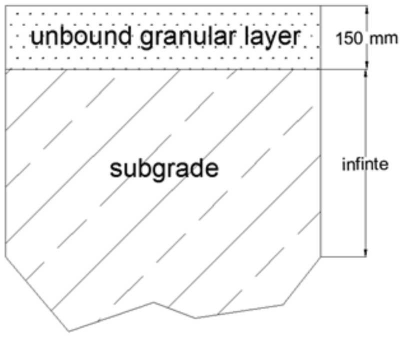


Figure 4.22. CIRCLY pavement design

Table 4.6. CIRCLY design parameters

DESA	4×10^3 [a]
Project reliability	85% [b]
Traffic Multiplier	1 [c]
Subgrade Thickness	0.00 [d]

a. Minor road with two lanes, b. based on Austroads pavement design for unbound pavements, c. chosen traffic multipliers, d. 0.00 represents an infinite depth

CIRCLY analysis was conducted on the designed pavement to satisfy the cumulative damage factor (CDF<1) of the designed pavement as specified in Austroads (Austroads 2001). Table 4.7 summarises the results of the analysis, which shows the minimum depth required for the treated base layer as 350 mm and 369 mm for S1 and S2, respectively. They revealed a substantial reduction of material (22% and 23% reduction for S1 & S2 respectively) for the base layer from pre-stabilisation to post-stabilisation. However, it should be noted the values obtained from the CIRCLY analysis suggest the stabilisation of the pavement at a depth of 350 mm and 369 mm for S1 and S2, respectively, which was not followed at the site due to the limitation in the quantity of the imported materials. As mentioned earlier, the imported material was just acquired at a 150 mm depth to the roadbed.

Table 4.7. Results of the CIRCLY analysis

Soil ID	Thickness of layer (before stabilisation)	Thickness of layer (after stabilisation)	Difference in layer depth
S3	451mm	350mm	101mm
S4	479mm	369mm	110mm

4.4.2.2. Weighted Average CBR (Japan Model)

Japanese model was also incorporated for the pavement design to assess the benefits of enzyme-based stabilisation. This model refers to the Japan Road Association formula, which determines the equivalent subgrade strength (Austroads 2001). This model determines the required CBR to be achieved by pavement layers for an average weighted CBR of 5, which is currently being used as a rule of thumb by road contractors from experience. The equivalent subgrade strength is based on the following equation (Equation 4.1).

$$CBR_m = \left[\frac{\sum_i h_i CBR_i^{\;0.33}}{\sum_i h_i}\right]^3 \leq 20 \qquad \textbf{\textit{(Eq. 4.1)}}$$

Where CBR_i is the CBR value in layer thickness h_i... and $\sum h_i$ is taken up to a depth of 1.0m

As shown in Table 4.8, the weighted average CBR for the treated soil obtained from the Japanese model exceeds 5.0, which is currently being used as a rule of thumb by road contractors from experience. Therefore, it can be seen from the current study that the treated soil provides adequate strength to satisfy the constructed road to operate as lightly trafficked unbound road.

Table 4.8. Results of the CIRCLY analysis

Material	**Layer**	**Depth (m)**	**CBR per layer**	**Weighted average CBR**
S3(non-treated)	Unbound layer	0.15	2.6	2.57
	Subgrade	0.85	2.6	
S3 (treated)	Unbound layer	0.15	71.5	5.63
	Subgrade	0.85	2.6	
S4 (non-treated)	Unbound layer	0.15	2.4	2.38
	Subgrade	0.85	2.4	
S4 (treated)	Unbound layer	0.15	103.5	6.11
	Subgrade	0.85	2.4	

As seen above, the immediate impact on the pavement from the enzyme-based soil stabilisation has been effective. Monitoring of pavement segments was conducted to assess the performance of the road under operational traffic loads for up to two years post-construction to verify the effectiveness of the selected enzyme stabilisation.

4.4.3. Pavement Monitoring

Styles (2019) report the results of the trial road construction using a triple bottom line approach, which bases the evaluation taking into account the economic, social and environmental impacts of the pavement segments. Observations on parameters such as dust, roughness and cross-sections of the pavement are assessed in a two-year time frame. The initial observations report that all segments were smooth and stable, except for segment 9, which showed signs of immediate unravelling post-construction. However, it was reported that the enzyme stabilised S1 soil (segment 3) concerned the motorists during wet periods. Dust observations conducted by Styles (2019) is based on Boyd and Van Cauwenberg (1980) which scales the dust produced from 0 to 5, with zero being extreme dust conditions with severe visibility restriction which takes 1 to five seconds to improve. Table 4.9 refers to the summary of the testing conducted as reported by Styles (2019). As seen from the results (Table 4.9), cement and bitumen foam treated segment displays the highest resistance to dust formation by the clear margin. The enzyme treated segments 3 and 5 were rated 1.9 and 2.6, respectively. Travelling on segment 3 produced thin dust clouds which affected visibility and drifted past roadway, whereas segment 5 was mildly better.

Table 4.9. Review of the trial road reported in Styles (2019)

Segment	**Additive Unit rate ($)/m^2**	**Maintenance cost at the end of the trial ($)**	**Rating**	**Roughness rating**	**Remark**
1	22.97	255.3	4.1	Fair	Good dust suppressant
2	10.64	254.49	2.6	Poor	Rough

3	16.95	713.87	1.9	Bad	Poor rideability High maintenance costs High loss of shape
4	15.10	69.11	1.3	Poor	Rough
5	18.14	281.32	2.6	Fair	High loss of shape
6	4.71	139.85	2.7	Fair – Good	
7	11.89	48.78	2.9	Fair – Good	Low maintenance costs
8	10.38	-	3.2	Fair – Good	No maintenance required throughout the trial
9	4.83	375	2.5	Poor	Regrade required within seven months of treatment

Styles (2019) assesses the loss of material based on a simple yet disciplined visual measurement method. In this method, loose materials from a select section (measuring 1 m x 0.5m) of each treated segment were brushed to form a circle of 300 mm diameter. The depth of the formed circle was used as an indicator of materials loss. Once again, the findings from the test show that segment 3 (S1 soil type) reported the highest loss in materials. Segment 5, the other enzyme treated segment, was also reported to have shown the highest loss of the second soil type. Yet again, segment 1 was reported to be the most effective form of treatment. The roughometric readings were also used to analyse the rideability of the pavement post-treatment which graded the enzyme treatment segment 3 as “bad” and as segment 5 as “fair”.

Based on the results of the trail road treatment, it was evident that stabilisation was more effective on S2 soil type. It was also evident from the tests that segment 8 reported the best treatment which comprised of screened soil with 5% non-dispersive clay. These findings from the trial road stabilisation and monitoring suggest that although enzyme alone treated samples are likely to show immediate improvement in the strength of the soil, as reported in Fig 4.21, durability is not guaranteed. The immediate effect of the enzyme is based on the increased CBR of the soil, which in turn help decrease the pavement thickness, as shown in Table 4.7. By monitoring the health of the pavement post-treatment, it can be seen that the enzyme treated section has minimal effect on the longevity based on economic as well as pavement rating. Weighted economic scores reported by Styles (2019) highlight that both the enzyme treated segments were among the least economic segments based on the unit rate as well as maintenance costs. Based on the long-term ineffectiveness of the treatment by enzyme alone could render the treatment being inadmissible as an ideal option.

However, it can be said that the ineffectiveness, as seen here, could be due to a few reasons. Firstly, as shown in Table 4.7, CIRCLY analysis recommended the use of the stabilised pavement thickness as 350 mm and 369 mm. This recommendation was not able to be implemented in the treatment due to the limited quantity of the stabilised material available. As mentioned above, the treated section was only stabilised to 150 mm depth which could have played a vital part in decreasing the longevity of the enzyme stabilised segment. Styles (2019) also report in-house and contracting partnering issues as another reason for the ineffectiveness of the enzyme treated segment. For example, the improper methods deployed by the contractors such as the use of the multi-wheeled roller instead of self-propelled 15-tonne steel vibrating roller. The use of this type of roller did not help achieve the desired compaction levels of segment 3 (enzyme treated S1). Cross fall specified requirement of 6 ± 0.5% was also not met by the cross-road grader in many segments during the construction. Secondly, the reasoning for the effectiveness of the other additives could also be due to the nature of the chemical reaction that takes place within the soil fabric and the additives which could produce new cementitious compounds. As reported in Section 4.3.3, treatment with enzymes on the soil has not shown to produce any new or cementitious products. This would suggest that for the treatment to have shown effectiveness in the trail road construction, the depth of the stabilised layer should have been greater than 350 mm (S1) or 369 mm (S2) as recommended by the CIRCLY analysis. This would also suggest that investigation into the combination of the enzyme with secondary additives need to be explored when depth requirements could not be met to support enzyme-based soil stabilisation further. The effect of combining enzymes with additives such as fly ash and fibres has been reported in limited literature, albeit showing positive response (Khan and Sarker 1993, Correa et al. 2015).

4.5. Summary

Though soil stabilisation using enzymes is commonly being tested/applied, the application of enzyme-based additive for soil stabilisation is site specific. This research work aimed to identify the optimised mix proportions of a novel enzyme-based additive by identifying its mechanism in stabilising fine-grained field soil which is dominant in Victoria, Australia. A systematically controlled 4-Stage test program has been executed by identifying required sample preparation measures for stabilised soils. A series of macroscopic and microscopic tests were conducted on stabilised soils under various measures of stabilisation to understand and evaluate the effect of soil stabilisation on strength as well as on compaction characteristics of the soil. This chapter investigates the soil stabilisation effects using enzyme-based additive, which is being applied to construct unpaved roads in Australia and worldwide.

The key findings from the research study can be identified as the following:

- Results from the Stage 1 tests showed that the enzyme-based soil stabilisation was not affected by the oven drying effects up to the temperature of 40 ºC and the use of soil oven dried at higher temperatures could influence the stabilisation possibly due to irreversible changes the soil undergoes with the increase in temperature.
- Enzyme stabilised soils demand less water for compaction, i.e., there is a reduction in the stabilised soil OMC in comparison to the OMC of the control sample showing a shift to the wet side of the curve. This was revealed by the Stage 2 stabilisation test results, which showed no significant increase in strength for stabilised samples prepared on the basis of the OMC of controlled samples.

- The results from XRD and EDS in Stage 3 tests revealed no change in composition in the treated sample compared to the control sample suggesting neither chemical reaction nor new compound formation occurred by the presence of enzymes in soil. Micro-CT scans provided a distinct difference in the amount and distribution of pores between the samples, revealing that the stabilisation mechanism of this enzyme is mainly based on densification which is supported by the detailed compaction study reported in Stage 4.
- Results from Stage 4 tests showed that the increase in the stabiliser efficiency is significantly larger (up to 500% increase in CBR strength) when stabilisation is performed at stabilised optimum moisture compared to reference optimum moisture content of the soil. Thus, identifying the mechanism of stabilisation has enhanced the optimised strength of stabilised soil from 15% increase up to 500% increase compared to the strength of the control sample.

Having conducted a detailed investigation on the stabilisation effects of enzyme-based additive, the study identified that DMR 1:500 at 1% provides the optimum strength for the tested fine-grained soil. It also revealed that AMR of 7% at DMR 1:500 could ensure easier and uniform mixing of the additive in the soil. These findings will assist the road construction industry in constructing sustainable and cost-effective unpaved roads using the enzyme-based additives based on a sound understanding on the effect of this form of additives. From the case study conducted to assess the benefit of pavement stabilisation using non-traditional additives, the following conclusions were drawn:

- Enzyme-based soil stabilisation has an immediate effect on the soil based on the strength benefits of the soil from the treatment
- However, the true effectiveness of the stabilisation is dependent on a consistent and disciplined approach taken during the implementation of these additives.
- Investigation on the combining of multiple additives should also be investigated to evaluate the benefits of these form of stabilisers.

Enzyme-based soil stabilisers have been successfully used in ground applications for the past 30 years. However, the successful application of a given enzyme-based additive is case specific that depends on soil type, soil condition, and operational loads. As a result, the contractors incur a substantial cost in terms of time and money for preliminary lab tests, which may determine the suitable mix proportions to utilise in the field application. A sound understanding of the stabilisation mechanism of these additives can minimise these costs in addition to yielding the optimised benefits from the stabilisation process. This chapter investigated the stabilisation effects of a novel enzyme-based additive, commercially known as Eko-Soil, which is being applied to construct unpaved roads in Australia and worldwide. The research methodology followed within the chapter assisted in identifying the optimised mix proportions of the additive by unveiling its mechanism of stabilisation for a fine-grained field soil, which is dominant in Victoria, Australia. Series of experiments were conducted under a 4-Stage test program that included macro-scale mechanical tests to micro-scale imaging tests to unveil stabilisation effects and mechanism of stabilisation. The identified mechanism has facilitated enhancement in the efficiency of enzyme-based soil stabilisation significantly compared to the strength of non-stabilised soil. The chapter also includes the application of the additive into a pavement in a correct construction sequence followed by the monitoring and assessing of the stabilised pavements based on a triple bottom line method based on socio-economic and environmental impacts of stabilised non-traditional additives on a trial road in Victoria. The enzyme stabilisation has shown significant improvement of the road performance as was evidenced by the test results, which were based on site soil obtained before and after stabilisation. However, it can be seen from the field tests conducted that the stabilisation process, which involves enzymes could benefit from combining with other additives. The efficacy of combining secondary additives is explored in the next chapter (Chapter 5) of the thesis.

Chapter Five.

Enzymatic Fly Ash Optimisation for Sustainable Road Stabilisation

5.1. Introduction

As highlighted in Section 4.5 (Chapter 4), the lack of efficiency in enzyme alone stabilised soil in producing long term benefits call for the investigation in understanding the effectiveness of combining it with secondary additives. Effectiveness of combining secondary additives with traditional chemical additives has been highlighted in Section 2.2.1.2 (Chapter 2). Notable combinations include rice husk ash (RHA) and fibres with either cement or lime (Basha et al. 2005, Anggraini et al. 2015, Lekha et al. 2015). However, when it comes to the use of the enzymes, studies exploring its combinations with secondary additive is quite limited. Section 2.4.3 (Chapter 2) presents a summary of cases of stabilisation processes within the literature which utilize enzymes. Within the section (Section 2.4.3), merely 3 of the cited literature investigate this combined effect of enzymes and a secondary additive such as fly ash, cement and fibres (Khan and Sarker 1993, Correa et al. 2015, Guthrie et al. 2015) producing a varying level of effectiveness. This chapter deals with the investigation into combining enzyme additives with secondary additives. The choice of the secondary additive is based on several social-economic factors that have been investigated and reported in this chapter.

The use of cement as a secondary additive could be recommended as a suitable method of increasing the efficiency of enzyme-based soil stabilisers. However, this combination defeats the reasoning for the call to use non-traditional additives which mainly revolves around the contribution to the global CO_2 emission of the cement industry. As reported in Chapter 4, the cement industry contributes significantly to the global greenhouse gas emission and is the third-largest energy-consuming industry in the world (WBSCD 2018). Environmental and sustainability impacts could be positively affected by the decrease of relying on cement-based additives as construction materials where other alternatives could be used. It should also be understood that the increase in global population has elevated the need for energy and resources, which inherently has increased the amount of waste generated globally. With the increased cases of many landfills around the world reaching near capacity, the call for proper waste management systems through recycling and reusing has been at an all-time high. The increase in the population has also led to the rise in the volume of construction work, which in turn produces a considerable amount of waste materials as well as contributes to the rise in the emission of greenhouse gases. Studies show that cement processing accounts for 5 to 8% of the global CO_2 emission (Scrivener and Kirkpatrick 2008, WBCSD 2018) though the process carbonation can substantially benefit over time by uptaking emitted CO_2 (such as 43% of the CO_2 produced between 1930 to 2013 have been reabsorbed by cement carbonation as showed by Xi et al. 2016). However, there is still a need to decrease relying on cement-based additives as construction materials where other alternatives could be used instead. Enhanced attention is being devoted to the current construction industry by recycling/reusing waste materials to support engineering works. The recycled and the by-product materials include recycled foamed glass, construction and demolition materials and even beverage and agricultural wastes (Arulrajah et al. 2015, 2017, 2018; Kua et al. 2016, Mohammadinia et al. 2018, 2019a, b). Research works in these fields have led to the utilization of these products in engineering works. For example, since 2011, the road authorities in Victoria (Australia), have permitted the use of glass fines and crushed glass in varying pavement applications (VicRoads 2019). Furthermore, the use of fly ash has also attracted significant attention in the recent past due to its growing concerns for waste management, vast availability as well as benefits in ground improvements. Although fly ash is currently being used in various applications of construction, its utilization rate falls significantly below the generation rate that it contributes to around one-fifth of the entire Australian waste stream (Millington 2019). Reported statistics also highlight that out of 12 million tonnes of coal ash produced per annum, only 44% is recovered from the dumps and of which only half is used for beneficial purposes (Millington 2019). The work presented in this chapter investigates the effectiveness of utilizing fly ash as a potential secondary additive to be used with enzymes.

Fly ash, depending on the type of the coal burned, contains pozzolans that can instigate chemical reactions which could potentially increase the strength of existing soils. Fly ash is a by-product of power plants that source their energy from coal and often causes considerable financial and environmental liabilities for the energy companies for its disposal. Studies on the sustainable the use of fly ash have been reported in the literature, mainly as a substitute for natural resources (Kim et al. 2013, Wang et al. 2017, Zanoletti et al. 2017, Phummiphan et al. 2017). Investigations on the effect of fly ash as a partial substitute for cement in recycled aggregate concrete revealed a minor reduction in strength and slightly lower yield strength but better flowability, lower plastic viscosity and much higher chloride resistance, contributing to the sustainability of the by-product (Kim et al. 2013). Wang et al. (2017) reported that the life cycle sustainability assessment (LCSA) model of a concrete structure with fly ash demonstrates significant improvement in sustainability of concrete in the short term but not long term due to the potential shortened service life of the structure. However, the study emphasized that the use of fly ash could heavily reduce the social, environmental and economic impacts, for example, the effective use of all the fly ash available in China could mean savings of up to 150 bn CNY and 560 million tons of CO_2. Zanoletti et al. (2017) highlighted that the embodied energy of coal fly ash (CFA) is lower than other carbon-based surfactants, and the adsorbent properties of CFA can be effective in removing high concentration of Sodium Dodecyl Sulphate (SDS) which allows its implementation as a sustainable material in the field of anionic surfactant removal. In addition, studies highlight that fly ash can be used as an additive on the development of sustainable pavement base materials (Phummiphan et al. 2017). The increased utilization of fly ash holds the key to the reduction of its waste disposal issues as well as decreasing the need to produce calcium-based additives like cement for soil stabilisation. Cementing effects of fly ash have been well utilized by the application of fly ash in highway embankments since 1950. Five reactions could occur within a carbon-based binder and soil (Sargent 2015). Cationic exchange is one such reaction in which there is an exchange of Calcium ions with the metallic ions within the soil. In flocculation/agglomeration, restructuring of negatively charged clay particles occurs due to its surrounding by positively charged cations. In hydration, an exothermic reaction occurs which occasionally causes boiling of the pore water allowing ionic exchange, flocculation and pozzolanic reactions which help in the formation of cementitious compounds. In pozzolanic reactions, the main mechanism involves the transportation of calcium hydroxide by water to combine with silicate and aluminate clay minerals to form calcium silica hydroxide (CSH) and calcium aluminate hydroxide (CAH). Lastly, carbonation is another potential reaction which occurs within this admixture which involves the reaction between carbon dioxide from the air (penetrated through pores) and calcium hydroxide to form calcium carbonates. Pozzolanic reactions could be explained as follows:

$$Ca^{2+} + \ 2(OH)^{-} + \ SiO_2 \rightarrow C - S - H \qquad \textbf{\textit{(Eq. 5.1)}}$$

$$Ca^{2+} + \ 2(OH)^{-} + \ Al_2O_3 \rightarrow C - A - H \qquad \textbf{\textit{(Eq. 5.2)}}$$

The effectiveness of fly ash for subgrade treatment can be largely credited to the pozzolanic reactions which occur between the soil and the calcium-based additive. Pozzolanic reactions involve the transportation of calcium hydroxide by water to combine with silicate and aluminate clay minerals to form calcium silicate hydrate (CSH) and calcium aluminate hydrate (CAH) (Ismaiel 2006, Sargent 2015). Literature highlight the impact of both calcium and alumina content in affecting the rigidity, strength, and flexural fatigue of pavement materials. Where there is a lack of calcium-rich precursors, silica and alumina rich precursor such as fly ash has been proven effective in producing aluminosilicate gels (Provis et al. 2012, Mohammadinia et al. 2019). Despite its cementing effects, reports show that the improvements in geotechnical properties by fly ash alone are not adequate for its use in pavement and foundation design (Sharma et al. 2012). However, the use of fly ash has been reported as being effective to some extent in the literature, especially when combined with other additives. Kolias et al. (2005) reported the formation of a significant amount of tobermorite in fly ash and cement in clay soils which led to a denser and more stable structure of the samples, resulting in improvement in strength (compressive, tensile and flexural), modulus of elasticity and California bearing ratio (CBR). Furthermore, comparing pavement structures incorporating these stabilised subgrades with conventional flexible pavements show enhanced financial benefits of these additives due to the reduction in asphalt thickness. Chen et al. (2009), investigated the influence of SO_3 content on cement and fly ash stabilised crush stones which showed improvements of up to 120% in UCS of the material with 7.2% SO_3 when compared to the admixture with 1.8% SO_3. Brooks (2009) showed the potential benefits of the admixture fly ash and rice husk ash (RHA) with significantly increased UCS and CBR where fly ash and RHA contents were up to 25% and 12% respectively. Sharma et al. (2012) reported improvement in plasticity index and compaction limits of the soil, as well as optimum UCS and CBR strength improvement of fly ash and lime, treated samples at 20% fly ash and 8.5% lime. The authors also stated that the pozzolanic reaction overpowers the cationic exchange capacity as the stabilisation mechanism in fly ash soil interactions. In this pozzolanic reactions, calcium from lime and fly ash reacts with soluble alumina and silica from clay and fly ash in the presence of water to produce stable calcium silicate hydrate (CSH) and calcium aluminate hydrate (CAH) which generates long term strength gain and improves the geotechnical properties of soil (Sharma et al. 2012). Within the literature, many other combinations along with the fly ash such as fibres and ground granulated blast furnace slag (GGBFS) have also been trialled showing adequate strength gain (Yilmaz 2015, Sharma & Shivapullaiah 2016, Phummiphan et al. 2018). However, cement remains to be the main additive that is combined with fly ash for most of the stabilisation work to date (Neramitkornburi et al. 2015a, b; Chompoorat et al. 2018, Chompoorat et al. 2019, Yoobanpot et al. 2020).

As seen from the literature, fly ash poses as a potential alternative as a sustainable and effective additive to divert from traditional ground treatment additives, especially when combined with other additives, which could speculate the positive efficacy when combined with enzymes. Eko-Soil was reported in the preceding chapter as facilitating the effective stabilisation of clayey soils through densification and the reduction of the affinity for water. The previous chapter highlight that the due to the reduction in the affinity for water, the optimum moisture content (OMC) of the stabilised material decreases (Shifts to the left) whereas the maximum dry density (MDD) increases (shifts upwards) producing stronger material (up to 300% improvement in load-bearing capacity) with lesser compaction energy. Kushwaha et al. (2018) reported that enzymes of same key ingredients have the potential to effectively modify the subgrade quality of marginal soils to be used as highway embankments materials, however, not enough to be used as subbase or base course materials. It should also be noted that despite the efficacy of enzymes to provide significant improvement in soil strength, comparative studies on calcium based non-traditional additives and enzyme report relatively inferior benefits of enzyme treated additives over the calcium based additive counterparts. Thomas et al. (2018) report that alkali-activated ground granulated blast furnace slag (GGBS) is a more effective treatment option to observe strength gain of soil with UCS of alkali-activated GGBS treated soil 1.15 times that of OPC treated soil and 5.5 times that of enzyme treated soil.

Effectiveness in combining enzymes with fly ash has also been reported in the literature such as Khan and Sarker (1993), which report an increase in strength of clay and fly ash mixture as well as fly ash and lime mixtures effectively by increasing the surface roughness of fly ash and kaolinite minerals. Eujine et al. (2017a, b) also report the noticeable effect of combining enzyme and lime “enzymatic lime” on soil stabilisation, with the enzyme additive assisting to increase the rate of the reaction between the modified clay and lime in which CSH and CAH gel is formed with no delay in the initial reactions. Eujine et al. (2017a) also hypothesized that the enzymes have the potential to cater for further reactions by replacing existing aluminium cations with calcium cations in the presence of Rhodasurf (the active ingredient in Terrazyme).

The chapter incorporates the enzymatic fly ash and lime stabilisation using Eko-Soil, which has proven (as reported in Chapter 4) to be effective in stabilising the research soil. The current research investigates the effect of combining fly ash with enzyme-based additives as a means to contribute to a further improvement in soil strength as well as a means to mitigate the global waste management issue caused by the disposal of this calcium-based by-product of burning coal. In this chapter, a series of experiments were conducted in a comprehensive five-part testing method using enzyme stabilised soil with fly ash and lime as secondary additives. Optimised dosage of fly ash and the enzyme stabiliser is investigated in Part 1 of the investigation. Part 2 and 3 assessed the effect of time and the effect of lime on the strength of the treated soil, respectively. A series of chemical and microscopic analysis was conducted in Part 4 to understand the reaction mechanism that facilitated the strength gain of the treated soil by the stabilisers. Furthermore, the application and the benefits of this form of treatment is illustrated to demonstrate the significance and benefits of the research conducted in practical applications.

The methodology of the study conducted within this chapter has been designed to provide insight on enhancing the effectiveness of enzyme stabilised soil, as reported in Chapter 4. The study also holds potential benefit to the road construction industry by providing effective use of this waste material which has considerable environmental effects. The outcomes from the project could not only benefit the construction of sustainable roads using enzymatic fly ash stabilised soil but will also assist in fly ash waste mitigation effort by effectively using them in engineering works.

5.2. Experimental Procedure

The research methodology in this chapter was conducted in a four-part experimental plan to investigate the benefits of combining the enzyme with secondary additives for the selected field soil. The research questions to be covered in this chapter include the following:

- Are there notable physical and mineralogical changes induced by enzyme-based soil stabilisation?
- How much of the additive is required to see effective strength benefits on soil?

- How to quantify the strength gain of soil due to enzyme-based soil stabilisation?
- Can the efficacy of enzyme-based stabilisation be enhanced by combining with other non-traditional additives?
- What is the time-dependent effect of enzyme-based soil stabilisation?

Firstly, experiments were conducted to obtain the optimised values of the fly ash and enzyme admixture based on its effect on the strength of the soil. Time-dependent strength of optimised stabilised soil was then investigated in Part 2 using samples prepared under different curing periods up to 28 days. The effects of lime incorporation into the enzymatic fly ash were assessed in Part 3. Chemical tests and microscopic imaging tests were conducted in Part 4 on the optimised samples to understand the stabilisation mechanism of the enzyme-based soil stabilisation with the combination of fly ash and lime. The following section presents a full description of the materials and test procedure adopted in this research work.

5.2.1. Materials Used

The materials used for the experimental analysis within the study has been detailed in Chapter 3 of the thesis. The natural soil classified as Lean Clay (CL) has been tested with varying dosages and applications of Eko-Soil. The process of refining and preparing the soil for testing has been reported in detail in Section 3.1.1 (Chapter 3), along with the soil's physical and chemical properties. Section 3.1.2 (Chapter 3) details the properties of the tested enzyme. The fly ash used for the research is a commercial additive available from Cement Australia. The detailed description of the general chemical and physical characteristics of the material is reported in Section 3.1.3 (Chapter 3). Hydrated lime used in this study was another commercial product acquired from Lime Group Australia, whose detailed description is reported in Section 3.1.4 (Chapter 3).

5.2.2. Sample Preparation and Tests Conducted

The samples preparation methods and the tests align well with the procedure reported in Chapter 4. Consistency in the sample preparation and test procedure was followed for repeatability and reliability of the conducted work. The soil was sieved to 2.36 mm and cleared of any visible impurities. The sample preparation and testing were performed based on Australian Standards (AS1289.1.1 2001). Compaction and density curves required for the samples were conducted based on AS1289.5.1.1 (2017), which utilizes standard compaction effort. In the case of the treated samples, the following methods were adopted. All the powdered additives were added to the soil based on the dry unit weight of the soil and mixed mechanically as well as by hand ensuring to attain visual homogeneity prior to adding varying levels of moisture and curing for a minimum of 24 hours for the soil to reach equilibrium before compaction. The moisture content of the soil was determined using an OHAUS moisture analyzer which helps identify universal moisture content of tested substance. The data was used to determine the geotechnical moisture content of the soil. In the cases where the enzyme and fly ash were combined, the relevant powdered additives were first added to the soil before adding the liquid stabiliser based on the AMR and DMR. The preparation of the samples for mechanical testing was based on the revised specimen preparation method reported by Rauch et al. (2003). Mechanical tests conducted included standardized UCS testing on compacted cylindrical samples (105 mm diameter, 115 mm Height) and CBR testing based on Australian Standards, AS5101.4 (2008) and AS1289.6.1.1 (2014), respectively. Samples were prepared with a criterion of achieving a minimum of 90% optimum density and a maximum of 2% allowance to either wet or the dry side of the OMC. The samples not meeting this criterion were re-casted to achieve uniform sample preparation. For the cases where enzyme based stabiliser was used, the powdered additives were added to the soil based on the dry unit weight of the soil and prepared at the optimum moisture content (OMC) – Application Mass Ratio (AMR) + 2% to allow for unavoidable moisture loss and left to reach equilibrium for a minimum of 16 hours in a bag ensuring the excess air was squeezed out before sealing the bag (Fig 5.1). The required enzyme AMR is then added to the soil using the required Dilution Mass Ratio (DMR) and mixed using a laboratory mixer as well as my hand to attain a high degree of homogeneity prior to compaction for UCS and CBR tests. The UCS samples were covered with a thin layer of cling wrap as well as a layer of aluminium foil during the curing process (Fig 5.2). The unsoaked CBR samples were cured in a plastic sealable bag to ensure no significant loss of moisture during the process (Fig 5.3). An example of the calculation for determining the moisture is as follows:

$\boldsymbol{Sample\ calculation}$:

$To\ prepare\ 4400g\ of\ wet\ soil\ to\ OMC\ of\ 15.4\%\ with\ the\ following\ additive\ contents$:

$OHAUS\ moisture\ reading = 4.15\%$

$$Total\ moisture\ in\ soil = \frac{4.15}{100} \times 4400 = 182.6\ g$$

$Therefore\ dry\ soil = 4217.4\ g$

$$Fly\ ash\ content = 15\% = \frac{15}{100} \times 4217.4 = 632.6\ g$$

$$OMC = 15.4\% = \frac{15.4}{100} \times 4217.4 = 649.5\ g$$

$$therefore\ enzyme\ required = 1\%\ AMR = \frac{1}{100} \times 4217.4 = 42.2\ g$$

$$Lime = 2\%\ AMR = \frac{2}{100} \times 4217.4 = 84.3\ g$$

$therefore\ moisture\ to\ be\ added = 649.5 - 182.6 - 42.2 = 424.7\ g$

**(please note that this is the universal moisture content)*

Typical curing time adopted for sample testing (except in Part 2) was 4-days, similar to the curing conditions highlighted in the preceding chapter. The selection of curing time of 4 days is also justified based on the Australian Standard (AS1289.6.1.1 2014) which suggests a minimum curing time of 24 hours for unsoaked cases and at least four days for soaked cases. All the tests were conducted in at least duplicates.

Figure 5.1. Curing of soil specimen to reach equilibrium

Figure 5.2. Curing of UCS samples in triplicates of one parameter

Figure 5.3. Curing of unsoaked CBR samples in duplicates for various parameters

5.2.3. Mix Design and Specimen Preparation

Varying levels of fly ash content has been used for the additive content, as reported in the Results section (Section 5.3) of the chapter. The selection of AMR and DMR for enzymes used in the current study is based on the findings reported in Chapter 4, which recommends the usage of DMR 1:500 at 1% dry unit weight of the soil. The effect of the use of enzyme at a higher AMR of 7% dry unit weight of the soil is also investigated to ensure easier and uniform mixing of the additive in the soil allowing the enzymes to cover more sites within the soil in lesser time. Hence, the AMR of the enzyme used in the current research study will focus on 1% & 7% cases. DMR of the enzyme was selected at 1:500 & 1:100 to cover the optimised strength dilution and higher enzyme concentration, respectively. It is to be noted that the DMR-AMR ratios of 1:100 - 7%, 1:500 - 1% and 1:500 - 7% are referred in this chapter as E1, E2 and E3 respectively. Selected quantities of powdered dry fly ash were added to the soil, based on dry unit weight of the soil, before adding moisture. The selection of quantities of fly ash within the study was based on reported literature that showed positive spectrum for fly ash stabilized soils (Khan and Sarker 1993, Kolias et al. 2005, Sharma et al. 2012). The fly ash contents investigated for efficacy in the current study are 5%, 10%, 15% and 20%. The quantity of hydrated lime used in this study is based on the pH test conducted in accordance with Eades and Grim (1966) and Texas Department Of Transportation Standard TEX-121-E (TxDOT 2002) as well as optimised dosages reported in the literature (Kolias et al. 2005, Sharma et al. 2012). All specimens were prepared at OMC determined from the compaction tests for each additive content and compacted using a standard hammer. The UCS samples were demoulded, wrapped around in a thin plastic film and wrapped again in an aluminium foil stored in a curing room with temperature set between 21 ℃ to 24 ℃ and 50% humidity for the desired curing duration. The CBR samples, along with the moulds, were also covered using plastic bags to preserve moisture content and kept in the curing room prior to testing.

5.2.4. Testing

5.2.4.1. Mechanical Testing Parameters (Part 1 – 3)

Summary of the tests conducted in Part 1 to 4 of the current study is listed in Table 5.1 – 5.4. A number of mechanical tests were conducted in Part 1 on the controlled soil, soil fly ash mixture and soil enzymatic fly ash admixture. Unsoaked CBR tests were conducted on all the enzyme stabilised soil with varying fly ash contents ranging from 0% (control) to 20%, as shown in Table 5.1 to identify the optimised enzymatic fly ash content. After identifying this optimised enzymatic fly ash content, UCS tests and soaked CBR tests were conducted on optimised soil and additive mixture. Both UCS and CBR of the samples were tested mechanically using a Shimadzu 50 kN apparatus using a strain rate of 1 mm/min based on AS5101.4 (2008) and AS1289.6.1.1 (2014), respectively. The tests were conducted at least in duplicates and in some cases triplicates with specimens. In Part 2, the DMR and AMR combinations with the optimised fly ash content were subjected to a time-dependent strength test where samples were prepared to test for curing times ranging from 4 days, 14 day and 28 days as summarized in Table 5.2. Tests in Part 3 investigate the effect of adding lime to the optimised enzymatic fly ash case in terms of UCS and soaked and unsoaked CBR (Table 5.3).

5.2.4.1. Chemical Testing Parameters (Part 4)

Chemical and imaging tests were conducted in Part 4 to unveil the reaction mechanism of the additives (Table 5.4). In this Part, a number of novel technological and non-invasive imaging techniques were also included. To investigate the composition of the admixture, thermogravimetric analysis (TGA) was conducted by analysing the change in physical and chemical properties of the treated samples with variation in temperature by measuring the change in mass with the increase in temperature. TGA was conducted using a Pyris 1 TGA (Perkin Elmer) in a nitrogen atmosphere under a flow of 30 ml/min and heating rate of 10 °C per min, varying the temperature from 25 °C to 850 °C. Samples for TGA analysis were analysed at least four days after preparation with a maximum of seven days based on the availability of the apparatus. Post-CBR tested samples at selected DMR/AMR combinations were subjected to pore-structural and microstructural analysis. Cubed samples of 20 mm were attained from the middle of the tested specimens and analysed for differences in its microstructure and pore-structure. Scanning electron microscope (SEM) with secondary electron imaging was used to examine microstructure change within the treated samples using FEI Quanta 200 ESEM in which shaved loose particles from control, as well as stabilised samples, were carbon coated prior to analysis. The micro CT scans (µCT) were used to investigate pore connectivity and pore-structure of both control and stabilised specimens. By scanning the samples at 20 µm resolution at 100 kV and 100 µA using a copper filter with 1000 images recorded during a complete scanning rotation. Finally, the CTAN software was used to analyse the porosity of tested specimens. The mechanistic analysis was conducted in Part 5 to illustrate the application of research results and to identify the benefit of the additive in pavement stabilisation in road design and construction.

Table 5.1. Summary of tests conducted in Part 1

Test Name	**DMR/ (AMR)**	**Fly ash (FA) (%)**	**No. of Tests (including repetitions)**
Compaction[1]	Control	0%, 5%, 10%, 15%, 20%	5
	1:100 (7%)	0%, 5%, 10%, 15%, 20%	5
	1:500 (1%)	15%	1
	1:500 (7%)	0%, 5%, 10%, 15%, 20%	5

CBR[2]	Control 1:100 (7%) 1:500 (1%) 1:500 (7%)	0%, 5%, 10%, 15%, 20% 0%, 5%, 10%, 15%, 20% 15% 0%, 5%, 10%, 15%, 20%	10 10 2 10
CBR[3]	Control 1:100 (7%) 1:500 (1%) 1:500 (7%)	0%, 15% 0%, 15% 15% 0%, 15%	4 4 2 4
UCS[4]	Control 1:100 (7%) 1:500 (1%) 1:500 (7%)	0%, 15% 0%, 15% 15% 0%, 15%	6 6 3 6

[1] *Determination of OMC/MDD using standard compaction*

[2] *Unsoaked CBR using standard compaction tested after 4-day curing*

[3] *Soaked CBR using standard compaction tested after 4-day curing*

[4] *Unconfined Compressive Strength using standard compaction after 4-day curing*

Table 5.2. Summary of tests conducted in Part 2

Test Name	**DMR/ (AMR)**	**FA (%)**	**Curing time (days)**	**No. of Tests (including repetitions)**
CBR[1]	Control	15%	4*, 14, 28	6
	1:100 (7%)	15%	4*, 14, 28	6
	1:500 (1%)	15%	4*, 14, 28	6
	1:500 (7%)	15%	4*, 14, 28	6

[1] *Unsoaked CBR using standard compaction*

** 4-day test results obtained from Part 1*

Table 5.3. Summary of tests conducted in Part 3

Test Name	DMR/ (AMR)	FA (%)	Lime (%)	No. of Tests (including repetitions)
pH test	Control	0%	0, 2, 4, 6, 8, 10	6
CBR[1]	Control 1:500 1%	15% 15%	2 2	2 2

[1] *Unsoaked CBR using standard compaction*

Table 5.4. Summary of tests conducted in Part 4

Test Name	DMR/ (AMR)	FA (%)	No. of Tests (including repetitions)
TGA[1]	Control 1:100 (7%) 1:500 (1%, 7%)	0%, 15% 15% 15%	2 1 1
SEM[2]	Control 1:100 (7%) 1:500 (7%)	0%, 15% 15% 15%	4 2 2
µ-CT[3]	Control 1:100 (0%, 7%) 1:500 (7%)	0%, 15% 0%, 15% 1%, 15%	2 2 2

[1] *Thermogravimetric Analysis*

[2] *Scanning Electron Microscopy of treated and untreated samples for soaked and unsoaked samples*

[3] *Pore distribution and analysis with duplicates*

5.3. Results and Discussion

5.3.1. Part 1: Optimisation of Enzymatic Fly Ash Stabilisation

5.3.1.1. Effect on Compaction Characteristics

The results of the compaction characteristics of the enzymatic fly ash stabilised soil mixes are presented in Table 5.5 and Fig 5.4 – 5.7. The effect of fly ash alone can be seen from the results for control samples. As shown in Fig 5.4, the increase of fly ash content in the soil samples causes an increase in OMC in general (except for the initial drop) while decreasing the MDD. This trend is commonly seen in fly ash stabilised soils (Bera & Kundu 2016, Mohajerani et al. 2017). The decrease in maximum dry density could be attributed to the introduction of more fines (in the form of fly ash) which has a lesser specific gravity that occupies the voids in the soil. The increasing OMC from the increase in fly ash content could be due to the affinity of water caused by the increase in the amount of these newly introduced fines. The effect of adding the enzyme to this fly ash stabilised soil can be seen from Table 5.5 and Fig 5.5 – 5.7. As seen in the case of fly ash stabilised soil, there is a similar trend of increasing OMC with fly ash increment at every enzyme dosage. However, the combined action of fly ash and enzyme produces a plateauing effect on the increasing trend in the OMC. It should also be noted that the addition of enzymes (E1, E2 and E3) on this fly ash stabilised soil has decreased the increase in OMC when compared to soil stabilised by fly ash alone. For example, on the natural soil, the OMC increases from 16% to 16.6%

with the increase in fly ash while the addition of enzyme exhibits a lesser increase from 15.8% to 16.1% on the natural soil. The enzyme treated fines which have replaced soil fabrics have less water absorption capacity resulting reduction of OMC compared to control soils. Although, reduction in the affinity of water is observed in the enzymatic fly ash stabilised soil, no increase in MDD is observed. Such compaction behaviour observed for enzymatic fly ash stabilised soil revealed that the stabilisation mechanism is not controlled by densification, which was the fundamental mechanism for enzyme-based soil stabilisation, as reported in Chapter 4. However, the compaction characteristics of the treated soil do not prove that there is no densification occurring; instead, it suggests that this mechanism is not the dominating reason for strength improvement of the treated soil, i.e. cationic exchange as well as the flocculation/agglomeration reactions as reported by Sargent (2015) could still be occurring albeit not to a dominant level compared to the other chemical reactions occurring within the soil.

Table 5.5. Compaction characteristics of the enzymatic fly ash soil admixture

	Control					**1:100 7% (E1)**				
Fly Ash (%)	0	5	10	15	20	0	5	10	15	20
OMC	17	16	16.2	16.4	16.6	15	15.8	16	16	16.1
MDD	1.79	1.77	1.76	1.75	1.76	1.81	1.77	1.76	1.76	1.76
	1:500 7% (E2)					**1:500 1% (E3)**				
Fly Ash (%)	0	5	10	15	20	0	5	10	15	20
OMC	14.6	15.8	16.2	16.2	16.2	14.6			15.4	

MDD	1.81	1.79	1.77	1.76	1.75	1.8			1.75	

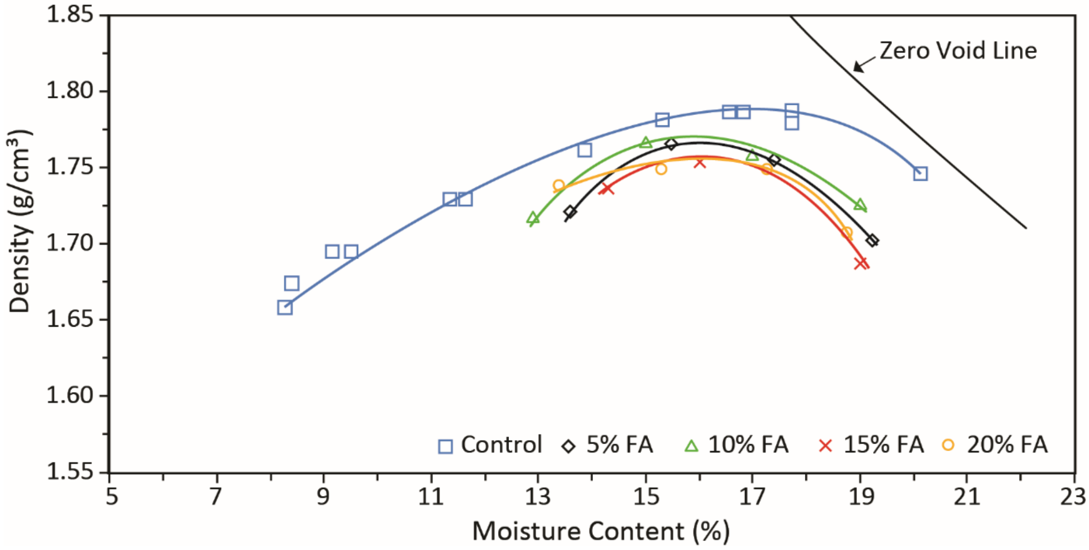


Figure 5.4. OMC/MDD curve of fly ash treated soil

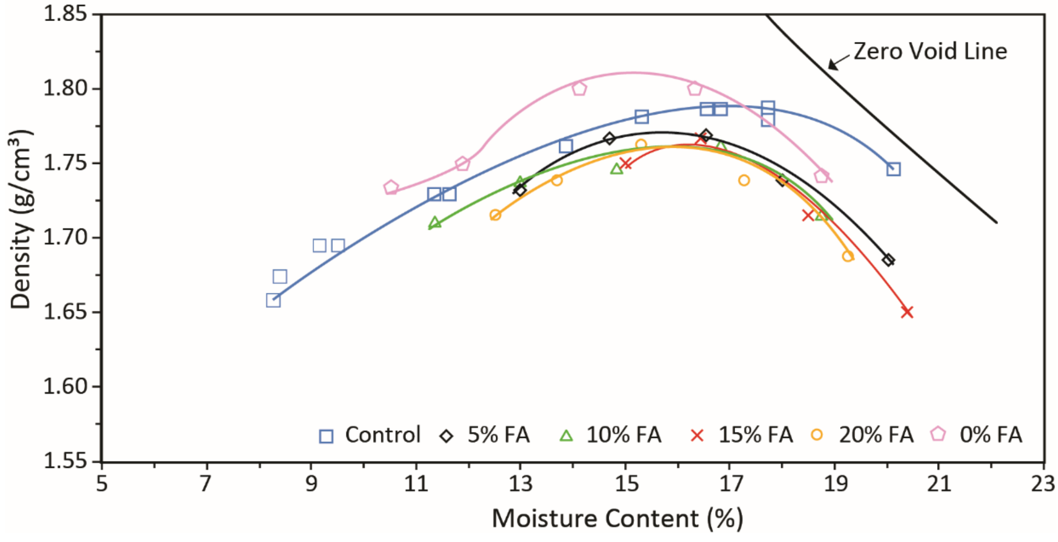


Figure 5.5. OMC/MDD curve of E1 enzymatic fly ash treated soil

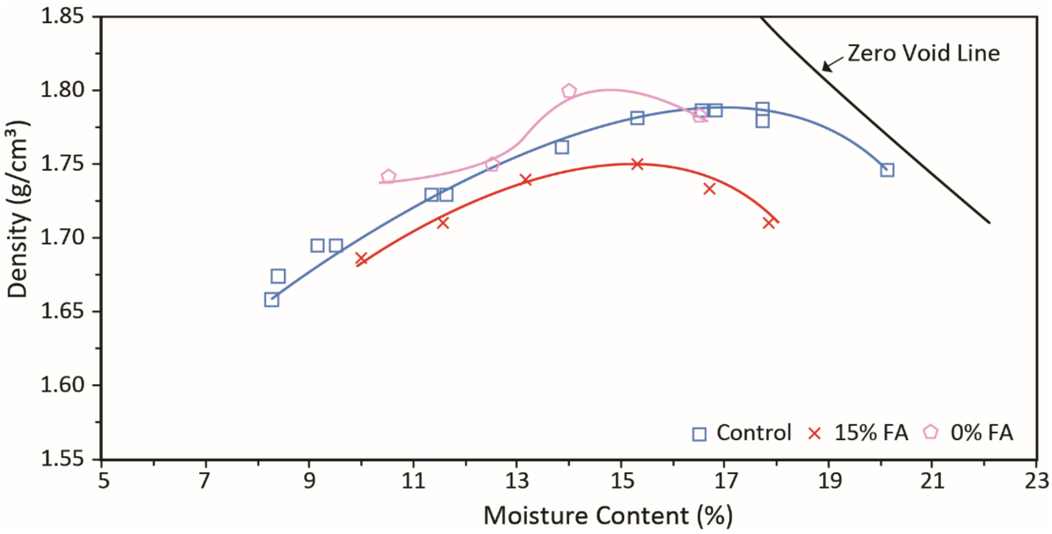


Figure 5.6. OMC/MDD curve of E2 enzymatic fly ash treated soil

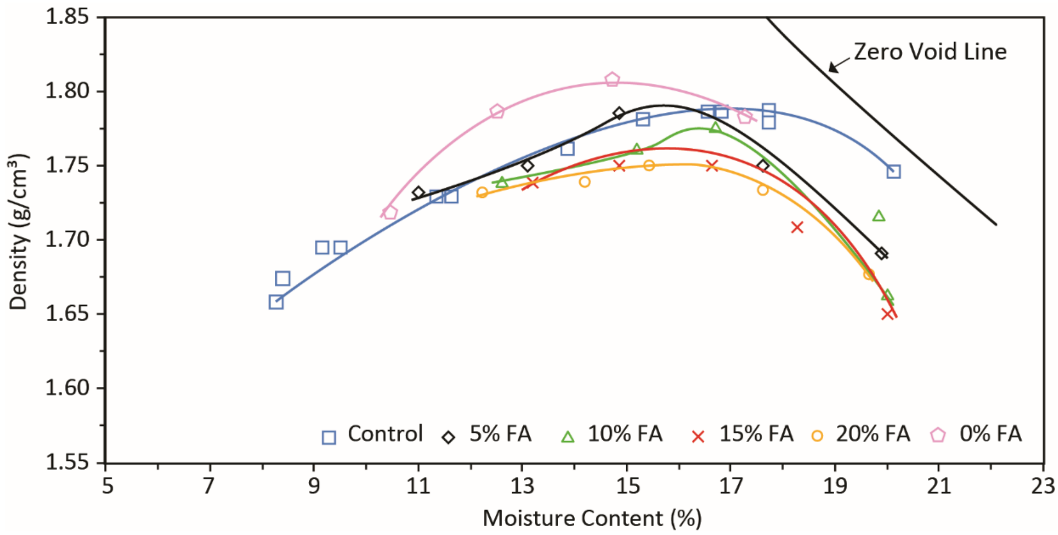


Figure 5.7. OMC/MDD curve of E3 enzymatic fly ash treated soil

5.3.1.2. Optimisation

The enzyme and fly ash contents were selected by conducting a series of mechanical tests. Firstly, the unsoaked CBR tests were performed to determine suitable fly ash content. Having identified this fly ash dosage, further tests (soaked CBR and UCS) were conducted to optimise the enzymatic fly ash stabilisation. Results of the tests are shown in Fig 5.8 – 5.10.

From Fig 5.8, it can be seen that the fly ash provides strength improvement of confined soil samples as revealed by the CBR test results. Results show that increasing fly ash content has enhanced the mechanical behaviour of soil considerably. Addition of 5% fly ash has increased the CBR from 4.5% to 30%, which is over 500% increase in strength compared to the untreated sample. However, at 5% fly ash, the combination of each dilution of enzymes and fly ash yielded lower strength than that of fly ash stabilised soil in all the tested enzyme concentrations, i.e., CBR of E1, E2, and E3 at 5% fly ash is 25% whereas CBR of soil and 5% fly ash is 30%. The combination becomes effective beyond 10% of fly ash addition and fully effective at 15% fly ash, where E1, E2, and E3 treated fly ash soils attain higher strength than the fly ash alone cases. These results reveal that the use of enzymatic fly ash could enhance CBR up to 45% (900% increase) at 20% fly ash compared to the control soil. It should also be noted that enzymatic fly ash is only effective at fly ash contents greater than 10% where the strength gain is higher compared to the fly ash alone stabilised soil. A fly ash content of 15% can be selected as the minimum quantity required as it provides distinct strength improvement at all DMR/AMR combination. The selected fly ash content (i.e. 15%) also aligns with the typical strength of subgrade stabilisation required (5% CBR) for constructing unsealed roads. (AGPT06/09 2009).

Results of soaked CBR and UCS tests (at 15% FA) conducted to obtain optimum enzyme content are shown in Fig 5.9 and 5.10, respectively. In general, enzyme treated soil has shown improvement (50% increase in CBR) in soil strength under soaked condition compared to controlled soil condition whereas the addition of fly ash

(15%) to the soil has shown an improvement of 300% (i.e. soaked CBR increase from 2% to 8%). However, combining both the additives has resulted in further improvement in soil strength. For example, the addition of fly ash into enzyme stabilised soil improved the soaked CBR up to 10% from 8% (i.e. 25% further increase in soaked CBR) with a similar increase in CBR strength irrespective of the changes in enzyme dosage.

On the other hand, UCS testing on enzymatic fly ash treated samples does not show significant improvement in strength compared to fly ash stabilised samples even though a mild UCS increase at 1:500 1% (E2), as shown in Fig 5.10. The reason for this behaviour could be due to the nature of testing. i.e. UCS testing method was not deemed as a method to give a conclusive quantitative analysis of strength improvement as the test does not replicate field conditions where the pavement stresses are confined. Similar reasoning is used in Chapter 4 to explain the differences in the effective AMR on CBR and UCS samples. It is also understood that, in the absence of confinement force, specimens which are stabilised with fly ash could be highly susceptible to failure and strength loss from slaking (Makusa 2013). Thus, the enzyme provides no added benefit to fly ash stabilisation in terms of UCS strength testing. The reasoning for this is further explained in Part 4 of this chapter. It should also be noted that the slight dip in strength of the soil treated with 1:100 1% (E1) could be due to the difference in the mineralogical composition of the natural soil. From these results, the optimisation DMR/AMR of the enzyme for fly ash stabilisation can be either 1:500 1% (E2) or 1:500 7% (E3). E1 is not ideal as it showed less effectiveness in 10% fly ash content. As reported in the previous chapter

(chapter 4), both E2 and E3 are recommended enzyme mix with the higher AMR, ensuring better mixing of the enzyme with the soil. The higher AMR could also mean that there is a higher amount of reactive agents available to neutralize various sites simultaneously. The strength increase due to enzymatic fly ash stabilisation has been further elaborated in Part 4 using microscopic analysis test results. However, an improvement in strength up to 76% can be observed with no addition of enzyme compared to control soil strength.

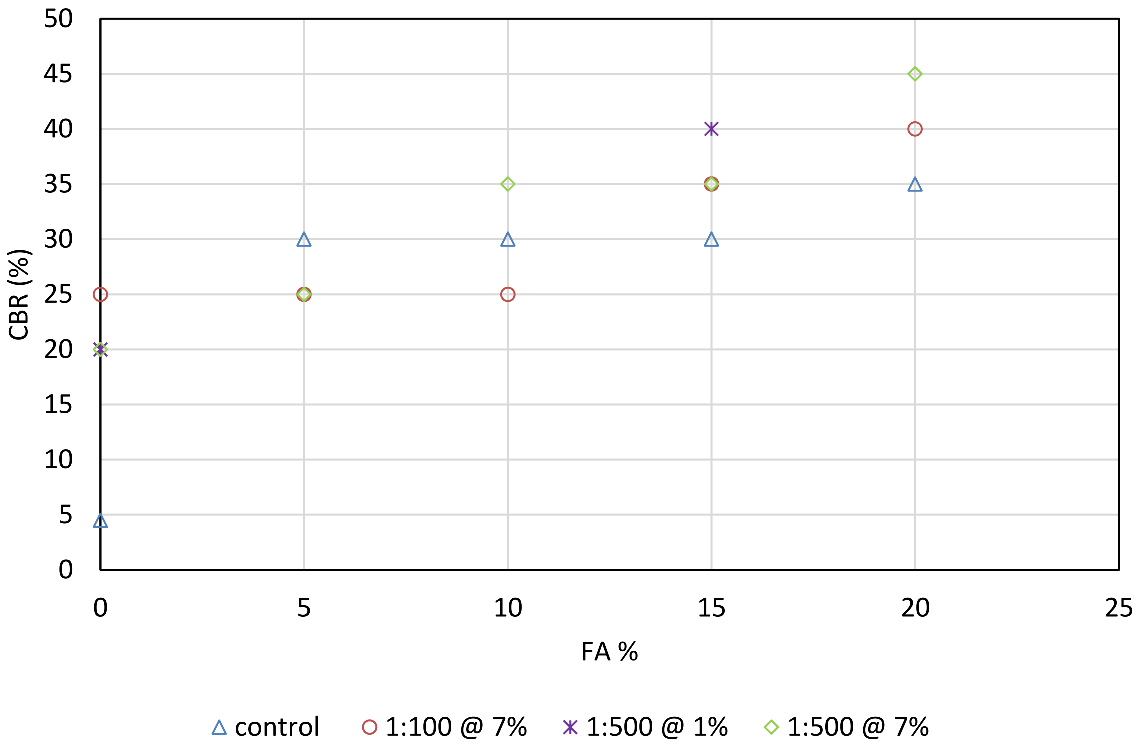


Figure 5.8. Unsoaked CBR of enzymatic fly ash treated soil

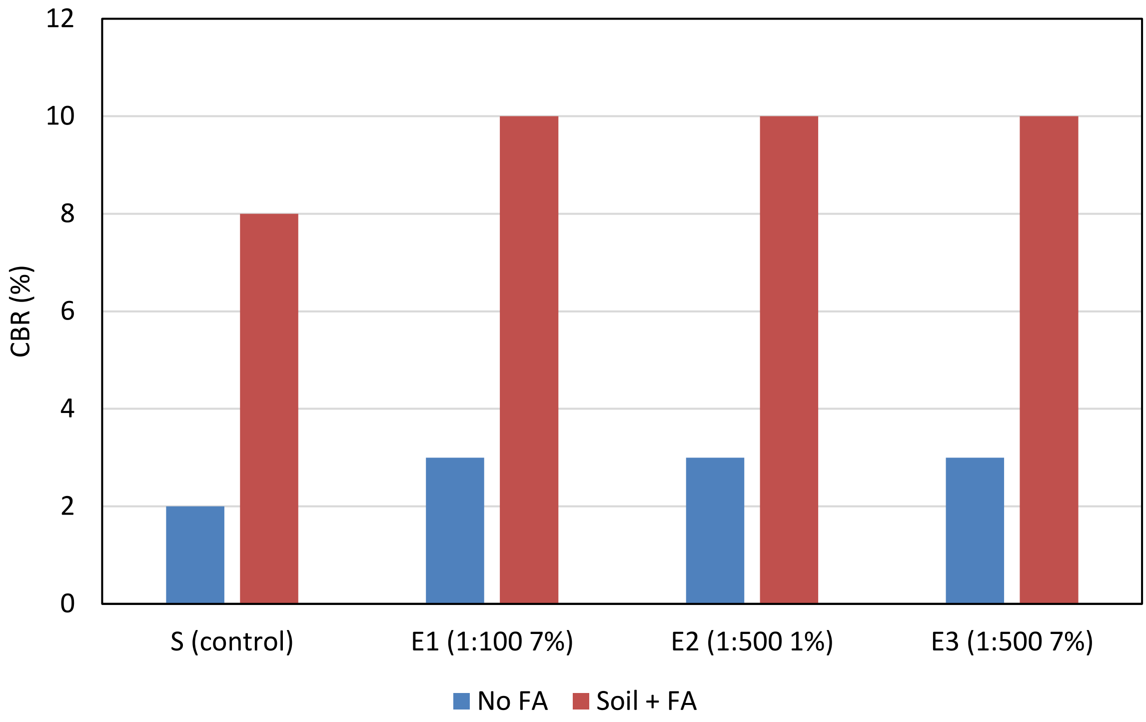


Figure 5.9. Soaked CBR of enzymatic fly ash treated soil

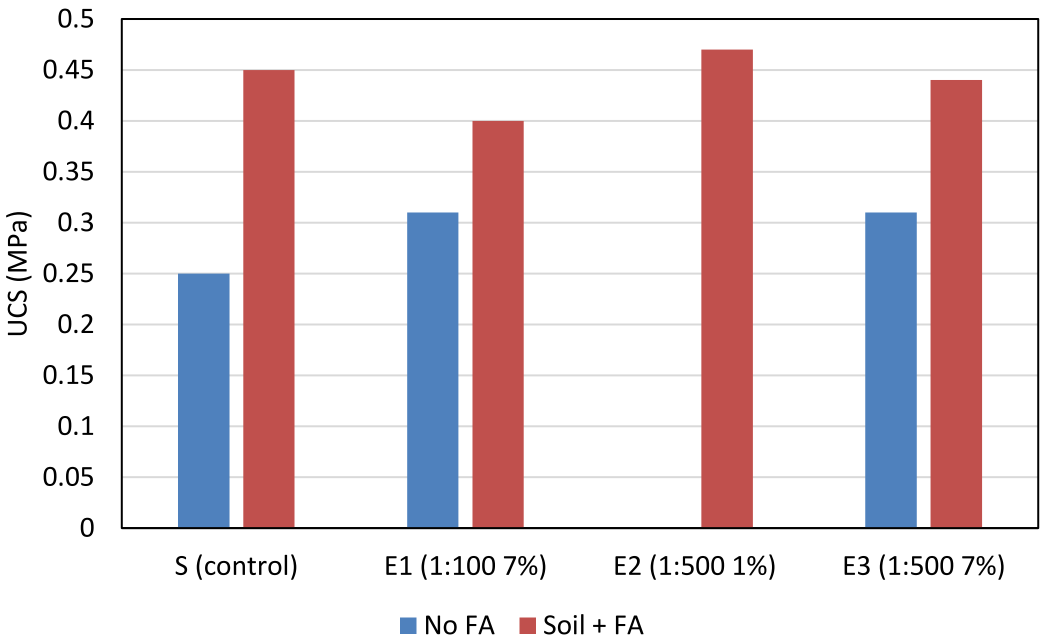


Figure 5.10. UCS of enzymatic fly ash treated soil

5.3.2. Part 2: Effect of Time on Enzymatic Fly Ash Stabilisation

The efficacy of fly ash and enzymatic fly ash stabilisation up to 28 days can be seen in Fig 5.11. The results of the time-dependent strength tests show that the increase in strength with enzymatic fly ash can be observed within four days of treatment whereas the strength of the fly ash alone stabilised samples takes around 28 days to achieve similar strength. As expected, based on its fundamental definition, the enzyme works as a catalyst in increasing the strength of the soil at a shorter time. The enzyme acts to decrease the activation energy required by the soil and fly ash to instigate the pozzolanic reaction. Activation energy is defined as the kinetic energy, or velocity required by the molecules to initiate a reaction. Enzymes are commonly defined as proteins which reduce the activation energy of the reaction process. Tolleson et al. (2003) hypothesize the catalysis as the mechanism of strength gain in all enzyme treated soil samples. Eujine et al. (2017a) report similar accelerated strength improvement stabilised with enzymatic lime on a kaolinitic natural soil due to the formation of cationic bonds at an accelerated rate due to the low activation energy required for the new modified clay lime reactions. The enzymatic fly ash stabilisation managed to yield the optimum benefits of the stabilisation in 4 days with no further improvement in strength seen after this testing period which suggests indifferent chemical reactions to the 28-day cured fly ash stabilised soil. The positive efficacy of this enzymatic fly ash additive is also seen in Fig 5.11 irrespective of the rate of dilution and application of the enzyme as well as the curing time. These results suggest that a low concentration of the enzyme can yield the optimum benefits of fly ash stabilisation in a short time frame without losing its capacity with time. Such behaviour is highly beneficial for road constructions industry since it can minimize the disruptions to traffic. From these results as well as the results from the preceding section (Section 5.3.1.2), it can be seen that E2 presents a suitable enzyme AMR/DMR combination which could provide adequate strength improvement for the treated soil with a higher DMR that provides the most cost effective and sustainable soil mix.

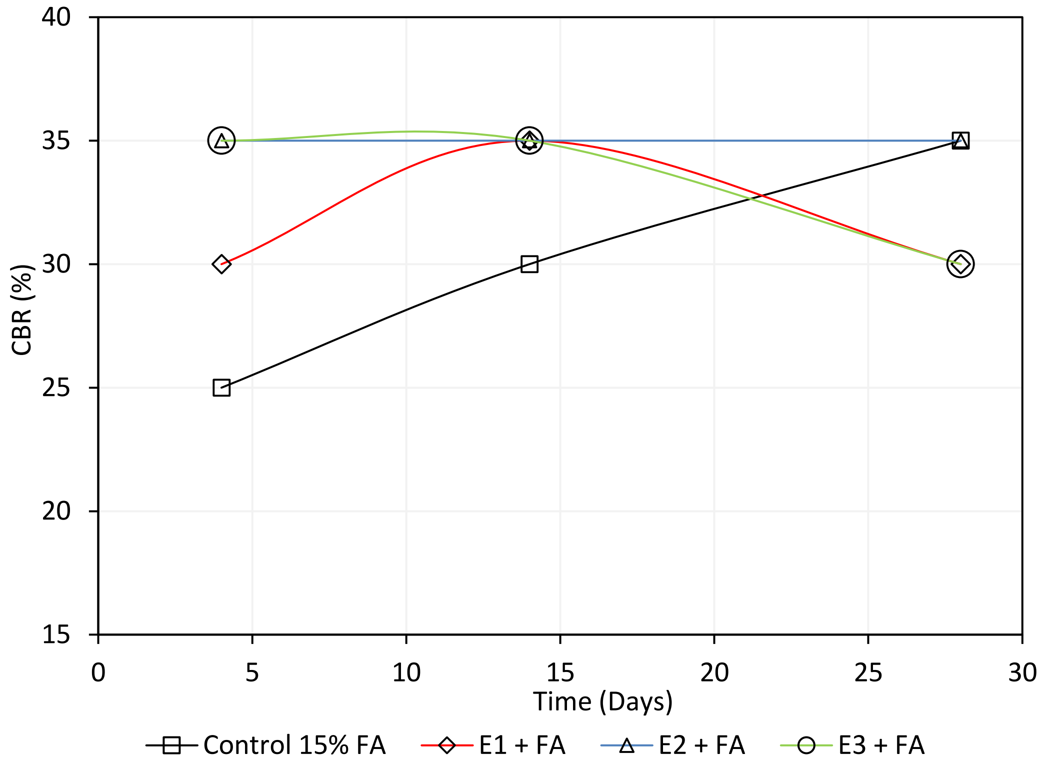


Figure 5.11. Time-dependent test

5.3.3. Part 3: Efficacy of Enzymatic Fly Ash and Lime Stabilisation

The use of activators with fly ash additives has been vastly covered in the literature. The use of these activators is known to facilitate an accelerated rate of strength development, and higher compressive strengths which ultimately improves the engineering performance of fly ash amended soil (Rao and Asha 2012). Rao and Asha (2012) explain that the addition of lime replaces the exchangeable ions of the soil with calcium ions. This increase in the exchangeable ions contributes to the increase in flocculation of clay particles while transforming the soil to a granular less plastic soil. The increase in pH accommodated by the introduction of lime further promotes the dissolution of the siliceous and aluminous compound from the soil lattice. The reaction between the dissolved compounds with the calcium ions produces C – S – H and C – A – H gels which coat the soil particles and crystallize to form interparticle bonds. Other works have also reported the efficacy of improving the strength of fly ash amended soils using sodium sulphate (Na_2SO_4) and lime ($Ca(OH_2)$ (Wilińska et al. 2019). Wilińska et al. (2019) report faster development of the pozzolanic activity by these activators based on the shortened induction period and a more intense C-S-H precipitation period. Hydrated lime was used as a stabilising agent along with the fly ash and enzyme to investigate the effect of lime on the efficacy of enzymatic fly ash stabilised soil samples. The results from the pH tests revealed that the minimum dosage of lime to be 2%, as shown in Fig 5.12 and 5.13. This minimum lime content was determined based on the amount of lime required to maintain the pH of the soil lime slurry between 12.30 to 12.40 (Eades and Grim 1966, TxDOT 2002). As seen in Fig 5.13, the pH values of the soil with the tested levels of the lime plateau around 13, which suggest that the minimum lime content is lesser than 2%. However, as the test was conducted by increments of 2% with 2% being the lowest added amount of lime, this was chosen as the minimum lime content. Selection of the activator of such low content is also ideal as it supports the scope of predominantly using recycled materials (in this case, fly ash) with the enzyme. The target moisture content and maximum dry density of the lime samples (enzymatic and non-enzymatic) were based on further compaction tests conducted, which revealed the OMC and MDD as 17.4% and 1.69 g cm^{-3}. Tests were conducted at this selected lime content (2%), and the strength results (CBR & UCS) are compared among the control soil, fly ash stabilised soil and fly ash + lime stabilised soils for enzymatic and non-enzymatic stabilised soils as shown in Fig 5.14 – 5.16. Addition of lime has significantly increased the soil strength for both enzymatic and non-enzymatic stabilised soils. For example, unsoaked CBR increased from 30% to 45% (50% increase) for fly ash stabilised soil without the presence of the enzyme, and an increase from 40% to 50% (25% increase) can be noted for enzymatic fly ash stabilised soils (Fig 5.14). A similar increasing trend for CBR strength can also be observed from the soaked tests, as shown in Fig 5.15. It should be noted from the CBR test results that the presence of enzyme has considerably increased the fly ash-lime stabilisation by 10% and 40% through unsoaked CBR (45% to 50%) and soaked CBR (13% to 18%) respectively. These results revealed the effectiveness of lime and fly ash as suitable secondary additives to enzyme-based stabilisation. However, UCS results showed no strength improvements on soil stabilised using lime for both fly ash and enzymatic fly ash soil stabilisation (Fig 5.16). Even though the enzymatic stabilisation has improved the UCS from 0.25 MPa to 0.31 MPa compared to fly ash stabilisation, the addition of lime has not contributed to gain significant additional strength. The friable nature of samples due to the addition of lime has failed the samples easily due to no lateral constraint in UCS tests.

Figure 5.12. Lime content determination process

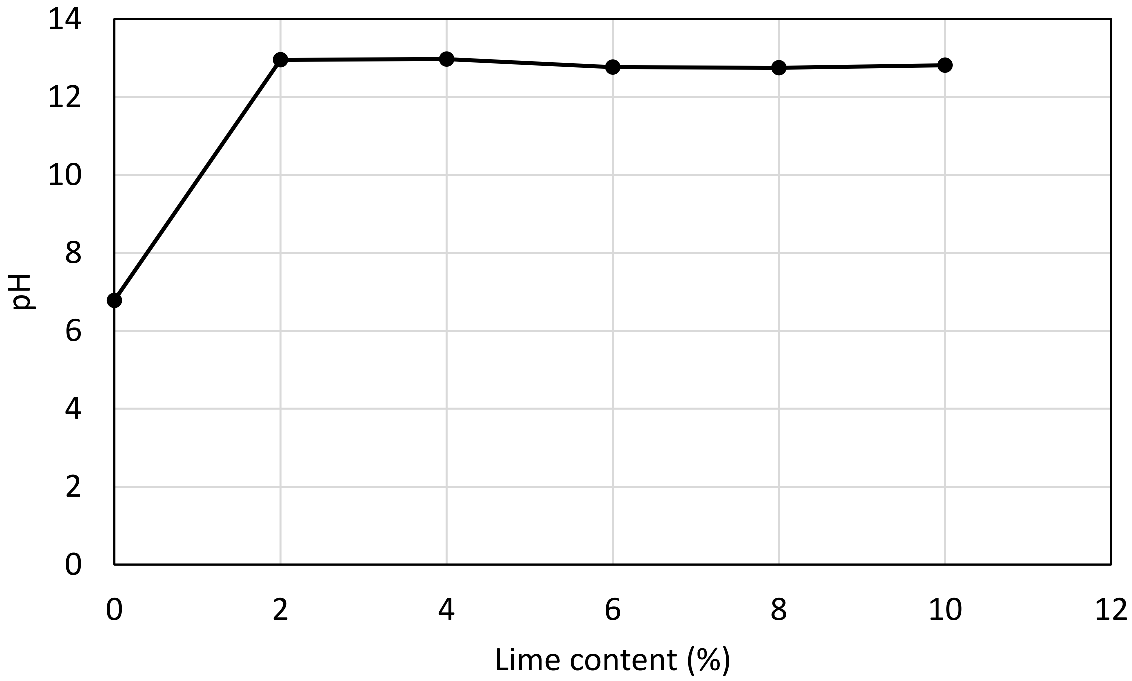


Figure 5.13. Minimum lime content test

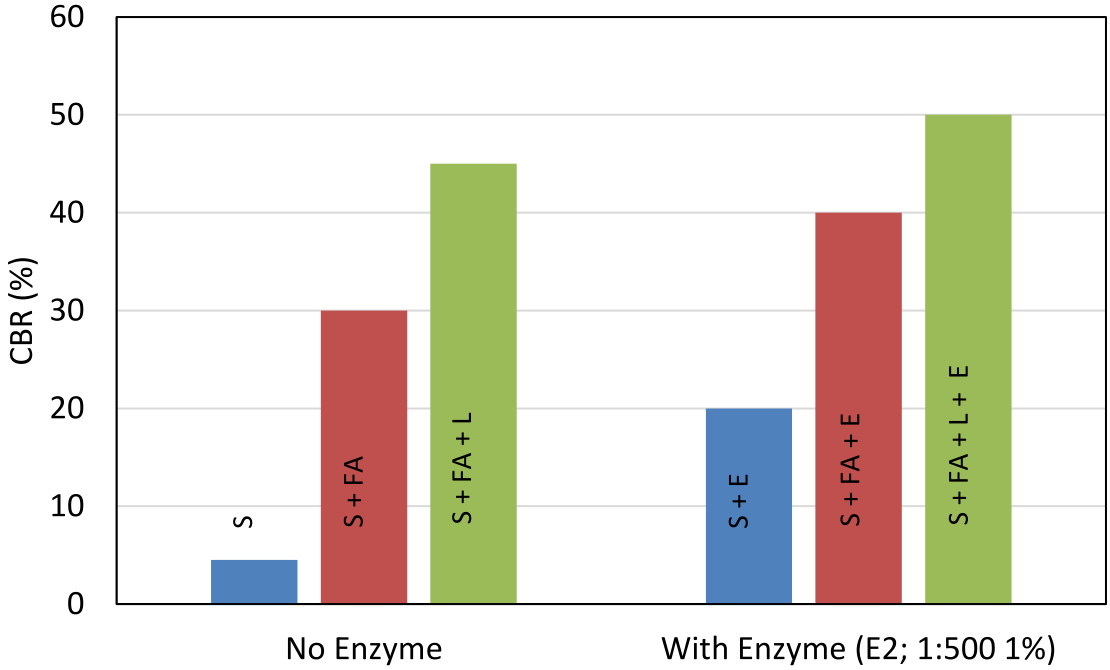


Figure 5.14. Unsoaked CBR tests conducted on compacted samples

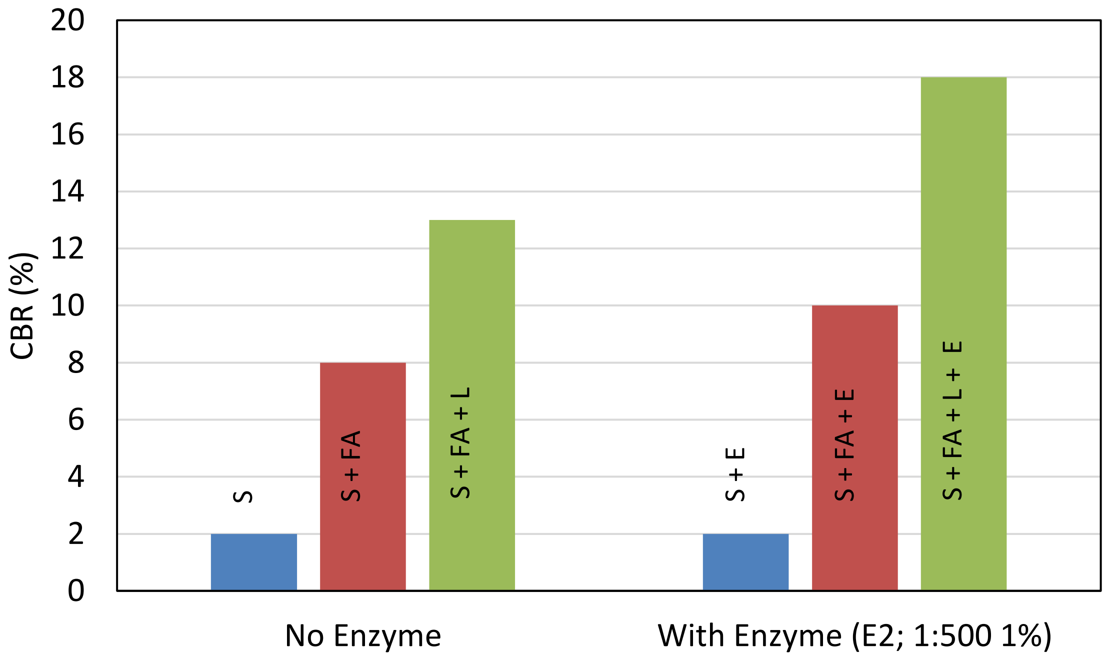


Figure 5.15. Soaked CBR tests conducted on compacted samples

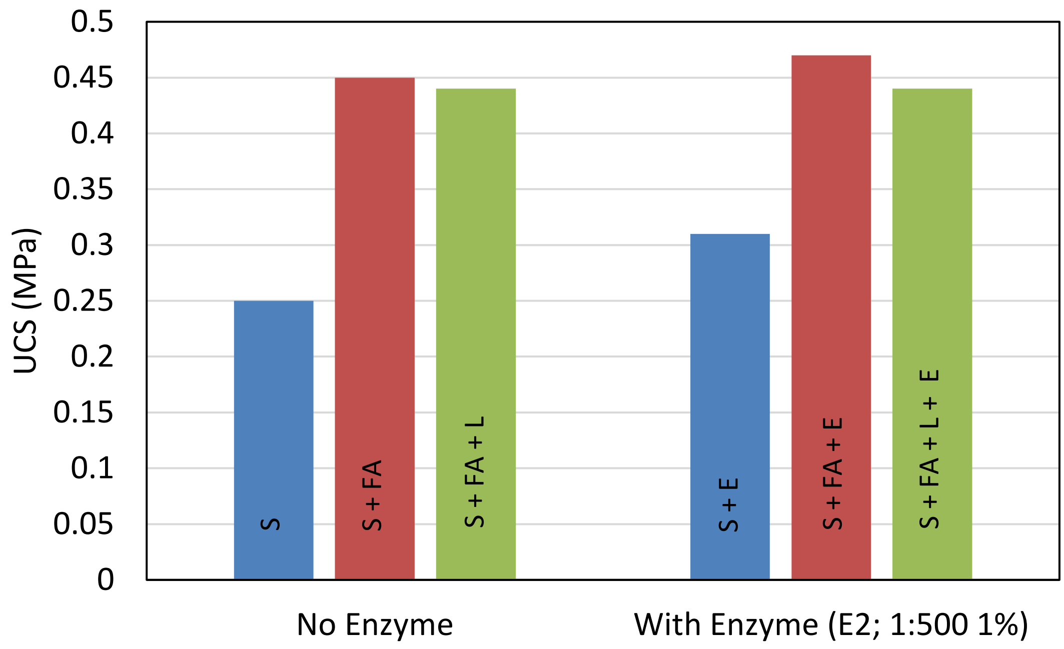


Figure 5.16. UCS tests conducted on compacted samples

5.3.4. Part 4: Unveiling Mechanisms

5.3.4.1. Chemical Analysis

Results of TGA conducted on different stabilised samples are presented in Fig 15.17 in comparison with the non-treated soil. Yoobanpot et al. (2020) highlight that zones pertaining to CSH lie between 50 °C and 200 °C, whereas the CAH and CASH main weight loss occurs between 200 °C to 300°C which was not evident from the 7-day prepared samples. However, the results from this study reveal that there is a distinct difference in the weight loss in all the treated cases when compared to the control soil, especially at larger temperatures (> 500 °C). The changes in weight under stabilisation will reflect either new formations or loss of entrapped interlaminar water in the stabilised samples (Al-Mukhtar et al. 2012, Jiang et al. 2015). It should also be noted that the weight loss of soil treated with the enzymatic fly ash do not differ significantly compared to the fly ash stabilised soil, suggesting that no new or different form of dehydroxylation occurs between the two cases. The mass loss in the untreated soil between the temperature range of 460 – 660 °C is from the dehydroxylation of clay minerals in the soil while the lime and fly ash stabilised soil has also depicted dehydroxylation of the portlandite at 390 to 460 °C like the previous investigations using lime (Maubec et al. 2017) and fly ash (Sharma et al. 2012) stabilisation. These new formations resulted from fly ash, and lime has enhanced the strength considerably for stabilised soil samples compared to controlled soil. From the TGA results, it can be seen that there are no significant changes in fly ash treated and enzymatic fly ash treated soil which suggests that the changes in the pore-structural and microstructural analysis could be the reason for the increase in strength reported in Fig 5.8. However, the distinct difference in mass loss of the soil treated with fly ash + lime and enzymatic fly ash + lime suggests that new formations occur at an accelerated rate due to the less activation energy required by the enzyme treated samples.

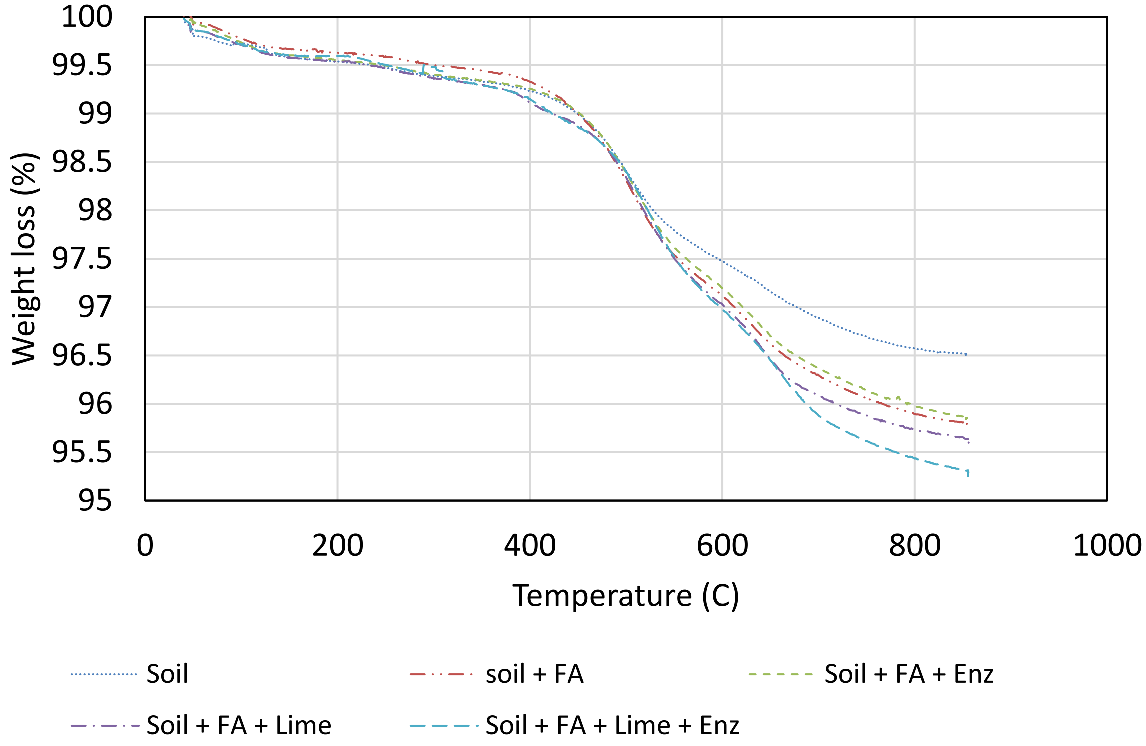
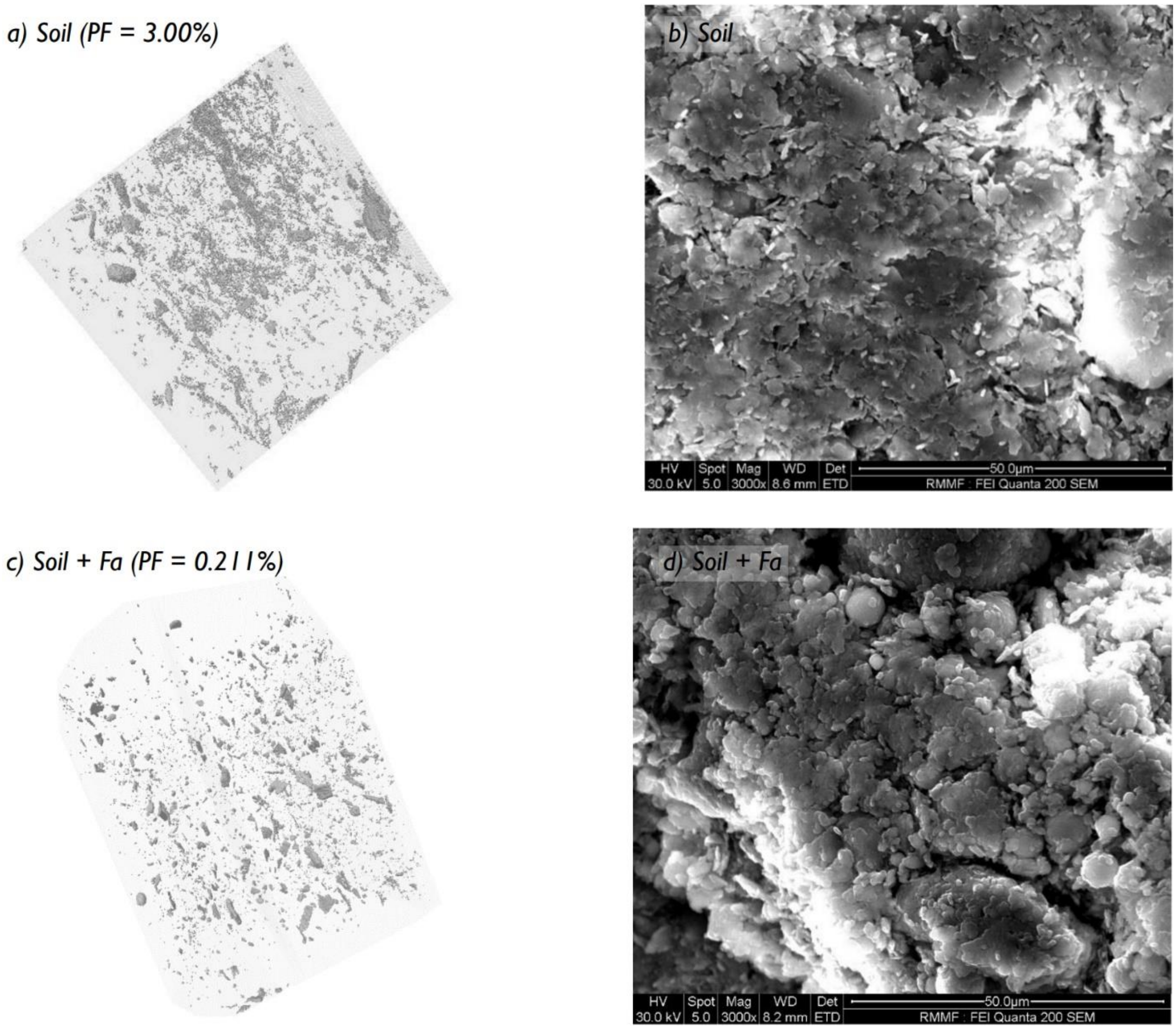
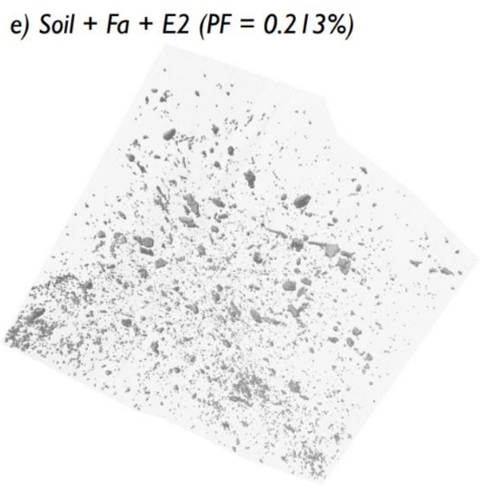


Figure 5.17. TGA conducted on research materials

5.3.4.2. Pore and Microstructural Analysis

Images from the pore-structure and microstructural analysis of stabilised and controlled samples identified using X-ray aided Computer Tomography and SEM are shown in Fig 15.18. For pore-structural analysis, 2 cm cube samples, cut from the middle of treated and untreated unsoaked CBR tested sample were subjected to X-ray radiation at 360^{o}. These scanned images were reconstructed using Skyscan NRecon to form a 3D representation of the sample highlighting the porosity factor (PF) of the treated samples. This PF is based on the pixel intensity of the sample voids, in which the scanned image is binarized as a black or white pixel where the black pixels represent the sample voids. The PF represents the total volume of these black pixels in the 3D sample. In general, there is a decrease in the PF of all the treated samples. A substantial reduction (93%) can be observed in the PF (Fig 15.18) from controlled soil to fly ash stabilised soil as the PF decreases from 3% to 0.21%. The reduction in porosity in the soil could be due to various reasons. Firstly, in the fly ash stabilised soils, this reduction in the pores could be due to the cementitious CSH and CAH formed from the fly ash treatment of soil. The reduction in porosity of fly ash stabilised samples can also be seen in SEM images. It is to be noted that the samples prepared for SEM scans were prepared from the loose shavings from the 2 cm cube used for CT scans. The samples were prepared and tested within seven days of preparation (based on the availability of the equipment). As seen from Fig 15.18d, treatment of fly ash in soil has resulted in clumpier sections (compared to 15.18b), which would support the reduction in the porosity factor observed from the CT scans. The introduction of the enzyme to this fly ash stabilised samples reveals more readily formed clumps as seen in Fig 15.18f (compared to 15.18d), indicating the accelerated formation of the clumps, which result in rapid strength gain. It should also be expected that the addition of lime into the enzymatic fly ash stabilised soil tends to increase pH instantaneously (as seen in Fig 15.13) which readily supports pozzolanic reactions where CSH and CAH gel covers the pores. From these results, it can be hypothesised that enzymes can decrease the activation energy required for the fly ash to commence the pozzolanic reaction which is supported by the increased rate of strength gain as seen in Fig 15.9. However, combining lime to this enzymatic fly ash admixture increases the amount of free lime available in the soil, which further facilitates pozzolanic reactions that fill the pores in the soil with a gel-like CSH and CAH. The mechanism of the filling of pores with these formations is different from the mechanism identified in Chapter 4 for enzyme alone stabilisation. This can be understood by the considerable reduction in the MDD of enzymatic fly ash stabilised soils due to the addition of finer materials compared to the enzyme alone stabilisation. These results show that hydration and pozzolanic reactions dominate flocculation and cationic exchange reactions occurring within the admixture.





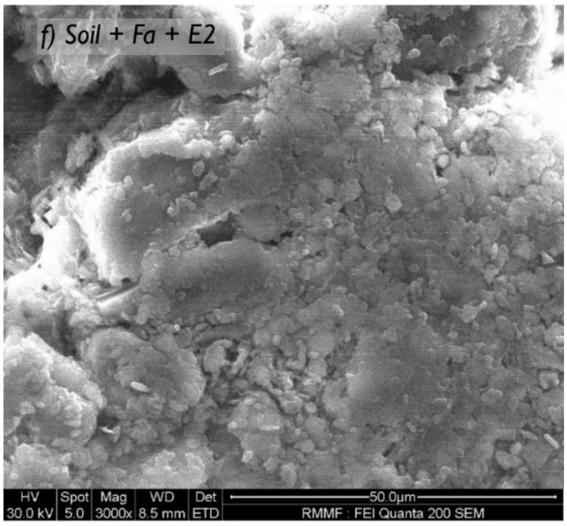


Figure 5.18. Pore-structural and micro-structural images of compacted samples

It is evident from the results that the treatment incorporating enzyme and lime in fly ash treated soil is very effective to enhance the bearing capacity of the soil. Comparative study on this admixture treatment to current literature reporting the effect of fly ash and lime show varied rate of effectiveness. For example, Sharma et al. (2012) report 176% (2.06% → 5.7%) improvement in CBR with the treatment of fly ash and lime in a similar USCS classified soil. Treatment of the soil in this study with the fly ash and lime admixture is seen to have improved the CBR on control soil by 550% (2% → 13%). Increase of up to 800% (compared to control soil) is seen with the addition of enzyme to this mix, which highlights the benefit of this form of additive. Kolias et al. (2005) report improvement of CBR of varying degrees on two CL classified soils. Kolias et al. (2005) based on a 90-day cured and 24hr soaked CBR test design report strength improvements of 2900% (2% → 60%) and 1800% (10% → 190%). As can be seen from these results, the strength benefits from stabilisation are largely governed by the soil type in addition to the additive used in the stabilisation process. Results obtained from the current study revealed that enzyme could help to increase the efficacy of fly ash soil treatment by decreasing curing time required to attain the current reported strength because of the catalytic nature of the additive.

5.4. Application and Benefits

5.4.1. Application

Results obtained from the lab testing program were applied to evaluate the efficiency of enzyme-based stabilisation with secondary additives in road pavement design. Mechanistic pavement design was conducted using the CIRCLY 6.0 software, which is the current method of practice in Australia for pavement designs. Two approaches are commonly adopted to determine the pavement thickness of unsealed roads in Australia (ARRB 2020). The first approach is the implementation of a single base or wearing course layer over the subgrade with nominal thickness ranging from 100 mm to 150 mm which is suitable for roads with low traffic volumes with light vehicles. The second approach involves the provision of the layer of 50 mm to 100 mm less thickness required for sealed pavements. The second approach is suitable for roads likely to be affected by wet climatic conditions and/or poor drainage, with higher traffic volumes, and with significant heavy vehicular traffic. The implication of the additives on the pavement design was conducted by comparing a typical unsealed pavement with an unsealed pavement stabilised with the additive mix proposed in the study. The pavement designed in the study is classed as rural “Class 4” road based on Austroads classification (AGPT05/19 2019). The designed road can be further classed as a “4B – minor road” which carries average daily traffic (ADT) of 50 – 150 vehicles. This class of roads are designed to connect the local centre population to the primary network. Typically, these classes of roads have a minimum of 5.5 m width with the operating speeds varying from 30 – 70 km/hr based on the terrain. Generally, roads of this classification consist of three layers, the subgrade and a separate base course and wearing course layer. However, pavements which consist of a single combined base layer and wearing course layer is also commonly used in Australia as well as other nations (ARRB 2020). Adhering to these requirements, four pavements were analysed using CIRCLY to determine the pavement thicknesses of the wearing course/base materials. Analysis of the pavement was conducted for a pavement layered such that the subgrade has 3% CBR, with a layer of stabilised subgrade of a certain height and a 20 mm unbound granular material as a wearing course layer. Pavement A was designed incorporating two layers; the untreated subgrade and the wearing course layer, whereas pavements B – D were based on a three-layer pavement, i.e., subgrade, stabilised materials, and the wearing course material. Pavement B incorporates the subgrade stabilised with fly ash, pavement C incorporates fly ash stabilised soil with enzyme and pavement D covers stabilised material using fly ash and the enzyme mix with lime. The additive content used for fly ash, enzyme and lime are 15%, E2, and 2%, respectively. Results of soaked CBR were used in the CIRCLY analysis. CIRCLY adopts a Cumulative Damage Factor (CDF) concept to predict the total damage on the pavement. In this method, the pavement strain response to vehicle loading indicates the effect on the pavement's service life. Damage factor for an i^{th} loading is defined as the number of repetitions of a given strain (n_i), divided by its allowable strain (N_i). When the sum of all the damage factors exceeds 1, the pavement is presumed to have reached its design life (Wardle 2010). Modifications to layer thickness or stiffness can be used to achieve satisfactory CDF. Parameters used in the CIRCLY pavement analysis are shown in Table 5.6.

Table 5.6. Parameters used for the CIRCLY

Description	Value
Pavement DESA	13.0×10^{3} (ARRB 2020)
Project reliability	80%
Stabilised material Elastic modulus	Wearing course – 200 Mr Stabilised layer – 180 Mr Control layer – 30 Mr (Heukelom and Klomp 1962)
Poisson's ration	0.35 (Austroads 2006)
Max size of wearing course material	19 mm (ARRB 2020)
Wearing course material	Class 2/3 crushed rock (AlexFraser 2020)

Table 5.7. Pavement thickness analysis using CIRCLY

Pavement	Stabiliser used	Layer	Thickness (mm)	CDF
A	N/A (control soil)	Wearing course	500	-
		Subgrade	Infinite	4.16×10^{-1}
B	15% FA	Wearing course	250	-
		Stabilised subgrade	250	3.05×10^{-1}
		Subgrade	Infinite	5.11×10^{-1}

C	FA + E2	Wearing course	225	-
		Stabilised subgrade	250	9.19×10^{-1}
		Subgrade	Infinite	9.42×10^{-1}
D	FA + E2 + Lime	Wearing course	225	-
		Stabilised subgrade	250	9.19×10^{-1}
		Subgrade	Infinite	9.42×10^{-1}

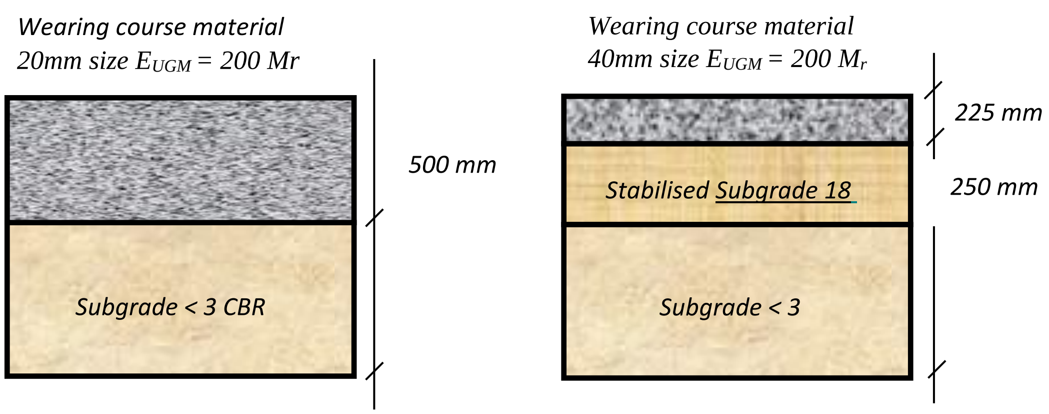


Figure 5.19. a) Untreated Pavement A requires 500 mm wearing course layer; b) Reduction of 275 mm thickness of wearing course layer in Treated Pavement D

Results of the analyses showing the change in the layer thickness are shown in Table 5.7 and Fig 5.19. As seen from the analysis results, pavement A highlights the need to incorporate a base thickness of minimum 500 mm to keep the cumulative damage factor to an acceptable level. Fly ash treatment of the first 250 mm of subgrade has significantly reduced the wearing course material from 500 mm to 250 mm (50% decrease). The addition of E2 to this mix has the potential to further reduce the thickness of the wearing course material from 250 mm to 225 mm (10% decrease). It should be noted that the addition of lime to enzymatic fly ash does not make a further reduction in wearing course layer thickness which could be due to the limitations in decreasing wearing course layer thickness at the chosen modified subgrade thickness. The 55% decrease in the thickness of the pavement layers (500 mm → 225 mm) is from the increased bearing capacity of the soil due to the pozzolanic reactions occurring in the soil, which decreases the porosity of the soil. This significant decrease in wearing layer thickness translates to savings in terms of natural resources to serve as a road base. As seen from these results, all the tested additives have the potential to reduce the pavement layer. It should be noted that the 225 mm thickness of the layer as found from the CIRCLY analysis refers to sealed pavements. As mentioned earlier, the current practice for determining the thickness of the unsealed road pavement layers in Australia is based on an approach of providing a layer of 50 mm to 100 mm lesser than that of a sealed road. Based on this criterion, if top 250 mm of the subgrade is stabilised with enzymatic fly ash on an unsealed pavement, it would only require a wearing course layer/base course layer with a minimum thickness of the 125 mm to a maximum of 175 mm. This further adds to the sustainability of the pavement by decreasing the need for natural resources in the form of wearing course material. It should be noted that the addition of lime to enzymatic fly ash does not make a further reduction in wearing course layer thickness which could be due to the limitations in decreasing wearing course layer thickness at the chosen modified subgrade thickness. As seen from these results, all the tested additives have the potential to reduce the pavement layer. A significant reduction in thickness is evident in the upper layer, which could directly result in considerable cost savings by reducing demand for importing quarry materials for the road base.

5.4.2. Benefits

This study showed that fly ash could be combined with enzyme-based additives to increase the efficiency of enzyme-based soil stabilisers. As reported in Section 4.5 (Chapter 4), further exploration into this combined effect of secondary additives needed to be conducted to induce chemical reactions within the soil enzyme composite to facilitate long term stabilisation effect to the soil. From Section 5.1, it can be seen that attention should be devoted to the use of green stabilisers or recycled materials to provide sustainability benefits to these ground improvement techniques. This study supports that fly and lime, when added to enzyme stabilised soil, provides considerable mechanical benefits as well as sustainability impacts compared to enzyme alone stabilised cases. Sustainability issue addressed by the incorporation of the additive can be based on the environmental perspective on two factors. Firstly, in terms of savings from a global CO_2 emission perspective by decreasing the need to produce calcium-based stabilisers for pavement stabilisation. Secondly, the reduction in the contribution to the nation's waste stream. However, it should be noted that the sustainability of the product is heavily dependent on the distance between the fly ash source and construction location. McLellan et al. (2011) highlighted the significance of increasing the utilization rate of geopolymers to improve their sustainability impacts by decreasing transport costs. For example, currently, in Australia, due to the significant distance between collection sites (source of fly ash) to construction sites along with the lower utilization rate would decrease sustainability based on the mode of transport used. In contrast, ordinary Portland cement (OPC), due to it being an established product holds an advantage over these geopolymers on this regard. Therefore, increasing implementation of this type of binder further increases its sustainability impact. This study also showed that by applying these additives in the pavement design, the demand for natural resources in the form of wearing course materials is also reduced. This could mean savings in terms of the cost related to the wearing course material as well. For these reasons, the use of the enzymatic fly ash along with the minimum amount of lime can be recommended as an effective means of stabilising weak and problematic soils on the basis of the chemical reactions that take place within the soil fabric.

5.5. Summary

This chapter aimed to investigate methods of improving the efficacy of enzyme-based soil stabiliser using secondary additives for ground improvement while further ensuring sustainability impacts are positively affected. Waste materials and by-products were considered as potential secondary additives as currently, the improper management of waste materials poses a significant threat to society. It is critical to invest in cement alternatives which can provide cost effective and eco-friendly methods of ground improvement. The utilization of waste materials for ground improvement has been actively investigated in the past and encouraged as a means to promote sustainable road constructions. This research identifies the versatility of enzyme as an additive which can facilitate the increased utilization of fly ash, a waste by-product from burning coal in power plants. The combination of enzymes and fly ash improves the sustainability of both additives by proving to be effective in providing eco-friendly solutions for ground issues. Despite being proven effective in engineering works, the stigma revolving around the use of either fly ash or enzyme is yet to be used to its full potential even though it can efficiently be utilized to support sustainable engineering constructions. This study aimed to identify the efficiency of improving enzyme-based stabilisation (as shown in Chapter 4) by using fly ash. It also unveiled the stabilisation mechanism and effectiveness of the combined additives on the fine-grained field soil subjected to testing within this thesis. Further combination of lime to the additives is shown to facilitate pozzolanic reactions similar to that of cement. The research aimed to produce optimised values for the effective utilization of the additives for enhancing the resilience of the infrastructure. Following conclusions can be drawn from the results of detailed lab test program conducted in the current study:

- Part 1 of the chapter suggests that densification is not the dominant mechanism in enzymatic fly ash-based soil stabilisation based on the compaction characteristics. Additive content of 15% (w/w) and E2 has been identified as the suitable fly ash and enzyme contents. Strength gains of up to 400% (soaked CBR), 680% (unsoaked CBR) and 88% (UCS) can be observed at this additive combination.

- Part 2 of the chapter show that the addition of enzyme-based stabilisers accelerates strength gain of fly ash stabilised soils to achieve peak strength within four days as opposed to 28 days in soils stabilised by fly ash alone by decreasing the activation energy required to start the pozzolanic reaction between fly ash and soil.
- Part 3 and Part 4 results support that a lime content of 2% is suitable to activate pozzolanic reactions which increase the strength of fly ash alone stabilised soil up to 550% (soaked CBR), and 900% (unsoaked CBR). Addition of 2% lime to enzymatic fly ash further increases the strength by up to 800% (soaked CBR) and 1000% (unsoaked CBR) due to the formation of hydration products such as calcium silica hydrate (CSH) and calcium aluminate hydrates (CAH).
- Part 5 results suggest that pavement stabilised with the combined additives decreases the thickness of the wearing course layer of the pavement by up to 60% (500 mm → 225 mm) which could directly correlate to savings in terms of the overall cost of the pavement along with sustainability impacts such as reduced carbon footprint in producing the additive as well as waste mitigation of fly ash binders.

It is evident from the results that both fly ash and lime are very effective to enhance the bearing capacity of the soil. Chapter 4 alludes to the potential benefit of combining enzyme with other additives. The findings reported within this chapter support that calcium-based by-products such as fly ash have a pronounced effect of benefiting soil strength. The outcomes of the research have the potential to benefit the road construction industry by providing effective use of sustainable, eco-friendly products, especially waste material in engineering construction. While this research assists in confirmation of the findings previously reported in the preceding chapter, it could also significantly assist in enhanced fly ash waste mitigation as well as the development of alternative stabilisation practices in the road construction industry. Chapter 6 investigates the long-term effect of the treatment based on durability as well as the computational and finite element modelling methods.

<u>Chapter Six.</u>

Durability and Performance Investigation of Enzyme Stabilised Soil

6.1. Introduction

The investigation on the efficacy of enzymes as a non-traditional soil stabiliser on fine grained soil has shown benefits in improving the immediate strength of soil, as reported in Chapter 4. This improvement in strength has been reported with no significant chemical changes or new cementitious formations but rather a decreased affinity of water which helps in a stabilised clay platelet. Further studies, as reported in Chapter 5, have shown larger strength improvement can be gained when combining enzymes with fly ash and lime due to facilitating chemical reactions which in turn produce cementitious materials within the soil fabric. Once again, this strength improvement is seen immediately, especially by the role that the enzymes play in decreasing the activation energy required for the reaction to take place. This chapter evaluates the long-term effect, performance, and the durability of the enzyme-based stabilisation.

The durability of the road is essential in determining how much money is spent on it. It is estimated that over $5.6bn will be spent between the financial year 2013 – 14 to 2022 – 23 for the repairing of many roads across Australia (Infrastructure 2019). The Australian road network comprises of over 817,000 km of road with over 57% of these being unpaved. The designing of these unpaved roads to inappropriate standards place a significant financial burden to the nation. Therefore, it is imperative that when pavements are designed or stabilised to mitigate strength issues, it is designed after accounting for the durability of these pavements. Although there are tests that could be easily conducted on paved roads to assess their durability, their applicability for unpaved roads can be seen as limited. The major difference between a paved and an unpaved road is layer specifications. Flexible paved roads usually have a subgrade layer covered by a subbase course layer, followed by a base course layer and topped with a surface course layer. Unpaved roads, on the other hand, doesn't necessarily have much protection apart from a wearing course layer, which in most cases is gravel. The issue with these types of pavements is that it often has a poor ecological record. Harsh weather conditions may affect it by causing washout of materials through erosion, pollute waterways, jeopardise the life of the pavement and even cause casualties. As mentioned in the previous chapters, soil stabilisation can work as a suitable method to mitigate these issues.

The efficacy of these stabilised unpaved roads is often based on their CBR strengths. The Japan Road Association's approach model is a good example to determine the general life service of a pavement based on its CBR. This model has been widely used by Australian engineers, more commonly since the method was presented in the 2001 Austroads interim design guide. With this method, a weighted subgrade strength from the stratified layers of the subgrade is attained. The method is used for subgrade of 1 m of the underside of the subbase, which experiences vertical stratification (Vorobieff and Murphy 2003). This method is used in the industry to determine equivalent design subgrade strength CBR_m based on the stiffness of the supporting soil depth and is represented by Equation 6.1.

$$CBR_m = \left[\frac{\sum_i hiCBRi^{0.33}}{\sum_i hi}\right]^3 \leq 20 \qquad \textbf{\textit{(Eq. 6.1.)}}$$

$$where\ CBR_i\ is\ the\ CBR\ value\ in\ layer\ thickness\ h_i\ and \sum hi\ is\ taken\ upto\ a\ depth\ of\ 1.0\ m$$

Mechanistic pavement design is also considered as an acceptable pavement design approach in Australian construction practices, where CIRCLY is used to assess the cumulative damage factor (CDF) obtained from the design layer thickness and its CBR. These methods are used in practice to gauge failure criterion. However, this method, much like the Japan Road Association's approach model only indicates whether the pavement can withstand the applied loads and does not provide much understanding on volumetric and mass related changes occurring in the pavement layers. The durability of pavements could be addressed either by experimentally investigating the fundamental behaviour of the materials used for the pavement design or by a computational analysis which involves modelling and assessing pavement performance and responses based on numerical modelling. Both these methods (i.e. fundamental and numerical assessments) of durability assessment can be seen reported in various literature as highlighted below.

6.1.1. Performance Evaluations Based on Experimental Analysis

Wetting-drying, freezing-thawing and leaching tests of soil specimens are a few among the most common laboratory testing procedures which can be conducted to understand the fundamental behaviour of materials and to quantify its resistance to external conditions. As understood from the literature, soils, especially ones with a considerable amount of fines, are prone to moisture-induced volumetric distress which can compromise the health of pavements constructed with these soil types. As investigated and reported in the previous chapters of this thesis, chemical stabilisation, can significantly alter fundamental microscale characteristics of the soil, which in turn would provide adequate resistance to load and enhance the functionality of the raw material. Chemical stabilisers modify the soil properties through cationic exchange, flocculation and agglomeration, pozzolanic reactions and hydration (Prusinski and Bhattacharja 1999). Densification mechanism of enzyme-based additives, which was demonstrated in Chapter 4, also provides effective stabilisation for weak subgrades. This chapter provides the results of experiments conducted to evaluate the durability performance of subgrades stabilised primarily by using enzymes as well as a combination of enzyme and the secondary additives investigated in Chapter 5. It also elaborates on literature covering key experiments and their outcomes on assessing the durability performance of stabilised soils.

The use of test methods such as wetting-drying, freezing-thawing, and leaching tests have been investigated as a potential method of quantifying durability in lime and cement stabilised samples within the literature. Prusinski and Bhattacharja (1999) recommend conducting of these tests to indicate how lime and cement stabilisers help maintain strength properties of soil when subjected to extreme conditions. However, the study highlights that the testing does not necessarily indicate the stabiliser's resistance to changes in PI, shrinkage limits and load-bearing values. The study concludes that both cement and lime introduce changes to the material properties of the soil which could increase the durability of the specimens, provided, proper care is maintained during specimen preparation in terms of applying optimum content and procedure. The study also points out that the skewed results as reported within other available literature which favour one additive over the other (lime or cement) could be because of the discrepancies in sample preparation methods which highlight the need to have consistent sample preparation method in determining the durability effect of these additives. The study also highlights leaching of calcium as a concern with lime stabilised soils and recommends the use of lime content which is 4 – 5% greater than the one identified using (Eades and Grimm 1966) to inhibit detrimental leaching of lime.

As mentioned earlier, Freeze-thaw and wet-dry cyclical testing has been commonly conducted and reported in various other literature to assess durability. Miller and Zaman (2000) conducted these tests to compare the qualitative durability of Cement kiln dust (CKD) treated soil. CKD, similar to fly ash, is the dust particle collected and removed as an industrial waste during Portland cement manufacturing. Miller and Zaman (2000) point out that treatment with CKD proves to be an effective method of stabilisation. However, due to the difference in the properties of CKD based on the source material and the type of collection used, the additive efficacy and strength gain are harder to be quantified. Durability results from the study highlight the extreme nature of wet-dry cyclic tests and emphasise that the testing is not representative of the in-situ conditions. However, the study shows that despite the extreme nature of the testing, the experiment provides a relative comparison of additive performance. Relative to the untreated soil, the CKD treatment improved resistance of soil to wetting and drying. However, the nature of the testing was deemed too extreme even in treated cases, as many didn't survive the 12-cycle plan. On the other hand, freeze-dry cyclical tests have shown to be not as extreme. All treated samples survived the 12 freeze-dry cycles. Samples subjected to freezing and drying cyclical tests decreased in moisture content significantly, which facilitated the increase of UCS at the end of the 12 cycles. The method of testing for freezing and thawing were based in parts from ASTM D559 (2003) and D560 (2003). However, the practicality of the testing should be limited to areas in which the subbase becomes saturated and are susceptible to ice formations and subsequent heaving. The study also highlights the importance of developing realistic durability testing procedures which could prove to be effective in long term performance prediction of stabilised soil. This suggests that when assessing the durability of specimens, selection or designing of tests should be based on two factors. Firstly, the applicability of the tests, i.e., areas subjected to saturation of the subbase and the layer is susceptible to ice formation and heaving. Secondly, based on the severity of the tests, i.e., wetting and drying might be too severe, whereas freezing and drying might not be severe enough relative to the field conditions.

Khalife et al. (2012) investigated the effects of the hydrated lime, class C fly ash, and CKD on the durability of two fat clays at the end of wetting-drying and freezing-thawing tests. In the study, specimens subjected to these cyclical tests were further subjected to various strength tests. The study reported that the level of decrease in UCS sample was influenced by soil type and type of stabiliser. This study reports indicative results which show a significant reduction in the strength of the specimens subjected to first freeze-drying cycle due to the combined effect of pore structure and increase in moisture content. Yet again, the aggressive nature of the wetting-drying test is highlighted here with treated and untreated sample disintegration reported during the first cycle. On the other hand, resilient modulus samples subjected to both the cyclical tests provide a better understanding of the effectiveness of the stabilisers due to the nature of testing. The study also highlights the superiority of 6% lime in stabilising the soil over 10% class C fly ash and 10% CKD.

The aggressive nature of wetting and drying tests can be noted in the majority of the available literature. Parsons and Milburn (2003) and Milburn and Parsons (2004) highlight the superiority of cement over all the other tested stabilisers when utilising wetting and drying cyclical testing. One of the stabilisers subjected to the durability study in the investigation included commercial enzymes which produced the least effective resistance to durability tests while also only providing modest strength gain compared to the other additives. The study also reports that the freeze-drying results show the most soil loss in the case of lime stabilised soils. Several other studies exist which utilises and compares both wetting-drying and freezing-thawing method to assess the durability of soils (Hoy et al. 2017, Chang et al. 2017, Rezaeimalek et al. 2018, Jiang et al. 2018). This would support the argument that the current standard practice of wetting and drying is not an effective form of assessing the stabilisation effects of the additives tested within the scope of this research. However, from these studies, it can be seen that it could be beneficial to modify testing conditions to attain indicative comparative results. For example, the curing methods, curing time and even mode of testing can be altered when testing, which in turn can provide a better understanding of stabilisation effects in the long run.

The investigation into the shrinkage and swell characteristics of stabilised soil has also been reported in the literature as indicating its effectiveness to degradation resistance (Puppala et al. 2013). However, as it is understood that these characteristics are heavily dependent on the type of soil, the durability of low plastic soils might not be easily assessed. The study reconfirms the notion that high plastic soils are mostly highly expansive. A follow-up study uses correlations to predict swell and shrink related displacements in soils. Puppalla et al. (2014) use soil plasticity, and compaction characteristics as independent variables to introduce models that rely on seasonal compaction moisture content variations to estimate infrastructure swell and shrinkage strains. The study highlights the potential of supplementing laboratory experiments with numerical analysis to analyse the response of pavement structure subjected to weather conditions. While further studies are recommended in the cited literature to reduce the scatter observed in test results on the developed correlation, it was shown that the introduced model represent true upper and lower bound predictions for swell movements in the field. The numerical model presented in the cited study also provided reasonable vertical swell and strain predictions which highlight the effectiveness of numerical analysis for assessment of the durability of stabilised materials.

6.1.2. Performance Evaluation Based on Numerical Methods

As highlighted above, a common method employed to assess the durability in current practice, especially incorporating time in the form of loadings, is performance modelling of structures. While experimental analysis as reported in various chapters within this thesis provide a simplified understanding on the response of pavement structure on a fundamental level, it is often limited to specific cases which limit its validation subject to environmental, operational loading and material conditions. The use of numerical analysis could provide to be an effective solution as it can be developed and adapted to different conditions as long as the estimated values obtained by the study can be validated in practical cases. The numerical modelling of granular pavement materials can be conducted using two approaches. The first approach utilises modelling assuming linear elasticity of the pavement materials, whereas the second approach considers the stress dependency of the granular material, which assumes nonlinear elastic behaviour. While several comparative studies have been conducted on the efficiency of constitutive models based on these two approaches, it is hard to single out just one approach over the other to provide a better understanding of the pavement performance. Programs such as CIRCLY and KENLAYER manipulate the linear elastic theory, which is bound to the Hooke's law constitutive model to calculate the stress, strain, and displacement in all points of the medium. Whereas, there is also a variety of constitutive models that have been developed for nonlinear analysis.

Lekarp et al. (2000 a, b) present a summary of the structural response of unbound aggregates on pavements. Lekarp et al. (2000a) summarise from an extensive literature review that the unbound granular material shows complex elastoplastic behaviour when subjected to repeated loading which has to be investigated further at a microscopic, particulate level rather than macroscopic levels. In the study, the investigators report disagreements in the general scientific community when it comes to the impacts of certain parameters which influence the response of the granular materials. For example, the study highlights that while all researchers agree the resilience of these materials is heavily influenced by applied stress and amount of moisture present in the material, there are some disagreements on the nature of the impact of factors such as aggregate type, particle shape, fines content, maximum grain size has on the resilience response. Resilience response, as gathered from literature, is generally defined by resilient modulus and Poisson's ratio. Lekarp et al. (2000a) also discuss the two approaches of computational modelling of resilient behaviours, based on linear and nonlinear response profile of materials while highlighting the complexity in the field of research which pertains to pavement response prediction. Lekarp et al. (2000b) investigate the permanent strain response of unbound aggregates which report that resilient response is more often investigated in the literature over long term performance assessment due to the nature of the tests which are time-consuming and require separate specimens for each set of stresses. Various factors that affect the permanent strain development has been identified within the cited study as stress levels, principal stress reorientation, number of load applications, moisture content, stress history, density, fines content, grading and aggregate type. Al-Qadi et al. (2010) and Wang and Al-Qadi (2013) explains the importance of employing a nonlinear anisotropic 3D FE model when simulating asphalt and granular layer pavement response under dynamic loading showing that exclusion of cross anisotropic stress-dependent behaviours of the granular layer could significantly affect the accuracy of predicted fatigue and rutting potential of asphalt layers. The studies also highlight that the modulus distribution within the granular layer is not only affected by the wheel load and pavement layer thickness, but also by the temperature and vehicular speed due to the viscoelastic behaviour of the asphalt surface layer.

Predominantly, the response of pavement structure is predicted using elastic multilayer analysis which assumes linear elastic properties of the paving and subgrade materials when subjected to static loads. However, in reality, pavements are subjected to both static and dynamic loads. This, in turn, could reflect in a significant difference between predicted and measure pavement responses. Zaghloul and White (2016) investigate the effect of dynamic loads at various speeds on the pavement using ABAQUS, a 3D, dynamic finite element method (3D-DFEM). The findings of the study highlight the drawback in the use of multilayer elastic analysis which could lead to inaccurate prediction in pavement response while incorporating the effect of dynamic loading is shown to predict the deflection with 99% confidence level. Sensitivity analysis conducted in the study also reveals that the cross-section and loading parameters have a significant effect on pavement response. Cross-section parameters include shoulder width, pavement-shoulder joint, subgrade type, various material properties and deep foundation type whereas loading parameters included in the study include axle load and spacing, number of axles and wheels, time and rate of loading. The effects of temperature on pavement response have also been reported in the study due to its impact on the properties of asphalt. Similar findings, in terms of the consideration of the nonlinearity in granular layers for accurate modelling of flexible pavement, have also been reported in Sahoo and Reddy (2010) which highlight differences such as 35% higher vertical strain over subgrade and 44% increase in surface deflection in nonlinear models compared to values obtained through linear elastic analysis. The study also recommends the use of these nonlinear models for safer designing of low volume roads. Comparison of linear and nonlinear models using axisymmetric and 3D FE analyses for flexible pavement performance have also been reported in the literature indicating that proper characterisations of nonlinear stress-dependent geomaterials significantly affect the accuracy of critical pavement response (Kim et al. 2009). No significant differences are reported on linear and nonlinear models analysed in both axisymmetric and 3D FE analyses when the same modulus defined in axisymmetric stress conditions are used. However, it was reported that drastic changes were recorded between axisymmetric and 3D FE model when using a model developed from triaxial test data assuming equal minor and intermediate stresses ($\sigma_2 = \sigma_3$) and the universal model for 3D analysis employing additional intermediate stress (σ_2) and the octahedral shear stress (τ_{oct}) instead of deviator stress (σ_d). For example, when intermediate principle stresses were taken into consideration in 3D modulus development, lower asphalt concrete tensile stresses were obtained.

Ghadimi and Nikraz (2017) compare a linear constitutive model with nonlinear constitutive models K – θ, Uzan – Witczak, and Lade – Nelson. The study ranks the Uzan – Witczak model to produce “stiffer” (lesser deflection and strain) behaviour compared to all other models with Lade – Nelson model showing softest behaviour. Uzan – Witczack model also reports the highest increase in the elastic modulus of the base layer with loading and stress increments due to the model’s nature of dependency on deviator and bulk stresses. However, the distribution of the elastic modulus is more uniform in Lade – Nelson model as it accounts for considerable lateral distribution of stresses over Uzan – Witczak model which reports a more localised representation of the stresses. The study concludes the effectiveness of all these models in predicting the performance model, highlighting that although a change in properties of the layer might result in different results, the general trend of the mechanical behaviour would remain the same.

Effects of other phenomena such as shakedown and soil asphalt interaction (SAI) on pavement response has also been investigated (Ghadimi et al. 2016). Shakedown theory refers to change in response of the granular materials when they are subjected to a cyclical load which exceeds the layer’s yield criterion. Lekarp and Dawson (1998) also recommended considering the effect of shakedown, in earlier works, which report ultimate equilibrium condition as a result of low shear stress ratios and gradual failure at a higher shear stress ratio which speculates the need for a threshold stress ratio where a change of behaviour occurs. Ghadimi et al. (2016) highlight the importance of the inclusion of these critical design parameters in pavement design also highlighting the advantages of dynamic simulations over static loadings. The dynamic loading resulted in 50% lower surface deflections, 15% lesser tensile strain, 65% and 50% lesser compressive stress and strain at the top of subgrade compared to the static loading. The study also highlights the effective reduction in pavement thickness when incorporating interactional forces between the asphalt and base layers which present a more realistic prediction of pavement response. It is also concluded from the study that in a long-term dynamic simulation, the effects of shakedown and SAI could help achieve realistic simulations. While SAI has a positive effect of rut performance of flexible pavement due to the decrease in the compressive strain at the top of the subgrade, it should be noted that SAI has an adverse effect on the fatigue performance of the pavement structure due to the increase in tensile strain at the bottom of the asphalt layer in dynamic conditions. The diverse nature of the research on pavement performance prediction highlights the complexity of this field of study. Excluding the aforementioned research, other literature also exists highlighting the effect of various other parameters such as delamination of the asphalt layer, which increases strain responses, as well as bedrock depth which is commonly neglected in various studies which have proven to contribute to increased surface deflections (Li et al. 2017). Tyre-pavement interactions have also been investigated in studies to understand the effects of pavement responses (Wang et al. 2012). Coffey et al. (2018) report the effectiveness of designing haul roads using the finite element method of analysis over the currently used axisymmetric linear elastic modelling. The study reports minor variations between field and modelled deflections among the linear elastic modelling approaches and highlights that more accurate predictions can be obtained from a finite element method. However, the study also highlights that the simplicity, reduced calculation time and repeatability of linear elastic models such as S77-1 (Pereira 1977) method could still be advantageous and provide adequately accurate predictions for given vehicle designs and therefore should not be completely delegitimised.

As seen in the preceding chapters, the true mechanism and efficacy of non-traditional additives such as enzymes and its combined effects with calcium-based additives such as lime and fly ash is continuing to be understood. In this chapter, investigations are conducted on the durability of these additives based on experimental as well as computational analyses. Firstly, as a secondary study to the strength test reported in

(Chapter 4), the tested samples were demoulded, disturbed and then recompacted to see if the treated soil retained the strength gained from time and effective recompaction treatment. This will assist in elaborating the initial insight on the durability of the recompacted specimen on certifying the ability of stabilised samples to retain strength upon reinstated. Secondly, a novel wetting and drying cyclic test is conducted in this chapter to further assess the durability of a pavement stabilised with all additives tested in this research. Namely, enzymes, fly ash and lime. The durability in this section of the chapter is reported based on the volumetric change of the stabilised sample as well as the loss of mass. This testing is conducted to determine how treated specimens perform against the aggressive environment which replicates real pavement issues. Strength tests based on the UCS of the wetting and drying samples are also conducted for quantitative analysis of the strength deterioration. Lastly, general pavement analysis is undertaken on pavement stabilised by these additives in terms of performance evaluation using Finite Element Modelling to assess the feasibility of implementing this sort of stabilisation methods.

6.2. Experimental Methodology

A series of experiments and numerical analyses were conducted under a 3-stage test program to assess the durability of the stabilised samples. The research questions aimed to be addressed in this chapter include the following:

- Can the efficacy of enzyme-based stabilisation be enhanced by combining with other non-traditional additives?
- What is the time-dependent effect of enzyme-based soil stabilisation?

- How durable is enzyme-based soil stabilisation?

The durability of the materials has been tested in various forms. Firstly, durability tests on the compacted samples were conducted as secondary strength tests to what was reported in Section 4.4.4.1 (Chapter 4) of the thesis, i.e., the post-CBR and UCS tested specimens from Section 4.4.4.1 (Chapter 4) were used for durability analysis in the first stage of the chapter. These samples were demoulded, disturbed, hand mixed and assessed for moisture content before recompacting. On recompaction, new CBR tests were conducted after a 7-day curing period. The tested sample was demoulded, disturbed, remixed and recompacted and tested for 14 days. The same methodology was followed to attain the 28-day recompaction results. Secondly, a novel experimental design was utilised to assess the durability of the stabilised samples based on a few modifications on a soil testing standard through exposing newly stabilised samples to wetting and drying environments which replicate real pavement conditions. Lastly, the general pavement assessment was conducted to determine the benefits of stabilising pavement subgrades using this method in terms of performance (loading). This section of the chapter provides a detailed explanation of the methodology, along with the rationale for the test procedure adopted in this work.

6.2.1. Materials Used

Same soil has been used for durability assessment similar to the soil used in previous chapters. As explained in the previous chapters, the soil has been refined to particles passing 2.36 mm sieve to attain consistency and increase the reactivity of soil. Eko-soil is used at DMR, ranging from 1:100 to 1:900 and AMR ranging from 1% to 7%.

Fly ash contents used in this study are 0% (control) and 15% for the treated cases which were proven to be effective as pointed out in Section 5.3.1.2 (Chapter 5). The use of lime will also be limited to 2% based on the results from Section 5.3.3 (Chapter 5), which highlights the selected amount as required quantity to increase the pH of the soil to promote the pozzolanic reactions. Sample preparation methodology is as presented in Section 6.2.2 covering recompaction tests (Section 6.2.2.1) and for modified wetting and drying cyclical tests (Section 6.2.2.2).

6.2.2. Sample Preparation

6.2.2.1. Recompacted samples

The oven dried soil was used to prepare both UCS and CBR samples, as reported in Section 4.3.2 (Chapter 4). The moisture content of the soil was determined using an OHAUS moisture analyser which helps identify universal moisture content of tested substance. The data was used to determine the geotechnical moisture content of the soil. To prepare both these CBR and UCS samples, water was added to the soil to OMC – AMR + 2% and allowed to reach equilibrium in a sealed container for a minimum of 16 hours with the extra 2% added to account for the unavoidable moisture loss during the sample preparation process. Sample calculations on determining dry and liquid contents for the soil is shown in the preceding chapters in Section 4.2.3 (Chapter 4) and Section 5.2.2 (Chapter 5). The CBR samples were prepared at control, 1:100 7%, 1:500 7% and 1:900 7% whereas the UCS was prepared at control, 1:100 7% and 1:500 7% where the samples were all left to equilibrate for at least an hour. The high AMR was to ensure more enzyme was available to neutralise clay particles quicker and easily as opposed to lower AMR. All these stabilised samples were tested in duplicates after four days of curing in a sealed plastic bag in a temperature control room. The results have been reported in Section 4.3.4.2 (Chapter 4). In stage 1 of this analysis, the tested CBR samples were extruded from moulds and disturbed. The soil specimens were mixed well with the hand as well as mechanical means to ensure visual consistency. All the disturbed samples were then compacted again, making note of a noticeably lesser sample than the previous samples and tested yet again after a 7-day curing period following same curing process. This set of data was identified as the first set of recompaction dataset. Similarly, the samples were disturbed yet again and recompacted and tested after a 14-day curing period and 28-day curing period. Moisture content was monitored at every stage of this testing. The same procedure was followed on the UCS samples. Testing parameters for the mechanical testing have been reported in Section 6.2.3 of this chapter.

6.2.2.2. Modified Wetting-Drying Testing

This phase of the investigation was conducted to explore the durability of stabilised samples compared to the control soil. A total of six forms of soil were investigated in duplicates. The first three types are control soil, soil + fly ash, soil + fly ash + lime. The last three types are the combination of the first three types with the enzyme, i.e., soil + enzyme, soil + fly ash + enzyme, soil + fly ash + lime + enzyme. Modified wetting – drying cyclic test was adopted for this stage of testing. As reported in the earlier section of this chapter (Section 6.1), wetting and drying cyclic test is a very aggressive testing method with the literature and available standard recommending the test for cement-treated soil specimens. Although this form of testing is considered as an aggressive testing method, the testing does replicate extreme harsh conditions that pavement is subjected to during its lifecycle. For this reason, efforts were made to modify the testing to produce analytical results. For this testing, all six types of samples were prepared by premoistening soil to OMC – AMR + 2% and allowed to reach equilibrium prior to the addition of the stabilisers. In the cases involving fly ash and lime, the soil was mixed with this admixture before the pre-moistening process, ensuring the sample was mixed well mechanically and by hand to make it look visually uniform and consistent. After all the desired mix design samples were prepared, they were compacted to UCS specimens. These specimens after 4-day curing were recorded of its mass and volume before the testing process. The testing process was derived from ASTM D559 (2003). As mentioned earlier, this standard refers to the wetting and drying cycle for compacted soil-cement mixtures. Cement gives binding properties to the soil, which helps the sample hold together. However, because the tested specimens in this research do not contain a cement binder, these UCS specimens do not have any form of confinement. Section 6.3.2 of this chapter presents the preliminary tests conducted to select the sample preparation methods utilised for the durability test which explains the need for a form of confinement for the compacted sample subjected to wetting and drying. A rubber membrane was used to allow for the confinement of this sample. After measuring the mass and the volume of the compacted samples, the sample confined by this membrane is placed on a porous disk and submerged in water for an hour before drying in the oven at 70 ℃ for 23 hours completing a cycle of 24 hours. This is performed to simulate a pavement which is susceptible to the rise and fall of the groundwater level as well as surface wetting and drying from the environmental conditions. The brushing of the specimen with a steel wire brush was not conducted during the testing due to the difficulty in doing so because of the rubber membrane. It was deemed that the testing procedure without the inclusion of wire brushing was sufficient to attain an indicative effect of resistance by the treatment. Six cycles were conducted for this test monitoring the mass and volume changes at different sections of the cycle. UCS test of the sample was conducted at the end of the 6^{th} cycle to assess the strength. All six types tested for this phase was conducted in duplicates.

6.2.3. Testing

The mechanical testing in this research has been conducted at least in duplicates or in some cases, triplicates. All the samples were prepared to the OMC and MDD, which was achieved during the characterisation phases as recorded in the previous chapters. The specimens were compacted using standard compaction method. Samples that were not 90% of the optimum density or more than a maximum of 2% allowance to either side of the OMC were recast within the set boundaries for uniformity and repeatability in testing. The mechanical testing was conducted using Shimadzu 50 kN universal tester using a strain rate of 1 mm/min. The CBR and UCS testing were conducted in accordance with Australian Standards (AS5101.4 2008, AS1289.6.1.1 2014). Summary of the tests conducted and the timeline of testing in Stage 1 is shown in Table 6.1 And Fig 6.1. In the case of the durability testing, the compacted UCS samples were covered with rubber membrane and weighed prior to the wetting cycle.

The schematic diagram showing the procedure is shown in Fig 6.2. The volume of the sample was identified using Archimedes principle. For this, a special water tank of known volume (240 mm x 240 mm base) was prepared using acrylic laser cut and glued together, ensuring no leakage (Fig 6.3a). After filling the tank to a reference height of the water, the specimen was lowered into the tank, ensuring a complete immersion of the sample. After the sample has rested and the water seemed to have come to a still, the rise in water level was recorded, which was then used to calculate the volume of the specimen. This specimen was transferred to a water tub and submerged. The water tub had a porous base plate with designated spots for the specimens to be placed. The porous disks allowed the water to flow into the sample from below as well. The samples were submerged for an hour, and at the end of this one-hour wetting period, the sample was taken out and tilted on its side to allow for loss of free and loose materials on the top and bottom of the specimen. The sample weight was recorded prior to the drying process. This same method was repeated for another five cycles. At the end of the drying process in cycle 6, the sample was taken out of the rubber membrane and tested for the UCS strength. The testing summary and the order of the experimental testing condition has been presented in Fig 6.2 and Table 6.2.

Table 6.1. Summary of lab test and conditions in Stage 1

Test Name	DMR & AMR	No of Tests (including duplicates)
CBR	Control	2
	1:100 7%	2
	1:500 7%	2
	1:900 7%	2
UCS	Control	2
	1:100 7%	2
	1:500 7%	2

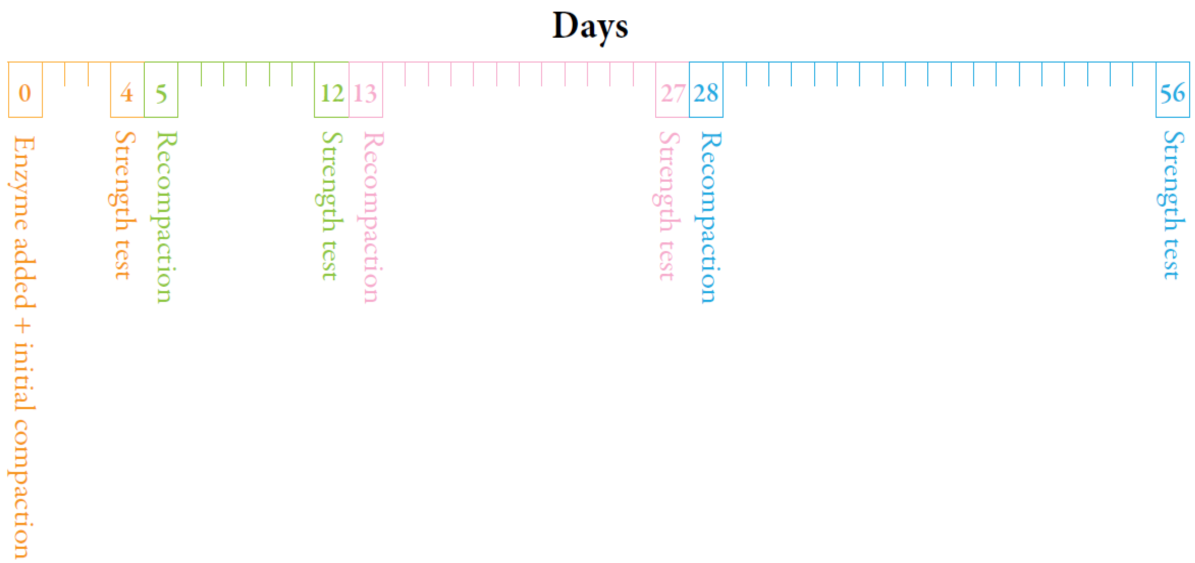


Figure 6.1. Stage 1 testing timeline

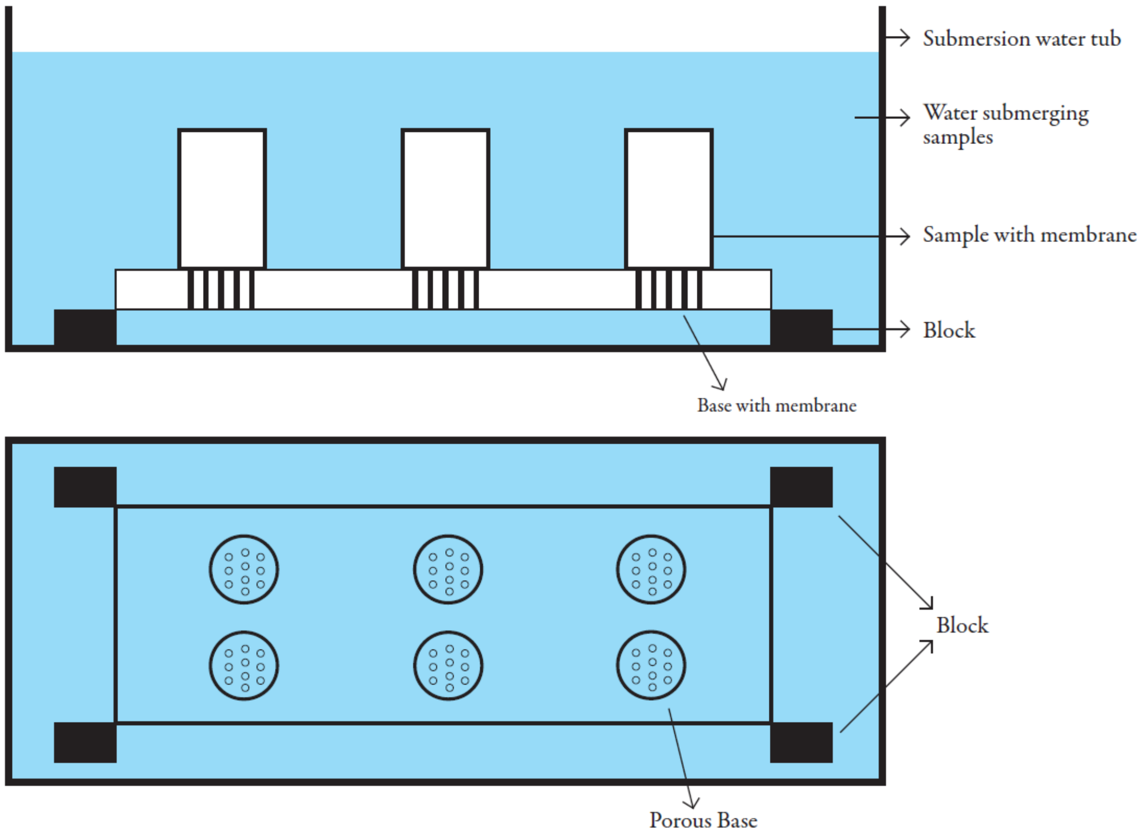
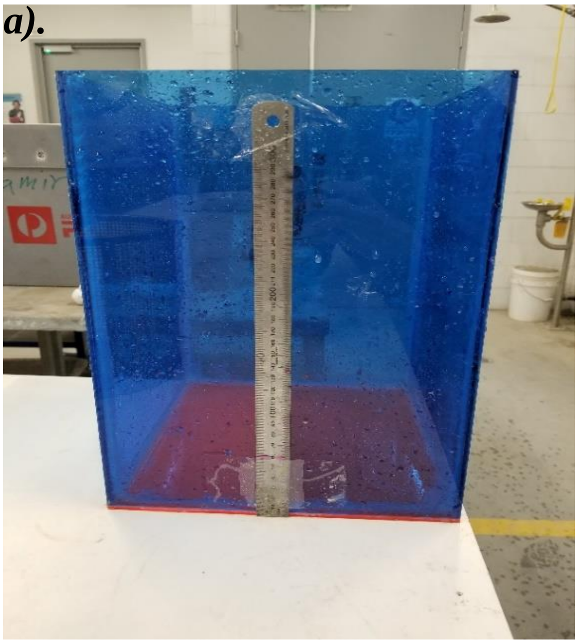


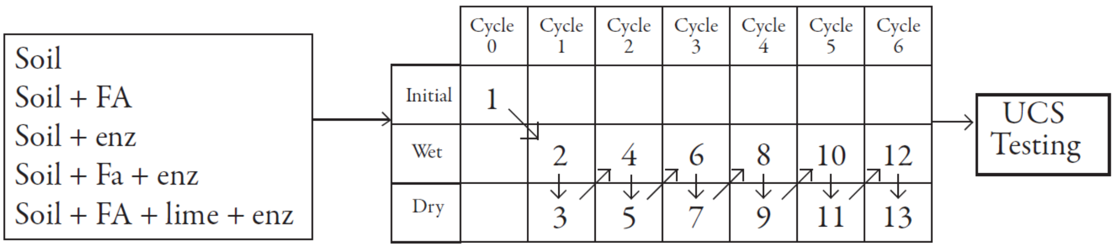
Figure 6.2. Modified wetting - drying cyclic test schematic diagram (Stage 2)



c).

Figure 6.3. a) Water tank (240 mm x 240 mm base) used for volume calculation; b) Porous base plate with designated spots for sample placement; c) Test setup

Table 6.2. Order of experimental testing condition in Stage 2



6.3. Modelling Methodology

Finite element analysis was conducted in this study to assess the performance of the pavement subjected to dynamic loading. Validation of the numerical model was conducted using experimental results and test data reported from the literature. Model parameters used for the modelling included the results obtained from CBR testing as well as the shear parameters obtained from Direct shear test. Direct shear tests in the study were conducted on the basis of Australian standards (AS1289.6.2.2 1998). Shear tests conducted was based on the methodology followed by Pooni et al. (2020). The shearing rate used for the analysis was 0.025 mm/min until the specimens reached the maximum allowable horizontal displacement. Normal stress applied during the testing included 50, 100, 150 and 200 kPa to reflect typical pavement stresses (Jouve and Guezouli 1993). Results of the shear parameters as well as the pavement geometry, is described in detail under Section 6.3.1. It should be noted that the scope covered in this section of the study is not to develop a model that will effectively predict pavement response, instead to utilise an effective model from the literature to compare the performance of control and stabilised pavement.

Performance modelling was conducted on ABAQUS/CAE, where a 3D model was used to simulate the pavement subjected to loading. As mentioned earlier, the model used for simulating the application of subgrade stabilisation was verified using model approach reported in the literature. The model, as reported in Pooni et al. (2020), was developed to predict the performance of unsealed pavements constructed on stabilised fine-grained soil subgrade subjected to loading and moisture cycles. The analysis was conducted using a 3D model to simulate the stress and deflection of pavements upon traffic loading. In all analyses, the pavement subgrade depth was kept constant, and the initial density is considered as the maximum dry density across the model thickness.

6.3.1. Parameters Used for Pavement Design.

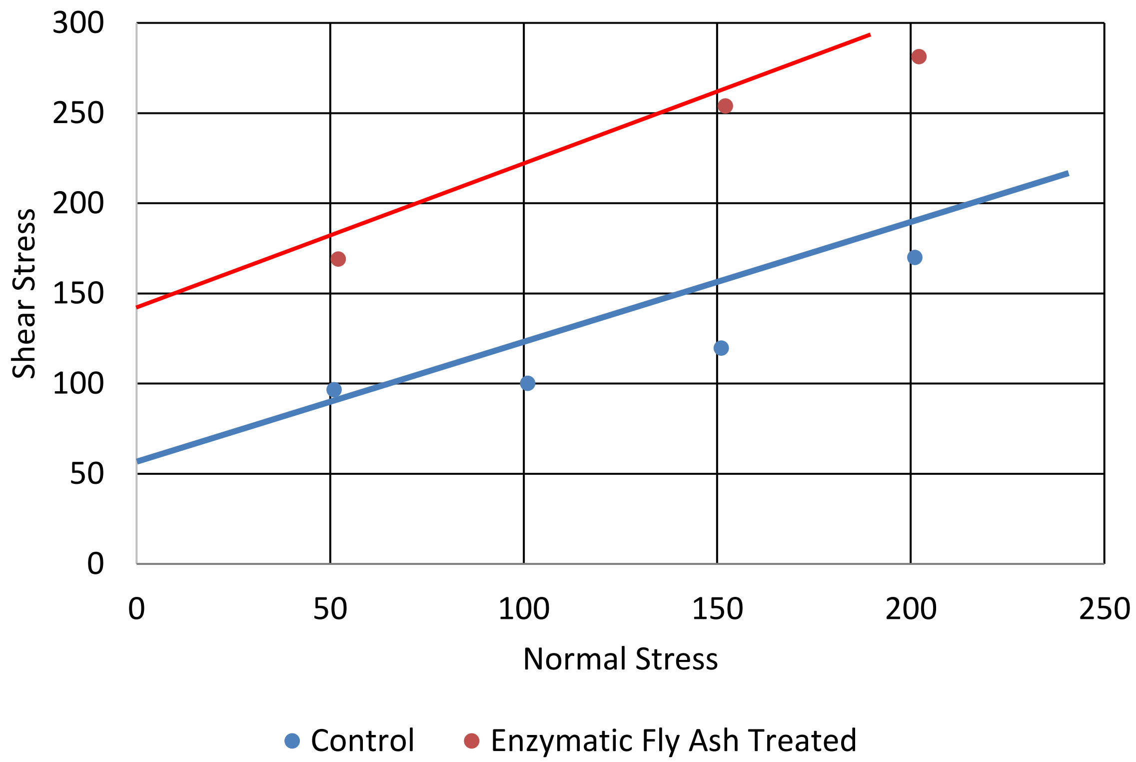


Figure 6.4. Effective shear strength parameters attained from direct shear test

Table 6.3. Effective shear strength parameters attained from direct shear test

	Effective Friction Angle	Effective Cohesion (kPa)	Dilation Angle
Control	25.59	27	0.69
Enzymatic fly ash stabilised	36.80	128.8	7.35

Fig 6.4 and Table 6.3 shows the effective shear strength parameters determined using direct shear tests conducted within the study. In this part of the study, the control soil is compared with soil treated with enzymatic fly ash and lime admixture. The additive

contents used are enzyme (1:100 1%), fly ash (15%) and lime (2%). As seen from the results, the cohesion of the soil is significantly increased with the admixture treatment. Friction angle is also seen to increase from 25.59 to 36.80. The improvement in the shear parameters can be translated to the general strength improvement reported in the preceding chapter (Section 5.3.3). While the pozzolanic reactions occurring within the soil could be speculated as to the cause for the increase in the ϕ', it could also be due to the formation of clay aggregates and resistance to slippage from an increased matric suction on the dry side of the compaction curve (Cokca et al. 2004). Cokca et al. (2004) also highlight the increase of C' from the increase of granular features by the treatment. Other parameters used for numerical modelling include modulus values of the control and treated soil samples. The resilient modulus for the specimens was derived from the CBR values reported in Section 5.3.3 of the preceding chapter. It should also be noted that the design approach followed for the numerical modelling was based on the CIRCLY design parameters reported in Section 5.4.1 (Chapter 5). Table 6.4 presents the modulus parameters utilised for the study. The conversion into the resilient modulus is based on Heukelom and Klomp (1962) in which the resilient modulus of the specimen is ten times the soaked CBR. This method is used in current practices as a simplified and time-efficient alternative to laboratory resilient modulus testing. Wearing course material was selected to be Class 2/3 crushed rock with a maximum size of 19 mm with the MDD of 2.24 g/cm^3 (ARRB 2020, AlexFraser 2020). Compaction characteristics of the specimens used for the modelling were based on the results reported in Section 5.3.1.1 (Chapter 5) as shown in Table 6.5. The pavement to be designed is classified as a “4B minor road” with some critical parameters, as shown in Table 6.6. The classification of the road was based on AGPT-06 (AGPT06/09 2009). Table 6.7 provides the thickness of the layers of both the pavement analysed for this study. The permeability of the natural soil was assumed to be $8x10^{-5}$ mm/s.

Table 6.4. Modulus parameters employed for the study

Wearing course material/base layer	200 M_r
Stabilised material	180 M_r
Control material	20 M_r

Table 6.5. Compaction characteristics employed for the study

	OMC (%)	**MDD (g/cm$^{3-)}$**
Control	17	1.79
S+FA+Lime +Enz	17.4	1.695

Table 6.6. Road classification used for the study

Class	**HV**	**HV Growth**	**ESA/HV**	**ESA**
4B Minor (30 – 100 AADT)	10%	2%	0.5%	$13.0*10^3$

Table 6.7. Pavement thickness attained from a CIRCLY approach

Pavement	**Stabiliser used**	**Layer**	**Thickness (mm)**
Control	N/A (control soil)	Wearing course	500
		Subgrade	Infinite

Treated	Fa + E2 + Lime	Wearing course	225
		Stabilised subgrade	250
		Subgrade	Infinite

6.3.2. Cyclic loading model

As highlighted in the introduction of this chapter, it is important that the performance-based analysis needs to be conducted based on approaches that account for the pavement layers which are subjected to dynamic loading rather than static loading to provide a more realistic representation of the pavement response especially in terms of pavement rutting. For this reason, the numerical modelling conducted in this study investigates the effect of cyclic 80 kN Standard Axle Dual Tyre (SADT) load on the pavement. The model approach followed by Pooni et al. (2020) has been validated in the literature based on comparisons of compressive stresses and strain as well as surface deflections from Ghadimi et al. (2016) and Bodhinayake (2008), respectively. The model is subjected to cyclic loading in the form of a triangular compressive pulse with an amplitude of 750 kPa at a loading and unloading time of 0.25 s, as shown in Fig 6.5. The base and subgrade are modelled with unbound granular particle properties emulating nonlinear elastoplastic material using the Mohr-Coulomb model. Based on the verification reported in Pooni et al. (2020), this modelling approach is capable of simulating realistic loading effects from moving traffic. The similarity in the soil type between the soil tested within this research and Pooni et al. (2020), warrants the use of this triangular compressive pulse cyclical loading.

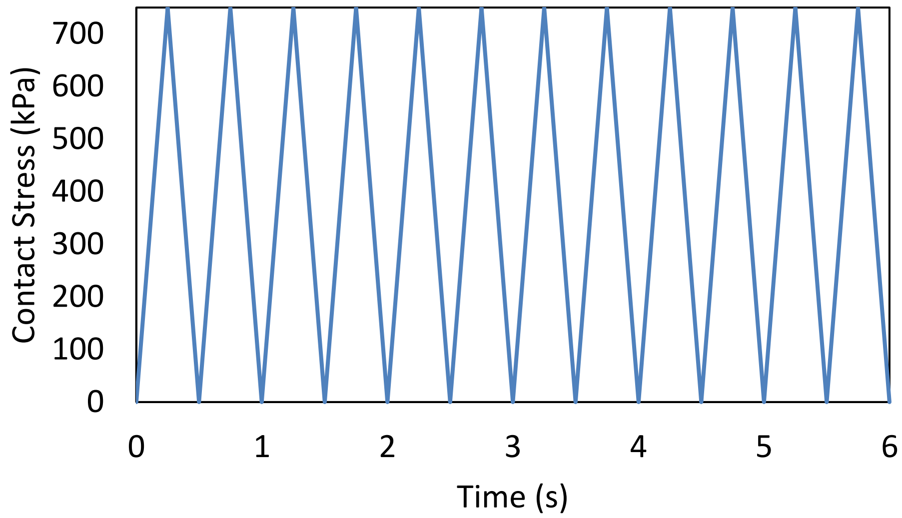


Figure 6.5. Loading cycle applied for dynamic loading within the study.

6.3.3. Pavement Geometry and Numerical Simulation

Model pavement designed in this study is a rural class road with a subgrade CBR of 3. The design consists of two lanes with a total width of 6.2 m, 2:1 batter slopes and 5 m subgrade depth. The simulation is conducted on half the model to facilitate quicker but effective computational time with two design approaches employed to compare the pavement response. The base material thickness was based on the CIRCLY approach. In design approach 1, the base thickness of 500 mm is provided over the in-situ soil subgrade whereas design approach 2, the base material thickness is reduced considerably to a thickness of 225 mm after stabilising the in-situ subgrade to a depth of 250 mm. As mentioned earlier, the additives employed for stabilisation of the subgrade are enzymatic fly ash with lime. Fig 6.6 shows the schematic representation of the pavement geometry. An eight-node brick reduced integration elements (C3D8R) and an eight-node brick trilinear displacement and pore pressure elements (C3D8P) were used to represent base material and control and stabilised subgrade materials, respectively. To eliminate the effect of boundary conditions, the soil boundaries were assumed to be smooth and located far from the traffic loads. The mesh is coarsely distributed towards the boundaries whereas finely distributed near the tyre loadings. The loading is in the form of contact pressure with an equivalent circular area with a load of 80 kN from SADT configuration. Fig 6.7 shows the mesh distribution of the 3D model.

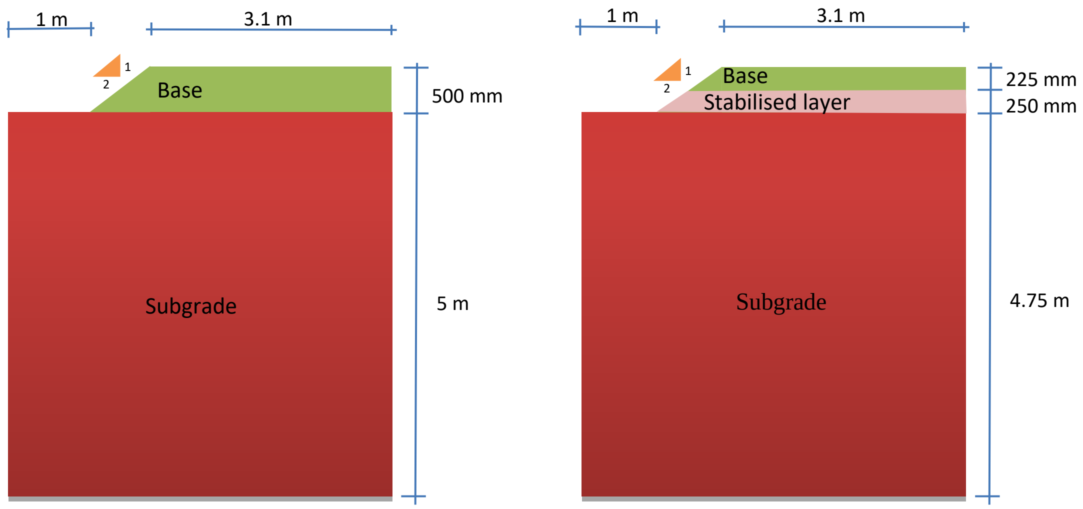


Figure 6.6. Schematic representation of untreated vs treated pavement

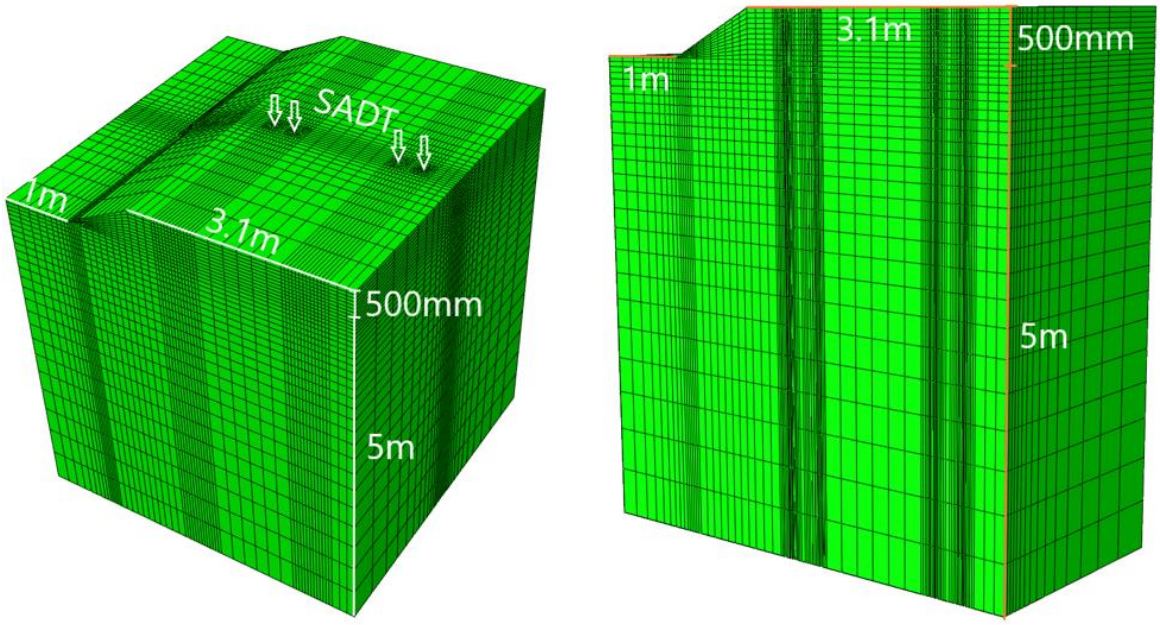


Figure 6.7. Mesh distribution of 3D model

6.4. Results and Discussion

The results of the experimental and modelling approach are presented in a 3-stage investigation. The results of the effect of time and re-compaction on soil strength are firstly presented. Durability assessment based on the novel wetting and drying cycles is then presented. Lastly, the results from the general pavement assessment using the numerical modelling approach are presented.

6.4.1. Recompacted Samples

The treatment of soil enzyme has been shown to improve the strength of the soil through densification by decreasing the affinity for water, as reported in Section 4.3.4.1 (Chapter 4). This form of testing was conducted to see whether this reduction in the affinity for water is affected by the disturbance as well as time. As seen in Fig 6.8, there is no decrease in CBR strength with the recompaction and time effects. It can be seen clearly that the effect of the enzyme treatment remains even months after treatment. It should be noted that by the end of the experimental phase, it had been at least 56 days since the enzyme was added to the soil. It can be seen that the soil had retained the strength attained from the treatment. This behaviour is commonly seen in all the treated DMR/AMR combinations showing that enzyme-based treatment could hold long term benefits in retaining the strength bearing capacity after treatment. This could also mean that enzyme treatment of lesser dilutions can attain strength closer to that of higher concentration with time. For example, the 1:900 treated samples achieved similar strength of around 30% CBR with 1:100 and 1:500 treated samples with time. The 1:900 treated sample took between 14 to 28 days to gain the strength of the higher concentrated samples. Another noteworthy observation is the improvement in the strength of the untreated samples with time and recompaction as well. This could be due to the drying out of the samples and a larger number of compactions helping it attain closer compaction with every compaction cycle. It could also be due to the increased density of the tested sample. As mentioned before, with every re-compaction, around 100 g of soil would be lost to compute the moisture content. Despite this factor, it should be noted that with the case of the treated samples, higher strength improvements compared to the control samples could be seen with greater compaction and time. It could be understood that the enzyme treatment has decreased the affinity of the water and still retained its strength value with time.

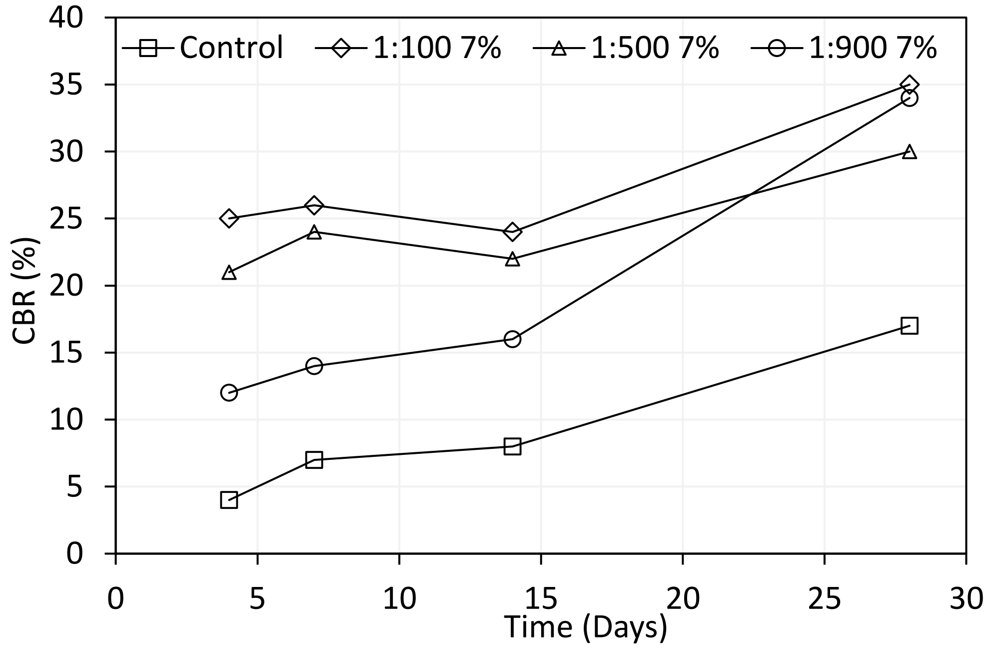


Figure 6.8. Time and recompaction effect on CBR

UCS testing of 1:900 specimens was not conducted as it was seen from the CBR testing that the 1:900 would behave similarly to the other DMR/AMR combination with time. As seen from the UCS tests in Fig 6.9, the UCS strength also improves with increased time and recompaction cycles. Once again, the increase in strength could be due to the increase in density of the sample with lesser of the soil available for compaction with every compaction cycle. From the UCS testing of the recompacted specimens, it can be seen that the untreated samples attain strength as high as the treated samples. This would suggest that the drying of the sample could play a major part in the strength improvement of UCS samples. As seen, this drying effect could only be seen in UCS tested samples which compromise the understanding of effectiveness on the durability of enzyme treated subgrades. It could even suggest that this testing method is not very effective in understanding the effective for the study of pavement designs. CBR is, however, a more reliable technique to assess the efficacy of enzyme with time as it provides confinement to the tested sample emulating typical pavement characteristics. From these results, however, it can be hypothesised that the reduction in the affinity for water may not be affected due to disturbance or time.

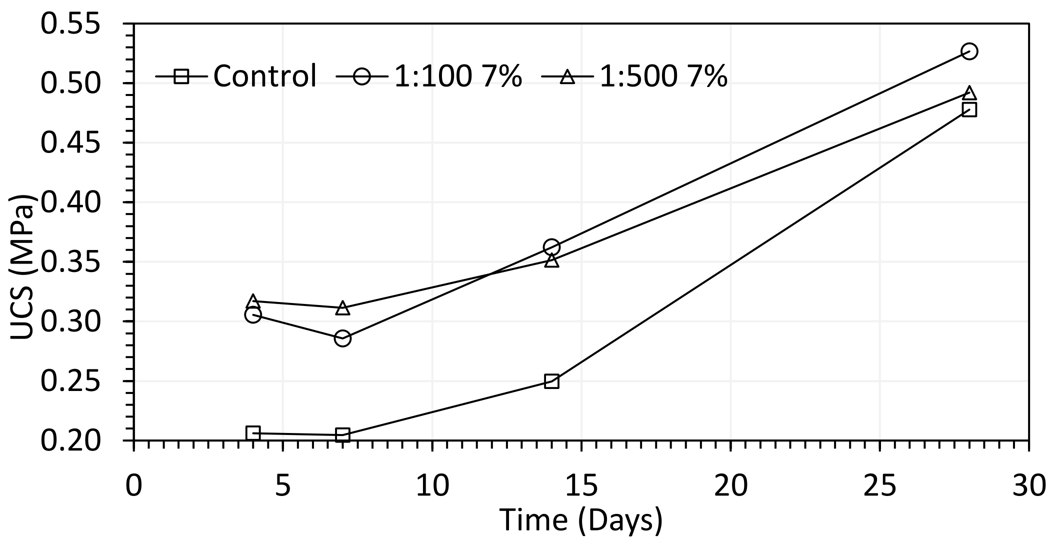


Figure 6.9. Time and recompaction effect on UCS

6.4.2. Modified Wetting-Drying Testing

As explained in the experimental procedure, the wetting and drying cycle was a modified version of ASTM D559, which is a testing standard to assess the durability of soils treated with cement. As part of this durability assessment, six treatment options have been compared against each other which are, control (untreated), fly ash alone treated, fly ash + lime treated, enzyme alone treated, enzyme + fly ash treated, and enzyme + fly ash + lime treated. However, due to the omission of a cement binder in the treatment plan, a rubber membrane was used. However, tests were also conducted on UCS specimens without a rubber membrane to see how they fare through this harsh test. As shown below in Fig 6.10, the samples, when immersed in water within the first 10 minutes, was seen to have collapsed due to the lack of confinement to the sample. This shows the aggressive nature of the test as well as gives an insight into how much addition of cement could affect the sample. However, because cement is not being tested here, modifications were made to the testing procedure by providing much-needed confinement to produce quantifiable results. This could be in the form of a membrane. It can be seen (Fig 6.10) that the failure in the samples occurs in the form of washing of the soil particles from the side. A rubber membrane has been introduced into the testing design to limit the lateral washing of the soil particles. The flow of the water within the sample is limited to move from the top of the sample to the bottom of the sample, i.e., the exposed areas of the samples. It is to be noted that these specimens were placed on a porous acrylic plate (Fig 6.3b) in order to allow the flow of water. The use of this method is to simulate the flow of water within a pavement. This approach for modified wetting and drying testing of fine-grained soil has also been proven effective in Pooni et al. (2019).

Figure 6.10. a). UCS samples without membrane before wetting cycle 1; b). UCS samples without membrane after 10 minutes of immersion in cycle 1

ASTM D-599 (2003) specifies the duration of the wetting cycles of 5 hours. The current experimental methodology utilises a preliminary test on sample (with membranes) immersion for 1 hour, 5 hours and 24 hours, as shown in Fig 6.11. Modifications to the duration of wetting cycles can also be seen utilised in various literature (Guney et al. 2007, Kalkan 2011, Aldaood et al. 2014, Pooni et al. 2019). However, the soaking hour of 1 hour was adopted in this study as it was believed to have given measurable changes in volume and mass within this selected time. A short soaking time could mean a quicker change in environmental cycles which could be added to the aggressive nature of this form of testing. The selection of the wetting and drying cyclic duration is to simulate the trend found in the typical climate report for the Greater Melbourne region which shows a warmer and drier spell (BOM 2018, 2019, 2020).

Figure 6.11. Samples confined by rubber membrane subjected to immersion

The results for the weight loss at the dry and wet states of the samples are shown from Fig 6.12 – 6.17. The dry state refers to the mass of the sample at the end of one drying cycle, whereas the wet state refers to the mass at the end of a wetting cycle. The results are based on the average weight loss of the duplicates of all the types tested in this stage. As mentioned earlier, the mass loss was compared between the sample after the drying cycle as well as the wetting cycle. Please note, "S" refers to the control soil; combinations with fly ash, lime, and enzyme are denoted by "FA", "L", and "Enz", respectively.

As seen from the results of the dry state mass loss (Fig 6.12 – 6.14), the additives tested have affected the sample to varying degrees. All the samples can be seen losing around 5% of its original mass within exposure to the first cycle. However, the efficacy of each additive was different, as seen in the following cycles. The control samples lose up to 15.5% of the original mass by the end of the 6th cycle whereas the enzyme treated sample report around 14.7% mass loss, as shown in Fig 6.13, which highlights a negligible effect of adding the enzyme to the soil. Although the enzymes were proven effective in strengthening the soil by facilitating densification of the compacted samples (Chapter 4 Section 4.3.4.1) from this testing, it can be seen that enzymes might not be able to provide a binding effect to the soil specimen, especially when subjected to harsh environmental condition. For example, the enzyme treatment facilitates the aggregation of clay particles due to the decrease in double layer water of the clay in the soil stratum, which in turn improves the bearing capacity of the soil. However, when exposed to harsh wetting conditions, the water can make its way in between the aggregated particles rendering them weak. Samples treated with fly ash and enzyme is seen to have the least effect in mass retention. As seen in Fig 6.13, fly ash treated samples lose 20.8% mass whereas the enzyme and fly ash composite loses 24.5% mass which supports the finding that enzyme does not provide binding force to the weak strut, formed by the addition of fly ash. This is understandable as the addition of the fly ash could make the soil more friable, making it prone to be a brittle failure. This would also suggest that the pozzolanic reaction is not fully effective within the four days of treatment, and accelerated strength gain, reported in Section 5.3.2, could be because enzyme helps the reduction of double-layer water around the clay particles which would, in turn, would force a tighter matrix of clay particles. This would also support the enzyme treated case, which shows that the lack of confinement could have an adverse effect on the treatment in terms of strength after this form of testing. However, with the lime treatment, there is no increase in mass loss after the first cycle. The disparities between the enzyme treated fly ash and lime with the control soil, fly, and lime shows a negligible change in mass of the soil. The reaction mechanism, in this case, would be primarily due to the pozzolanic reactions happening within the soil matrix producing the Calcium Silica Hydrate (CSH) and Calcium Alumina Hydrate (CAH) gels spread across the soil matrix.

A similarity in the changing trend of the sample mass can be seen in the wet states to that of the samples in the dry state. However, in the wet state (Fig 6.15 – 6.17), the samples treated with lime showcase lesser mass loss (around 1.5%) at the end of the first cycle. This could suggest that the readily available moisture could facilitate the pozzolanic reaction and for more CAH and CSH gel formations, which in turn help hold the specimen together. At the end of the 6^{th} cycle, fly ash and lime treated sample only lose up to 2.7%. The mass loss for enzymatic fly ash and lime composite is reported to be 3.9%.

The changes in the volume of the samples both in the dry state as well as the wet state were also investigated in the study. As seen in Fig 6.18 – 6.23, the volume of the samples deteriorates considerably with every cycle, which could be from the loss of mass at each cycle. Fly ash treated sample proved to be the least resistant sample to volumetric changes caused by the change of the soil to a more friable state. Lime stabilised cases proved to be the most effective form of stabilisation which could be credited to the pozzolanic reactions that take place within the sample. However, the enzyme treated case, in this instance seems to be ineffective, once again due to the no cementitious formations in the soil and because of densification alone cannot withstand the aggressive nature of the testing.

The investigation into the change in mass and volume of the soil samples shed insight into the efficacy of the stabilisers. Mass and volumetric changes are shown as being used as effective parameters to measure durability. The general trend of enzyme treated soil show similarity with control samples both in terms of mass loss and volumetric change. This would suggest that the enzyme does not provide any durability to the treated soil when exposed to harsh environmental conditions. Based on the densification mechanism of these enzymes, as reported in Section 4.3.4.1 (Chapter 4), the enzymes help decreases the porosity and cause aggregation of available particles. However, it should be noted that despite decreasing clays affinity for water, an excessive quantity of water could still compromise and damage the newly modified clay matrix. With the case of the fly ash treated samples, a higher loss in mass is seen when treated with fly ash. This is understandable as the addition of the fly ash could make the soil more friable, making it prone to be a brittle failure. The accelerated strength gain reported in Section 5.3.2 (Chapter 5) could be because enzyme helps the reduction of double-layer water around the clay particles which would, in turn, force a tighter matrix of clay particles with fly ash. This theory is also supported by the fact that fly ash alone treated samples achieving similar strength of enzyme treated fly ash with time. In the case of the combination of fly ash and lime admixture on the soil, there is a significant reduction in the mass loss recorded. The reaction mechanism, in this case, would be primarily due to the pozzolanic reactions happening within the soil matrix producing the Calcium Silica Hydrate (CSH) and Calcium Alumina Hydrate (CAH) gels spread across the soil matrix. However, once again, with the addition of enzyme to this treatment does not produce any notable difference in the loss of mass suggesting no benefit of the additive in wet conditions. While enzymes might not provide effectiveness in these harsh environments, as shown in Chapter 4 and Chapter 5, they can still be effectively used to strengthen bearing capacity of raw materials as well as decrease the activation energy to facilitate pozzolanic reactions.

UCS tests were conducted on the samples subjected to the wetting and drying cycles. It should be noted that the UCS tested were conducted on samples after removing the rubber membrane to take away the confining force provided by the membrane. It should also be noted that the samples were tested for strength at the end of the drying stage at the end of cycle 6. As seen from the results (Fig 6.24 – 6.25), the treatment has a varying effect on the strength of the soil. The enzyme treated soil is shown to increase the strength by 73% to 0.73 MPa, which could be credited to the reduction of voids in the soil post-treatment. However, with the fly ash treated samples, the strength of the soil reduces drastically to 0.33 MPa, a reduction of 18% of the control soil due to the soil becoming more brittle. This reduction in strength can be decreased with the addition of enzyme to 0.38 MPa (reduction of 4.5%) which could also be due to the introduction of the fly ash into the smaller voids in the soil courtesy of the reduction in the double layer of water. However, with the introduction of lime to this fly ash treated soil has been shown as increasing the strength of the soil by 180% to 1.1 MPa. The addition of enzyme, in this case, could be seen decreasing the strength of the soil to 0.89 MPa, which is still 120% times the control soil strength. However, it should be noted that the UCS strength tests reported here are not comparable with the ones reported in previous chapters (Sections 4.4.4.1, 5.3.1.2, and 5.3.2) as the UCS reported in the previous chapters are conducted on samples which have been cured in room temperatures, and not oven dried. The drying effect would significantly alter the results to show improvements. However, as reported earlier in the chapter, the comparison of UCS tests on the samples which are subjected to the wetting and drying cycle seems to have provided indicative results on the stabilisation effect.

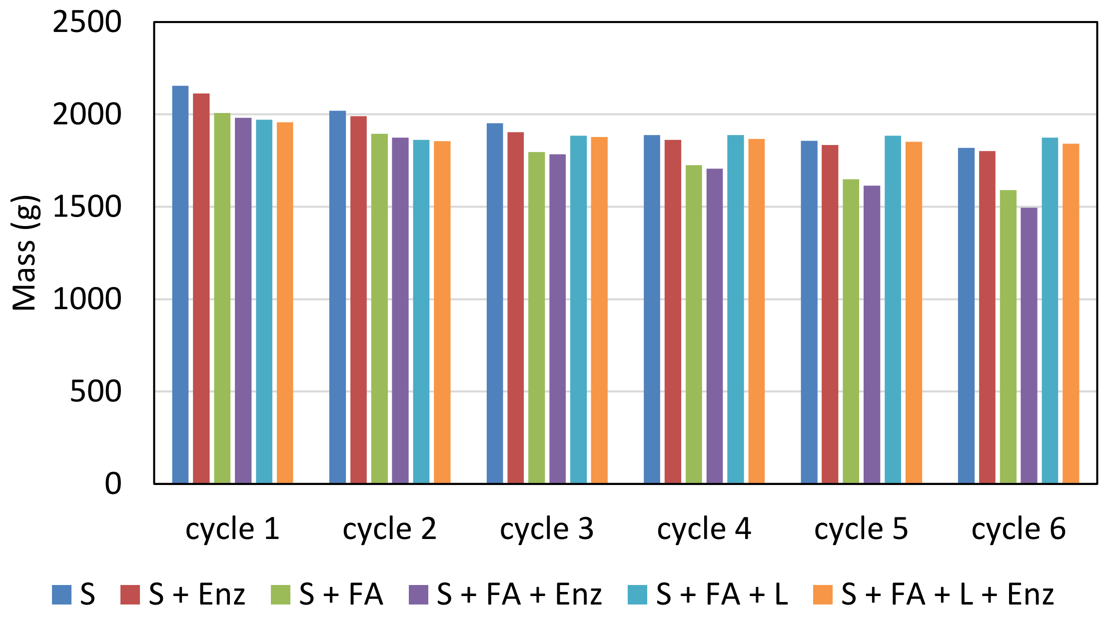


Figure 6.12. Dry state mass loss of samples

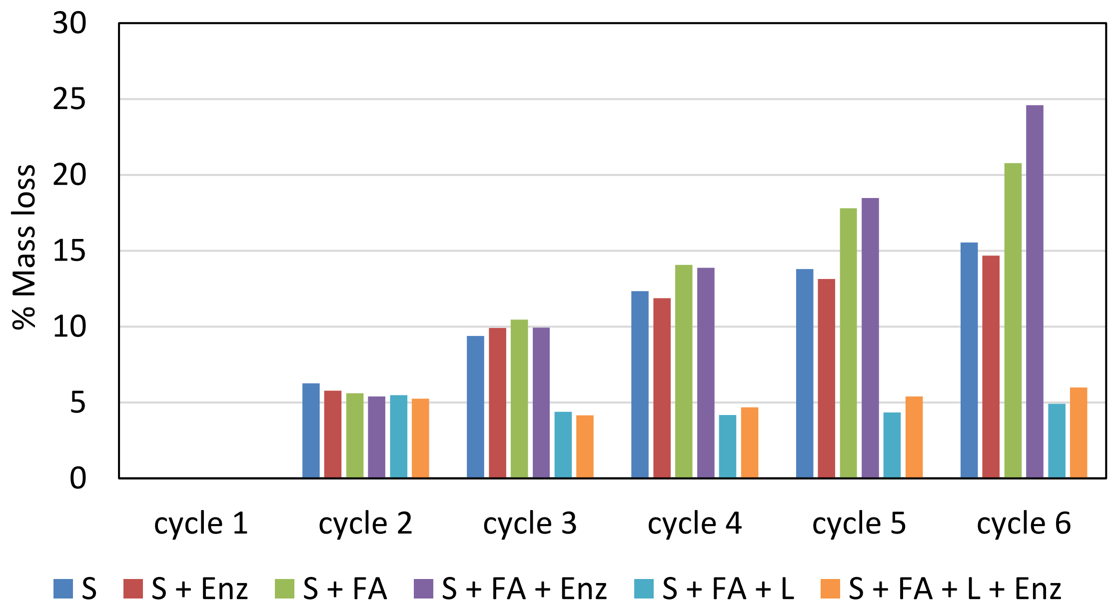


Figure 6.13. Dry state % mass loss

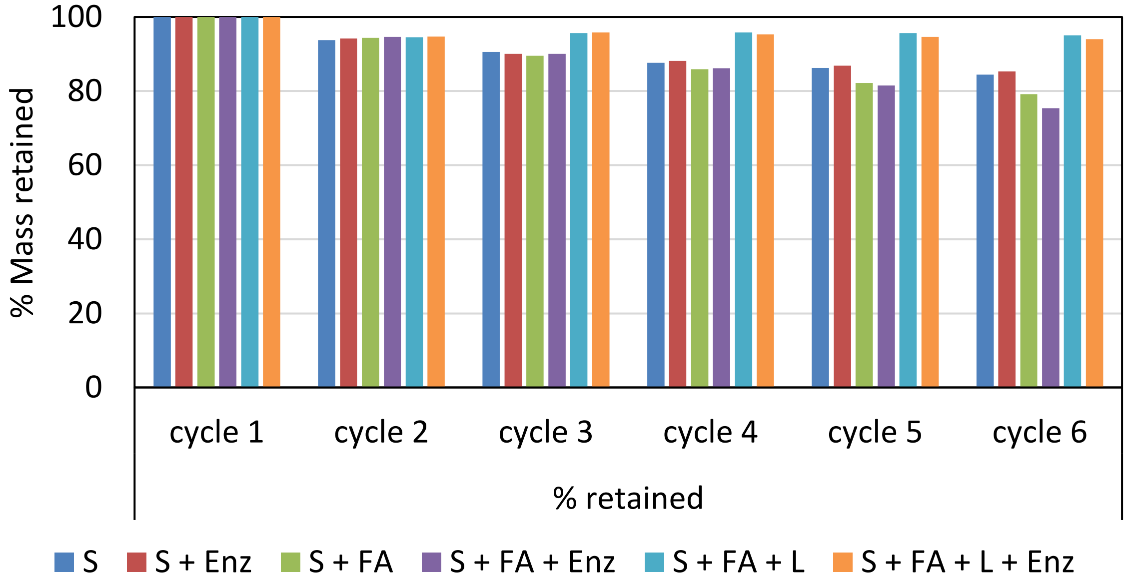


Figure 6.14. Dry state % mass retained

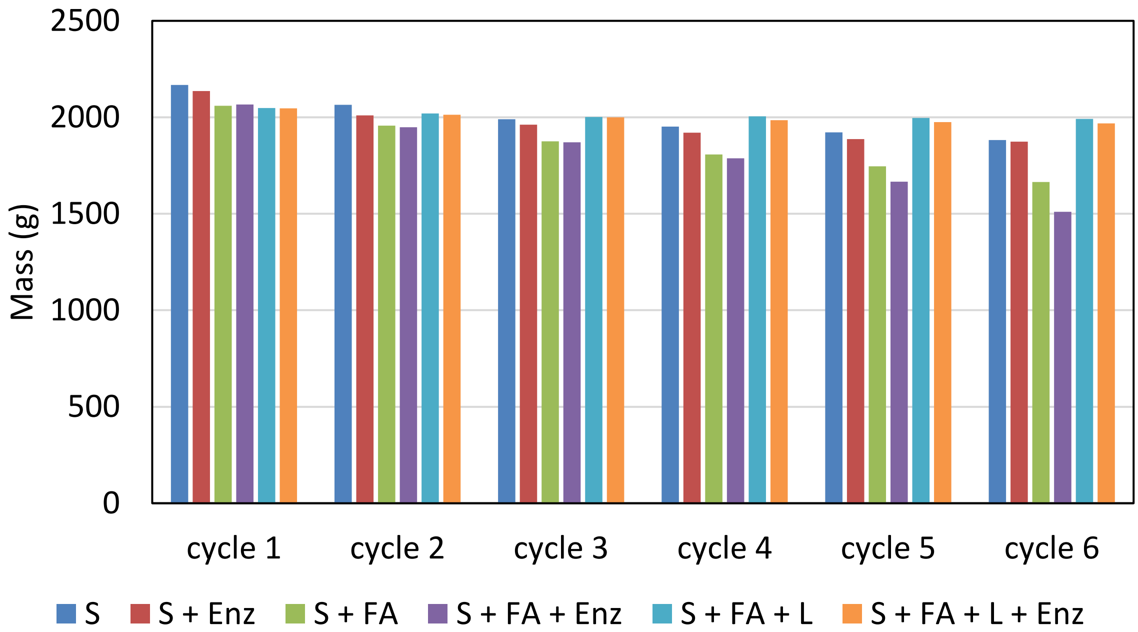


Figure 6.15. Wet state mass loss of samples

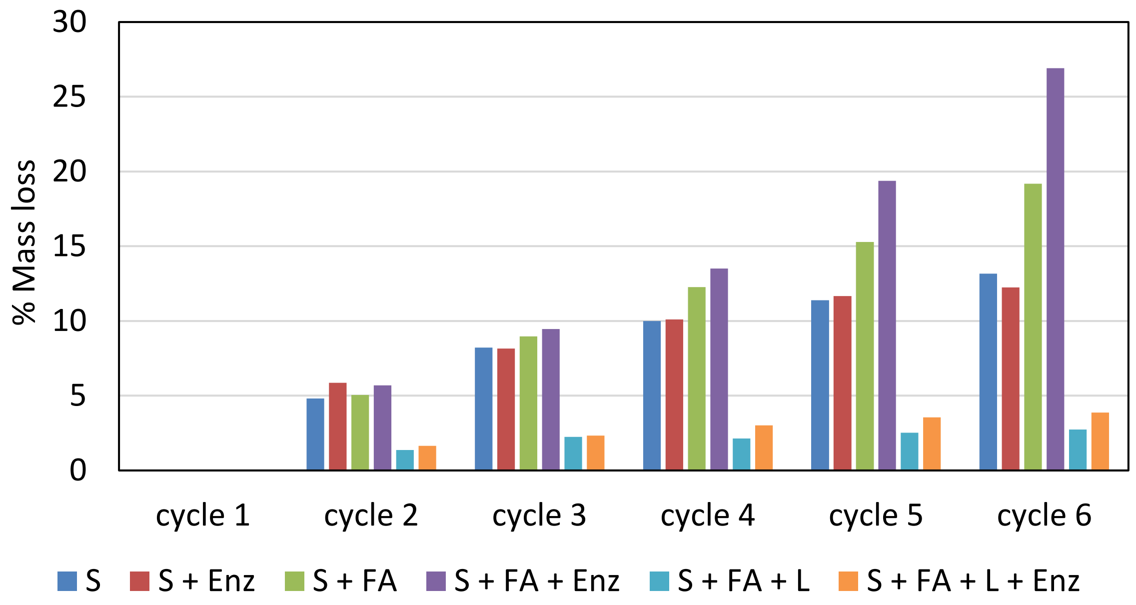


Figure 6.16. Wet state % mass loss

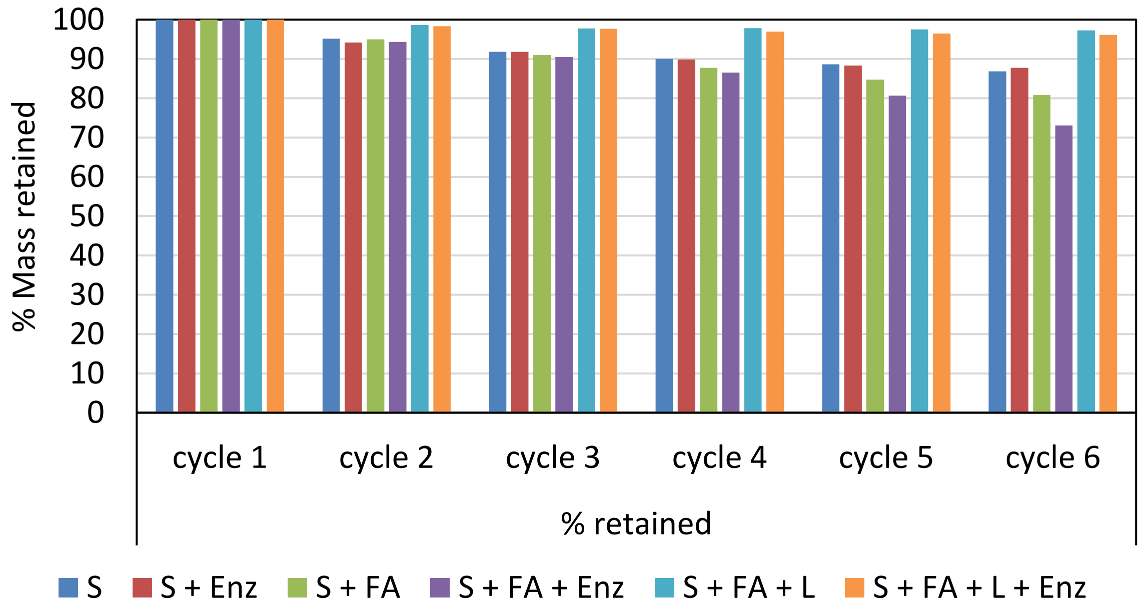


Figure 6.17. Wet state % mass retained

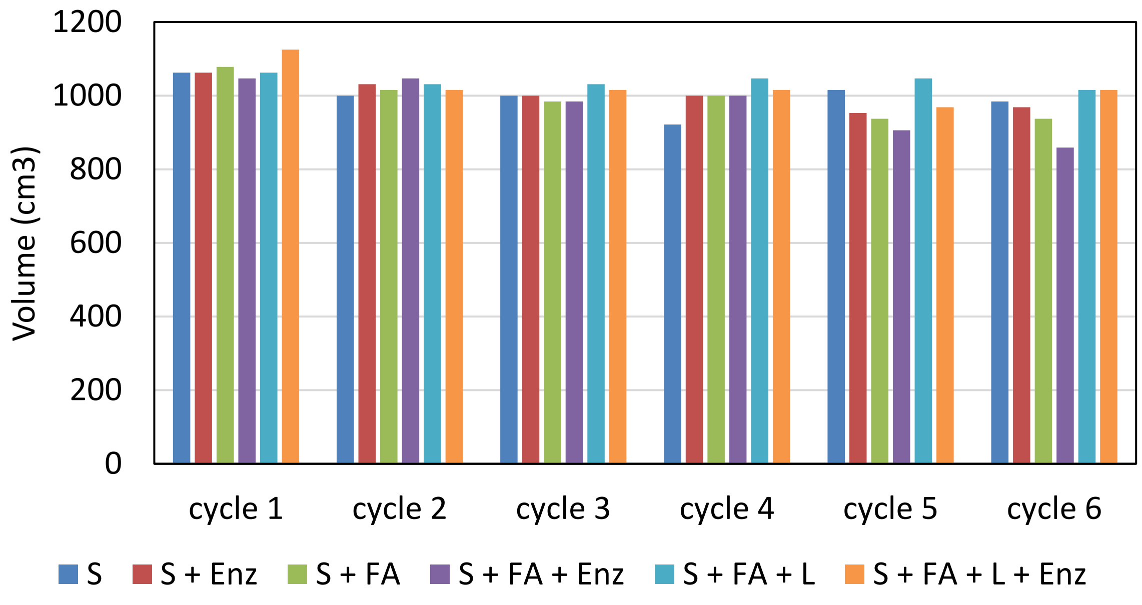


Figure 6.18. Dry state volume change

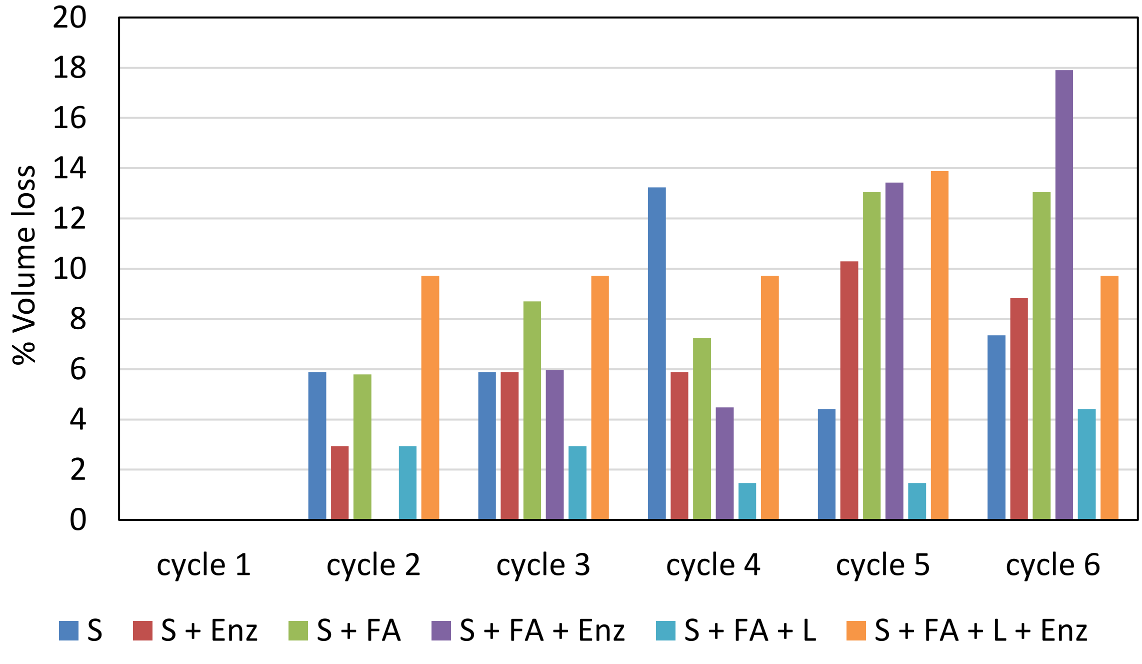


Figure 6.19. Dry state % volume loss

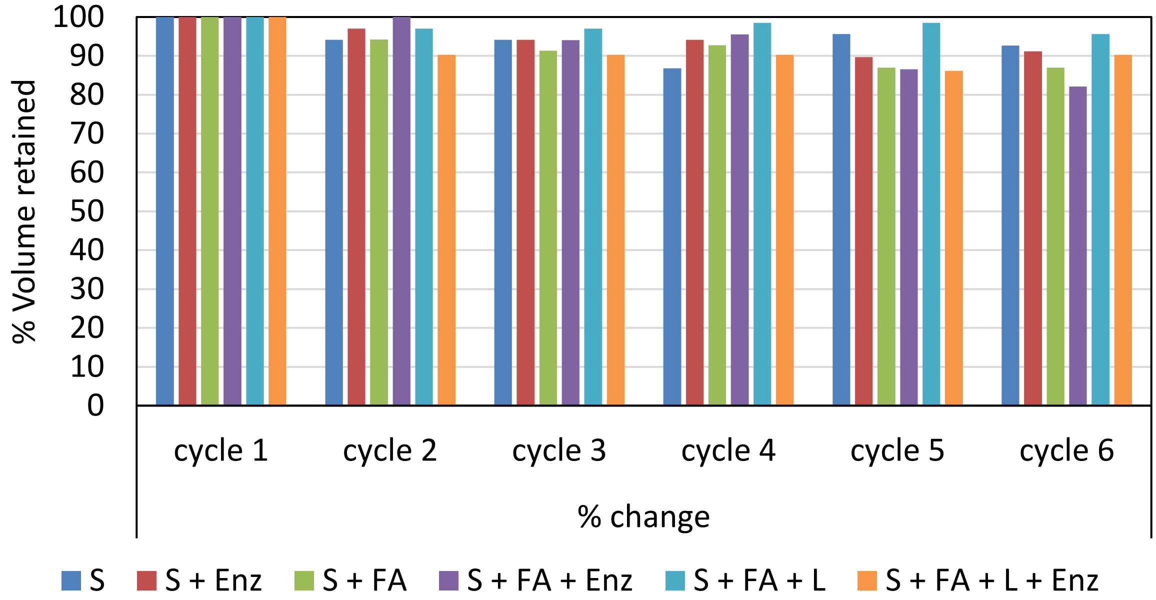


Figure 6.20. Dry state % volume retained

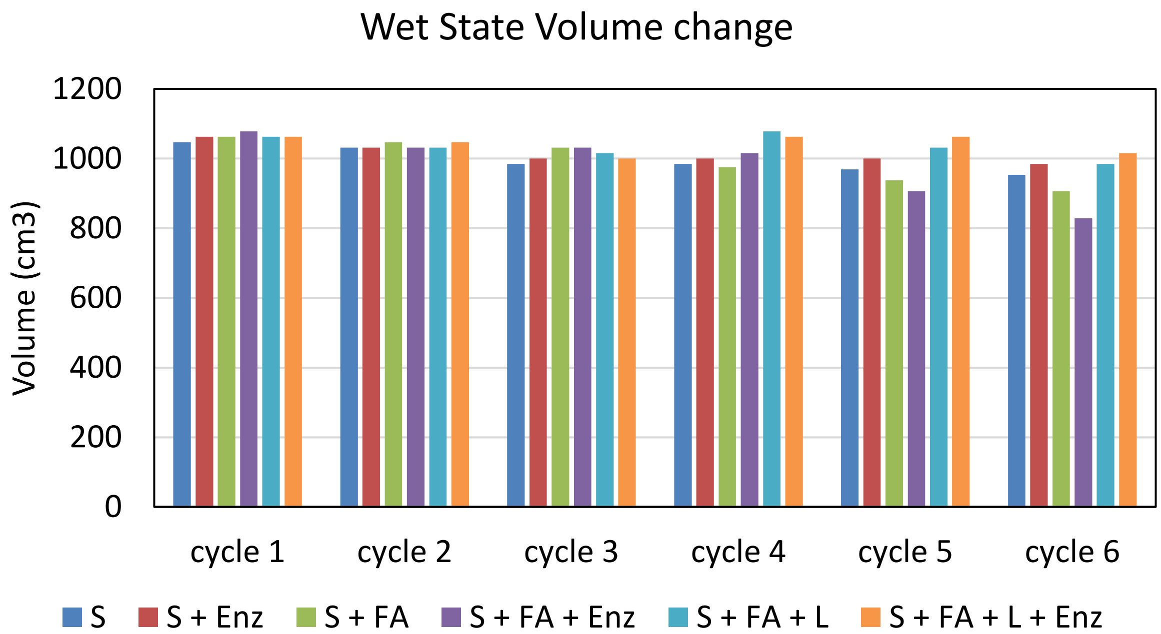


Figure 6.21. Wet state volume change

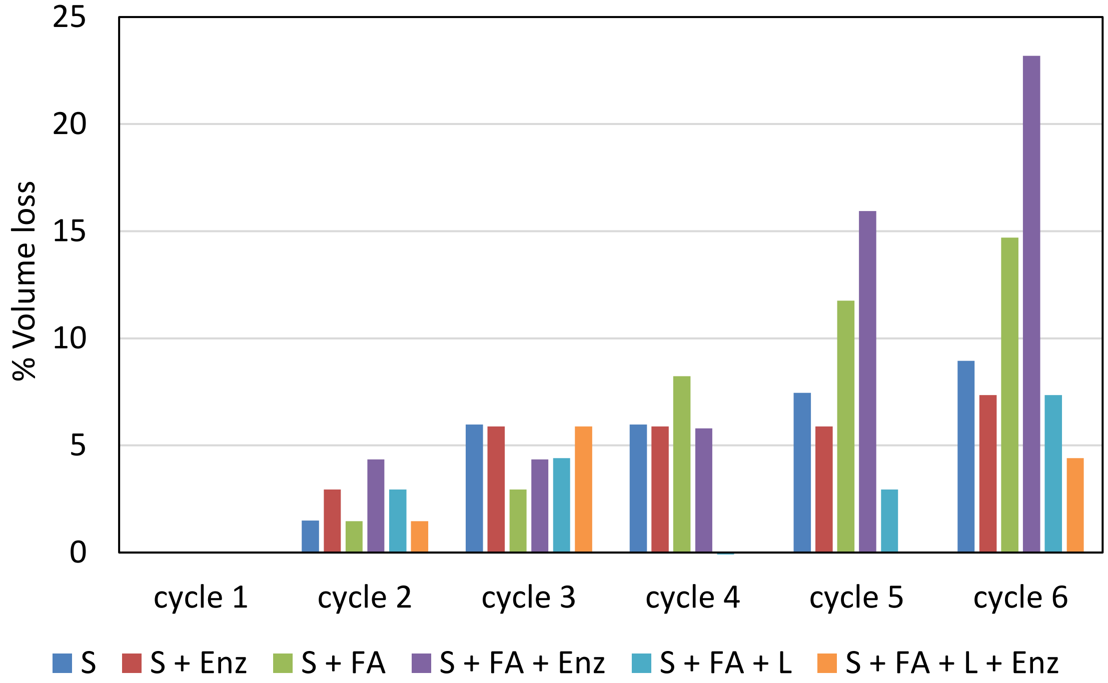


Figure 6.22. Wet state % volume loss

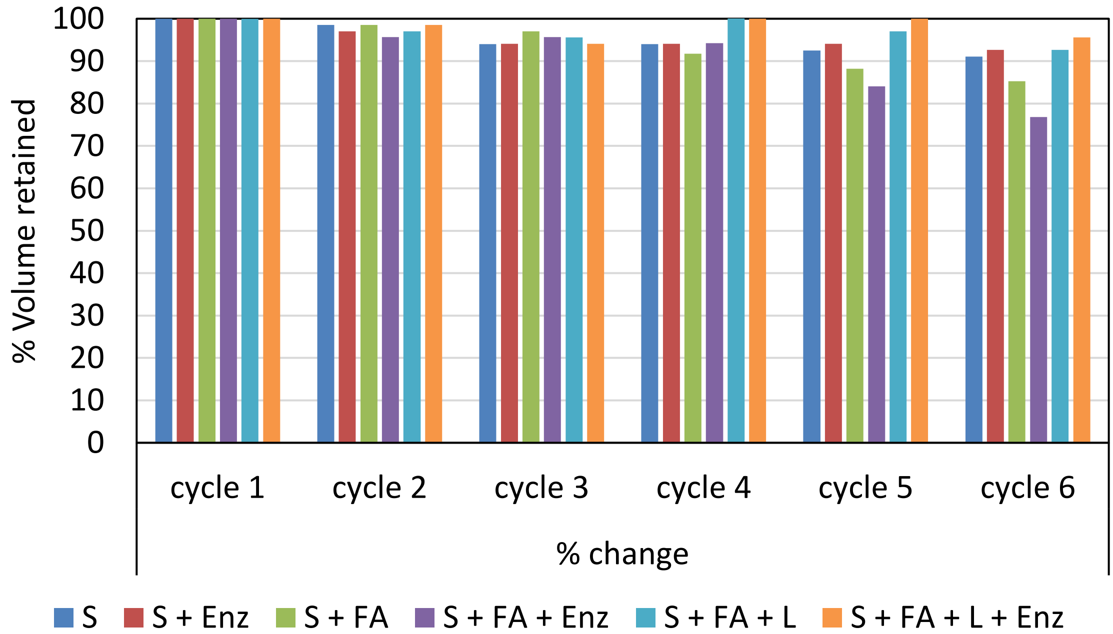


Figure 6.23. Wet state % volume retained

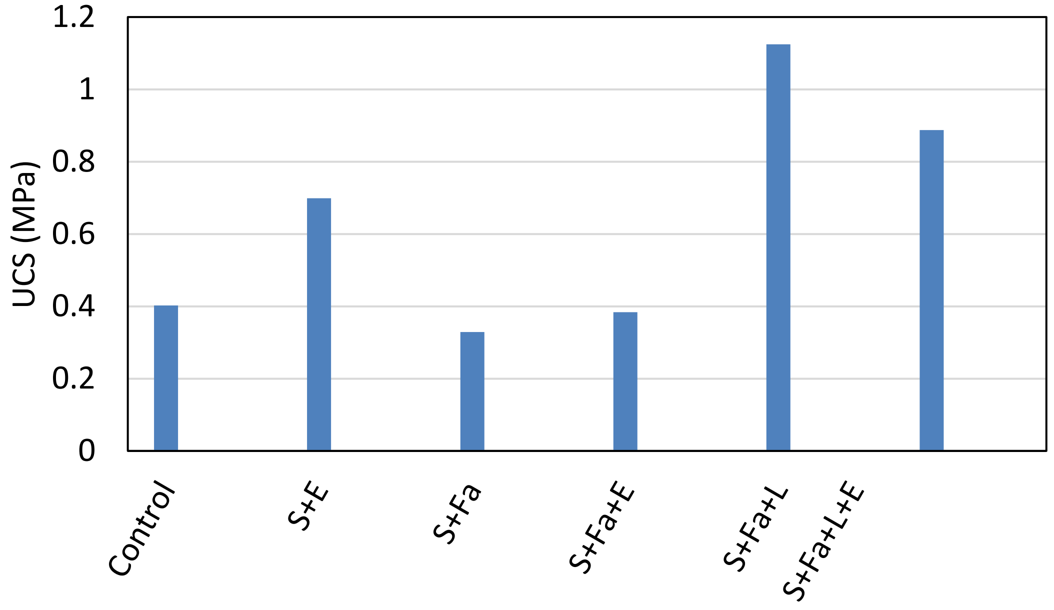


Figure 6.24. UCS strength after cycle 6

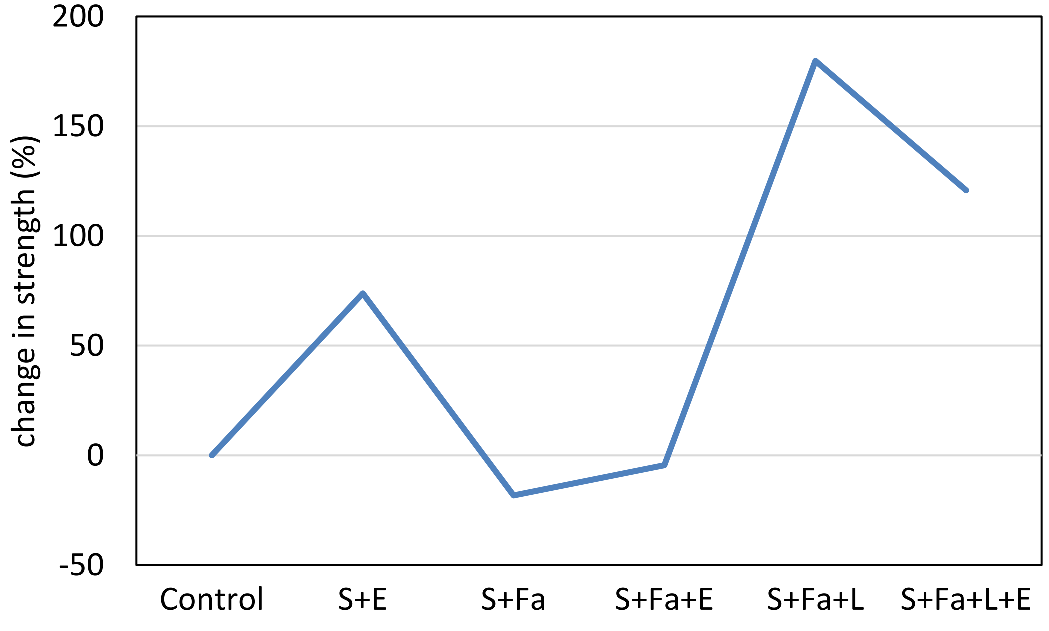


Figure 6.25. Strength variation indicative of the control soil sample

6.4.3. Pavement Performance Assessment

This section reports the results of the findings on pavement performance based on the numerical analysis conducted in this study. As mentioned in the preceding section,

(Section 6.3), the input parameters required for this analysis were based on the experimental data collected. The pavement performance evaluated within the study is a comparative study on untreated pavements and pavement treated with the combination of enzymatic fly ash and lime. Results of the numerical model application are reported in this section. As seen in Fig 6.26 and Table 6.8, the effect of stabilisation is pronounced from the results. It should be noted that plastic strain has not been reported in Table 6.8 as the model uses CIRCLY based approach in which both the models incorporated base layer thickness which was sufficient to withstand the subjected traffic load without causing material yielding. For example, the cumulative damage factor assessed using CIRCLY provides the minimum thickness requirement of the base layer to avoid yielding. If for instance, a value lower than the required base layer thickness was implemented in this pavement modelling, the material would have been prone to yielding and subsequent plastic strain. The CDF of the pavement and the thickness has been reported in Section 5.4.1 (Chapter 5). Cyclic loading approach resulted in a 30% reduction in stress and a 44% reduction in the elastic strain. In contrast, no difference was noted in terms of subgrade deflection compared to static loading approach in the untreated pavement. Similarly, upon treatment, at the bottom of the base layer, cyclical loading approach resulted in a significant discrepancy of up to 93% reduction in stress and 99.7% reduction in the elastic strain as well as 88.3% reduction in subgrade deflection. This would suggest that designing of pavements to counter the response of static loading would result in the overdesigning of a pavement, a phenomenon which has been heavily emphasised within the available literature (Ghadimi et al. 2016). These findings support the use of cyclic loading for pavement design, and for this reason, the results of dynamic loading will be used for the remainder of the analysis.

Table 6.8. Numerical modelling outputs

Loading	**Treatment**	**Stress (kPa)**		**Elastic Strain**		**Subgrade Deflection (m)**
		Top of SG	**Bottom of B**	**Top of SG**	**Bottom of B**	
Static	Control	-75.72	-75.72	-2.65E-03	-2.65E-03	-6.80E-03
	Stabilised	-37.28	-105.37	-1.31E-03	-5.90E-03	-1.79E-03
Cyclic	Control	-51.8802	-51.8802	-1.49E-03	-1.49E-03	-6.80E-03
	Stabilised	-12.1176	-7.36988	-5.51E-05	-1.53E-05	-2.09E-04

From the analysis conducted under cyclic loading, it can be seen that the treatment would result in 86% reduction in stress and 99% reduction in the elastic strain at the bottom of the base layer as well as 97% reduction in subgrade deflection. Comparing the stress as well as strain within the pavement, the treatment reduces these critical parameters which could effectively reduce rutting. The response of the pavement material is significantly affected by the treatment, which would also increase the life cycle of the pavement. The distribution of the stress and deflection within the pavement also suggests the effectiveness of the treatment. From Fig 6.26, within the stress distribution, it can be seen that the untreated pavement can undergo stress of up to 19.5 kPa (compression) towards the bottom of the 500 mm cover. In contrast, the treated section undergoes this level of stress at a lower point within the subgrade. It can also be seen that the treatment supports an even distribution of the load towards the shoulder of the pavement, whereas a stark contrast of stresses is noticed at the shoulder of the untreated pavement. It should also be noted that the lowest point within the subgrade experience significant variation in the stress reported such as 1.42 x 10^2 kPa in the untreated pavement compared to 1.16 x 10^2 kPa in the treated section. The distribution of the deflection within the pavement also highlight the effectiveness of the stabilisation. As seen in Fig 6.27, control pavement experiences a downward deflection of up to 6.9 x 10^{-3} m from the top of the base to within the top 20% of the subgrade in contrast to a significantly lower deflection within the entirety of the treated pavement. It can also be seen that downwards deflection is more localised to underneath the load applied within the pavement structure. Hence, from the numerical study conducted using calibrated models, it was seen that enzymatic fly ash and lime could be effectively utilised to design unsealed pavements ensuring adequate performance.

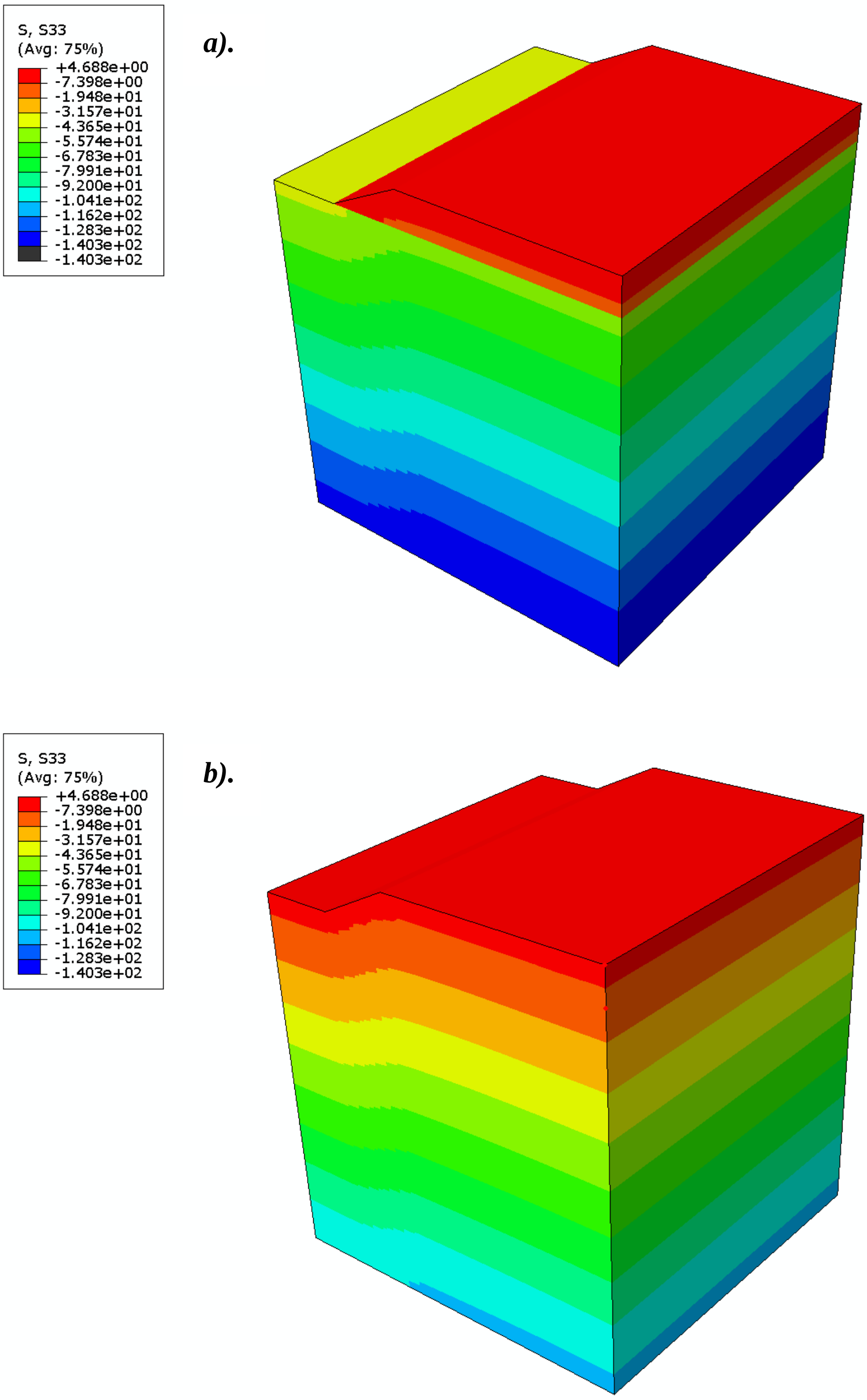


Figure 6.26. Stress distribution a) Untreated pavement, b) Treated pavement

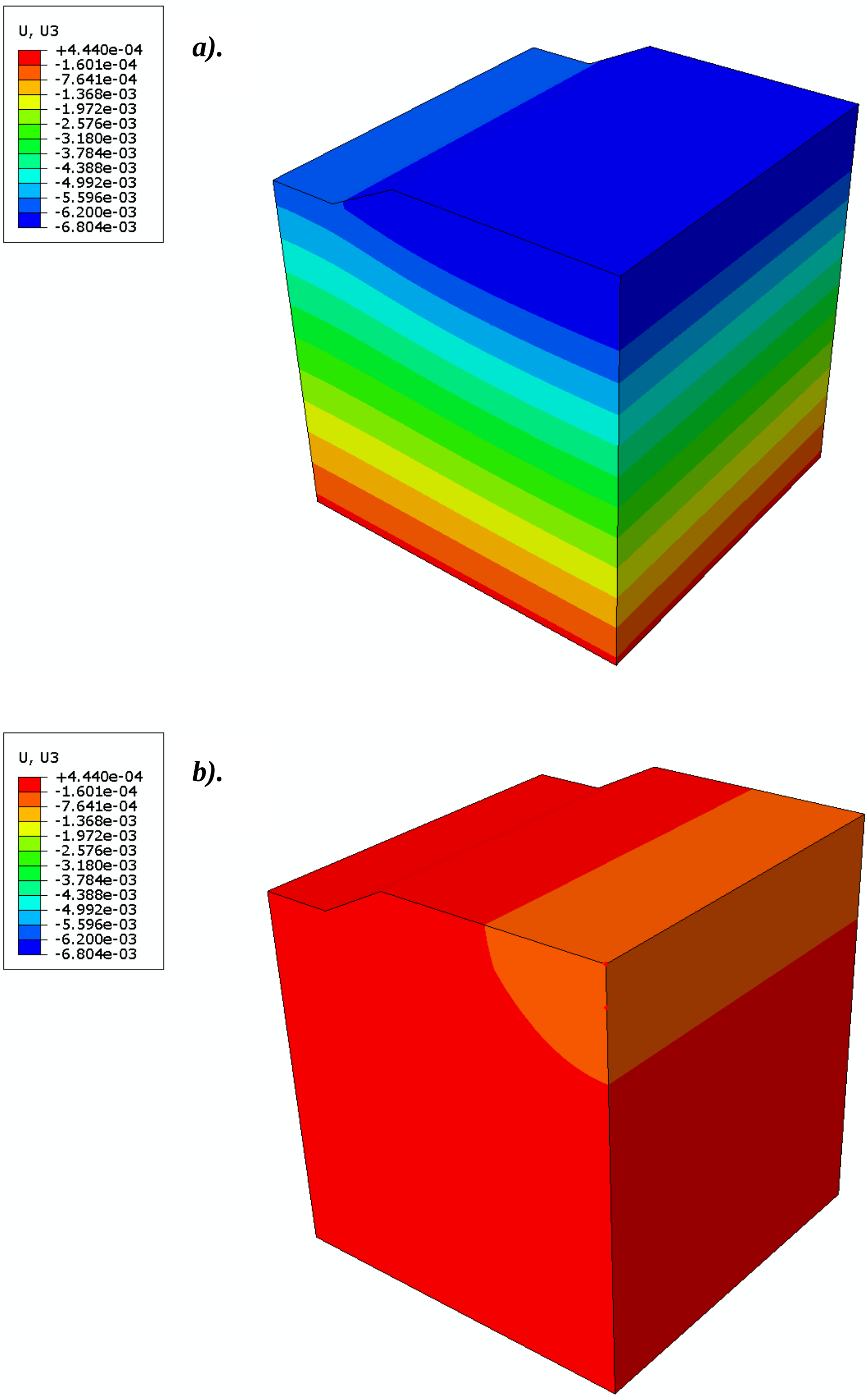


Figure 6.27. Deflection distribution. a) Untreated pavement, b) Treated pavement

6.5. Summary and Conclusions

This chapter evaluates the durability as well as the performance of pavements treated with the additives investigated within the scope of the research. As reported in the preceding chapters, the use of enzymes is of growing interest worldwide, especially to cater to the growing environmental concerns caused by the sole reliance on calcium-based additives. Chapter 5 unveils the efficacy of combining enzymes with fly ash, a waste by-product of thermal power plants which cause further environmental issues relating to their disposal. Chapter 5 also promotes the positive effect of combining the additives with lime to facilitate further pozzolanic reactions to increase the bearing capacity of the soil. This chapter evaluates the durability of these additives based on a 3-stage analysis. Firstly, the effect of recompaction and time of stabilised materials is investigated to understand how long the strength benefits induced by the treatment would last. Secondly, a novel test method is followed to investigate the resistance of the combined treatment to extreme environmental conditions. Lastly, performance modelling is employed to understand the effect of the treatment on loading conditions that pavements are subjected to. The following conclusions could be deduced from this chapter:

- Enzymes provide a positive stabilising effect to the soil as seen from the experimental data attained. It shows that the enzyme provides some benefit in retaining strength more than that of the control soil. The effect of enzyme treatment remains even months after treatment and is unaffected by further disturbance.
- Enzymes treated soils with higher dilution takes longer time to be fully effective. This is seen in the case of 1:900 treated samples. From the analysis of the 1:900 cases, it was seen that within 28 days, the strength attained was in the range of 1:100 and 1:500 tested samples.

- All samples, including the control samples, increase in strength with recompaction and time. However, the treated samples exhibited higher strength compared to the untreated case, which highlights the positive effect of enzymes.
- Enzymes were proven effective in strengthening the soil by facilitating densification of the compacted samples. However, enzyme alone might still not be able to provide a binding effect to the soil specimen, especially when subjected to harsh environmental condition.
- Samples treated with fly ash and/or enzyme is seen to have the least effect in mass retention when subjected to extreme wetting conditions due to the friable nature of the fly ash additive. The ineffectiveness of enzyme and fly ash in waterproofing the material causes the disintegration of the sample upon extremely larger moisture conditions. The inclusion of lime into the design mix has been proven effective in retaining mass in both the dry and wet states of the sample, especially due to the readily available moisture facilitating pozzolanic reactions. Similar trends were also identified in terms of the effect of volume change of materials.
- The UCS tests conducted following the wetting and drying cyclic test show varied response with the addition of enzyme in soil. While the addition of enzyme to the natural soil is seen effective in increasing the strength of the natural soil by up to 73% and increasing the efficacy of fly ash stabilised soil, it is not shown to be very effective in increasing strength of fly ash and lime admixture.

- Soil treated with enzymatic fly ash and lime show significant improvement in shear strength (i.e. cohesion and friction angle of the soil) when compared to the control soil in the absence of moisture variation. Numerical analysis of pavement subjected to loading at non-extreme moisture conditions shows that the increase in the shear parameters subsequently leads to the effective stabilisation of the pavement. i.e. the improved behaviour has resulted in a reduction in base layer thickness, which shows a reduction in pavement stress and strain as well as economic savings from the reduction of materials used for the pavement construction.

Chapter Seven.

Conclusions and Recommendations for Future Studies

7.1. General

The investigation conducted and reported in this thesis aims at unveiling the potential benefits of commercial enzymes for fine grained soil stabilisation by understanding its behaviour and mechanism to a microscopic level. The general overview of the problem addressed within research has been discussed in Section 1.2 (Chapter 1), which highlights the need for proper design strategies to overcome issues faced by many unsealed roads worldwide. The importance of unsealed roads is also reported within the section (Section 1.2 of Chapter 1) as they cover over 50% of the total Australian road network. The increased unsealed road proportion is not just common to the Australian road network. Instead, it can be seen that the US has more than 2.2 million km of unsealed road and Brazil, the world's fourth-largest road network has less than 10% of its roads paved. While common issues faced by these unpaved roads include dust control, washouts and pavement deformations, it is understood that stabilisation of these pavements seems like a potential method of mitigating these issues. The scope and the objectives of the research have been discussed in Section 1.3 (Chapter 1), which reports the importance of uncovering the benefits of environmentally friendly soil stabilisers such as enzymes to address sustainability factors. The outcomes of the research include designing of unsealed pavements stabilised using eco-friendly additives which would result in enhanced resilience of the infrastructure.

Chapter 2 presents a comprehensive literature review on the topic of the investigation while providing in-depth state of the art knowledge of soil stabilisation. It can be seen within the review that stabilisation can be subdivided into mechanical as well as chemical forms. Mechanical stabilisation is often seen used in conjunction with the most effective form of stabilisation, chemical stabilisation. Section 2.2 of the chapter provides an overview on the understanding of the mechanism of stabilisation as well as the parameters that affect its efficacy. The parameters include soil type, primary additives type, secondary additives, application rates, and curing time. Case studies highlighting the efficacy of these additives has also been presented within this section of the chapter. Section 2.3 and section 2.4 details in-depth analysis of non-traditional additives reported in the literature with particular emphasis given to enzyme-based additives. Enzyme soil stabilisers, as reported in the literature, have reported mixed results in terms of efficacy on soils. Case studies on these have also been presented. From this in-depth analysis, the potential benefits of the additive can be seen. However, its efficacy is heavily dependent on the soil type used, with fine grained soils showing maximum effectiveness. Dilution Mass Ratio (DMR) and Application Mass Ratio (AMR) are also crucial in effective stabilisation of fine-grained soil, as highlighted in the literature review. Some enzyme stabilisers reported in the literature have also been referred to as “bio-enzymes” which speculates the need to factor in the enzyme type for effective stabilisation. Sample preparation method, curing time and conditions have been highlighted within the literature as being crucial in determining the effectiveness of the additive. Based on this review, the following research questions were addressed within the research:

Table 7.1. Research Questions addressed in the current study

#	Questions
1	How do enzyme-based soil stabilisers affect soil behaviour?
2	What is the response spectrum of soils that has a positive effect on enzyme-based soil stabilisation?
3	Are there notable physical and mineralogical changes induced by enzyme-based soil stabilisation?
4	How much of the additive is required to see effective strength benefits on soil?
5	What are the sample preparation methods as well as the conditions required to attain a positive effect on enzyme stabilised soil?
6	How to quantify the strength gain of soil due to enzyme-based soil stabilisation?
7	Can the efficacy of enzyme-based stabilisation be enhanced by combining with other non-traditional additives?
8	What is the time-dependent effect of enzyme-based soil stabilisation?
9	How durable is enzyme-based soil stabilisation?

The above research questions have collectively helped attain the objectives of the current PhD study.

- Understanding the efficacy of enzymes as a stabiliser on various fine-grained subgrades based on its mechanism.

- Understand the efficacy of enzyme-based additives when combined with other additives.
- Quantification of time dependant strength on fine grained soil stabilised with enzyme-based additives and determining the performance pavements incorporating these materials when subjected to operational loads.
- Contribute to knowledge in designing of unsealed pavements stabilised with enzyme-based additives

Characterisation tests and its results conducted on the research materials utilised within the study are reported in Chapter 3. The soil subjected to testing was classified as fine grained, low plastic, lean clay with low compressibility and swelling potential. The enzyme was a commercial additive with the market name “Eko-Soil” whose active enzymes include Lipase, Amylase and Protease. The fly ash used in the study as a secondary additive to be used in conjunction with enzyme-based additives was a commercial additive classified as Class F fly ash. Lime additive also used in conjunction with enzymatic fly ash was a commercial product attained from Lime Group Australia.

Chapter 4 – 6 covers in detail the specific objectives, methodology and findings. The conclusions and observations are summarised in Section 7.2. This section is followed by the recommendations for future work in Section 7.3.

7.2. Conclusion

7.2.1. Suitability of Enzyme as Fine Grained Subgrade Stabiliser for Unpaved Roads

Chapter 4 aimed to answer research questions 1, 3, 4, 5, and 6 to unveil the stabilisation effect of Eko-Soil on the selected soil. The effect of thermal influence on the soil is conducted to identify a suitable oven drying temperature to change the soil into a workable condition from an initially saturated state (Section 4.3.1). It is observed that samples dried at temperatures greater than 40 °C can have an adverse effect on the stabilisation. This adverse effect of enzyme stabilised oven-dried soils at high temperatures (>40 °C) might be due to the soil undergoing irreversible changes within the structure. Following this stage of testing, optimisation tests were conducted on samples (control as well as treated) prepared at OMC of the control sample. These tests revealed that stabilisation was only effective at DMR 1:500. An increase in strength of up to 15% was seen in soils treated at this DMR. While chemical analysis of the treated samples shows no significant change, which suggests no new formations between the control and the treated samples, imaging techniques employed within the study reveal distinct differences in the amount and distribution of pores among the two. Further analyses on the compaction characteristics of the treated samples show a reduction in the OMC and increase in MDD of the soil, which facilitates a densification mechanism. The observations from the tests show results of up to 500% increase in CBR on soil treated with 1:500 DMR enzyme. 1% has been identified as a suitable AMR to see this increase in strength. However, it was also concluded that a higher AMR (7%) could mean the added enzyme would take lesser time to travel within the soil stratum facilitating more efficient stabilisation.

The application process of enzyme-based soil stabiliser on an unsealed road has been presented in Section 4.4.1 (Chapter 4) of the thesis. This process summarises the construction stages of an unsealed road using the developed enzyme-based stabilisation method. The enzyme-based additive is then compared with other non-traditional additives to provide indicative results relative to these additives. During the construction stages, the samples from the control and treated section were collected and compared against each other using laboratory tests. Laboratory tests show a significant benefit to the load-bearing capacity of the soil post-treatment. The pavement design analyses were conducted based on the strength parameters attained from the laboratory tests using a CIRCLY method as well as weighted average CBR model. The efficacy of the enzyme stabiliser in reducing the thickness of the unbound granular layer is seen in the CIRCLY analysis. Increase in the weighted average CBR is also seen from the treatment. However, it has been reported that monitoring of the pavement shows that although treatment using enzyme produces an immediate effect in the load-bearing capacity, the durability of the treatment needs to be tested. Another aspect to be aimed at understanding could be the effect of combining the enzyme with secondary additives to increase the durability aspect of the additive.

In conclusion, the observations and findings reported in Chapter 4 highlight that enzymes have the potential to improve the load-bearing capacity of the soil due to the additives ability to facilitate densification mechanism in fine grained soils (Research Question 1). While no chemical reactions occur within the treated sample, changes in compaction characteristics are seen (Research Question 3). DMR 1:100 at 1% or 7% can be used to effectively increase the strength properties of the soil (Research Question 4). Oven drying the soils at 40 °C is seen effective at increasing the workability of the clayey soil without any adverse effect to stabilisation (Research Question 5). Increase in strength by up to 500% in CBR is seen with the treatment (Research Question 6).

7.2.2. Effectiveness of Combining Enzyme with Secondary Additives

Chapter 5 picks up on the recommendation provided in Chapter 4 of combining enzymes with secondary additives. The introduction reported in Section 5.1 summarises the immediate need to invest in green soil additives. The use of fly ash as a secondary additive to supplement enzyme-based soil stabilisation to produce higher strength is investigated in this chapter. As highlighted within the chapter, the use of fly ash alone as a potential green soil stabiliser has been covered extensively throughout the literature review. Literature also provides anecdotal evidence on the combination of fly ash with lime and other additives. This would further support the reasoning to investigate the effect of combining the enzyme with fly ash. Section 5.3.1.1 reports that although there is a decrease in OMC with the treatment of enzymatic fly ash, there is a decrease in MDD due to the introduction of fines in the form of fly ash. For this reason, it was deduced that densification would not be the dominant mechanism facilitating strength gain in enzymatic fly ash stabilised soil. Results from the study identified 15% as a suitable fly ash content (Research Question 4) which can be combined with the enzyme (1:500 1%) to accelerate the pozzolanic reaction by decreasing the activation energy required to instigate the reaction (Research Question 3, 4 and 8). This enzymatic fly ash mix is shown to increase the strength of soil by up to 400% (soaked CBR), 680% (unsoaked CBR) and 88% (UCS) (Research Question 5). Addition of 2% lime is also shown to have further facilitated pozzolanic in fly ash stabilised soils (Research Question 4). This addition of lime to enzymatic fly ash can increase the soil strength up to 800% (soaked CBR) and 1000% (unsoaked CBR) due to the formation of hydration products such as calcium silica hydrate (CSH) and calcium aluminate hydrates (CAH) (Research Question 5). Application of the treatment to the soil based on the CIRCLY analysis has shown to decrease the thickness of wearing course material by up to 60%. This could mean financial savings as well as a reduction in the carbon footprint of the pavement, which further adds to the sustainability of the pavement (Research Question 7).

7.2.3. Durability and Performance of Enzyme Treated Soils

Chapter 6 investigates the performance and durability of the pavements stabilised with the additives subjected to testing within the study. The introduction within the chapter highlights the importance of conducting durability and performance tests in determining the long-term effect of soil stabilisers. The durability and performance have been assessed within the chapter in both an experimental and a modelling point of view. In the experimental analysis, firstly, the effect of time and recompaction on enzyme alone stabilised samples are investigated. This testing procedure is followed to gain insight on how long the effect of the enzyme remains within the soil based on strength tests. Secondly, a novel testing method is introduced in the study to provide indicative results on the stabiliser. This novel testing method is a modified model of testing of the wetting and drying test, which is commonly tested on cement-treated soil specimens. An in-depth description of the method has been reported in Section 6.2.2.2. Performance testing conducted on the basis of simulations has also been reported in this chapter. The notable observations include long term effect of enzyme event months after the treatment. It shows that these enzymes provide benefit in retaining strength more than that of the control soil. While the control samples also demonstrate strength gain with time and recompaction, the treated soils exhibited far greater strength gains (Research Question 1). The strength tests also show that although higher diluted samples (DMR 1:900) might not show immediate strength gain, with time (up to 28 days), these samples can attain strength values similar to those treated with greater concentrations (DMR 1:100 and DMR 1:500) (Research Question 8). Even though there is a significant increase in strength by the treatment of soils with the enzyme-based additives, it should still be noted that no binding force is provided by the treatment which allows the failure of samples with harsh environmental conditions. Fly ash stabilised samples were seen to have an adverse effect on the durability of the samples despite strength improvements which could be attributed to the change in the nature of the soil to a more friable material. The enzyme was also not seen to provide significant durability to harsh conditions too. However, the addition of lime to the mix has shown a significant benefit to strength as well as durability in the testing regime (Research Question 9). Enzymatic fly ash and lime are effective at improving cohesion and friction angle of the soil, which could subsequently lead to the effective stabilisation of the pavement. Simulation on the pavement modelled using dynamic loading is confirmed from the study as being an effective method to design realistic pavements. The reduction in base layer thickness is supported with the treatment and proven effective, which shows a reduction in pavement stress and strain as well as economic savings from the reduction of pavement layer Research Question 1, 7, and 9).

7.3. Contribution to the Field of Knowledge

- Enzyme based soil stabilisation mechanism was discovered for a fine-grained that is common to the Melbourne geology using a series of lab tests that analyse micro to macro behaviour of soils.

- Optimised mix design for enzymatic soil stabilisation was identified and trialled in fields for a soil which is common in Melbourne.

- Effect of temperature on soil prior to soil stabilisation using enzymes was investigated covering a wide range of practical temperatures.

- Enzymatic fly ash soil stabilisation mechanism was unveiled using a comprehensive lab test program that includes physical, chemical, mechanical and microscopic tests.

- Combined stabilisation using enzymes and secondary additives was optimised to devise sustainable benefits including waste mitigation and reduced need to quarry wearing course materials.

- A novel testing method was introduced to evaluate the durability of enzyme-based soil stabilisation.

- The durability and performance of enzymatic soil stabilisation was assessed using practical pavement evaluation tools and 3-D full-scale numerical models.

- Lab-based investigations were verified in field trials for enhanced reliability of research outcomes.

7.4. Recommendations for Future Studies

This study aimed to provide fundamental knowledge on the soil stabilisation mechanism of a commercial multi-enzyme additive with secondary additives. The study also investigated the effect of parameters such as time, sample preparation techniques, and additive contents. While this study has uncovered breakthrough on the effectiveness of the additives and the parameters that affect its efficacy, it should be noted that there are still avenues which could be explored about this additive in future studies. The following recommendations could be drawn from this study:

- This investigation was limited to understanding the effectiveness of the additive on fine grained soils. Although it is hypothesized that the enzyme-based additives might not be effective in stabilising granular materials alone, future studies could explore the combination and optimisation studies to obtain design mix of enzyme treated fine grained and granular admixture soils.
- The sample preparation for treatment was prepared using soils oven dried at 40 °C as it was observed that soils dried at higher temperature has an adverse effect on the strength gain. Due to the scope of this study being devoted to

understanding the mechanism of enzymes and did not include an in-depth analysis of what caused the adverse effect of higher temperature, investigating this may be worthwhile.

- The comprehensive testing conducted and reported within the thesis could suggest the investigation regarding dilutions have been vastly covered. The study recommends the use of 1:500 dilution as an effective rate of dilution. For further studies, testing on other dilution may not be worthwhile. However, tests on the optimal lime and fly ash content are advised to be conducted as these might be soil specific.
- From the strength tests conducted within this research, it is quite evident that CBR tests provide a more realistic and useful analysis in regard to the effectiveness of pavement analysis and design. The mechanical testing considered for this study was mainly UCS and CBR. Use of repeated triaxial test should also be considered in future studies. However, the time and cost associated with that test could hinder the practicality of the test. The use of non-destructive and non-intrusive testing methods could also be considered in future studies to quantify stabiliser effectiveness.
- A study cited in the literature review includes the effectiveness of enzymatic lime. Comparative study on the effectiveness of enzymatic fly ash as well as enzymatic lime could prove to be effective in truly recommending a superior stabiliser. However, it is to be understood that the sustainability of the additive might be compromised if lime is preferred over fly ash due from lime production contributes to higher greenhouse gas emission. On the other hand,

fly ash, being a waste product, would not contribute to carbon emission as much.

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Appendix

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1. **Renjith, R., Robert, D. J., Gunasekara, C., Setunge, S. & O'Donnell, B.,** 2020. Optimization of Enzyme-Based Soil Stabilization. ***Journal of Materials in Civil Engineering***, 32, 04020091.

2. **Renjith, R., Robert, D. J., Setunge. S., Costa, S., Mohajerani, A.,** 2020. Optimization of Fly Ash Based Soil Stabilization Using Secondary Admixtures for Sustainable Road Construction. ***Journal of Cleaner Production,*** *294, 126264.*

3. **Renjith, R., Robert, D., Fuller, A., Setunge, S., O'Donnell, B. and Nucifora, R.**, 2017. Enzyme based soil stabilization for unpaved road construction. In ***MATEC Web of Conferences*** (Vol. 138, p. 01002). EDP Sciences.

4. **Robert, D., Renjith, R., Setunge, S., O'Donnell, B., Hewawasmge, D., Tan, E** 2016. Assessing the stabilizing mechanisms of unbound road pavements. ***Civil Engineering Conference in The Asian Region CECAR 7***. Waikiki, Oahu, Hawaii.