THE EFFECT OF BIOLOGICAL EXUDATES ON THE MECHANICAL PROPERTIES OF GRANULAR SOIL

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

By

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ABSTRACT

This study explores how biological processes can be used to enhance the geotechnical characteristics (shear and permeability data) of soils. In particular, the creative use of microbial substances to efficiently improve the mechanical properties (shear parameters) of soils is studied. The research studies give an excellent opportunity for understanding the principles of biological processes in geotechnical engineering development. Specifically, biofilm behaviour is a part of the interactions between geotechnical engineering and the biological process of microorganism growth. The main research questions of this study are how biofilm affects the shear parameters of granular soil under low normal stresses. To that end, this study concerns experimental work to explore the effect of accumulated bacterial biopolymer (biofilm) on the shear response of well-graded silica sand. A comparison is achieved between biotreated samples with un-biotreated samples as well as comparing with clean dry and saturated samples under the various testing conditions are considered. To address the objectives of this study, experimental work was conducted using an adapted direct shear test procedure that enables proper tests to investigate the impact of biofilm on the shear strength in a saturated condition. Moreover, these tests were performed at a displacement rate of 0.5 mm/min, and at various normal stresses (1.0, 4.1, 8.89, 16.2, and 25.0 kPa). The soil samples (defined as biotreated samples) were prepared to encourage biofilm growth and the production of exopolymeric substances (EPS) by supplying a glucose rich nutrient. The control samples produced by delivery of a glucose free nutrient (defined as standard samples). All samples were prepared and tested in triplicate. Furthermore, this study

explain the influence of the biofilm on a poorly graded silica sand and a sea sand. The effect of various testing rates on the biotreated and standard sample was also investigated. The important finding of this study was that the potential impact of biofilm on densification on preloading biotreated sand samples. The growth of biofilm increases the ability of samples to densify under applied normal stress during incubation period compared with control samples. All biotreated samples show larger peak stress than the shear stresses of standard specimens. These differences may be because of the differences of loss on ignition content in both sample types. A biotreated sample contains higher biomass than that in the standard samples. The amount of formed biofilm in the biotreated poorly-graded silica sand and sea sand was significantly more than in the well-graded silica sand. This biofilm has had a similar effect on peak stress of both the well and poorly graded.

DEDICATION

I dedicate this thesis to my Lord on Earth for permanent inspiration and support

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In the Name of Allah, the All-Merciful, the All-Compassionate.

The praise is due to Allah the All-Powerful, the All-Knowing, the All-Wise, the All-Generous, the Noble and the Compassionate, for giving me the strength, patience and perseverance to complete this work to its best and pursuit of my dreams.

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Am: attometer, 1 attometer =1.0 am = 10^{-8} angstrom.

ASTM: American Society for Testing and Materials.

BS: British Standard.

CBR: California Bearing Ratio

CD: consolidated drained

CSLM: confocal scanning laser microscopy

CTC: 5-cyano- 2, 3-ditolyl tetrazolium chloride

CU: consolidated undrained

DST: Direct shear test

dy/dx: the maximum slope of the measured dilation-displacement response

EPS: extracellular polysaccharide substances

EPS-CM: extracellular polysaccharide-Culture Media

ID: relative density

I_R: relative dilatancy index

K: hydraulic conductivity

LOI: Loss on Ignition test

LTSEM: low-temperature scanning electron microscopy

MICP: Microbial induced carbonate precipitation

OD: optical density test.

$Ø_p$: Peak internal friction angle

$Ø_{res}$: Residual internal friction angle

P': effective stress at failure

PBS: Phosphate buffered saline

pH: hydrogen ion concentration

RHD: Relative Horizontal Displacement.

SD: Standard deviation

Tm: (1.0 “tetrameter=$1.0x10^{+22}$ angstroms).

UCS: unconfined compression strength test

UMB: ultra-micro-bacteria

V: volume of sand sample

α_p: Peak dilation angle

ΔH_o: *the differences of sample height at peak stress, mm.*

Δx: horizontal displacement at peak stress mm.

ρ_{min}: minimum sand density

Ψ: angle of dilatancy

Chapter 1

INTRODUCTION

1.1 Background

Many traditional approaches are used to improve the mechanical properties of soils. These approaches often include a mechanical and or chemical treatment processes and consequently are problematic in terms of sustainability, for example mechanical compaction process results in emissions of CO, CO_2 and other pollutants such as chemical grouting as well as noise. The use of some chemical materials for stabilisation of soil may also act an as toxic material leading to deterioration of the biodiversity of the soil, such approaches are also expensive (Yunus et al, 2014), (Ivanov and Chu 2008a). Therefore, the development of techniques harnessing microbiological processes for enhancement of the physical properties of soil is attractive as a potentially low cost low impact solution for soil improvement (Ivanov and Chu 2008a), (Singh et al, 2006).

Regarding biological application for soil improvement, successful microbiological processes depend on different factors. For example, the desired type of microbial metabolism, interactions with other existing microbes in the ecosystem, type of soil, nutrients availability, and depth of the subsurface, pH, temperature, pressure, ionic concentrations, and the presence of oxygen or other oxidants may all play a role. These parameters require understanding from the point of view of the microbiology, chemistry, geology, hydrology, and geotechnical engineering to allow investigation of the impact of soil biota on engineering properties of soils and in exploring biological processes for soil treatment and improvement, (Ivanov and Chu 2008, and Mitchell and Santamarina 2005). These studies introduced the fundamentals

of an environmentally benign technology development, indicating that successful microbiological processes can be developed and implemented for soil improvement. These processes have a wide application to different geotechnical problems, such as slope stability, excavation, tunnelling in cohesionless soils, controlling dusting and soil erosion, improving the bearing capacity of foundations, mitigation of liquefaction risk, reducing under-seepage of dams and cut-off walls, and facilitating desiccation and dewatering.

The inspiring opportunities for innovative use of biological processes to develop the physical characteristics of the subsurface such as shear strength, permeability, erosion resistance, liquefaction resistance and stiffness. Biological soil treatment processes have been recently investigated by many researchers such as Khatami and O'Kelly (2013), Cabalar and Canakci (2011), Ahmed and Hussain (2010), Banagan et al (2010), Ivanov and Chu (2008), Mitchell and Santamarina (2005) and Perkins et al (2000). Interdisciplinary research studies are enabled by the confluence of microbiology, biochemistry, geochemistry, and geotechnical engineering; this novel field has the potential to meet environmentally friendly and sustainability requirements for developing bio-remediation methods that enhance soil to support new and presenting infrastructures.

The engineering challenges in developing beneficial applications of microbiological processes include determining suitable approaches and inducing the desired method over a time frame of engineering interest. Interactions between microorganisms and mineral of soil particles have typically been studied extensively only by biologists and geologists. Cooperative multidisciplinary research among biologists, chemists, geologists, and geotechnical engineers is required to understand the potential of

microbiological soil improvement technologies. Most exopolymeric substances have high molecular weight containing chemical action groups with electrical charges (Sutherland 2001). These groups actively interact with soil particles. Therefore, it is expected that exopolymeric substances will have an effect on a soil behaviour and engineering properties in different ways such as improving the shear strength of the soil to reduce damage due to erosion and collapses.

The wide activities of microorganisms in soil environments have been well recognised (Lavelle et al, 2006). An important product formed by bacterial colonies or biofilms is extracellular polysaccharide substances (EPS) (Sutherland 2001), which are exuded by microorganisms for protection purpose and to make a more hospitable ecosystem for the microbiological community (Maier et al. 2000).

The hypothesis of this current study is that near to the surface in riverine and marine environments, biofilms play a significant role in stabilising sediments, and increasing the resistance to erosion (DeJong et al, 2013). Many organisms release extracellular polymers into their surroundings (Yallop et al, 2000). The Exopolymers have the potential to enhance sediment stability without the environmental risks of typical soil stabilisers. These Exopolymers are high molecular weight polysaccharides produced by microorganisms of soil (Nugent, 2011). The stability of bed sediment in an aquatic environment is dependent on the balance between hydrodynamic forces that cause erosion and the forces within the sediment that resist it (Grabowski et al. 2011). Biofilms formation happened when microorganisms adhere to a surface and excrete EPS as part of their metabolism. The slimy nature EPS develops

further attachment of other particles, thereby forming a biofilm that can affect the physical properties of soils (Banagan et al, 2010). A sediment matrix can result in sediment cohesion that increased the stability of the sediment. The presence of EPS in aquatic environments may enhance the colonisation of sediments by forming strong bio-mats (Vignaga 2012). Beach gravels at Montrose on the east coast of Scotland are cemented by microbially colonised biofilms which provide a significant mechanical strength, (Braithwaite and Gribble 1998).

This study is a promising opportunity to enhance soil in a way that is compatible with environmental friendly requirements. The novelty of this research is using a *Beijerinckia indica* microorganism that has a biopolymer producer to improve the mechanical properties of sand.

The shear strength of soils is of special relevance among geotechnical soil properties because it is one of the essential parameters for analysing and solving stability problems (calculating earth pressure, the bearing capacity of foundations, slope stability or stability of embankments and earth dams). The direct shear test is one of the most common laboratory test that is often used to determine the shear strength of soils.

The shear response of biotreated sand specimens subject to biological activities and growth of biofilm will be evaluated by comparison with control sand samples. The control samples, prepared in the same manner of biotreated samples, will have limited biological activity due to suppling a nutrient that does not contain glucose. Microbial processes produce extra polysaccharide substance (EPS) which causes aggregation of soil particles during bacterial growth (Ahmed and Hussain 2010). Therefore, the adhesion

behaviour may be developed by the formation of EPS on the sand grains. The development of internal friction and dilation angles will also be significant to study.

The main purpose of this thesis is to characterise how bacterial polymer (biofilm) change the shear strength of well-graded silica sand and to provide geotechnical explorations of the effect of the biofilm on the shear characteristic of poorly graded silica sand and composite well graded natural beach sand. A preparation method of sand samples will be presented by mixing each sand sample with a known volume of bacterial solution and daily feeding by pumping a nutrient to develop an exopolymeric substance EPS over the incubation period. Then the samples are tested using direct shear apparatus.

The nutrient solution and minerals liquids (nutrient glucose free) will be pumped four times a day for biotreated and control sand samples, respectively, over incubation period for two weeks. The quantity of medium calculated as 1.5 pores volume. These tests performed according to experimental plan design.

Besides performing the main experiment, a dry and saturated clean sand preload and without preload are tested using the direct shear test to compare the shear outcomes of these tests with the shear response of the main experiment. In addition, the impact of sand gradation and particle shape are studied in this research, Appendix C. In addition, the rate of the test will be changed to investigate the impact of biofilm presence on a drain condition of biotreated samples during testing which may effect on the shear stress-strain behaviour of sand. The dimension of sample was (60X60X45 mm). The displacement rates of the tests will be 0.1, 0.5, 2.0 mm/min.

1.2 Thesis aim and objectives:

The main aim of this research is to explore the influence of bacterial biopolymers, excreted by some organisms, on the mechanical properties of granular soil at low stress conditions. The main objectives of this study are to:

- Develop a reliable procedure of direct shear testing under low normal pressures on biotreated sample.
- Examine the influence of accumulated biofilm, under various applied normal stresses, on the shear stress-strain behaviour of sand.
- Investigate the effect on biofilm development and shear strength of the angularity and particle shape of sand and various rates shearing.

1.3 Thesis Outline

The thesis has been divided into seven chapters including chapter 1 (Introduction). A brief description of the thesis chapters is presented below:

Chapter 2: This chapter describes the existing literature relevant to the biological enhancement of soil. The chapter comprises four main sections. The first section includes an introduction to the use of biopolymers to improve the mechanical properties of soil.

The second section discusses the general behaviour of biofilm in the soil and covers the formation of biofilm, the factor influencing transport and growth of biofilm, and biological approach strategies.

The third section considers the application of direct shear testing at low normal stresses.

The final section covers the biological enhancement of soil and includes the effect of natural polymers on soil and sediment properties. The impact on the hydraulic conductivity and shear strength by using natural and artificial polymers is discussed.

Chapter 3: This chapter describes the experimental apparatus, the program of planned experiments, and the method of preparation and testing of the sample and adapting of the direct shear test. The chapter consists of eight sections.

The first section includes the plan of all required experiments. The second section presents the characteristic of silica sand and bacterial strain used (*Beijerinckia indica*). The third and fourth sections involve the culture of the microorganism. The design and development of a direct shear box, loading system and fluid system are covered in the fifth section. The sixth section comprises the preparation of the sand sample by using a wet pluviation technique as well as the assembling of loading system. The seventh section covers adapted direct shear apparatus and testing procedure, correction procedure for internal friction angles because of using low normal stresses in these tests. The eighth section presents the standard techniques used in the study. These include characterisation techniques such as sieve analysis, Proctor test, particle density test, minimum density, elements constitution of sand, permeability test as well as techniques to estimate biomass content via loss on ignition as well and biological methods which include determining cell number-optical density relationship, CTC procedure, serial dilution and finally cell number counting.

Chapter 4: This chapter considers the optimum conditions for biofilm production in the sand. Four main sections are be presented in this chapter. The first section introduces the investigation to identify the most suitable nutrient. The second section describes permeability test for clean and biotreated sand to evaluate hydraulic conductivity and loss on ignition. The third section describes the biological experiments which carried out to enhance biofilm formation by exploring the effect of pH variation of medium and change some of the chemical composts of nutrient on the growth of biofilm. In addition, the comparison of pH variation and measuring of optical density between the using of tap water and deionised water. The fourth section shows the summary of all of these experiments.

Chapter 5: This chapter introduces a direct shear test procedure reliable at low normal stresses. The chapter includes four sections; the first section shows the introduction of all required direct shear test to develop the standard procedure that can be used in the main experiments. The second section deals with the effect of using jacking screws on the consistency of direct shear test results. This section also describes the special procedure of direct shear test for the main biotreated experiment. The third section considers the rate of shearing during the direct shear test and the final section summarises the outcomes of the tests.

Chapter 6: The overall results of the experimental work are presented and analysed in this chapter. The outcomes of all tests such as the main biotreated experiments, the compared the result of dry and saturated, and preloading dry and saturated are described. Further testing and more comparisons exploring

a poorly graded silica sand, natural sand, and the influence of rate of test on the shear parameters are presented.

Chapter 7: This chapter presents the conclusions and recommended future work to expand this study.

Chapter 2

Literature Review

2.1 Introduction

Whilst few reviews have been presented that cover the full extent of this study a number of reviews exist in the literature that are very relevant. For example Umar et al (2016) have reviewed the concept of using biological process in soil improvement, and DeJong et al (2013) reviews the effect of biofilm presence in aspects of soil behaviour including adhesion, dilative behaviour, shear friction strength, and internal friction angle. In addition, the influence of some artificial polymers on development of soil properties were reviewed by Panda et al (2016). Building on these existing works this chapter describes the existing literature relevant to the biological enhancement of soil. The chapter comprises four main sections. The first section includes an introduction to the use of biopolymers to improve the mechanical properties of soil. The second section discusses the general behaviour of biofilm in the soil and covers the formation of biofilm, the factor influencing transport and growth of biofilm, and biological approach strategies. The third section considers the application of direct shear testing at low normal stresses. The final section covers the biological enhancement of soil and includes the effect of natural polymers on soil and sediment properties. The impact on the hydraulic conductivity and shear strength by using natural and artificial polymers is also discussed.

2.2 Use of biopolymers to improve the mechanical properties of soil.

Application of biopolymers is a novel approach in ground improvement and so, there are few reported studies directly related to the impact of bacterial polymer

presence on the behaviour of soil under different effective normal stress. However, some authors have investigated the effect of biofilm presence in aspects of soil behaviour including adhesion, dilative behaviour, shear friction strength, and internal friction angle - a good review of these was given by DeJong et al (2013).

In addition, the influence of some artificial polymers on development of soil properties was reviewed by Panda et al (2016) who summarised the impact of a range of artificial polymers on the different types of soil such as high and low plasticity clay, montmorillonite, kaolinite, and sand – clay mixtures. The authors presented several soil properties which were investigated in the reviewed studies by conducting different tests. For instance, swelling tests, sorption, X-ray Diffraction, unconfined compression strength test UCS with polymer content, and curing time, plasticity, compressibility, desiccation cracking, and erosion resistance. Overall, the authors revealed that some polymers enhanced and increased the UCS, and whilst others were effective for volumetric control. They concluded that plasticity index can be reduced by using another kind of artificial polymers.

Wloka et al (2004) and Sutherland (2001) reported that biofilms have been found in a very wide range of natural environments as well as these biofilms provide their component microbial cells with an almost infinite range of constantly changing micro-environments. The basic components of biofilms are microorganisms, extracellular polymeric substances (EPS) and water (up to 98%). The EPS matrix is the metabolic yield of bacteria and consists of different types of polysaccharides as stated by both Garrett et al (2008) and Wloka et al (2004) who also summarise the factors influencing transport and growth of microbial biofilm.

The most important issue is understanding how the bacterial polymer enhances the shear properties of soil. A general review of biological enhancement of soil has been presented in this chapter. Perkins et al (2000) investigated the effect of biofilm on the stress- strain-time characteristic of Ottawa sand. They reported that biofilm has an insignificant impact on the sand strength and stiffness. However, the biofilm increases the time-dependent creep deformation. A finite – element model was developed to predict a creep deformation (Perkins et al, 2000).

As reported by Meyer-Reil (1994), the presence of biopolymers have a positive effect on erosional characteristics of sediment and therefore makes biofilm technology potentially of used for soil improvement purposes. The microorganisms dominate the modification and decomposition processes of material in sediments (Meyer-Reil, 1994). The bacterial cells in the microbial colonies become an immobilised at particles surfaces. These cells, become embedded in an organic matrix of extracellular polysaccharides (Meyer-Reil, 1994).

Although the major focus of the current study is the effect of biofilms on the shear characteristics of sand, other approaches are also reviewed such as soil bio-remediation, soil bio-cementation using MICP, permeability control of sand. Ramachandran et al (2001) reported that calcite precipitation induced by *Bacillus pasteurii* could meaningfully increase the compressive strength of remediated cracks that filled with the microorganism. It was also found that

biotreated sand exhibited a substantial increase in compressive strength and stiffness value comparison with standard specimens (Ramachandran et al, 2001). Biological treatment has been already used in other fields. Scott et al

(1988) investigated the resuscitation, by nutrients, of starved cells that were already injected through rock pores to be grown, this study was in the context of blocking of the rock pores by these cells to increase oil recovery efficiently. Macleod et al (1988) showed that because of the smaller size of starved cells, such cells penetrate further into artificial rock core and with nutrient stimulation the starved bacteria grow and produce biopolymer that may reduce the core permeability.

2.3 General Biofilm behaviour in soils

In this section, a brief description of biofilm matrix as well as the stages of forming extracellular polymeric substance EPS and the mechanism of EPS adhesion to the surfaces are reported. The factors that effect on the transport and growth of microbial biofilm are also demonstrated.

2.3.1 Biofilm matrix and EPS

Percival et al (2011) defined microbial biofilm as microbial colonies stuck firmly to a substratum and enclosed within an extracellular polymeric substance (EPS) excreted by the microbial cells themselves. Various kinds of wet surfaces can be a suitable ecosystem for the growth of the biofilm. Biofilm can be developed in natural, and man-made environments under various conditions, forming on moist surfaces, plant roots and locations of living animals In addition to bacterial components, biofilm consist of non-cellular materials such as mineral crystals, corrosion particles and clay or silt particles as stated by Percival et al (2011). As shown in Figure 2-1, Molobela (2010) presented the stages of biofilm growth and these stages are considered in turn below: The formation of biofilm starts when the attachment

of a single cell to a substratum is the first step. In the second step, by the production of EPS, the attachment may be fixed by adherence of the cells to the substratum through the surface. Molobela (2010) also described that the third phase is the growth of bacterial cells which develop tight attachment micro colonies. These colonies depended upon available nutrients on the surface or from the water column above the substratum. In the fourth phase, a proportion of cells or fragments of a micro colony detach and disperse from the biofilm matrix and spread biomass in the presence of water, (Singh et al, 2002). The biofilm stability is gained partially by cell-cell interaction, and also partly from the EPS mass surrounding and embedded in biomass of the biofilms. Eventually, the biofilms may partly release cells into the surrounding environment to attach to other substratum and start a new life cycle, (Prakash et al, 2003). Figure 2-1 shows the schematic diagram of biofilm attachment and formation process in *X.axonopodis* pv.*citri* as reviewed by Li and Wang (2011).

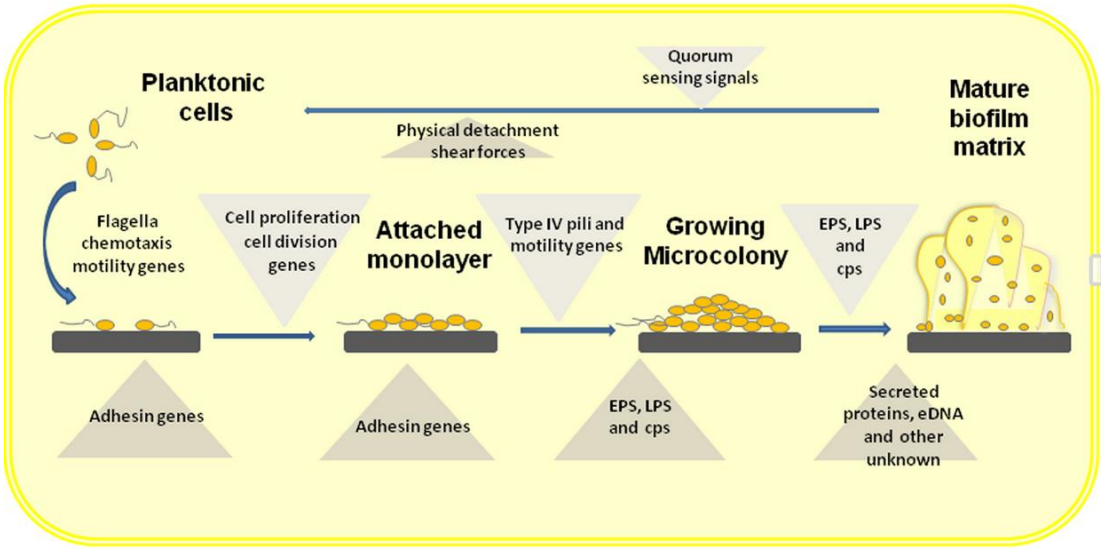

Figure 2-1. Schematic simulation of the multistage biofilm formation process in *X.axonopodis* pv.*citri (reproduced from Li and Wang, 2011)*

As Stoodley et al (1999) state, biofilms are notoriously hard to eradicate. It is vital to understand how biofilms are formed and behave to predict and ultimately control biofilm processes. In early 1990's, biofilm research developed when confocal scanning laser microscopy (CSLM) showed that biofilms made complex structures which could facilitate nutrient exchange.

Vu et al (2009) indicated that the microorganisms produce an extracellular polymeric substance (EPS), which is a complex combination of biopolymers polysaccharides, in addition to proteins, nucleic acids, lipids and humic substances. EPS consists of microbial aggregates and forms the structure and architecture of the biofilm mass. The crucial function of EPS includes the mediation of the initial attachment of cells to different surfaces and protects against ecological stress and desiccation, as Vu et al (2009) explained. Garrett et al (2008) have shown that the viscous behaviour of EPS can affect biofilm adherence to surface of soil particles (collector's surface). Therefore, the EPS improves the mechanical stability and adhesive capacity of microbial cells to surfaces.

2.3.2 Factors influencing transport and growth of microbial biofilm

There are some factors may affect nutrient delivery or bulk bacterial transport and growth of biofilm within a soil mass. The transportation of biopolymer happens when the biofilm becomes mature and the micro colonies continue to grow in volume, and a bacteria in proximity to the adhesion or living surface have difficulty in gaining access to nutrients from the external environments as Prakash et al (2003) reported. Only the bacteria located in the upper layers of the colony can continue multiplying (Giaouris and Nychas, 2006).

Another reason for biopolymer transportation is that the biofilm may be exposed to harsh conditions or shear forces from the flow of water causing detachment and separation of the biofilm.

Overall, different factors may significantly affect bacteria survival in the soil matrix, as indicated by Kavazanjian and Karatas (2008), and Mitchell and Santamarina (2005); these include: the type of microbial metabolism desired; interactions with other microbes present in the environment; soil type (particle size distribution and bulk density); depth below ground surface; the availability of nutrient; the presence of water; pH (affects surface charge, adsorption, and dissolution); redox potential (to gather energy); temperature (affects reactions within cells as well as physicochemical properties such as diffusion and viscosity); pressure; the concentration of ions; availability of oxygen and other oxidants; the presence of predatory microorganisms may limit desired bacterial population, the limitations of available space.

Umar et al (2016) stated that the central challenge, of establishment of successful continuous bacterial activities, is to overcome the limitations of mass transfer and effectively transport the nutrient or reagents to deeper parts of the area to be treated.

2.3.3 Biological approach strategies

Two primary strategies are required to develop the technique of a bio-mediated improvement of soil (DeJong et al, 2013). The first strategy is bio-augmentation where bacteria solution is pumped into the soil. The second one is a bio-stimulation where the native bacteria are stimulated. Over more than three decades, many investigators developed these strategies. Bio-

stimulation is increasingly used in the geoenvironmental field (Martinez, 2012).

In general, bio-augmentation is less favourable than bio-stimulation because of the introduction of exogenous (non-native) microbes. In some cases, this strategy needs permission and high cost. Also because of microbial filtration, it is practically difficult to have uniform application in the subsurface. Moreover, the proliferation of microbes may stop or they may become dormant when the environmental conditions are incompatible with the growth of such bacteria as stated by DeJong et al (2013).

Bio-stimulation is preferable to be used because it is based upon the stimulation and growth of native microbes, which are compatible with the environment of the subsurface, and reduce the permission difficulties. Nevertheless, there are numerous problems encountered when applying bio-stimulation, including attaining uniform treatment across a site, and accommodating the increased time combined with stimulation and growth as presented by DeJong et al (2013). A bio-stimulation technique has been developed for biofilm growth. It has been presented that the improvement of soil properties can be maintained with infrequent nutrient treatments. Eventually, larger organisms can also play a role in changing the mechanical properties of soils (DeJong et al, 2014).

2.4 Shear strength in soil.

The shear strength is typically defined as the shear stress that a soil can withstand due to the mobilised friction between soil particles (mineral composite of particles), interlock between particles (shape and density) and

adhesion. The typical responses of soil under all shear test methods are described by Budhu (2007) as:

- High density soils show high peak shear and high volume expansion.
- At critical state, the critical shear stress and critical void ratio depend on applied effective normal stress.
- High effective normal stress is high critical shear stress and low critical void ratio.

As noted by Budhu (2007), in the direct shear test, shear bands or shear zones may be developed in the shearing plane during direct shear testing as shown in Figure 2-2. The definition of the shear bands is they represent localized failure zones. The development of these bands depends on the boundary conditions of tested soils such as the homogeneity of the soil, the grain size, uniformity of loads, and initial density. On the basis of the shear stress - horizontal displacement relationship, the soil sometimes described as 'peak shear stress soil' or 'critical state shear stress soil (Budhu, 2007). For peak stress soil, the strain-softening response results from localised failure zone (shear bands). However, for critical state (non-peak) stress soils, the shear stress is reached failure when no further change in shear stress under continuous shearing at constant normal stress. Bagherzadeh and Mirghasemi (2009) pointed out that the mobilized internal friction angle in the shear zone of sample, they showed that the middle third of sample is assumed as the shear zone. They have shown that the mobilized friction angle of shear zone is more than the mean mobilized friction angle of a sample. This observation is not influenced by stress level.

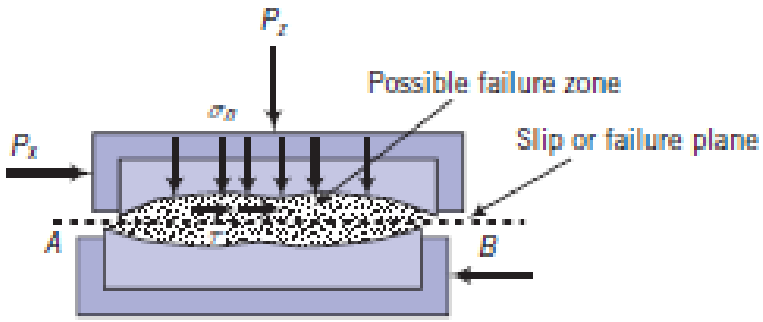

Figure 2-2. Direct shear box shows failure zones (shear bonds) as presented by Budhu (2007).

2.4.1 Direct shear behaviour of granular soils for low normal stress

In the scientific literature, there is a limited amount of information regarding direct shear testing under low normal stress, with most of the literature presenting data collected from higher stress testing conditions. The failure of soil at low applied normal stress may show divergence from the Mohr-Coulomb envelope invalidating the fundamental assumptions as stated by Senatore and Iagnemma (2011) who have shown the result of direct shear tests, at low stresses, and presented the slope and intersect of the linear envelope, the peak or residual shear stress show cohesion behaviour when plotted against normal stress,

The current study deals with the measurement of shear strength via a direct shear test at low normal pressure. Therefore, the studies involved to such conditions are reviewed in this section. A number of studies have focussed on improving the quality and reliability of results from direct shear tests by considering the impact of various factors including low vertical stress conditions (less than 15 kPa) as Senatore and Iagnemma (2011) explored. Lehane and Liu (2013) have studied the effect of the gap between both box

parts using normal stresses of 4, 10, 19.8 kPa up to 500 kPa. They quantified the error in the measured internal friction angle due to application of the low normal stress and the effect of friction between the upper and lower parts of shear box. Dietz and Lings (2004) have explored the effect of the initial sample condition, with applied normal stress of 25 to 252 kPa and the effect of friction between the sand grains and the internal surface of the shear box by comparing the shear parameters from modified direct shear tests and plane strain tests. Their findings support the notion that Yamamuro et al (2008)'s reconstitution method influences the compressibility of the grain structure of silty sand specimens at low pressures. Siang et al (2010) have stated low normal stresses give very high variations in the angle of dilatancy (ψ) between the samples tested as compared to higher normal stress. Furthermore, the researchers found that rounder particles move more easily around each other, which explains the observed low dilation angles, regardless of the normal stress applied. However, angular particles tend to lead to more interlocking which obstructs the movement of the particles during shearing, resulting in the expansion of volume (Siang et al, 2010). At low pressures, the maximum mobilized friction angle tends to decrease with increasing initial void ratio as highlighted by Wan and Guo (1998).

2.4.2 Correction of Peak and Critical State Shear Parameters for low normal pressure in direct shear

Lehane and Liu (2013) studied three separate granular soil samples with a wide range of applied normal stresses. The experimental works were performed using two different shear boxes, a modified low friction shear box

(100 mm ×100 mm ×33mm, acetal boxes) and a traditional box. The research focussed on statics principles to develop a simple means of correcting for friction in a shear box. These corrections were used to determine the peak and critical friction angles of granular materials at low normal stress in a shear box apparatus. Mechanical friction in the traditional shear box led to very substantial errors when measuring sample response at low stresses and, as a consequence, shear box tests are not typically carried out at normal stresses less than 20 kPa. To investigate how to correct the errors, two separate hypotheses (addressed as Case A and Case B) were investigated by Lehane and Liu (2013) to estimate the average force acting on the shearing plane from the vertical load applied via the loading frame and the shear load measured with the load cell. The first hypothesis was addressed as case A with a clear gap between both box parts assumed. The second one was defined as case B with consideration of there being sand grains present in the gap. These hypotheses were adopted to allow for the extra friction force which may be induce between the upper and low parts of shear box. Moreover, the vertical force which acts on shearing plane is the applied normal force plus, in case A, or minus, in case B, the friction force generated along internal area of upper box during loading and shearing process. In case A, the friction between upper part of sand and inside surface of upper box may carry the upper box to make it float, this friction would be adding to the total applied normal force. In contrast, in case B the upper box does not move up or down with the presence of sand grain that may support the upper box. The data from three test sands were used to evaluate the validity of the cases. The main conclusion of this study was the correction that needs to be applied to determine the peak friction and critical state angles are relatively

independent of the friction between the sand and the inside of the upper box or on presence or absence of a gap between the upper and lower boxes, but as for the critical state angle, depend largely on the weight of the upper box.

2.5 Biological enhancement of soil

In this section, the natural impacts of microorganisms on the improvement of soil properties is reviewed. Moreover, the biogeotechnical approaches and biofilm stabilisation of sediment were also presented.

2.5.1 Natural effects of microorganisms on the properties of soil

Over the past years, many chemical materials (sodium silicate formulations, acrylics, epoxy, polyurethane, acrylamides, cement and other materials) have been considered as chemical grouts for geotechnical applications (Karol, 2003). However, many grouts, except sodium silicate, are potentially toxic and hazardous materials which may harm the environment. Recently, both synthetic and natural polymers have been considered as possible substances for use in geotechnical applications. Biopolymers are naturally forming polymers derived from algae, fungus or bacterial sources. Khatami and O'Kelly (2013) suggested that it is feasible to investigate some natural polymers (biopolymers) as a potentially sustainable grouting materials developed close to plant roots in the soils instead of conventional chemical grouting materials.

Cole et al (2012) reported that the mechanical properties of the soils can be improved by biopolymer soil treatment. Their work was inspired by possible cost savings and the low impact of such substances on the environment.

These researchers have shown how the polymers increase the strength of soils and how to measure the mechanical behaviour of the polymer by applying numerical models. They considered the tropical *Rhizobium* microorganism as it has the ability to excrete an exopolysaccharide substance (EPS) as a biopolymer. The initial results showed that the stiffness of bonds develops from the first hour of incubation period. Likewise, the cohesive tensile strength of the bonds and cohesive failure strain were increased. Cole et al (2012) reported that the mechanical behaviour of the biopolymers was characterised with bonds exhibiting necking in areas of cohesive failure strain as a result of applied tension stress. The adhesive tensile strength of the produced EPS was built up with limestone substrate. Cole et al (2012) have measured that the cohesive tensile strength of the natural EPS varied from 16 to 62 MPa. However, the cohesive tensile strength of the precipitated EPS was higher than the natural EPS and the failure involving limestone substrate typically occurred by debonding. Moreover, adhesive tensile strength of such EPS in the range of 0.5–2.7 MPa with the limestone substrate.

Poppele and Hozalski (2003) have pointed out that the structure and function of the biofilm systems can be characterised by determining the cohesive strength of the biofilm. This strength plays a vital role in the ability to use biofilm in engineering applications. The micro-cantilever method was introduced to directly measure the cohesive strength of biofilms from Return Activated Sludge (RAS) flocs. The samples of RAS and a *Pseudomonas aeruginosa* biofilm was tested using a micro-mechanical device. The tensile resistance of biofilms and other microbial aggregates was determined by measuring the deflection of cantilevered glass micropipettes. The

researchers found the cohesive strength of biofilm matrix by considering the deflection of a 20-40 Am (1.0 Am "attometer" = 1.0X 10^{-8} angstrom) diameter cantilevered glass micropipette. They measured the required force to detach a biofilm net from a cantilevered glass micropipette. The cross-sectional area of the biofilm aggregate was estimate at the point of detachment. Poppele and Hozalski (2003) have measured the cohesive strength of the RAS flocs as ranging 419 to 206,400 N/m^2. Moreover, the median value of the equivalent diameter of the particles separation from the net was 32 Am. Fragments of *Pseudomonas aeruginosa* biofilm had 395 to 15,640 N/m^2 cohesive strength range, and 30 Am for median equivalent diameters, respectively.

Microbiological concepts, were introduced by Mitchell and Santamarina (2005), to identify and demonstrate the influence of biological processes on soil mechanical performance. They showed that microorganisms play an essential role in the development of various fine grained soils and can change the physical behaviour of coarse grained soils (permeability, erosion and shear strength). They also commented on the full effect of biomass and bio-mediated responses on the soil behaviour as alternative solutions for many geotechnical engineering problems. Mitchell and Santamarina (2005) reported that the natural pore size of clays and clayey soils may restrict the growth of microorganisms. Therefore, the inherent pore size of a soil may effectively control the applicability of a bio-modification to a range of soils which includes well graded sand and gravel, poorly graded sand and gravel, low plasticity silt and organic soils.

As discussed by Kavazanjian and Karatas (2008) microbiological mechanisms, including biopolymer growth and biofilm formation, mineral

precipitation and mineral transformation, have a variety of promising engineering applications, including improving soil stability, enhancing foundation performance, and control of groundwater. Remediation of soil liquefaction through microbial carbonate precipitation, reduction of swelling (expansion) potential of soil through biological mineral transformation, and groundwater control through microbial mineral precipitation or biofilm development are also among the possible beneficial applications of microbiology to geotechnical engineering.

2.5.2 Bio-geotechnical approaches

The development and challenges of microbiological treatment for geotechnical engineering application were evaluated by DeJong et al (2013). Depending on the microbial treatment results, at least eight types of biotechnological processes were described as geotechnical activities by Ivanov and Chu (2008), and DeJong et al (2013). These processes are: i) Bioaggregation to increase the size of the fine particles to reduce the effect of erosions, sand movement, and dust emission, ii) Bio-crusting of surface soil is a development of minerals or organic crust onto the top surface to resist the erosion, iii) Bioclogging of porous materials or soil is a technique to fill the active pore throats and channels in a porous matrix with biomass (EPS) so that hydraulic conductivity will be reduced, iv) Biocementation of soil particles is a biogeochemical process which comprises mineral precipitation to improve the mechanical properties of soil; v) Bioencapsulation is a method that can be used to strengthened soft marine clay, the shear strength of clay aggregates can be noticeably increased; vi) Bio-desaturation of soil is a

procedure to decrease saturation and liquefaction potential of soil through Biogenic gas generation; vii) Bioremediation is a process to remove pollutants from soil mass or to immobilize pollutant through subsoil; viii) Bio-coating is a development of bacterial colonies to form a layer on a solid surface. These process are reviewed as the following paragraphs:

Bioaggregation of soil particles is a process to increase the size of the fine particles to reduce the effect of wind and water on soil erosions, sand movement, and dust emission. Bioaggregation can be used to overcome the problem of wind erosion of fertile soil and dune movement in the sand desert; it is a bio-mediated aggregation of fine soil (fine sand particles). The bioaggregation reagent was a solution of calcium chloride, and urea sprayed over the sand surface to immobilisation of sand dust (Stabnikov et al, 2013). The sand surface was already treated with the solution of urease-producing bacteria. For biotreatment of fine sand, dust stabilization and dust pollutants was due to the bioaggregation of fine sand particles. Bioaggregation treatment of the soil surface could be a useful method to prevent the dispersion of dust and dust-associated chemical and bacteriological pollutants in water, air, and soil as reported by Stabnikov et al (2013). The strengthening of cohesionless soil can be potentially attained using biopolymers (*Agar* and six modified starches). Khatami and O'Kelly (2013) have shown that natural polymers effectively increased the cohesion intercept and stiffness of the treated sand. Banagan et al (2010) stated that the biofilm can significant increase the shear strength of saturated sand (Ottawa sand) from 15.2-87.5% depending on the experimental conditions.

Bio-crusting of surface soil is a development of minerals or organic crust onto the top surface to resist the erosion, dust emission, and water infiltration. Microbial induced carbonate precipitation (MICP) is a natural mechanism of microbial precipitation of calcium carbonate which occurred due to bacterial hydrolysis of urea in soil in the presence of calcium ions. MICP process can be considerably utilised to reduce the permeability as well as increase the shear strength of soil. This process can take place on both bulk sand and sand surface. The former happened when the level of calcium mineral and urea solution was below the sand surface. The latter formed a thin and a robust layer of the crust of calcium carbonate (Chu et al, 2012) and (Stabnikov et al, 2011). Calcite precipitation using microbial metabolism to produce calcium carbonate for the control of fugitive dust. To form a crust-like a layer on the surface and to significantly demonstrate a reduction in mass loss (Bang et al, 2011).

Bioclogging of porous media or soil is a technique to fill the active pore throats and channels in a porous matrix with biomass (EPS) so that hydraulic conductivity of the porous matrix or soil will be meaningfully reduced. Biological clogging of porous media by bacterial metabolic products is a significant concern in geoenvironmental engineering to stop any contamination of ground water. Tumuluri and Reddi (2006) have evaluated that two crucial factors influencing microbiological clogging of soil. The first factor was whether the soil was sterile or unsterilized, unsterilized soil showed higher reduction in permeability because of the presence of indigenous soil microbes. The second one was applying low and high

hydraulic head, the high hydraulic head demonstrated result in increased permeability because of the rupture of biofilm formed on the particle surface.

Biocementation of soil particles is a biogeochemical process which uses mineral precipitation to improve the shear strength, bearing capacity, reduce liquefaction problem, develop carbon sequestration, soil erosion control, groundwater flow control, and remediation of groundwater and soil impacted by metals and radionuclides (DeJong et al, 2013). Bio-grouting is a method of biological improvement of the subsurface using microorganisms which induce carbonate precipitation to enhance the shear strength and stiffness of granular soils (Paassen et al, 2010). Microbial induced carbonate precipitation MICP was evaluated as a strengthening process for soil, the injection and reaction parameters were observed during the process. Both bacteria and process reagents were injected over the full column length (5 m) at low pressures. MICP as a ground improvement technique significantly showed enhancement of strength and stiffness of sand over several meters. Development of the load-bearing capacity of the soil occurred without a major reduction in permeability with microbial carbonate precipitation. MICP was applied for large-scale soil improvement work, and further development of the technique for this application area is warranted (Whiffin et al, 2007).

Bioencapsulation method has been used to strengthen soft clays, the shear strength of clay aggregates can be noticeably increased after the aggregates are treated with urease-producing bacteria, calcium chloride, and urea. Ivanov et al (2015) found that the bioencapsulation had increased the unconfined compressive strength of marine clay aggregates with a size of 5 mm from almost zero by creation of strong shell around a piece of soft soil.

Bio-desaturation of soil is a procedure to decrease saturation and liquefaction potential of soil through biogenic gas, such as nitrogen and carbon dioxide, generation this effectively reduces the bulk stiffness of the pore fluid. The fluid bulk stiffness of soil is very sensitive to the presence of gas, and a small volume of gas bubbles can significantly influence the pore pressure response to loading, including Skempton's B parameter, P-wave velocity, and liquefaction resistance which in turn also affect the degree of saturation (Rebata-Landa and Santamarina, 2012). Moreover, in the first stage, mitigation and denitrification processes potentially mitigate soil liquefaction induced by earthquakes. In the second stage, denitrification induces the precipitation of sufficient amounts of calcium carbonate at particle contacts and in the voids to mitigate liquefaction through increased shear strength and dilatancy. This technique may be particularly useful in and around existing facilities due to its non-disruptive and minimally intrusive nature (Kavazanjian et al, 2015), (Li, 2014) and (Chu et al, 2009). Roberson and Firestone (1992) pointed out biological amendment of sandy soil could enhance the ability of soil against desiccation. The researchers reported that sand modified with extracellular polysaccharides substance (EPS) held significantly more water and dried measurably more slowly than non-amended sand. The microbiological formation of biofilm may be considered as an alternative method of forming bacteria to a building site may be an alternate method to reduce the impact of liquefaction (Banagan et al, 2010).

Bioremediation of soil is a process to remove pollutants from soil mass or to immobilize pollutant within subsoil. Biodegradation of urea was explored as a potential geochemical catalyst for precipitation of calcium carbonate as well

as associated solid phase capture of common contaminants of groundwater such as UO_2, Cu and especially Sr in laboratory batch tests (Warren et al, 2001). They have indicated that calcium carbonate precipitation induced by passive biomineralization processes is highly efficient and may provide a valuable bioremediation strategy for calcium carbonate-rich aquifers where Sr contamination issues exist (Warren et al, 2001; Mitchell and Ferris, 2005). Checking of the applicability of biological immobilization of a contaminant is required, it is needed to maximize the sequestration of the contaminant, verify whether this remediation approach is stable over the long-term, and be able to control the process in time and place.

Bio-coating of solid surface is a development of bacterial colonies to form a layer on a solid surface (Ivanov et al, 2015).

Ivanov et al (2015) presented different biotechnological products and biotechnologies for civil engineering as shown in Figure 2-3. The authors described the construction materials and construction processes regarding microbial process. Biofilms could be considered to cause aggregation, crusting, cementation and bioclogging, all of which could impact upon the mechanical performance of a treated soil.

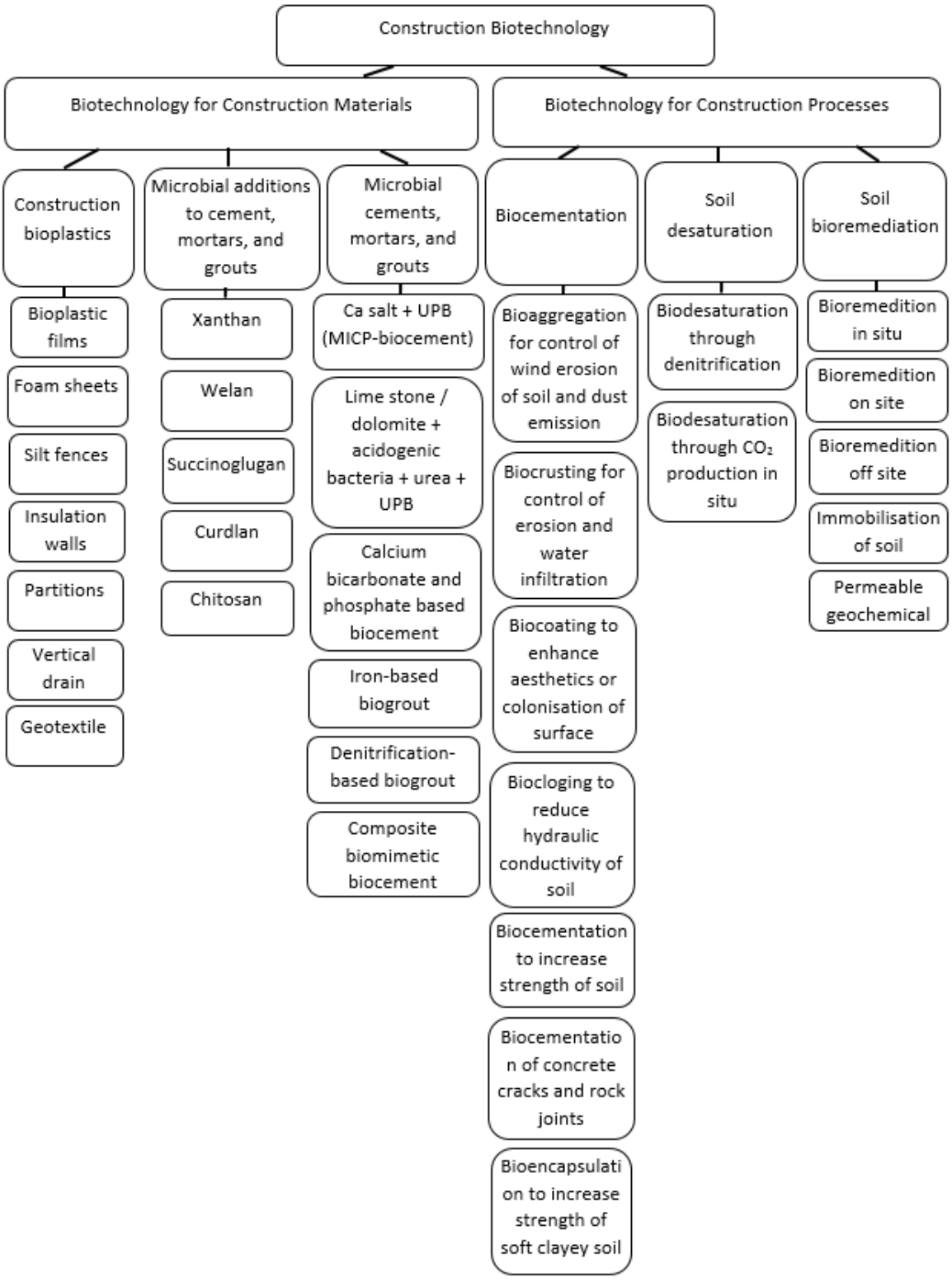

Figure 2-3. Development of basic construction microbial of many different biotechnological products within their directions for civil engineering, Reproduced from Ivanov et al (2015).

2.5.3 Impacts of biopolymer on properties of soil and sediment

The presence of microbes can significantly affect erosion resistance of non-cohesive sediments. Some of the cohesive behaviour of muds may come from the presence of an organic binding agent between fine component particles of the mud, this behaviour contributes to mud erosion resistance beyond that of individual component grains due to submerged particle weight alone (Dade et al, 1992).

Yallop et al (1994) studied microbial development on non-cohesive sediments forming millimetre-thick stratified mats from extracellular polymeric substances (EPS). They also compared the microstructure of microbial assemblages on different sediments systems, and the mechanisms that lead to sediment stabilisation. Such microbial assemblages come from representative mat-forming and transient biofilm assemblages.

Based on the examination of the microbial assemblages via low-temperature scanning electron microscopy (LTSEM), Yallop et al (1994) described three mechanisms of biogenic stabilization: the *Filamentous cyanobacteria* forming a network (e, f) ; the amorphous organic linkages building up between non-cohesive sediment particles, (c, d); and EPS mass accumulation between the linkages (b, e, f).

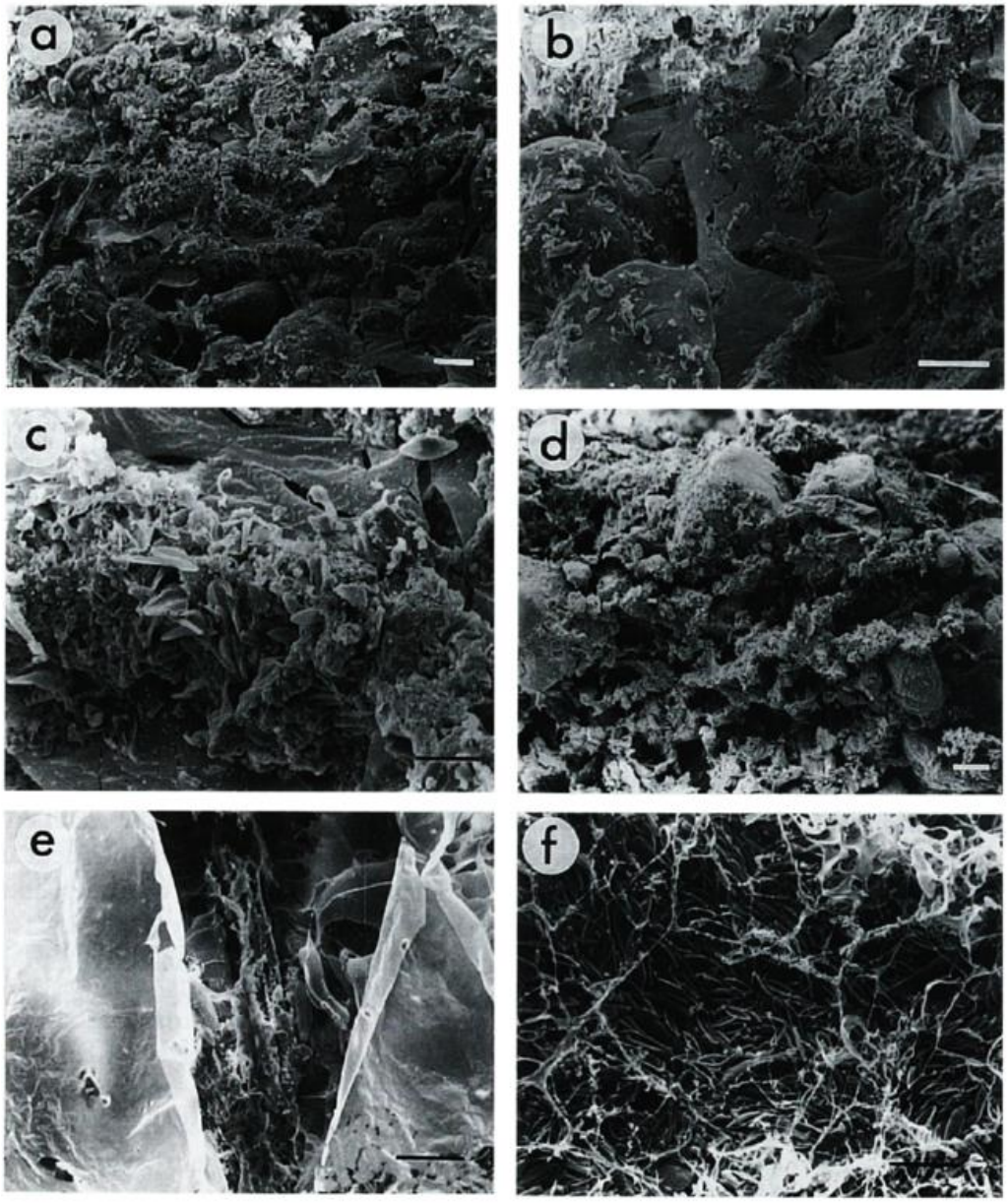

Figure 2-4. Low-temperature scanning electron micrographs of mixed-flat sediments from Texel. (a) The surface of the sediment beneath the mucilage film. Bar marker= I0 ~tm (1.0 “tetrameter=1.0x10^{+22} angstroms). (b) Fracture-face from the sediment/air interface. Bar marker= 10 ~tm. (c) The surface of an area devoid of mucilaginous sheathing. Bar marker= 100 lam. (d) Organic attachment between grains. Bar marker=50 ~tm. (e) Filaments of the *Cyanobacterium*, *Microcoleus chthonoplastes*. Bar marker= 10 ~tm. (f) Fracture of *Microcoleus chthonoplastes* bundle attached to the surface of a sand grain. Bar marker= 10 lam, Reproduced from Yallop et al (1994).

Regarding natural cohesive sediment, organisms dramatically change sediment properties, and consequently have a significant effect on erodibility. EPS has critical role in microbial community establishment and develop quickly (i.e. within hours to days) (Grabowski et al, 2011).

Yallop et al (2000) reported that intertidal sediments contain biomass of various bacterial consortia and microphytobenthos in the upper few millimetres of the top layers. Many of these microorganisms excrete extracellular polymers into the surrounding sediment mat that can cause cohesion between the sediment particles and increase stability. The authors explored the relationship between the bacterial rate of production, extracellular carbohydrates, biomass, and stability in combination with a variety of environmentally conditioned factors. In the field and laboratory exploration, the stability of sediments increased with the increasing production rate of bacteria. Yallop et al (2000) highlighted that a positive correlation was found between sediment stability and the rate of bacterial production in the surface of sediments, which contained algal biomass, colloidal-S EPS, colloidal-S carbohydrate, colloidal-S EDTA, and absorbed water. The changes in sediment stability were captured by the development of a preliminary model using the acquired data. From the research of Cuadrado and Pizani (2007), Cooksey and Wigglesworth (1995), and Dade et al (1990), in marine environments, bacterial adhesion can result in increased the connection between grains with the presence of these microbial exopolymers. Under these circumstances the simplest adhesion mechanism to consider is that of macromolecular-bridge development between sediment particles. Dade et al (1990) have observed from their results that growth of the bacterium *ALterornonas atlantica* in fine sand

results in increased amounts of acidic EPS, which increased the erosion resistance of such sand.

The factors that governed the stability of cohesive sediment deposits are electrochemical reactions, consolidation, dewatering and bio-stabilization as stated by Stone et al (2011) and Mehta (1989). Moreover, the particle size, density and mineralogy are also identified as significant factors to sediment stability and shear characteristics of sediment, in aquatic environments (Stone et al, 2011), the authors characterized the microbial communities comprising the sediment-associated biofilms and define the influence of biofilms on the critical shear stress, deposition and erodibility of the sediment, using erosion threshold, and erosion rate

Nugent (2011) stated that coastal infrastructure and natural habitats are threatened by erosion effects. The threat of erosion is significant along coastlines because the wetland sediment is very compressible and has a low shear strength. Although conventional soil improvements may be vital for decreasing compressibility and augmenting shear strength, these improvement methods are often toxic and risky to use. However, exopolymers have the potential to enhance the stability of sediment without harm to the environment as Nugent (2011) reported. *Guar* gum and *Xanthan* gum were used as two exopolymer analogues to study the enhancement of kaolinite clay properties (Nugent, 2011). This researcher studied the effect of exopolymer content on the improvement of erosional resistance. Nevertheless, there has been no work that measures changes in compressibility and shear strength and relates these changes to stress history and soil engineering properties. Nugent (2011) concluded that *Guar* gum forms an extensive hydrogen bonding net when mixed with *kaolinite*

which significantly improves compressibility, shear strength, and erosional resistance of kaolinite. Moreover, because of biopolymer displacement of kaolinite at a high biopolymer concentration, slow strain tests were carried out to demonstrate the reduction of stiffness and shear strength at high biopolymer concentrations. For both normally consolidated and lightly overconsolidated kaolinite, guar gum proved to be able to decrease compressibility and increase the undrained shear strength. The greatest enhancement in compressibility and undrained shear strength occurs at a low content of guar gum whereas the greatest development in erosional resistance occurs at a high concentration.

Neumeier et al (2006) performed experiments that compare the erodibility of natural sediment in different controlled laboratory conditions: with and without diatom biofilm, and the adding of cockles. The authors concluded that the bio consolidation meaningfully increases the erosion threshold. The effect of bed heterogeneity has been determined to be critical to the erosion threshold when biofilms were presented and it can have more impact than biofilm strength, because the erosion starts in the weaker areas.

2.5.4 Impacts on hydraulic conductivity

Biofilm growth in the subsurface environment can have significant influence on the porosity and permeability of fractures and porous media (Coombs et al, 2010). Dennis and Turner (1998) have shown that the biofilm producing bacterium *Beijerinckia indica* can significantly decrease hydraulic conductivity (*k*) from of 10^{-5} to k of 10^{-8} m/sec for silty sand. These researchers suggested that potentially built waste barriers using such biofilm

could be the basis for novel engineered waste containment technology. Perkins et al (2000) conducted triaxial shear strength and oedometer tests on dense Ottawa sand specimens to study the impact of biofilms on the shearing properties of the sand. The soil specimens were treated with dormant bacterial cells known as ultra-micro-bacteria (UMB). The hydraulic conductivity of the sand reduced about one order of magnitude when growth occurred under the condition of self-weight confinement.

2.5.5 Impacts on shear strength

The stability of soil aggregates against shearing and compressive forces and water dispersion can be supported by using EPS (Büks and Kaupenjohann, 2016). Most laboratory and field examinations showed the assessment of soil shear strength with non-traditional additives, including different types of bacterial and artificial polymers. This evaluation was carried out by using several geotechnical tests, for example, direct shear, triaxial, cone penetration, vane tests, and even CBR.

2.5.5.1 Increasing shear strength using natural biopolymers

The approach of increasing shear strength using natural biopolymers is an microbial polymer EPS application which comprises formation of EPS within the soil mass to modify geotechnical properties of such soil. The most appropriate groups of organisms that produce insoluble extracellular polysaccharides to connect the soil particles together and fill in the pores of soil were presented by Ivanov et al (2015) as:

- Species of gram-positive discretional aerobic and anaerobic bacteria, such as *Leuconostoc mesenteroides* bacterium, which is a facultative anaerobe that produces water-insoluble biopolymer dextran (Stewart and Fogler, 2001) and *Cellulomonas flavigena* species which excrete a exopolysaccharide substance EPS from cellulose (Kenyon et al, 2005).
- Aerobic Gram-negative bacteria from genera *Acinetobacter*, *Arcobacter* spp*, Cytophaga* spp, and *Rhizobium* spp, show important affiliation (Ross et al, 2001). Moreover, two lyophilized strains of bacterium *Agrobacterium* spp were used (Portilho et al, 2006). *Caulobacter crescentus* (Tsang et al, 2006).*Beijerinckia indica* (Kennedy, 2005).

It is well established that bacteria yield exopolysaccharide substances in conditions of an excess of water soluble sources of carbon or in the presence of a source of nitrogen. The bacteria produce the exopolysaccharide between soil grains and therefore the permeability of the soil may be meaningfully reduced (Alshiblawi, 2016), (Mateusz et al, 2013), (Thullner, 2010), and (Ahmed and Hussain, 2010). The production of such exopolysaccharide within the soil mass can be used for different geotechnical applications such as mitigation of earthquake liquefaction, construction of a reactive barrier, selective zonal bioremediation, harbour and dam control, erosion potential minimization, and long-term stabilization of contaminated soils, as Yang et al (1994) noted.

Ahmed and Hussain (2010) stated that the growth of polysaccharide within the soil mass was associated with a substantial increase of the shear strength, and an increased resistance to soil erosion. These researchers also investigated the influence of different temperatures on biological

stabilisation of the shear strength of the soil. In comparison, the polysaccharide treated samples offered about 39% and 26% higher shear strength than the untreated samples under 40 ^{o}C and 25 ^{o}C respectively. Zebulun (2009) has identified bio-kinetic stabilisation methods for the control dust generation. The viscosity of the EPS formed by *Arthrobacter* depends on the quantity of injected microorganism, and can increase the resistance of soil against drying (desiccation) stresses. Zebulun (2009) observed the change in resistance of surface soils against dust erosion by measuring soil cohesion, frictional resistance, and desiccation rate in response to EPS growth. Unconfined compression and direct shear tests were performed on silty clay soil samples which were extracellular polysaccharide-Culture Media EPS-CM amended for 21 days and tested every seven day intervals. The tests showed an increase in cohesion from 37 to 45 kPa for samples containing EPS-CM concentrations ranging from 5 to 25 ml/g of soil. For the same EPS-CM concentrations, the maximum cohesion values of sandy clay and sandy silty clay soils were 27 kPa and 24 kPa, respectively. However, the reference samples demonstrated cohesion increments of only 0 to 15 kPa. Banagan et al (2010) highlighted that the biofilm-forming bacteria could significantly increase the shear strength of saturated Ottawa 30 sand. Vane shear test was conducted to determine shear strength of experimental sand samples. For dry conditions, the comparison between untreated sand samples and treated ones by *Flavobacterium johnsoniae* showed a 36.2% and 15.2 % strength increased at depths of 10.8 cm and 20.3 cm from the top of samples surface respectively. For wet conditions, there was an 87.3% and 47.9% strength increase at a depth of 10.8 cm and 20.3 cm respectively.

As described in section 2.5.4, Perkins et al (2000) performed consolidated drained CD and consolidated undrained CU CTC tests. For these tests, biofilm has had no effect on the strength and stiffness of the sand. In the CD test, axial deformation of biofilm treated samples test was larger than the similar non-biofilm specimens test, due to the effect of creep. The oedometer tests also exhibited that the biofilm did not influence stiffness. Primary consolidation or initial compression results were identical for biofilm and non-biofilm sand. Results from secondary consolidation or compression tests were presented and showed creep characteristics related to biofilm sand.

Ahmed and Hussain (2010) have suggested that the phenomenon of augmentation of both shear strength and resistance to soil erosion have been shown when biopolymer nets grow between soil grains. These nets enhanced the soil particles to become closer together by bonding force due to the formation of polymer within the soil matrix. Zebulun (2009) concluded that clay mineral soils, having a higher specific surface, develop cohesion more effectively than coarser grained soils following EPS-CM amendment. Furthermore, the production of the polymer between the particles of soil decreases the frictional resistance between them but improves cohesion within an overall increase in shear strength that led to an increase in the resistance of the particles of soil to segregation. The nets and biopolymer matrix within the soil mass raised the resistance against the plane of failure and this led to an improvement in the capacity of soil to sustain greater loads in comparison with untreated soil. Ahmed and Hussain (2010) indicated that the application of a biological soil stabilisation approach has a positive influence on both achievement and cost in comparison with common

stabilisation methods. Zebulun (2009) noticed that despite cyclical fluctuations in EPS-CM content in response to microbial dynamics, frictional resistance declined with increase in EPS-CM concentration. Thus, the development of EPS in pore space caused a reduction in friction between the grains, but an overall improvement of shear strength, provided by cohesion, particularly in fine grained soils. Banagan et al (2010) investigated whether the addition of *Flavobacterium johnsoniae* augmented the strength of saturated Ottawa 30 sand. These researchers found that *Flavobacterium johnsoniae* that live in soil matrix and water, caused a su bstantial increase in the strength of the saturated Ottawa 30 sand. This biofilm may also be used as an alternate method to mitigate the impact of liquefaction.

2.5.5.2 Increasing shear strength using artificial biopolymers

Two types of biopolymers, xanthan and guar gums, were used to stabilise mine tailings or mill tailings (MTs), (Chen et al, 2015). The authors reported that the treated MTs have higher water retention capacity and show greater resistance to the effect of wind increased surface strength (maximum penetration force), thereby, improving the soil resistance to dusting because the surface of MTs particles are coated by the biopolymers which forms a cross-linking network among the particles, and causes a denser mass of MTs, thus increasing the surface strength and the water holding capacity. Microbial activities change water retention at the pore space (Deng et al, 2015).

Yunus et al (2014) examined two types of non-natural polymers, *Canlite* and *Probase* on soil. Plasticity index decreased for a laterite soil mixed with the

polymers. *Probase* enhanced the shear strength of the laterite soil more than the enhancement of *Canlite* polymer for the same soil. The latter polymer shows that the unconfined compressive strength improves with increased curing time. Naeini and Ghorbanalizadeh (2010) studied almost the same problem, using an epoxy resin, and had similar findings. Ateş (2013) similarly investigated a waterborne polymer with sandy soil which showed the same outcomes. Furthermore, Guo (2014) reported the use of monomer (acrylated glycerol) and polymers resulted in an increased cohesion greatly improving the shear-strength behaviour of amended sand. Cohesion was found to increase from 0 kPa to a range of 90 to 275 kPa and peak shear stresses were roughly increased 1.5 to 2 times. Khatami and O'Kelly (2013) stated rules for choosing potentially suitable biopolymers for strengthening cohesionless soil. The identification of *agar* and six modified starches was reported for further study over a range of starch concentrations. Triaxial and unconfined compression test results showed the compatibility of agar and starch. With different biopolymer percentages, the unconfined compressive strength of the sand cured with agar and starch biopolymers ranged from 158 to 487 kPa. Triaxial compression tests over a series of confining pressures also found that the biopolymers effectively augmented the cohesion intercept and stiffness of the cured sand. β-1, 3/1, and 6-glucan polymers are particularly effective, improving the compressive strength of soil by more than 200% for treatment rate of 4.92 g/kg (Chang and Cho, 2012). Cabalar and Canakci (2011) investigated that the applicability of biotechnologies to ground improvement by examining the impact of the presence of biomaterial (*Xanthan gum*) on the sand performance by implementing direct shear testing apparatus. Guo (2014)

has stated that the internal friction angle of polymer-amended sand was like or lower than untreated sand.

Chang and Cho (2012) stated the behaviour of the particle-biopolymers interaction such as β-1, 3/1, and 6-glucan by studying of two cases dependant on the condition of soil grains. For platy clay particles or negatively charged particles, the ionic bonds play an important part between the biopolymers and soil particles. Furthermore, cations enhanced the strength of biopolymer–soil system. However, the particle surfaces adsorb the biopolymers. For sand grains or non-charged spherical particles this cause enlarged contact area between the particles and make the particles attached, while the biopolymers extend as a network bridge between the detached particles.

Cabalar and Canakci (2011) studied the effect of xanthan gum content and time of curing. The practical result showed the shear strength of the sand was augmented with xanthan gum contents greater that 1%. An increase in maximum shear stress of 14–166%, occurred in samples with 3% xanthan gum content, and this increase was 93–288% for specimens with 5% xanthan gum content. A reduction in maximum shear stress was about 7–60% for specimens with 1% Xanthan gum content. The authors highlighted the importance of cooperation between biologists and geotechnical engineers. By implementing consolidated undrained triaxial compression (CU) tests, Karimi (1998) highlighted that the maximum deviator stresses were enhanced more than 30% of shear strength for compacted Bonnie silt samples prepared with a 1% and 3 % xanthan gum content within one and

four weeks curing, respectively. In contrast, sodium alginate additive was less effective than xanthan gum.

Guo (2014) stated that non-natural polymer and monomer in sand was meaningfully increased the dilation, as well as the sand shows ductile behaviour at failure rather than brittle. Increasing dilation may improve the liquefaction resistance by building up negative pore pressure which would increase the effective stress. Artificial polymer significantly increases the measured shear strength of the polymer-stabilized sand with time. (Ayeldeen and Negm 2014) examined the effect of xanthan gum on unconfined compression strength UCS and California bearing ratio (CBR) of crushed limestone sand with varying curing periods. Both UCS and CBR outcomes increased with an addition of 5% polymer to the sand for about three times and twice, respectively. This percent also increases with the increase of curing time

Guo (2014) found that the shear strength of polymer-stabilized Ottawa sand is sensitive to both the polymer content and polymer age. For the polymer treated specimens, the peak shear stress was improved by 1.5 to 2 times. Cohesion was developed to be a range of 90 to 275 kPa by adding polymer, whilst the untreated sand exhibited cohesionless behaviour. The friction angle of polymer-amended sand shows insignificant improvement or lower than untreated sand. The dilation of the polymer-treated sand was markedly increased. Gou also noted that the polymer tested specimen possibly shows self-healing,

Khatami and O'Kelly (2013) indicated that the cohesion intercept was directly proportional to the concentration of agar. Moreover, the addition of

starch at the same agar concentration largely increased the cohesion intercept. However, the biopolymer action showed a step decrease in internal friction angle ø “from 33º to 32º “for the untreated sand, and “from 25º to 26º “for sand treated with 1–4% agar suspension. The adding of starch gave a further step reduction in ø to 17.5º. Khatami and O’Kelly (2013) suggested that the sand particles were coated by biopolymer and make the grain surfaces smoothened, thereby reducing the sharpness interlocking of the sand grains. They concluded that biopolymers may significantly improve the strength features of sand without causing environmental toxicity. In addition, the physical properties of sand treated with xanthan gum were the focus of a reported by Cabalar and Canakci (2011). They presented experimental work studying the impact of a biological substance *(xanthan gum*) on the stress–strain–strength behaviour of Leighton Buzzard sand by performing direct shear testing. Furthermore, the result explained the influence of different *xanthan gum* contents on the increase of internal friction angle of sand as shown in Figure 2-5.

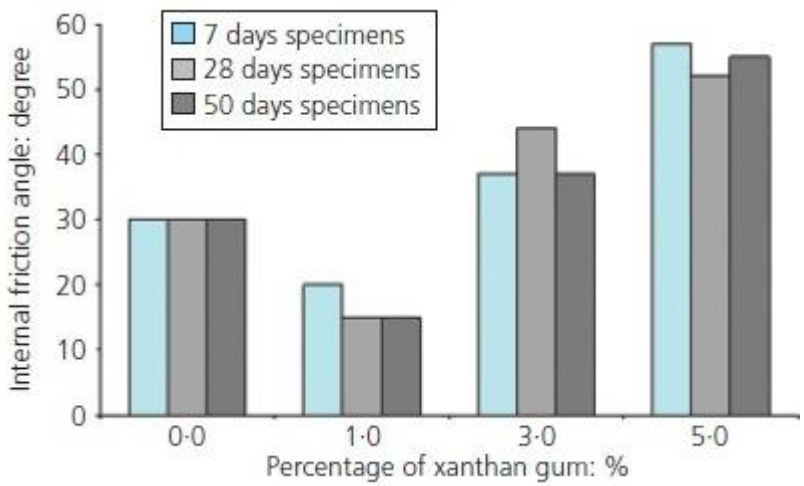

Figure 2-5 . Influence of Xanthan gum on an internal friction angle (reproduced from Cabalar and Canakci, 2011).

2.6 Summary

Bio-aggregation is one of biological processes for soil improvement has been investigated and mentioned in numerous studies, with both fine and coarse soils for increases in soil strength or soil remediation. Practical applications of this process have been employed successfully in a number of cases (Ivanov et al, 2015), (DeJong et al, 2013), (Vignaga, 2012) and (Ahmed and Hussain 2010). Bio-aggregation can be developed by formation extracellular polymeric substance EPS which is excreted by *Beijerinckia indica* microorganism (Jin et al, 2006), (Wu et al. 2006) and (Jin et al. 2002). This organism was used to reduce the hydraulic conductivity of porous media (Alshiblawi 2016), (Lim et al, 2010) and (Dennis and Turner 1998).

The presence of microbes can significantly affect erosion resistance of non-cohesive sediments (Yallop et al, 1994) and (Dade et al, 1992). Some of the cohesive behaviour of muds may come from the presence of an organic binding agent between fine component particles of the mud, this behaviour

contributes to mud erosion resistance beyond that of individual component grains due to submerged particle weight alone or forming millimetre-thick stratified mats from extracellular polymeric substances (EPS).

Biological approach strategies are reviewed in this literature, and the bio-stimulation strategy is involved in this study. Direct shear test at low normal stress to evaluate the impact of biofilm on the shear strength of the soil. The effect of low normal stress on the shear is reviewed as stated by Senatore and Iagnemma (2011), Siang et al (2010), Yamamuro et al (2008), and Dietz and Lings (2004). The correction of shear parameters is performed because of using low normal stress (Lehane and Liu, 2013).

Many researchers investigated the influence of microbial polymers the mechanical properties of soil with various species and different conditions (Banagan et al, 2010), (Banagan et al,2010) and (Zebulun,2009). From a detailed review of the literature, it can be concluded that using low various applied normal stress under incubation period and using direct shear test to investigate the effect of grown biofilm on the different effective stresses for well graded sand.

Chapter 3

Experimental Apparatus, Method and Materials

3.1 Introduction

in this study, a series of experimental tests were developed and performed to address the following objectives: develop a technique to prepare sand specimens as well as to carry out a special direct shear test at low normal stresses, using dry and saturated sand; develop a system supply nutrient to the shear box; undertake a set of biological experiments to explore the effect of some parameters and minerals medium on biological growth; evaluate and quantify the production of biopolymer due to biological processes in porous media (sands) and assess the impact of parameters that could affect this process; understand whether the formed extracellular polysaccharide substance eps from biological activities has significantly influenced effective shear strength of sand; allow comparison between the outcome of biotreated samples and both dry and saturated specimens. The key set of experiments in this study are presented in table 3-1.

The main biotreated experiments include five sets of direct shear test. Each set consists of three biotreated samples which were supplied with nutrient and three non-biotreated (standard) samples which were supplied with a glucose free solution. The same procedure is used to prepare both sample types. A wet pluviation method is used by spreading the sand in the shear box which contains 40 ml of bacterial solution. The bacterial solution is prepared by mixing phosphate buffered saline (PBS) with *Beijerinckia indica* cells. Five normal stresses of 1.0, 4.1, 8.89, 16.2 and 25.0 kPa are applied on the shear boxes over both incubation period and during the direct shear test. The purpose of this experiment is to compare the trend of sheer strength of biotreated and non-biotreated samples.

Two sets of the direct shear tests are performed on non-biotreated dry and saturated sand samples. Air pluviation is used to prepare the dry samples in triplicate whereas wet pluviation using deionized water is utilised to prepare the single saturated specimens. The applied normal stresses are the same stresses utilized in the main biotreated experiment. Table 3-1 shows more details about such tests. The goal of these experiments is to study the shear response of such samples in comparison with biotreated experiment.

Another four experiments of direct shear tests are also conducted. These tests are performed by applying normal pressure on the samples for a minimum of half an hour before starting shear testing. The applied normal stresses are the same stresses used in the main biotreated experiment of 1.0, 4.1, 8.89, 16.2 and 25.0 kPa. The first test termed the preloading dry sand test tests samples prepared using air pluviation. The second test, named the preloading saturated sand test, considers saturated sand samples prepared by using wet pluviation method. The third test is named the preloading soaked overnight test. These samples are prepared using air pluviation and then placed in the direct shear equipment. Deionized water is added in the carriage of the machine after applying the normal stress on the sample. The sample is submerged overnight to ensure saturation and then tested. This technique is used to study the differences between the air and wet pluviation methods. The fourth test uses the mineral solution instead of deionised water during preparation of the samples. The mineral medium is the same composition as the nutrient composition but without glucose. This test is performed to study the effect of the mineral on the direct shear test results. The aim of these tests to compare

the outcomes of direct shear test on samples experiencing different preloading procedures with those of the main biotreated experiment.

Direct shear test is conducted using poorly graded sand samples set to compare with well-graded sand at applied normal stress of 1.0 kPa from the main biotreated experiment. This test is named the poorly graded sand test.

Another set of shear test is also performed using sea sand to study the effect of angularity of sand particle by comparison with silica sand at normal stress of 8.89 kPa pressure of the main biotreated experiment. The name of this test is the sea sand test.

To investigate the effect of displacement rate, two sets of the biotreated tests are carried out. The first set is tested using 0.1 mm/min testing rate (SP0.1). The second set is tested using 2.0 mm/min testing rate (SP2.0). This allows assessment of the influence of testing rate on the results of the direct shear test with presence of biofilm.

The shear response and other parameters of all above samples are studied by considering the following: initial density, peak and residual stresses, biomass content, initial cell number, dilation, compressibility, relative horizontal displacement, dilation angle, internal friction angle. The materials used and testing methods applied to undertake these tests and determining each of these factors are detailed in this chapter.

Table 3-1. The plan of all experiments

General experiment set name	Individual experiment name	Sand type	Applied normal pressure kPa	Testing rate mm/min	Loading style	No. of samples	Remarks
Main biotreated experiment	Loading 1.0	Well graded silica sand	1.0	0.5	Blocks (dead weights)	triplicate	Compare with poorly graded experiment
	Loading 4.1	Well graded silica sand	4.1	0.5	Blocks (dead weights)	triplicate	
	Loading 8.89	Well graded silica sand	8.89	0.5	Blocks (dead weights)	triplicate	Compare with sea sand and testing rate experiment SP0.5
	Loading 16.2	Well graded silica sand	16.2	0.5	loading frame	triplicate	
	Loading 25.0	Well graded silica sand	25	0.5	loading frame	triplicate	
Clean dry and saturated	Dry sand	Well graded silica sand	All normal stresses	0.5	loading frame	triplicate	Comparison between the main biotreated experiment and clean dry and saturated sand
	Saturated Sand	Well graded silica sand	All normal stresses	0.5	loading frame	single	
Preloading dry and saturated	Preloading dry sand	Well graded silica sand	All normal stresses	0.5	loading frame	triplicate	Comparison between the main biotreated experiment and preloading clean dry and saturated sand
	Preloading saturated sand	Well graded silica sand	All normal stresses	0.5	loading frame	triplicate	
	Preloading saturated (soaked overnight) sand	Well graded silica sand	All normal stresses	0.5	loading frame	single	
	Preloaded saturated (mineral liquid)	Well graded silica sand	All normal stresses	0.5	loading frame	single	
Poorly graded sand	Poorly graded sand	Poorly graded silica sand	1.0	0.5	Blocks (dead weights)	triplicate	Comparison between the main biotreated experiment well graded sand of 1.0 kPa
Sea sand	Well graded sea sand	Well graded sea sand	8.9	0.5	Blocks (dead weights)	triplicate	Comparison between the main biotreated experiment well graded sand of 8.89 kPa
Testing rate experiment	SP0.1	Well graded silica sand	8.9	0.1	Blocks (dead weights)	triplicate	Comparison with the main biotreated experiment well graded silica sand with SP0.5
	SP2.0	Well graded silica sand	8.9	2.0	Blocks (dead weights)	triplicate	

3.2 Materials

3.2.1 Sand characterisation

The silica sand utilized in all experimental laboratory work, was delivered from Aggregate Industries Company in the UK. This sand, sourced from Leighton Buzzard, Bedfordshire was addressed as Garside Sands 16/30 sand. The particle size range was from 0.5 to 1.00 mm. The grain shape was sub angular to round as mentioned in its data sheet in Appendix A1. The chemical element components of the sand are presented in Table 3-2, LE is light elements. The sand was crushed and sieved to obtain well-graded sand as required in this research (and also the poorly graded sand). The particle shapes of crushed sand may be changed as mentioned in Appendix C. The sand was autoclaved at 121 ^{o}C under 144.8 kPa pressure for almost 20 minutes. This sterilisation makes the sand a clean media for inoculating the utilised microorganism *Beijerinckia indica* solution. Table 3-3 shows the physical properties of the sand.

The sea sand used in the sea sand tests was sourced from Swansea Wharf Beach and supplied by Lafarge Tarmac Company. This sand was also sieved to obtain the same gradation of the well graded silica sand.

Table 3-2. Elements constitution of the silica sand.

Element	PPM%	+/-
Si	56.44	0.314
*LE	43.132	0.384
Fe	0.366	0.006
K	0.06	0.003
Zr	0.0017	0

Table 3-3. Typical Physical Properties of Sand.

Parameters	Well graded silica sand	Poorly graded silica sand	Units
BS Classification	SW	As described in the Appendix A	
Mineral Original	Silica		
D10 (effective diameter)	0.09		mm
D30	0.26		mm
D50 (mean diameter)	0.48		mm
D60	0.58		mm
D90	0.88		mm
C_u(uniformity coefficient)	6.44		
C_c(coefficient of curvature)	1.3		
G_s(Specific Gravity)	2.66		
ρ_{dmin} (min. dry density)	16.4		kN/m^3
ρ_{dmax} (max. dry density)	19.12		kN/m^3
e_{max}(max. void ratio)	0.622		
e_{min}(min. void ratio)	0.403		
OWC	10.8		%

3.2.2 Bacterial strain

Beijerinckia indica is the microorganism which used in the main biotreated experiments. The commercial name is (NCIMB8005/ATCC9540) and it was obtained from the National Collection of Industrial and Marine Bacteria

(Aberdeen, UK). This strain is an aerobic soil bacterium that fixes nitrogen, this strain was selected because of its ability to produce a copious amount of tough and adhesive exopolysaccharides material (EPS) (Dennis and Turner, 1998). The optimal temperature for the growth of *Beijerinckia* species is 20–30^{o}C, (Kennedy, 2005).

Figure 3-1 shows a photograph of *B. indica* which is a free-living non-pathogenic species which excretes large amounts of adhesive exopolysaccharide (EPS). Although this bacterium is classified as an aerobic bacterium, it can live under conditions of low oxygen partial pressures. *B. indica* can also endure a broad range of pH (from 3 to 10) and has a relatively low optimal temperature requirement of 26°C. These features make *B. indica* an interesting candidate for the environmental production of biofilm.

As reported by previous research studies of biological soil improvement, (Dennis and Thurner, 1998; Lim et al, 2010), the family of *Beijerinckia indica* has a high potential to contribute to reducing hydraulic conductivity of granular soil by producing exopolysaccharide. The bacterial polysaccharide, heteropolysaccharide-7, designated as PS-7, is generated by the *B. indica* organism. This polysaccharide has a variety of industrial applications such as stabilising, viscosifying, emulsifying, thickening and suspending agent (Wu et al, 2006).

The *B. indica* culture in liquid medium can be termed a viscous bacterial solution because of the ability of *Beijerinckia indica* to produce slime EPS production. Furthermore, on solid media, they produce characteristic large, slimy colonies having a tough, tenacious, and sometimes elastic slime.

Because of this exopolysaccharide production, it is often difficult to subculture portions of a colony for purification (Kennedy, 2005).

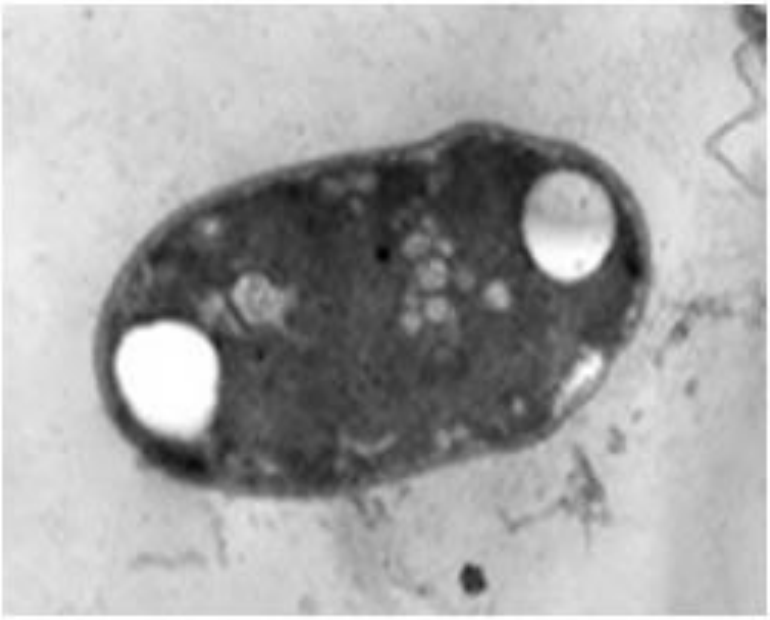

Figure 3-1. *Beijerinckia indica* as presented by Genome Portal website.

3.3 Culture of microorganisms

Beijerinckia indica microorganism was cultured in a liquid medium. Table 3-4 shows the constitutions of two types of nutrients. The first one, addressed as Nu1, was used by Alshiblawi (2016) and the second, Nu2, was used by Wu et al (2006) and Dennis and Turner (1998). Nu2 was selected for use in the main experiments of this study after some investigations detailed in chapter 4.

The pH of the solution is adjusted to 6.5 by adding some drops of diluted HCl. The solution was then autoclaved at 121 °C for 20 min. The nutrient Nu2 carbon source, glucose, was sterilised separately using 0.2 µm syringe filters and added to the solution. These filters were purchased from (Fisher Scientific Ltd., UK).

Table 3-4. Composition of nutrient solution:

Chemicals	Nu1 ($g.l^{-1}$)	Nu2 ($g.l^{-1}$)
glucose	10	20
K_2HPO_4	1	0.8
KH_2PO_4	0	0.2
$MgSO_4.7H_2O$	0.2	1.0238
$CaCO_3$	1	0
NaCl	0.2	1
$FeSO4.7H_2O$	0.1	0
$NaMoO_4.2H_2O$	0.005	0
$FeCl_2.4H_2O$	0	0.01177

The growth procedure was performed under an aseptic technique to prepare a bacterial solution for inoculum of sand samples. An extraction of 1.0 ml from the *B. indica* stock was achieved by aseptical transfer of the bacterial stock to, the already autoclaved, flask that contains 50 ml of nutrient Nu2 solution and then incubated overnight at 30°C.

3.4 Culture condition and preparing bacterial solution

From the solution of *Beijerinckia indica* which was already prepared, a 1.0 ml was inoculated for two 500 ml flasks, each flask containing 200 ml of culture medium. The period of incubation, with shaking, was 24-48 hours at 30°C. The cultures were aseptically transferred to 50ml polypropylene centrifuge tubes to

separate and extract the bacterial cells from old nutrient liquid because the cells are more viscous and higher density than the nutrient solution. The cells are separated at the bottom of the tube, to allow harvest of bacterial pellets, by centrifuging the grown culture in the 50ml polypropylene centrifuge tubes at 3200 rpm for 20 minutes using Heraeus Varifuge 3.0 centrifuge machine. After removing the supernatant, phosphate buffered saline (PBS: For 1.0 litre; 8 g of NaCl, 0.2 g of KCl, 1.44 g of Na_2HPO_4, 0.24 g of KH_2PO_4) is added to the tube and stirred using a vortex mixer which is then followed by a repeat of the centrifuge process, this activity is done to wash the cells. The extracted cells in all eight tubes are mixed with 400 ml (PBS) to prepare the bacterial solution. Six tubes were used for the experiment, and two tubes were kept as a reserve.

3.5 Direct shear apparatus design

A bespoke apparatus was designed and developed to allow the direct shear experiments to be undertaken. The aims of the project is performing sets of direct shear tests on the biotreated and non biotreated silica sand alongside other supported tests. there are some requirements of the tests that can be listed that need to be achieved: six traditional size (60x60x4.5 mm) shear boxes are required for this study, three boxes for biotreated and the other three ones for standard samples; the sand samples of direct shear test are prepared in saturated case by using wet pluviation technique as will be mentioned in the next section in this chapter; all prepared samples are incubated for two weeks and they are tested at various low normal pressures; six light weight loading frames are needed to apply the normal stresses over incubation period; a pumping system and draining waste tubing are required to avoid any

contamination of the sand samples; for pumping system, the nutrient needs to be pumped to the top of samples through the loading pad, and at the same time a vacuum applied at the bottom of the shear box to allow evacuation of the waste. This technique will be described in more detail in the next section; slotted stainless steel squares are required to lock the shear boxes to prevent any probable rebound the samples over transferring. This plate needs to be suitable for installation on the shear box sample before realising the applied normal stress during transfer the sample to the direct shear test after elapse the incubation time.

Six units of the apparatus with their loading frames, as shown in Figure 3-2, were manufactured. A PVC grey plastic box was used as a main box. The shear box under loading and incubation period would be placed in the main box. The main box was made with (124.5X89X109.4 mm) dimensions. The box is made up of two compartments, the upper compartment is occupied by the shear box, and the lower part is used as a basin to collect extra waste liquid that may pass through sides around of shear box over the incubation period. A (60X60X45 mm) traditional square shear box was made of acetal plastic. This plastic resists the reaction of chemical compositions of nutrient medium and does not produce toxic materials from chemical reaction of the medium that may kill or harm the organism. Each box has lower and upper parts; it also is shown in Figure 3-2.

Two perforated plastic grid squares were fabricated, one of them was used below of sand specimen and the other on the top surface of the sample, the thickness of each one was 3.22 mm, the purpose of these squares was to allow

water to move out to the top and bottom of the sample during the shearing test, Figure 3-2.

Also, a PVC loading pad was fabricated. Two holes were made through the loading pad to deliver the nutrient through these holes. An upper container with a 5 cm diameter and 2.0 cm height was made as shown in Figure 3-3. A 3.0 mm internal diameter of silicon tubeing was used to connect between the holes in the loading pad and inlet tube which connect to the nutrient bottle. The nutrient liquid was pumped to the top of the sample during the incubation period as shown in Figure 3-2.

A stainless-steel plate with dimensions 80X80X2 mm was used to lock the samples while moving them from loading system to direct shear equipment to prevent rebounding of samples, Figure 3-2.

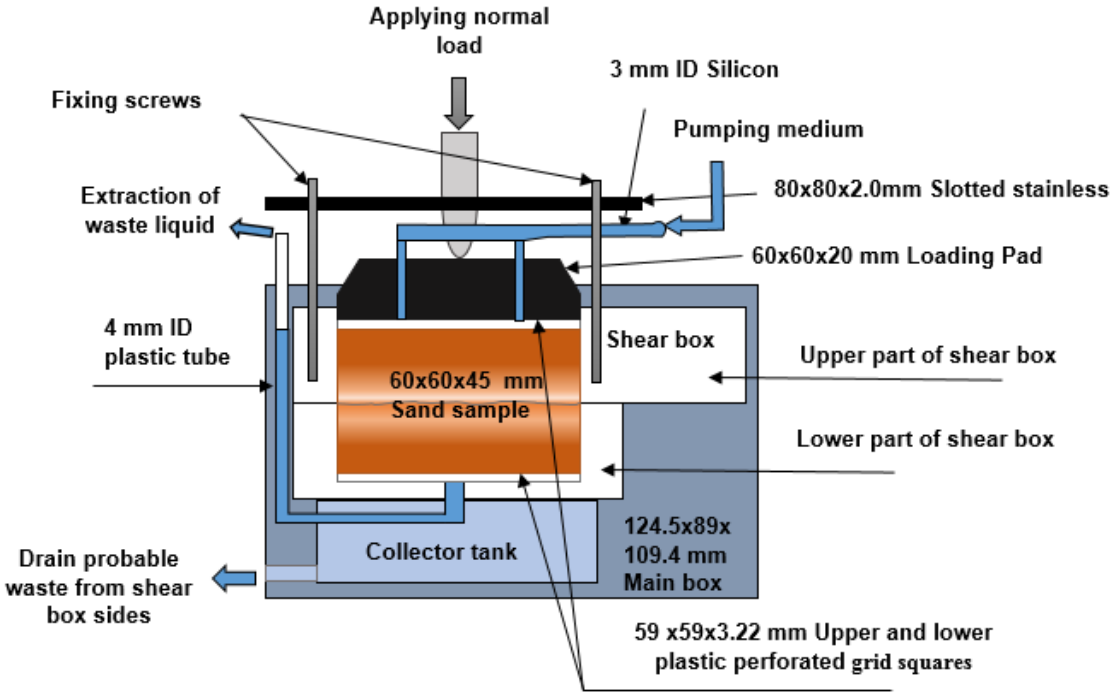

Figure 3-2. Schematic of main and shear boxes detail.

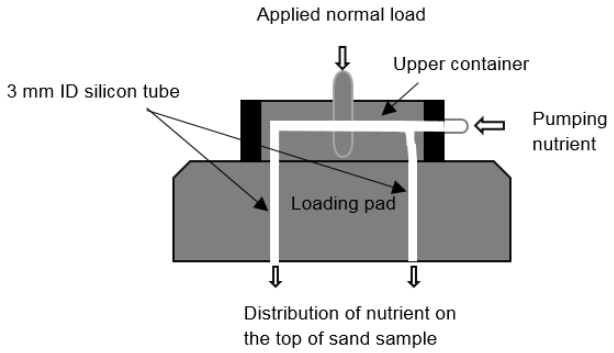

Figure 3-3. PVC loading pad and nutrient distributor.

3.5.1 Loading system

Figure 3-4 schematically depicts the loading system used to apply the vertical normal load on to the sample. Each single box comprises a light loading frame, made from aluminium (13x10x1.6 mm channel cross section) and stainless steel threaded rod (7.8 mm diameter), as illustrated in Figure 3-4, the frame weighed about 266.3 g. Loading hook is weighted 113.26 g, and weight blocks are also shown in Figure 3-4. A desk frame was made from slotted angle bar using to place the six boxes on it over the incubation period, Figure 3-4.

3.5.2 Fluid system

The nutrient was pumped to the sand sample by connecting the silicon nutrient feeding tube to the PVC loading pad as illustrated in Figure 3-4, to supply the nutrient to the top of sand samples. At the same time a vacuum was applied at the bottom through a hole which is in the lower part of the sample. This technique was used because the flow system was not closed. Also it was found that without applying a vacuum at the bottom the presence of the side gap between the two parts of shear box allowed the pumped nutrient to leak from the system by passing through the gap. Details of the fluid system for biotreated and standards samples are shown in Figure 3-4.

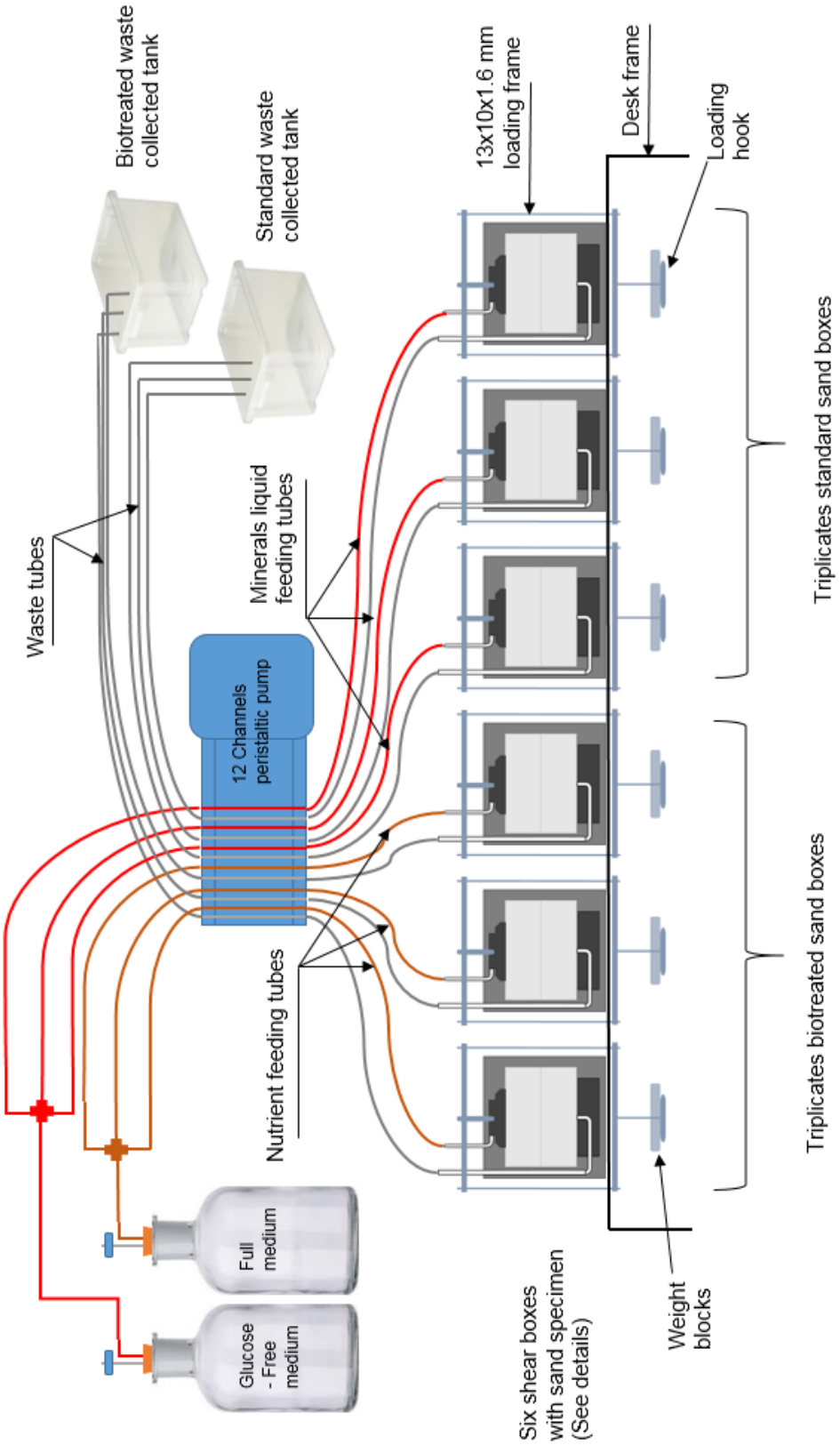

Figure 3-4. Schematic diagram for the main biotreated and standard shear boxes experiment.

Some challenges were encountered using the apparatus. Firstly, for the preparation of six identical samples, a preparation procedure was implemented to produce repeatable and consistent sand samples. Secondly, the technique of medium delivery to the boxes of sand samples over incubation time required some testing and fine tuning. The medium was pumped to the top of the specimen and at the same time, was removed, via application of a vacuum from the bottom. Thirdly, the samples need to be kept under compression to prevent rebound process while moving the samples from loading apparatus to shear test equipment. Also, the placing of the conventional shear boxes in the direct shear machine required carefully installation.

3.6 Preparation of sand specimens

For the main biotreated experiment, two sets of three shear boxes were prepared using the wet pluviation method to construct the layer of deposited sand. One of these sets was for standard samples (mixing the sand with a bacterial solution but without pumping nutrient containing glucose), and the other set was for biologically treated specimens (mixing the sand with a bacterial solution and pumping nutrient containing glucose). The volume of bacterial solution is equivalent to 1.5 times (40 ml) the pore volume of the sand specimen which insures that the sand specimen is in a fully saturated state initially.

The procedure of this preparation is also applied for preparation of saturated samples using deionised water. For dry samples, air pluviation is used by spreading dry sand in the shear box without using water.

All parts of six shear boxes were sterilised using virkon (supplied from VWR international) solution with a concentration of 10 g/litre overnight, and they were immersed in deionized water for at least two hours and then rinsed by replacing the deionized water three times before using them in the experiment.

3.6.1 Wet pluviation

The procedure of preparation starts by placing a thin layer of glass wool over the hole that is in the bottom of the shear box to prevent any fine sand particles to pass through to the pumping system. A 60x60 mm lower perforated grid plastic square was then placed, after blocking the bottom hole using a sterilised stainless plug. Pouring of 40 ml (1.5 pore volume) of the bacterial solution in the sterilised shear box is then undertaken. The bacterial solution has already been prepared from a culture of *Beijerinckia indica* organism with known initial cell numbers per gram of dry sand (as described in section 3.8.8.4). After that, 230 g of sterilised dry sand was poured using a suitable funnel moved horizontally by hand to spread sand particles in the shear box uniformly. Moreover, the sand particles fall from a constant height to result in a consistent sand density for all prepared samples. Subsequently, the samples were shaken for 1.0 minute using a small table shaker, brand IKA, KS, 130 basics with 640 min^{-1} swivel motion. This step was used to remove any trapped air bubbles from the sand matrix. The perforated plastic square and perforated plastic loading pad were placed over the top surface of sand sample. Then the prepared shear box specimen is placed in the plastic main box, the height of loading pad

concerning the top surface of the shear box was measured before applying a load in the incubation period.

3.6.2 Installation of the shear box in the main box

The stainless-steel plug was removed from the lower hole and a 4 mm internal diameter transparent tube attached via a plastic elbow connector to the vacuum pump. Each prepared shear box was placed in its main box which contains a reservoir tank at the bottom to collect any extra drained water from around shear box sides. This reservoir tank was connected to plastic boxes under loading frame inside the incubator. The main box can be placed on the desk after setting the aluminium loading frame to apply the desired or required weight blocks load, as illustrated in Figure 3-4.

3.6.3 Loading and incubation of samples

For the 1.0 kPa loading test a 0.36 kg weight, as a first loading, is applied on the top surface of the prepared sand in the shear boxes for both sets of specimens (treated and standard) over two weeks of the incubation period. Full nutrient solution (minerals and glucose) is supplied for treated specimens during this period to keep bacteria growing and producing EPS. However, the same minerals solution without glucose is delivered to the standard samples. The incubation temperature is 30 ^{o}C. This procedure is repeated with the appropriate load applied for the other normal loads of 4.1 kPa, 8.89 kPa, 16.2 kPa and 25.0 kPa.

3.7 Adapted direct shear apparatus and testing procedure

Some particular techniques were implemented to improve the repeatability of the direct shear test for biotreated and non-biotreated samples, these are described below.

3.7.1 Direct shear test case

The direct shear tests were conducted under saturated conditions. Therefore, the carriage of the shear box has been filled with deionized water to the top of sample, and the level of water was kept constant during testing. The placing of perforated plastic squares at the top and the bottom of sample allow adequate drainage over the testing. The amount of material passing a 0.063 mm test sieve was less than 5.0% in the well graded sand to avoid segregation of fine particles.

Before starting these tests, two jacking screws were used to raise the upper part of the box, these had previously been fully screwed in to avoid any disturbance in saturated sand specimens during placing process. Various applied normal stresses (1.0 kPa, 4.2 kPa, 8.89 kPa, 16.2 kPa, 25 kPa) are used to study the impact of biopolymer at various effective stresses. These stresses correspond to the stresses that have applied over incubation period for each specimen.

3.7.2 Testing of dummy sample

A dummy sample was tested before commencing any of the main tests as it was observed, after a series of repeated tests, that the first sample regularly exhibited a higher shear resistance than the subsequent ones, at

low normal stresses, when it had not be used for some time. It is not clear why this occurs but it is possibly due to some variations in lubrication in the connection and hinges of the load cell of the direct shear instrument which are overcome after initial use.

3.7.3 Making a gap

A gap between upper and lower part of the shear box was established before testing for all samples to eliminate the friction between the box parts that may show larger shear result than the actual outcomes. After removing the main screws, two jacking screws have been used to make a 0.4-0.6 mm gap between the upper and lower parts of the shear box by raising the upper part. After a series of direct shear tests, this size of the gap was found that achieved the best consistency of shear behaviour (detailed in Chapter 5). In addition, it is mentioned in the BS 1377:7:1990 of direct shear test, using jacking screws showed best and more reliable result.

3.7.4 Using a special linear potentiometric transducer LVDT

A special Linear potentiometric transducer, 10 mm travel for vertical deformation, LVDT (*Novo Technik*, TR-0010) was used after removing the inside spring because the spring applied the equivalent of 400 g extra load on the samples when fully compressed. The calibration work is described in appendix A2.

3.7.5 Manufacturing of Frame load and weight blocks

A loading frame was made to use in the direct shear test machine instead of using of automatic applying pressure that is provided by the yoke of DST

machine itself. The direct shear equipment provides 25.0 kPa as a minimum applied normal stress, but the current study needs maximum normal stress of 25.0 kPa. The weight of frame was 1440 g which apply 4.1 kPa. For the lowest stress of 1.0 kPa, a small block was used including the weight of loading pad of 78.0 g without using loading frame. Moreover, 36 weight blocks were made from mild steel; these blocks were weighed 2775 g each. The loading frame and weight blocks were used to apply normal load on the samples.

3.7.6 Calculation and Correction of Shear Parameters

As stated by Lehane and Liu (2013), in general, mechanical friction in the traditional shear box leads to very substantial errors when measuring sample response at low stresses and, as a consequence, shear box tests are not typically carried out at normal stresses less than 20 kPa. Lehane and Liu (2013) investigated two separate hypotheses, addressed as case A, clear gap between the two parts of shear box, and case B, the presence of sand grains in the gap to estimate the average force F_n acting on the shearing plane from the normal load applied via the loading frame F_t. A simple means of correcting was developed for the mechanical friction in upper part of shear box. These corrections were used to determine the peak and residual friction angles of granular materials at low normal stresses in a shear box apparatus. A schematic view of a shear box arrangement is shown in Figure 3-5. The applied normal stress in this study are low stresses of 1.0, 4.1, 8.89, 16.2 and 25.0 kPa. Therefore, this correction may be reliable to apply on the direct shear outcomes by applying case B. The

presence of jacking screws tips may be considered as the presence of sand grains between two parts of shear box. The upper part was carried by the screws tips. In addition, if the Poisson's ratio equals 0.2 as the authors assumed. The percentage of friction force which generated in the internal surface of shear box was 8.5 to 9.3 % of the applied normal stress F_n as stated by Lehane and Liu (2013) and Bareither et al (2008). A full procedure of correction is presented in appendix A3.

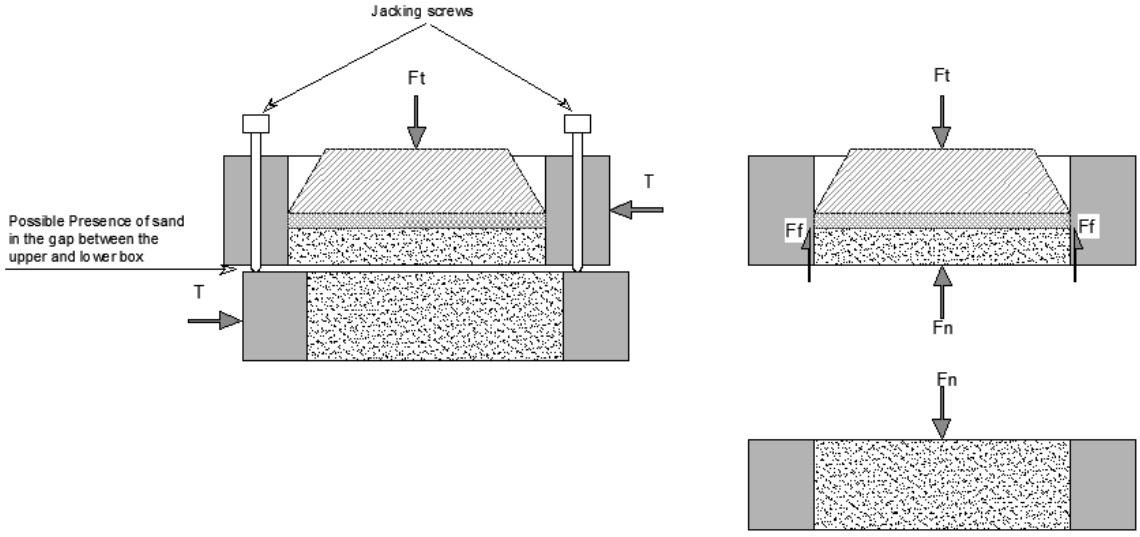

Figure 3-5. Modified schematic view of shear box (Reproduced from Lehane and Liu 2013).

3.8 Analytical techniques

3.8.1 Sieve analysis

A well graded silica industrial sand was used in all main experiments (except the poorly graded and sea sand test). Before use, the sand was crushed by machine (*LABTECH ESSA LM1-P*) in the concrete laboratory of Cardiff Engineering of School. The crushed sand was sieved into several fractions

from 0.063 to 0.6 mm. A well-graded sand was then prepared by mixing the required percentage of each fraction according to BS 1377-2:1990 item 9 (the poorly grand sand was prepared in the same way). The gradation of sand is illustrated in the Figure 3-6. The coefficient of curvature (Cc), and uniformity coefficient (Cu) were found for the classification purpose.

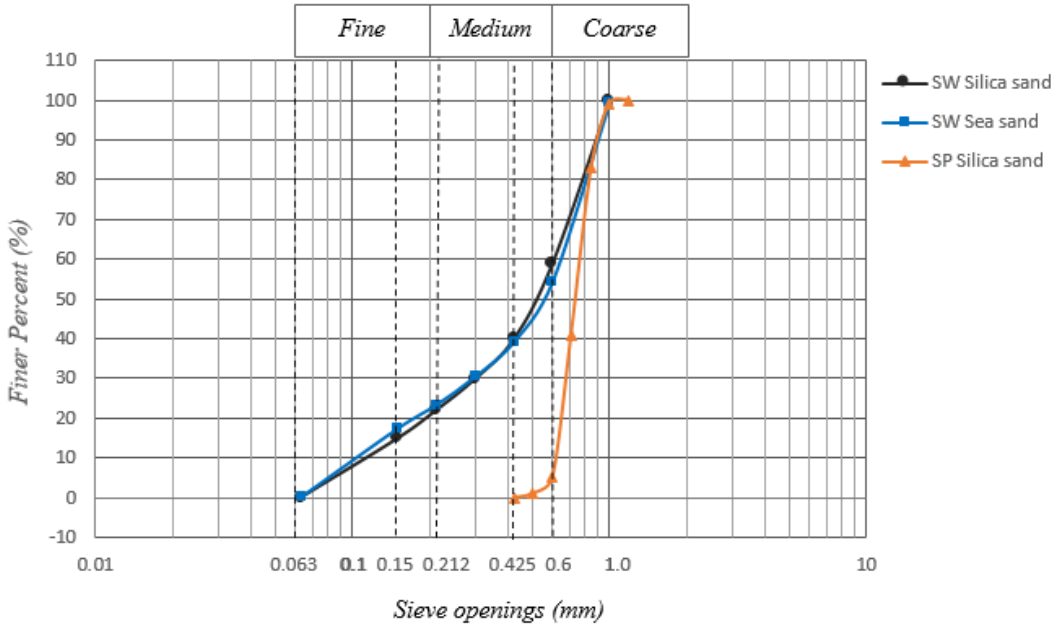

Figure 3-6. Gradation of crushed sand (SW), Sea sand (SW) and uncrushed (SP).

3.8.2 Proctor test

A standard proctor test was conducted on the silica sand to determine the maximum dry density and optimum water content as shown in Figure 3-7. This test was performed according to BS 1377: part 4:1990.

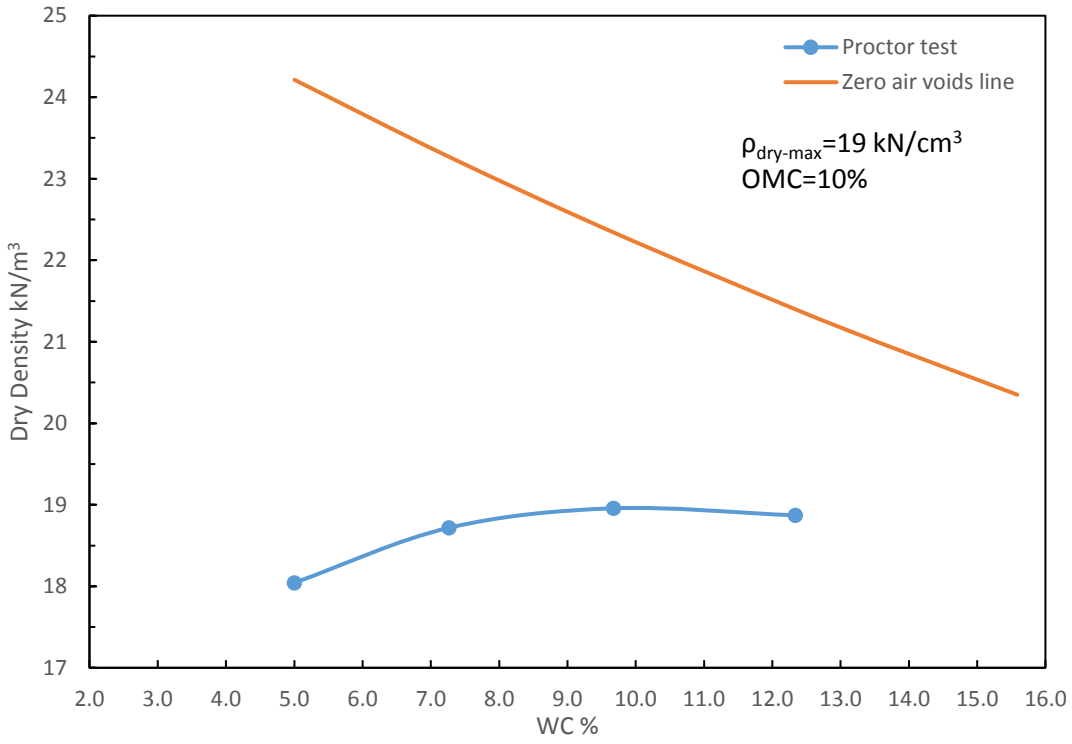

Figure 3-7. Standard proctor test.

3.8.3 Particle density test

The specific gravity or particle density of different soil samples can be defined as the mass of a soil sample in a given volume of particles. This test was carried out using a pycnometer as specified by British Standards BS1377: Part 2:1990. The test results showed that the particle density of sand is 2.660±0.001.

3.8.4 Minimum density of sands test

This test was conducted to determine the minimum density of the silica sand, according to BS 1377:4:1990. The procedure comprises shaking a 1000 cm^3 which cylinder contains 1000 g sand and measuring the volume of sand ten times. The resulting minimum density is determined as follows:

$\rho_{min.}$ = 1000/V

3.8.5 Elements constitution

The INNOVX system machine was used to determine the chemical composition of the sand. This machine is in the Characterisation Laboratories for Environmental Engineering Research (CLEER) of Cardiff Engineering of School.

3.8.6 Hydraulic conductivity test

Constant and falling head permeability tests were conducted on the silica sand, and the resulting coefficients of conductivity for both tests are compared in this study as shown in the next chapter. It is well known that the constant head method is typically used for granular soils, but the hydraulic conductivity test is conducted with special apparatus of the sand sample columns. Therefore, the falling head test is also performed to compare the resulting permeability coefficient for both methods.

A Mariotte bottle was used to provide constant head for undertaking a constant head permeability test for the silica sand. The bottle is sealed at the top, with a side hole that is exposed to the atmosphere. The level of this hole represents the constant level of water in this experiment, as shown in Figure 3-8. The water head is measured between the top hole at the base of the Mariotte tube to the outflow tube as addressed as datum as shown in Figure 3-8. This head is maintained at a constant value during testing. The amount of flow can be determined by measurement of the drop in height of water in the Mariotte bottle over a known time.

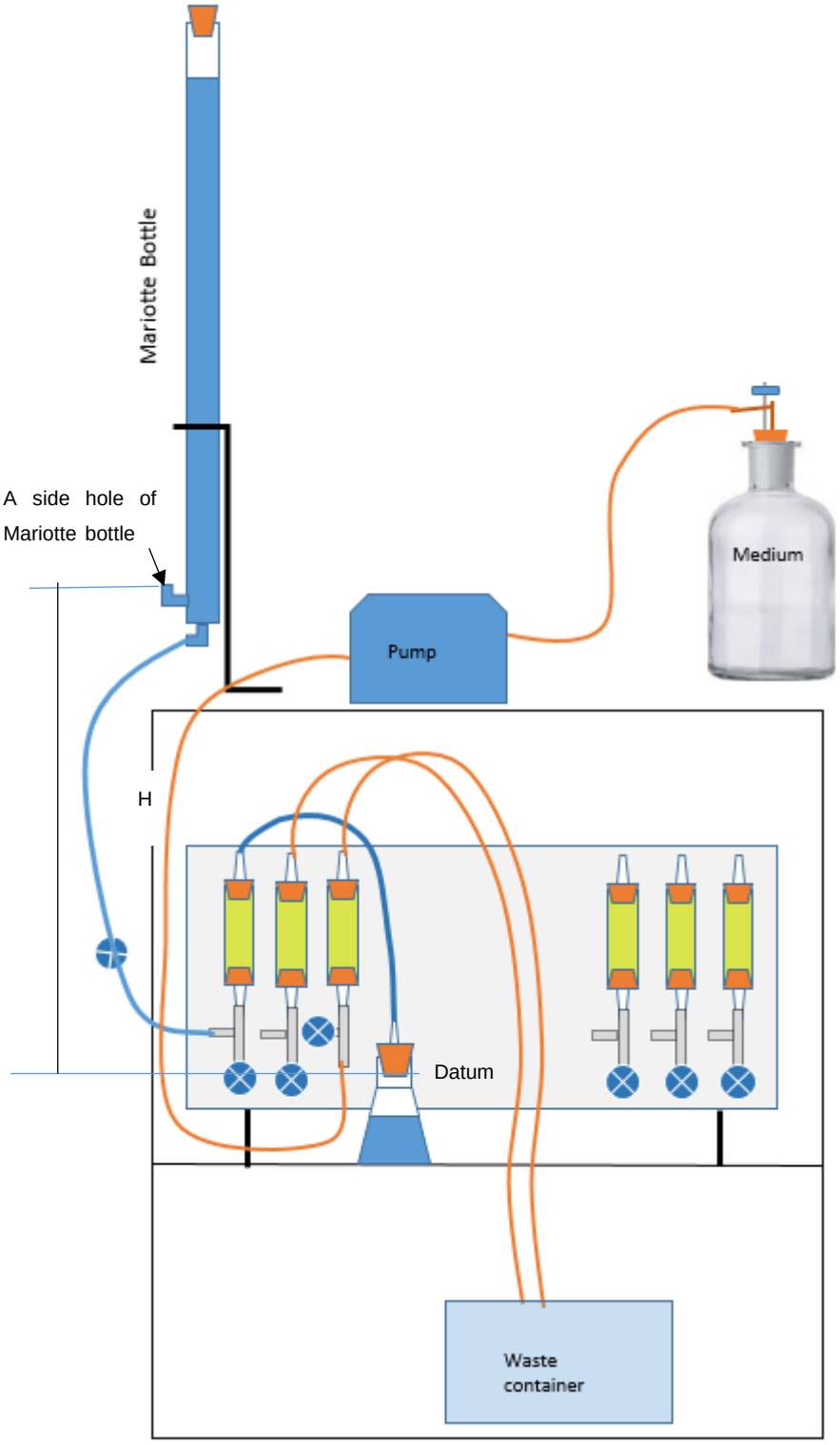

Figure 3-8. Schematic of used Mariotte bottle.

3.8.7 Estimation of biomass content

After the direct shear test is completed, biomass content of tested samples was estimated via a loss on ignition test. The actual amount of generated biofilm in sand samples can be used to understand the impact of these contents on shear results. The loss on ignition method is used to estimate the amount of organic materials in the sample. This approach was achieved according to BS 1377-3-1990. The existing biomass was investigated in three layers (top surface, shear plane and bottom layer) for each sample to estimate the amount of developed biofilm over incubation time in biologically treated and standard samples for all project experiments.

3.8.8 Number of cells – optical density relationship

The concentrated bacterial suspended of *Beijerinckia indica* cells was measured by taking optical density. 1.0 ml of culture was aseptically transferred into a disposable plastic UV cuvette (Fisher Brand). The optical density was measured at 600 nm wavelength incident light for beijerinckia indica (Dedysh et al, 2005). Hitachi U1900UV VIS spectrophotometer was used to measure the optical density at every 2 to 3 hrs. The growth profile of *Beijerinckia indica* solution versus elapsed time was plotted in Figure 3-9.

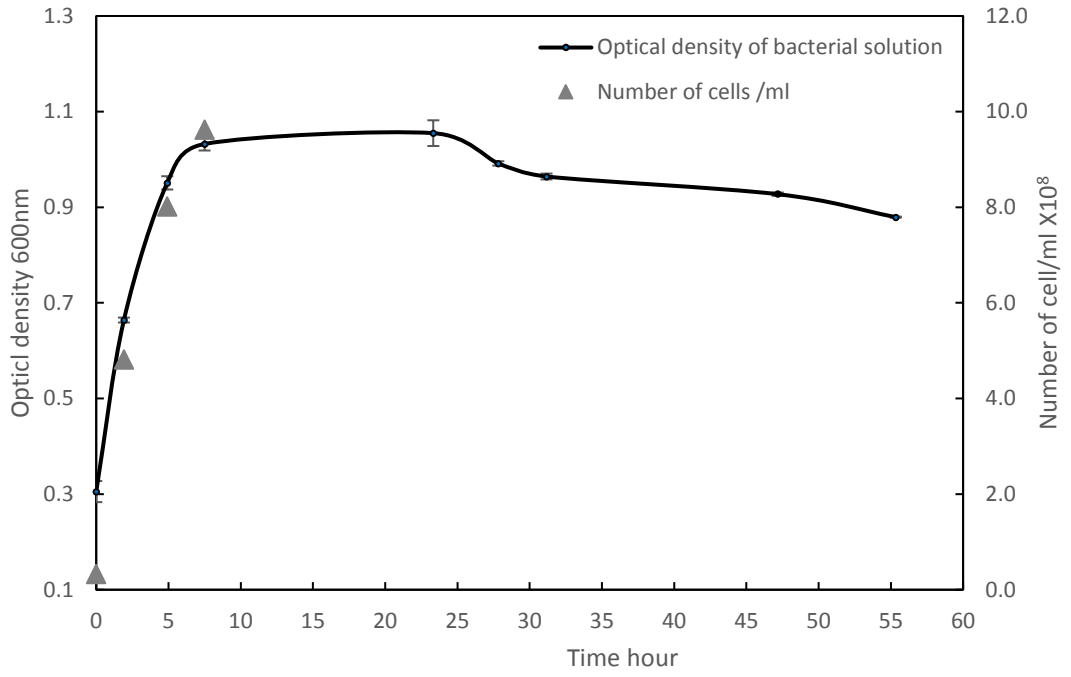

Figure 3-9. Optical density and number of bacterial cells against elapsed time.

3.8.8.1 CTC procedure:

5-cyano- 2, 3-ditolyl tetrazolium chloride (CTC), supplied from *Sigma-Aldrich, UK*, which has been used to evaluate the respiratory activity of many bacterial populations derived from the environmental source. The cells respiring via the electron transport chain will absorb and reduce CTC into an insoluble, red fluorescent formazan product. Cells not respiring or respiring at slower rates will reduce less CTC, and consequently produce a less fluorescent product, giving a semi-quantitative estimate of healthy bacteria.

3.8.8.2 Preparation of Working Solutions

Rodriguez et al (1992) presented a CTC procedure that has been adopted in this study. 15.5 mg of CTC (5-cyano- 2, 3-ditolyl tetrazolium chloride) was dissolved in 1.0 ml of deionised water to obtain final concentration 50mM. Subsequently, a 10µl of stock of CTC has been added to 90 µl to deionised water to get 5mM. Most protocols recommend staining with a 5 mM staining solution (final concentration). This solution was stored in 4°C and used within one week.

3.8.8.3 Serial dilution

In order to dilute the bacterial solution for cell counting purpose, a serial dilution was performed by preparing at least 5 dilutions from 10^{-1} to 10^{-5} dilution factor. 90 µl of Phosphate Buffered Saline has been added to each of five sterilised micro centrifuge tubes. 10 µl of the *B. indica* bacterial solution was added to the first 90 µl of PBS tube. This incolated solution was mixed by vortex mixer. Then, transferring 10 µl from the first tube to the second one and do the same steps for other tubes. Each tube contains 90 µl of the bacterial diluted solution with known dilution factor. Adding 10 µl of CTC solution which already prepared to each tube and mixing gently by vortex mixer. These five tubes were incubated at 30°C for 2 to 4 hour to allow the live cells to reduce the dye to fluorescent CTC-formazan.

3.8.8.4 Cell number counting

After the incubation period, 10 µl of dye solution has been taken from the micro centrifuge tube which has the best dilution that gives 30-300 countable cell number. The 10 µl has been dropped on a microscopic glass slide and was covered with a glass cover chip. The slides were analysed under a *Nikon Eclipse LV100* microscope with a Nikon DSFi1digital camera used for epifluorescence counts with a 20x lens. The CTC-formazan present in the living cells was excited at 365 nm and emitted red light at 650nm wavelength.

The cell numbers were counted for ten images of different regions and the average calculated. The image area was determined in μm^2 unit by considering the number of pixels. Then, the image area was converted to mm^2 unit. The equivalent area calculated by divided the area of glass chip (18*18=324 mm^2) by the image area in mm^2 unit. Multiplying the resulted area by the average number of the cell to find the total cells in 10 µl. Then, multiply the cell number by the dilution factor to get a number of cells in 10 µl. Finally, the number of cells in 1.0 ml can be determined by multiply the cell number by 100. A relationship between the numbers of live cells with optical density was presented as shown in Figure 3-10.

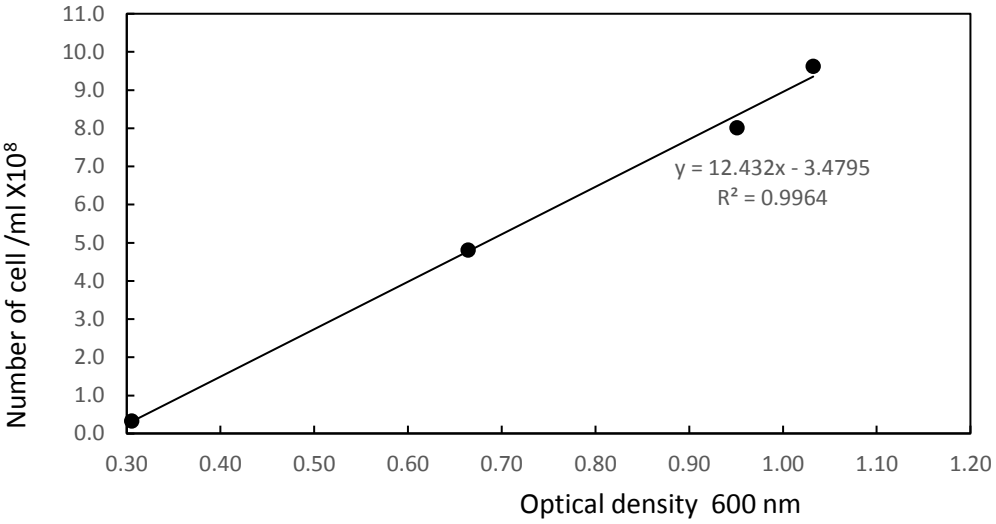

Figure 3-10. The relationship between number of cells and optical density of *Beijerinckia indica.*

The initial number of live bacterial cells, determined via CTC procedure in the bacterial solution is presented in Figure 3-11. This solution, prepared as mentioned above, was used for the preparation of all the biotreated and standard samples and so only a single value of cells number was determined for each experiment.

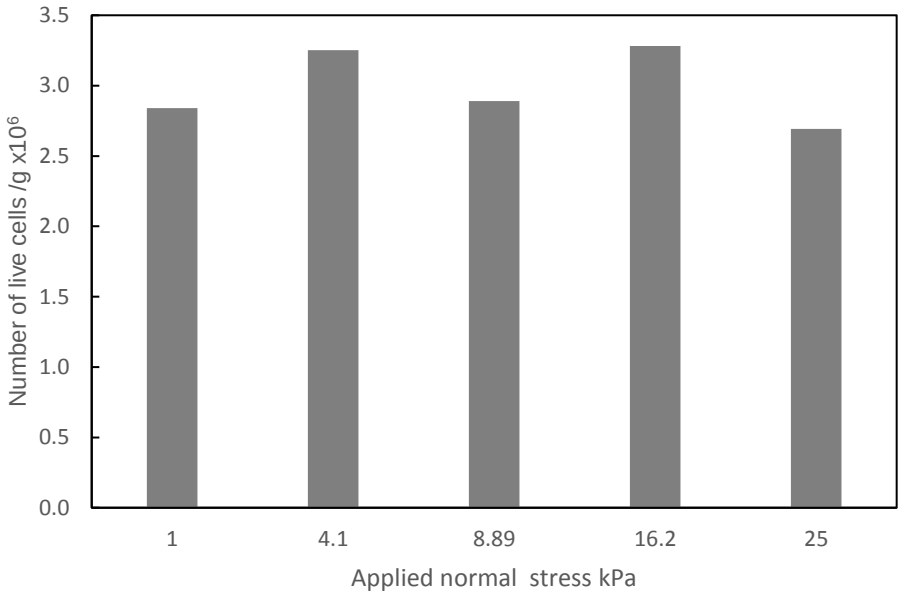

Figure 3-11. The prepared number of live bacterial cells for each experiment.

3.9 Summary

In this chapter, the specimen preparation conditions and experimental method of direct shear test at low normal stress for various tests are explained in detail. The modified direct shear apparatus, loading system as well as fluid system which applied on the both biotreated and standard samples are clarified.

the procedures conducted for determining the basic physical properties of the sands, such as the particle size distribution, the compaction characteristics, particle density, minimum density, elements constitution for silica sand and hydraulic conductivity. For counting of number of bacterial cells, working bacterial solution was prepared and CTC procedure was carried out on the solution. Loss on ignition is the essential test was performed to estimate the amount of developed biomass in biotreated and standard samples.

Chapter 4

Optimum conditions for biofilm production in sand

4.1 Introduction

This chapter aims to investigate the optimum conditions for biofilm development in the silica sand detailed in chapter 3 and in particular considers the selection of the most appropriate nutrient solution by assessing two candidate chemical compositions (Nu1 and Nu2) previously reported in the literature. Hydraulic conductivity and loss on ignition tests were undertaken to evaluate the effect of grown biofilm on the silica sand when using both nutrient Nu1, and Nu2. The impact of biofilm on the hydraulic conductivity of the silica sand and as well as the percent of loss on ignition was compared to investigate whether Nu1 or Nu2 gives a higher percentage of the biomass. Some additional experiments were also performed considering the amendment of the nutrient solution with $MgSO_4.7H_2O$, yeast extract, Magnesium carbonate ($MgCO_3$) and $CaCO_3$ to investigate the effect of such chemicals on the amount of biofilm produced in the sand matrix. Also, the variation of nutrient pH with time was measured to study whether this influenced the growth of biofilm.

The permeability test is a common laboratory test to explore biological clogging in porous media as stated Alshiblawi (2016), Perkins et al (2000), Dennis and Turner (1998) and Taylor and Jaffe (1990). Many researchers concluded that the permeability coefficient of sand could be significantly reduced through bacterial treatment such as biofilm plugging with reductions of one to three orders of magnitude of hydraulic conductivity because of biofilm plugging reported by Seki (2013), Pintelon et al (2012) and Dennis and Turner (1998). Therefore, permeability test was performed in this study to evaluate the impact of the presence of grown biofilm in the sand mass on the permeability

coefficient. In addition, loss on ignition test was undertaken to estimate the amount of formed biofilm.

In section 4.2 constant and falling head hydraulic conductivity tests conducted on a clean well-graded sand and a biotreated sand using deionized water and PBS are presented. A reduction in hydraulic conductivity indicates the presence of bioclogging due to growth of biofilm (Alshiblawi, 2016), (Dennis and Turner, 1998). Therefore, this test was performed to assess the production of biofilm in biotreated specimens.

In section 4.3 a set of biological growth experiments are reported to improve the growth of the bacteria. Some modifications of chemical concentration of nutrient were carried out to produce the optimal nutrient which may form the maximum amount of biofilm. Therefore, this exploration was carried out to study the influence of using buffers ($MgCO_3$ and $CaCO_3$) on the pH variation which may impact on the growth of the *Beijerinckia indica* (Becking, 1961). These buffers have different solubility in water- the solubility of $MgCO_3$ is 0.6 $g.l^{-1}$, whereas the solubility of $CaCO_3$ is 0.013 $g.l^{-1}$ (Aylward and Findlay, 2002). Furthermore, the impact of room temperature on the pH variation of nutrient with the time was also studied. The effect of initial pH of the medium was also investigated by preparing four bacterial solutions with different initial pH values to monitor how the pH changes with the time as well as how bacterial growth responds to variations of pH. Triplicate data were collected and analysed. A further experiment is also achieved to explore the influence of the concentration of some chemicals on the growth of *Beijerinckia indica*. These chemicals were a part of the medium components such as $MgSO_4.7H_2O$ and yeast extract. The effect of such substances was compared with the regular

(standard) nutrient medium. Finally, tap water was used to prepare the nutrient instead of using deionized water to understand the effect of trace elements in tap water on the growth of bacteria.

4.2 Permeability Tests:

For the permeability tests, three 6.76 cm length and 2.57 cm diameter columns of saturated well graded standard sand were prepared. The prepared density of sand was 18.3 kN/m^3 for all three specimens for each test. This density is similar to that prepared for the main biotreated shear boxes. Both constant and falling head permeability tests were conducted on the columns of sand samples. These tests were run after immersing the sample in water for 24 hrs. Each test was performed by supplying deionized water to the bottom of the columns. The purpose of performing these tests is to compare the measured permeability coefficients of constant and falling head tests when supplying water from the bottom to the top of samples. In the biotreated sample, the movement of water from the bottom to the top of the sample allowed CO_2 bubbles to move to the top of the sample and escape. This is important as CO_2 gas may be generated by biological processes and may reduce the degree of saturation.

4.2.1 Permeability test of clean sand

Hydraulic conductivity tests were carried out on clean sand by supplying deionized water to the bottom of the columns. The results shows only a slight difference between constant and falling head tests with values of hydraulic conductivity of $1.84X10^{-5}$ and $1.70X10^{-5}$ m/s respectively as

shown in Figure 4-1. Therefore, the constant head and water supplied from the bottom were chosen for the next biological experiment. The supplying of water from bottom of the sample helps to rid of any probable CO_2 bubbles to the top of sample as well as the constant head method commonly used for granular soil.

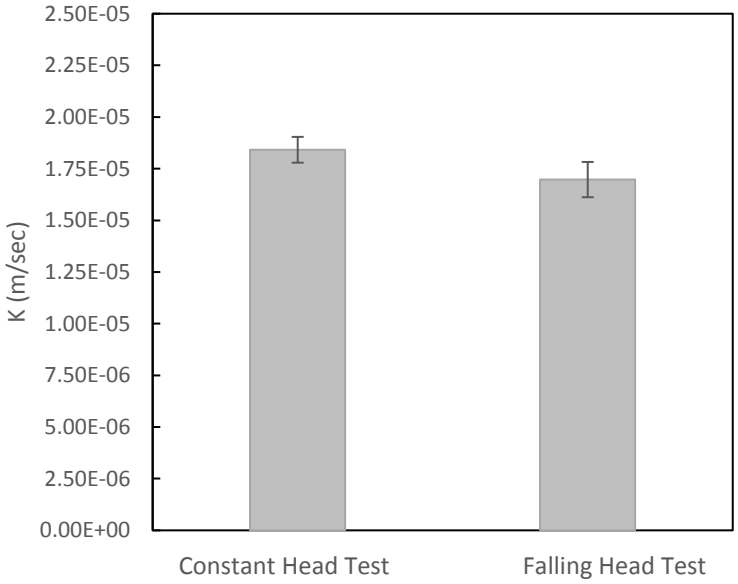

Figure 4-1. Comparison between constant and falling heads tests water supplied from the bottom to the top.

4.2.2 Evaluation of nutrient medium

Two types of nutrient were used in a bioclogging experiment to identify which nutrient results in the greatest impact on coefficient of permeability and formation of biofilm in the sand sample. The chemical components of both nutrient media are indicated in Table 3-4 (Section 3.3). The hydraulic conductivity test was performed in triplicate for each nutrient. Six columns were prepared by pouring 12.0 ml of phosphate buffered saline (PBS) solution containing suspended *Beijerinckia indica* into the 6.76 cm long and 2.57 cm diameter column, and then placing 70.0 g sand

using wet pluviation technique. The column was then shaken for 1 minute using a vortex mixer. Sterilised PBS was delivered to the bottom of each column to determine the initial hydraulic conductivity after incubation for 2 hours. Then the prepared columns were incubated for one week at 30 ^{o}C. Nutrient solution (1.5 pore volume, or 8 ml) was pumped to all six columns four times a day, three with Nu1 and three with Nu2 solution. The final hydraulic conductivity was measured after incubation and is shown alongside the initial value in Figure 4-2. Solutions Nu1 and Nu2 show approximately the same initial and final hydraulic conductivity with a significant decrease observed in both case in comparison with initial permeability (Figure 4-2).

The loss on ignition from samples across the column specimens (Figure 4-3) has been used as an indication of biomass content, and demonstrates that use of the Nu2 nutrient resulted in a substantially larger production of biofilm compared to that of the Nu1 nutrient. Therefore, Nu2 was selected for use in the main biological experiment of the current study.

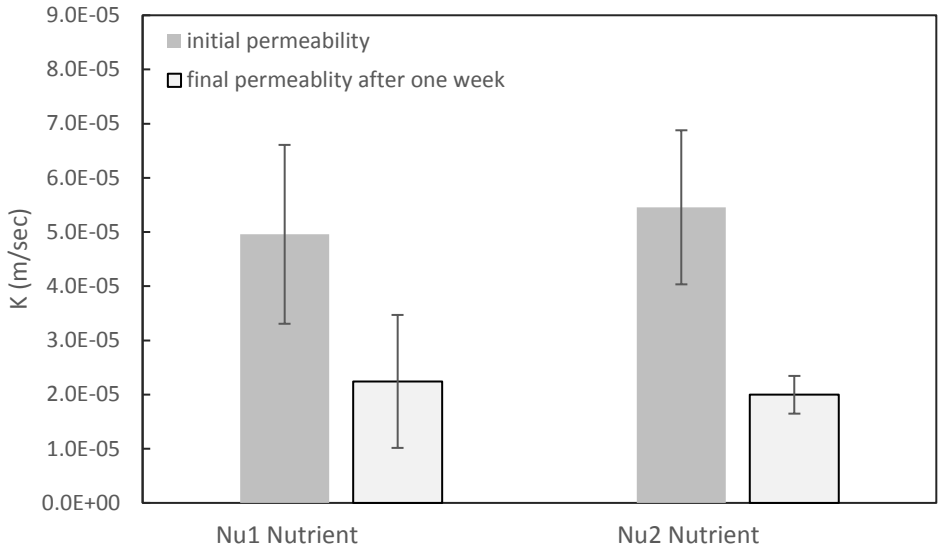

Figure 4-2. Initial and final permeability coefficient values for both nutrients.

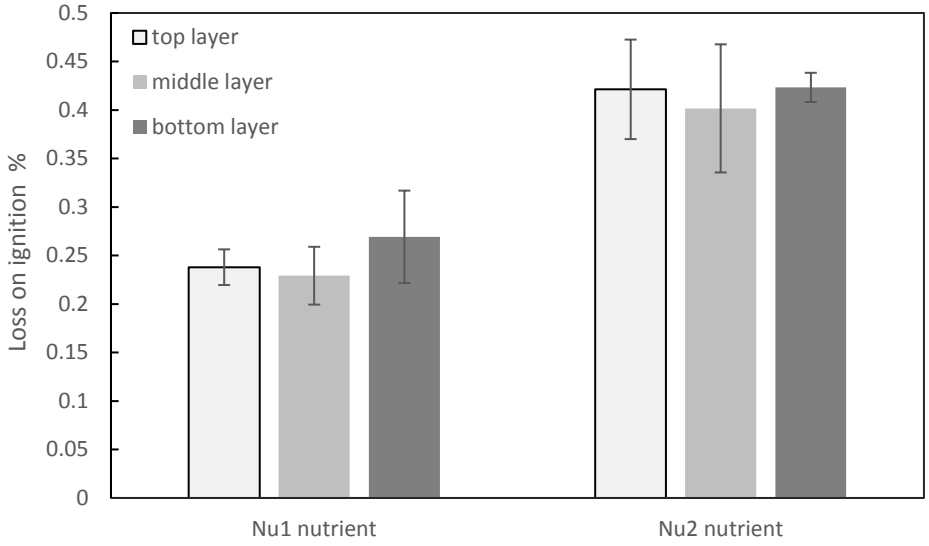

Figure 4-3. Biomass Percent for both nutrients.

4.3 Enhanced biofilm formation

In an attempt to increase the amount of biofilm production, a further set of experiments were undertaken and are described in this section. The impact of controlling pH of the medium, is investigated by adding buffers both with

and without stirring of the medium. The effect of pH variation with time on the growth of *Beijerinckia indica* was investigated by measuring the optical density of the bacterial solution. The impact of amendment of nutrient minerals on the bacterial cell growth was also explored. Furthermore, the study of bacterial growth with using nutrient which is prepared with tap water as well as deionized water is undertaken to investigate the impact of trace elements.

4.3.1 Impact of temperature on pH Variation of nutrient

This section describes how the pH of nutrient solution Nu2 changes over time at room temperature and reduced temperature (5-8 ^{o}C), to explore whether such changes may occur in bacterial growth experiments. The variation in pH was minimal, from 6.81 to 6.97 and from 6.62 to 7.10 for room and refrigerator temperature respectively.

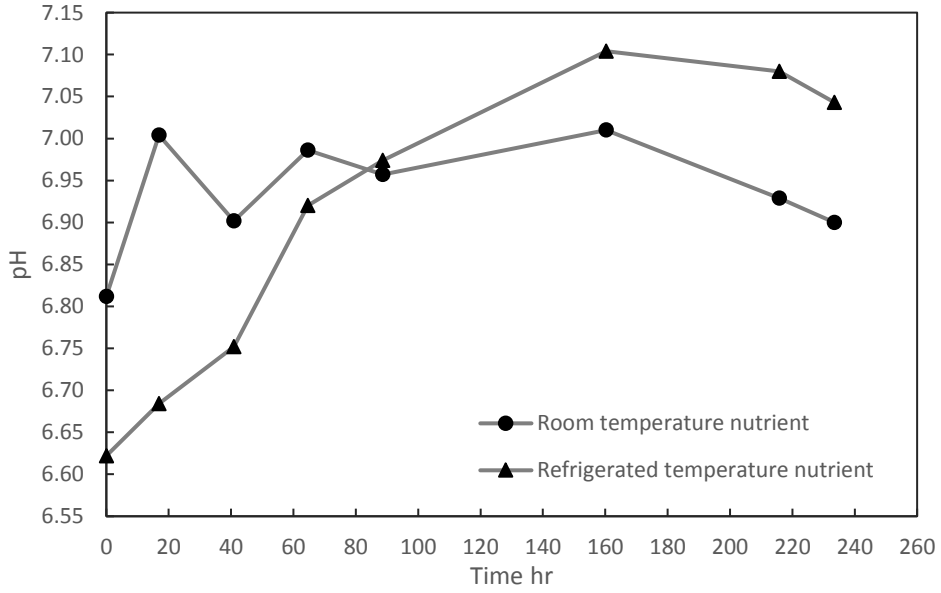

Figure 4-4. Variation of pH value with time for nutrient solution at room temperature and under refrigerated conditions.

4.3.2 Effect of pH variability on bacterial growth

The response of *Beijerinckia indica* to the nutrient medium pH was explored using nutrient media amended to have different initial pH values using HCl and NaOH as required. The values of pH were 3.13, 4.93, 6.56 and 9.00. The variation of pH and optical density of the four solutions with time are presented in Figure 4-5 and Figure 4-6. In general, the pH values dropped up to about 19 hours and then showed only slight variations until the end of the test except with an initial pH of 3.13 where no significant change was observed. In the latter case, no growth in optical density was seen, whilst growth curves at initial pH of 4.92, 6.56 and 9.00 showed similar behaviour with substantial growth in the first 19 hours. It can be seen that the solution of pH 9.00 has a higher rate of growth than the others. It is possible that the production of organic acids and CO_2 generation caused the observed pH changes. After 19 hours, there are only relatively small changes in optical density with pH 4.92 and 6.56 tests, but the pH 9.00 medium had a drop in optical density from 1.7 to 1.2. This medium has a stationary phase for a small period before optical density decreases – dead cells are also recorded by optical density, and so it may be that cells lysed. The pH of the nutrient Nu2 was chosen to

be 6.56 for the further biotreated experiments, because this value shows increasing optical density even though different rates of growth.

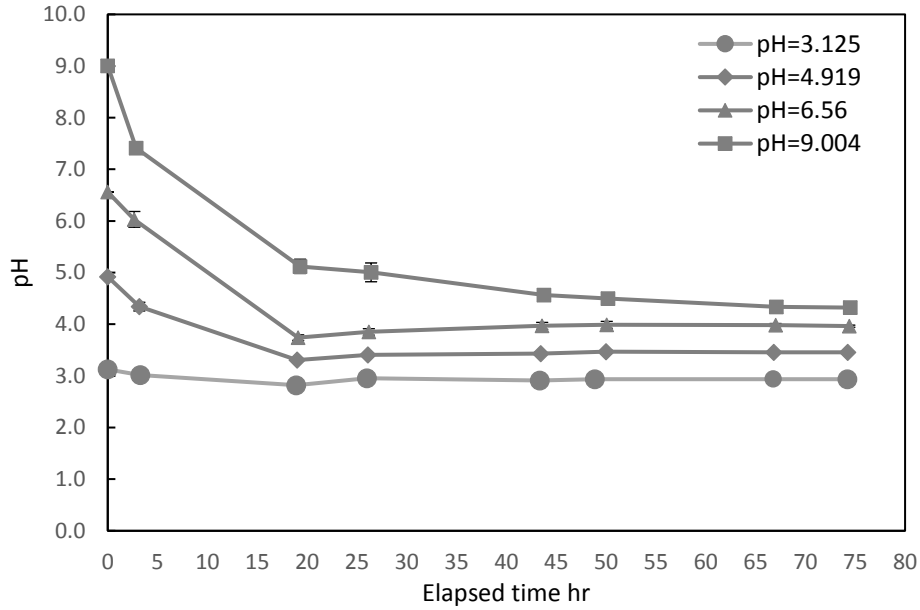

Figure 4-5. pH variation of *B. indica* solution with time.

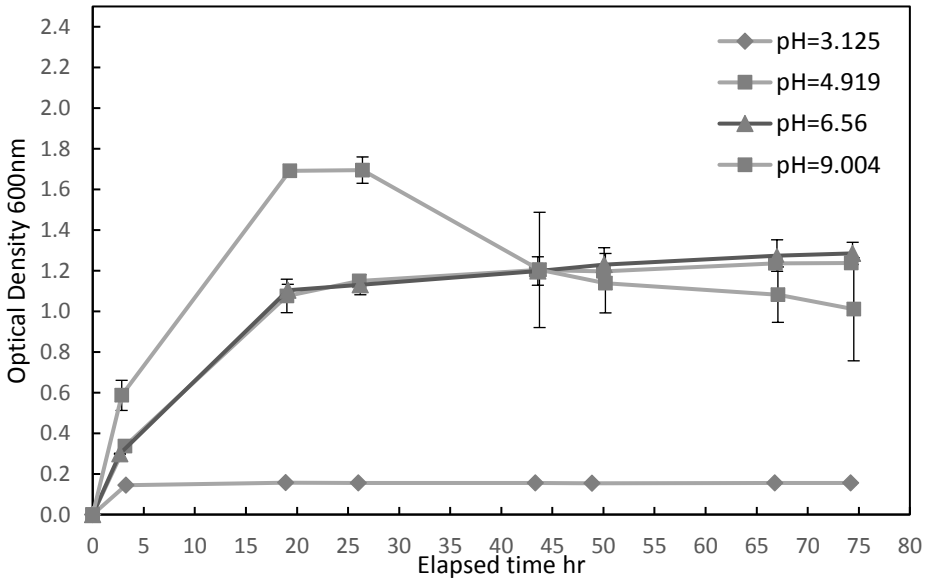

Figure 4-6. The optical density of *B. indica* solution with time.

4.3.3 The effect of adding buffer on the nutrient pH

Previous results suggested that a decrease in pH led to reduced growth of bacteria. The amendment of regular nutrient Nu2 by adding $CaCO_3$ as a buffer to control the pH of the medium was studied initially in terms of its effect on medium pH only. This buffer is known to have a very low solubility in water (13.0 $mg.l^{-1}$ at 25 ^oC) as such most of the buffer is present in sediment at the bottom of the container. Therefore, the reading of nutrient pH for both stirring and non-stirring cases was compared. Two samples (1.0 litre each) of regular medium Nu2 were prepared separately and then sterilised. Then 1.0 g of sterilised $CaCO_3$ powder was added to each sterilised medium. Becking (1961) highlighted that the presence of this buffer in the nutrient during sterilising might remove trace elements which are essential to the organism growth from the medium. One of the samples was allowed to settle whilst the other was stirred to be cloudy (turbid solution) over the experiment period. Calcium carbonate is an inert and relatively insoluble substance, which is often used for the detection or neutralisation of acid produced by micro-organisms. The impact of turbid nutrients on bacteria growth was studied by using such buffer as shown in Figure 4-7, the readings of pH were taken for both stirred and non-stirred mediums to investigate the effect of stirring on the variation of pH value over elapsed time under room temperature. Figure 4-7 shows that the effect of stirring was insignificant in relation to the pH of the medium over the elapsed time, as might be expected because of very low solubility of $CaCO_3$ in water. On the other hand,

adding of this buffer increasing the pH from about 6.9 to 7.34 at zero time as shown in Figure 4-7.

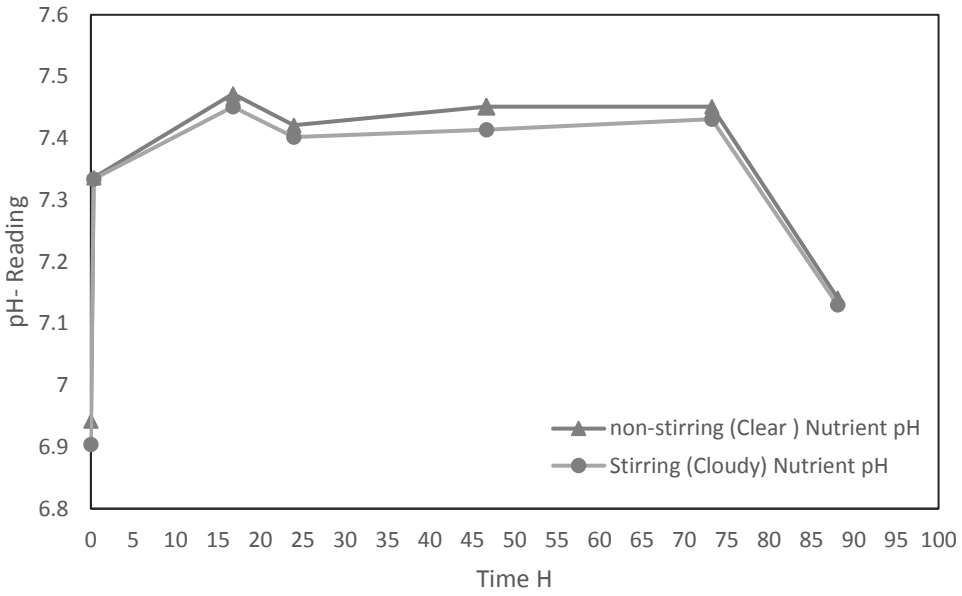

Figure 4-7. pH reading of Nutrient with $CaCO_3$ Variation with time.

Subsequently, a bacterial growth experiment was performed by preparing three samples using clear (unstirred) medium and three samples using turbid medium, each with a 200ml Erlenmeyer flask size containing 100 ml bacterial solution which was described in the introduction section of this chapter. The optical density was measured after leaving the samples without shaking for at least one hour to allow any suspended $CaCO_3$ particles to settle. As illustrated in Figure 4-8, it can be seen that the maximum value of optical density happened after around 28 hours for both media. The maximum optical density of using the stirred medium was 1.85. This is slightly greater than the optical density of using the non-stirring solution at this time which could be caused by the presence of $CaCO_3$ or possibly slightly greater cell growth. As shown in Figure 4-9 the pH in both solutions decreased

more slowly than was observed previously in Figure 4.5, but a similar final pH was reached. The stirring of the bacterial solution may increase dissolution of $CaCO_3$ thereby buffering the solution and resulting in an increase in the pH. Therefore, the rate of optical density may be higher than the rate of increase of non-stirring solution.

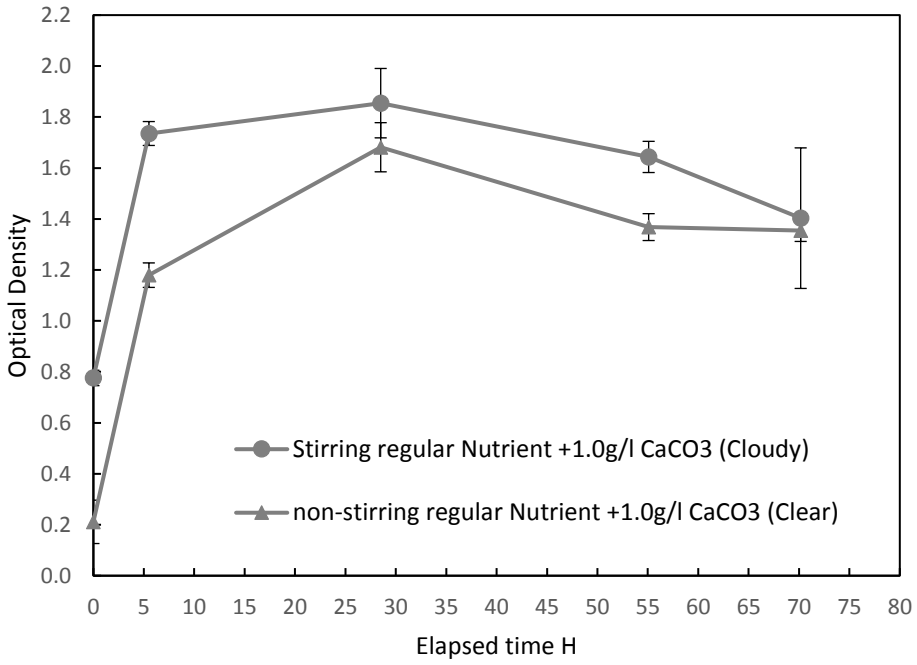

Figure 4-8. Optical Density of the bacterial solutions using stirring and non-stirring nutrient with the elapsed time.

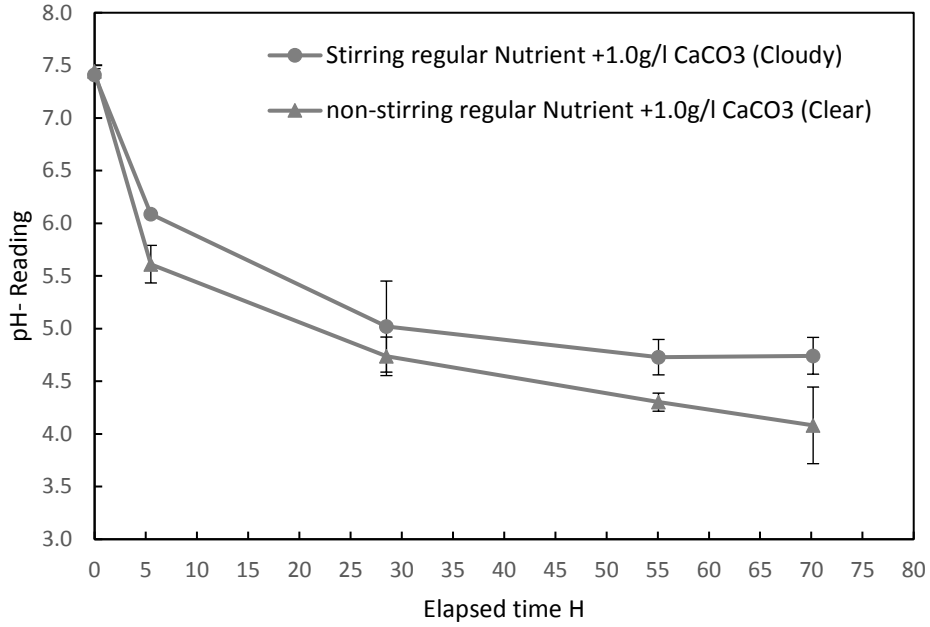

Figure 4-9. pH variation of the bacterial solutions using stirring and non-stirring nutrient with time.

4.3.4 The effect of chemical concentration

In the remainder of this section, experiments were conducted to study whether the amendment of some nutrient chemicals and further attempts at pH control had an effect on the growth of biofilm are presented. Effects on the pH and the impact on the growth of bacteria are investigated. For this experiment, five different nutrient solutions containing *B. indica* were prepared in triplicate. For each solution three 250 ml Erlenmeyer flasks were prepared containing 100 ml bacterial solution each based on some modification of the regular nutrient components. One set of solutions was used as a control, containing the regular Nu2 medium. The following amendments were made to the remaining four sets: a 50% reduction in $MgSO_4.7H_2O$ concentration (i.e. to 0.5 g/l). because the magnesium is the

required mineral and necessary for optimum growth as stated by Becking (1961) and Jensen (1954); reduction in the concentration of yeast extract to 50% (i.e. 0.5 g/l). Cole et al (2012) stated that the using of yeast extract causes production of a copious amounts of EPS; amendment to the regular nutrient Nu2 by adding 0.1 g/l magnesium carbonate ($MgCO_3$) to control nutrient pH using a more soluble buffer; adding of 1.0 g/l calcium carbonate ($CaCO_3$) to the regular nutrient to control nutrient pH using less soluble buffer; the $MgCO_3$ is more soluble in water than the $CaCO_3$. The solubility of $MgCO_3$ is 0.6 $g.l^{-1}$, whereas the solubility of $CaCO_3$ is 0.013 $g.l^{-1}$ (Aylward and Findlay, 2002).

The variation of pH and optical density were measured over a 50-55 hour incubation period. As shown in Figure 4-10, the range of initial pH readings for all media was 6.3 to 7.5. After around 27 hours of growth, the pH values drop to between 4.2 and 4.7. However, the largest optical density (shown in Figure 4-11) was measured at about 21 hours for both the mediums of reduced 50% of $MgSO_4.7H_2O$ and regular nutrient with 0.1 g/l $MgCO_3$ amendment. The reduction by 50% of yeast extract negatively affects the development of optical density otherwise the comparison between the standard nutrient and other mediums shows little difference regarding growth behaviour. Therefore, the standard nutrient could be used in the main biological experiments.

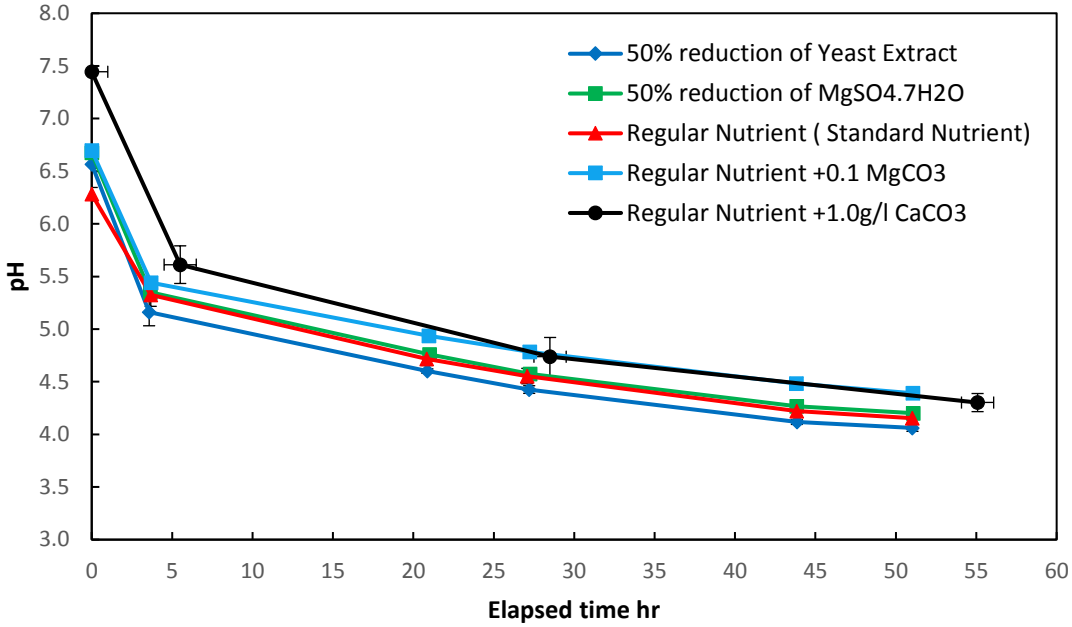

Figure 4-10. Variation of pH of the bacterial solutions with the time.

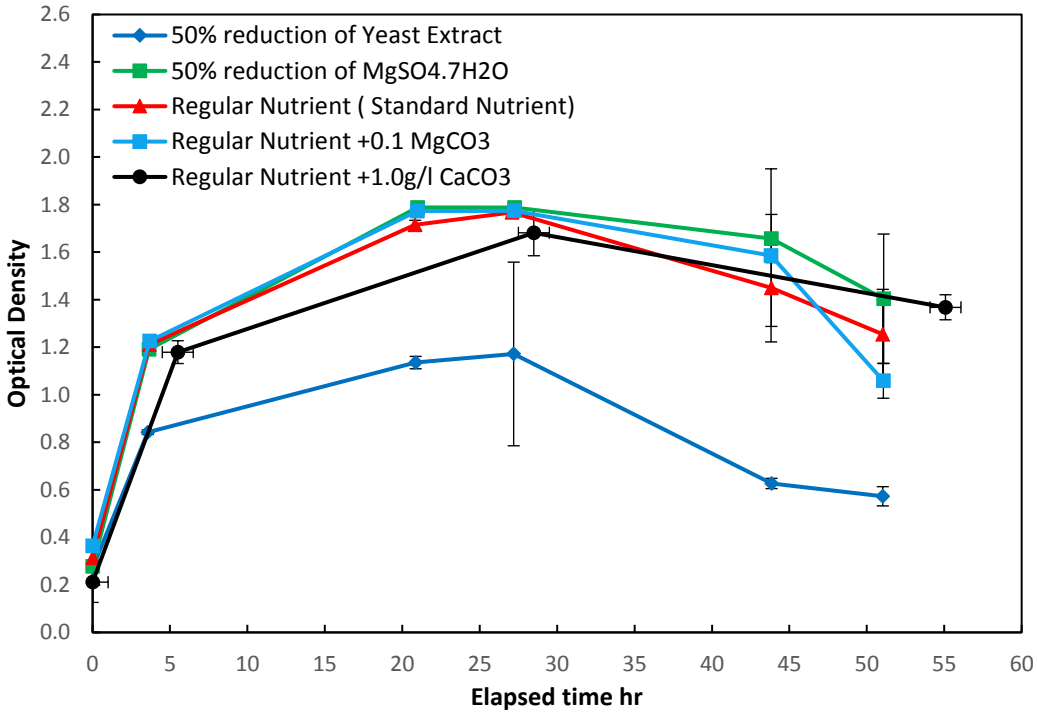

Figure 4-11. Variation of optical density of the bacterial solutions with time.

4.3.5 Using tap water instead of deionized water in medium

In this section, an investigation of using tap water instead of using deionized water in the preparation of nutrient solution is made. Two more nutrient

solutions containing *Beijerinckia indica* were prepared in triplicate. Each solution was prepared in three 250 ml Erlenmeyer flasks containing 100 ml bacterial solution. The solution was prepared using tap water one with $CaCO_3$ and the other without $CaCO_3$. This experiment explored the influence of effective elements in tap water and the same time the influence of alkalinity in this medium on the growth of bacteria . A comparison was made with a similar experiment with deionised water. Figure 4-12 shows that the effect of $CaCO_3$ on pH in either tap water or deionized water is not significant. The nutrient medium with tap water has a much slower decrease in pH than that with deionised water. Figure 4-13 shows that with tap water optical density peaked after about 30 hours at a higher value than that observed with deionised water. In addition, with deionised water the optical density declined after the peak. The slower pH reduction with tap water appears to positively affect bacterial growth.

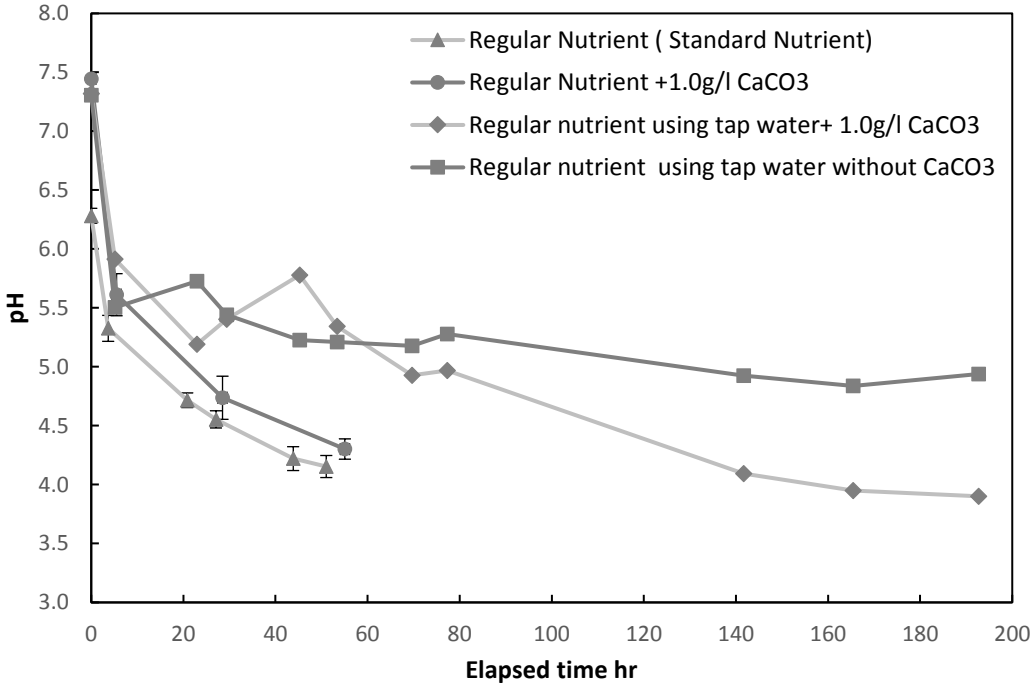

Figure 4-12. Reduction of pH for different used nutrients.

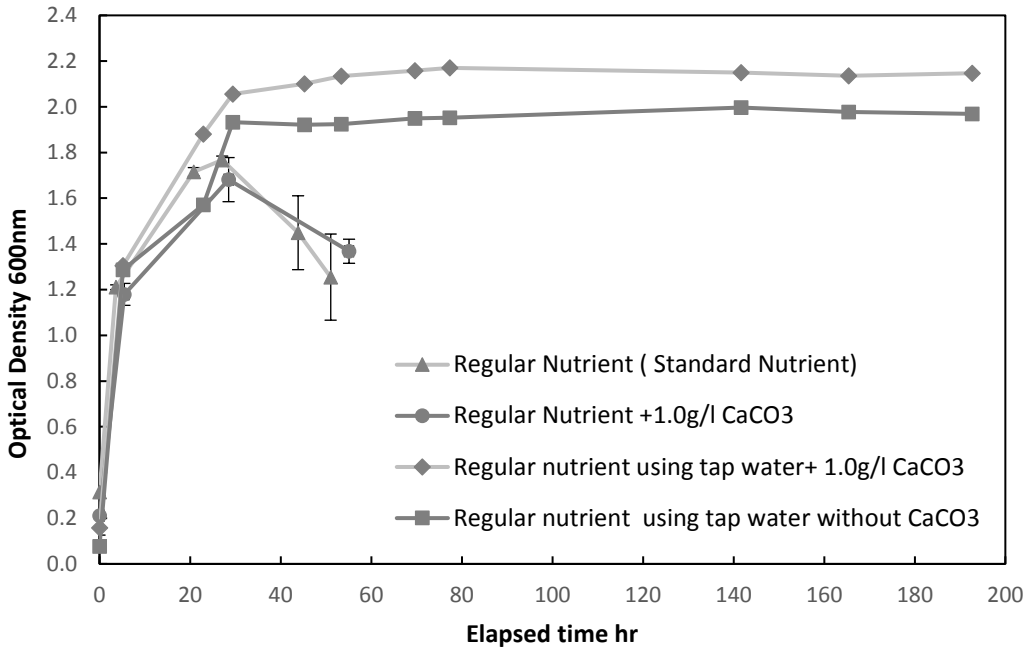

Figure 4-13. Optical density behaviour with different used nutrients.

4.4 Summary:

The main finding of these experiments is the possibility of using the same nutrient medium as used by Dennis and Turner (1998) for utlising of *Beijerinckia indica* microorganism. Before choosing this medium, further biological experiments and conditions of preparation were explored as mentioned above to obtain the optimal medium which produced higher amount of growth. The Nu2 medium produced higher biomass than the Nu1 without modification of chemical concertration. In addition, it is not necessary to use any buffers although using tap water in the medium offered advantages over deionised water.

Chapter 5

DEVELOPMENT OF A RELIABLE PROCEDURE FOR DIRECT SHEAR TEST UNDER LOW VERTICAL STRESSES

5.1 Introduction

The main aim of this chapter is to develop a consistently repeatable direct shear test procedure which is valid for use with biotreated samples under very low normal stresses. To attain this aim, a series of direct shear tests, based on the procedure described by BS 1377-7:1990, were conducted using dry silica sand. These tests were performed by using a light weight traditional direct shear box (60x60x4.5 mm) made from acetal plastic, and applying very low normal stress (1.0 kPa) during testing. Four aspects of the standard procedure are investigated which are i) the use of jacking screws before and during the tests, ii) the size of the gap established between the two halves of the shear box, iii) the mass of sand used and iv) the rate of testing.

In section 5.2 the use of jacking screws before and during the tests is investigated. The height of the gap between the two parts of the shear box was also evaluated to determine the gap height which produces the most consistent (repeatable) results. Moreover, another direct shear tests carried out to determine the suitable amount of sand can be used in the main biotreated experiment and other supporting tests.

The initial densities, peak stress, and residual stress, and their variables for all tests are presented by box and whisker diagram to evaluate the accurate method.

Finally, in section 5.3, the results of a targeted review of the relevant literature are also presented in relation to testing rates typically used with sands.

Following this series of direct shear tests, an experimental procedure is proposed as a standard method for use in the remainder of this study.

5.2 The effect of using jacking screws

To investigate the use of jacking screws in the test, three techniques were used: firstly, tests are performed using the jacking screws to raise the upper part of the shear box and then keeping the screws in place in the upper part of the box during the shear test, thereby, maintaining frictional contact between the screw tips and the upper surface of low part of the shear box. These samples were named as sample set A; the second technique, also used the jacking screws to raise the upper part of the shear box, but the screws are retracted, after making a gap between the two parts of the shear box and removed from the shear box before testing. These samples were addressed as sample set B; the third procedure was directly performing the direct shear test without using any jacking screws. These samples were described as sample set C.

Sets A and B were tested using 150 g sand mass but were repeated as sample set D and E by using 230 g of sand.

1.0 kPa was used as the applied normal pressure for all sets and the rate of test was 0.5 mm/min. This pressure was the minimum normal stress used in the main experiments of this study.

5.2.1 Retaining jacking screws during testing

A set of eleven specimens of 150 g of dry well-graded silica sand (Sample Set A) were tested using direct shear apparatus to investigate the effect of the presence of jacking screws during testing on the consistency of the measured shear stress and dilation behaviour of the tested specimens. The average prepared initial density and standard deviation of the specimens was

17.8 ± 0.2 kN/m^3. The average of the height of sand in upper shear box part was 6.08 ± 0.22 mm. The shear stress versus relative horizontal displacement ((RHD=horizontal displacement /specimen length)*100) are presented in Figure 5-1. As observed from the figure, the tests exhibit an initial peak stress for the most of eleven specimens. Thereafter, the shear stress increases with different rates to reach ultimate stress at further horizontal displacement. The definition of failure, as Bareither et al (2008) noted, is either a peak stress or an ultimate stress. Here, shear stress increases until the slope reaches a minimum after which the shear stress increased at a constant rate with further horizontal displacement. The observation of gradual increasing shear stress at large displacements are believed to be due to particle-box interactions (Bareither et al, 2008). The friction stress may be induced between the inside surface of the shear box and sand grains over testing. The exaggerated particle- box interaction may happen due to a greater number of particles moving within the shear zone. The shear failure may occur in this zone. The thickness of the shear zone depends on the size of the sample, gradation of soil, the rigidity of loading pad, the uniformity of applied normal stress (Moayed and Alizadeh, 2006). The initial peak shear stress was highly variable, as was the overall behaviour at higher RHD. In this test, the jacking screws were used to make a gap between both shear box parts and maintained raising the upper part of shear box during shear test. Therefore, the majority of friction occurred between the tips of jacking screws and the lower part of the shear box. Figure 5-2 presents dilation behaviour curves of the specimens, it can be seen under the low normal pressure of 1.0 kPa the sand directly exhibit dilation at the initial stage

of shearing (no initial contraction so the sand is behaving as a dense material).

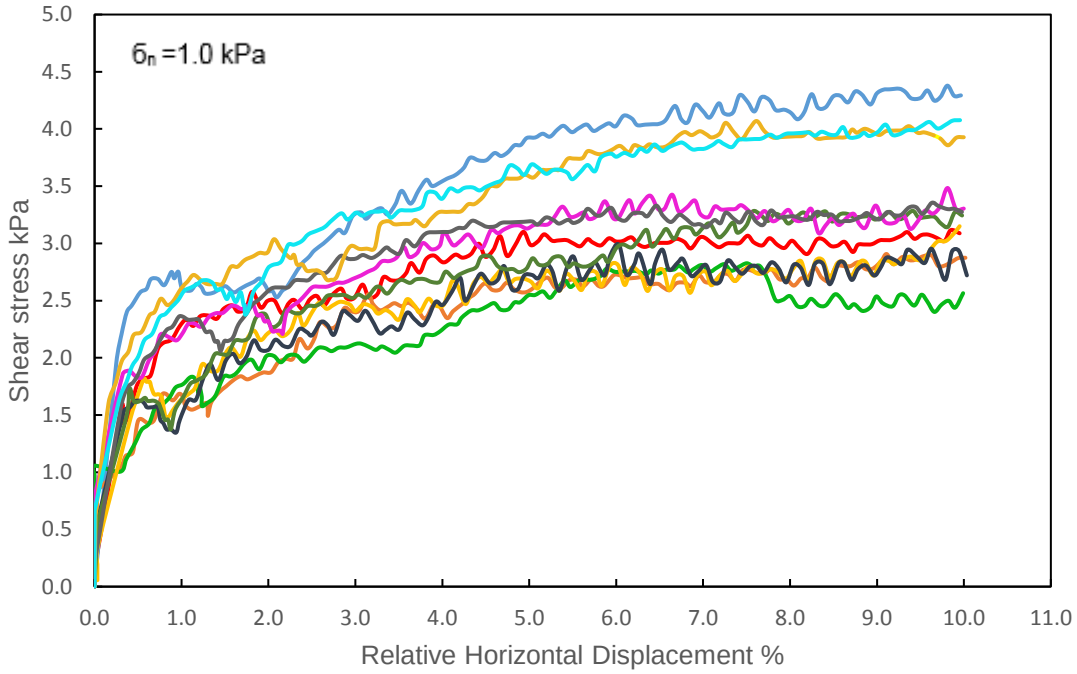

Figure 5-1. Shear strength relative horizontal displacement, (maintaining jacking screws).

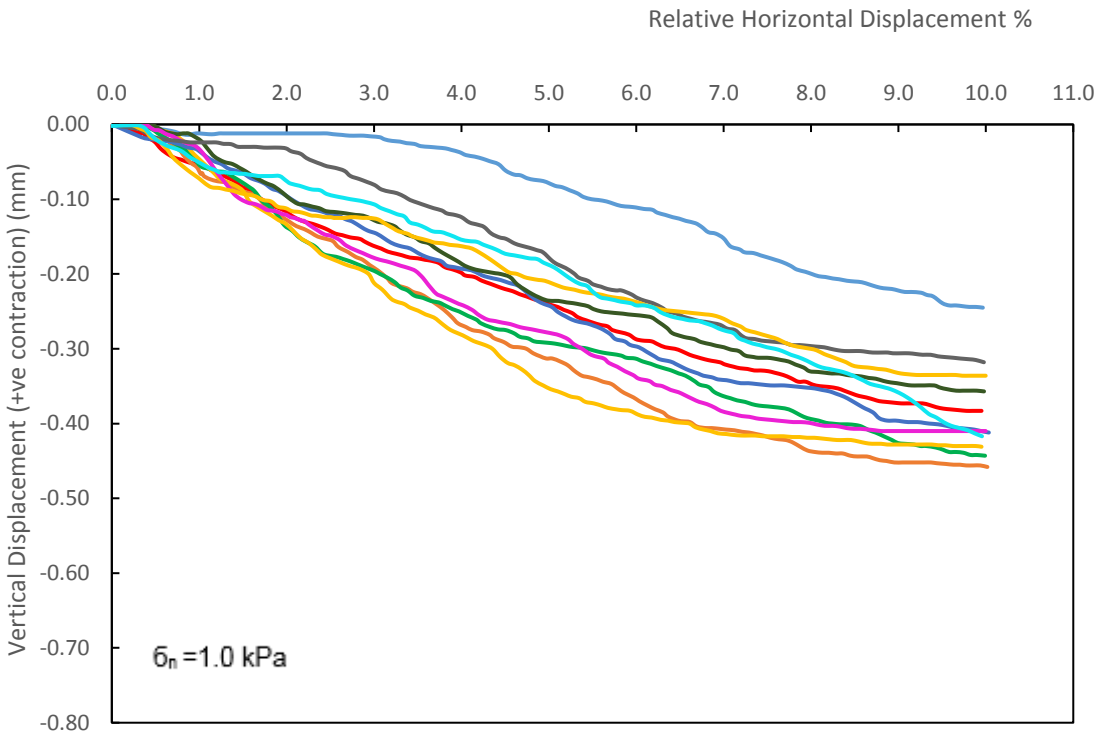

Figure 5-2. Dilation – relative horizontal displacement, (keeping jacking screws).

5.2.2 Retracting jacking screws before starting test

These experiments were conducted by using jacking screws to make a gap and then retracting the screws over testing as suggested in BS 1377-7:1990. A series of another eleven specimens of the dry well-graded sand (Sample Set B) were tested using the direct shear method to study the influence of gap between the upper and lower shear box parts on the resulting consistency of performance under shear. In this case, if the induced friction between sand particles and the upper box is enough to support the weight of the upper box, the gap space between the upper and lower parts of the shear box is maintained. The average height of sand in the upper shear box part was 5.45 ±0.46 mm. For this case, the vertical stress on the shearing plane is increased by the weight of the upper box (Lehane and Liu, 2013). Even if

the internal friction is insufficient to support the upper part of the box, the specimen dilation could lift the upper box in the early stages of shearing, and with sufficient internal friction, a gap between the box parts can be maintained. The existing friction on the inside of the upper box increases with the applied stress and therefore the possibility of a gap remaining throughout shearing is higher at larger applied stresses and for lighter shear boxes (Lehane and Liu, 2013).

The average initial density of the specimens was 18.4±0.3 kN/m^3. This density is slightly higher than the prepared density in the first approach in section (5.2.1). The resulting curves of shear stress and vertical displacement versus relative horizontal displacement (RHD) are shown in Figure 5-3 and Figure 5-4 respectively. As observed in the figure, some samples exhibit non-peak stress and there is considerable variability in the results, similar to the previous approach.

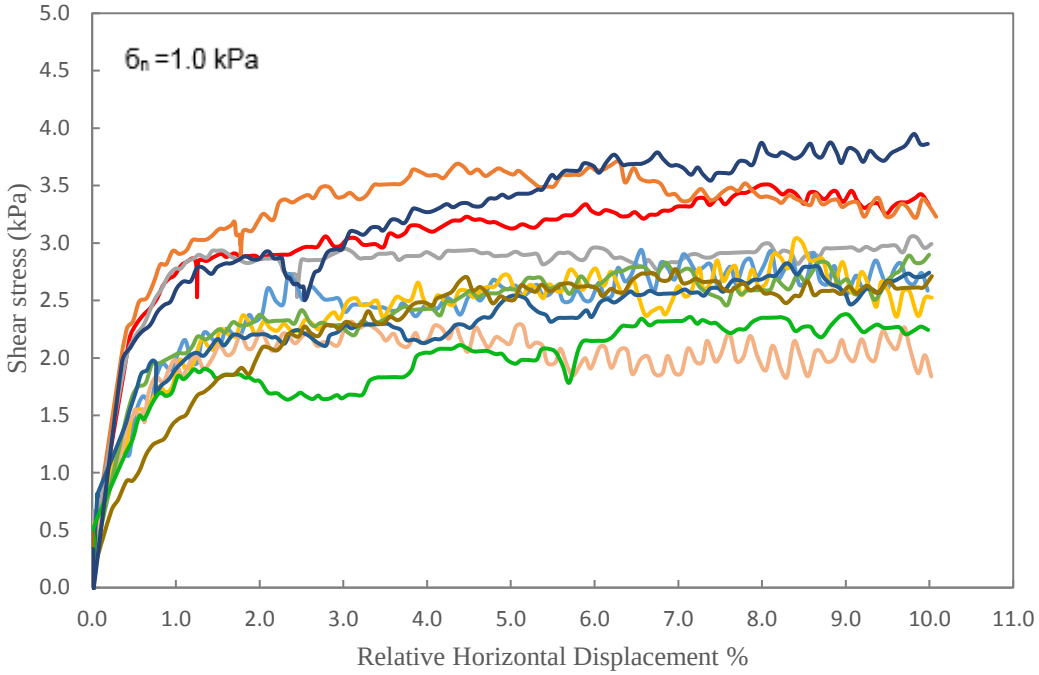

Figure 5-3. Shear strength relative horizontal displacement, (retracting jacking screws).

Figure 5-4 depicts the dilation behaviour of tested samples. It can be seen that the dilation in this approach shows that the sand exhibits higher dilation and more variation than the tested sand in the first approach (section 5.2.1).

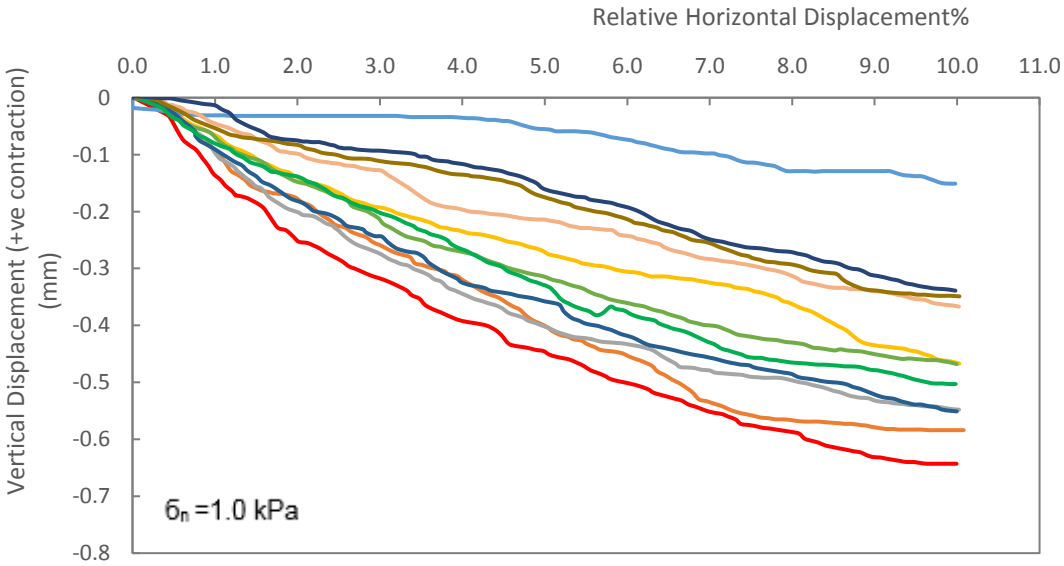

Figure 5-4. Dilation - relative horizontal displacement, (retracting jacking screws).

5.2.3 Testing without using jacking screws

This set of direct shear tests was performed without using jacking screws at all. Again, eleven specimens of the dry well-graded sand (Sample Set C) were tested to explore the consistency of the shear behaviour of sand specimens. The average prepared density of the specimens was 18.4±0.2 kN/m^3. The average of the height of sand in upper shear box part was 5.86±0.38 mm. The relationship between shear stress and RHD is presented in Figure 5-5. Most tests show similar behaviour, with stress increasing monotonically towards the ultimate stress, although a slight deviation after the initial rapid stress increase was observed in some. Again, there was considerable variability in results.

Overall, the tests carried out on sample sets A, B and C show high variability in the relationship between shear stress and lateral displacement. It is suggested that the reason for this may be the use of a small amount of sand of 150 g, as the uppermost surface of the sand was close to the shear plane and this surface was often not horizontal after testing, which may impact upon shear performance. Therefore, later tests were carried out with 230 g of sand.

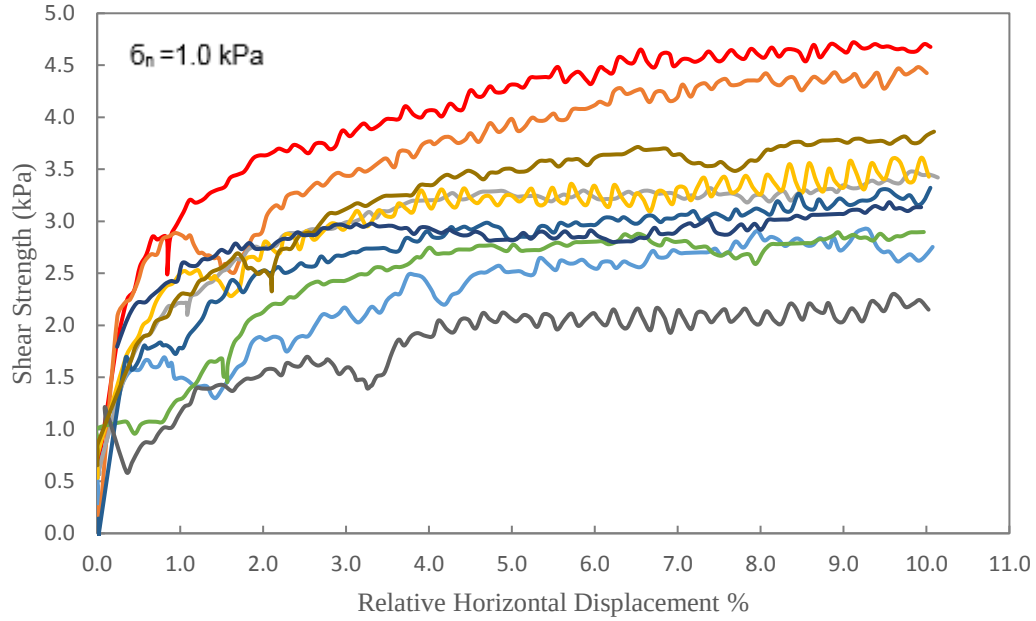

Figure 5-5. Shear strength relative horizontal displacement, (without using jacking screws).

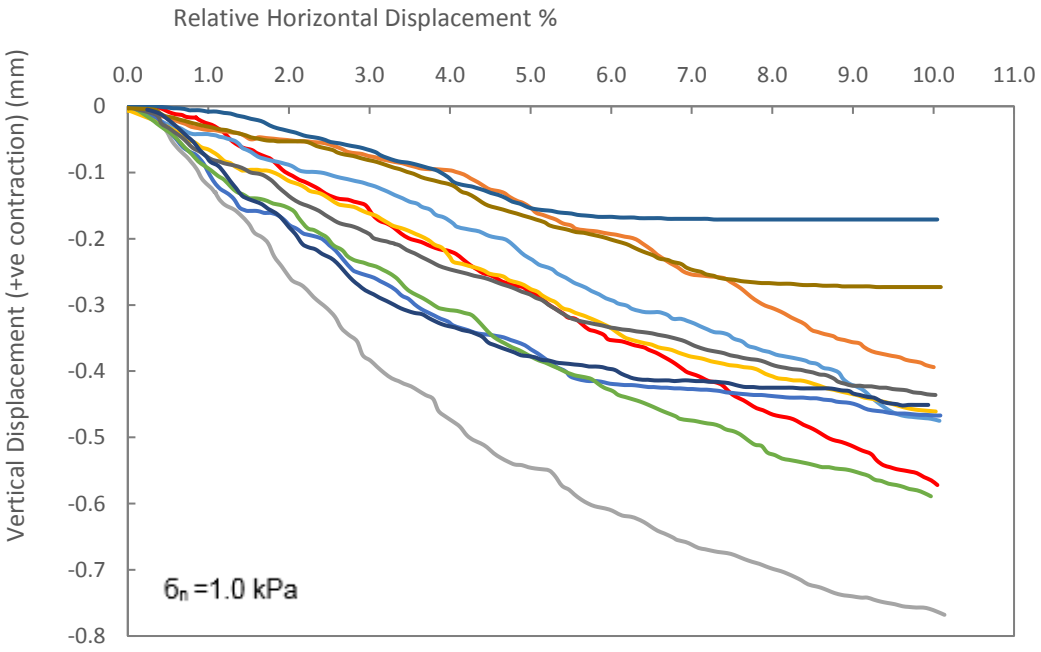

Figure 5-6. Dilation - relative horizontal displacement, (without using jacking screws).

5.2.4 Effect of sand mass on repeatability of direct shear test behaviour

This section explores the effect of increasing the mass of sand used, from 150 g to 230 g, on direct shear test behaviour. The normal pressure was applied 1.0 kPa again for all tests. The first set of eleven specimens (sample set D) was carried out by using the jacking screws to establish a gap and then leaving them in place over testing. The average initial density of these samples was 17.7±0.04 kN/m^3. The average height of sand in the upper part of a shear box of 18.14±0.08 mm. Various heights of gap were achieved between the two halves of the shear box with the presence of jacking screws, thereby, the friction happened between the top surface of the lower box part and both screws tips during testing. Figure 5-7 depicts the shear strength of these tests. All specimens exhibit peak stress, and after a softening stage (post peak), the stress slightly increases with horizontal displacement. This set of testing was performed with various gap measurements between two box parts up to 0.7 mm. The effect of small versus larger gaps was considered by categorising the different tests based on the gap size, of less than 0.3 mm and between 0.3 and 0.7 mm. Overall, there is little difference in shear behaviour corresponding to the gap height, but specimens with a gap between 0.3 and 0.7 mm tended to be more consistent compared to those with a gap from 0.2 to 0.3 mm (or less than 0.3 mm). Less dilation was exhibited by specimens with the larger gap size (Figure 5-8), and there is a

suggestion that dilation behaviour was more consistent in this group too.

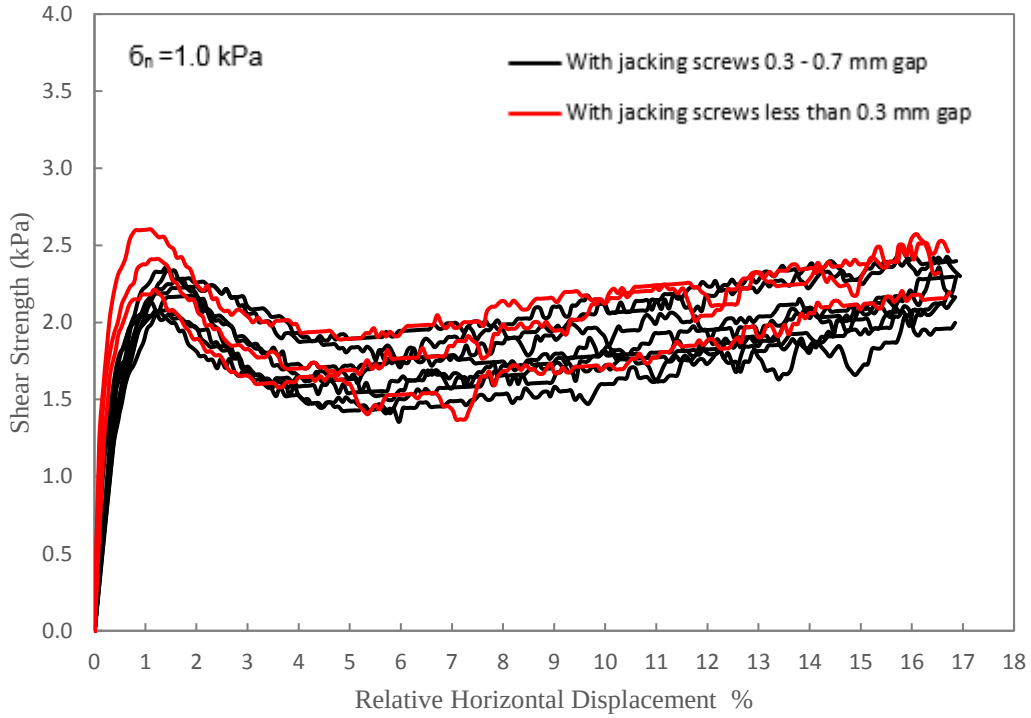

Figure 5-7. Shear strength relative horizontal displacement, (maintaining jacking screws).

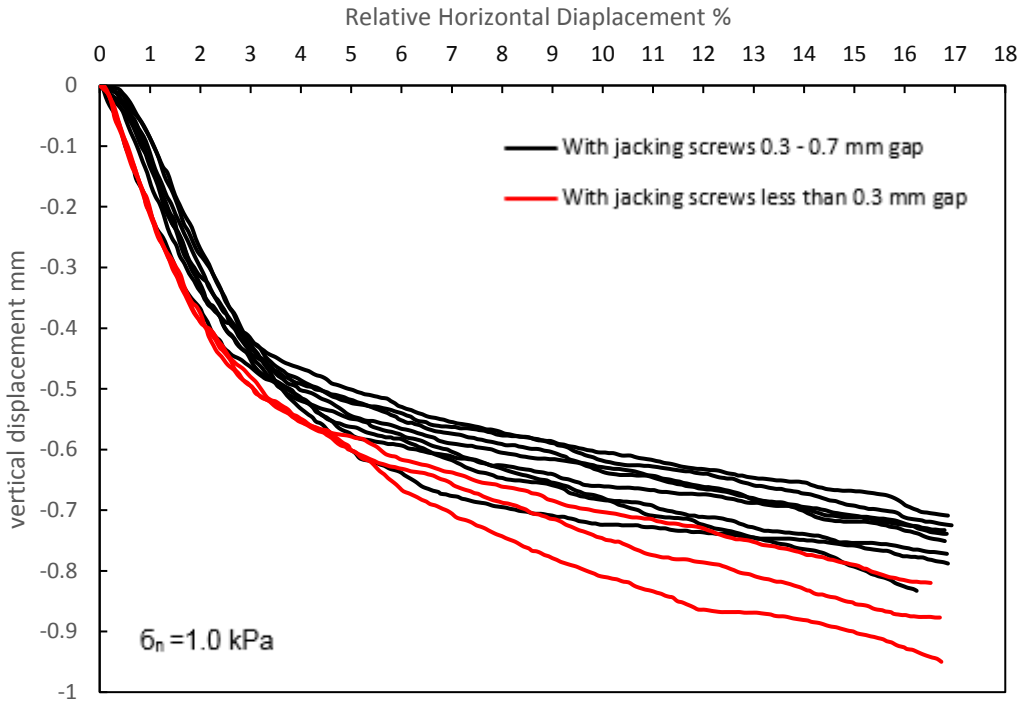

Figure 5-8. Dilation - relative horizontal displacement, (maintaining jacking screws).

The same experiment was repeated on another set of twelve samples (sample set E) but the jacking screws were removed after raising the upper part of the shear box. The average height of sand in upper part of box was 18.04 ±0.3 mm. The additional mass of sand in the upper part of the shear box may provide enough friction to maintain the gap during testing. The average initial density was 17.7 ± 0. 1 kN/m^3.

The average peak stress is 2.49±0.169 kPa for the gap from 0.2 to 0.7 mm, whilst for a gap of more than 1.0 mm the average peak shear stress is 2.47 ±0.304 kPa (as shown in Figure 5-9).

Figure 5-9 demonstrates a wide variation in peak shear strength and subsequent behaviour from sample set E, especially when the gap was more than 1.0 mm. Likewise, the dilation behaviour was also highly variable (Figure 5-10).

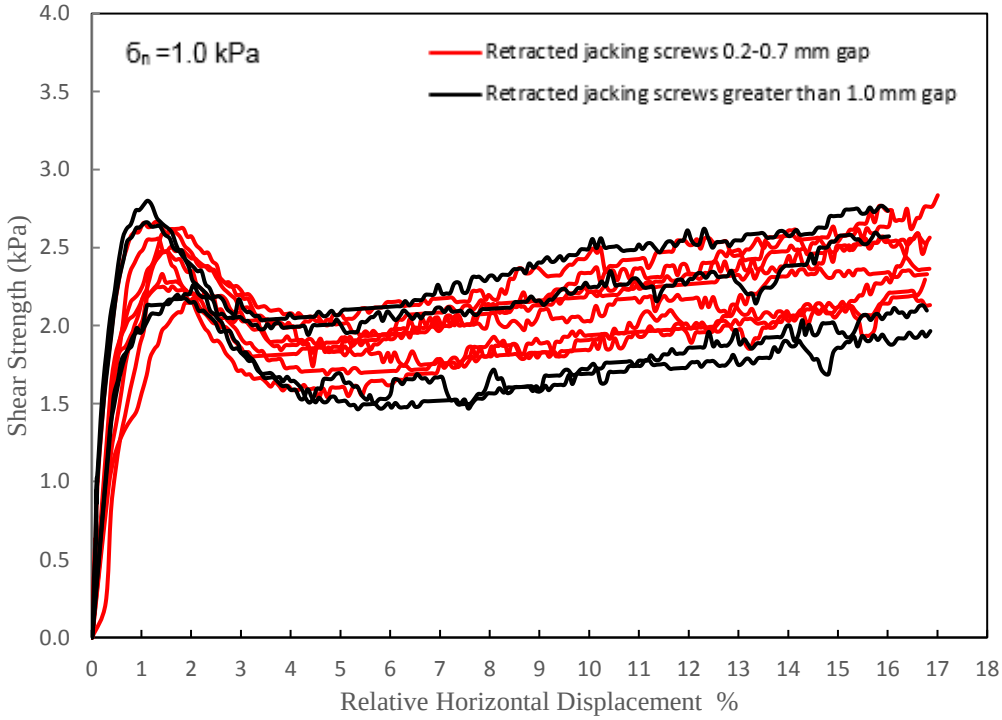

Figure 5-9. Shear strength relative horizontal displacement, (retracted jacking screws).

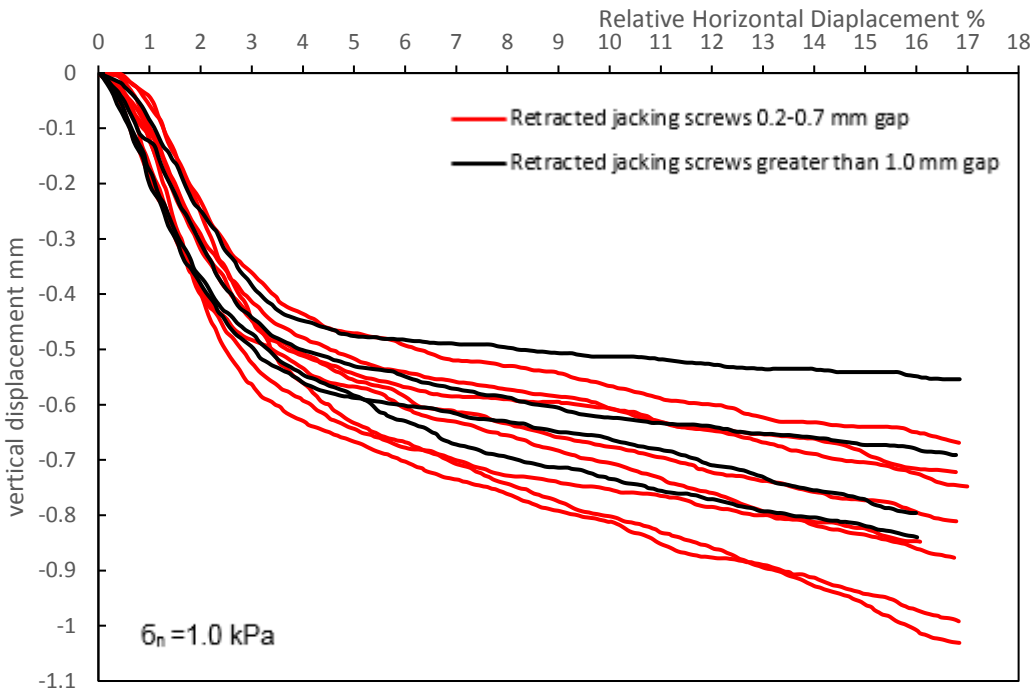

Figure 5-10. Dilation - relative horizontal displacement, (retracted jacking screws).

Using 230 g of sand provides a sand height in upper part of the box from 17.5 to 18.5 mm whilst 150 g provides a range of 4.0 to 7.0 mm of sand height over the shearing plane, as presented in previous sections. With only 1.0 kPa normal pressure applied, this may cause a plowing effect (Bareither et al, 2008) where the sand surface rises at one end of the shear box and drops at the other over the testing period. The use of 150 g sand shows critical state failure (non-peak behaviour) and high variability in the shear stress – horizontal displacement relationship as shown in Figure 5-1, Figure 5-3, and Figure 5-5. However, the using 230g exhibits peak stress and less variability than the using of 150g of sand as shown in Figure 5-7, Figure 5-9. Therefore, 230 g was chosen for use in the main experiments of this study.

5.2.5 The validation of the experimental procedure

Based on the results presented in the preceeding sections a further series of experiments were performed to validate this method which used jacking screws to make a gap from 0.3 to 0.7 mm between the two parts of the shear box. Two sets of seven direct shear tests (Sample Sets F and G) have been conducted on dry and well-graded sand with jacking screws present (F) or retracted (G). The applied normal stress was again 1.0 kPa and the amount of dry sand was 230 g. A density of 17.7 ± 0.00 kN/m^3 was measured for the tests keeping the jacking screws in place and 1.76 ±0.01 g/cm^3 for the test where the jacking screws are retracted. The average height of sand in upper box was 18.1 mm from the top specimen surface for both cases. In both cases, the gap between the two box parts ranged from 0.3 to 0.7 mm. The average peak shear stress with jacking screws present was 2.24 kPa ± 0.11 kPa, whilst with them retracted it was 2.47 ±0.16 kPa (Figure 5-11). It can be seen that the former offers slightly less variability in shear behaviour than the latter (SD of 0.11 rather than 0.16). With the former, despite the presence of friction between jacking screw tips and the top surface of lower box part, it shows peak stress of about 10 % less than the second case. The vertical displacement behaviour was considerably more consistent when jacking screws were left in place (Figure 5-12).

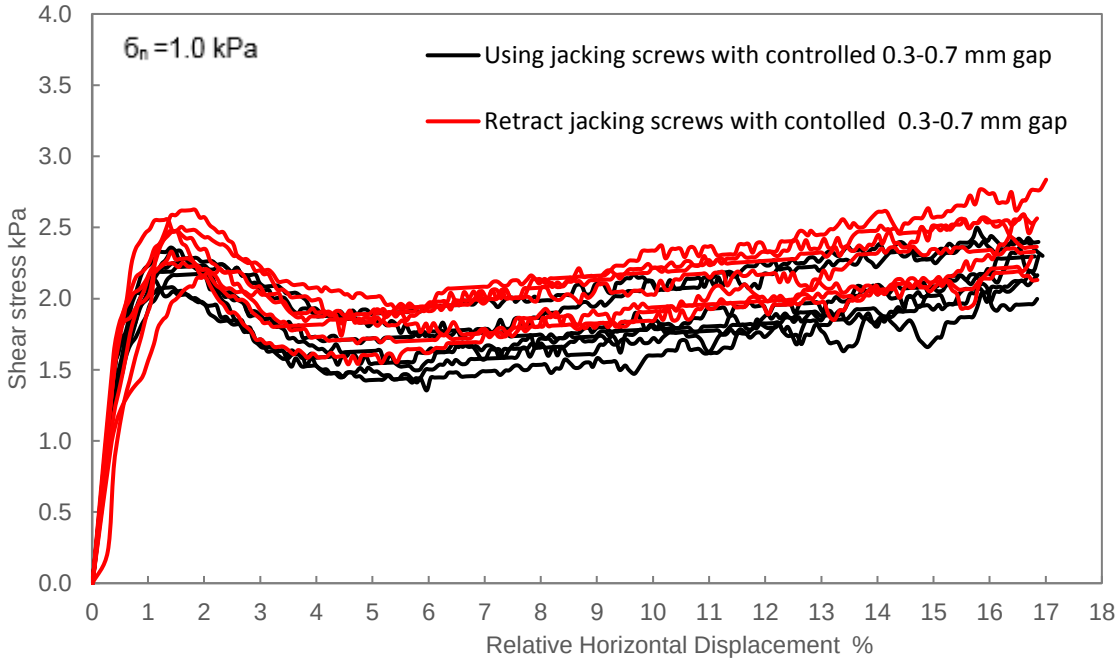

Figure 5-11. Shear strength relative horizontal displacement.

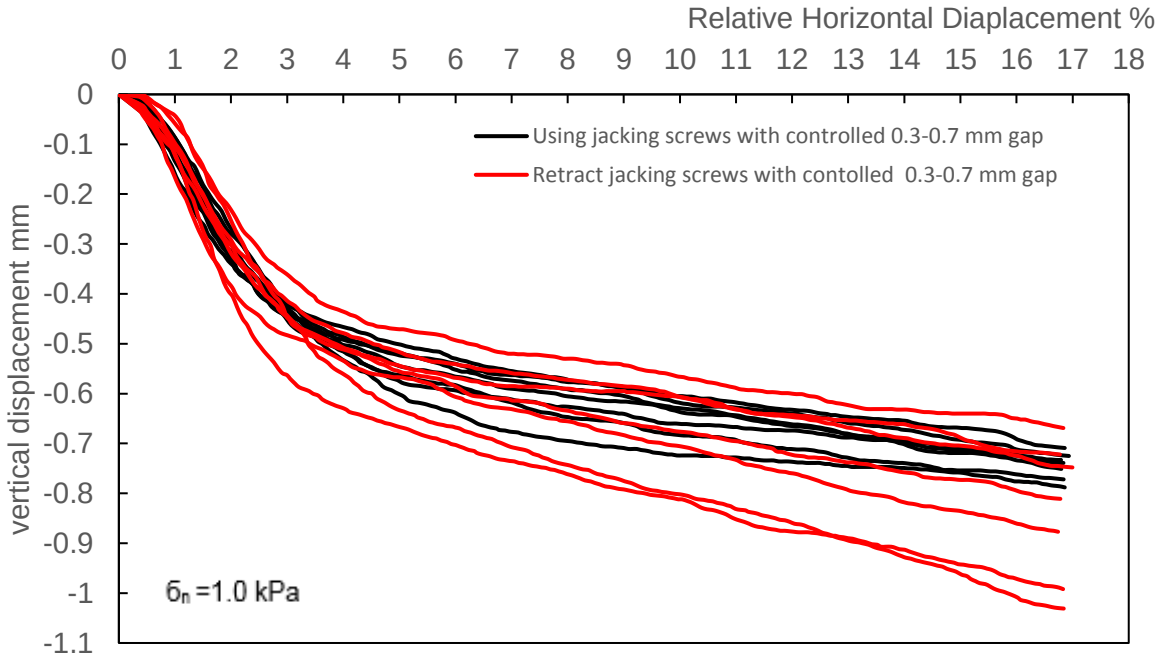

Figure 5-12. Dilation - relative horizontal displacement.

A simple comparison of initial density, peak and residual stresses between all tested cases from sample sets A to G are presented using box and whisker plots (Figure 5-13, Figure 5-14 and Figure 5-15) respectively.

On box and whisker plots, the height of the box shows the inter-quartile range, and the central line indicates the median of the data. The whiskers show the upper and lower maxima.

Figure 5-13. Box and whisker plot of initial density of samples. for all sample sets. It can be seen that the density of the sample of A, D, E, F and G have approximately the same average of the density of about 1.76 g/cm^3, whilst sample sets B and C exhibit a higher average density of about 1.84 g/cm^3.Sample set A shows higher variability than the other samples. Sets A and B had the largest variability whilst sets D to F had the lowest variability and were most consistent in their initial density.

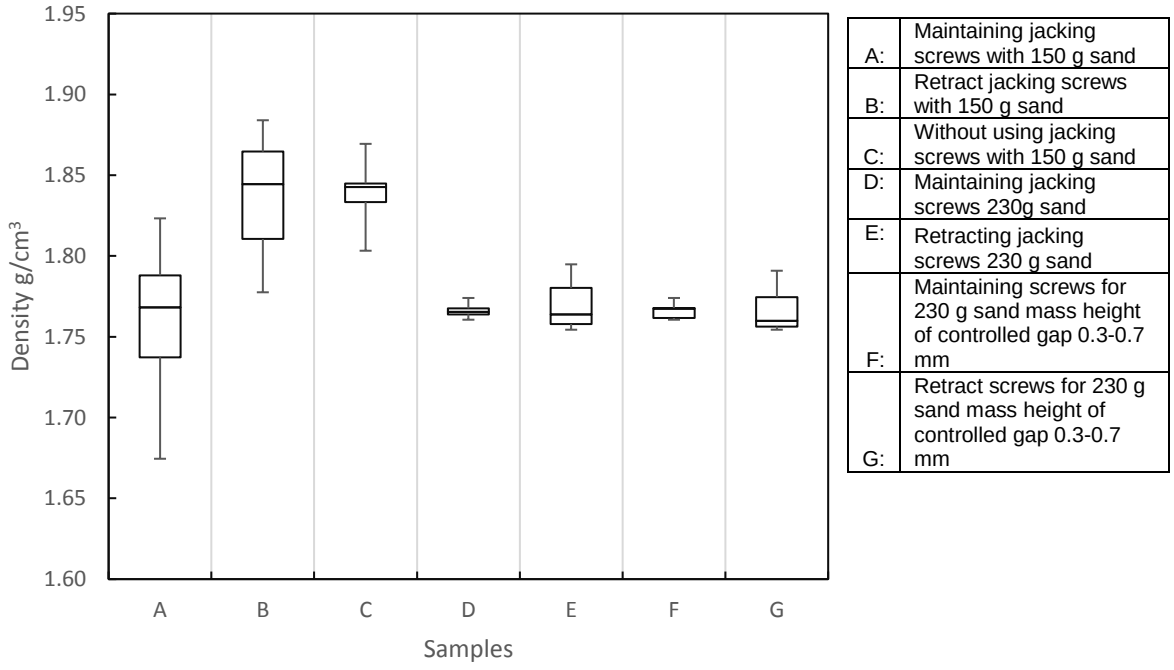

Figure 5-13. Box and whisker plot of initial density of samples.

Figure 5-14 shows failure stress data in a box and whisker format. In the three groups of tests (A/B/C, D and E, F and G), the first of each group kept the jacking screws in place during testing, whilst the second retracted the jacking screws. In sample set C jacking screws were not used at all. In sets A, B and C, 150 g of well-graded sand was used. In the remaining tests, 230 g of sand was used. In all three groups, the first set had lower variability and slightly lower failure stresses overall than the other sets. Therefore, it is concluded that using jacking screws and keeping them over testing would be more repeatable than the other conditions. The variability in sets D and F was lower than in set A, which indicates that a higher sand mass may be better. It is concluded that keeping jacking screws, with 0.3 to 0.7 % mm gap and 230 g of sand is the most reliable method to be used in the main experiments.

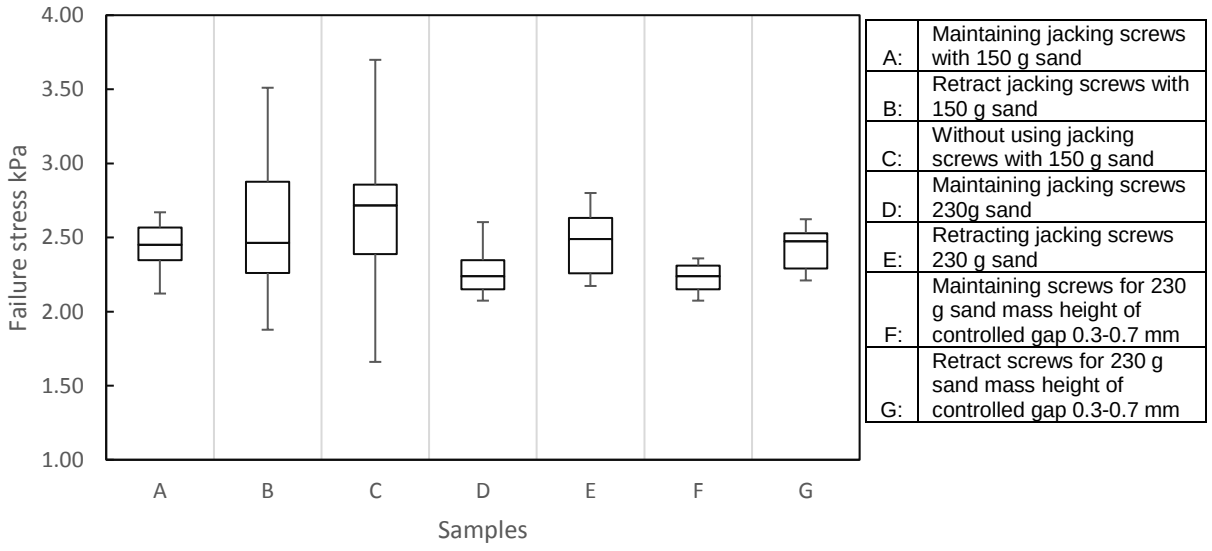

Figure 5-14. Box and whisker plot of shear failure stress.

Figure 5-15 shows the residual stress of all experiments, the average stress of D and F tests are approximately similar, but the first test less variability. Moreover, the tests of E and G nearly demonstrated a similar residual stress, but the test G less variability.

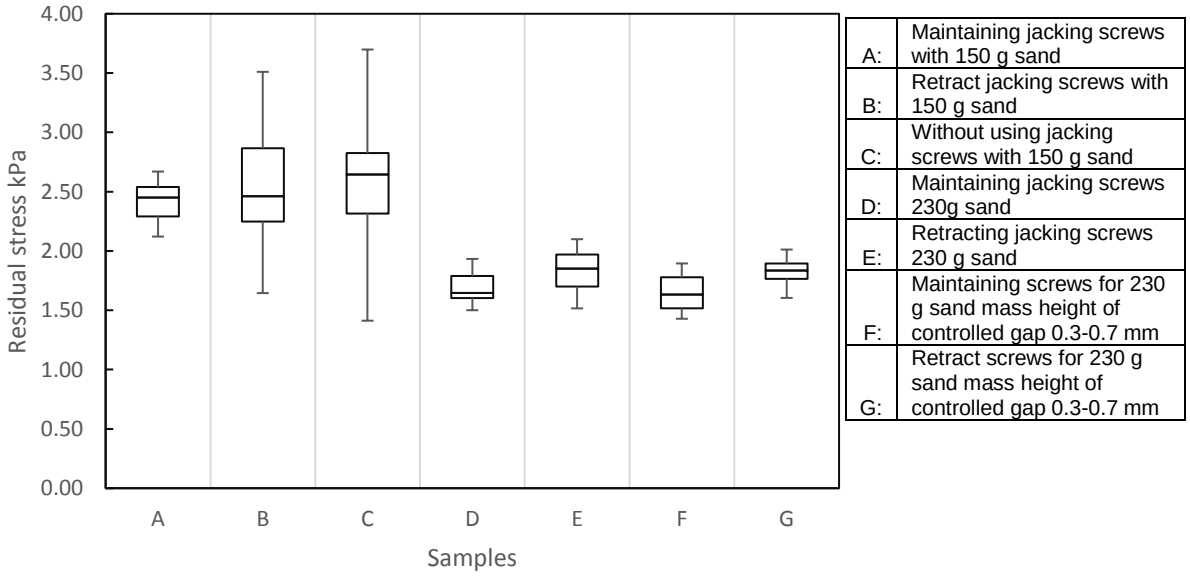

Figure 5-15. Box and whisker plot of residual stress.

5.3 Rate of test

According to ASTM D3080/D3080M-11 for well-graded and poorly graded sands, the required time of failure is 10 minutes, and the failure displacement is 5.0 mm, therefore the equivalent rate is 0.5 mm/min. British Standards (BS 1377-7 1990) note that the failure should occur within 5 to 10 minutes, thereby the rate of testing is equivalent to 0.5-1.0 mm/min. Relevant literature was reviewed to explore what other researchers report as an appropriate rate in their studies and to consider possible appropriate rates to test samples containing biofilm.

Four measures of test rate are mentioned in the scientific literature namely: the rate of displacement (mm/min), strain rate (%/s), required time to reach failure (min) and finally, the maximum required displacement (mm). As shown in Table 5-1 shearing rates range from 0.1 to 2.0 mm/min for wet and dry sand. Based on the standards and literature a rate of 0.5 mm/minwas chosen for the main experiments, but other rates were also tested.

Table 5-1. Literature review of typical shearing rates.

Reference	testing rate	Unit	Method	Soil type
Li et al (2016)	0.01	mm/min	Simple shear	Leighton Buzzard sand
Kwan et al (2016)	0.25	%/s	Simple shear	Monterey and Washed Mortar sands
Mamo et al (2015)	0.0069	%/s	DST	poorly graded sand
	0.0014			
	0.035			
	115		triaxial	dry Poorly graded sand
	1000			saturated the Poorly graded sand
Watanabe and Kusakabe (2013)	0.005-250	%/s	Triaxial	dry and wet Toyoura sand
Seminsky (2013)	2.0	mm/min	DST	mix gravel and Ottawa sand
Kalhor (2012)	0.133	mm/min	DST	Silty clay
Dadkhah et al (2010)	1.0	mm/min	DST	clayey sand
Thermann et al (2006)	0.5-0.05-0.005	mm/min	DST	mix clay silt sand 4,32,64% respectively
Edil et al (2006)	0.24	mm/min	DST	sand
Moayed and Alizadeh (2006)	0.9	mm/min	DST	Silty sand
(ASTM D 3080-98 2003)	10	min	DST	SW, SP (<5% fines)
(Yasufuku et al, 2003)	2.0-0.2-0.02	mm/min	DST	frozen sand
(BS1377:7: 1990)	5.0-10	min (quick test)	DST	Sand

5.4 Summary and conclusions

The investigation presented in this chapter has shown that using jacking screws with a gap from 0.3 to 0.7 mm between shear box halves and a sample mass of 230 g enables a good consistency and repeatability of result. Unlike, using 150 g of sand mass demonstrates high variability of shear result and the shear stress increases for further displacement after post peak because of plowing effect (Bareither et al, 2008). The height of sand in upper is about 6.0 mm may cause plowing during shearing test. Despite BS 1377-7:1990 suggested retracting the jacking screws during direct shear test, retaining the screws during the test shows consistent measured shear stress and dilation behaviour of the tested specimens. The rate of shearing was chosen to be 0.5 mm/min.

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Chapter 6

RESULTS AND DISCUSSION OF BIOLOGICAL EXPERIMENTS IN SAND AND COMPARISON WITH DRY AND SATURATED SAND

6.1 Introduction

The main biotreated experiment is the key experiment in this study. This experiment includes five sets of well-graded silica sand samples. Each set comprises three biotreated well-graded silica sand samples and three non-biotreated (standard) samples tested under direct shear at one of five applied normal stresses: 1.0, 4.1, 8.89, 16.2 and 25.0 kPa. The normal stresses were applied to the samples over an incubation period as well as during a direct shear test.

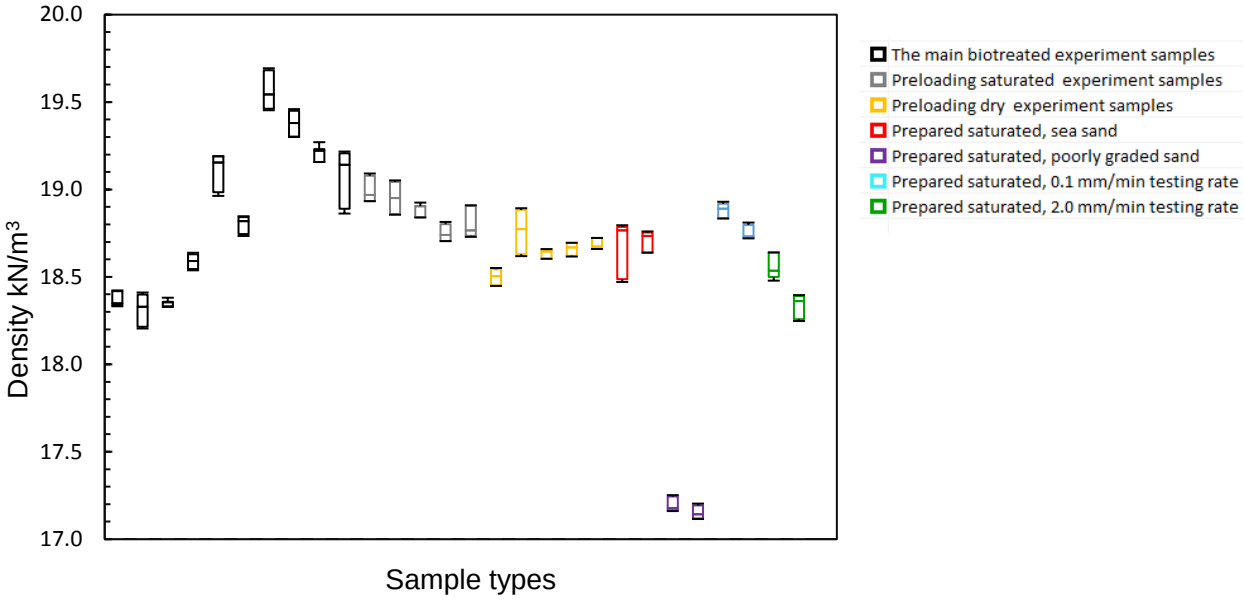
The biotreated experiment was performed by using *Beijerinckia indica* to form a biofilm or bacterial polymer EPS within the sand matrix during an initial incubation period. This EPS may influence the shear characteristics of the sand such as peak and residual stresses, dilation behaviour, peak and residual dilation angles, peak and residual internal angles, as well as sand compression. A comparison was made between the shear outcomes of biotreated samples and standard ones. In addition to the main biotreated experiment described above, a series of additional abiotic tests were performed for further investigation into various aspects including: two sets of direct shear tests performed on a clean dry and water-saturated sand, applying the same normal stresses and using the same sample preparation as in the main set of biotreated experiments. The presence of biofilm may change the hydraulic behaviour in the sand and so these tests provide information on performance under different saturation conditions; to more accurately mimic the main biotreated experiments, two further sets of tests were carried out on preloaded clean dry and water-saturated samples to study the effect of preloading process on the

behaviour of tested sand; one set of saturated samples was tested using mineral nutrient medium instead of deionised water to eliminate the effect of the medium used in biotreated and standard samples; it was hypothesised that wet pluviation may have caused particle size segregation, and so an air pluviation method was used to prepare some samples which were then soaked in water overnight - results were compared to wet pluviation methods; a number of additional experiments with biotreated and standard samples were carried out to study the following effects: a set of six samples (three biotreated, three standard) of a poorly graded sand were tested in the same way as the main biotreated experiment (well-graded sand) at a normal stress of 1.0 kPa. The amount of formed biofilm in both sand masses and the effect of such biofilm on the shear parameters are considered; the effect of particle shape on the shear response was explored in biotreated and standard tests, using a sea sand, which was more rounded than that the silica sand. A set of direct shear test was conducted on a well-graded sea sand at a normal stress of 8.89 kPa, the same testing rate (0.5 mm/min) and using similar biological treatment preparation used in the main set of biotreated experiments; finally, the impact of the shearing rate with and without biotreatment was studied. Two sets of direct shear experiments were performed on well-graded silica sand, applying the same normal stresses and using the same biological preparation used in the main set of biotread experiemnts. Shearing rates of 0.1, and 2.0 mm/min as well as a test rate of 0.5 mm/min, (as used in the main biotreated experiment) are considered all with an 8.89 kPa normal pressure.

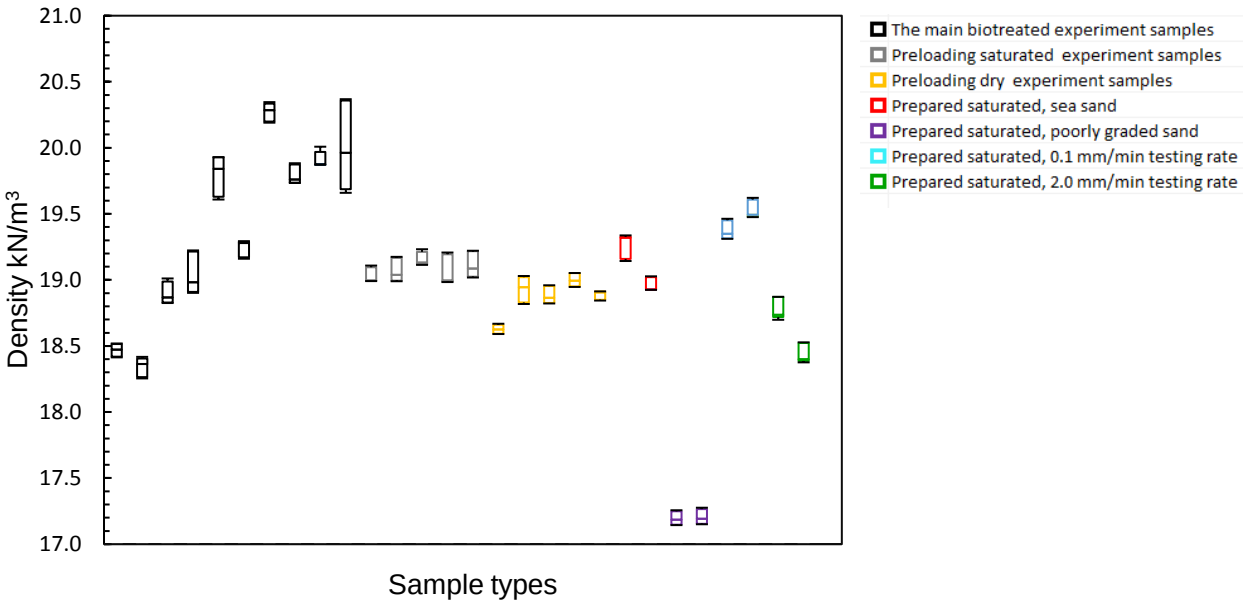
All experiments are described in more detail in chapter three, section 3.1.

6.2 Initial density

In this section, the dry density of all tested specimens has been compared to determine the repeatability of the sample preparation method, and to understand whether any differences in initial conditions may have affected the test outcomes. Figure 6-1 (a) and (b) present the density after sample preparation and that after the incubation stage with preloading (i.e. just before direct shear testing). Numerous research studies have concluded that the behaviour of sands can be significantly influenced by the initial state of the soil. The method of sand packing affects the shear behaviour as indicated by Oliveira et al (2012), Dave and Dasaka (2012), Della et al (2011), Yamamuro et al (2008), and Vaid and Negussey (1984). Therefore, it is important to present the variation of prepared dry density (using dry sand mass 230 g) of each specimen because this may affect the outcomes of shear tests. Figure 6-1 presents data as box and whisker plots, where the box height indicates the interquartile range and the line across the box represents the median. The key criteria for comparison between the samples are the median and the height of the box.



(a) Sample density after preparation.



(b) Sample density just before testing (final density)

Figure 6-1. Box and whisker plot of sample density.

For the main biotreated experiment, the increasing sample density, as shown in Figure 6-1b (after incubation and preloading), correlates with increasing applied

normal stress, apart from at the highest normal stress of 25 kPa where the density was similar to that of 16.2 kPa. However, this final density may depend on the differences in initial prepared density for each sample as illustrated in Figure 6-1a, which shows similar behaviour.

For dry and saturated clean sand subjected to preloading, the density is closer for all samples after preloading compared with that after preparation and does not appear to be related to applied normal stress.

The prepared dry density of poorly graded sand was less than the density of well-graded ones. The preparation of such samples was achieved by using 230g of poorly graded sand mass to keep the amount of sand mass for all tests. The density of poorly-graded sand was approximately 5.5 % less than the minimum density of the other samples as shown in Figure 6-1b. This sand may occupy a larger volume than the same amount of well-graded sand, because the poorly graded sand has more pore volume.

In order to compare the shear parameters between the main biotreated experiment and the saturated and dry sand samples, the comparison of the density after preparation (air pluvation for dry samples and wet pluvation for saturated samples) is presented separately in Figure 6-2 – it is not presented in Figure 6-1 as there was no preloading applied in these tests. The saturated samples were prepared and tested in single sample while the dry samples were in duplicate. It can be seen that the dry samples show the lowest density. It is well known that the dry density would be less than saturated density because of using air pluviation technique for dry samples rather than wet pluviation for

the preparation of saturated specimens as well as the effect of shaking for 1.0 min for saturated sample more effective than dry sample preparation.

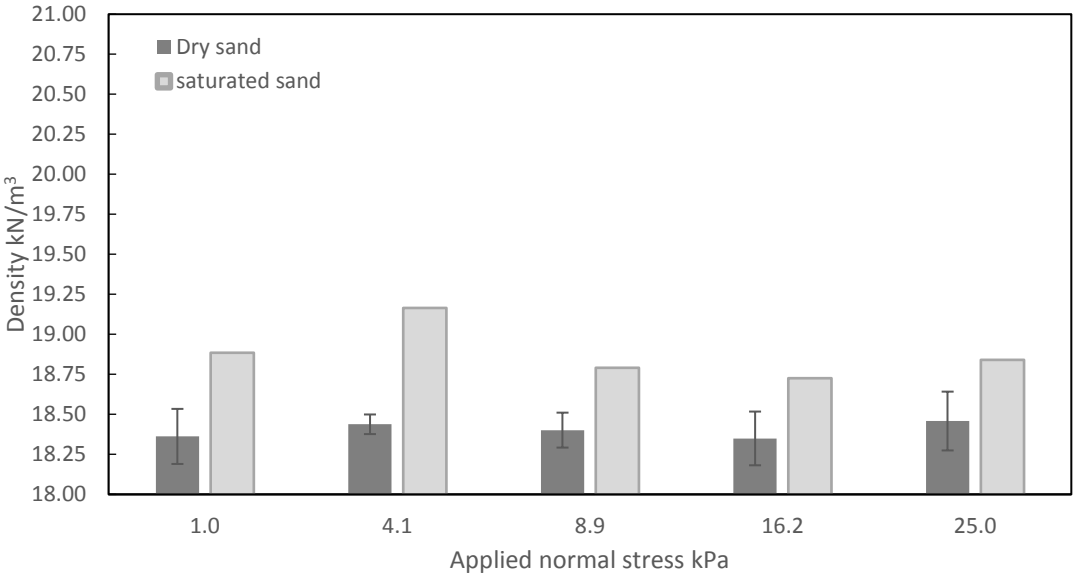


Figure 6-2. The prepared density of dry saturated of clean sand samples.

Figure 6-3 shows in more detail the average density after preloading of biotreated and standard samples that were part of the main biotreatment experiment showing increasing density with normal stress up to 16 kPa normal stress. There is also some indication at larger normal stress that biotreated samples had larger densities after growth of biofilm than standard samples suggesting that biofilm may cause increased compressibility.

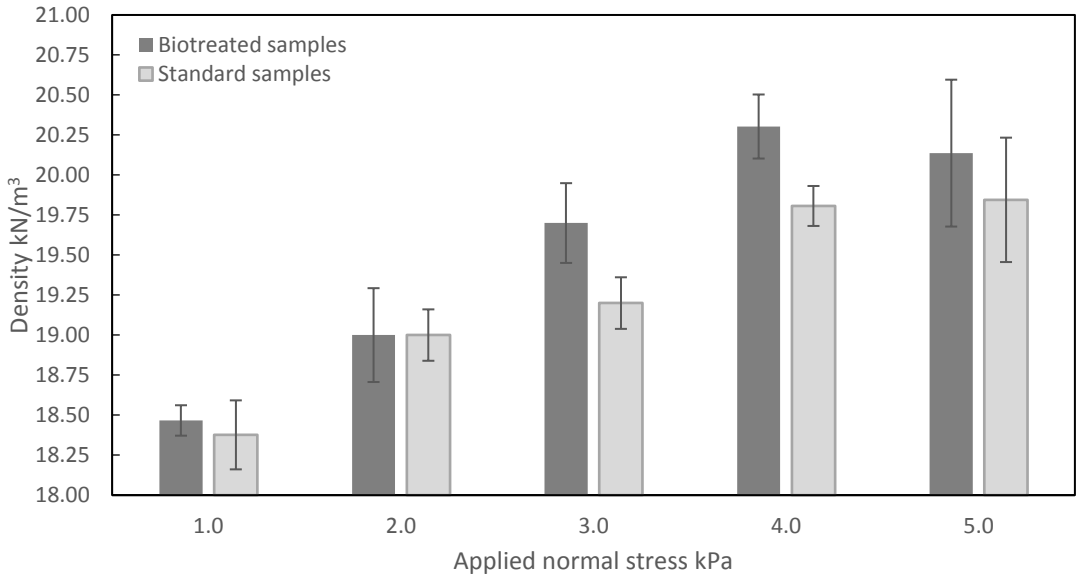


Figure 6-3. Density after preloading of biotreated and standard samples.

The potential effect of biofilm on increased densification during preloading is shown in Figure 6-4 which shows the ratio of the density after preloading and incubation (causing biofilm growth) to that found after initial preparation. A value greater than 1 would indicate that biofilm growth and preloading have densified the sample, whereas for standard samples the effect would only be caused by preloading (no biofilm growth). For a loading of 1.0 kPa, biotreated and standard samples behaved similarly. However, the other loadings indicate that the growth of biofilm caused the sand to be densified under loading compared with the standard samples. This biofilm may work as a lubricating agent which helps the sand particles to compact under loading (Perkins et al, 2000). The variability of samples at 25.0 kPa and 4.1 kPa was high, may depend on the variability of initial density of this loading.

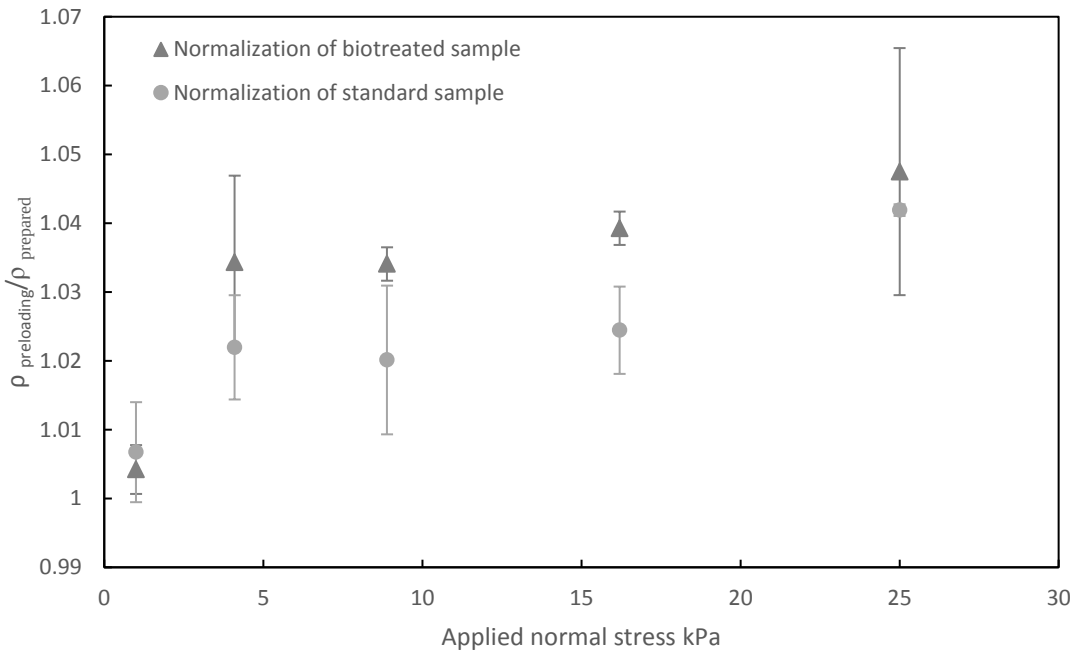


Figure 6-4. The normalization of the preloading density to the prepared density with the effect of biofilm presence.

6.3 Shear data of biotreated and standard results

The direct shear tests for all sets of biotreated and standard specimens under vertical pressures of 1.0, 4.1, 8.9, 16.2, and 25 kPa investigated the impact of accumulated bacterial polymer on shear behaviour of the sand samples by comparing with standard specimens. This polymer is thought to grow between silica sand particles during the incubation stage.

These tests study the differences of peak and residual stresses between the biotreated and standard specimens. In addition, the amount of biomass within the sand mass was identified by using the loss on ignition (LOI) method as described in chapter three. Also, dilation behaviour, dilation angles, relative

horizontal displacement (RHD) at peak stress, internal friction angles, and compression of samples over the incubation period were studied.

6.3.1 Shear response in direct shear testing

Shear stress and vertical displacement versus the relative horizontal displacement for biotreated and standard samples are presented in Figure 6-6 to Figure 6-10. Overall, the key point of all figures is the peak stress in biotreated specimens is consistently larger than in the standard samples. However, the residual stresses are very similar. After the peak stress, a subsequent decrease in shear stress occurs, then the shear stress starts to increase again at further horizontal displacements in all tests, especially in the 1.0 and 25 kPa loading tests. The interaction of sand particles and the shear box may cause this increase of shear stress at larger displacement after post peak stress, as observed in Figure 6.6 to Figure 6-10. The interaction may happen by the particle to particle force concentrations at the front of the upper shear box and back of the lower box during the direct shear test. Thus, particle movement may create force concentrations that are transferred to the particle-box interface, increasing the measured shear resistance as discussed by Bareither et al (2008) (Figure 6-5).

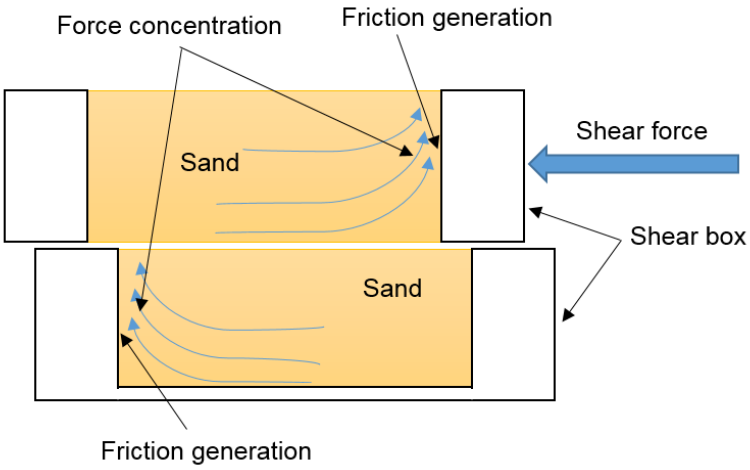
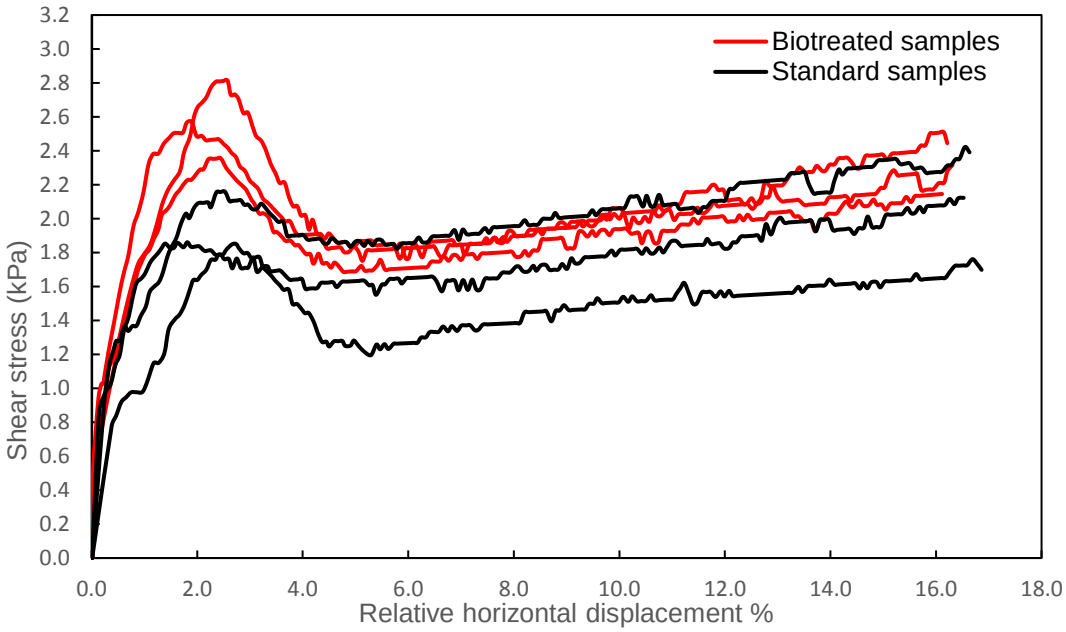


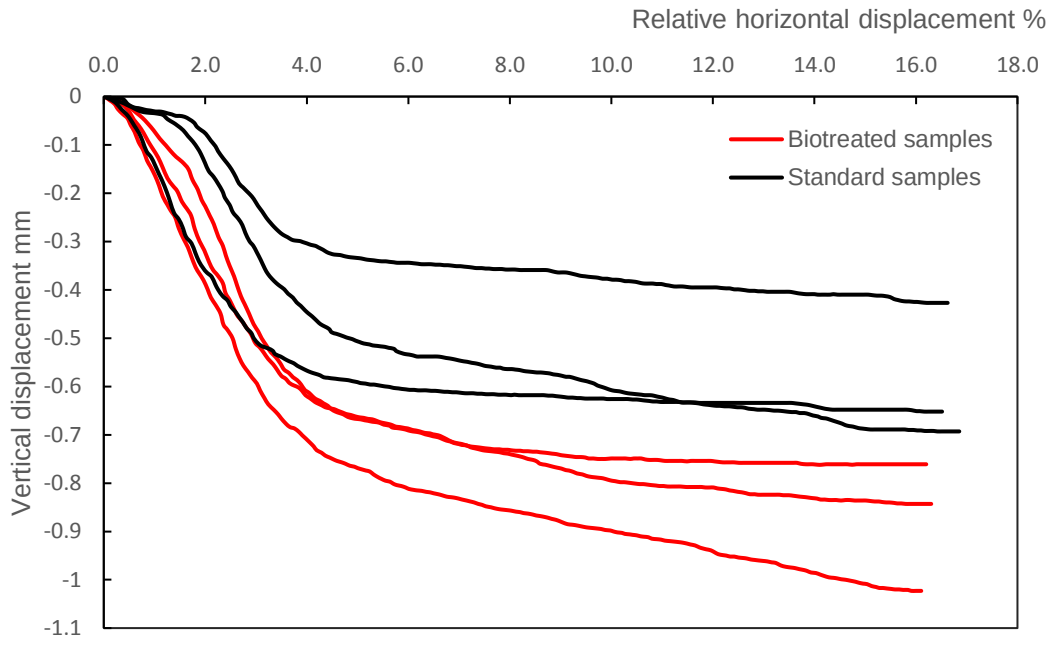
Figure 6-5. Schematic diagram for explanation of interaction friction by Bareither et al (2008).

Overall, the shear stress of biotreated samples steeply increases to reach the maximum peak stress. In the post peak (softening) stage, the shear stress rapidly decreases to reach a residual state for biotreated samples whereas the shear stress more gradually decreases to similar residual values for standard samples.

The volume change gradually increases at larger horizontal displacement for 1.0 kPa loading (Figure 6-6 b). In the 25.0 kPa loading test, the dilation decreases at further displacement, thereby, the sand tends to show contraction (Figure 6-10 b). On the other hand, tests at 4.1, 8.89, 16.2 kPa show little volume change after about 6-8 % of displacement (Figure 6-7 to Figure 6-9). So a critical state (named as a residual stress in this study) occurs at this percent of displacement as defined by Budhu (2007) and Bareither et al (2008).

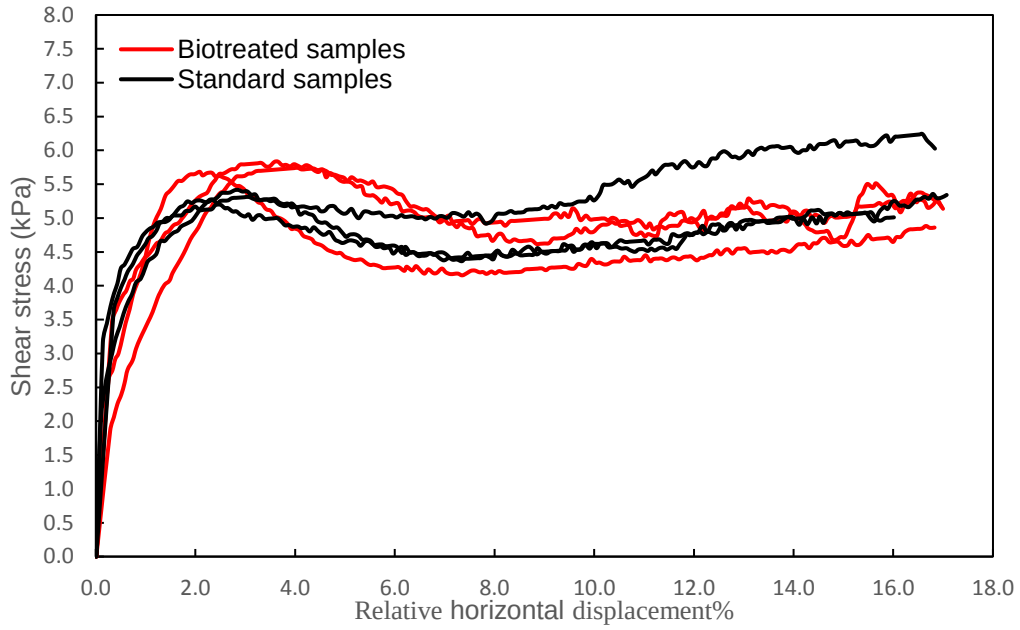


(a) Shear stress

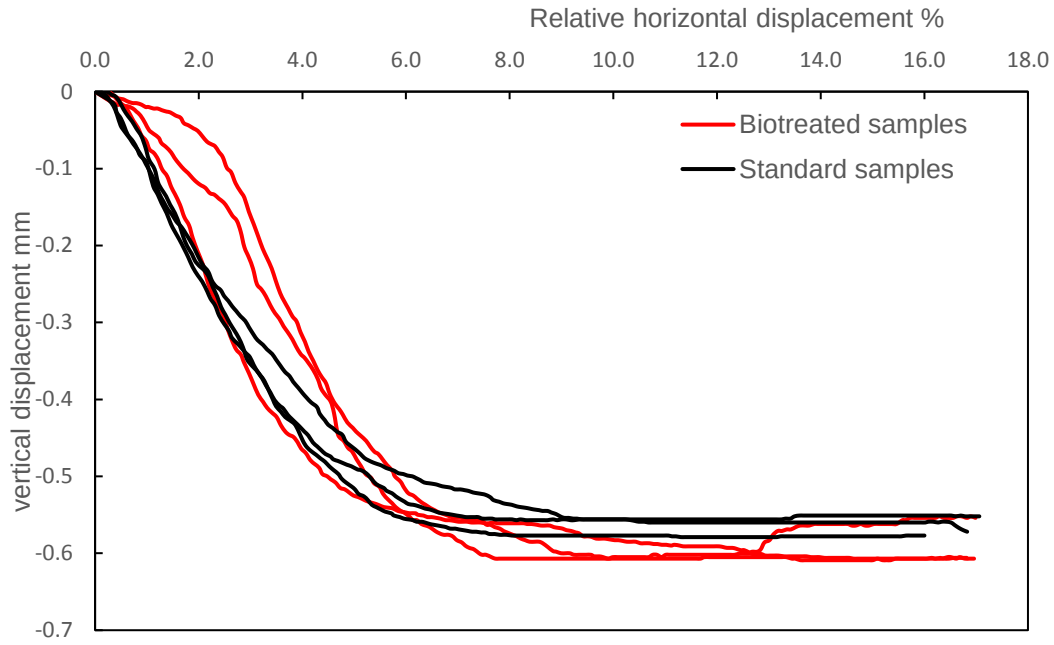


(b) Vertical displacement

Figure 6-6. Shear stress (a) and vertical displacement (b) versus relative displacement for a vertical loading of 1.0 kPa.

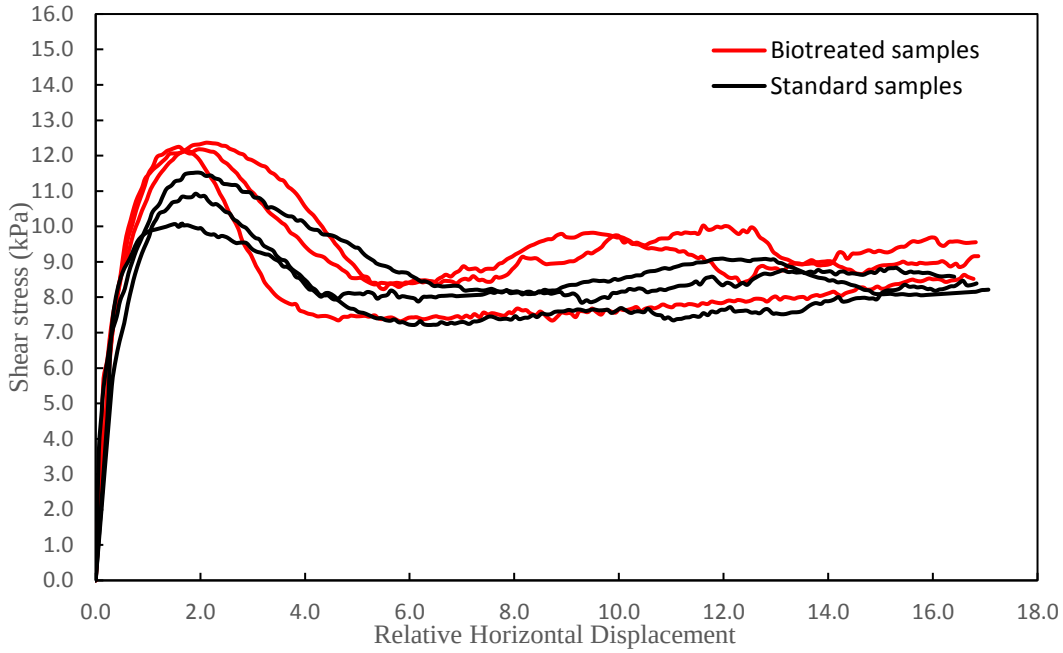


(a) Shear stress

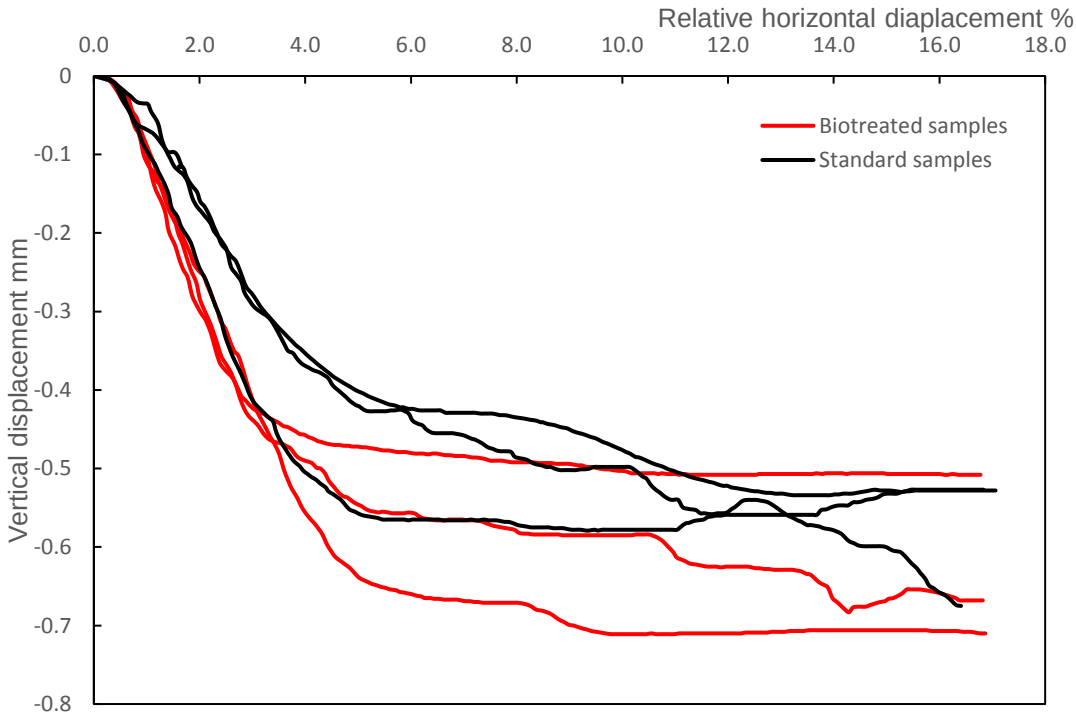


(b) Vertical displacement

Figure 6-7. Shear stress (a) and vertical displacement (b) versus relative displacement for a vertical loading of 4.1 kPa.

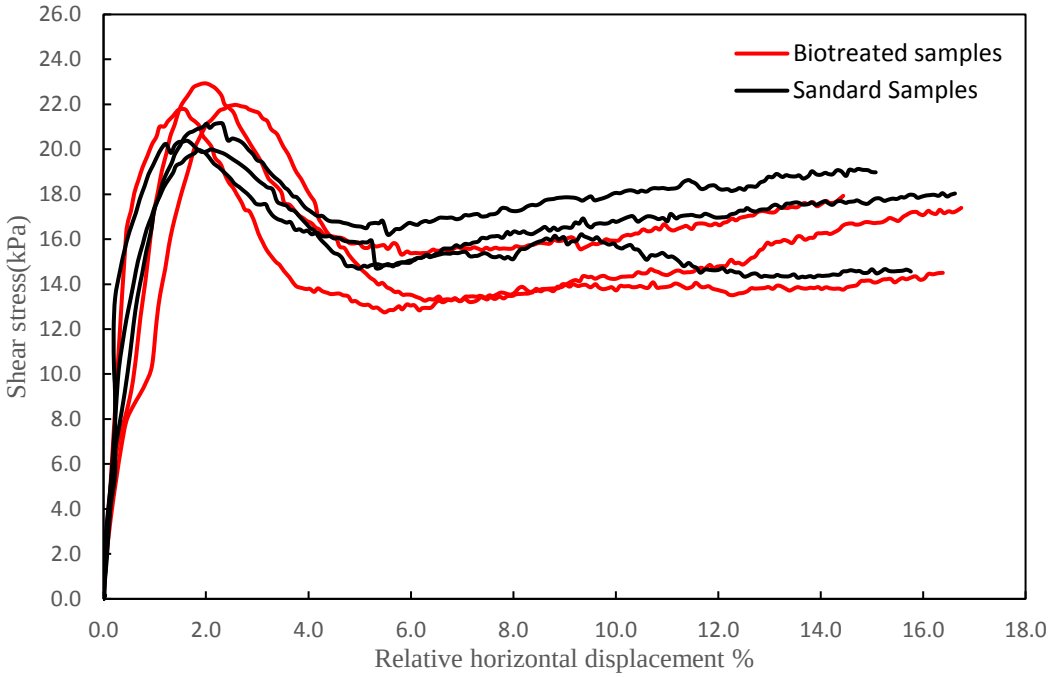


(a) Shear stress

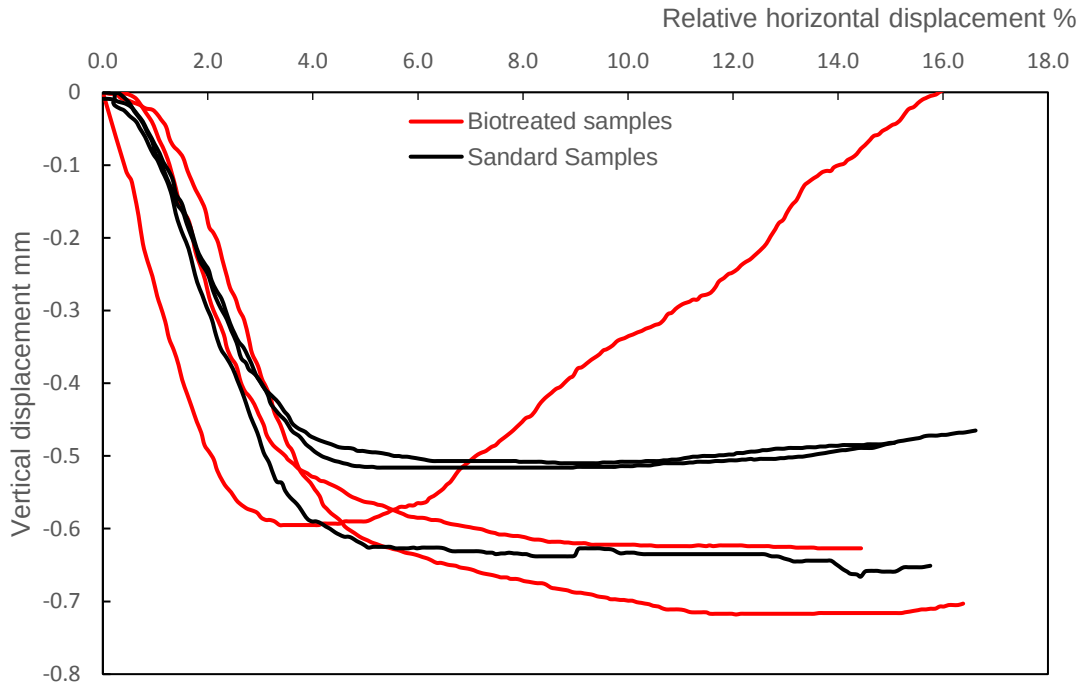


(b) Vertical displacement

Figure 6-8. . Shear stress (a) and vertical displacement (b) versus relative displacement for a vertical loading of 8.89 kPa.

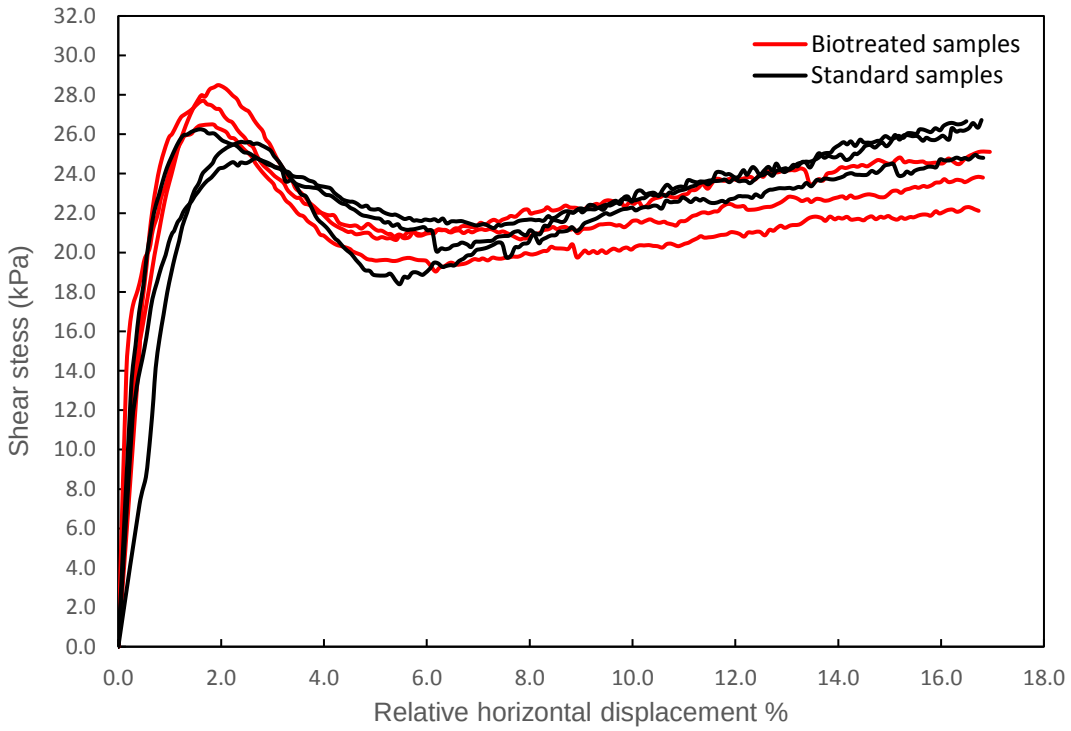


(a) Shear stress

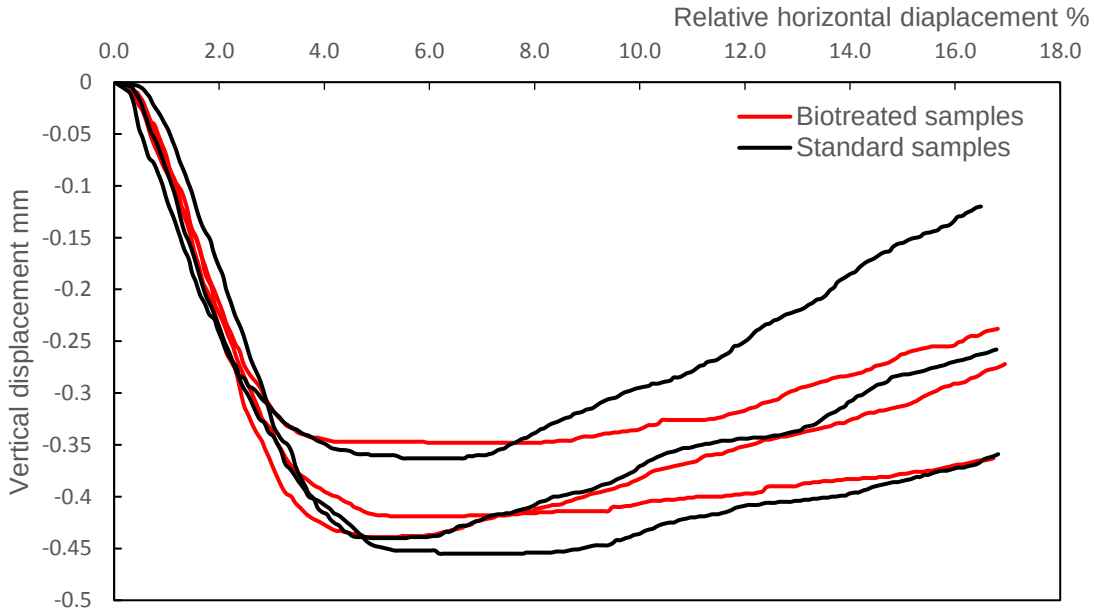


(b) Vertical displacement

Figure 6-9. Shear stress (a) and vertical displacement (b) versus relative displacement for a vertical loading of 16.2 kPa.



(a) Shear stress



(b) Vertical displacement

Figure 6-10. Shear stress (a) and vertical displacement (b) versus relative displacement for a vertical loading of 25.0 kPa.

6.3.2 Analysis of direct shear test

Figure 6-11 depicts peak stress of both biotreated and standard specimens versus normal stress. The normal stress was applied by using weights above the specimen for 1.0, 4.1, and 8.89 kPa, whereas a loading frame was utilised to apply vertical stresses of 16.2 and 25.0 kPa. Another test was conducted by applying 16.2 kPa using weights to investigate the effect of loading method on the outcomes. The peak stress in the loading frame test was approximately 3.2% and 1.2% less than that using weights for biotreated and standard samples, respectively, thereby, the effect of loading style was considered insignificant.

All experiments exhibited larger peak stress for the biotreated samples than for the standard ones, which suggests that the growth of biofilm in biotreated specimens contributes to soil strength, possibly by affecting sand grain aggregation. Under 1.0 kPa loading, the peak stress of biotreated sand was 30% larger than the peak stress of standard samples. For the higher loads, biotreated peak stress around from 8% to 13% greater than the peak stress of standard specimen.

As discussed earlier, the biotreated and standard samples differed in their density at testing, with increased densification of the sample after the preloading and incubation period in biotreated samples compared to standard samples (Figure 6-4). This densifying effect may positively influence the shear response of silica sand in the biotreated compared to the standard samples and may at least partially account for the observed differences in direct shear tests. In this case, the grown biofilm works as a lubricating agent

which causes the sand particles to compact further under normal loading (Perkins et al, 2000, Körstgens et al, 2001, Çabalar et al, 2009).

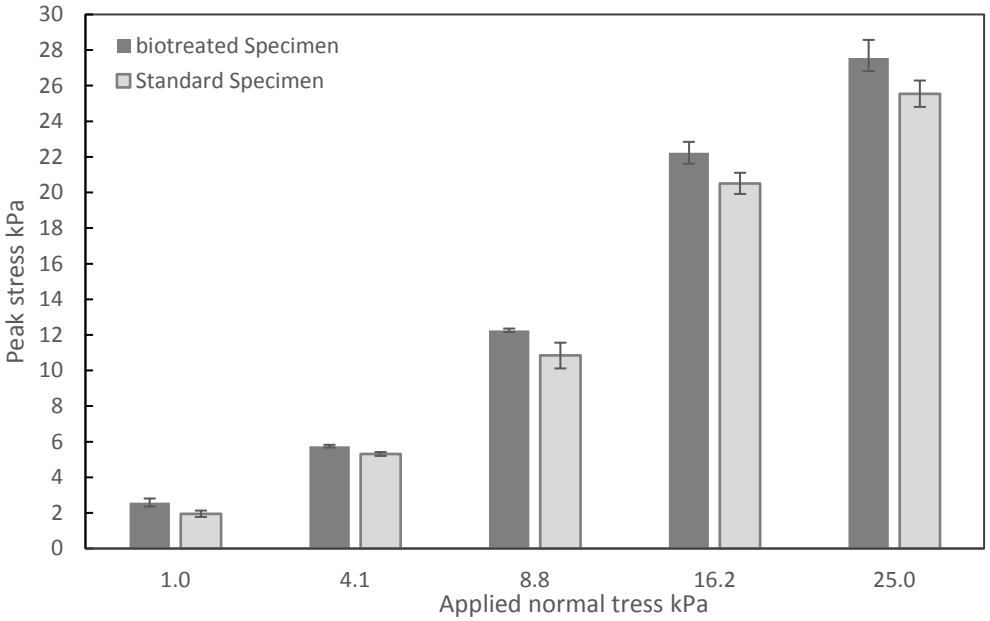


Figure 6-11. Peak stress of biotreated and standard specimens for different applied normal effective pressures.

The relative horizontal displacement (RHD) at peak stress was determined to explore the influence of biofilm presence on the horizontal displacement at the peak stress and is shown in Figure 6-12. It can be seen that there is no clear influence of biofilm on the RHD at peak stress.

Figure 6-13 shows the residual stress of both biotreated and standard samples. It can be seen that the residual stress of biotreated specimens is very similar to that of the standard samples, except at a normal stress of 16.2 kPa where the residual stress of standard specimen is about 10% greater than the biotreated samples. These results indicate that the presence of biofilm has an insignificant effect on the residual stress.

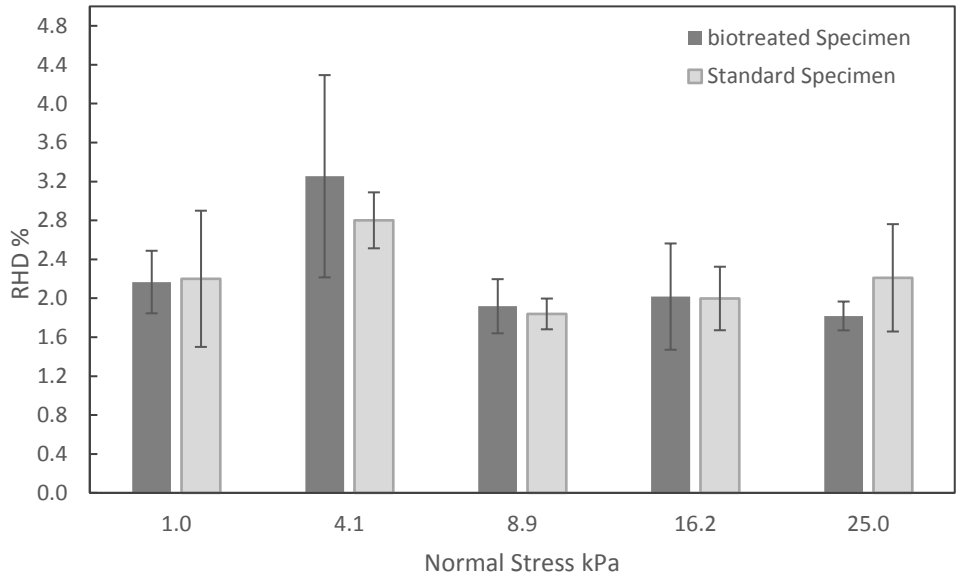


Figure 6-12. Relative horizontal displacement at peak stress versus applied normal stress.

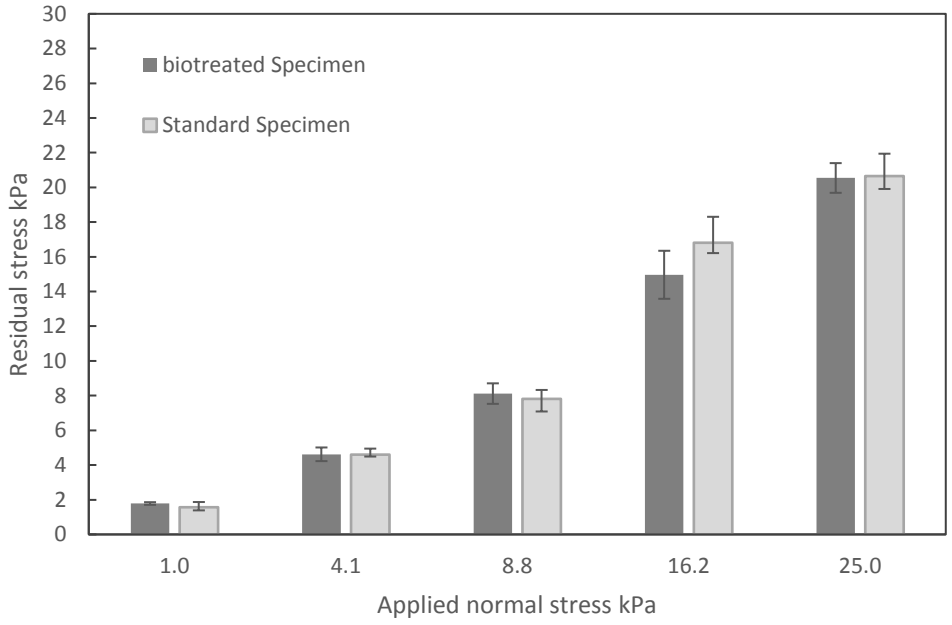


Figure 6-13. Residual stress of biotreated and standard specimens for different applied normal pressures.

6.3.3 Biomass content

As mentioned in section 3.8.7, the amount of biomass was quantified by using the loss on ignition method. The average loss on ignition was determined at the top, middle and bottom of each biotreated and standard sample as shown in Figure 6-14. It can be observed that the top layers of all biotreated samples show much more loss on ignition than the middle and bottom layers. The reason for this issue may be because the top layer is directly exposed to oxygen. However, the amount of biomass at middle and bottom layers are approximately similar. The loss on ignition at the middle layer is likely to be the most relevant biomass content regarding influencing the shear response of samples because the middle layer of the sample was exposed to shearing during testing.

The amount of biomass in biotreated specimens was demonstrated to be substantially higher than the biomass content of standard samples.

As shown in Figure 6-14, it can be noticed that some loss on ignition was also found in the standard specimens. This is likely to be because such specimens were also initially prepared with a bacterial suspension, as well as due to the presence of yeast extract from the nutrient medium supplied to the standard samples. In comparison with the standard samples, the loss on ignition in the middle layer of biotreated samples was between 2.5 and 3.9 times higher than that in the standard samples.

Generally, regarding the effect of applied normal pressure on the specimens over the incubation period on the amount of EPS growth in the sand matrix,

it is to be noted that there are no obvious trends. Non-biotreated controls have relatively consistent values, and differences between biotreated specimens are quite small - there is a slightly higher LOI at lower normal load, but this may be natural variability. As depicted in Figure 6-14, the applied normal stresses do not affect the growth of biofilm. These stresses may be carried by sand grains only, thereby no excess pore water pressure is generated under the loading for sandy soil.

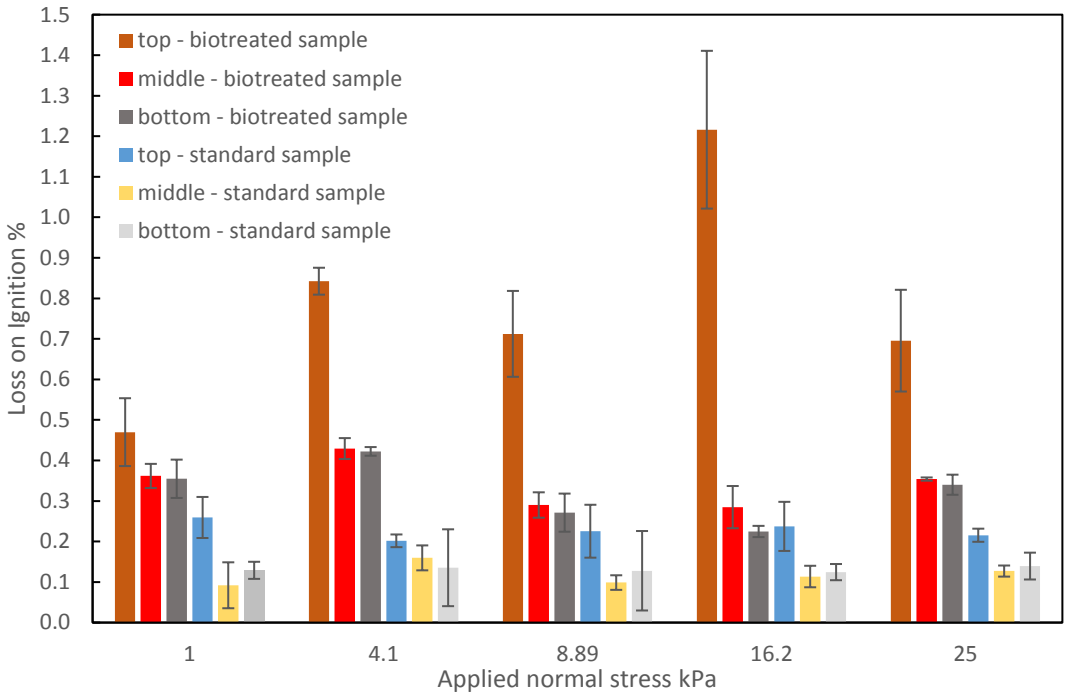


Figure 6-14. Loss on ignition (% by weight) as a measure of biomass in sand samples versus applied normal pressure.

Figure 6-15 describes the relationship between initial number of live bacterial cells per unit mass of sand and loss on ignition percentage for biotreated and standard samples. As shown in the figure, the initial number of live bacterial cells per unit mass of sand does not appear to affect the final loss on ignition values. However, an increase in the number of live bacterial cells may

positively influence the amount of biofilm formed in the top layer of biotreated samples. Regarding the biomass measured in middle and bottom layers, there is no clear correlation with cell numbers, and other factors (such as oxygen availability) are likely to be important.

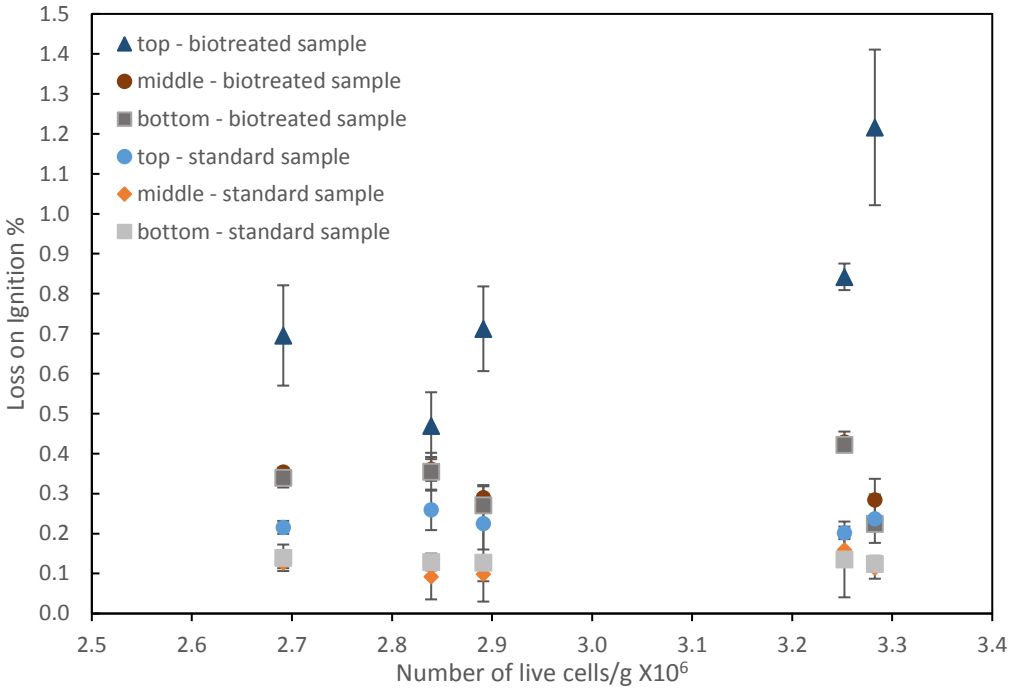


Figure 6-15. The relationship between initial numbers of live bacterial cells per gram of sand with formed biomass.

6.3.4 Discussion a dilation

Figure 6-16 shows the dilation (vertical displacement) at peak stress under various applied normal stresses for biotreated and standard sand samples. It is no clear impact of biofilm accumulation on the dilation behaviour, while the presence of biofilm may be expected to lubricate the sand particles, this may limit the effect of interlocked grains on movement during shearing on dilation behaviour (Perkins et al, 2000) that is not apparent in the results. In general,

irrespective of the different applied load most samples show variable dilation behaviour (Figure 6-16).

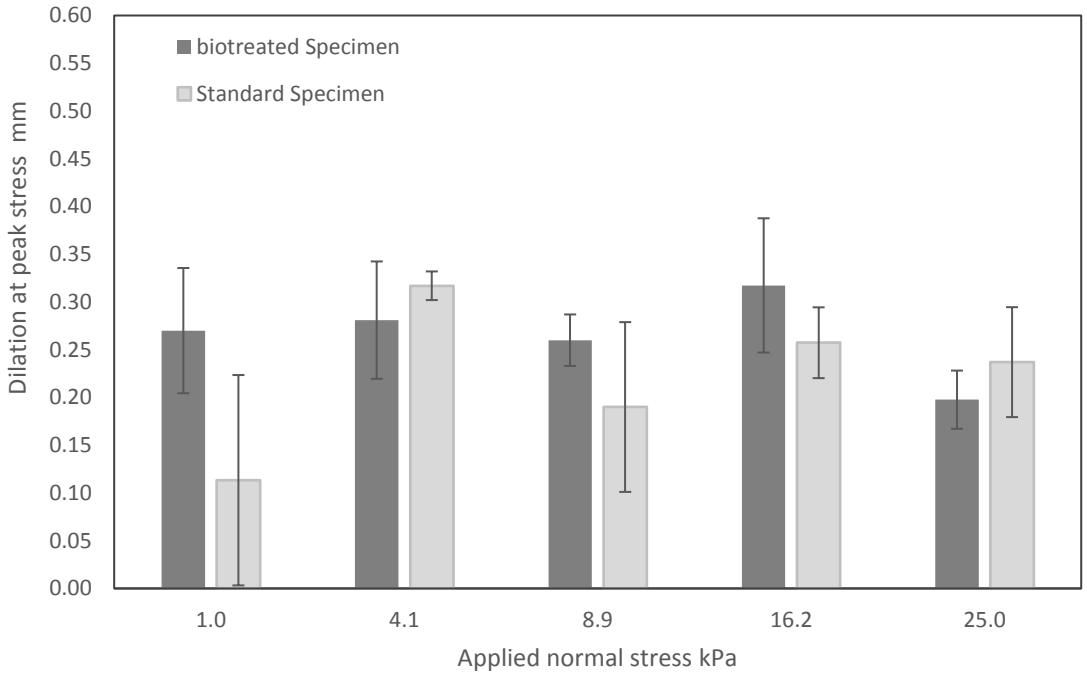


Figure 6-16. Dilation of biotreated and standard samples versus applied normal pressure.

Dilation angle (α) is a function of the negative change in sample height to the change of horizontal displacement as defined by Budhu (2007) and Bolton (1986). It can be determined at peak stress as α_p:

$$\alpha_P = \tan^{-1}\left(\frac{-\Delta H_0}{\Delta x}\right)$$

ΔH_o: the differences of sample height at peak stress, mm.

Δx: horizontal displacement at peak stress mm.

Figure 6-17 depicts the variation of the peak dilation angle for both sample types. It can be seen that dilation angle decreases with increasing normal

stress especially at the highest stress tested case of 25.0 kPa loading. Hsu (2005), Hosseini and Jesmani (2013), and Bolton (1986) suggested that dilation angle decreases with increasing normal stress.

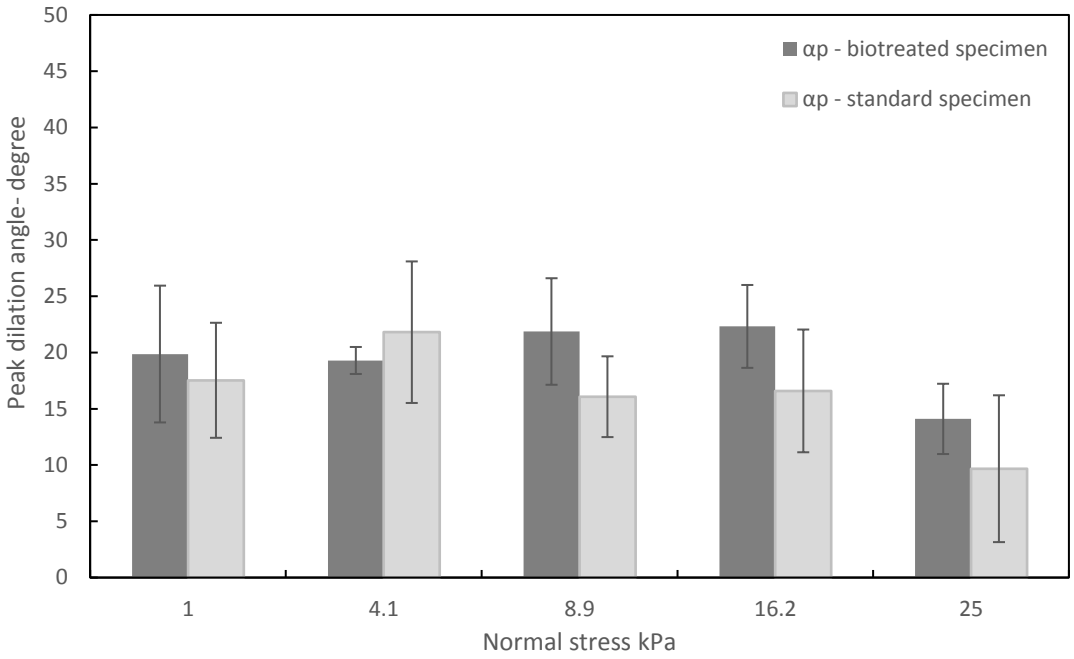


Figure 6-17. Peak dilation angles for biotreated and standard samples.

The individual internal friction angles for each normal stress, both measured and corrected for low normal stress as stated by Lehane and Liu (2013), for biotreated and standard samples were determined. The correction accounts for the friction between sand particles and the internal surface of upper part of the shear box which may be significant at a low normal stress. The procedure of the correction was demonstrated in section 3.7.6. Figure 6-18 and Figure 6-19 present the comparison between measured and corrected peak and residual friction angles of biotreated and standard samples. The

peak friction angle of biotreated specimens is greater than the angle of standard specimens. Although the amount of EPS was small, this content positively influences the peak effective friction angle, in comparison with the peak friction angle of standard specimens. Conversely, the residual effective friction angle of biotreated samples is, in general, very similar to that of standard samples. It can be seen that the friction angle decreases with increasing normal stress. That is because dilatancy decreases with increasing normal stress, and therefore friction angle decreases as reported by Hsu (2005), Hosseini and Jesmani (2013), and Bolton (1986). The correction was significant for the applied normal stress of 1.0 and almost 4.1kPa. However, the correction was less significant for the rest of loading because the overlapping of error bars appear for the loading of 8.89 kPa to 25.0 kPa.

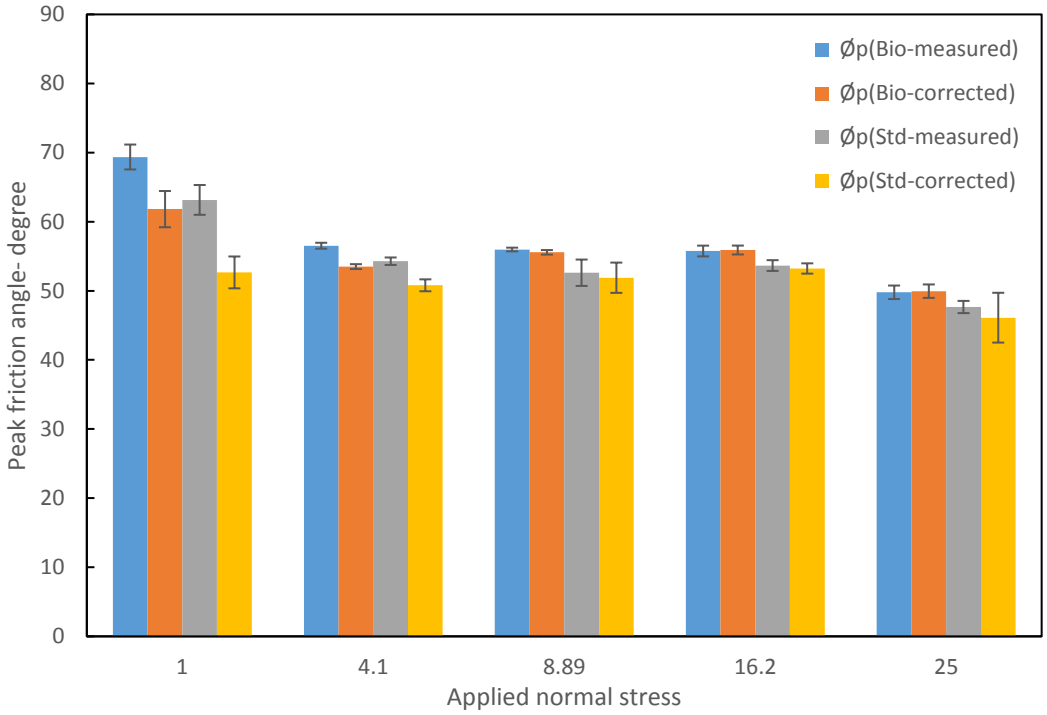


Figure 6-18. Measured and corrected peak internal friction angles for biotreated and standard samples.

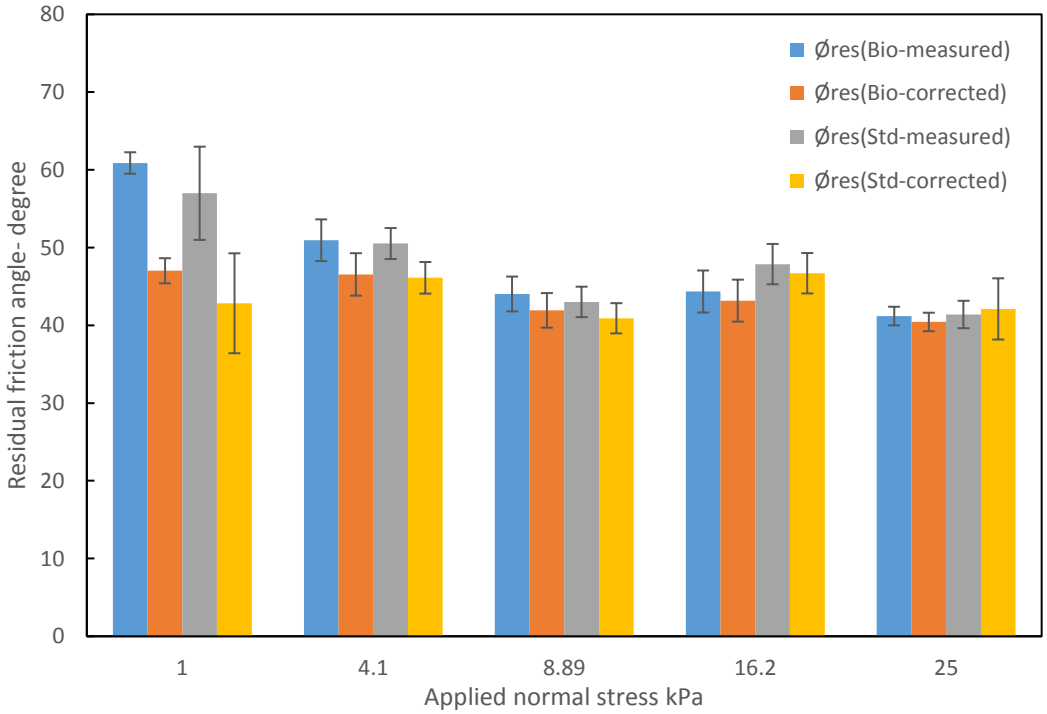


Figure 6-19. Measured and corrected residual frictional angle.

6.3.5 Coulomb's failure relationship

Coulomb's failure criterion is commonly presented to show the linear relationship of shear strength with applied normal stress acting on the sheared plane or failure plane. Figure 6-20 illustrates the failure envelope lines of peak and residual stresses for both biotreated and standard samples, which suggest a lack of linearity. The reason of the poor of linearity comes from the reduction of peak stress at 25.0kPa loading. The actual peak stress for biotreated and standard samples are 27.56 and 25.55 kPa, but the expected stresses to attain the linearity of failure envelope are about 34.0 and 32.0 kPa respectively, whereby the loading of 25.0 kPa shows around 24% less peak stress than the expected stress for both samples. Considering the first four applied stresses only, the failure envelope is linear and suggests very low cohesion. As mentioned above, the peak stress for all biotreated samples was higher than the peak stress of standard ones for all loading stages, and this has previously been at least partly attributed to an increase in densification as a result of the growth of biofilm. Regarding the Mohr-Coulomb formula, besides the improvement of friction characteristics of biotreated sand, the development of cohesive properties of such sand may also be considered by the growth of EPS which consists of a high viscosity substance. This substance can form polymer bridges between the microorganisms and surface of the sand particle, to connect them (Garrett et al, 2008). However, there is no distinct difference in cohesion with these specimens. The residual stress has an almost identical trend for both biotreated and standard samples.

In terms of residual stress, the biotreated samples are consistent with standard samples, and appear to have a more linear failure envelope as shown in Figure 6-20. On the other hand, at 16.2 kPa loading, the residual stress is larger for standard sample compared with the biotreated specimens by about 12 %. The average density of the standard sample is 2.8 % less than the density of biotreated specimens at 16.2 kPa loading. The residual stress is highly affected by initial void ratio and applied normal pressure (Imam et al, 2005).

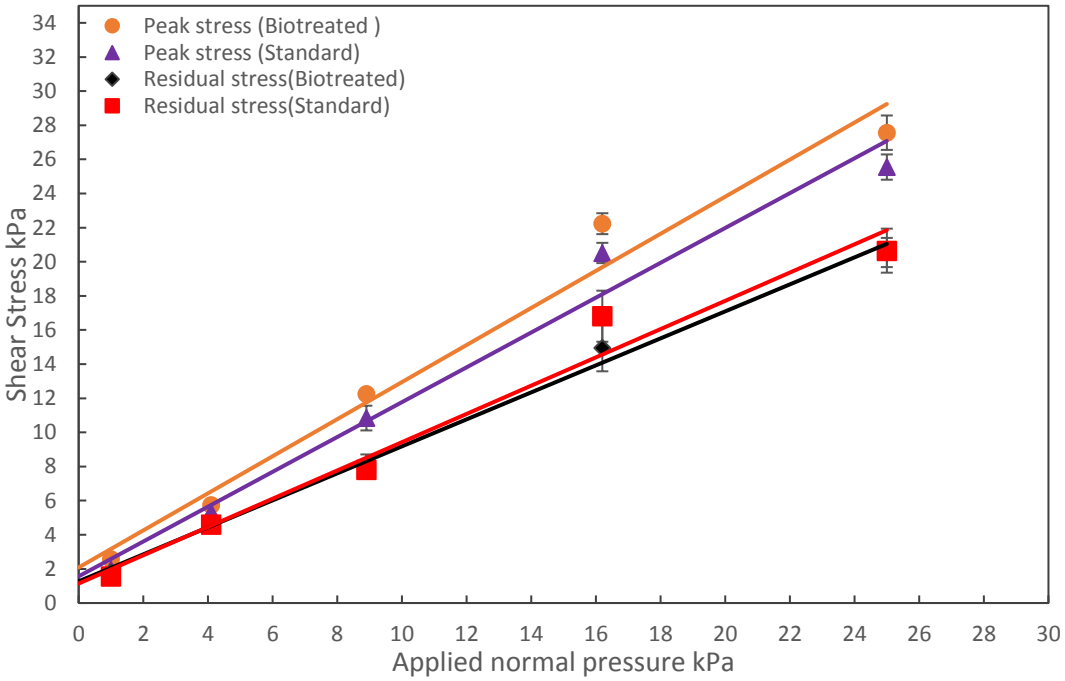


Figure 6-20. Linear regression of Coulomb's failure envelope for main experiments.

Figure 6-21 shows the ratio of peak stress of biotreated samples to that of standard ones versus a similar ratio for loss on ignition. This demonstrates that there is a positive relationship suggesting that an increase in biopolymer leads to an increase in peak strength relative to standard samples. At 1.0 kPa

loading, the peak stress with biotreated sand is about 30% larger than for the standard samples as already mentioned whilst the biomass content in the biotreated specimen is about 3.93 times more than that in the standard samples. The increase in peakstress is about 8-13% larger than the standard sample peak stress when the amount of the biomass content in the biotreated specimen is about 2.5-3.0 times more than that of the standard samples.

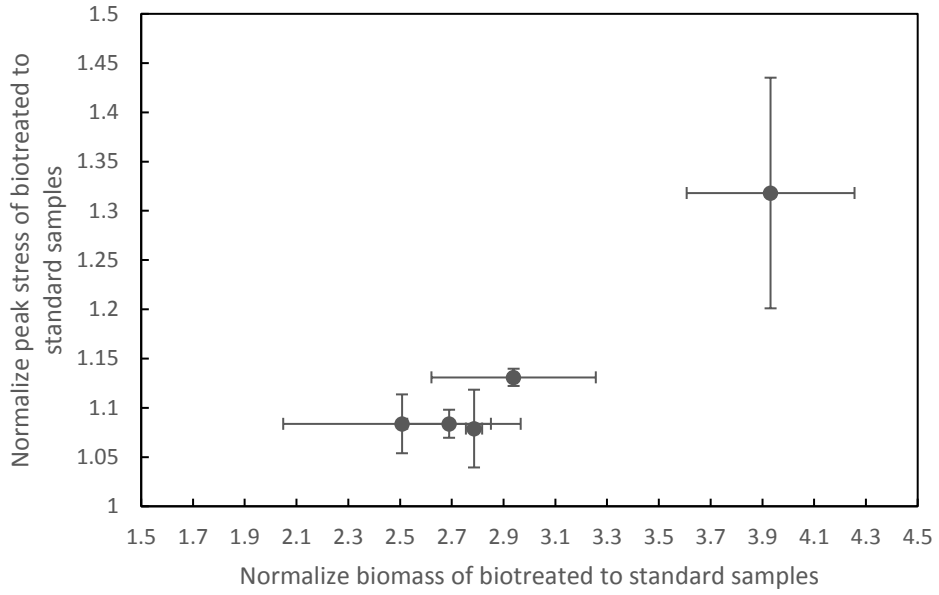


Figure 6-21. Normalization of peak stress of biotreated to standard samples – normalization of biomass.

6.4 Shear behaviour of dry and fully saturated clean sand

Direct shear tests were performed on dry and saturated clean sand samples, with the same applied normal stresses used in the main biotreated experiments. These tests were conducted to provide baseline behaviour of the silica sand

under different moisture conditions, which may help to explain the non-linear peak stress behaviour of biotreated and standard samples at 25.0 kPa.

6.4.1 Comparison of peak and residual stress

Figure 6-22 depicts the actual values of peak stress for all tests in these experiments. The saturated sample shows similar behaviour as biotreated and standard samples with linear behaviour up to 16 kPa but deviation from this behaviour at 25 kPa whereas the dry samples have linear behaviour. It is likely that biotreated and standard samples are also saturated, or near saturated, and so this behaviour may be due to an effect of moisture. Dry samples had an approximately linear failure envelope across all applied normal stresses. This behaviour also indicates that the reduction in final density observed with biotreated and standard samples at 25.0 kPa is not a clear reason for declining of peak stress because the saturated clean sand samples have approximately the same density across all stresses (Figure 6-2). Figure 6-23 shows the linear trend of actual peak stress for only the first fourth normal stress.

The actual residual stresses for all samples at all applied normal stresses are presented in Figure 6-24. The actual stresses exhibit almost linear behaviour except for the stress of standard sample which was explained above. The biotreated and dry samples have mostly identical linear regression as shown in the figure. However, saturated clean sand exhibited a higher slope of the failure envelope. In a saturated case, density change during shear is achieved by expelling or by taking in water, and effective-stress change is

brought about through an increase or a decrease in pore water pressure (Terzaghi et al, 1996), thereby, the water may drain out causing an increase in the effective stress (Terzaghi et al, 1996). As shown in Figure 6-24, the residual stress of saturated sand was about 14.0% higher than the expected stress at both loadings of 16.2 and 25.0 kPa. The factors which affect the residual stress are confined stress or peak stress, which is also higher than expected for the saturated sand at 16.2 kPa loading, and the final density of the samples at both loadings are similar. The residual stress highly affected by initial void ratio and applied normal pressure (Imam et al, 2005).

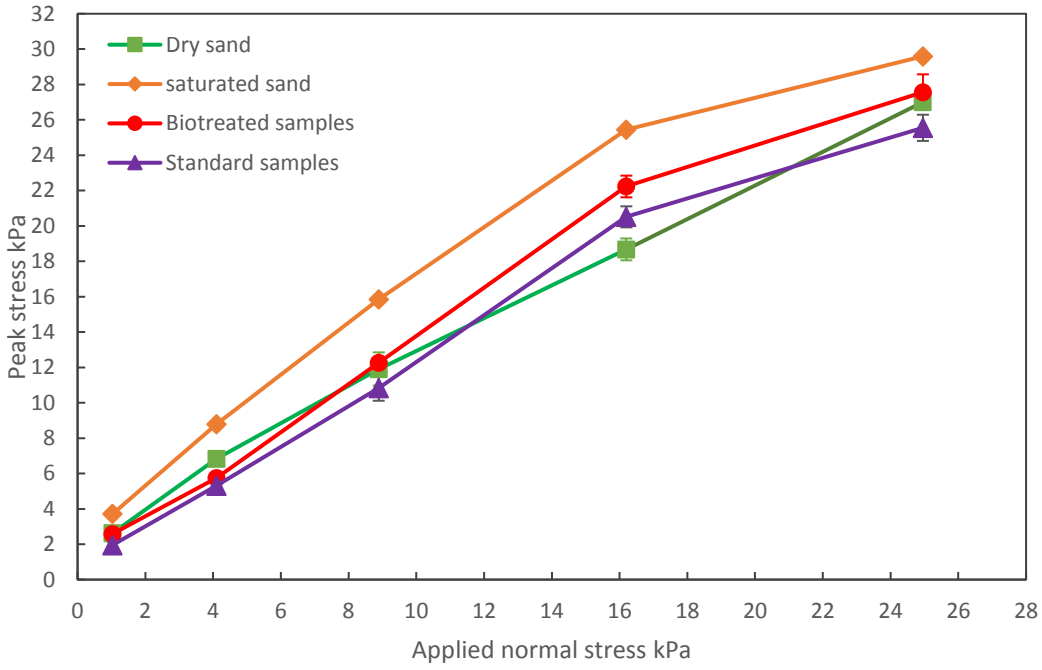


Figure 6-22. Actual peak stress with normal stress relationship.

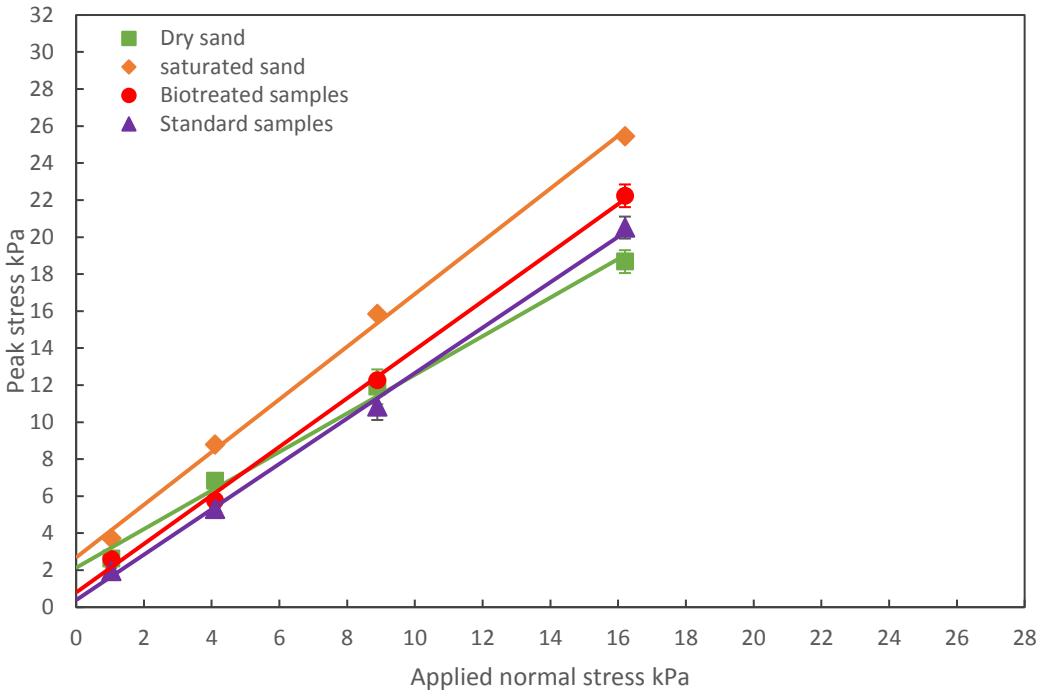


Figure 6-23. Linear Coloumb's failure of the envelope of peak stress with normal stress after eliminating the fifth loading.

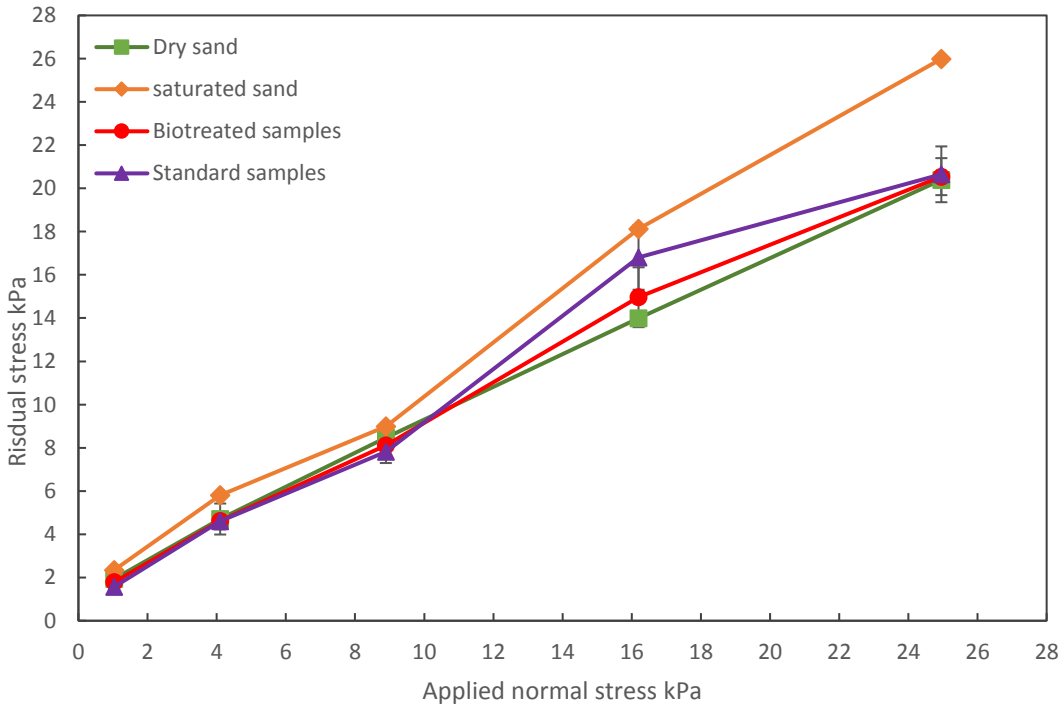


Figure 6-24. Actual residual stress with normal stress relationship.

6.4.2 Dilation and friction data

The peak dilation angle is obtained as the maximum slope of the measured dilation-displacement response (dy/dx) (Lehane and Liu, 2013). Roy and Campanella (1997), and Hosseini and Jesmani (2013) believe that internal friction angle of coarse-grained soils is composed of two basic elements, friction angle and dilatancy, where dilatancy angle is an indicator of volume changes of the sample during shear and in the case of expansion is considered positive. Figure 6-25 shows the peak dilation angle versus applied normal stresses. The peak and residual corrected angles were shown in Figure 6-26 and Figure 6-27, to compare these angles of biotreated samples with the standard, dry and saturated sand samples versus applied normal stress. Overall, it can be seen from the Figure 6-25 and Figure 6-26 that peak dilation angles and internal friction angle appear to decrease with increasing normal stress and decreasing relative compaction. Dilatancy decreases with increasing normal stress, and therefore friction angle decreases as stated by Hsu (2005), Hosseini and Jesmani (2013) and Bolton (1986), but the effect here may be relatively weak because of the low normal stresses employed. The varying of peak dilation angles is a part of the reason for nonlinearity (Barton, 2008). During the direct shear test, the sand becomes loose enough to be in a critical state with zero dilation in a residual stage (Bolton, 1986).

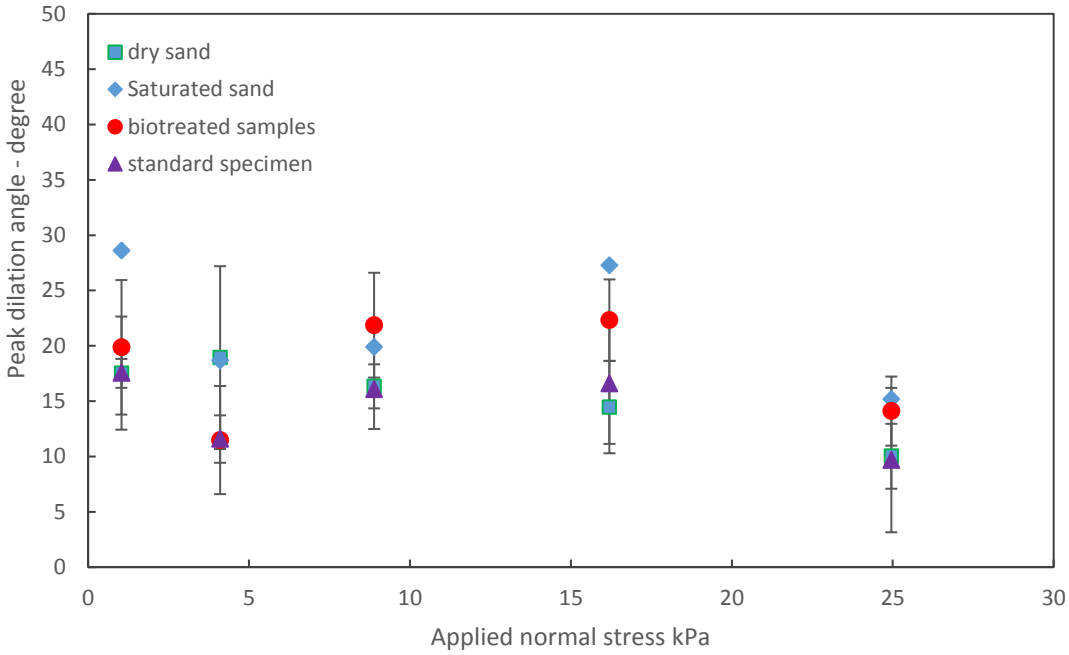


Figure 6-25. Observation of peak dilation angle for each loading test.

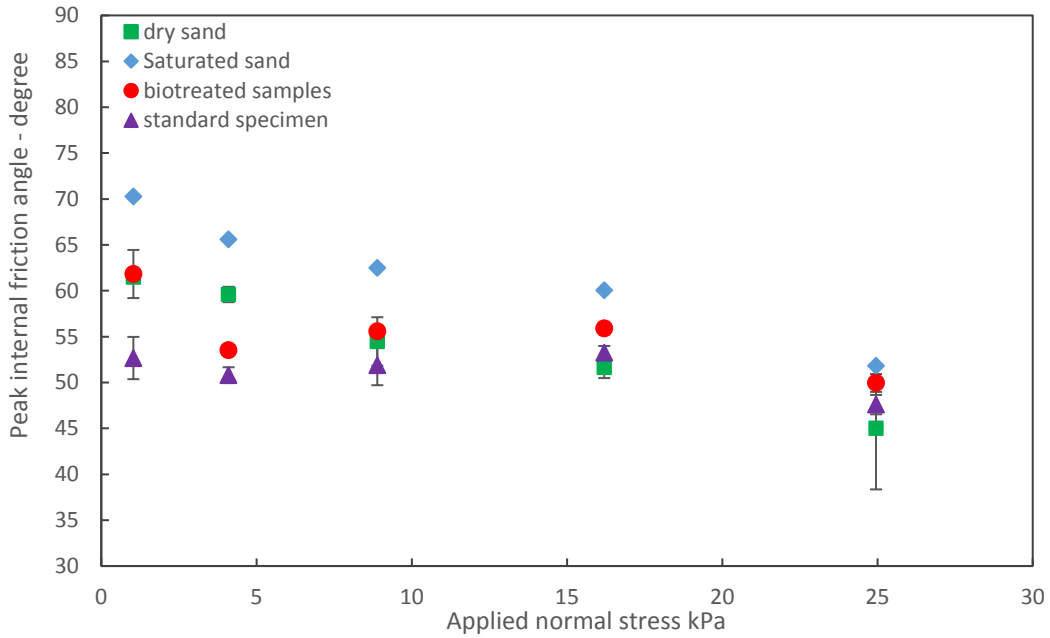


Figure 6-26. Peak internal friction angle versus applied normal stress.

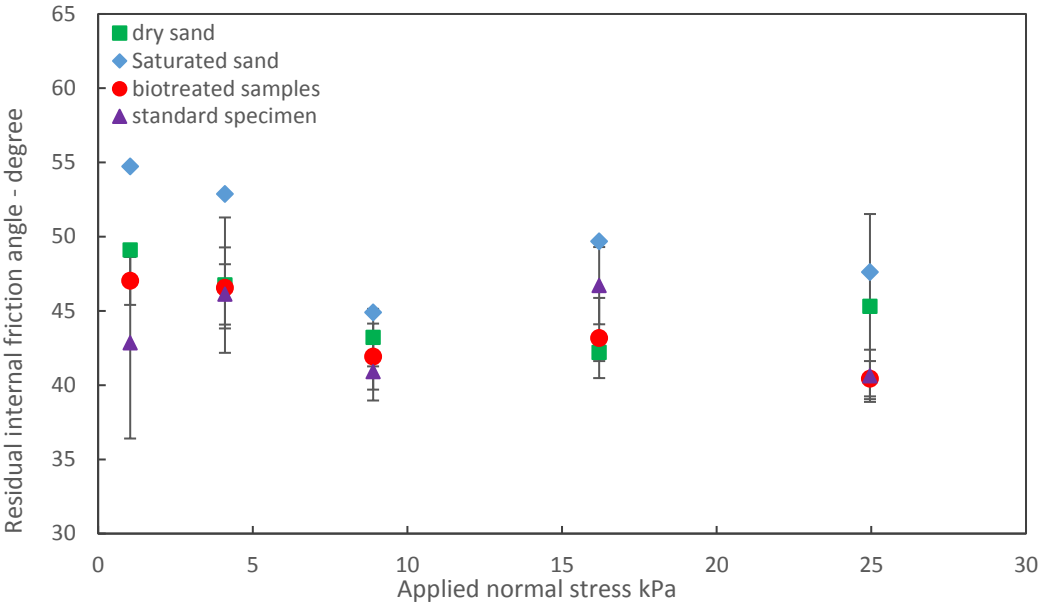


Figure 6-27. Residual internal friction angle versus applied normal stress.

6.5 Effect of preloading on dry and saturated direct shear performance

The experimental study involved performing a series of direct shear tests on dry and saturated sand samples that had been subjected to preloading at the appropriate stress for a minimum of half an hour before the shear test. In contrast, biological experiments were exposed to the load for two weeks during incubation but as sand responds quickly to compression, there was no need to apply the load for a long time. Otherwise, preparation was in the same manner that was utilised in the main biological experiments and in the dry and saturated samples discussed in the previous section. Two additional sets of preloaded saturated samples were prepared, one using a mineral medium instead of deionized water and the other prepared dry and soaked overnight in deionized

water. The latter was conducted to overcome the effect of the differences in preparation due to air and wet pluviation that may impact on the packing of sand particles during preparation, in particular that settlement of sand into and through water might lead to segregation of particle sizes.

The graphs of shear stress – relative horizontal displacement for all tests of this study are presented in appendix B, with peak and residual values only considered here.

6.5.1 Analysis of direct shear test

The peak and residual stresses of the four preloaded treatments are compared with the biotreated experiment and standard samples in this section. Figure 6-28 shows the peak stress versus applied normal stresses whilst Figure 6-29 shows similar data but with linear regression of failure envelope. All preloaded, wet pluviated clean sand samples approximately exhibit a linear failure envelope line over all stresses, with some variability. The preloaded saturated samples have higher peak stress than the biotreated and standard samples whilst the peak stress of dry samples are lower, although these do not exhibit the deviation from linear behaviour at 25.0 kPa and gradually diverge with increasing normal stress. The mineral medium-treated sand failure envelope lies between that of the dry and saturated samples. Despite the preparation of saturated and the mineral medium treated samples being the same (wet pluviation), the saturated samples show significant larger peak shear stress than samples with mineral medium. The linear regression for samples dry pluviated, preloaded and

soaked overnight appears to diverge from linear behaviour at 25.0 kPa, as seen in the main biological experiment samples.

It is possible to compare between the preloaded saturated samples with deionized water and mineral medium because they are prepared and tested in the same way. The significant difference in peak stress, as mentioned above, may be related to the effect of dissolved minerals on the behaviour of fine particles of silica sand. The fine grains of silica sand have a negative charge (Ingles and Metcalf, 1972). According to BS 1377:3:1990, fine sand ranges from 0.06 to 0.2 mm, so the percent fine sand in these tests was 22%. Therefore, the presence of ions in the sand matrix from the mineral medium may affect the shear response of the sand.

Plots actual of residual stresses versus normal stress are shown in Figure 6-30 and Figure 6-31 with the latter again showing linear regression. As depicted in Figure 6-31 the residual stress for all cases has approximately similar behaviour in this range of applied normal stress, with linear behaviour and a negligible cohesion intercept.

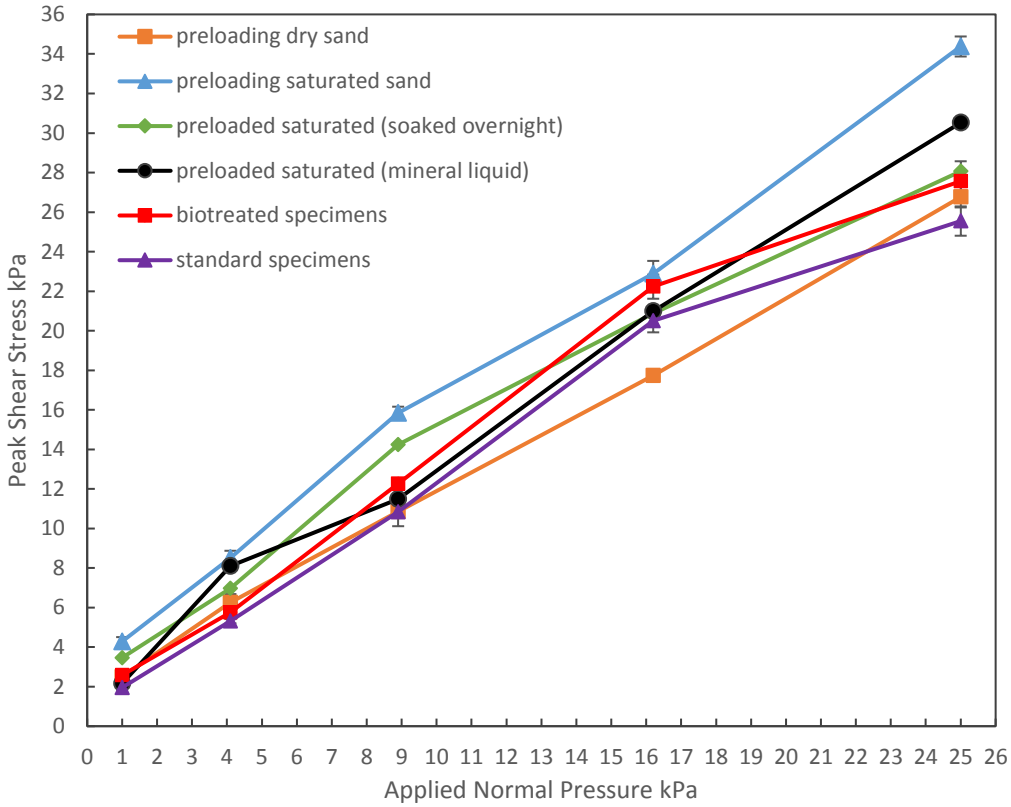


Figure 6-28. Comparison of actual peak stress of biotreated experiment and the four preloading cases.

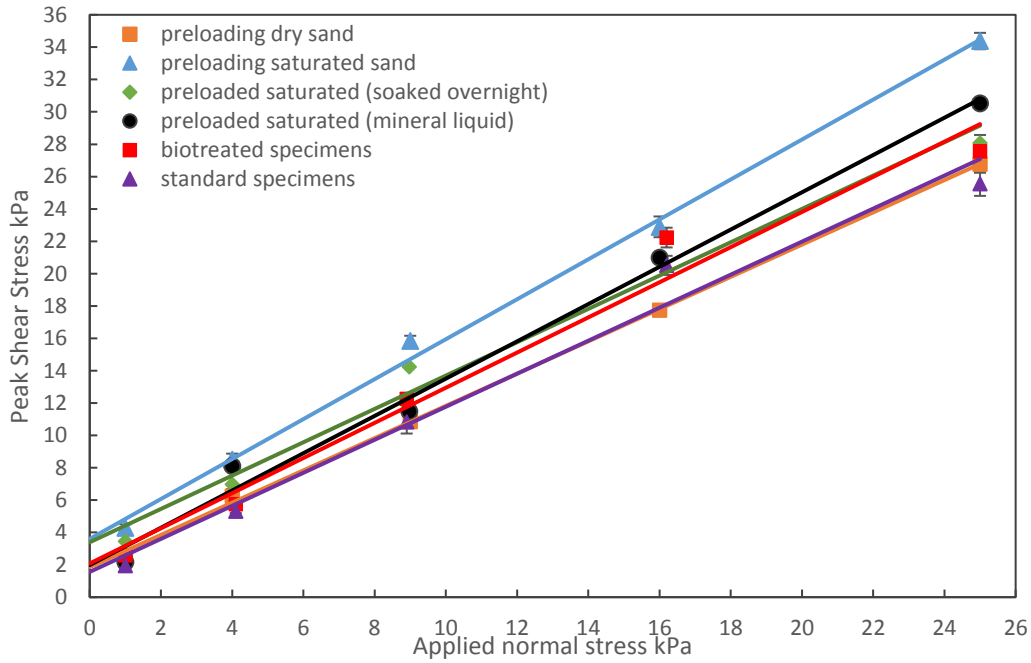


Figure 6-29. Comparison of peak stress Coulomb's failure envelope of biotreated experiment and the four preloading cases.

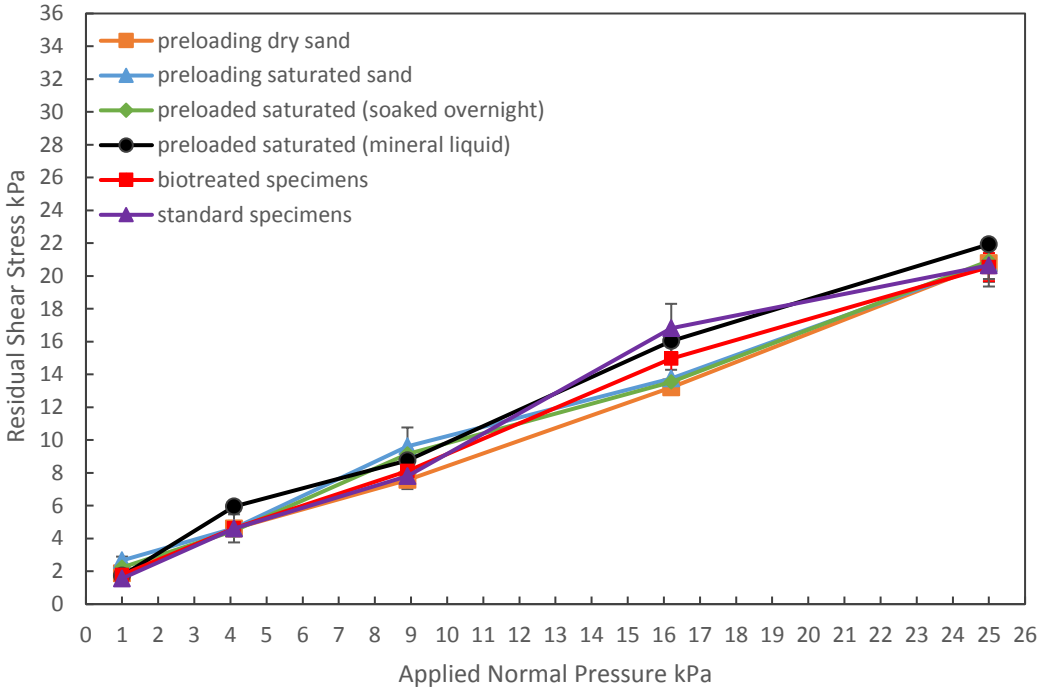


Figure 6-30. Comparison of acutal residual stress of biotreated experiment and the four preloading cases.

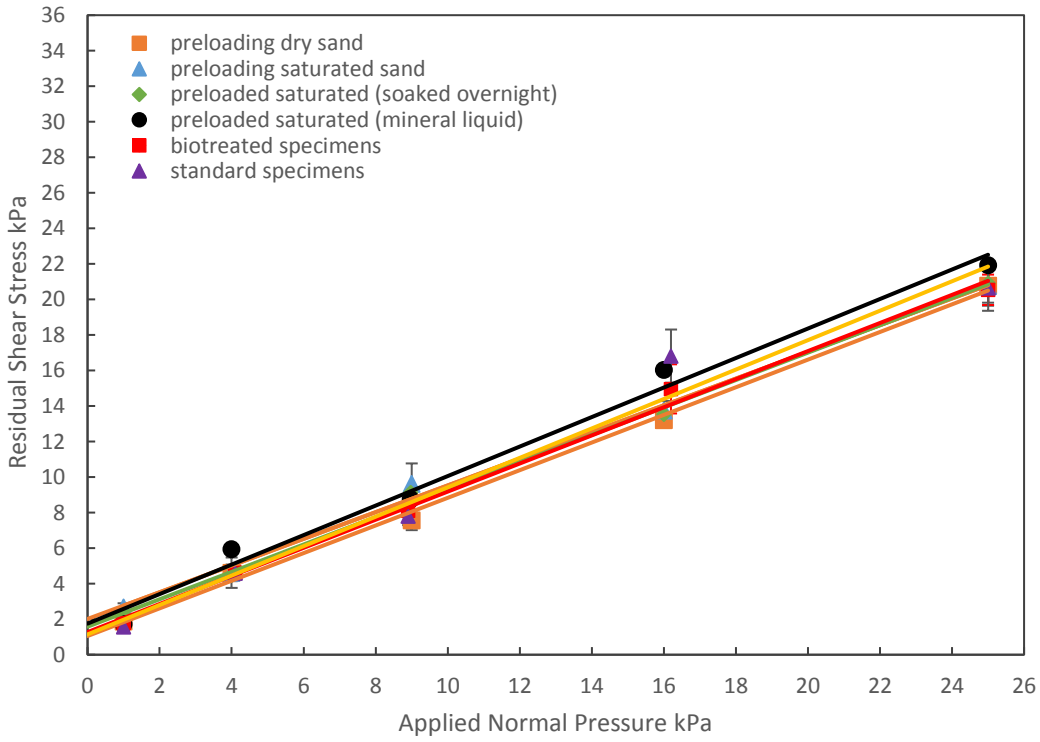


Figure 6-31. Comparison of residual stress Coulomb's failure envelope of biotreated experiment and the four preloading cases.

6.5.2 Dilation and friction behaviour

As described before, the peak dilation angles were defined by the variation of vertical displacement to the change of lateral displacement. Figure 6-32 indicates peak dilation angles versus applied normal stress. Overall, dilation angle decreases with increasing normal stress but with different rates depending on the normal stress. At low normal stress, there is a rapid reduction, followed by a more gradual reduction above 4 kPa. The variation of peak dilation and strain softening may depend on the confining pressure and initial void ratios (Wan and Guo, 1998).

Regarding friction angles, the peak and residual friction angles decrease with increasing normal stress as shown in Figure 6-33 and Figure 6-34. Generally, the angles slightly decrease with increasing normal loads.

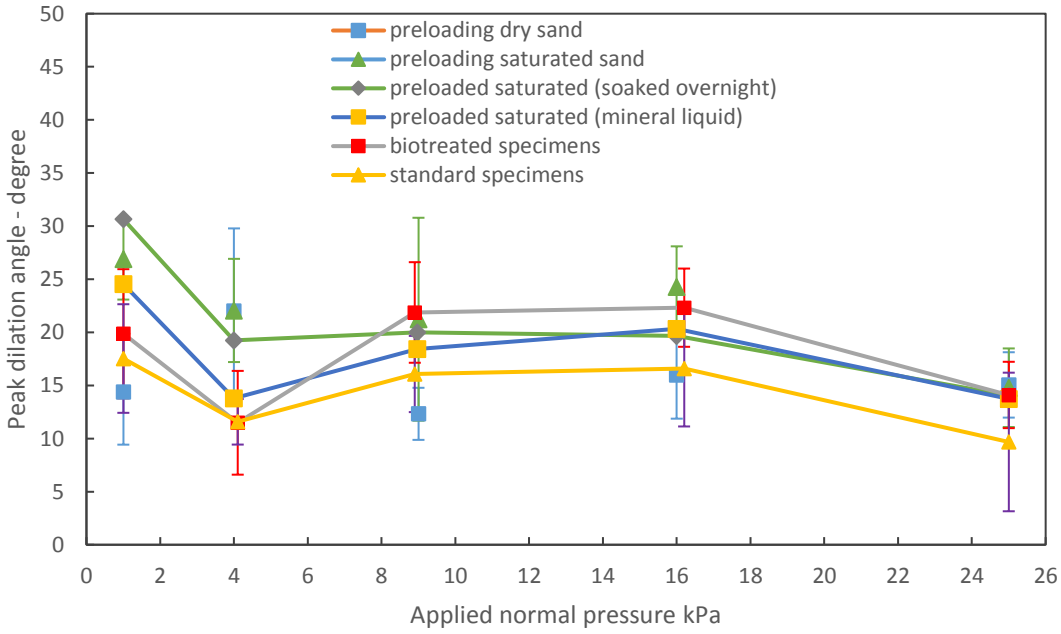


Figure 6-32. The effect of normal stress- peak dilation angle relationship.

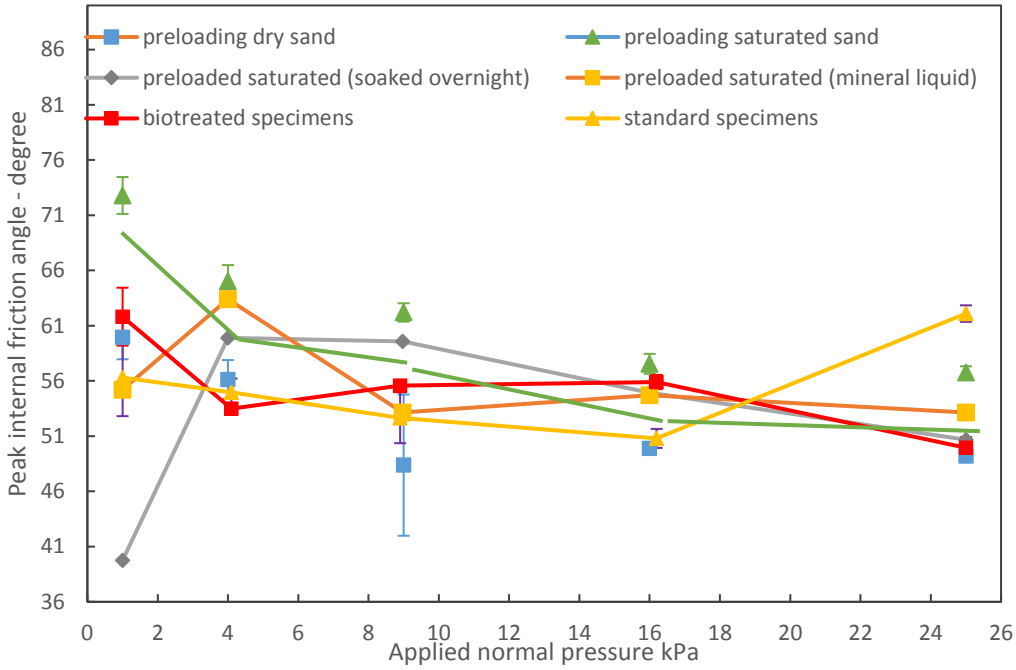


Figure 6-33. The observed variation of peak internal friction angle.

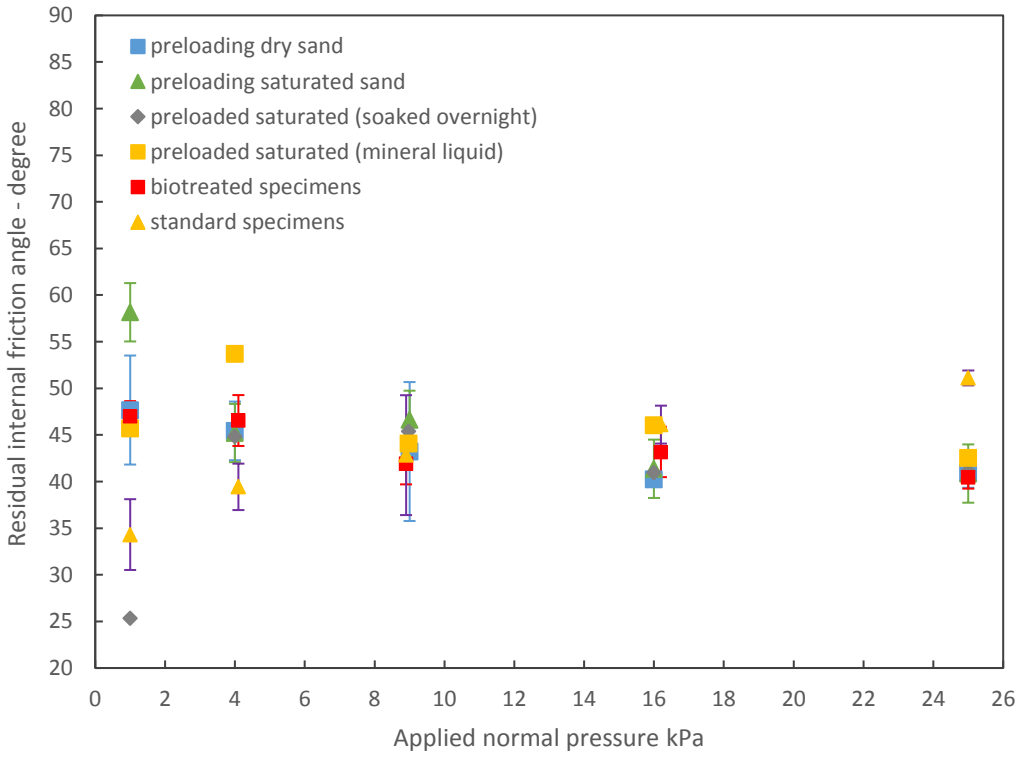


Figure 6-34. The observed variation of residual internal friction angle.

6.6 Effect of particle grading

The following section compares the results of tests on well-graded and poorly graded silica sand. The source of both sands are the same, thereby they have the same mineral composition. These experiments to understand the effect of biofilm on shear behaviour of both materials. Poorly graded sand has a larger pore volume than the well-graded sand, and the poorly graded sand may have less contact area between soil particles especially at a higher void ratio (Shipton and Coop, 2012). If biofilm affects soil behaviour by reinforcing contact points then there may be more effect of biofilm in a well-graded sand. Moreover, the transport of bacteria and nutrient through poorly graded sand would be expected to be quicker than in the well-graded sand. Jenneman et al (1984) stated that bioclogging occurred in high permeability zones since such zones obtain a greater portion of the nutrient flow. Therefore, a greater accumulation of biofilm may be expected in the poorly graded sand. Camper et al (1993) showed that the transport of bacteria through a porous medium is dependent on characteristics such as porosity, tortuosity, and particle diameter as well as the porous medium hydrodynamics, including interstitial pore velocity and dispersivity. Onur (2014) suggests that poorly graded soils have higher porosity and permeability values than well-graded soils in which smaller grains tend to fill the voids between larger grains. Devlin (2017) stated that because of the poorly sorted nature of the sediment, the estimated range of hydraulic conductivity is very high. The comparison was achieved by conducted a direct shear test on well-graded as well as poorly graded sand. The experiments were run at a normal stress of 1.0 kPa.

6.6.1 Biomass content

The amount of biofilm accumulation in the poorly-graded sand was approximately twice that in the well-graded sand for all layers of samples as demonstrated in Figure 6-35, although there is considerable variability in the poorly graded sand. The increasing of amount of biomass in poorly graded sand may be because such samples have larger pore spaces which increase access to nutrients and dissolved oxygen in the sand (Chou, 2007). The biomass growth occurs in the larger pore throats at the nutrient inoculum-interface (Stewart and Fogler, 2002). The microbial transport phenomena is affected by pore size, motility and porous medium hydrodynamics (Camper et al, 1993).

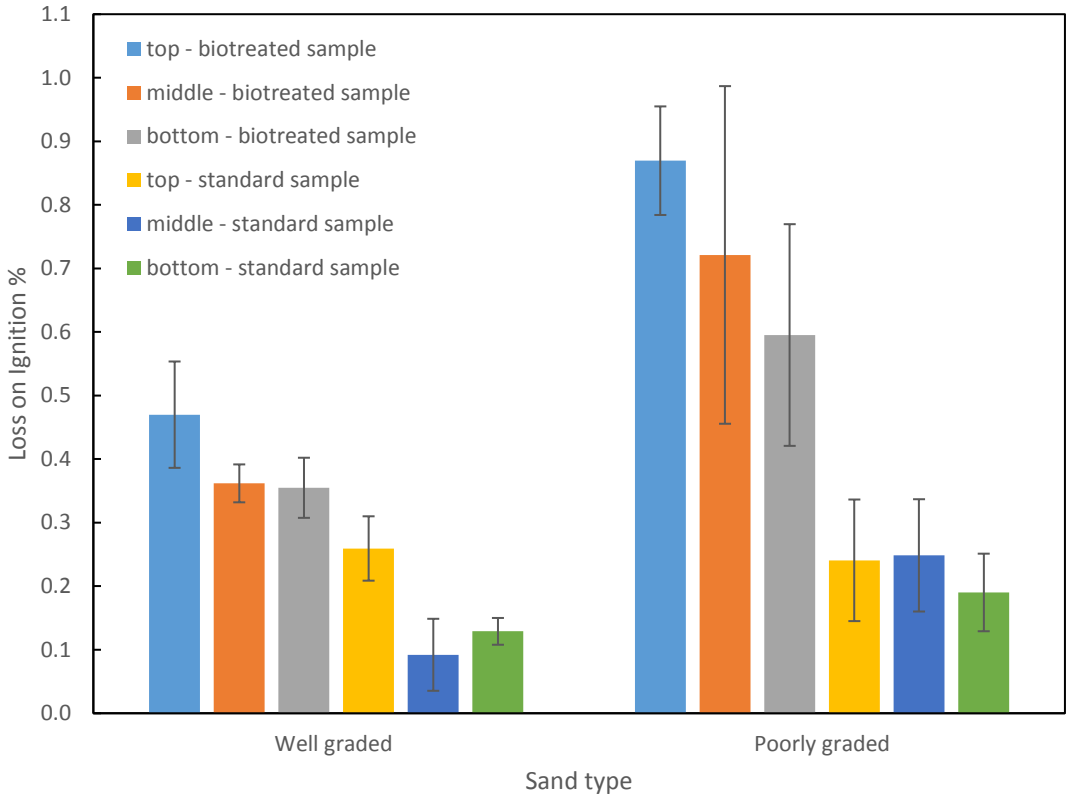


Figure 6-35. Observation of biomass content in well graded and poor graded sand.

6.6.2 Peak and residual stresses

Figure 6-36 depicts a comparison of peak and residual shear strength between the well-graded and poorly graded sand. The figure reveals that regarding peak stress, the grown biofilm has had a similar effect on both the well and poorly graded sands. In contrast, the residual shear strength for poorly graded sand shows larger than the residual stress of well-graded sand.

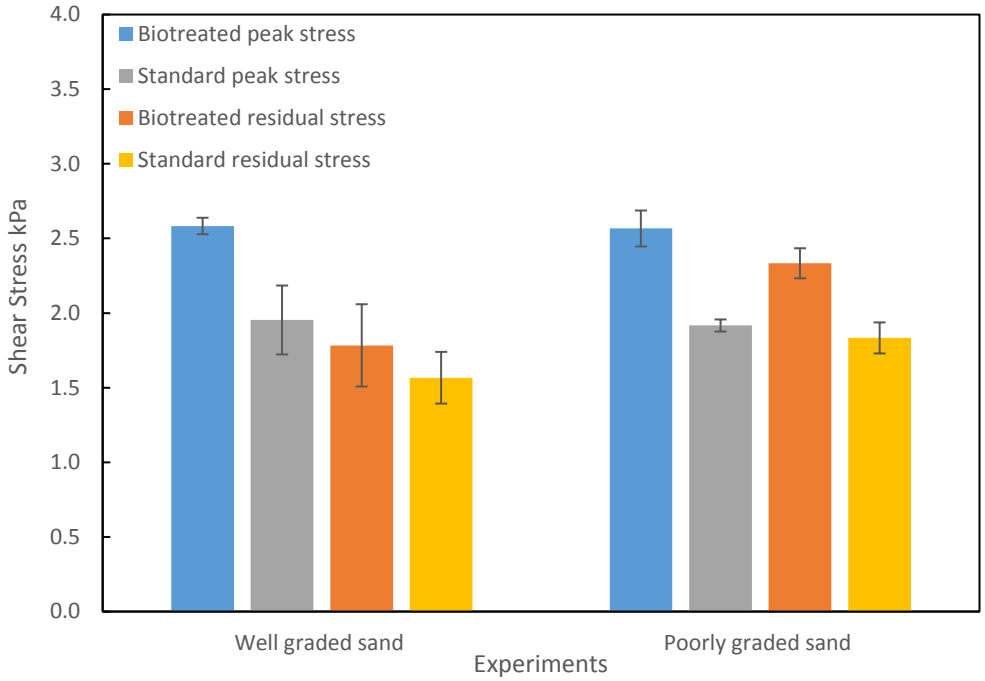


Figure 6-36. Peak and residual stresses for both sand grains.

6.6.3 Dilation and friction behaviour

The poorly graded sand exhibits a considerably larger dilation than well-graded sand for both types of samples as shown in Figure 6-37.

The compression of sand under 1.0 kPa loading and over incubation period was measured. Figure 6-38 indicates that there is very little difference in compression between the two biotreated specimens despite there being much more biomass with poorly graded sand, thereby the formed biofilm has no effect on the compression of biotreated samples. However, the standard samples have more compression than the biotreated samples. Both types of sand approximately show similar compressible values.

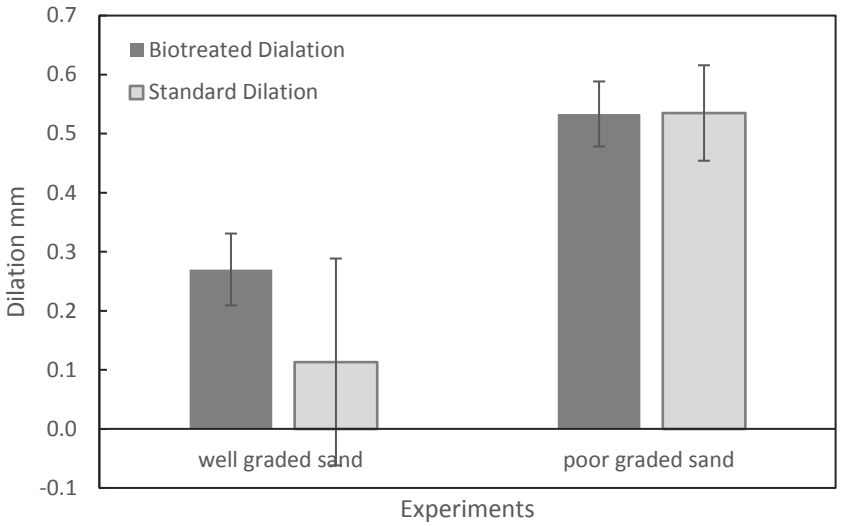


Figure 6-37. Dilation behaviour versus each experiment.

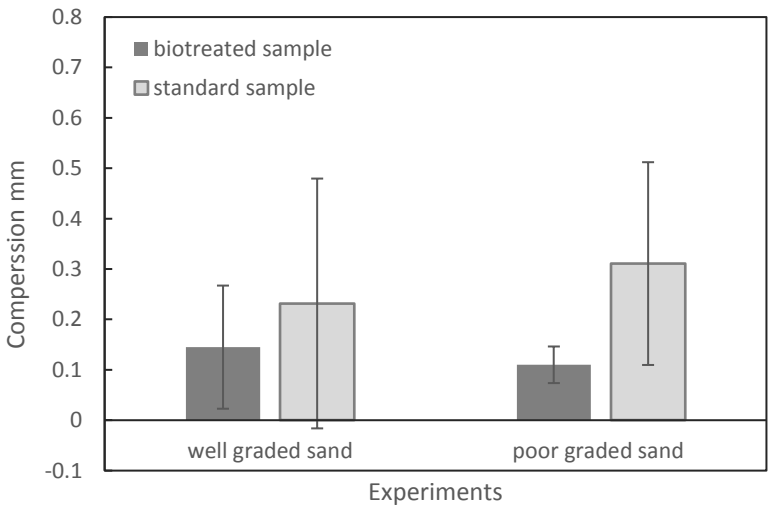


Figure 6-38. The measured compression of well and poor graded sand over two weeks.

The relative horizontal displacement when the shear stress reaches the peak value is also presented in Figure 6-39. It can be noticed that the standard poor graded sand has larger horizontal displacement at peak stress in comparison with well-graded sand.

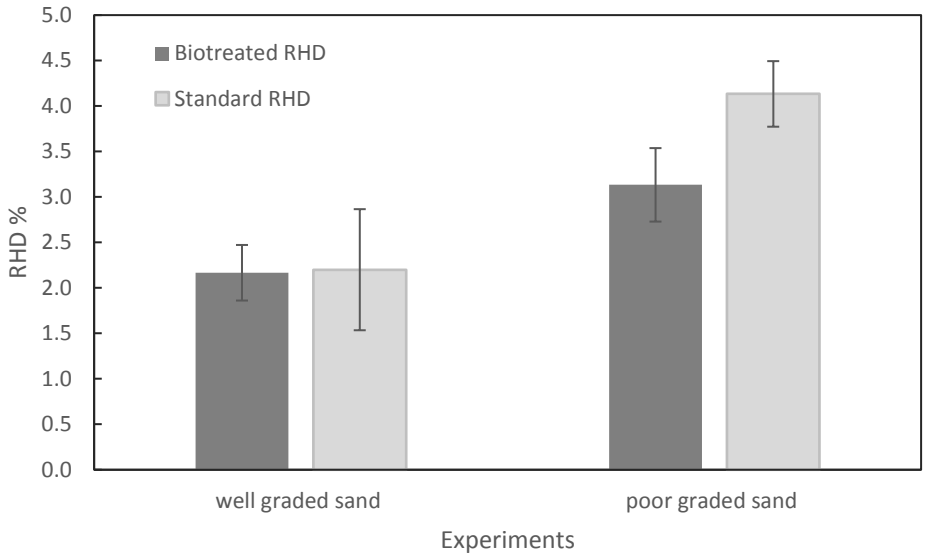


Figure 6-39. Relative horizontal displacement at peak stress for both gradations.

Figure 6-40 and Figure 6-41 show the peak dilation angle and peak and residual friction angles for well-graded and poorly graded sand respectively. It can be seen that peak dilation angles for both the biotreated and standard samples of poorly graded sand are slightly less than the angle of well-graded sand. Also, the same figure also presents the peak and residual friction angles, showing biotreatment causing an increase in peak friction angle compared to standard samples but there being no difference between sand types. Residual friction angles, however, show that poorly graded sands had higher values than well-graded, but that biotreatment had little effect.

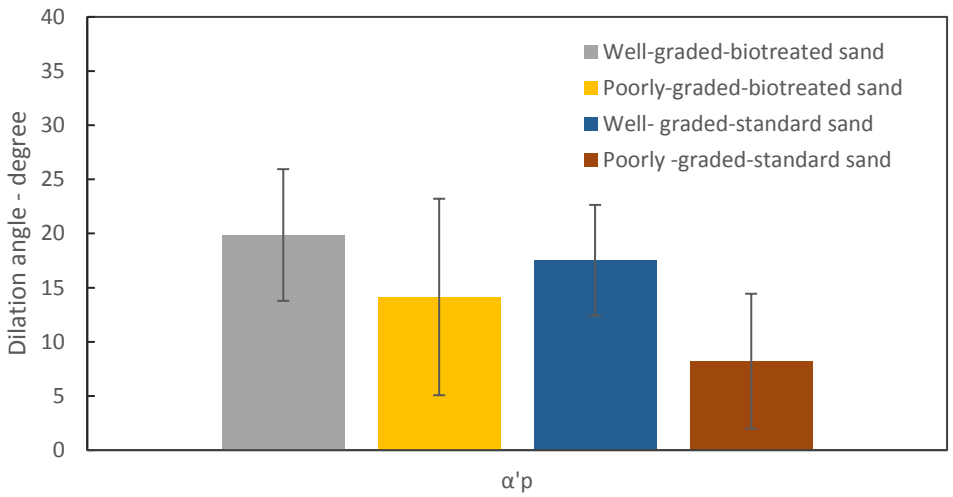


Figure 6-40. Peak dilation of biotreated and standard samples of well-graded and poorly graded sand.

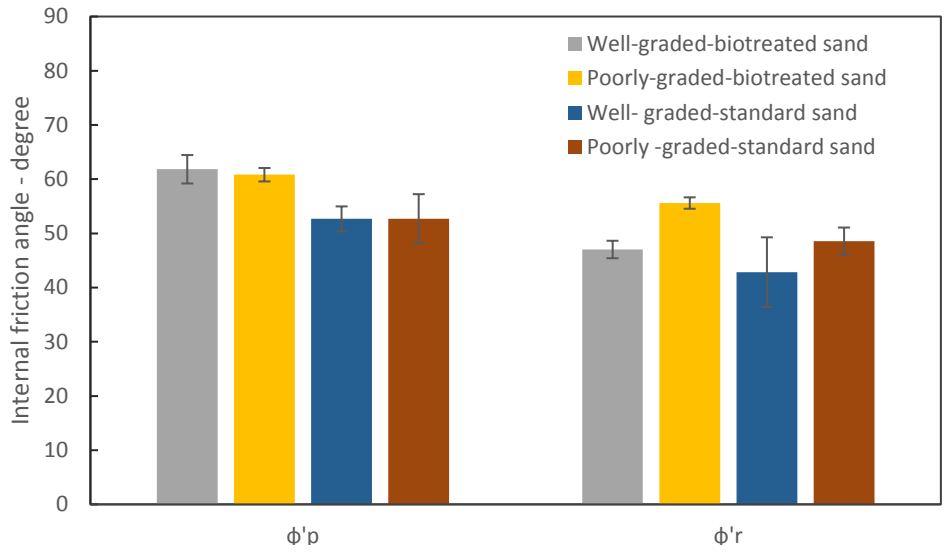


Figure 6-41. Peak and residual internal friction angles of biotreated and standard samples of well-graded and poorly graded sand.

6.7 Effect of angularity

The following section investigates the influence of particle shape on the growth of biofilm in a sand mass and the effect of such biofilm on shear behaviour via testing of the main well-graded silica sand (used in the main experiment in the current study) and well-graded sea sand. Although the original specification of the main silica sand grains is sub angular to round, such sand was crushed to produce well-graded sand, which may have resulted in some angular particles. It is well known that the grains of sea or beach sand were rounded. The image analysis was performed to measure the angularity of both sand types as presented by (Chandan et al. 2004), this analysis is mentioned in appendix C. The direct shear tests on both sand types were carried out by applying 8.89 kPa as a normal stress.

6.7.1 Biomass Content

Figure 6-42 illustrates the biomass (loss on ignition) in sea and silica sands. It can be clearly noticed that the biomass in sea sand was greater than that in silica sand for biotreated samples. The increase in the middle layer (shearing plane) of biotreated sea sand samples was around 68% more than in the silica sand. For the standard specimens of sea sand, the LOI was found to be the same as in biotreated silica sand. However, the sea sand was initially treated with HCl to remove any inorganic carbon from the sand mass, and no organic carbon was added during incubation.The sea sand was also heated at 550 ^{o}C for sterilization before use.

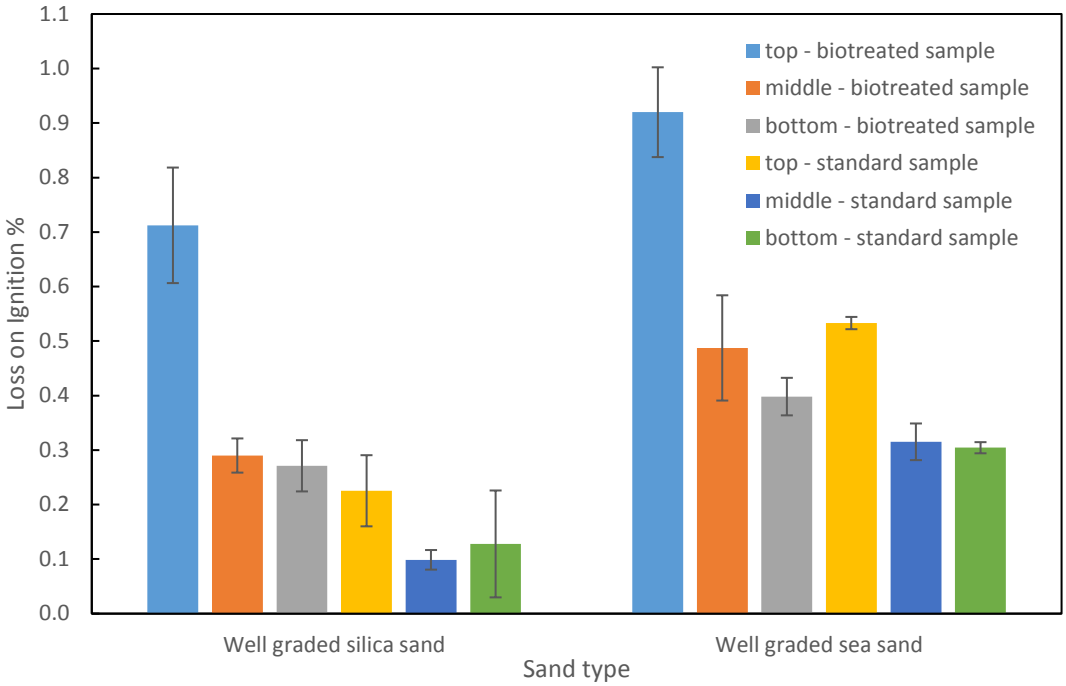


Figure 6-42. Observed biomass content.

Figure 6-43 indicates that biotreated and standard samples for both sand types have almost the same compression, but the biotreated samples are considerably more compressible than the standard samples which is attributable to the presence of biofilm. Biofilm may act as a lubricating agent (Perkins et al, 2000).

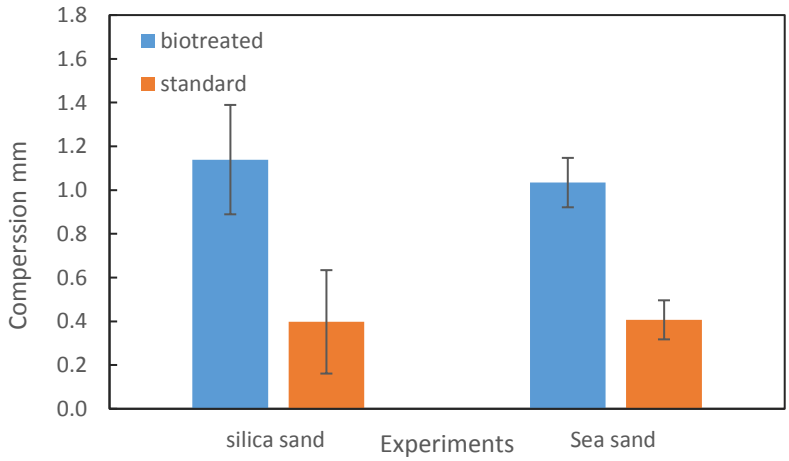


Figure 6-43. Compression of sands.

6.7.2 Peak and residual stress

The comparison of peak and residual stresses for both silica and sea sand tests is presented in this section. Figure 6-44 shows that the peak stress of biotreated and standard samples of silica sand are slightly larger than those of sea sand which can be attributed to the increased angularity of silica sand. The influence of biofilm on the peak stress seems similar in both grains of sand. The residual stresses for biotreated and standard samples in both sand types are similar.

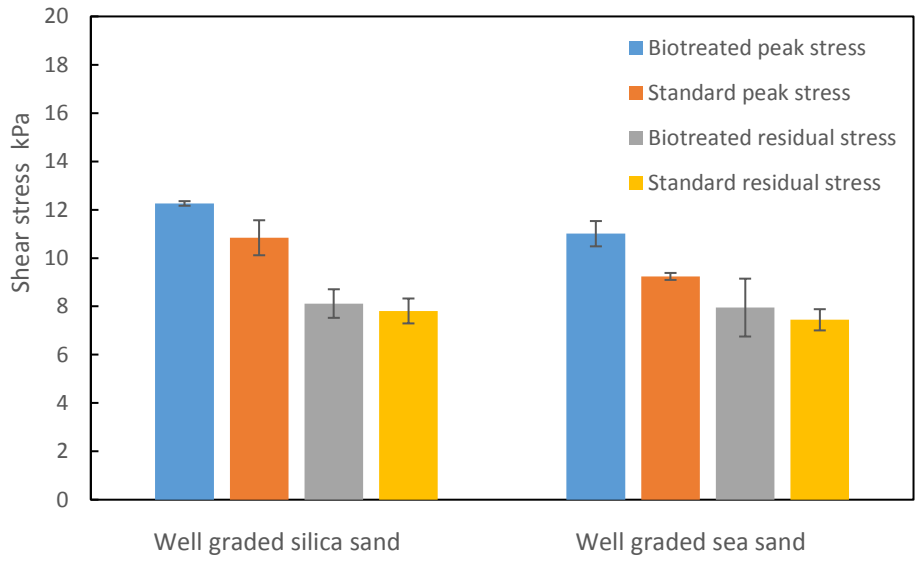


Figure 6-44. Peak and residual stresses for both sand types.

6.7.3 Dilation and internal friction angles

Figure 6-45 illustrates that both sand types have similar dilation behaviour, indicating an insignificant impact of particle shape on this property. The dilation of biotreated samples had almost similar for both sand types and

similar dilation was seen in the standard samples, although the latter was highly variable.

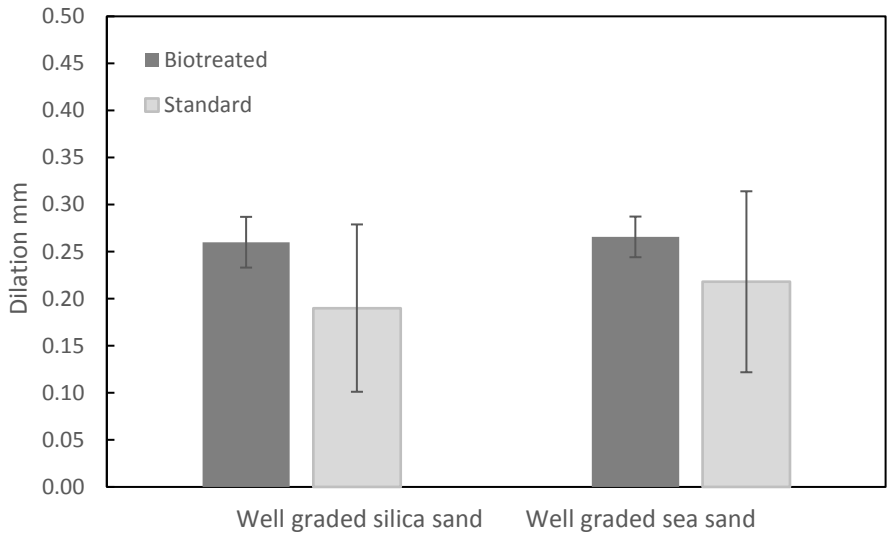


Figure 6-45. Dilation of silica and sea sands.

The relative horizontal displacement (RHD) at peak shear stress for this study is shown in Figure 6-46. All samples, biotreated or standard and both sand types, have similar RHD values at peak stress.

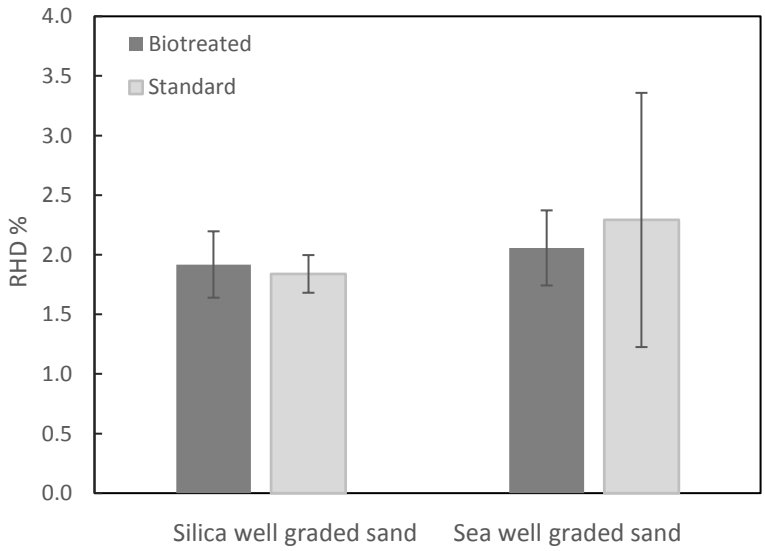


Figure 6-46. Relative horizontal displacement at peak stress for both sand Types.

To study the influence of biofilm on the peak and residual dilation and friction angles by comparison of these angles between silica and sea sands. Figure 6-47 reveals that the peak dilation angles of biotreated silica and sea sands are similar. Standard specimen dilation angles for both sand types are also similar, but are lower than the dilation angle of biotreated specimens. Regarding peak friction angle, the silica sand samples (both biotreated and standard) have a larger friction angle than the sea sand, as shown in Figure 6-48 which can be attributed to angularity. The peak friction angles of biotreated sands consistently larger than for the corresponding standard samples.

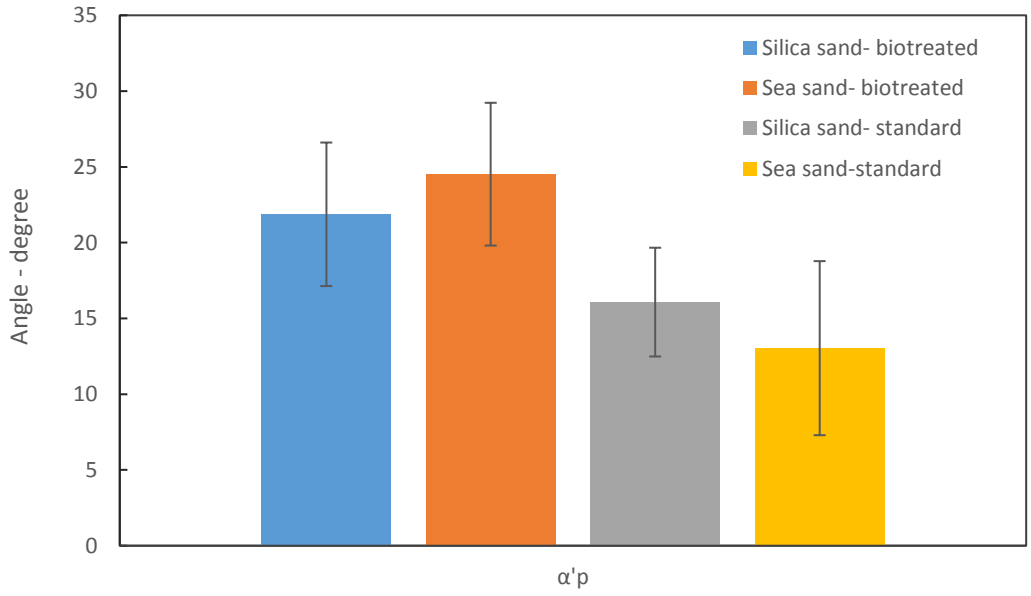


Figure 6-47. Peak dilation angle for silica and sea sands.

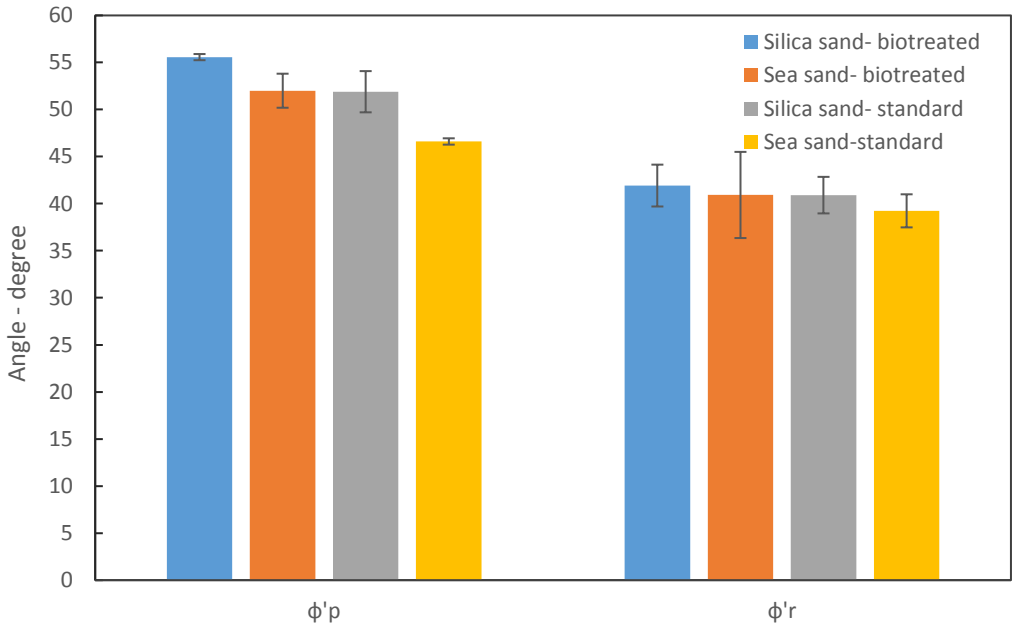


Figure 6-48. Peak and residual friction angles for silica and sea sands.

6.8 The influence of rate of testing

For granular soil, the rate of the test is not normally considered important during the direct shear test because the build-up of pore pressure in such drained material is unlikely. Therefore, the measured total shear stress is equal to the effective stresses in the case of drained direct shear tests according to BS 1377-7:1990. In this study, the presence of biofilm in the pore space may lead to a different response regarding a build-up of excess pore water pressure which may correspond to the rate of testing. Therefore, in this section, a series of tests is undertaken to study the impact of the testing rate on the shear response of biotreated samples.

The experimental study involved performing three sets of direct shear tests with different testing rates. The samples were addressed as SP0.1 (testing rate of 0.1 mm/min), SP0.5 (0.5 mm/min) and SP2.0 (2.0 mm/min). The following

sections compare the results of the direct shear test at different testing rates with an applied normal stress of 8.89 kPa.

6.8.1 Biomass Content

The amount of biofilm accumulation in the sand matrix for all the three sets is depicted in Figure 6-49. As shown in the figure, the amounts of loss on ignition are very similar, which is expected as they had identical conditions until testing.

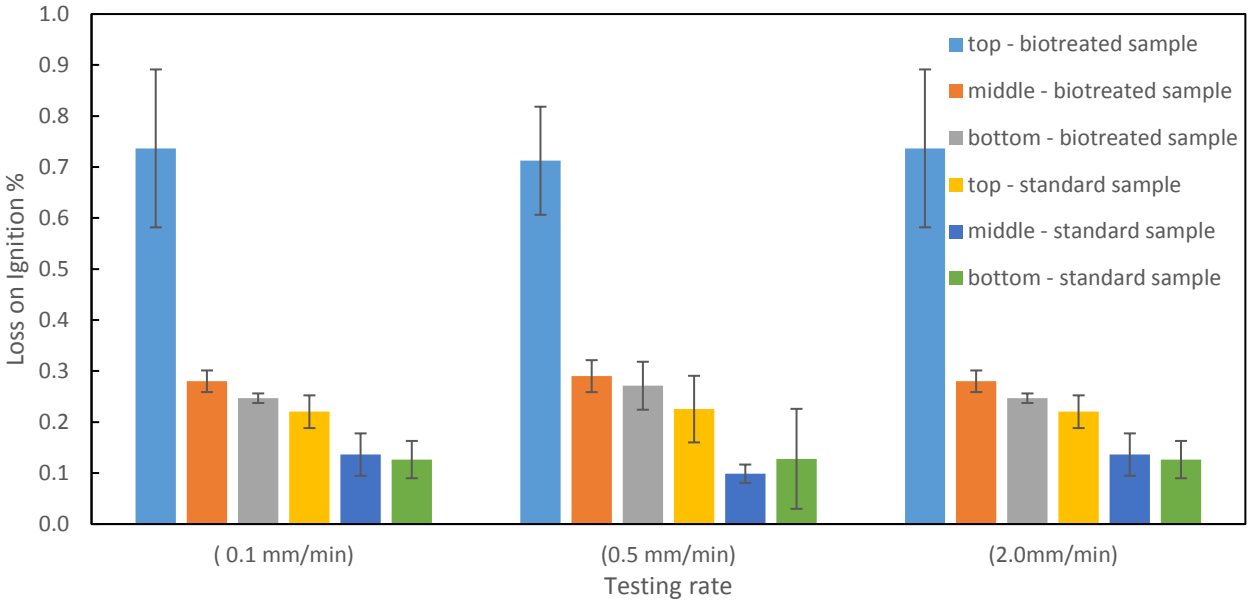


Figure 6-49. Measured biomass content.

6.8.2 Peak and residual stresses

Figure 6-50 illustrates the comparison of peak and residual shear strength among the different rates of testing. Peak stress in biotreated samples is similar for SP0.1 and SP0.5, while that in SP2.0 was higher. The peak stress in standard samples also increased with the rate of testing. SP0.1 and SP0.5

are below the rate mentioned in BS 1377:7:1990 (1.0 mm/min), and may allow sufficient time to dissipate the excess pore pressure. However, 2.0 mm/min, at twice the suggested testing rate from BS 1377-7:1990, is a relatively quick test for granular soil. It is hypothesised that this is because of the presence of biofilm blocking pore throats. In this case, the excess pore water pressure might be generated during the quick or relatively quick test. The presence of the accumulated biofilm may play a crucial role to increase the shear stress. The biofilm decreases the diameter of effective pore throat of granular soil or even block them (Jaiswal et al, 2014; Tang et al, 2013; Eljamal et al, 2008; Dunsmore et al, 2004). Measuring excess pore water pressure in the direct shear test is difficult. Moreover, Mamo and Dey (2014) highlighted that the shear parameters resulting from direct shear tests are susceptible to changes in testing rate. In saturated soils, density change during shear is achieved by expelling or by taking in water, and effective stress change is brought about through an increase or a decrease in pore water pressure. The soils of high density, such as dense sands tend to expand. Therefore, If the rate of loading is fast enough, a soil with a tendency to expand or dilate during drained loading, it will exhibit a decrease in pore water pressure during undrained loading, increasing effective stress (Terzaghi et al, 1996). The results demonstrate little differences between the residual shear strength of biofilm and non- biofilm samples.

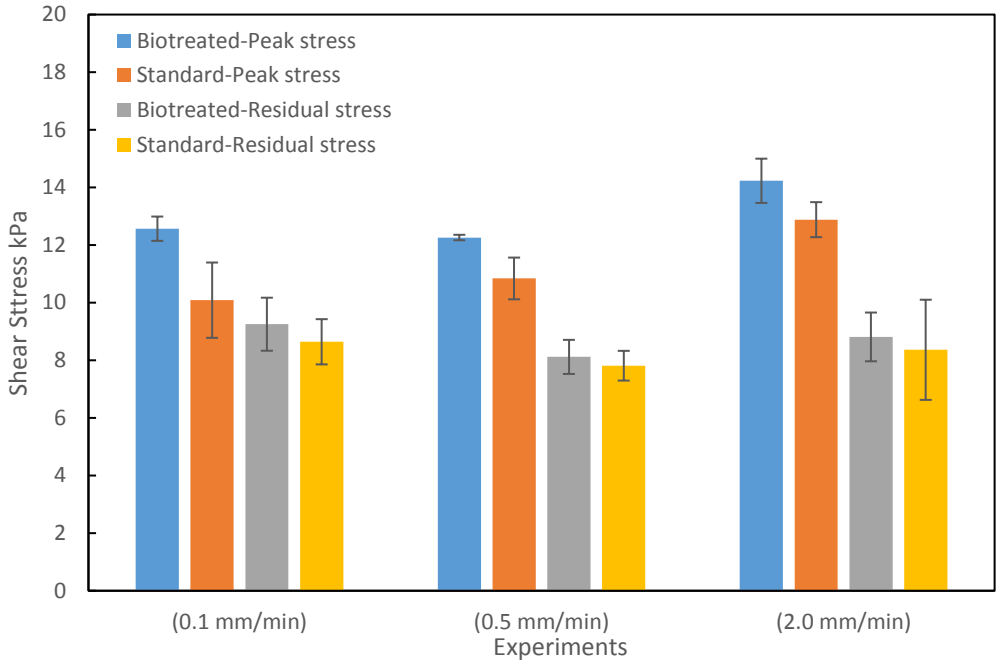


Figure 6-50. Peak and residual stresses for a different rate of testing.

6.8.3 Dilation behaviour and friction data

As Figure 6-51 reveal, biotreated and standard samples show that the dilation steadily increases with increasing the rate of testing. For SP2.0 samples, both biotreated and standard specimens show similar dilation increasing. Roy and Campanella (1997) and Bolton (1986) presented that the rate of dilation can be represented as the relative dilatancy index (I_R) which depends on the differences between peak and residual friction angle. Moreover, this index is also a function of the relative density and applied stress level.

It can be highlighted from the Figure 6-51. Generally, the increasing of dilation rate may be impacted by the rate of testing. Watanabe and Kusakabe (2013) reported that the rate of deformation may affect dilation characteristics, dilation increases as the strain rate increases. Also, Anim (2010) stated that

the mount of dilation in the mobilized zone increases as the strain rate increases.

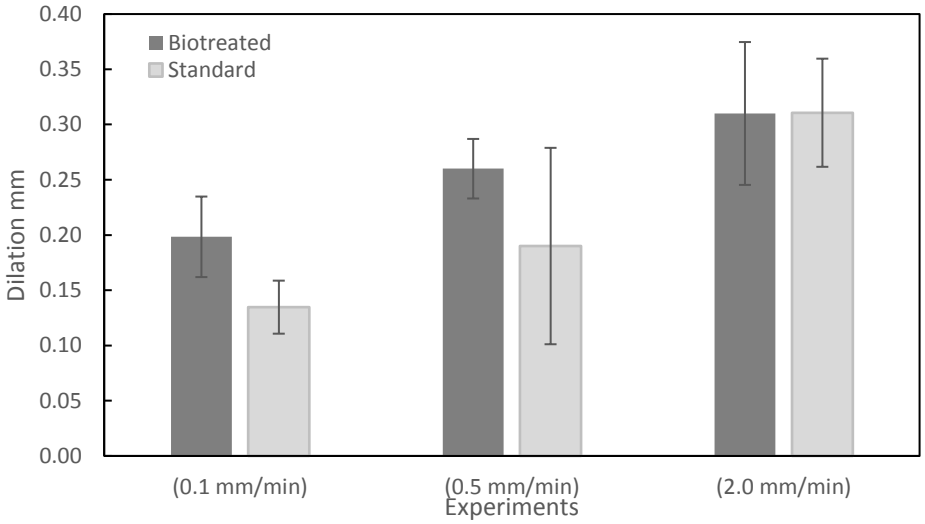


Figure 6-51. Dilation behaviour versus different rates of testing.

The relative horizontal displacement ratio at the peak stress is shown in Figure 6-52. It can be seen that all biotreated and standard samples reach peak stress at a similar RHD value on average.

To study the influence of biofilm on the peak dilation and friction angles, by comparison, the response of these angles to testing rate is assessed. Figure 6-53 reveals that the peak dilation angle of biotreated samples does not vary consistently with the rate of testing although standard samples exhibited a slight increase with increasing testing rate. The residual dilation angles were very small or zero.

Figure 6-54 shows the peak and residual friction angles. The peak friction angle increases with testing rate whilst the residual friction angle does not

significantly change. The internal friction angle increase with the increase in strain rate (Watanabe and Kusakabe, 2013).

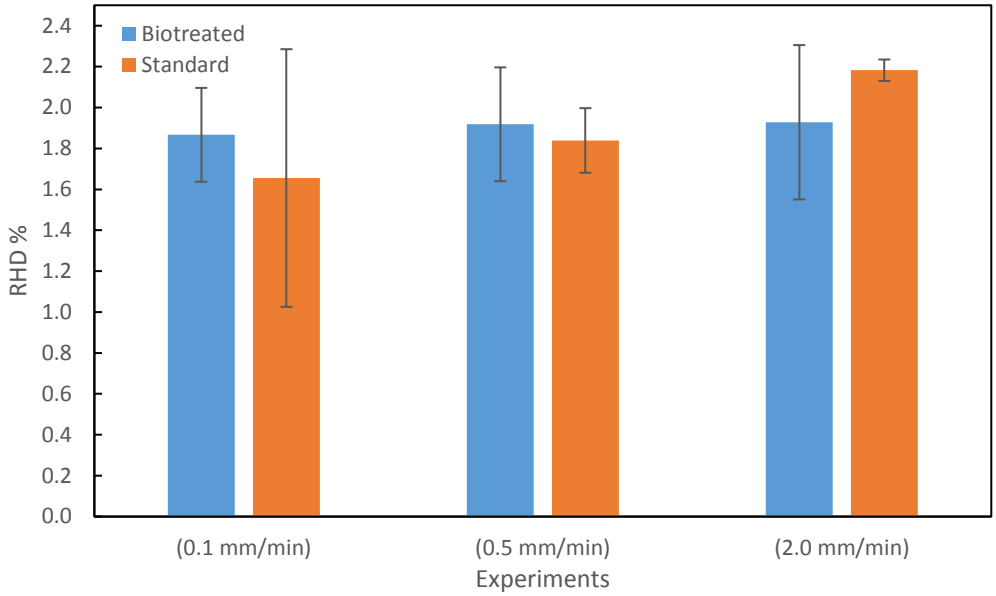


Figure 6-52. Relative horizontal displacement ratio versus different rates of testing.

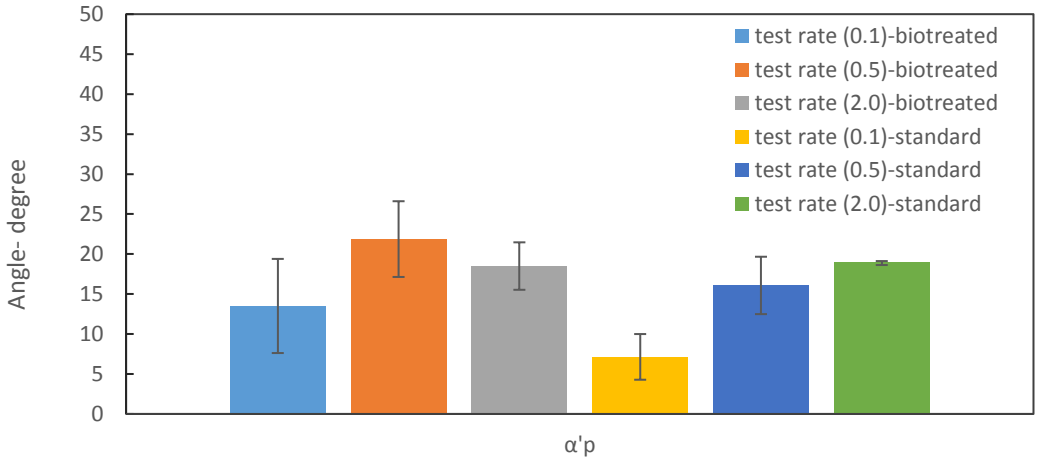


Figure 6-53. Peak dilation angles versus different rates of testing.

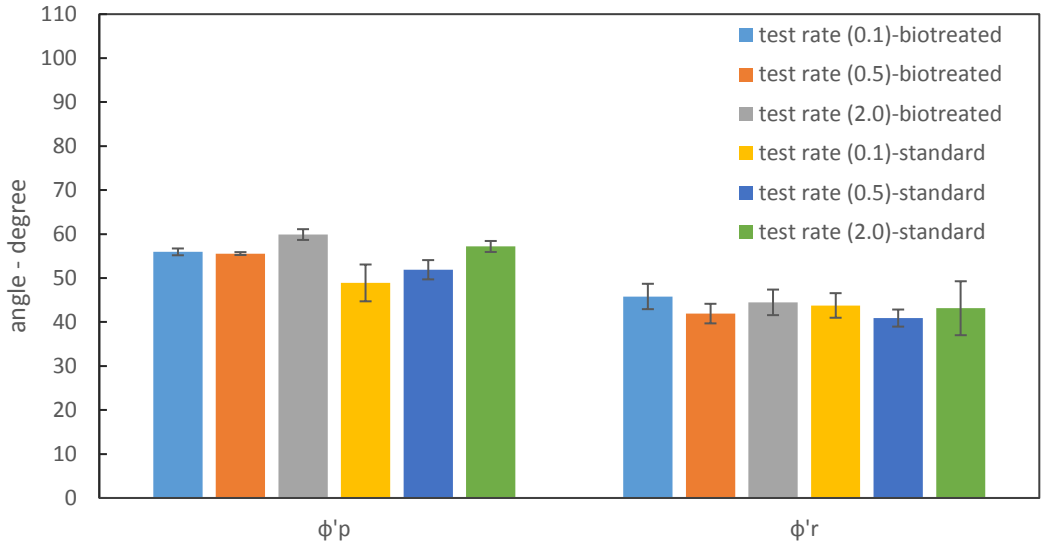


Figure 6-54. Peak and residual friction angles.

6.9 Summary

The major finding of this study is how biopolymer affects the shear behaviour of well-graded sand. The following points summarise the main outcomes of the biological treatment experiments:

- The biotreated samples show larger peak stress than the standard samples. At 1.0 kPa normal stress, the peak stress was 30% greater and for the other normal stresses, it ranged from 8-13% higher for biotreated samples.

Overall, the applied normal pressure on the specimens during the incubation period did not influence the amount of EPS growth in the sand matrix. The applied normal stress was resisted by the sand particles and no excess pore water pressure generated due to the drained behaviour of sand according to direct shear test procedure. There is experimental evidence to suggest that the biofilm causes the sand to be densified under loading compared with the

standard samples. It may work as a lubricating agent, causing the sand particles to compact together under loading (Perkins et al, 2000). There is also evidence to suggest that increasing biofilm increases peak shear strength, although this in itself does not indicate whether this effect was caused by increased densification on its own, or whether other strengthening effects are dominant.

The peak stress in both biotreated and standard samples exhibited a non-linear failure envelope due to the reduction of peak stress at 25.0 kPa loading. whereby the loading of 25.0 kPa shows around 24% less peak stress than the expected stress for both samples. This may have been caused by the final density of these samples being less than expected considering the generally increasing density at other stresses. Considering the first four applied stresses only, the failure envelope does exhibit linearity, suggesting very low cohesion.

- The residual stress has an almost identical trend for both biotreated and standard samples. Both sample sets have a linear failure envelope with similar values. Preloaded saturated samples have higher peak stresses than biotreated samples, and a higher angle of friction determined from a linear failure envelope. The preloaded dry samples also exhibit a linear failure envelope but with lower peak stresses and a lower friction angle than biotreated samples.

  The residual stress of all preloaded dry and saturated samples was similar and consistent with biotreated and standard samples.

- The amount of biofilm accumulation in the poorly-graded sand was approximately twice that in the well-graded sand at all depths – greater accumulation was again noted near the surface. There is considerable

variability in the poorly graded sand data compared to well-graded sand, however. The greater amount of biomass in poorly graded sand may because such samples have a larger pore volume and increased access to nutrients and dissolved oxygen (Chou, 2007) compared with the well graded sand.

For peak stress, the biofilm had a similar effect on both the well and poorly graded sands whilst the residual shear strength for poorly graded sand was larger than that of well-graded sand. Both sand types exhibited similar compression, but poorly graded sand had a slightly lower peak dilation angle whether biotreated or not.

- The loss on ignition in sea sand was greater than in silica sand, especially near the upper surface but also around the shearing plane. Both sands exhibited similar compression behaviour, with greater compression in biotreated samples. Increased angularity appeared to cause a slight increase in peak stresses (both with and without biotreatment) but residual stresses were similar. Dilation during testing was also similar, suggesting angularity had little impact.

- Increasing testing rate increased peak stress (at the highest rate) both in biotreated and standard specimens, suggesting the effect is not due to the presence of biofilm. Residual strengths were similar at all rates. Also with increasing rate, dilation appears to increase.

Chapter 7

CONCLUSIONS AND RECOMMENDATIONS

7.1 Introduction

The research reported in this thesis studied the ability of *Beijerinckia indica* to metabolically produce extracellular polymeric substances (EPS) which causes aggregation of soil particles during bacterial growth. Increased strength of soil may be caused by the presence of microbial polymer nets connecting sand particles as highlighted by Deng et al (2015), Chen et al (2015), DeJong et al (2013), Welling (2012), Khatami and O'Kelly (2013), Ahmed and Hussain (2010), Wloka et al (2004), and Donlan (2002). The filling of pore with biopolymer and increased densification may also have an effect. Previous studies by Ahmed and Hussain (2010) and Banagan et al (2010) determined that there was a substantial effect of biopolymer in soils in uncontrolled conditions and using simple measures such as shear vanes at apparently low confining pressures, whilst Perkins et al (2000) found little effect of biopolymer at large confining pressures in triaxial specimens. The use of the direct shear test at low normal stress allows the study of samples confined at low stress but under highly controlled conditions.

Therefore, this study has attempted to study the bio-densifying process in granular soil and the applied normal stresses that can affect biofilm interaction with sand grains, as well as to understand how factors such as biotreated poorly graded silica sand, biotreated well graded sea sand, and different testing rates affect the shear strength of the sand in biotreated and non-biotreated conditions.

The aim of this study were to evaluate experimentally the effect of bacterial growth on the shear strength of the sand, and the factors that can affect the bio-

aggregate process such as the grain size and bacterial growth conditions. The main conclusions from this work, based on the initial aims and objectives in already mentioned in chapter 1, are summarised:

- The first objective of developing a reliable procedure for carrying out direct shear tests at low normal pressure was achieved using jacking screws to minimise the friction between the two shear box parts and using a sufficient mass of sand.

- Examination of the influence of accumulated biofilm on the shear stress-strain behaviour of sand at low normal stress was carried out under a range of conditions. The biofilm appears to have a significant and repeatable strengthening effect under a range of conditions that may be at least partly due to increased densification of the sand when the biofilm is grown in samples under applied stress.

- The effect of biofilm on the shear strength of poorly and well-graded or rounded and angular sands as well as at a range of testing rates has been explored.

7.2 Conclusions

The major finding of this study is how biopolymer effects the shear behaviour of well-graded sand. For this purpose, the resulting shear strength was presented versus applied normal stress to understand the relationship. The following findings and conclusions can be drawn.

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7.2.1 Main biotreated experiment

Generally, for 1.0 kPa the biotreated sample show about 30% larger peak stress than the standard specimen, whereas the biotreated specimens at higher stresses between 4 and 25 kPa exhibited approximately 8-13 % larger peak stress than the standard samples.

Overall, the applied normal pressure on the specimens over the incubation period did not influence the amount of biofilm growth in the sand matrix, but there is evidence to suggest that the growth of biofilm increases the ability of sand to densify with increasing applied normal stress. The applied normal stress may be resisted by the sand particles (effective stress), thereby no excess pore water pressure would be generated under stress.

Relevant to Coulomb's failure criterion, the relationship of peak shear strength with applied normal stress for both biotreated and standard samples shows a lack of linearity. Although linear behaviour was observed up to 16 kPa normal stress, there is a reduction in peak stress at 25.0 kPa loading. Considering only the first four applied stresses, the trends of envelope are generally linear, and suggest a similar trend of increasing density under loading, whilst the corresponding density for samples at 25 kPa was less than expected based on this trend and may contribute to the lower than expected peak stresses. As mentioned above, the peak stress for all biotreated samples was greater than the peak stress of standard ones for all loading stages. Regarding the Mohr-Coulomb formula, besides the improvement of friction characteristics of biotreated sand, the development of cohesive properties of such sand may also be considered by the growth of EPS which

consists of a high viscosity substance. This substance can form polymer bridges between the microorganism and surface of the sand particle, to connect them together (Garrett et al, 2008). However, in this study, there is no distinct difference in cohesion with these specimens. This is likely to be because the amount of grown biofilm was very low, the maximum biomass was 0.43% at the middle layer of the 4.1 kPa loading biotreated samples.

In terms of residual stress, the biotreated samples show consistent behaviour, with the failure envelope behaving linearly and with almost identical values for both biotreated and standard samples.

The loss on ignition in the middle layer of biotreated samples was greater than in the standard samples by about 2.5-3.0 times. The upper layer has larger biomass content compared with the middle and bottom layers for all biotreated samples at all applied normal stresses.

Correcting the internal friction angle based on the method of Lehane and Lui (2013) was considered necessary because of the low stresses applied in these experiments. The correction was significant only at stresses of 1.0 and 4.1 kPa, but this correction was less important for the other normal stresses.

7.2.2 Comparison between of biotreated experiments and dry and saturated sand experiments without preloading

All samples were prepared in the same manner, and little variability in the prepared density for the different samples was found. The variability of the prepared dry density does not affect the linear trend of the Coulomb's failure envelope. On the other hand, the saturated samples have almost the same

prepared density for testing at all stresses but demonstrate similar behaviour as biotreated and standard samples, namely that the peak shear stress is lower than expected at 25.0 kPa loading and the failure envelope deviates from linear behaviour. Therefore, the final density of biotreated and standard samples may not be the single factor which affects the decrease in the peak stress at normal stress of 25.0kPa.

The standard samples showed the same failure behaviour of the peak stress for dry sand. However, the biotreated samples show a slightly steeper failure envelope. The fully saturated samples offered the largest shear resistance and are parallel to the biotreated trend.

The residual stresses of both biotreated and standard samples have mostly identical linear regression. Fully saturated samples have a residual envelope line with a steeper slope. However, this test was performed with a single sample, and so the variability in these tests could not be determined.

It was observed that the peak dilation angles and internal friction angles decreased with increasing normal stress and decreasing relative compaction. Dilatancy decreases with increasing normal stress, and therefore friction angle decreases as stated by Hsu (2005), Hosseini and Jesmani (2013) and Bolton (1986).

7.2.3 Comparison of preloading tests

The preloading of samples in both dry and saturated cases led to linear failure envelopes across all applied normal stresses, i.e. there is no deviation at 25.0 kPa loading as seen in the previous tests. The peak stresses of the

preloaded saturated samples were the highest observed, and higher than biotreated samples) whilst the preloaded dry samples were the lowest, slightly lower than the standard samples.

7.2.4 The effect of particle grading

The poorly graded sand has more capability to carry biofilm than the well-graded sand because the poorly graded sand has larger pore volume than the well-graded sand (Jenneman et al, 1984).

The peak stresses of biotreated specimens of both sand types are similar, but the residual stress for biotreated poorly graded sand is substantially larger than in the biotreated well graded sand. Furthermore, the residual stress of standard specimen of poorly graded sand is slightly larger than the residual stress of biotreated sample of well graded sample.

The dilation of both biotreated and standard poorly graded sand is substantially larger than the dilation of well graded sand. This behaviour is unusual because well graded sand should have larger dilation. It may be suggested that with very low normal stress (1.0 kPa), this load may have less effect on the control of the final density of the samples. The density plays a crucial role in the determining of shear behaviour of granular soil (Senatore and Iagnemma 2011). There is no clear differences in the compression in either sand types, either biotreated or standard samples. The RHD for standard samples of poorly graded sand was significant larger than with the corresponding samples of well graded sand. The difference in dilation angle between both sands as well as both sample types is insignificant.

As a result the peak friction angles are similar for the well graded and poorly graded sand in biotreated and standard samples.

7.2.5 The effect of angularity

Regarding peak and residual stresses for both samples of silica sand are slightly larger than the stresses of sea sand, this behaviour may the differences of angularity quantity between both sand types shown in Appendix C.

Both silica and sea sands show a similar dilation behaviour for biotreated and standard samples as well as the same compressibility, but biotreated samples have slightly larger compression than the standard ones. Overall, the peak dilation angle of silica sand is a little higher than the sea sand. The silica sand has a slightly greater peak friction angle than in the sea sand for biotreated specimens, but in standard samples it was considerably larger in the silica sand.

7.2.6 The influence of the rate of testing

Peak stresses are larger at a rate of test of 2.0 mm/min than at slower rates, there appears to be no difference between biotreated and standard samples.

Dilation behaviour slightly increases with the increase of testing rates, whereas the peak dilation angle or RHD at peak stress are not significantly affected by rate. As a result, the peak friction angle at 2.0 mm/min is slightly higher than that at 0.1 and 0.5 mm/min for biotreated and standard samples.

Residual friction angles are nearly equal for both types of samples and all test rates.

7.3 Recommendations and future work

This study explored the bioaggregation activity by biofilm grown in silica sand. Furthermore, the factors that have a significant influence on this activity such as the effect of sand gradation, particle shape, the rate of testing, normal stress and the optimal growth medium for the selected bacteria. A number of areas that would warrant further investigation are discussed below:

i) The amount of biofilm produced is low, and considerably lower than that found in other relevant studies. I think that developing methods to increase amount of biofilm and determining whether there is any impact upon cohesion, is important.

ii) Further exploration and confirmation of the impact of biofilm on sand densification under load.

iii) Long-term performance of biofilm-treated specimens – deterioration of the biofilm, how long does it take, can the biofilm be maintained and what conditions are required for this?

iv) A numerical model to help understand whether theories of how the biofilm affects mechanical properties of sand are correct or not.

v) Further exploration to develop better understanding of the behaviour of moisture in biotreated specimens – are these saturated or unsaturated? Drained or undrained?

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APPENDICES

APPENDIX A1: Specification of silica sand

AGGREGATE INDUSTRIES

Specialist silica sands

Garside Sands 16/30 Sand

A dried silica sand.

Applications

- Water filtration pool filters
- Potable and waste filters
- Synthetic sports pitches
- Adhesives, grouts, paints
- Pet dietry and aquaria.

Sectors

- Water filtration
- Sports and leisure
- Pet care
- Functional fillers and flooring.

Available in

- Loose: both bulk pressurised tankers and tippers
- Bulk bags (tote)
- 25kg and 12.5kg poly bags.

Mechanical analysis

Sieve size (mm)	Average % passing	Specification
1.18	100	100
1.00	99	98 - 100
0.85	83	60 - 90
0.71	41	10 - 75
0.60	5	0 - 25
0.50	1	0 - 5
0.425	0	0 - 1

Colour	Yellow	Also available in Natural White
Specific gravity	2.65	Details on request
Uncompacted bulk density	1.56 t/m³	
Nominal effective size	0.54 - 0.71mm	
Uniformity coefficient	<1.4mm	

Garside 16/30 sand

Size range:	1.00 0.50mm
Source:	Leighton Buzzard, Bedfordshire
Geology:	Lower Greensand of the Cretaceous period
Composition:	Quartz
Grain shape:	Sub angular to rounded

Garside Sands 16/30 Sand

Manufacturing standard

All aggregate Industries products are manufactured in accordance with ISO 9001 with factory compliance to ISO 14001.

Chemical analysis

Chemical analysis		%
Silica	SiO_2	98.29
Alumina	Al_2O_3	0.31
Titania	TiO_2	0.02
Iron	FE_2O_3	1.29
Magnesium	MgO	<0.01
Calcium	CaO	<0.01
Sodium	Na_2O	<0.01
Potassium	K_2O	0.00
Phosphate	P_2O_5	<0.01
Chromium	Cr_2O_3	0.0002
Manganese	MnO_2	<0.005
Loss on ignition @ 1000°C	L on I	0.19

The above figures only relate to one specific test.

Sustainability and local sourcing

Energy use: Aggregate Industries is at the forefront of sustainability and has committed to reduce carbon emissions by 20% by 2016 based on a 2012 base line.

Recyclable: 100% of the product can be recycled thus reducing the amount of material that is sent to landfill.

Manufacturing location: produced in the UK, with locally sourced materials under strict environmental and social legislation.

Responsible sourcing: Aggregate Industries is the first company in the world to achieve a BES 6001:2008 Responsible Sourcing Certificate from BRE Global. Products are assed on:

- quality management
- environmental management
- health and safety management
- greenhouse gas emissions
- minimising raw material usage
- labour practice
- biodiversity
- community engagement.

Policies

Aggregate Industries' policies on the environment and community, health and safety and sustainable solutions for different product applications can be viewed on our website www.aggregate.com

COSHH data

Full COSHH data on Garside Sands products is available on request. Please call the Technical helpline on 01525 237911.

Technical support

Detailed guidance and assistance with the preparation of specification and use of Garside Sands products is available through the sales offices. A free technical service is also available.

Please call Garside Sands Technical Services on 01525 237911

Garside Sands, Eastern Way, Heath & Reach,
Leighton Buzzard, Bedfordshire, LU7 9LF

Tel: 01525 237911
Fax: 01525 237991
Email: garside.sands@aggregate.com

www.aggregate.com

AGGREGATE INDUSTRIES

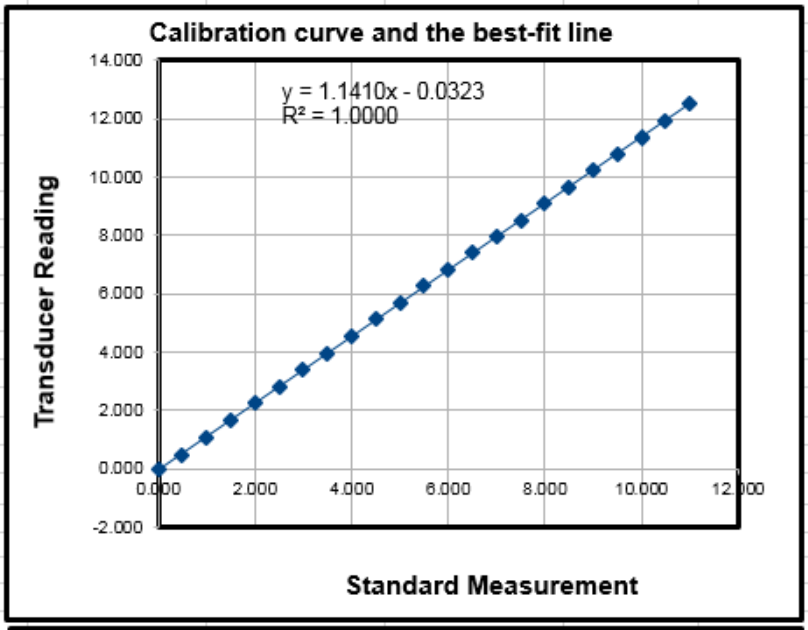
APPENDIX A2: Calibration of transducer

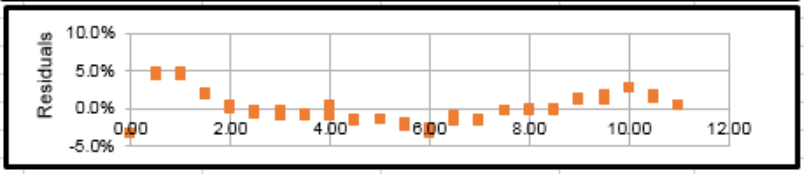
Analytical calibration using a simple linear curve fit, with error estimation

Calibration data	
Standard Measurement	Transducer Reading
0.000	0.000
0.500	0.492
1.000	1.062
1.500	1.660
2.000	2.248
2.500	2.823
3.000	3.396
3.500	3.970
4.000	4.528
4.500	5.116
5.000	5.687
5.500	6.266
6.000	6.839
6.500	7.397
7.000	7.972
7.500	8.531
8.000	9.097
8.500	9.666
9.000	10.225
9.500	10.797
10.000	11.350
10.500	11.934
11.000	12.514
11.000	12.514
10.500	11.935
10.000	11.354
9.500	10.793
9.000	10.224
8.500	9.669
8.000	9.099
7.500	8.530
7.000	7.969
6.500	7.401
6.000	6.847
5.500	6.268
5.000	5.687
4.500	5.120
4.000	4.541
3.500	3.973
3.000	3.401
2.500	2.827
2.000	2.251
1.500	1.663
1.000	1.066
0.500	0.495
0.000	0.000

Application to unknowns

Readings of the unknowns	Calculated concentration	Calculated error (σ)	Calculated error
1.0000	0.9047	0.005	0.57%
2.0000	1.7811	0.005	0.30%
3.0000	2.6575	0.006	0.21%
4.0000	3.5339	0.006	0.17%
5.0000	4.4103	0.006	0.14%
6.0000	5.2867	0.007	0.13%
7.0000	6.1631	0.007	0.12%
8.0000	7.0395	0.008	0.11%
9.0000	7.9159	0.008	0.10%
10.0000	8.7923	0.009	0.10%





Standard deviation of the residuals = 22.6%

	value	Error (σ)	% Error
Slope	1.1410	0.0009	0.08%
Intercept	-0.0323	0.0058	-18.09%

APPENDIX A3: Calculation of correction

Calculation and Correction of Peak and Critical State Shear Parameters

(Lehane and Liu 2012) examined three separate granular soil samples with a wide range of applied normal stresses. The experimental works were performed using two different shear boxes, modified shear box (100 mm ×100 mm ×33mm, Teflon boxes (Low friction) and traditional boxes. A simple means of correcting were developed for the mechanical friction in a shear box. These corrections were used to determine the peak and critical friction angles of granular materials at low stress levels in a shear box apparatus. General speaking, mechanical friction in the traditional shear box leads to very substantial errors when measuring sample response at low stresses and, as a consequence, shear box tests are not generally carried out at normal stresses less than 20 kPa. Two separate hypotheses (addressed as Case A and Case B) are investigated to estimate the average force acting on the shearing plane from the normal Load applied via the loading frame and the shear load measured with the load cell. For the three test sands, using data were assessed to be valid. A schematic view of a shear box arrangement is shown in figure 3-5 as a similar condition of the experimental to understand the discussion of laboratory work.

According to (Lehane and Liu 2012), this is the correction procedure of the calculations of experimental (1). To determine maximum shear stress and critical shear stress from shear stress versus horizontal displacement as a result of experimental (1);

Max Stress =2.979 kPa

Critical Stress =2.372 kPa

Applied Normal Stress = 0.986 kPa

Box Dimensions:

L1 =59.853 mm

L2 = 59.403 mm

Weight of Upper box (N) = 2.3 (230 gm)

Tp: Peak Load = Max stress* sheared area =2.979* (59.853*59.403/1000)=10.59 N

Tcs: Critical Load = Critical stress * sheared area =2.372* (59.853*59.403/1000)=8.43 N

Ft: Total Applied Normal Load = Applied Normal Stress * sheared area = 0.986* (59.853*59.403/1000)= 3.505 N

<u>There is a gap between upper and lower boxes by (jack screws) (no friction between boxes)</u>

It was addressed as **case A** by (Lehane and Liu 2012):

μ_{peak}=Tp/ (Ft + Weight of upper box)…….. (eq. 1a, Lehane and Liu 2012)

=10.59/ (3.505+2.3) =1.82445= tan (ϕ_p')

ϕ_p'= $\tan^{-1}$(1.82445) = 61.272 ^{o}

μ_{cs}=Tcs/ (Ft + Weight of upper box) …..(eq. 1b, Lehane and Liu 2012)

=8.43/ (3.505+2.3) =1.452= tan (ϕ_{cs}')

ϕ_{cs}'=$\tan^{-1}$(1.452) =55.454 ^{o}

<u>There are sand particles between upper and lower boxes</u>

It was addressed as **case B** by (Lehane and Liu 2012):

Assume Poisson's ratio of sand (ν) =0.2 (Lehane and Liu 2012)

K_r= ν/ (1- ν)

K_r =0.2/ (1-0.2) =0.25

Sheared area (A)=L1*L2=59.853*59.403/100=35.55448 cm^2

Height of sand through upper box= 8.9 mm (from measurement of experimental (1))

Inside area (Ai) = (59.853+59.403)*2*8.9/100=21.227 cm^2

μ_{sb}=0.38 (assumed by literature for plastic box) (Lehane and Liu 2012)

Normal force (Fn) =(1-(K_r * μ_{sb}*Ai/(2*A)))*Ft/(1+(K_r * μ_{sb}*Ai/(2*A)))…… (eq. 4, Lehane and Liu 2012)

=(1-(0.25*0.38*21.22/(2*35.55)))*3.505/(1+(0.25*21.22/(2*35.55)))=3.312 N

Max Friction Load (Ff) = (Fn+Ft)* K_r * μ_{sb}*Ai/ (2*A)…… (eq. 3, Lehane and Liu 2012)

= (3.312+3.505) *0.25*0.38*21.22/ (2*35.55))) = 0.193 N

Percent of Ft= Ff*100/Ft= 0.193*100/3.505=5.515 %

μ_p= tan ϕ_p'= (Tp- μ_{cs}*(upper box Weight+Ff))/(Ft-Ff)… (eq. 6a, Lehane and Liu 2012)

= (10.59-1.453*(2.3+0.193))/ (3.505-0.193) =2.1038

ϕ_p'=$\tan^{-1}$(2.1038) =64.576 ^{o}

μ_{cs} = tanϕ_{cs}= (Tcs- μ_{cs}*(upper box weight +Ff))/(Ft-Ff)… (eq. 6b, Lehane and Liu 2012)

=8.4335-1.453*(2.3+0.193)/ (3.505-0.193) =1.453

ϕ_{cs}'=$\tan^{-1}$(1.453) =55.456 ^{o}

Reduction Factor (RF) for only μ_{peak} for case A:

RF = (Ft + Weight of upper box) / Ft …… (eq. 7, Lehane and Liu 2012)

= (3.505+2.3)/3.505= 1.6561

Corrected μ_{peak}= μ_{peak}/RF=1.82445/1.6561=1.10165

ϕ_p'=$\tan^{-1}$(1.10165) = 47.77 ^{o}

Reduction Factor for critical state (μ_{cs}) for both cases (A&B) with gap and sand particles present between box parts:

RF= (Ft+ upper box weight)/Ft…….. (eq. 7, Lehane and Liu 2012)

= (3.505+2.3)/3.505=1.656

Corrected μ_{cs}= μ_{cs}/RF=1.4526/1.656=0.877

ϕ_{cs}'=$\tan^{-1}$(0.877) =41.25 ^{o}

Reduction Factor for (μ_{peak}) for sand particles present between boxes case B:

r= μ_{cs}/ μ_p=1.4526/2.10434=0.6903

RF=(r*upper box weight+ (0.9+0.1*r)*Ft)/Ft…….... (eq. 9b, Lehane and Liu 2012)

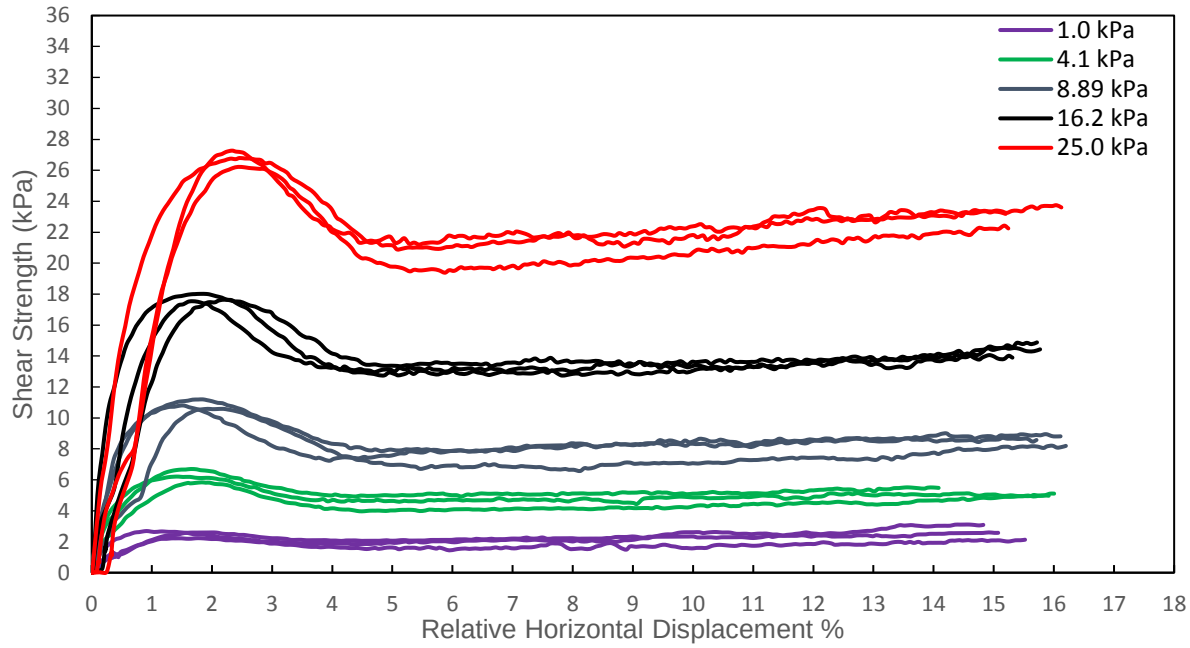
= (0.6903*2.3+ (0.9+0.1*0.6903)*3.505)/3.505=1.422

Corrected μ_p=2.1038/1.422=1.479

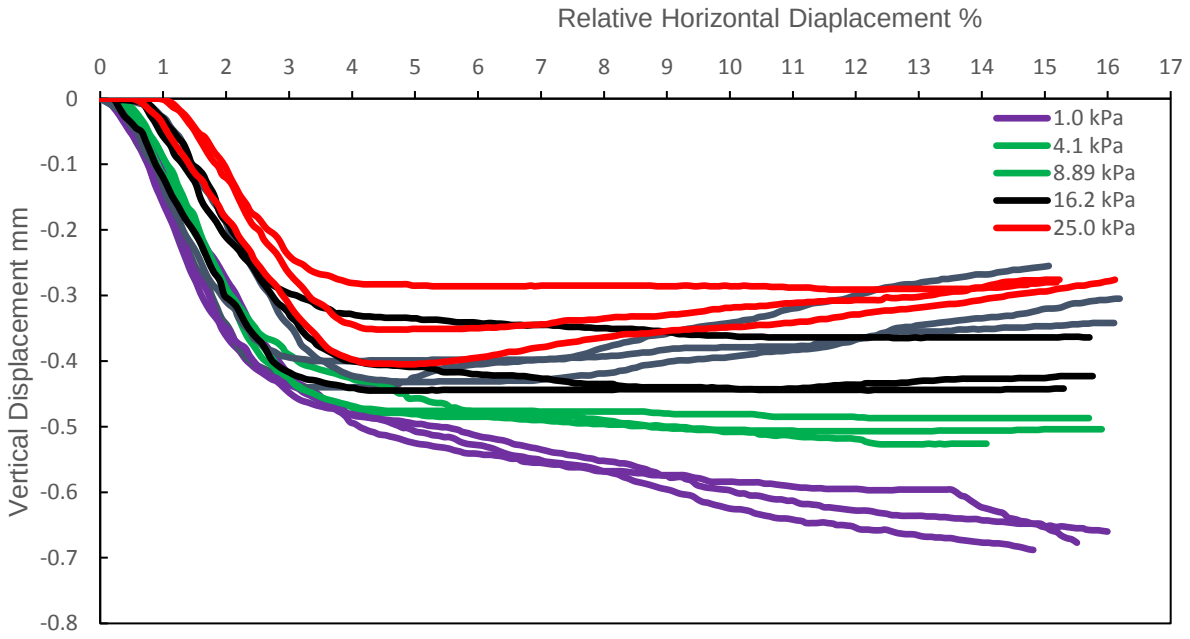
ϕ_p'=$\tan^{-1}$(1.479)=55.94 ^{o}

APPENDIX B: Typical shear results

A) Triplicate preloading samples of clean dry sand:



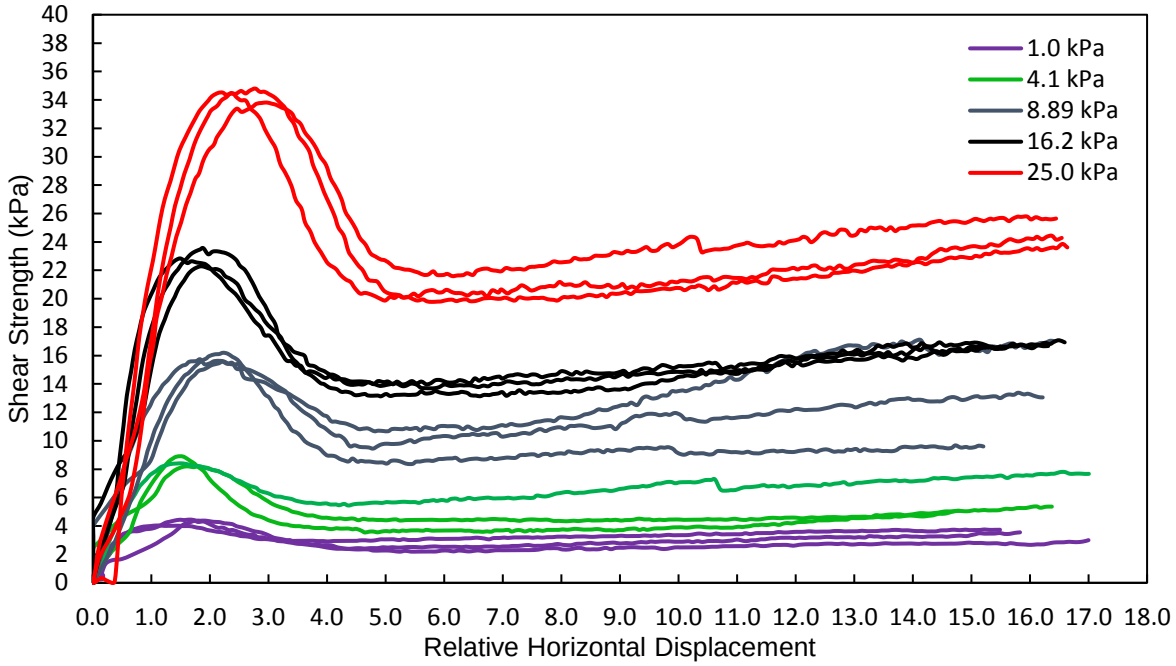
(a) Shear stress



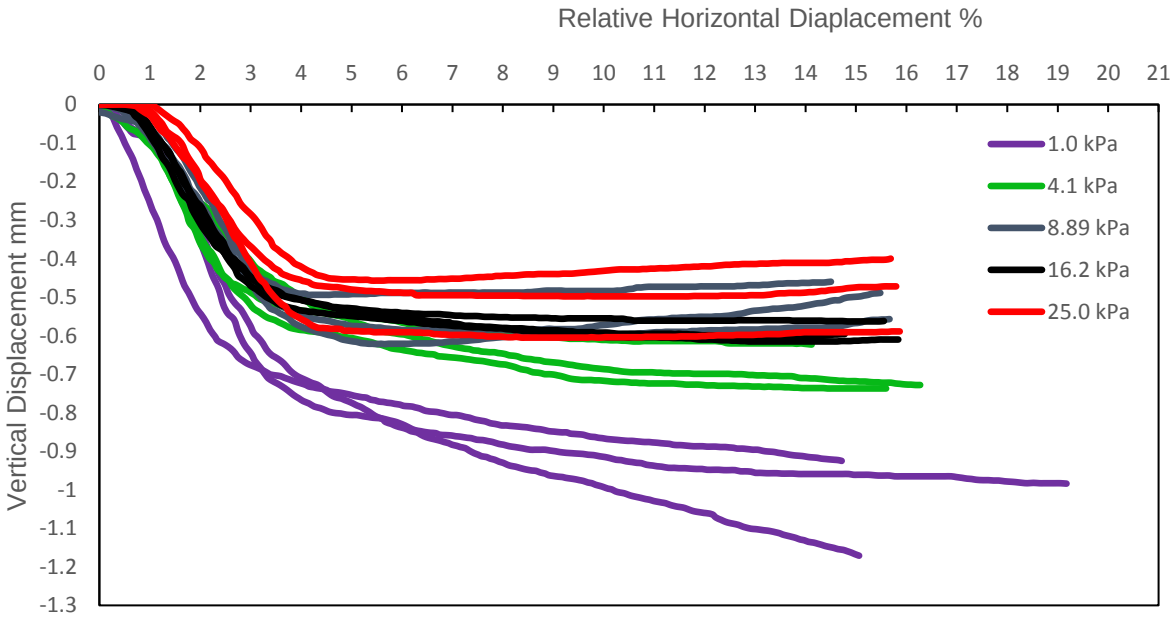
(b) Vertical displacement

Figure A. Shear stress (a) and Vertical displacement (b) versus Relative displacement for various vertical loading and rate test 0.5 mm/min.

B) Triplicate preloading samples of clean sand saturated:



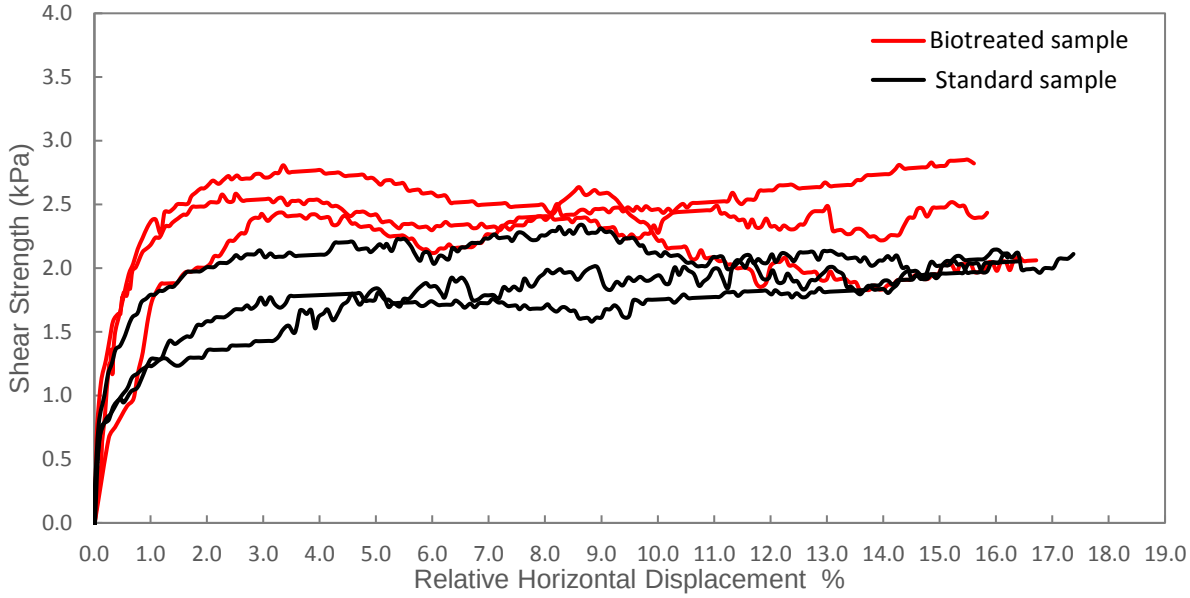
(a) Shear stress



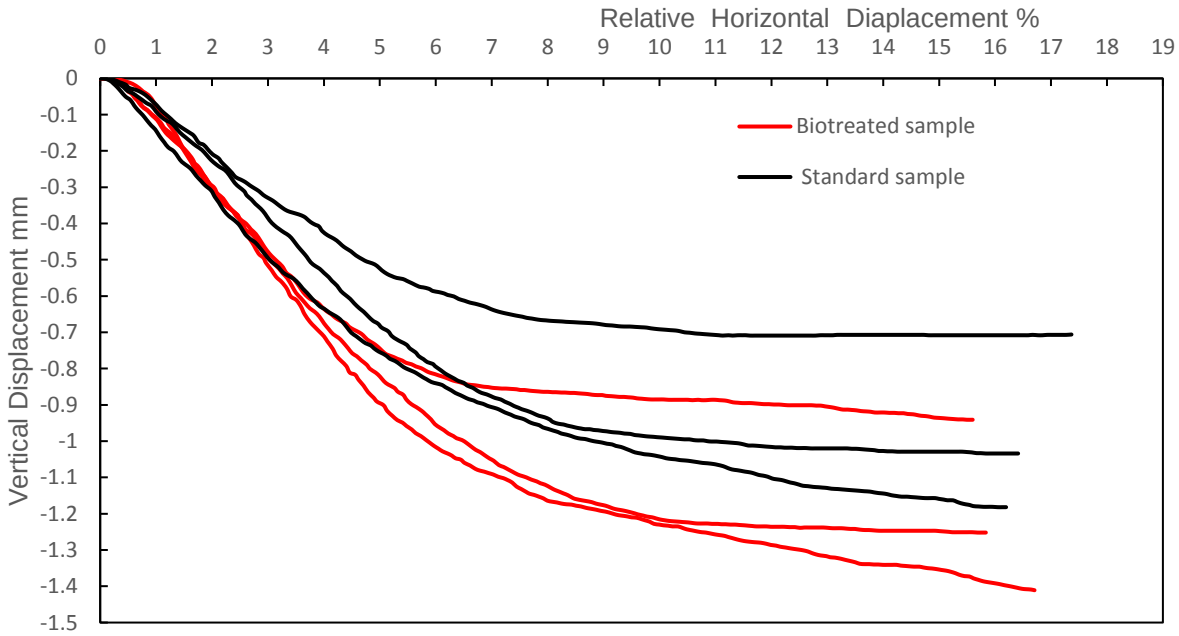
b) Vertical displacement

Figure B. Shear stress (a) and Vertical displacement (b) versus Relative displacement for various vertical loading and rate test 0.5 mm/min.

C) Poorly graded sand normal load 1.0 kPa, 0.5 mm/min



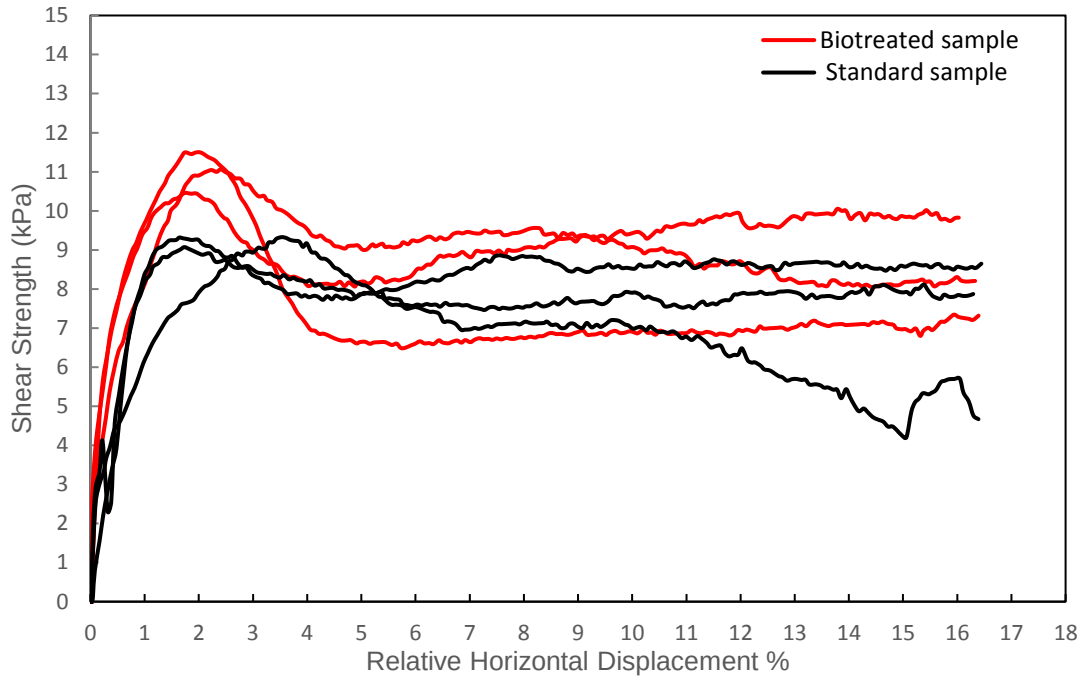
(a) Shear stress



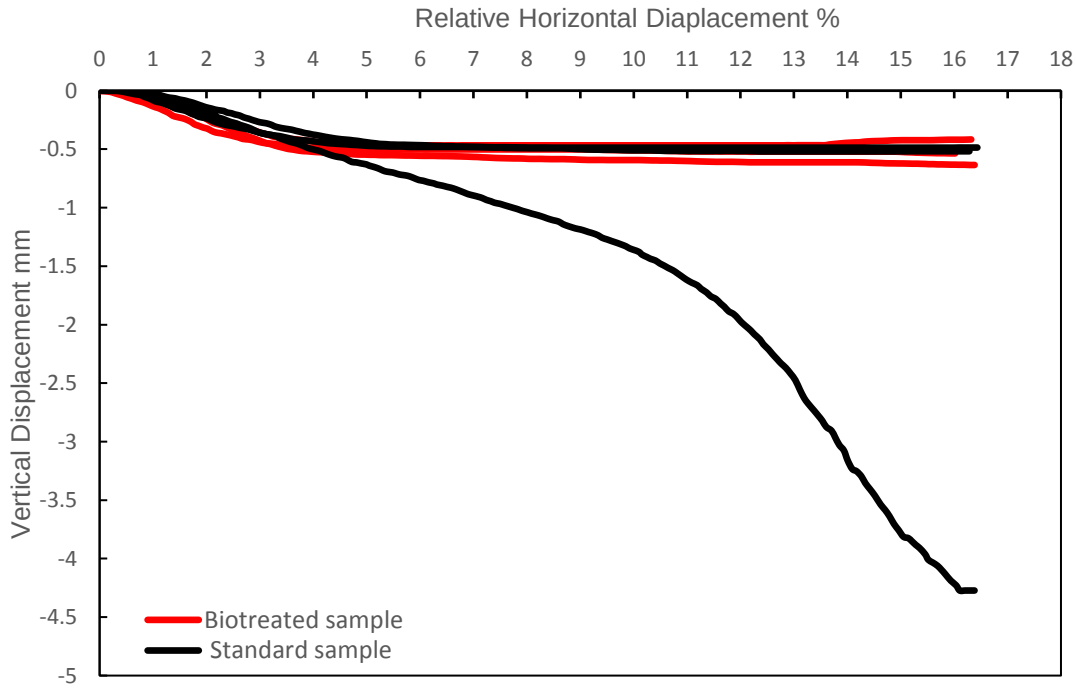
(b) Vertical displacement

Figure C. Shear stress (a) and Vertical displacement (b) versus Relative displacement for a vertical loading 1.0 kPa and rate test 0.5 mm/min.

D) Sea sand biotreated test 8.89 kPa and 0.5 mm/min



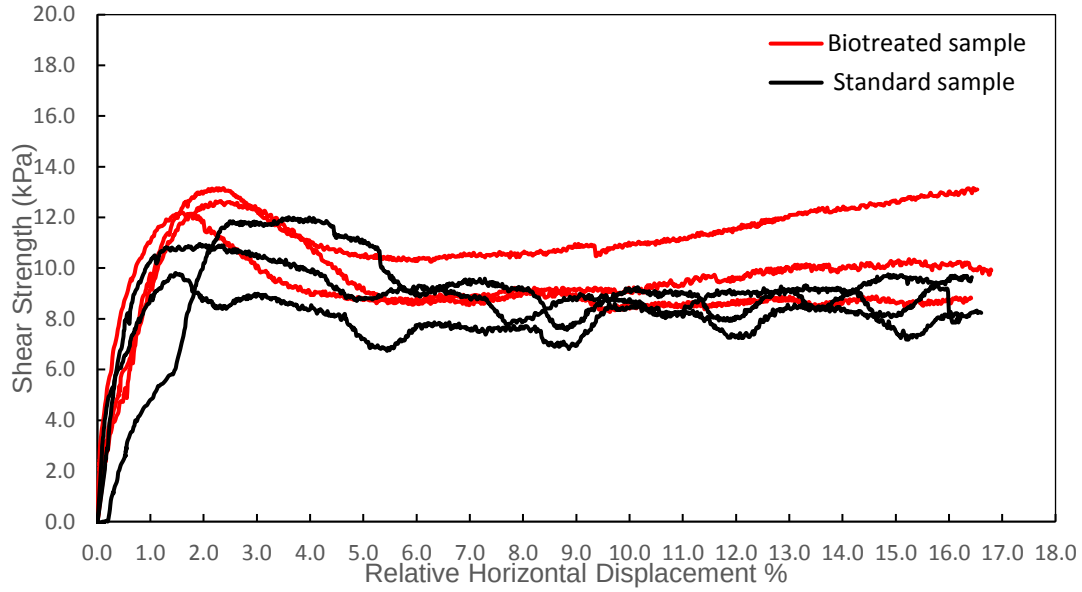
(a) Shear stress



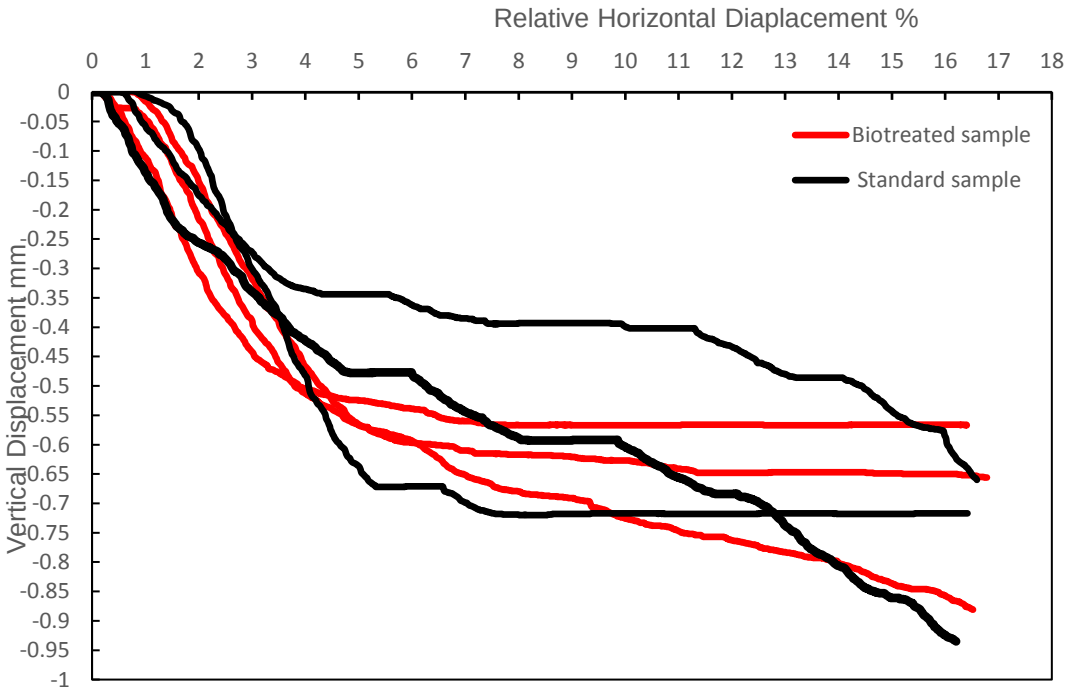
(b) Vertical displacement

Figure D. Shear stress (a) and Vertical displacement (b) versus Relative displacement for a vertical loading 8.89 kPa and rate test 0.5 mm/min.

E) Silica well graded sand biotreated test 8.89 kPa and testing rate 0.1 mm/min:



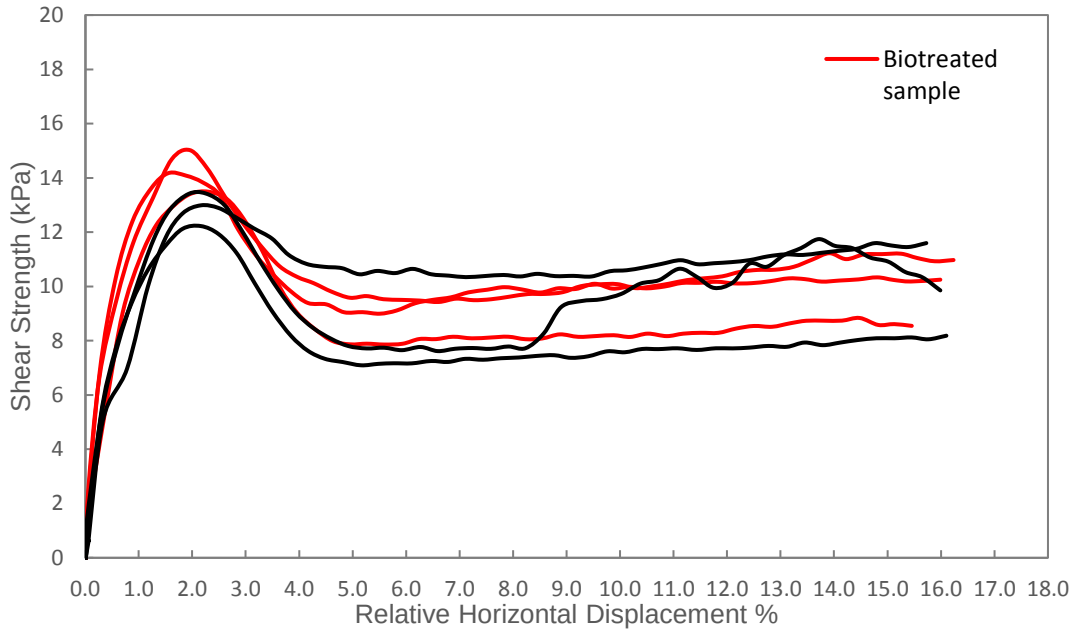
(b) Shear stress



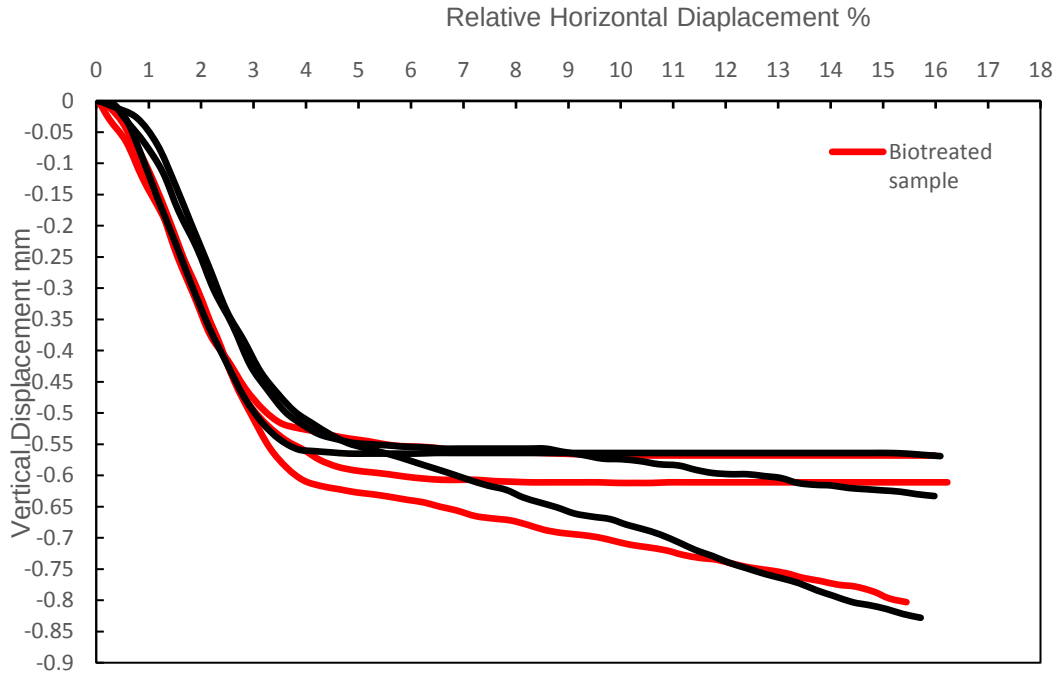
(a) Vertical displacement

Figure E. Shear stress (a) and Vertical displacement (b) versus Relative displacement for a vertical loading of 8.89 kPa and rate test 0.1 mm/min.

F) Silica well graded sand biotreated test 8.89 kPa and testing rate 2.0 mm/min:



(a) Shear stress

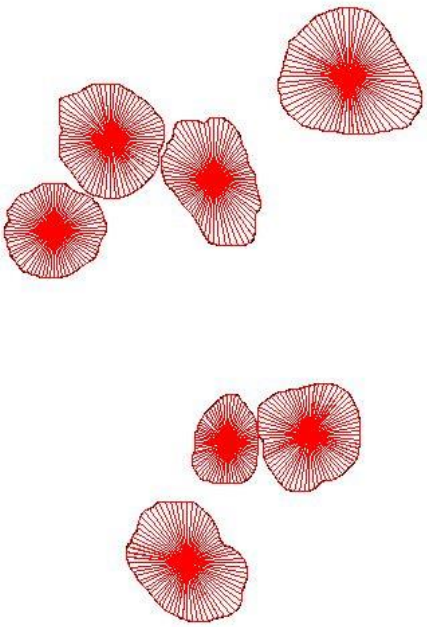


(b) Vertical displacement

Figure F. Shear stress (a) and Vertical displacement (b) versus Relative displacement for a vertical loading of 8.89 kPa and rate test 2.0 mm/min.

APPENDIX C: Image processing for grain shape

Silica sand :



particle	quantify particle angularity	Spericity	Shape factor	circulity	round	solidity
1	1104.8	0.95	0.90	0.89	0.89	0.99
2	1174.2	0.95	0.95	0.87	0.93	0.97
3	1354.0	0.77	0.70	0.76	0.61	0.95
4	1116.4	0.93	0.91	0.87	0.87	0.98
5	983.0	0.97	0.96	0.89	0.95	0.98
6	727.5	0.86	0.79	0.85	0.73	0.98
7	941.6	0.87	0.78	0.81	0.73	0.97

For the first particle:

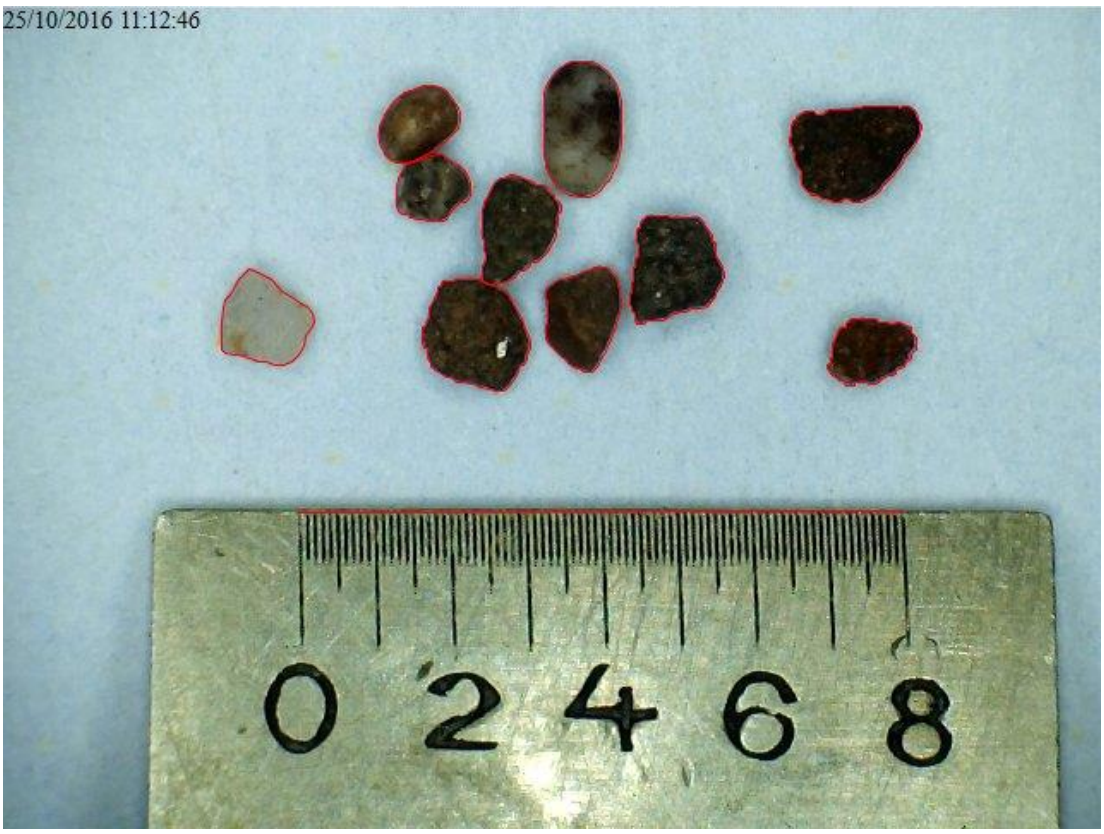
The coordinates from AutoCad particle-1

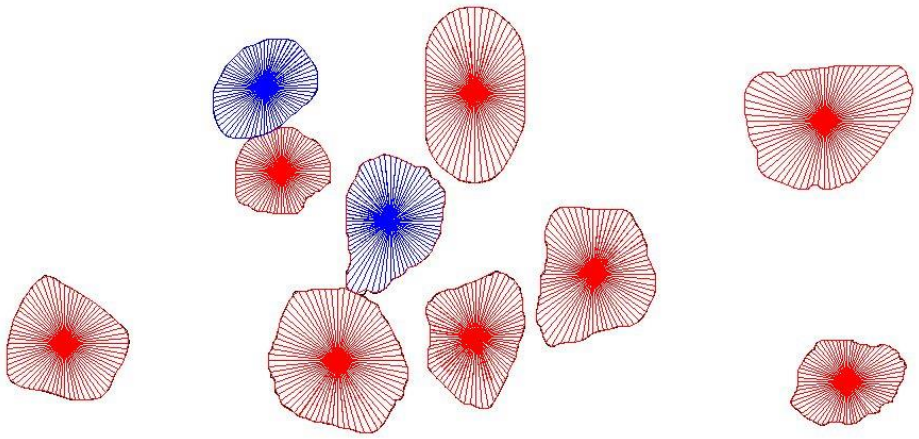
points	x	y	gradient x (Gx)	gradient y (Gy)	θ	
1	6.52	1.84				
2	6.51	1.88	-0.01	0.04	-75.9638	
3	6.49	1.92	-0.02	0.04	-63.435	12.52881829
4	6.47	1.96	-0.02	0.04	-63.435	1.02318E-12
5	6.46	2.08	-0.01	0.12	-85.2364	21.8014279
6	6.44	2.12	-0.02	0.04	-63.435	21.8014279
7	6.42	2.16	-0.02	0.04	-63.435	1.02318E-12
8	6.39	2.19	-0.03	0.03	-45	18.43496439
9	6.36	2.22	-0.03	0.03	-45	1.26477E-12
10	6.33	2.25	-0.03	0.03	-45	1.26477E-12
11	6.29	2.27	-0.04	0.02	-26.5651	18.43496439
12	6.26	2.3	-0.03	0.03	-45	18.43496439
13	6.22	2.32	-0.04	0.02	-26.5651	18.43496439
14	6.18	2.34	-0.04	0.02	-26.5651	0
15	6.13	2.35	-0.05	0.01	-11.3099	15.25513159
16	6.09	2.36	-0.04	0.01	-14.0363	2.726313297
17	6.05	2.36	-0.04	0	0	14.03625532
18	6	2.36	-0.05	0	0	0
19	5.96	2.34	-0.04	-0.02	26.56507	26.56507362
20	5.91	2.34	-0.05	0	0	26.56507362
21	5.87	2.32	-0.04	-0.02	26.56507	26.56507362
22	5.83	2.3	-0.04	-0.02	26.56507	0
23	5.8	2.26	-0.03	-0.04	53.13015	26.56507362
24	5.78	2.23	-0.02	-0.03	56.30998	3.179832806
25	5.75	2.19	-0.03	-0.04	53.13015	3.179832806
26	5.73	2.16	-0.02	-0.03	56.30998	3.179832806
27	5.71	2.13	-0.02	-0.03	56.30998	7.88702E-13

28	5.68	2.1	-0.03	-0.03	45.00004	11.30994203
29	5.65	2.07	-0.03	-0.03	45.00004	1.26477E-12
30	5.63	2.04	-0.02	-0.03	56.30998	11.30994203
31	5.61	2	-0.02	-0.04	63.435	7.125022367
32	5.6	1.96	-0.01	-0.04	75.96382	12.52881829
33	5.62	1.77	0.02	-0.19	-83.9911	159.9548857
34	5.6	1.68	-0.02	-0.09	77.47126	161.4623227
35	5.61	1.48	0.01	-0.2	-87.1377	164.6089261
36	5.67	1.46	0.06	-0.02	-18.435	68.70270398
37	5.71	1.45	0.04	-0.01	-14.0363	4.39870907
38	5.76	1.43	0.05	-0.02	-21.8014	7.765172577
39	5.79	1.41	0.03	-0.02	-33.6901	11.88866808
40	5.83	1.39	0.04	-0.02	-26.5651	7.125022367
41	5.87	1.37	0.04	-0.02	-26.5651	2.59348E-13
42	5.91	1.36	0.04	-0.01	-14.0363	12.52881829
43	5.96	1.35	0.05	-0.01	-11.3099	2.726313297
44	6	1.35	0.04	0	0	11.30994203
45	6.04	1.35	0.04	0	0	0
46	6.09	1.35	0.05	0	0	0
47	6.13	1.36	0.04	0.01	14.03626	14.03625532
48	6.17	1.39	0.04	0.03	36.86993	22.83367346
49	6.2	1.4	0.03	0.01	18.43496	18.43496439
50	6.24	1.42	0.04	0.02	26.56507	8.130109221
51	6.27	1.45	0.03	0.03	45.00004	18.43496439
52	6.31	1.47	0.04	0.02	26.56507	18.43496439
53	6.34	1.49	0.03	0.02	33.6901	7.125022367
54	6.37	1.52	0.03	0.03	45.00004	11.30994203
55	6.4	1.55	0.03	0.03	45.00004	0
56	6.42	1.58	0.02	0.03	56.30998	11.30994203
57	6.45	1.61	0.03	0.03	45.00004	11.30994203
58	6.48	1.64	0.03	0.03	45.00004	2.20268E-13
59	6.51	1.68	0.03	0.04	53.13015	8.130109221
60	6.52	1.72	0.01	0.04	75.96382	22.83367346
					angularity index(a)	1104.757796

Dl	1.025
Ds	0.914
Di	1
Spericity	0.954625
Shape factor	0.902785
circulity	0.886
round	0.891
solidity	0.985

For sea sand :

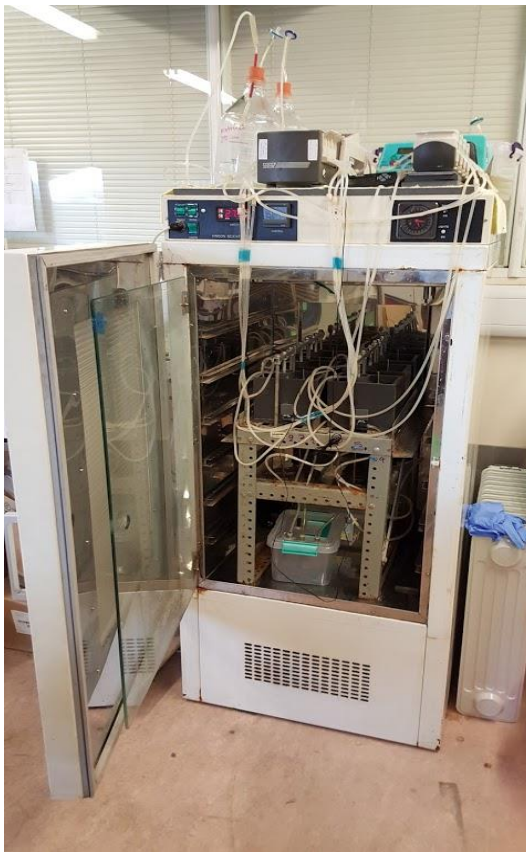




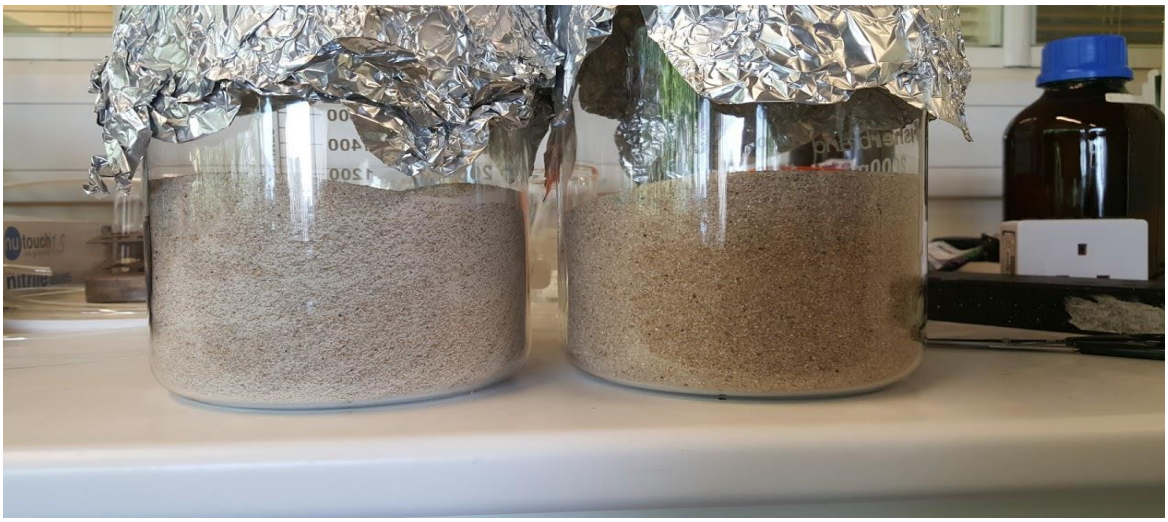
particle	quantify particle angularity	Spericity	Shape factor	circulity	round	solidity
1	810.5	0.94	0.93	0.92	0.90	1.00
2	1243.1	0.84	0.79	0.94	0.72	1.00
3	1247.1	0.93	0.88	0.90	0.85	0.98
4	1482.3	0.77	0.66	0.89	0.58	1.00
5	939.0	0.84	0.75	0.77	0.70	0.97
6	838.7	0.93	0.90	0.84	0.87	0.98
7	600.6	0.84	0.80	0.84	0.73	0.96
8	1388.2	0.89	0.89	0.81	0.83	0.96
9	1096.4	0.84	0.78	0.80	0.72	0.98
10	1274.6	0.85	0.78	0.73	0.73	0.94

APPENDIX D: Selected photos from the work

a) Sand Columns biological experiment



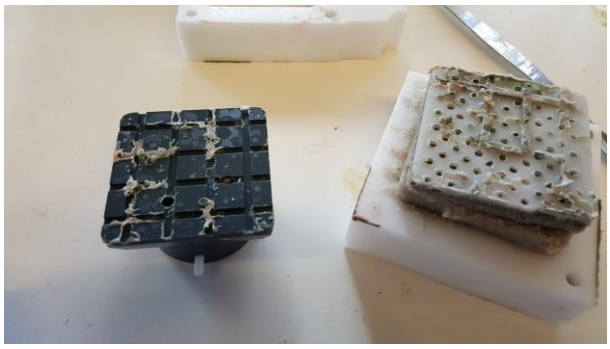
b) Shear box biological experiment



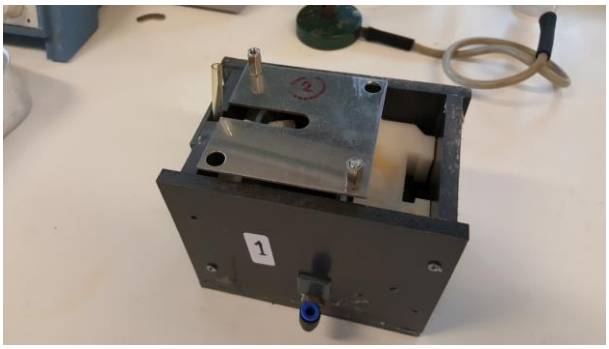
c) Silica sand, well graded sand (left), poorly graded sand (right)

d) Dried biotreated samples (left) and dried standard samples (right)

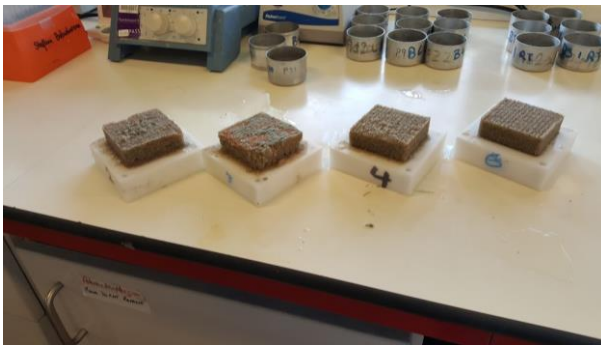
e) Preparation of biotreated samples



f) Biotreated sample after shearing test

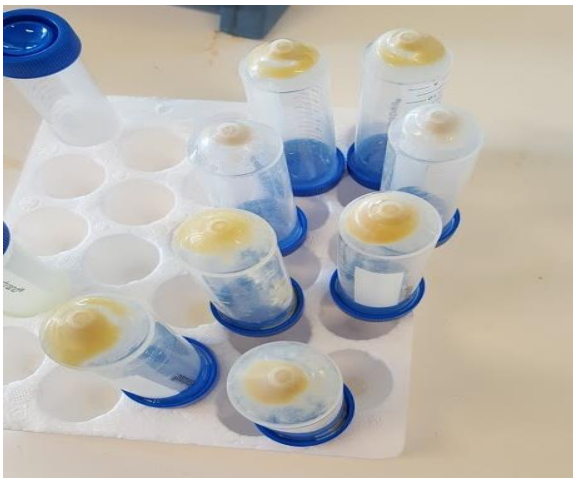


g) The main box, shear box and luck plate

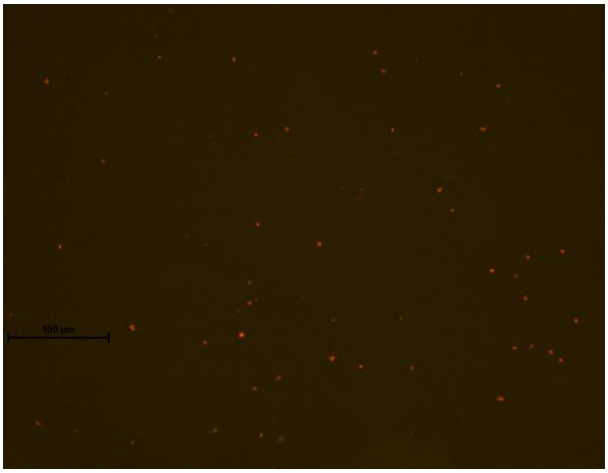


h) Disassembling the shear box after testing.

i) Bacterial solution with different initial pH.



j) Bacterial cells after centrifuging



k) Counting bacteria by CTC method under Nikon ECLIPSE LV100 microscope

m) Direct shear equipment using loading frame

l) Direct shear equipment using weight blocks