IMPROVING DEFORMATION CHARACTERISTICS OF SAND SOILS USING BIO-IMPROVEMENT METHODS

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ABSTRACT

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Utilizing biological processes is new and innovative method which can be used to improve engineering properties of soils in an environmentally friendly way. In this study, a recently introduced bio-improvement method named microbially induced calcite precipitation (MICP) is used to improve the strength and deformation characteristics of soft soils. For that purpose, ureolytic bacteria were utilized to improve the strength properties of soils. Ureolytic bacteria can induce enzymatic hydrolysis of urea, which results in production of free ammonium (NH_4^+) and bicarbonate (HCO_3^-) ions. In case of existence of free calcium (Ca^{2+}) ions in the environment, calcite ($CaCO_3$) precipitation may occur as the result of a reaction between HCO_3^- and Ca^{2+} ions. This reaction results in cementation of soil grains, which results in the improvement of engineering properties of soil. Within the scope of the study, both MICP's applicability and its effect on strength properties of soil were investigated by performing direct shear tests. More specifically, improvement efficiencies of *Sporosarcina pasteurii* (ATCC 11859), a widely studied bacterium, and *Bacillus licheniformis* (ATCC 14580), a relatively new introduced bacterium, were examined with samples prepared at different relative densities and with different number of injections. The improvement in the strength values are also supported with

the help of sophisticated imaging tools such as Scanning Electron Microscopy (SEM) and Energy Dispersive X-ray Spectroscopy (EDS) techniques.

Keywords: Microbially Induced Calcium Carbonate Precipitation, Direct Shear Test, Sporosarcina pasteurii, Bacillus licheniformis

ÖZ

ZEMİNLERİN DEFORMASYON ÖZELLİKLERİNİN BİYO-İYİLEŞTİRME METODLARI KULLANILARAK İYİLEŞTİRİLMESİ

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Zeminlerin mühendislik özelliklerinin iyileştirilmesinde çevre dostu bir yol olan biyolojik süreçlerden faydalanmak yeni ve yenilikçi bir yöntemdir. Bu çalışmada, "Mikrobiyal Tabanlı Kalsiyum Karbonat Çökelmesi" olarak adlandırılan ve bakteri kullanımıyla zemin iyileştirilen yöntemler, yumuşak zeminlerin dayanım ve deformasyon özelliklerinin iyileştirilmesi için kullanılmıştır. Bu tez kapsamında, zeminlerin dayanma gücü özelliklerinin iyileştirilmesi için üreolitik bakterilerden yararlanılmıştır. Üreolitik bakteriler, ürenin enzimatik hidrolizini teşvik ederek serbest amonyum (NH_4^+) ve bikarbonat (HCO_3^-) iyonlarının oluşumuna sebep olabilir. Bunun sonucunda ortamda serbest kalsiyum (Ca^{2+}) iyonlarının bulunması durumunda HCO_3^- ve Ca^{2+} iyonlarının reaksiyonu sonucunda kalsit ($CaCO_3$) çökelmesi gerçekleşebilir. Bu reaksiyon zemin taneleri arasında çimentolaşmaya sebep olarak zeminin mühendislik özelliklerinin iyileşmesini sağlamaktadır. Bu tez kapsamında mikrobiyolojik tabanlı kalsiyum karbonat çökelmesinin bir zemin iyileştirme yöntemi olarak uygulanabilirliği ve zeminin dayanma gücü özelliklerine etkisi direkt kesme deneyleri vasıtasıyla araştırılmıştır. Çalışmada daha önce çokça araştırılmış olan *Sporosarcina pasteurii* (ATCC 11859) ve literatürde kıyasla daha yeni olan *Bacillus licheniformis* (ATCC 14580) bakterilerinin farklı zemin sıkılık ve besleme sayıları altındaki iyileştirme verimlilikleri karşılaştırılmıştır. Uygulama görmüş zeminlerin

mukavemetinde görülen iyileşmeler, Taramalı Elektron Mikroskobu ve X-Işını Spektroskopisi teknikleri kullanılarak desteklenmiştir.

Anahtar Kelimeler: Mikrobiyolojik Tabanlı Kalsiyum Karbonat Çökelmesi, Direkt Kesme Deneyi, Sporosarcina pasteurii, Bacillus licheniformis

To my family…

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ABBREVIATIONS

ATCC	American Type Culture Collection
EDS	Energy Dispersive X-ray Spectroscopy
MICP	Microbially Induced Calcium Carbonate Precipitation
OD_{600}	Optical Density at 600 nm wavelength
SEM	Scanning Electron Microscopy

CHAPTER 1

INTRODUCTION

1.1. Overview

The requirement of altering engineering properties of soils to provide proper support for construction projects emerged various ground improvement methods ranging from mechanical, hydraulic, physical and chemical modifications to the ones through the use of inclusions and confinements. Among those, conventional ones include soil densification through vibration, compaction by preloading, dewatering, partial removal or replacement of insufficient soil, and the use of chemical additives such as cement, lime, etc. Although some of these improvement methods are nearly well-established, the environmental concerns over the chemical additives due to their toxic and hazardous drawbacks resulted in the ban of nearly all chemical grouts in some countries (DeJong et al., 2010).

Recently, the increased awareness of the public regarding the use of environmentally-friendly solutions instead of conventional ones have motivated the researchers to find new materials and methods. One of the promising alternatives was the use of nature's own agents. It is estimated that the microorganisms are active over 3 billion years and they are found to be involved in a diversity of processes, which also carries some particular geotechnical importance (Kohnhauser, 2007; DeJong et al., 2013). For example, in fine-grained soils, microfossils may form a significant portion of the soil and unexpected geotechnical properties may be obtained through their involvement. The reaction accelerating effect of the microorganisms considered to have a role in the swelling of soils containing pyrite. In addition to these, bacteria can accelerate calcite formation through specialized enzymes. Considering these observations, researchers

suggested that by intensifying the biological activity in a target zone, it is possible to strengthen the soil through binding the grains or increasing grain's surface roughness.

The promising properties of biological agents paved the way for new applications in different fields. The microorganisms have been utilized in different engineering applications such as strength improvement of ash bricks (Dhami et al., 2012), surface treatment of concrete to obtain more durable construction material (De Muynck et al., 2008), recovery of oil from oil fields by selective plugging (Ferris et al., 1996), wastewater treatment (Hammes et al., 2003), compressive strength improvement of concrete (Ramachandran et al., 2001).

The comparison between the chemical reactions and the ones involving microorganisms showed that microorganisms can accelerate the geochemical reactions by orders of magnitude (Stocks-Fischer et al., 1999). This study and a later study by Mitchell and Santamarina (2005), reviewing the importance of the biological processes, have been the pioneering works in the field of geotechnics. This promising process, later named as Microbially Induced Calcium Carbonate Precipitation (MICP), then attracted the interest of the researchers and the use of biological agents was considered to be a possible solution of many geotechnical problems such as settlement reduction (DeJong et al., 2010), high permeability (Al Qabany and Soga, 2013), liquefaction (Burbank et al., 2012), soil internal erosion (Jiang et al., 2017) etc.

As the laboratory scale experiments to outline the engineering properties of improved soil continue, the practical adaptation of the MICP to the field still remains as a challenging topic. The studies **(**van Paassen et al., 2010; van Paassen, 2011; Filet et al., 2012**)** dealt with enlargement of the applied volume encountered various problems. In addition, the importance of monitoring the progress brought the solution using non-destructive monitoring techniques. Unsurprisingly, these advancements also used laboratory-scale experiments to evaluate the improvement in the field.

To be able to design a generalized MICP based ground improvement method, a deep understanding of the background (i.e. biological processes and factors affecting the

processes) is required. All components of the biology based improvement mechanism including the soil, biological agent (bacteria, enzyme etc.), medium, injection characteristics (repetition number, application pressure etc.), temperature, level of pH etc. need to be investigated to obtain an efficient MICP process. To date, most of the studies in the literature have dealt with sand and acquire successful results. While Ottawa sand was the mostly examined soil, a few studies dealing with organic soil (Canakci et al., 2015), tropical residual soil (Soon et al., 2013), and gravel (van Paassen et al., 2012) also exist in the literature. A great number of the work has focussed to investigate the variation of the concentration of the components in the medium, flow rate, environmental conditions (Al Qabany et al., 2012; Martinez et al., 2013; Mortensen et al., 2011).

1.2. Research Objective

Within the above MICP based ground improvement framework and considering the problems encountered in the literature so far, this study aims to reach the following achievements;

- Proper evaluation of the performance of microbially treated soil in terms of shear strength parameters.
- Design of a direct shear test procedure for microbially treated soil for the above purpose,
- Comparison of the calcium carbonate precipitation ability of two strains of *Bacillus*, namely *Sporosarcina pasteurii* and *Bacillus licheniformis*, under different relative densities and treatment durations.

1.3. Scope and Method

Bio-geochemical processes involved in MICP is affected by a vast number of factors such as soil conditions (e.g. gradation, density, saturation degree etc.), temperature, the pH level etc. In addition, the knowledge from different fields including Biology, Chemistry and Civil Engineering is required. Therefore, some factors especially those

ones important from Geotechnical Engineering perspective were selected as the main focus of this study. In this sense, to investigate the direct shear strength improvement and the behavior of the microbially treated sand, two strains of *Bacillus*, namely *Sporosarcina pasteurii* (*S. pasteurii*) and *Bacillus licheniformis* (*B. licheniformis*), were utilized. The efficiency of the process is highly dependent on the geometric (grain size distribution, surface roughness etc.) and chemical (pH, organic content etc.) properties of the soil. In this study, poorly graded silica sand was used. Therefore, the obtained results could be only valid for this soil. Temperature is one of the most important environmental factors affecting the process. Within the concept of the study, the effect of temperature was not investigated, the experiments were performed under relatively constant temperature. Furthermore, to support the findings of soil testing, investigations in the microscale using Scanning Electron Microscopy and X-Ray Diffraction were performed.

1.4. Thesis Outline

The rest of the thesis will follow an outline as follows:

- Chapter 2 presents an overview of the literature related to MICP. The primary focuses of the chapter include the theoretical background of the process, treatment methods and their efficiency, results of laboratory and field tests, mathematical modeling of the MICP process.
- Chapter 3 gives details regarding the materials and methods used including the sample preparation scheme, the followed direct shear test procedure, the way of microscale investigations on specimens treated with and without bio-catalysis.
- Chapter 4 presents the results of direct shear tests applied on sand specimens treated solely with the chemical solution and included microorganisms having active urease enzyme.

Chapter 5 summarizes the study, highlights some challenges related to the laboratory experiments, provides the conclusions the thesis.

CHAPTER 2

LITERATURE REVIEW

The increasing demand for ample living spaces from society brings along the requirement for rehabilitation and construction of civil infrastructures. However, increasing construction activity is somehow limited due to inadequate soil conditions. To overcome this problem, geotechnical properties of incompetent soil can be altered by means of ground improvement (Karol, 2003).

Karol (2003) categorized the soil improvement methods by the type of the application as presented in Table 2.1. Some traditional soil improvement methods may be impractical; for example, the resulting settlements during dynamic compaction may damage the buildings around, or the use of cement, silicates and other chemicals in grouting methods may also be harmful to the environment. The disadvantages of existing methods and environmental concerns lead scientists to seek for environmentally friendly solutions, such as, nature's own processes for soil improvement.

Simulating and utilizing natural processes gave researchers the chance of producing environmentally-friendly options for different areas. It is evaluated that microbial organisms that have been utilized in bio-treatment of soils are more than 1.5 million years old and the processes that are controlled by those organisms have been dynamic since that time (DeJong et al., 2010). Considering its possible environmental advantages, utilizing biological processes have received increasing attention in the last decades. The area of microbiology and its derivatives have been applied in different fields of engineering including sand consolidation (Stocks-Fischer, Galinat, and Bang 1999), compressive strength improvement of concrete (Ramachandran et al., 2001), concrete durability improvement (De Muynck et al., 2007), wastewater treatment

(Hammes et al., 2003), selective plugging for enhanced oil recovery (Nemati et al., 2005), soil improvement (Whiffin et al., 2007; DeJong et al., 2010; Chou et al., 2011), etc. In particular, possible geotechnical applications include improvement of resistance against seismically induced liquefaction (Burbank et al., 2011), building settlement reduction (Martinez and DeJong, 2009), piping prevention (Jiang et al., 2017), soil stabilization preceding tunneling construction (van Paassen et al., 2012), and slope stabilization (DeJong et al., 2010).

Table 2.1. *An overview of soil improvement methods (Karol, 2003)*

Type	**Method**
VIBROCOMPACTION	Blasting
	Terra-probe
	Vibratory rollers
	Dynamic compaction or heavy tamping
	Vibro-flotation
	Hydro-compaction
COMPACTION PILES	Compaction piles
	Sand compaction piles
PRECOMPRESSION	Preloading
	Surcharge fills
	Electroosmosis
REINFORCEMENT	Mix-in-place piles and walls
	Strips and membranes
	Vibro-replacement stone
	Vibro-displacement stone
GROUTING AND INJECTION	Particulate grouting
	Chemical grouting
	Pressure injected lime and lime-fly ash
	Displacement or compaction grout

Table 2.1 (continued) *An overview of soil improvement methods (Karol, 2003)*

	Jet grouting
	Electrokinetic injection
MISCELLANEOUS	Remove and replace
	Moisture barriers
	Prewetting
	Structural fills

2.1. Microbially Induced Calcium Carbonate Precipitation

In the literature, many bio-mediated soil improvement techniques such as Microbially Induced Calcite Precipitation (MICP) (DeJong et al., 2006; van Paassen, 2009; Martinez et al., 2015), Enzyme Induced Carbonate Precipitation (Neupane et al., 2013), Plant-Induced Calcite Precipitation (Park et al., 2014) etc. have been studied. Among these techniques, MICP has been the subject of many studies for the last decade as interest in bio-mediated soil improvement is rising. There are many different microbial metabolic pathways that MICP may be achieved (DeJong et al., 2010) including urea hydrolysis (Stocks-Fischer et al., 1999; DeJong et al., 2010), denitrification (van Paassen et al., 2010; van der Star et al., 2009; O'Donnell et al., 2017), sulfate reduction (Peckmann et al., 1999), and iron reduction (Ivanov et al., 2010). Researchers stated that among these processes enzymatic urea hydrolysis seems to be the most advantageous one since it is the most energy-efficient one and the other mechanisms are slower at the rate of creating the conditions favorable to precipitation (DeJong et al., 2010; De Muynck et al., 2010). However, DeJong et al. (2010) also noted that there may be secondary benefits of different paths. For example, production of gas as a result of denitrification may lead decrease of saturation degree, hence a decrease of liquefaction potential.

MICP based on enzymatic hydrolysis of urea is one of the most common pathways used to improve engineering properties of soil. Jiang et al. (2017) outlined the chemical reaction network as follows:

- enzyme urease is synthesized through metabolic activities of bacteria
- urease catalyzes ureolytic reactions which end up with the decomposition of urea into ammonia (NH_3) and dissolved inorganic carbon (Eq. 1)

$$(NH_2)_2CO + H_2O \rightarrow 2NH_3 + CO_2 \quad (1)$$

- alkalinity increases around the bacteria as a result of Eqs. 2 and 3.

$$2NH_3 + 2H_2O \leftrightarrow 2NH_4^+ + 2OH^- \quad (2)$$

$$CO_2 + 2OH^- \leftrightarrow HCO_3^- + OH^- \leftrightarrow CO_3^{2-} + H_2O \quad (3)$$

- calcium carbonate precipitates on nucleation sites, i.e. bacterial cell surfaces, in the presence of calcium.

$$Ca^{2+} + CO_3^{2-} \leftrightarrow CaCO_3(s) \quad (4)$$

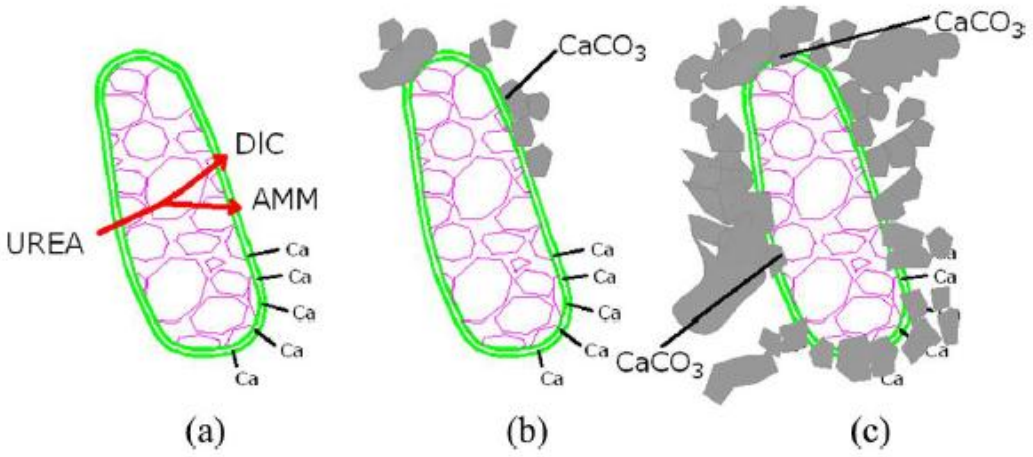


Figure 2.1. Calcium carbonate accumulation on the bacteria (Wang et al., 2017)

Having too many intermediate steps until calcium carbonate precipitation achieved is not desirable since the accumulation of intermediate products may lower the efficiency of the process. For example, in the denitrification process, four different enzymes are

involved. These enzymes have different localization, lifetime, regulatory mechanism, kinetics, and sensitivity to inhibitory factors. If the activity of any of these enzymes is inhibited for any reason such as occurrence/accumulation of inhibitive intermediates, the denitrification process may not be completed and intermediate products, such as nitrite and nitrous oxide, that are harmful to the environment may accumulate (van Paassen et al., 2010).

Stocks-Fischer et al. (1999) compared the microbiologically induced and chemically induced $CaCO_3$ precipitation and found that more calcium was precipitated in the presence of microorganisms. There are two mechanisms that bacteria involve in MICP (Stocks-Fischer et al., 1999; DeJong et al., 2006). First, bacterial cells provide nucleation sites as cations attach negatively charged bacterial cell surface. Then, the alkalinity of the environment around the microorganism increases due to microbial activity and this condition promotes calcium carbonate precipitation.

Utilizing bacterial activity gives us a chance to control the timing, rate, and spatial distribution of the chemical reaction that results in byproducts which improve the soil properties hence controlled the manipulation of soil properties can be possible (DeJong et al., 2009). On top of that, while sufficient strength improvement is achieved, permeability reduction may be limited since pore space is not completely filled with calcium carbonate. The slightly reduced permeability gives the opportunity to further applications.

Hammes and Verstraete (2002) stated that there are four main factors that influence the MICP process: (1) concentration of calcium ion, (2) concentration of dissolved inorganic carbon, (3) the pH, (4) availability of nucleation sites. In addition, there are several environmental factors that affect the performance of carbonate precipitation in soils such as compatibility of soil and bacteria, temperature, salinity, oxygen availability etc. (Al Qabany et al., 2013; Whiffin, 2004; Rebata-Landa, 2007; Mortensen et al., 2011).

Researchers also calculated the cost of bio-treatment. Some researchers compared the cost of bio-treatment with other mostly used techniques and others tried to find

alternatives to reduce the cost of the process. For example, Suer et al (2009) compared the cost of the biogrouting and jet grouting. The authors concluded that although production of urea and $CaCl_2$ required much energy, biogrouting was found to be cheaper than jet grouting with lower environmental impact (less water usage and less waste production). Ivanov et al. (2010) compared iron-based and calcium-based MICP in terms of permeability reduction. The results showed that iron-based precipitation has an economical advantage, however, calcium-based MICP showed more effective results in reducing permeability. Cuzman et al (2015) searched alternative growth media to find an economical solution for future big scale applications. The study showed that some of the tested dairy wastes were good alternatives for nutrient solutions of bacterial growth and urea fertilizer was a good alternative for pure urea.
The long-term relationship between precipitated calcite and environment is critical. The existence of calcite shows the environment has stable conditions for calcium carbonate precipitation and it can be interpreted that the newly precipitated calcite would be durable. The precipitated calcium carbonate is durable and dissolves very slowly unless the environment shows acidic characteristic due to flushes by acidic groundwater or acidifying process in the pores (e. g. degradation of biomass; van Paassen et al., 2010). Hata et al. (2011) observed that at high pH values calcite can maintain a crystalline structure but at low values the solubility of calcite increases. Authors pointed out that pH value of 5.5 as the threshold since calcite dissolves considerably when pH values are less than 5.5.

2.1.1. Bacteria Types and Application Methodology

To date, the researchers have studied the calcite precipitation performance of different bacteria. As discussed previously, environmental conditions have crucial effects on bacterial activity, and therefore, it is important to know the behavior of the bacteria in depth. In this section, information about bacteria strains studied in the literature and options for enhancing bacterial population, hence the activity in the soil matrix so that calcium carbonate precipitation can be obtained in a reasonable time is given.

Sporosarcina pasteurii (*S. pasteurii*) is the most frequently studied bacteria in the literature. The behavior of the bacteria under various conditions has been investigated. Mugwar and Harbottle (2016) studied the activity of *S. pasteurii* in the presence of metal ions. The authors explored that when urea hydrolysis process is active, a higher bacterial activity was possible at higher concentrations of metals. In addition, it was found that the concentration which inhibition of bacterial growth occurs was dependent on the metal. Whiffin (2004) evaluated the suitability of many microorganisms with urea activity. Considering biocementation and environmental constraints only two bacteria, *S. pasteurii* and *Proteus vulgaris* were examined in detail. One of the very important conclusions of this work is that when *S. pasteurii* cultured in a non-sterile environment for up to two days, the level of contamination was below 50% (w/v) of the inoculum.

Although *S. pasteurii* has been the most studied bacteria in the literature, the ureolytic bacteria are ubiquitous in soil. For example, *Bacillus licheniformis* (*B. licheniformis)* is a bacterium whom species have been widely used for commercial and agricultural purposes (Rey et al., 2004) and it was reported that ureolytic strains of the bacteria exist (Gaiero, 2014). Vahabi et al. (2015) examined *B. licheniformis* AK01 strain to evaluate its calcite crystal formation capacity. The strain's performance was compared with other bacteria including a strain of *S. pasteurii* (DSM-33) and better results were obtained. Studies (Helmi et al., 2016; Seifan et al., 2016) focused on $CaCO_3$ precipitation efficiency of *B. licheniformis* under various conditions also exist in the literature.

Bacillus sphaericus and *Bacillus megaterium* are some examples of other species appear in the literature. For example, DeMuynck et al. (2010) studied remediation of ornamental stone, Cheng et al. (2013) treated sand columns with various degrees of saturation, and Hataf and Jamali (2018) investigated the effect of fine grain percentage by utilizing *Bacillus sphaericus*. Soon et al. (2013, 2014) successfully treated tropical residual soil with *Bacillus megaterium*.

In the literature, researchers not only studied with bacteria having well-known behavior but also isolated bacteria from natural soil samples that can lead the MICP process (van Paassen et al., 2010; Canakci and Kilic, 2015; Phang et al. 2018).

The effects of interaction between ureolytic and non-ureolytic bacteria on the performance of calcium carbonate precipitation process got the attention of researchers. Gat et al. (2011) searched these effects using *S. pasteurii* (ureolytic bacteria) and *Bacillus subtilis* (non-ureolytic bacteria). The authors concluded their work that having increased number of nucleation sites provided by non-ureolytic bacteria accelerated the MICP process.

The effect of the living state of microorganisms was also studied by researchers. Chou et al. (2011) performed treatments using dead, resting, and growing cells of *S. pasteurii*. The study concluded that improvement obtained with growing cells were greater than the other conditions (Figure 2.2).

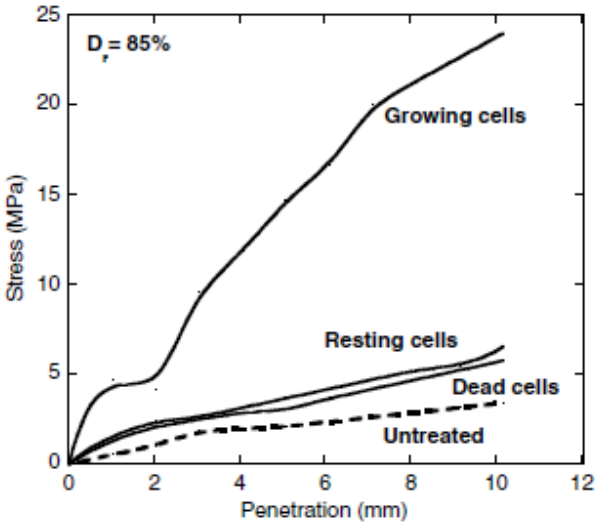


Figure 2.2. Effect of living state of microorganism on California Bearing Ratio Test results (Chou et al., 2011)

To initiate and maintain the biochemical process that leads to calcium carbonate precipitation, it is required to increase enzyme activity and microbial population to a required level. To achieve this demand two strategies are adopted in the literature: bio-stimulation and bio-augmentation. Bio-stimulation involves the injection of an enrichment reagent to stimulate population growth of native microorganisms and bio-augmentation includes increasing the population of urease-produce bacteria by injecting non-native bacteria. When ureolytic bacteria exist in the subsurface, the use of bio-stimulation is preferable since using native organisms decreases the engineering challenges of controlling the transport of cells in the subsurface (DeJong et al., 2009). On the other hand, in the absence of ureolytic bacteria in the subsurface and if the subsurface is suitable to augmentation (e.g. accessible, high permeability, uniform gradation), bio-augmentation may be useful (Mortensen et al., 2011). However, competitive conditions may affect the survivability of the introduced non-native organisms. In this study, bio-augmentation strategy is adopted, however, the results should also be representative of bio-stimulation.

Studies have shown that using both strategies can significantly improve geotechnical properties. Gomez et al. (2017) compared the improvement of 0.3 m thick sand layer in a tank having 1.7 m diameter treated by applying bio-stimulation and bio-augmentation methods. In the application of bio-augmentation method, *S. pasteurii* was injected. Biological analyses showed that the bacteria stimulated for the application of bio-stimulation method had distinctly different 16S ribosomal RNA (rRNA) sequencing. Cone tip resistance, calcite content and shear wave velocity measurements revealed that geotechnical properties were significantly improved in both tanks.

2.1.2. Soil and Microorganism Compatibility

Soil treatment using microbial activity requires the geometric compatibility of soil and bacteria as it affects the distribution of bacteria which in turn impacts the uniformity of treatment. The diameter of bacterial cells usually ranges between 0.5 and 3.0 μm

(Mitchell and Santamarina, 2005). Although the size of bacteria brings the ability to travel through many types of soil, movement of bacteria is restricted by the size of pore throats through which the bacteria must pass to move in the soil matrix. The fine fraction of soil greatly affects the pore throat size. Holtz and Kovacs (1981) proposed that the size of the pore throat can be estimated from mechanical sieve analysis as 20% of the soil particle size that corresponds to 10% passing. Therefore, the particle size relative to microbe size forms the lower bound limit of treatment by in-situ injection, however ex-situ mixing of bacteria and required nutrients may broaden the range of application (DeJong et al., 2010). Harkes et al. (2010) also stated that mixing the bacteria and treatment reagent can be another application strategy. Although this strategy may result in early precipitation of calcium carbonate, it can be used for the treatment of coarse-grained soils (such as in-situ mixing applications). On the other hand, use of this strategy on fine-grained soils can be problematic since early precipitated crystals may clog the injection wells which in turn spoil the distribution with accumulation at the injection region.

Apart from pore throat size, existence of sufficient particle-particle contacts per unit volume is another factor that affects the success of the process. While bigger pore throat size provides space the microorganism to move, it also means less particle-particle contacts hence less strength gain. Therefore, there should be a balance between these two contradicting properties. A comparison of soil particle size and the approximate limits of biotreatment methods are depicted in Figure 2.3.

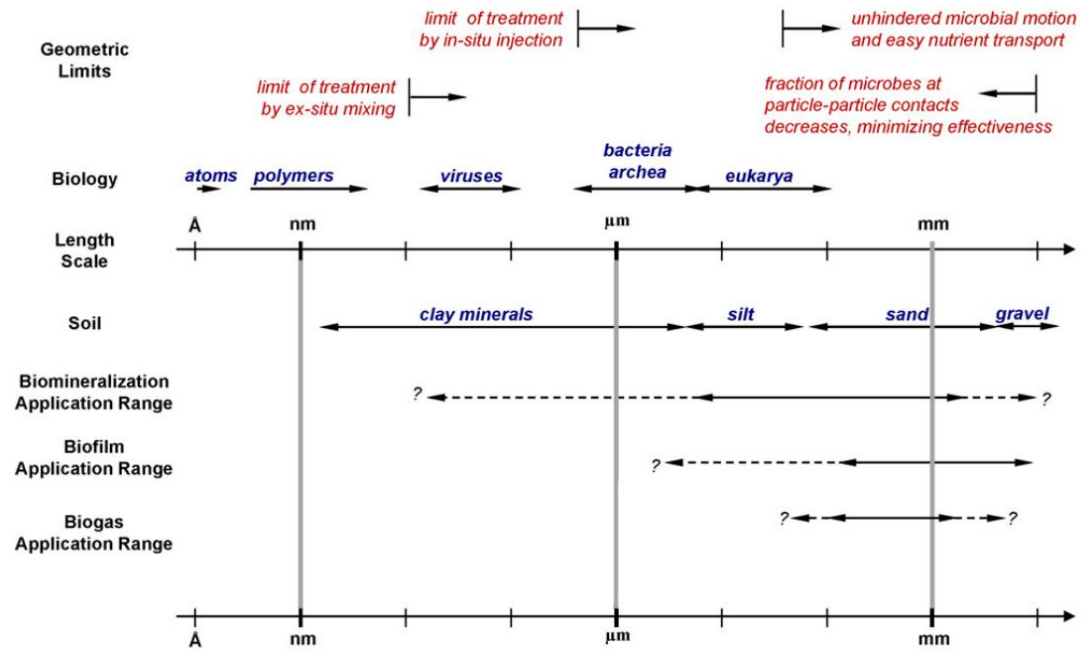


Figure 2.3. Comparison of typical sizes of soil particles and bacteria geometric limitations and approximate limits of various treatment methods (DeJong et al., 2010)

Rebata-Landa (2007) focused effect of grain size on MICP and concluded that grain size between 50 and 400 µm is the most optimal range as the bacterial activity is restricted in fine soils and for coarse soils, nutrient amount required to increase stiffness and strength is very large.

Throughout the years, various soils have been successfully treated with MICP by researchers. For example, van Paassen et al. (2012) treated gravel, Soon et al. (2013) used a tropical residual soil classified as silt, Canakci et al. (2015) studied MICP on organic soil.

Retention of microorganisms on the grain surfaces also affects the success of the treatment. Surface topography is one of the factors that affect the retention of microorganisms. Whitehead and Verran (2006) pointed out that the surface feature's dimension and shape (linear etc.) can promote retention of microbe since these properties increase the contact area between microorganism and surface. The salinity

of the environment also influences the cell's retention. Harkes et al. (2010) stated that an increase in salinity results in better adsorption of cells.

Mortensen et al. (2011) tested MICP on a variety of mineralogies (silica, calcite, feldspar, and iron oxide) and stated that MICP is possible within a variety of soil minerals.

2.1.3. Treatment Media and Efficiency of the Process

The application strategy of bacteria medium and treatment reagent has been the subject of several studies. The retention time of nutrients, the number of flushes, injection pressure, concentrations of components of the nutrient solution are some examples of investigated factors affecting the end-product.

Martinez et al. (2013) tested factors affecting the efficiency of MICP including flow direction, flow rate, and treatment media concentrations. The results showed that the use of a high urea to calcium concentration ratio (333 mM urea, 100 mM $CaCl_2$) allows the rise in pH associated with ureolysis to be unaffected by the pH lowering effect of calcium carbonate precipitation. On the other hand, low urea to calcium concentration ratio (50 mM urea, 50 mM $CaCl_2$) does not allow pH increase during the process, hence, limits the calcium carbonate production. The study also showed that application of media with high chemical ratio using stopped-flow, where media was pumped and followed by a rest period, was better than applying continuous flow, where the injection process continues all the time, to get uniform calcium carbonate precipitation. Martinez et al. (2011) also examined those two injection alternatives and observed that continuous flow caused the accumulation of calcium carbonate at locations close to the injection port.

Uniform distribution of precipitated calcium carbonate is important to get uniform strength improvement throughout the treated soil. Martinez et al. (2013) claimed that the distribution of microbes is the most effective factor to acquire uniform calcium carbonate precipitation. In the same study, it was outlined that when the augmentation technique is conducted, the initial concentration of microbes and retention time are the

most important factors on the distribution of microbes. To acquire a uniformly distributed microorganism profile, Harkes et al. (2010) injected different fluids following the injection of bacteria medium. The study showed that injection of cementation solution immediately after bacterial suspension prevents flushing out of bacterial cells. Moreover, homogeneity can be achieved through injection of fluids with different salinities since an increase in salinity results in increasing adsorption of cells. Therefore, the salinity of the suspension can be used to mobilize or immobilize the cells to have a uniform cell distribution.

Achal and Pan (2014) compared different calcium sources including calcium chloride, calcium oxide, calcium acetate, and calcium nitrate in order to observe the effects of calcium source on MICP. Calcium chloride as calcium source provided the most calcium carbonate precipitation which is followed by calcium nitrate. Moreover, the efficiency of natural sources as calcium source also took the attention of researchers. For example, Choi et al. (2016) investigated the efficiency of eggshell as a calcium source. Unconfined compression and permeability tests showed that using eggshell is as efficient as using calcium chloride (Figure 2.4).

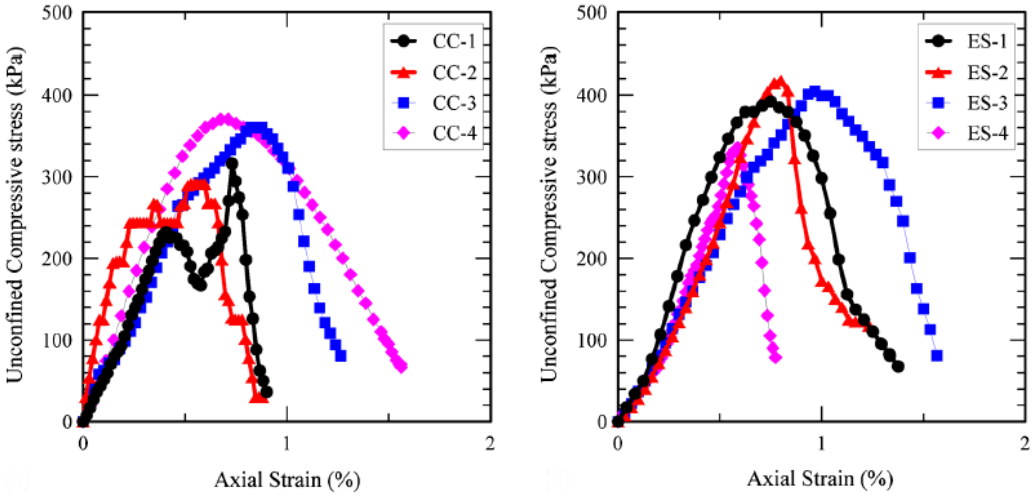


Figure 2.4. Unconfined compression strength test results of soils treated using (a) calcium chloride, (b) eggshell as calcium source (Choi et al., 2016)

Whiffin (2004) showed that temperature has a significant effect on urease activity. Findings of the study include that the urease activity of *S. pasteurii* increases proportionally with temperature in the range from 25 to 60 ^{o}C. At 70 ^{o}C the activity reaches its optimum and above that value activity drops considerably.

Owing to its importance, the effect of pH value on the calcium carbonate precipitation has been the focus of many studies. Stocks-Fischer et al. (1999) discovered that urease activity, hence precipitation rate remains high at pH ranging between 8.3 and 9.0. As a consequence of microbial activity, environmental conditions progressively shift to favorable conditions for precipitation especially in terms of pH. After the required conditions for calcium carbonate precipitation are satisfied, the rate of precipitation is regulated by the rate of microbial metabolic process and/or the available chemicals (DeJong et al., 2010). Instead of direct addition of a basic solution to increase pH, using ureolytic bacteria is preferable since the gradual increase of pH resulting microbial activity promotes a wider spatial distribution of calcite precipitated (Ferris et al., 2003).

Al Qabany and Soga (2013) investigated strength increase and permeability reduction under different urea and calcium chloride concentrations. The researchers reported that urea-calcium chloride solution with concentrations of 0.25 M and 0.5 M gave better chemical efficiency, defined as the percentage of urea-calcium chloride solution that precipitates as calcium carbonate. It varied from 70% to 100% efficiency than treatment with 1 M concentration solution with an efficiency of 20%. In addition, the use of a chemical solution with 1 M and larger concentration resulted in the precipitation of vaterite crystals (a less stable form of calcium carbonate). Furthermore, in the same study, researchers observed that the size of the calcium carbonate crystals increases with increasing concentration with corresponding inhomogeneous precipitation patterns.

The experiments performed on sand columns that have various saltwater concentrations showed that higher salinity results in a rapid increase in shear wave

velocity which indicates a high rate of calcium carbonate precipitation (DeJong et al., 2009).

The distribution of microorganism has a great effect on the distribution of precipitated calcium carbonate. While bacteria are injected, cells are filtered by the soil matrix. As a result of filtration, bacterial cell distribution follows a reduction through the injection path (Ginn et al., 2002), which directly affects the distribution of calcium carbonate since the rate of urea hydrolysis hence the rate of calcium carbonate precipitation depends on bacterial cell concentration. Some studies in the literature focus on this relation. For example, Chou et al. (2011) observed that increasing bacterial cell concentration results in an increase in calcite precipitation amount**.** Okwadha and Li (2010) concluded their work that the rate of ureolysis depends on bacterial cell concentration more than initial urea concentration in case of enough urea in the environment to maintain bacterial activity. The relation between bacterial cell concentration and the rate of urea hydrolysis is presented in Figure 2.5.

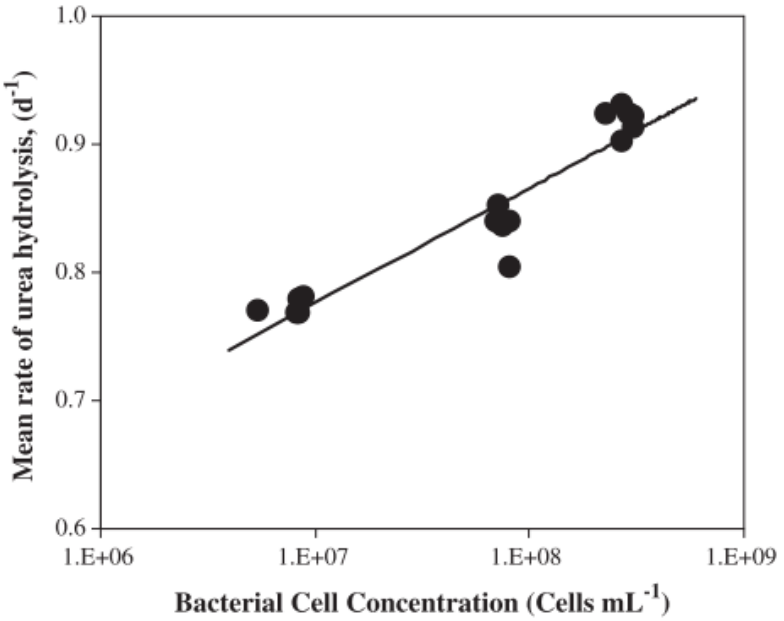


Figure 2.5. The relation between bacterial cell concentration and the mean rate of urea hydrolysis (Okwadha and Li, 2010)

2.1.4. Modeling

MICP is a complex process that is a combination of biological, chemical, hydrological, and mechanical processes. Modeling of MICP requires coupling of these processes. Modeling effort gives an opportunity to be able to detect technical challenges before application. To date, modeling attempts have focused on two aspects: mathematical modeling of biogeochemical processes and calcite distribution or mathematical modeling of mechanical behavior of biocemented soils (DeJong et al., 2013). Dupras et al. (2009) performed batch experiments to observe the variation of pH under different amounts of urea and various bacteria concentration as ureolysis reaction continues and the created model successfully captured the experimental results. Van Wijngaarden et al. (2010, 2011) modeled the transport of injected solution by assuming a homogenous bacteria distribution. Extending their previous works Van Wijngaarden et al. (2012) focused on modeling of the transport, adsorption, and fixation of the injected bacteria. Fauriel and Laloui (2011) interested in modeling the propagation of biogrout in soils. Researchers developed a bio-hydro-mechanical numerical model considering bio-hydro-mechanical couplings, transport, miscibility, bacterial growth and decay, and bacterial attachment and detachment. Martinez et al. (2014) utilized different treatment schemes including the type of flow, chemical concentrations, the source of flow etc. on half meter sand columns. The developed transport model had good agreement with the observed data (Figure 2.6).

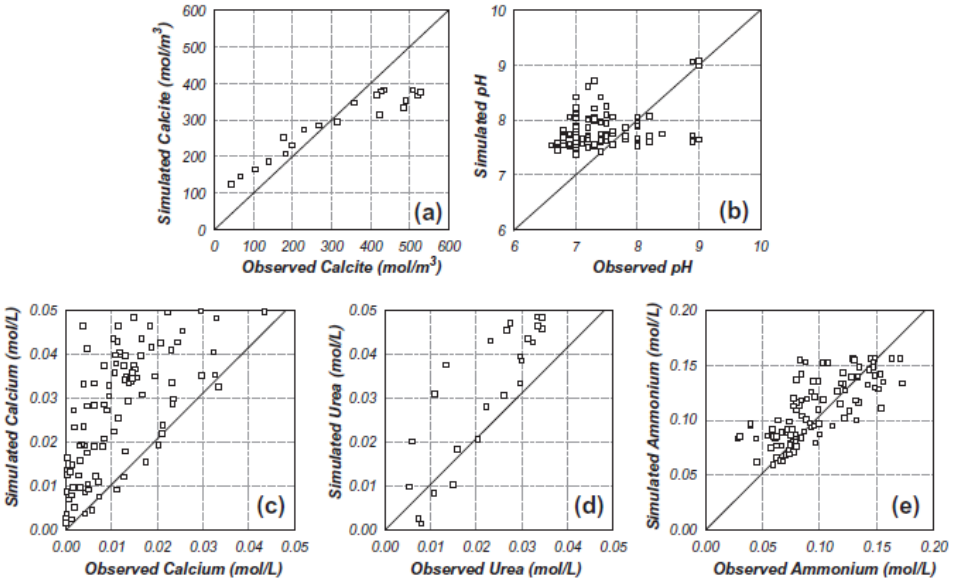
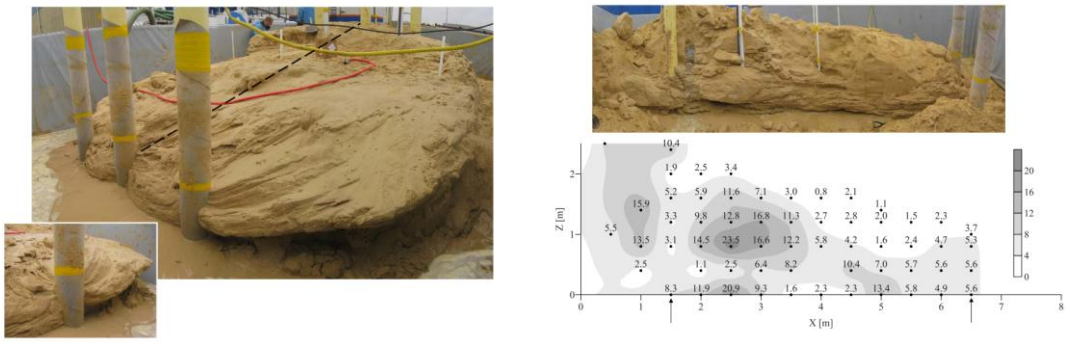


Figure 2.6. Simulated vs observed data (Martinez et al., 2014)

2.1.5. Large-scale Applications

To date, only a few large-scale experiments or field trials have been performed utilizing MICP to alter engineering properties of soil. The first field application of biogrout included applying MICP with bioaugmentation strategy for stabilization of gravel around horizontal borehole to enable horizontal directional drilling (HDD) for a gas pipeline installation in the Netherlands in 2010 (van Paassen, 2011). During the process, the biogrout procedure was applied at depths varying from 3 m to 20 m below the surface. The application was performed with wells placed such that 6 injection wells were surrounded by 14 extraction wells. In each step of the application, about 1000 m^3 of soil volume was treated. Each step of biogrout procedure included injection of about 200 m^3 of bacterial suspension followed by injection of 300 to 600 m^3 of cementation solution. Extraction wells were active until the extracted groundwater had the initial electrical conductivity and ammonium concentrations. The procedure was successfully applied so that HDD's and pipeline installations were successfully performed.

van Paassen et al. (2010) applied MICP to calcify 100 m^3 sand in a concrete container (8.0 m × 5.6 m × 2.5 m). The bacteria and cementation solution were given via three wells for both injection and extraction purposes. The cementation procedure included the injection of 5 m^3 of bacterial suspension followed by 5 m^3 of 50 μM $CaCl_2$ of which suggested enhancing bacterial retention by Harkes et al. (2010). Then about 96 m^3 of cementation solution containing urea and $CaCl_2$ (of 1 M concentration for both) was injected in 16 days. In the end, it was observed that most of the cementation took place within a distance of 2 m from the injection wells. Moreover, a block of soil having about 43 m^3 in volume with a shape clearly indicating the flow paths was successfully cemented (Figure 2.7).



(a) (b)

Figure 2.7. (a) the cemented sand body, (b) $CaCO_3$ content along the vertical cross-section passes through the middle of the cemented body (van Paassen et al., 2010)

Filet et al. (2012) also treated sand in a container. The treatment plan was included three injection lines having injection and extraction points with 5 m gap in between. The volume aimed to treat had dimensions of 1.5 m in high, 4.5 m in width, and 6 m in length. After the treatment process was completed 110 samples were taken to conduct unconfined compression tests. Only four samples had strength below 50 kPa and the average strength was about 200 kPa.

Although laboratory tests have addressed many questions well, scaling up the process still brings lots of challenges. The possible challenges when scaling up the process including but not limited to having uniform microorganism and treatment media distribution, handling of by-products, in-situ monitoring of the process, lifetime monitoring to observe durability, and the education of engineers and researchers.

2.2. Testing Microbially Treated Soils

To evaluate the efficiency of the treatment, it is required to test the samples. In the literature, the improvement in the sample was investigated by performing different methods. The review of these methods is presented in the following sections.

2.2.1. Monitoring the Process and Product

Examining the final product in micro-scale is crucial to evaluate the behavior in macro-scale. Scanning electron microscopy (SEM) is one of the mostly used tools in this field. By using this technique, researchers get a chance to observe $CaCO_3$ precipitation patterns, crystal sizes, even indications of microbial placement. Lin et al. (2016) provided good examples of precipitation alternatives, crystal size, and different morphologies with the help of SEM imaging (Figure 2.8).

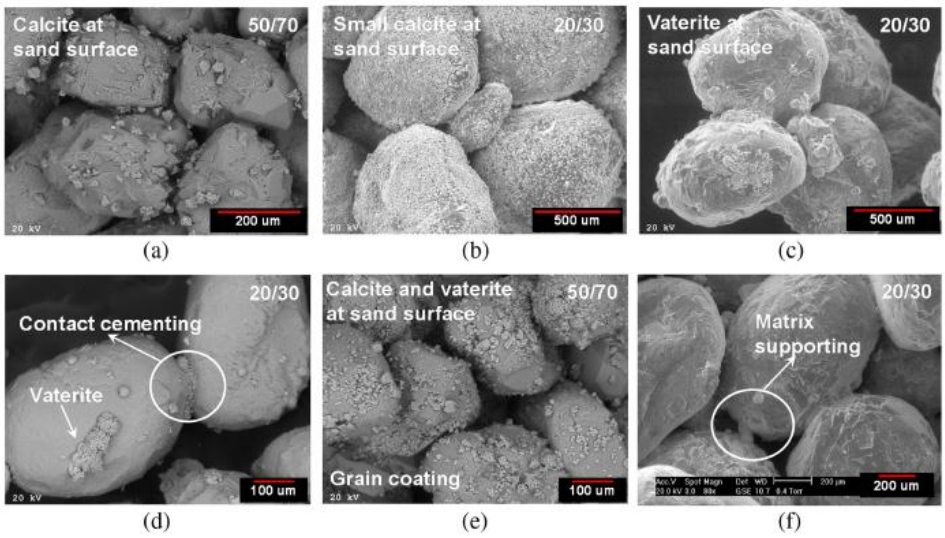


Figure 2.8. Scanning Electron Microscopy images showing examples of distribution alternatives and different $CaCO_3$ morphologies (Lin et al., 2016)

Other beneficial tools include Energy Dispersive X-Ray Spectroscopy (EDS) and X-Ray Diffraction (XRD). EDS provides a mapping of elements on an area. XRD technique is used to determine the crystalline structure so that the precipitated crystal can be identified.

Calcium carbonate content after MICP process is another important information to quantify the effectiveness of the process. Dissolving calcium carbonate by washing the soil sample with acid, such as hydrochloric acid (HCl) is a common way to determine precipitation amount. Choi et al. (2017) conducted a detailed study on the effectiveness of different calcium carbonate content measurement methods**.**

To control and manage the microbial activity as it alters the soil properties, monitoring of changes during MICP process is essential. The geophysical measurement methods (shear wave velocity, compression wave velocity, and resistivity) cause little or no strain so that they can be used without disturbing the treatment process (DeJong et al., 2010). Besides their advantages, the geophysical methods provide information about the change in the bulk properties, but the local changes cannot be captured. An example of shear wave data during various phases of treatment can be seen in Figure 2.9. In addition, the level of cementation can be evaluated using shear wave velocity measurements (Figure 2.10).

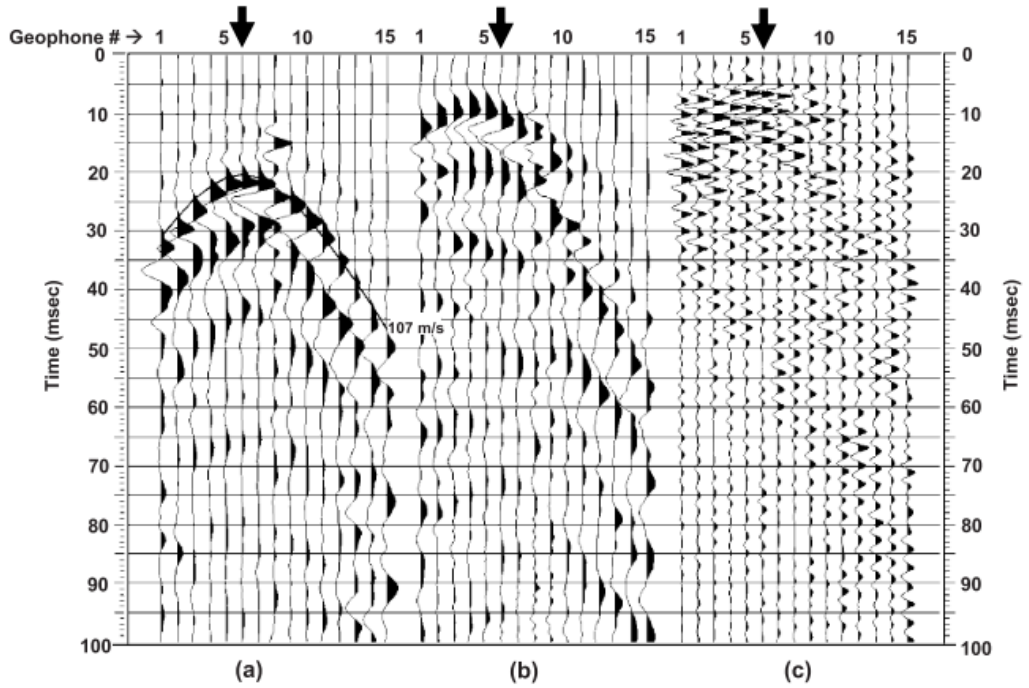


Figure 2.9. Shear wave data (a) before treatment, (b) after 1 day of injection, (c) at the end of the treatment process (van Paassen et al., 2010)

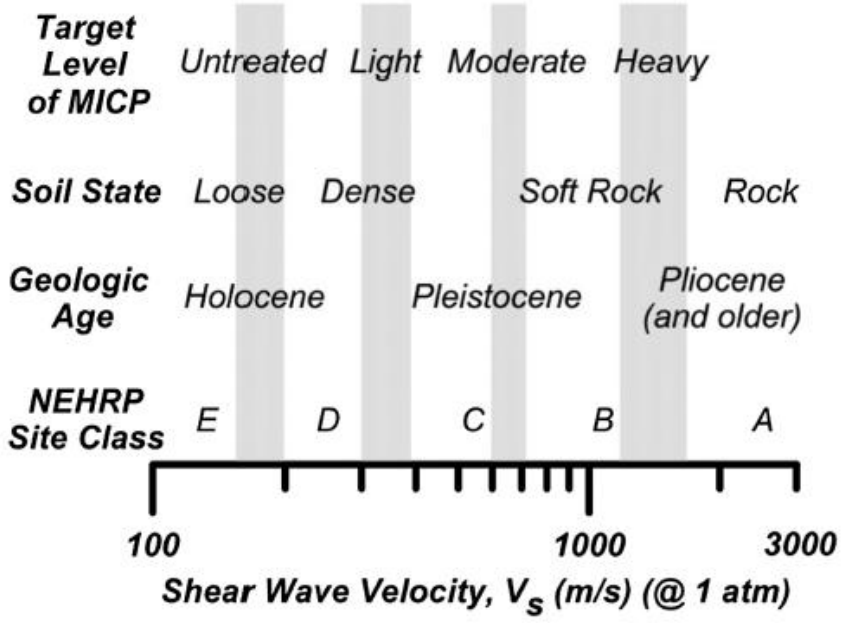
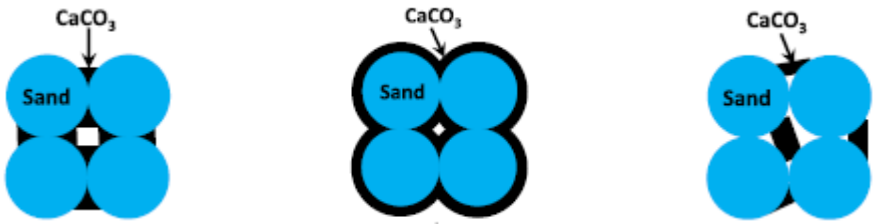


Figure 2.10. The relation between the level of cementation and shear wave velocity (Montoya and DeJong, 2015)

2.2.2. Strength Tests on Treated Soils

Performing strength tests on treated samples are important to assess the quality of the treatment. In the literature, the strength gain and behavior of the soil after bio-treatment was evaluated performing various strength tests under different loading conditions.

It was observed by many researchers that the distribution and location of precipitated calcite have a crucial impact on the strength and behavior of treated soil. Based on the engineering aspect, it is desired to have precipitation only on particle-particle contacts so that all precipitated material contributes to strength improvement. Another extreme calcite distribution alternative is grain coating where precipitation takes place on grain surfaces. This alternative may also contribute strength by increasing the particle angularity. While Martinez and DeJong (2009) also indicated these two distribution alternatives, Lin et al. (2016) proposed an addition to them named as matrix supporting. Figure 2.11 illustrates these calcite distribution alternatives.



(a) (b) (c)

Figure 2.11. $CaCO_3$ distribution alternatives: (a) contact cementing, (b) grain coating, (c) matrix supporting (Lin et al., 2016)

The location of calcite accumulation is an important factor affecting the engineering response of treated soil. Cheng et al. (2013) showed that at the same calcite content, a specimen with calcite accumulation intense at particle contact points results in more shear strength improvement.

DeJong et al. (2010) state that bacteria would rather position themselves near particle-particle contacts than exposed particle surfaces. According to the authors, this behavior can be attributed to reduced shear stresses and nutrient availability around particle contacts. In this way, the nature of microorganism behavior also contributes to a better strength gain.

In the literature, the effort to quantitatively evaluate the results of bio-improvement process includes: triaxial compression test (Cheng et al., 2013; Lin et al., 2016; Montoya and DeJong, 2015), cyclic triaxial shear test (Burbank et al., 2013), unconfined compression test (Al Qabany and Soga, 2013; Li et al., 2015; van Paassen et al., 2010; Whiffin et al., 2007), and California bearing ratio test (Chou et al., 2011). The direct shear tests in the literature are reviewed in detail in the next section.

In addition to the above ones, there are some remarkable studies in the literature. For example, DeJong et al. (2006) treated initially loose and collapsible sand with MICP and gypsum. Undrained shear strength tests revealed that MICP treated sand showed non-collapse behavior and shear behavior was similar to gypsum cemented sand. Another good example can be the bench-scale test that was conducted by Martinez and DeJong (2009). Medium-loose sand beneath a scaled shallow foundation model was treated. The results of the tests showed a five-fold reduction in the settlement as compared to untreated sand (Figure 2.12). Beside applications with using only bacteria, the effect of material addition and soil condition were also investigated. Li et al. (2015), for example, treated sand-fiber mixture with MICP. The results showed that up to an optimum fiber content the unconfined compression strength gradually increased. Cheng et al. (2013) identified the degree of saturation as a factor affecting the obtained shear strength after performing MICP under various degrees of saturation. Moreover, the unconfined compression test results showed that at lower saturation degrees higher strengths can be achieved (Figure 2.13a). Some unconfined compression test results in the literature can be seen in Figure 2.13b.

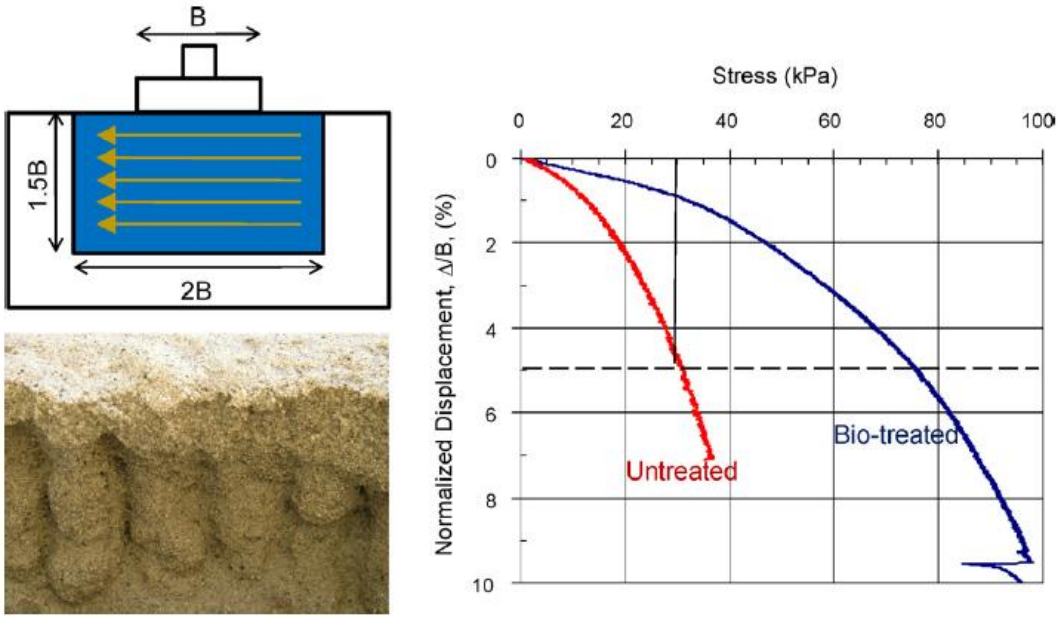
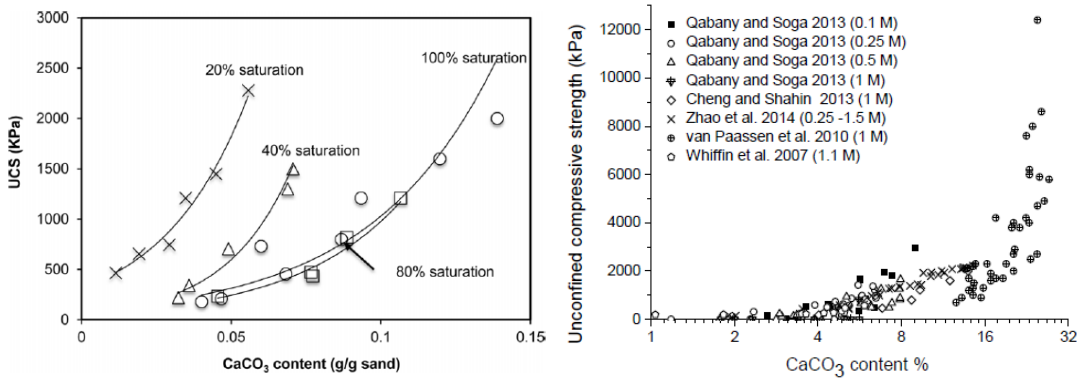


Figure 2.12. Results of bio-treatment experiments under a shallow foundation model (Martinez and DeJong, 2009)



(a) (b)

Figure 2.13. (a) Unconfined compressive strength variation of samples having different saturation degrees (Cheng et al., 2013), (b) relation between $CaCO_3$ content and unconfined compressive strength (Wang et al., 2017)

2.2.2.1. Direct Shear Test Results in the Literature

The aim of the presented study is to evaluate the direct shear strength and shear behavior of bio-treated fine sand, hence a detailed review of the literature dealt with direct shear behavior is presented in this section.

Chou et al. (2011) treated sand specimens prepared with different relative densities with *S. pasteurii*. Direct shear tests were performed under normal stresses ranging from 11 to 40 kPa to investigate the success of the process for dust control applications. Results of the tests showed that loose sand treated with growing cells had greater peak strength than other treatment schemes. Moreover, almost no improvement in peak strength obtained from any of the treatments for densely prepared specimens. According to the findings of the study, the authors classified the MIPC treated specimens as weakly cemented.

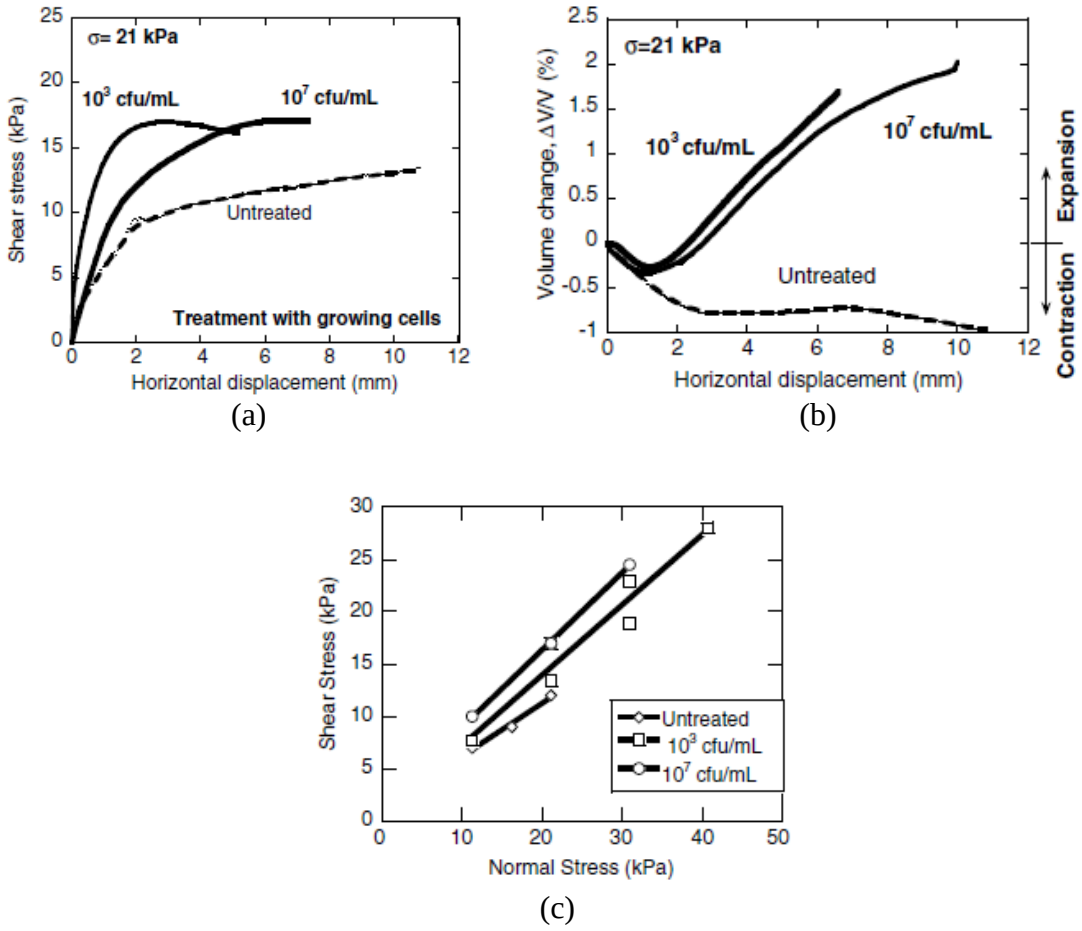


Figure 2.14. Direct shear test results for untreated and bio-treated sands (Chou et al., 2011)

van Paassen (2011) performed direct shear tests on gravel treated using MICP. Authors pointed out the relation between direct shear strength and the number of flushes. The results showed that as the number of flushes increases direct shear strength increases (Figure 2.15).

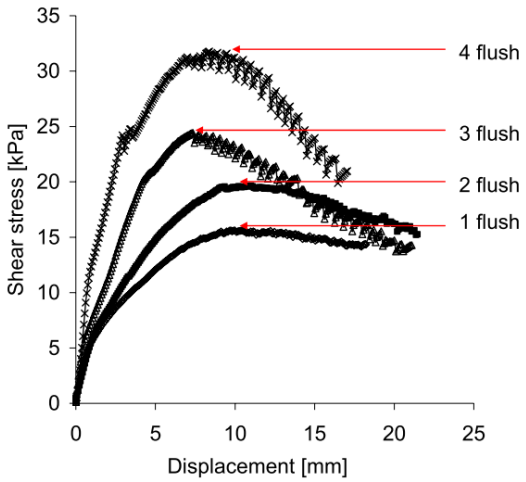


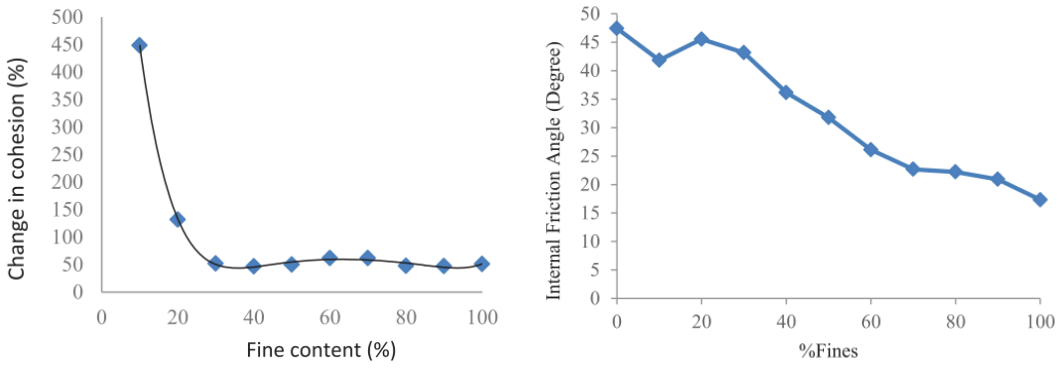
Figure 2.15. Correlation between shear strength and number of flushes obtained from treatment on gravel (van Paassen, 2011)

Canakci et al. (2015) treated soil specimens classified as peat with *S. pasteurii*. Treated and control (treated only with water) specimens were sheared at a rate of displacement of 0.02 mm/min under 15 kPa normal stress. While control specimen showed ductile behavior, treated specimen showed brittle behavior. In addition, despite loosely prepared, treated sample behaved as if it was dense soil.

Zamani et al. (2017) conducted undrained monotonic and cyclic direct shear tests on fine poorly graded sand and sand having 15% silt after treatment performed using *S. pasteurii*. The injection of cementation media took place every 12 hours and the injections continued until a shear wave velocity of 400 m/s obtained. Cyclic direct shear tests showed that MICP effectively reduced the liquefaction potential of silty

sand. In addition, the shear strength improvement was obtained for both sand and silty sand without having any clogging.

Hataf and Jamali (2018) treated soils with varying fines content utilizing *Bacillus sphaericus*. Although the shear strength of all samples treated with MICP was increased compared to untreated samples, a decrease in shear strength and cohesion was significant above fines content value of 20% (Figure 2.16a). In addition, the friction angle of both treated and untreated samples decreased with increasing fines content so the authors concluded that MICP has no effect on this parameter (Figure 2.16b).



(a) (b)

Figure 2.16. Change of (a) cohesion, (b) internal friction angle of treated soil with respect to fines content (Hataf and Jamali, 2018)

Cheshomi et al. (2018) conducted direct shear tests on sandy soil samples having various densities and treated with different injection numbers and injection frequency. *S. pasteurii* was the microorganism used to catalyze the process. Loosely prepared samples showed better improvement in terms of shear strength. Moreover, increasing treatment duration resulted in an increase in shear strength (Figure 2.17). However, injection frequency in a day did not show such a linear correlation. Injection two times in a day resulted in a bigger peak strength than injection one or three times in a day.

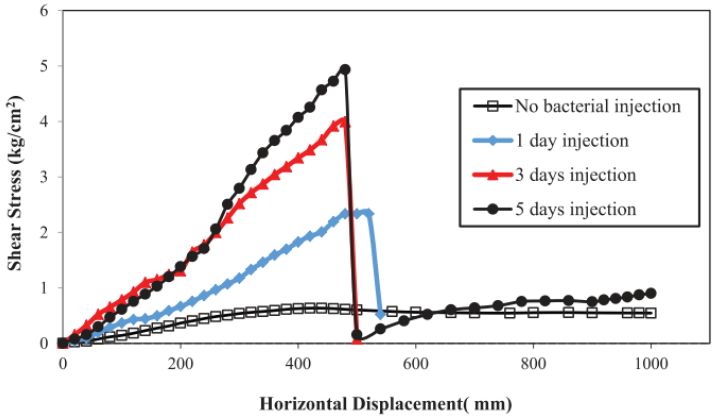


Figure 2.17. Effect of injection duration on shear strength (Cheshomi et al., 2018)

As explained in this chapter, there are numerous studies illustrating the positive effects of MICP from various perspectives. Within the scope of this thesis, the next chapter simply focuses on the main work for performing direct shear tests to outline the shear strength characteristics of the sand samples improved with MICP.

CHAPTER 3

TEST SETUP, SPECIMEN PREPARATION AND TREATMENT PROCEDURE

Quantification of the geomechanical properties of the treated and untreated sand specimens required the design of a test procedure as there is no standard testing method for microbially treated soils. Within this perspective, this chapter is devoted to presenting the microorganisms, and describing the soil used in the experiments and explaining how the strength test specimen preparation procedure was developed.

The materials and method used to perform the experiments were developed in a progressive way. The sample preparation procedure was built on the basis of the literature and the geomechanical testing was performed according to standard test method for direct shear test provided by ASTM (ASTM D3080). Together with all these stages for preparing the experiments, the observations made during the preliminary tests are presented in the following sections.

As mentioned in Chapter 2, there are many factors affecting different stages of MICP such as chemical concentrations, bacterial cell number, pH, temperature, etc. The decisions regarding the proper arrangement of those variables are important for obtaining sufficient calcium carbonate precipitation, hence, strength improvement. Small scale laboratory experiments such as observing calcium carbonate precipitation in syringes can lead the way in determining appropriate conditions. Saricicek (2016) performed such laboratory studies by utilizing MICP in sand columns prepared in syringes. In that study, two bacteria strains, *S. pasteurii* and *B. licheniformis*, were utilized with different chemical concentrations. Similarly, in the current study, the concentrations of the components of cementation solution used were determined based on the suggestions of Saricicek (2016).

3.1. Materials

3.1.1. Sand

In this study, quartz sand (Pomza Export Mine Industries & Trade Company), which was also utilized for a previous study (Ahmadi-Adli, 2014), was chosen in the experiments. The sand was uniformly graded (coefficient of uniformity, C_u=2.24). Based on the Unified Soil Classification System the soil was classified as poorly graded sand. The specific gravity of the sand was 2.66. The maximum and minimum void ratios were 1.0 and 0.62, respectively. The laboratory experiments were performed to find maximum/ minimum void ratios, specific gravity and grain size distribution according to relevant ASTM standards (ASTM D4254, ASTM D7382, ASTM D854, ASTM D6913) and results were reported by Ahmadi-Adli (2014). The properties of the sand are presented in Table 3.1. In addition, Figure 3.1 shows the gradation curve of the sand. Sterilization process (such as autoclave sterilization, washing with acid etc.) was not applied to sand prior to usage.

Table 3.1. *Properties of the sand used in the experiments*

Property	**Value**
D_{60} (mm)	0.202
D_{30} (mm)	0.14
D_{10} (mm)	0.09
C_u	2.24
C_c	1.08
Soil classification	SP
G_s	2.66
e_{max}	1.00
e_{min}	0.62
$\rho_{d,max}$ (g/cm^3)	1.648
$\rho_{d,min}$ (g/cm^3)	1.332

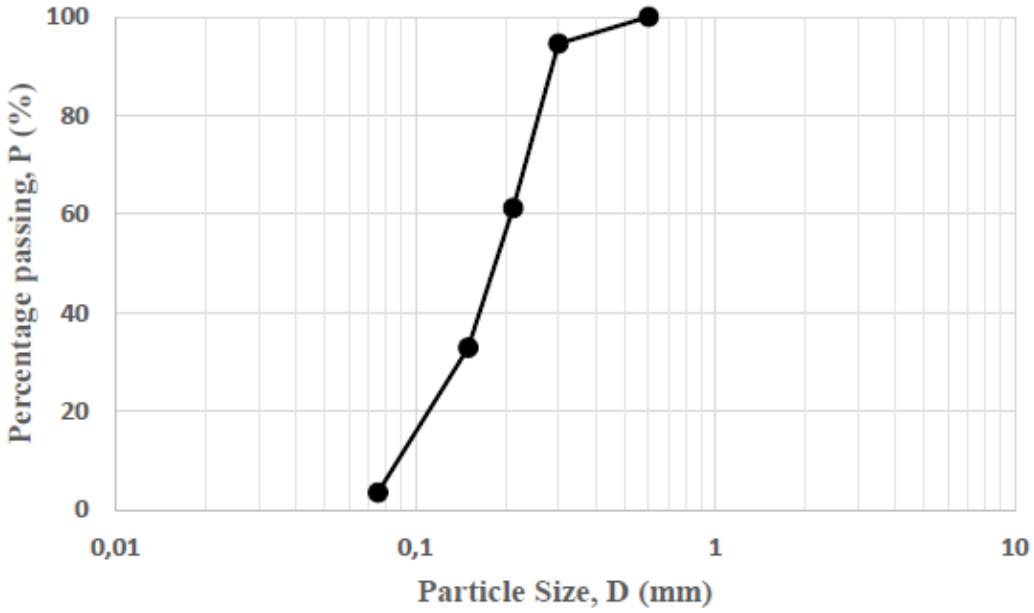


Figure 3.1. The grain size distribution of the sand (Ahmadi-Adli, 2014)

3.1.2. Bacteria and Growth Conditions

Two bacteria strains were utilized in this study, namely *S. pasteurii* [American Type Culture Collection (ATCC) 11859] and *B. licheniformis* [ATCC 14580]. Both strains have Biosafety Level of 1 indicating that bacteria are suitable to work in the laboratory conditions.

Separate media were produced for each of the bacteria. Table 3.2 provides a list of ingredients and their amounts. The culture medium (ammonium-yeast extract medium) of *S. pasteurii* contains 20 g yeast extract, 10 g ammonium sulfate ($(NH_4)_2SO_4$), and 15.75 g Tris buffer per liter of distilled water. The preparation process of this culture media can be outlined as follow:

- 20 g of yeast extract and 10 g of ammonium sulfate [$(NH_4)_2SO_4$] were dissolved in 100 ml of distilled water separately.
- 15.75 g of Tris buffer was dissolved in 800 ml of distilled water.

- All of the ingredients of the medium were sterilized (121 ^{o}C, 15 min) separately.
- After cooling, the ingredients were mixed in a 1000 ml sterile glass bottle under sterile conditions. pH measurement indicated a pH value of 9.0 providing appropriate conditions for the growth of *S. pasteurii*.

For the culture medium of *B. licheniformis*, 8 g of nutrient broth (*Merck*), which contains 5 g peptone from meat and 3 g meat extract, was dissolved in a liter of distilled water. Then the medium was poured into flasks and sterilized with autoclave (121 ^{o}C, 15 min).

Table 3.2. *Components of culture media of bacteria Sporosarcina and Bacillus licheniformis*

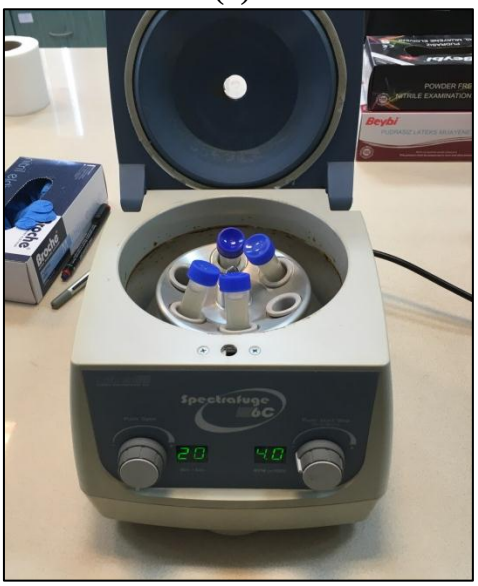
Bacteria	**Components**	**Amount (grams per liter)**
Sporasarcina pasteurii	Yeast extract	20
	$(NH_4)_2SO_4$	10
	Tris buffer	15.75
Bacillus licheniformis	Peptone	5
	Meat extract	3

Having the culture medium prepared, the cultivation of bacteria was performed under aerobic batch conditions. The stock culture of *S. pasteurii* was incubated at 30 ^{o}C with shaking (orbital shaker; *Zhicheng*, China) at 200 rpm for at least 48 hours. *B. licheniformis* was incubated at 37 ^{o}C at 200 rpm for 24 hours. The incubation process was maintained until an optical density (OD_{600}) of approximately 1 was obtained. The OD_{600} values were obtained using an UV-visible spectrophotometer (UV-5100, SOIF, China). Next, centrifugation (*Spectrafug 6C, LABNET*, USA) was applied to harvest the bacterial cells at 4000 g for 20 min using 15 ml tubes. All stages requiring the sterile conditions were performed under a laminar flow hood (*Esco*, USA). After successful centrifugation, the supernatant was removed, and the remaining pellets were placed at -20 ^{o}C to store the bacteria until use. Some stages of the process are shown in Figure 3.2.

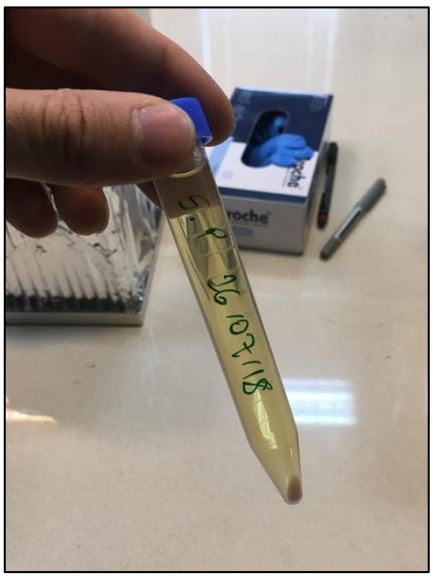
(a)

(b)

(c)


POWDER FRE
NITRILE EXAMINATION
Beybi
Brother
Spectrafuge
6C
20
40

(d)



(e)

Figure 3.2. (a) pouring ammonium-yeast extract into the flasks, (b) inoculation of Bacillus licheniformis from stock culture, (c) orbital shaker equipment, (d) centrifugation stage, € bacterial pellet at the bottom of the tube after centrifugation

3.1.3. Preparation of Urea Medium and Calcium Chloride Stock Solution

In this step, the cementation medium was prepared. All the media used in this study were prepared in METU Biological Sciences Department Microbial Ecology Laboratory. The medium consists of two solutions: urea medium and calcium chloride solution. The concentrations of the ingredients of the solutions were adopted from Saricicek (2016).

The urea medium was prepared in two stages. In the first stage, 10 g of ammonium chloride (NH_4Cl), 2.12 g of sodium bicarbonate ($NaHCO_3$), and 3 g of nutrient broth were dissolved in deionized water. Next, the solution was transferred to the flasks and autoclaved (121 ^oC, 15 min) for sterilization. The ingredient urea was not included at this stage since the heat applied during autoclave stage could degrade the urea. Consequently, in the second stage, 20 g of urea to prepare 1 L urea medium was dissolved in deionized water and then sterilization process using filters with 0.22 μm openings (*GVS*, USA) was performed. The filter-sterilized urea solution and

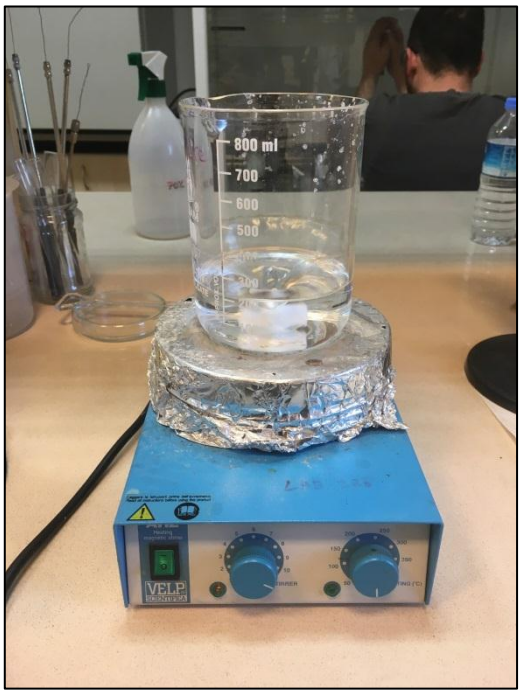
autoclaved media were mixed when the autoclaved media cooled down to around room temperature. The components of the urea medium and their concentrations are summarized in Table 3.3.

Table 3.3. *Components of urea medium*

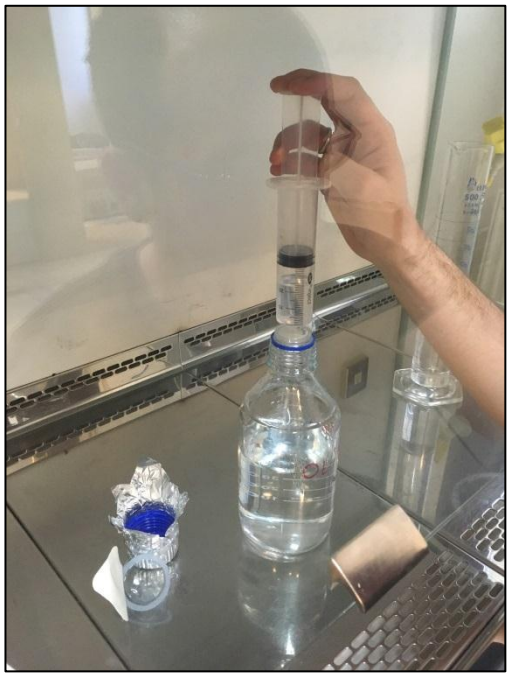
Components	Amount (g/L)	Concentration (mM)
Urea ($CO(NH_2)_2$)	20	333
Ammonium Chloride (NH_4Cl)	10	187
Sodium Bicarbonate ($NaHCO_3$)	2.12	25.2
Nutrient Broth	3	-

As the calcium source of the cementation solution, calcium chloride ($CaCl_2$) stock solution was prepared. 140 g of $CaCl_2$ was dissolved in 1 L distilled water. Then, the solution was autoclaved (121 ^oC, 15 min) for sterilization. Some stages of the urea medium preparation process are shown in Figure 3.3.

(a)



(b)



(c)

Figure 3.3. (a) dissolution of ingredients of urea medium except for urea with a magnetic stirrer, (b) dissolution of urea with magnetic stirrer, (c) sterilization of urea using the filter

3.2. Preparation of Direct Shear Specimens

In this thesis, the direct shear test was selected to evaluate the engineering properties of the untreated and bio-treated sand specimens. The primary challenge was to prepare the sand sample to be tested to quantify the geomechanical properties. In order to develop a laboratory specimen preparation procedure simulating potential field conditions, options revealed in the literature for different stages of the process were evaluated. In this section, how the details of the experimental procedure formed is outlined.

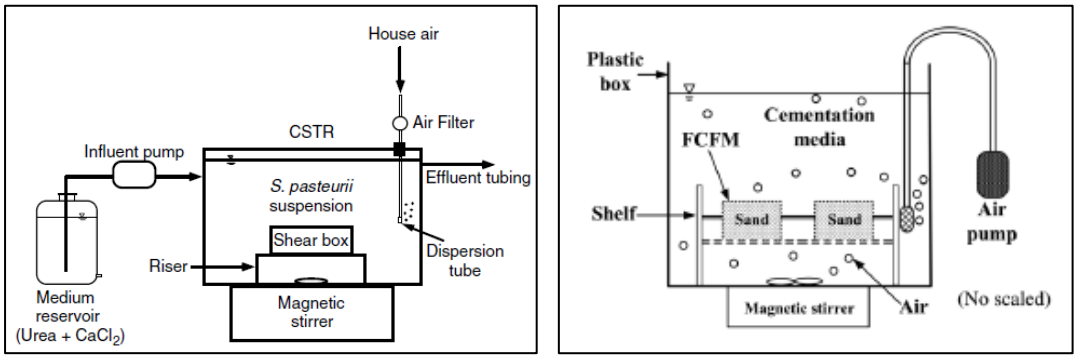
3.2.1. Development of the Specimen Preparing Procedure

Sample preparation procedure and details of testing were determined in a progressive way on the basis of the related studies in the literature as there is no standard test method for microbially treated soils. The alternative methods mentioned in the

literature and their engineering and scientific evaluation process within the context of this study is explained in the following sections.

Firstly, it was required to decide how the cementation medium would be applied to the specimen. To date, the researchers have focused on two alternatives: immersing the mold to the medium while periodically refreshing it or keeping in it without any addition (Chou et al., 2011; Zhao et al., 2014) and injection of medium through the sand body by gravity or using a peristaltic pump at certain time intervals (Whiffin et al., 2007; Harkes et al., 2010; Al Qabany et al., 2012; Canakci et al., 2015; Jiang et al., 2017). Some schematic drawings of the first alternative available in the literature are presented in Figures 3.4a and 3.4b. In the current study, injection of the medium by using a peristaltic pump was preferred. The reasons for this choice are that this procedure provides more control on injected medium volume and resembles the specimen preparation procedure to the grouting methods applied in the field.

For the molds, in which direct shear test specimens would be prepared, two alternatives were evaluated: preparing the specimen in the direct shear box and preparing it in an external mold. For the latter case, molds produced using geotextile material as suggested by Zhao et al. (2014) is an example (see Figure 3.5a-b). In the current study, it was decided to prepare the test specimens in direct shear boxes to eliminate the disturbance that may occur while transferring the specimen. However, preparing the sample in the direct shear box had the risk of corrosion which could affect the bio-geochemical reaction. Therefore, non-corrosive materials were attempted. At first, the costly economical option, coating an oxidable material with stainless material was utilized. Since during injections signs of corrosion were observed (Figure 3.6), it was assessed that the occurrence of corrosion was possible due to poor production quality. Later, a stainless steel box had to be utilized, even though it was not the most economical option. The manufactured boxes had the same dimensions as that of conventional circular boxes available in the METU Civil Engineering Soil Mechanics Laboratory (60 mm in diameter).

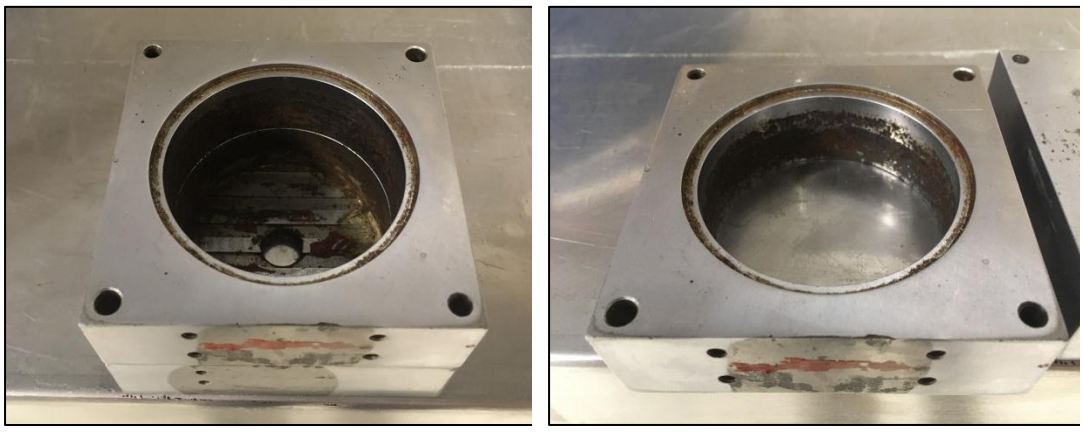


(a) (b)

Figure 3.4. Schematic drawings of the system used to prepare direct shear specimens by (a) Chou et al. (2011), (b) Zhao et al. (2014)

(a) (b)

Figure 3.5. (a) full contact flexible mold made of geotextile, (b) direct shear sample (Zhao et al., 2014)



(a) (b)

Figure 3.6. Corrosion observed on the primary mold options

The pilot tests were performed under dry conditions. The specimens were kept under 60 ^{o}C until no mass change was observed. At the end of the drying process, stiff crust layer was observed at the top of the specimens as can be seen in Figure 3.7. The direct shear tests under that condition gave unreliable results. Example of shear stress-horizontal deformation and vertical deformation-horizontal deformation relationships are presented in Figure 3.8. This behavior can be attributed to malfunctioning of normal load transfer on the soil due to the stiff crust, which was strongly attached to the upper half of the box. When the upper and lower halves of the boxes were separated to observe the shear plane, the sand body remained attached to the upper halve. When the separation screws were removed, halves of the boxes touched each other, which probably prevented the proper measurement of the shear strength of soil. It is important to note that abovementioned behavior was observed under the applied stress levels of 10, 20, 30 kPa, respectively. This may not be a case for higher stress levels**.** On the other hand, the drying process which includes only air drying was not preferred as it would take long time. Therefore, to eliminate the crust formation resulting from the applied drying process and to accelerate the experiments, it was decided to perform direct shear tests under saturated conditions.

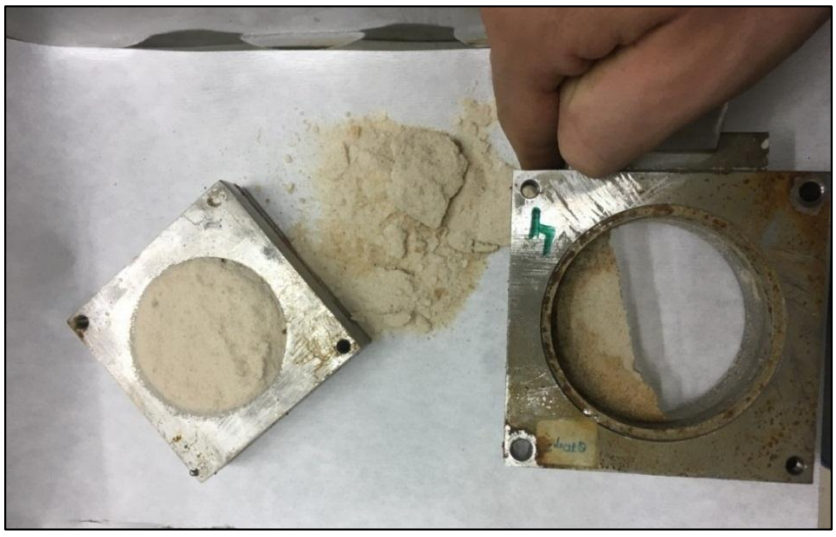
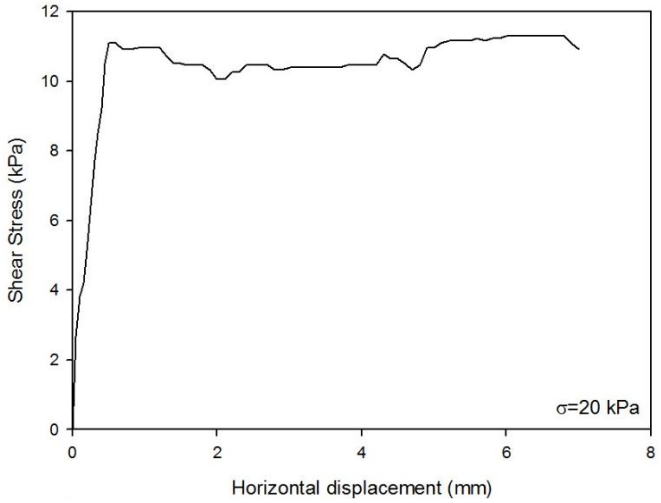
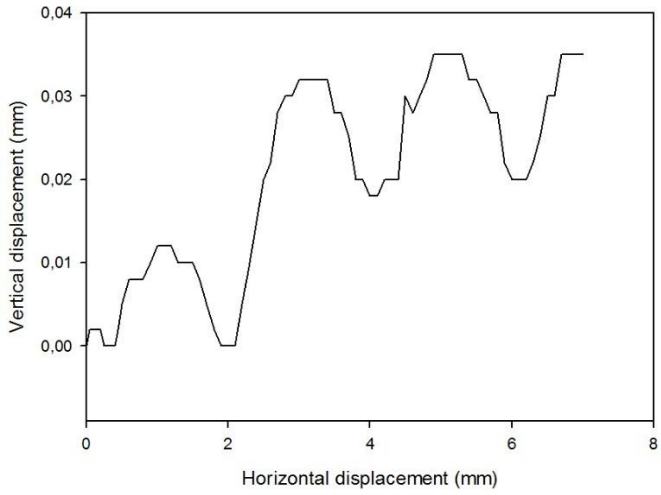


Figure 3.7. The crust formation at the top half of the direct shear box



(a)



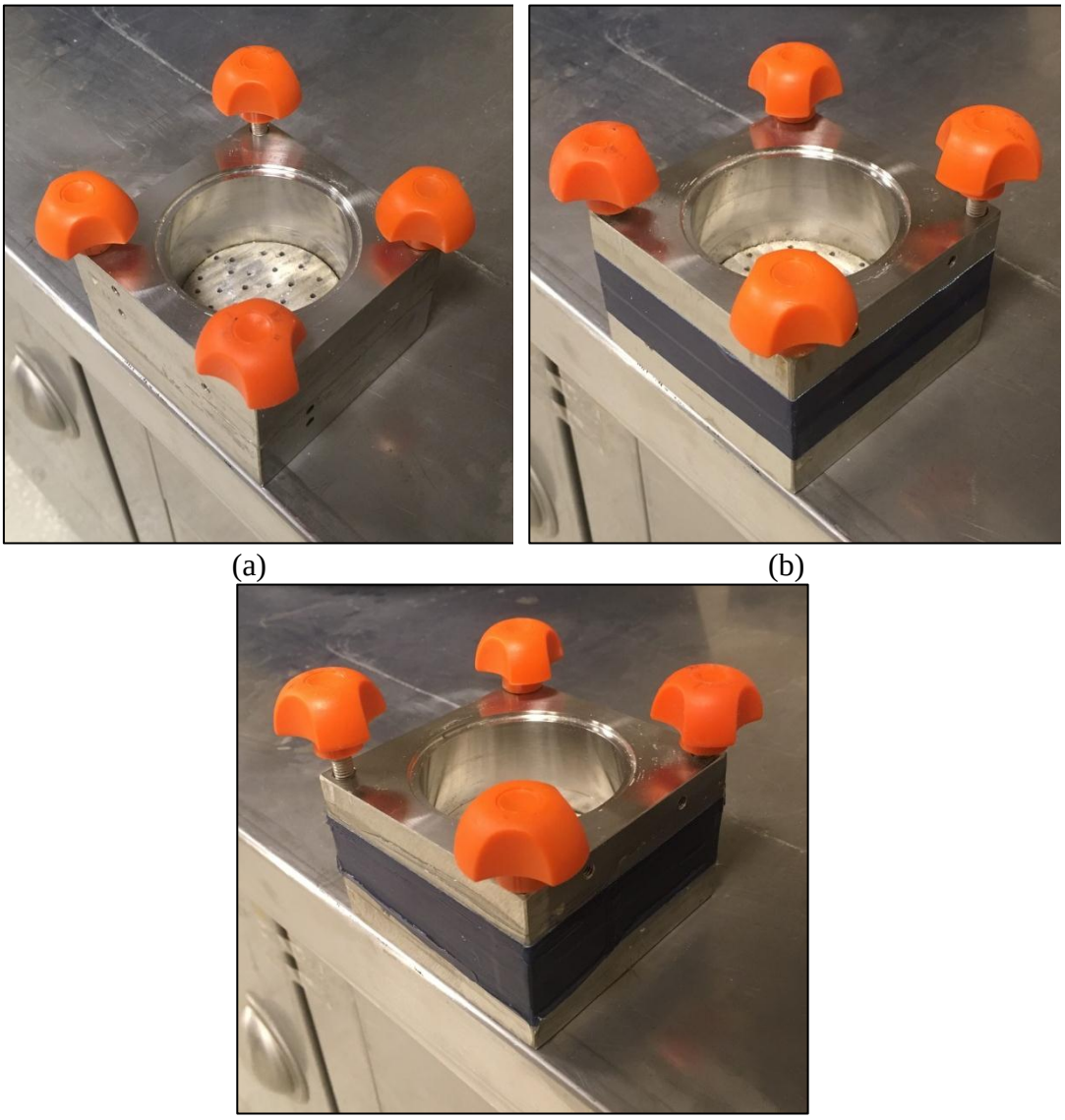
(b)

Figure 3.8. Example of (a) shear stress-horizontal deformation relationship, (b) vertical deformation-horizontal deformation relationship from preliminary test

3.2.2. Preparation of the Direct Shear Test Molds

In this study, stopped-flow strategy, where a volume of the reactants was injected and kept for a certain period of time, was followed. The molds were sealed to prevent drainage of the injected media as the reactants were supposed to remain in the mold. The sealing process was performed using a special band, which prevents leakage, and silicone. Direct use of silicone at the shear box's slipping plane was not preferred as

residue of silicone could affect the strength data, which generally remains there after cleaning of the mold. Therefore, a band was used, on the edge of which the silicone was applied. Figure 3.9 shows the stages of mold preparation against leakage. A sterilization process was not applied prior to usage of the molds.



(a) (b)

(c)

Figure 3.9. Mold preparation against leakage

Finally, an apparatus was also assembled to the mold where the silicone hose was connected. This connection can be seen in Figure 3.10.

Figure 3.10. Silicone hose connection to the direct shear test box

3.2.3. Preparation of Specimen with and without Bio-catalysis

An injection scheme with two stages was applied to prepare the bio-treated specimens. The injections were applied from the bottom of the specimen using a peristaltic pump (Longer Pump Dispensing Peristaltic Pump Model BT100-1F with DG-1-6 rollers). Before beginning the treatment injections, two pore volumes of deionized water was flushed through each specimen with an injection rate of 10 mL/min in order to saturate them. By doing so, a relatively controlled flow field was aimed (Martinez et al., 2013). For the rest of the injections, 1.1 pore volume of solution was flushed through the specimens. The additional volume was given to fill the voids in the mold such as pores in the porous stone. In the first stage of the injection, the bacteria were introduced to the sand specimen from the bottom with a flow rate of 5 mL/min. The first injection included bacterial cells and urea medium. The calcium stock solution was added to the urea medium just before the finishing of the bacterial solution to prevent early

precipitation of calcium carbonate. Harkes et al. (2010) also suggested injection of a solution with high salt content after introducing the bacterial solution as retention of bacteria in the soil matrix enhances with increasing salt concentration. In the second stage, the cementation solution was applied with an injection rate of 10 mL/min. The first injection of the second stage applied after six hours of the introduction of bacteria to allow the bacteria to attach to the soil matrix. The time between the other injections was three hours on the same day. The treatment schemes applied for procedures with 10 and 20 treatments are given in Tables 3.4 and 3.5, respectively. Finally, the preparation of the specimens without bio-catalysis followed the same procedure with treated specimens except for the introduction of the microorganisms.

After three hours from the last injection, distilled water was flushed through the specimens to terminate the chemical reactions by removing the substances not entering into the reaction. After that, the water level on the molds was monitored as water could evaporate while waiting for the shearing stage. When a drop of the water level was observed, water was added in order to avoid specimen from drying.

Table 3.4. *Treatment scheme for 10 injections*

Before treatment	**Day 1**	**Day 2**	**Day 3**
-The growth of bacteria and harvesting -Cementation media preparation -Mold preparation against leakage	3	4	3

Table 3.5. *Treatment scheme for 20 injections*

Before treatment	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
-The growth of bacteria and harvesting -Cementation media preparation -Mold preparation against leakage	3	4	3	4	Flushing with water + 1	2	3

3.2.4. Shear Test Procedure

To evaluate the strength of the specimens treated with and without introduction of the bacteria a direct/residual shear test machine was used (UTS-2060, UTEST) (Figure 3.11). During the test, the measurement for force, and horizontal and vertical displacements were recorded by calibrated linear variable differential transformers (LVDT).

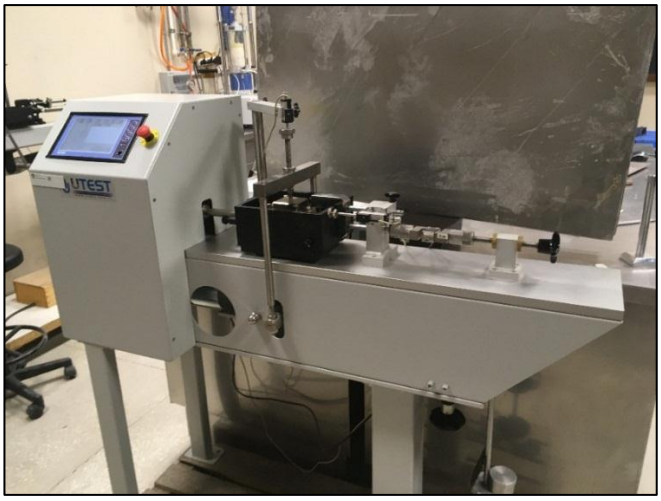


Figure 3.11. Direct shear test setup

At the beginning of the test, the specimens were immersed in the water and kept there for a minimum two hours. As stated before, the surface of the specimens was not allowed to get dry after injections ended. It was assumed that considering the saturation time recommended by ASTM for sandy soils, the specimens were saturated under these circumstances.

After the saturation stage, the LVDT's were placed and consolidation stage was started. The specimens were consolidated under the load that will be applied in the shearing phase. The change in the vertical displacement was monitored following the application of the consolidation load. When the system reached a steady state, the consolidation stage was terminated. Data obtained from consolidation phase were used to determine the shearing rate to obtain drained strength parameters.

Preliminary consolidation tests were performed to determine a common shearing rate. An example data illustrating the relation between root of time and vertical deformation is shown in Figure 3.12.

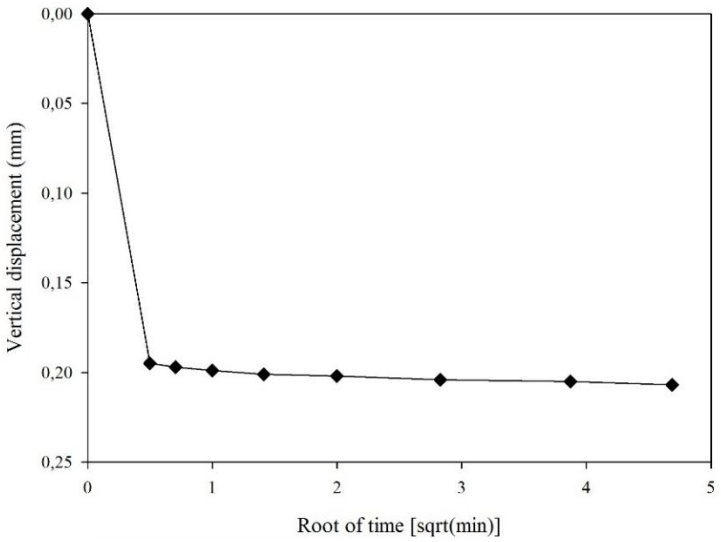


Figure 3.12. Typical root time versus vertical displacement plot of treated specimens

The data for vertical deformation versus time did not show a well-defined relation when time axis was plotted in log scale. On the other hand, plots drawn with axis scaled with respect to root of time showed a better relation. Therefore, to determine the time to failure Eq. (1) was used according to ASTM standard for direct shear test (ASTM D3080).

$$t_f = 11.6t_{90} \quad (1)$$

The rate of shearing was determined using Eq. (2).

$$R_d = \frac{d_f}{t_f} \quad (2)$$

Lateral displacement at failure,d_f, was estimated as 2 mm considering the pilot tests.

Finally, the well-known formula [Eq. (3)] was used to calculate shear strength:

$$\tau = c' + \sigma' tan\phi' \quad (3)$$

3.3. Scanning Electron Microscopy

Finally, in order to visually examine the microbially induced precipitation formations on the surface of the sand grains scanning electron microscopy (SEM) was used in this study. For preparation purposes, the samples were first oven-dried at 60 ^{o}C overnight and subsequently was subjected to a coating process for SEM analysis. After coating completed, the specimens were ready for SEM and energy dispersive X-ray spectrometer (EDS), which was used to analyze the chemical composition of samples.

Having successfully prepared the samples for testing, the next step is to perform the tests. The next section simply describes the results of the direct shear tests as well as the ones obtained from both SEM and EDS. The discussion is given together with the results.

CHAPTER 4

DIRECT SHEAR TESTS ON TREATED SAND SPECIMENS

To date, researchers have performed various geomechanical tests to quantify the improvement of the strength properties and the change in the behavior of the biologically treated soils as summarized in Chapter 2. These tests included the simple tests like permeability tests (Filet et al., 2012; Al Qabany and Soga, 2013; Dawoud et al., 2014), unconfined compression test (van Paassen et al., 2010; Oliveira et al., 2013; Montoya et al., 2013; Al Qabany and Soga, 2013), California bearing ratio test (Chou et al., 2011), direct shear test (Chou et al., 2011; Canakci et al., 2015; Zamani et al., 2017; Hataf and Jamali, 2018; Cheshomi et al., 2018) etc. and more complex tests such as static (DeJong et al., 2006; Ozdogan, 2010; Montoya and DeJong, 2015; Lin et al., 2016) and cyclic triaxial tests (Burbank et al., 2013). In this chapter, the strength characteristics of sand samples on which biomineralization process was applied are examined by performing direct shear tests. Direct shear tests are preferred, as their results are widely used for understanding the behavior of sand samples, and they are generally preferred when field applications are considered.

In order to experimentally examine the possibility of biomineralization through hydrolysis of urea by urease as a sandy soil improvement technique, the experiments in this study were divided into three components:

- determination of direct shear strength of a poorly graded sand under various conditions including treatment with two bacteria strains (namely, *Sporosarcina pasteurii* and *Bacillus licheniformis*), sand relative density (relative densities of 40 and 70 percent), and number of treatments (10 and 20 injections of cementation solution)

- assessment of the catalysis effect of bacteria by comparing the improvements with and without introducing bacteria,
- verification and quantification of mineral precipitation via scanning electron microscopy (SEM) and energy dispersive X-ray spectrometry (EDS).

In Chapter 3, the specimen preparation procedure was developed in the light of the literature as there is no standard test procedure for bio-treated soils. The developed procedure was followed during specimen preparation.

Three points, obtained through shear tests under various normal stress levels, were used to define the soil failure envelope. Moreover, all tests were run in duplicates to investigate the variations arising from the homogeneity concerns. Therefore, in total, six specimens were prepared for each combination of relative density, bacteria strain, and treatment number. The injection stage of the sample preparation can be seen in Figure 4.1. The shearing rate was decided by performing pilot compression tests on the treated specimens to assure drained conditions. A common shearing rate, 0.03 mm/min, was used for testing. After obtaining two peak shear stress values corresponding a normal stress level, the average of them was taken and one of the points forming failure envelope was acquired accordingly.

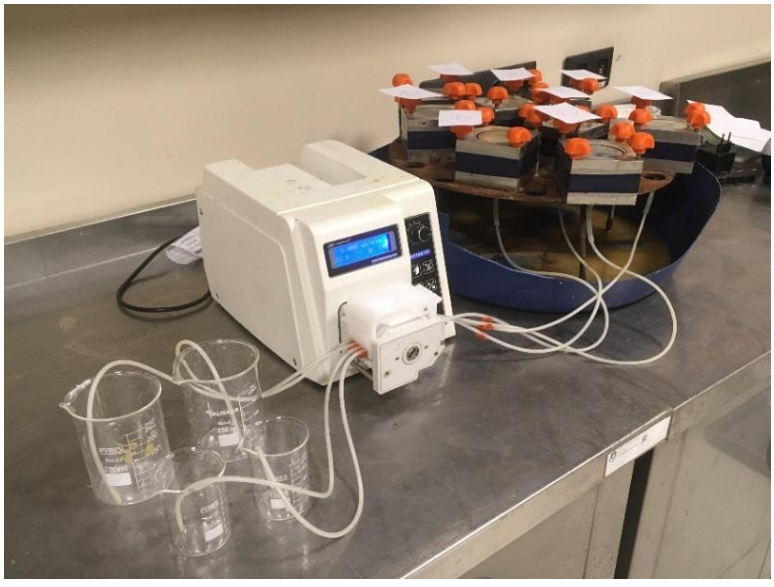


Figure 4.1. Specimen preparation stage

As mentioned in Chapter 2, the efficiency of the MICP process is highly dependent on the temperature. Whiffin (2007) studied the temperature effect on the urease activity of *S. pasteurii* and concluded the study that in the range of 25-60 °C the activity increases with temperature and reaches an optimum value at 70 °C. During specimen preparation, the temperature of the environment was in the range of 20-25 ^{o}C.

4.1. Direct Shear Test Results

In the following sections, the effects of the bacteria strain/condition, sand density and number of injections on strength improvement are discussed. To more clearly illustrate the results and understand the effects of individual variables, a notation is adopted including bacteria condition, injection number, and relative density, combined with "_" sign. For example, for the sample treated with *Sporosarcina pasteurii* by applying 10 treatments and prepared at a relative density of 40 percent is shown as sp-10_rd40. In addition, "bl" is used for *Bacillus licheniformis* and "nm" stands for treatments with no microorganism.

The results obtained from the direct shear tests for 10 and 20 treatment applications are summarized in Tables 4.1 and 4.2, which are provided for the strength parameters, friction angle (ϕ), and cohesion (c), respectively.

The small cohesion values obtained may be related to the sensitivity of the measurement system. No correlation was found between these values.

Table 4.1. *Summary of strength parameters for treated sand in the case of 10 treatment applications*

		Bacteria condition			
Relative Density		*No treatment and bacteria*	*Sporosarcina pasteurii*	*Bacillus licheniformis*	*No bacteria*
40%	Friction angle, ϕ	32,6°	36,6°	34,7°	32,3°
	Cohesion, kPa	0,9	1,1	0,6	1,5
70%	Friction angle, ϕ	37,5°	40,3°	38, 0°	37,6°
	Cohesion, kPa	0,6	0	0,7	0

Table 4.2. *Summary of strength parameters for treated sand in the case of 20 treatment injections*

		Bacteria condition			
Relative Density		*No treatment and bacteria*	*Sporosarcina pasteurii*	*Bacillus licheniformis*	*No bacteria*
40%	Friction angle, ϕ	32,6°	37,1°	36,3°	32,6°
	Cohesion, kPa	0,9	2,3	1,4	1,4
70%	Friction angle, ϕ	37,5°	39,9°	37,3°	37,2°
	Cohesion, kPa	0,6	0,4	1,8	0,2

4.2. Effect of Microorganism Strain and Condition on Strength Improvement

In this section, the effect of treatment using two bacteria strains, *S. pasteurii* and *B. licheniformis*, and treatment without introduction of microorganism on strength improvement are discussed.

As mentioned in Chapter 2, bacteria involve in the bio-cementation process with two mechanisms (Stocks-Fischer et al., 1999; DeJong et al., 2006). The first mechanism is

that bacteria act as a nucleation site as cations (such as Ca^{2+}) attach to the negatively charged cell surface. For example, Chou (2007) obtained strength increase when dead cells were used although the improvement was not comparable with the application where live cells were used. Secondly, microbial activity, i.e., hydrolysis of urea by urease, increases the alkalinity of the environment and promotes the precipitation of calcium carbonate. Furthermore, the urease activity, hence, the rate of ureolysis, determines the morphology of the precipitated calcium carbonate crystals. van Paassen (2009) states that while higher urea hydrolysis rates dominantly produce spherical vaterite, a less stable form of calcium carbonate, and amorphous calcium carbonate, low hydrolysis rates produce rhomboidal calcium carbonate.

The friction angles of the untreated sand having relative density values of 40% and 70% are 32.6 and 37.5, respectively. Tables 4.1 and 4.2 show that a significant strength improvement is possible when treatment using bacteria is applied and the primarily affected strength parameter is friction angle. This observation can be attributed to the $CaCO_3$ precipitation pattern. The precipitation may have occurred dominantly on the surface of the grains and increased their roughness which resulted in increasing the internal friction angle instead of cohesion (for further discussion on this topic see section 4.4). Besides, treatments with *S. pasteurii* have been more effective on the friction angles when compared to that of *B. licheniformis*. For example, the increase in friction angles of the sand treated with *S. pasteurii* after 10 injections and prepared at relative densities of 40% and 70% were 12% and 8%, respectively. In comparison, the increase was 6% and 2% for the sand treated with *B. licheniformis* under same conditions (see Figures 4.2 and 4.3). The peak shear stresses are also significantly increased when *S. pasteurii* used as the biological agent as it can be seen in Figures 4.2, 4.3, 4.4 and 4.5. The standard deviation values for the tests performed with specimens treated with *B. licheniformis* are higher than those of *S. pasteurii*. On the other hand, treatments without bacteria introduction had no effect on the friction angle for any of the relative density and number of injection combination. This behavior was expected as there is a significant difference in the reaction rates between microbially

and chemically induced $CaCO_3$ precipitation due to catalysis effect of urease (Stocks-Fisher et al., 1999). The failure envelopes of the untreated sand and sand treated without bacteria introduction are not shown in the same graph for the sake of clarity of the graphs as they overlap.

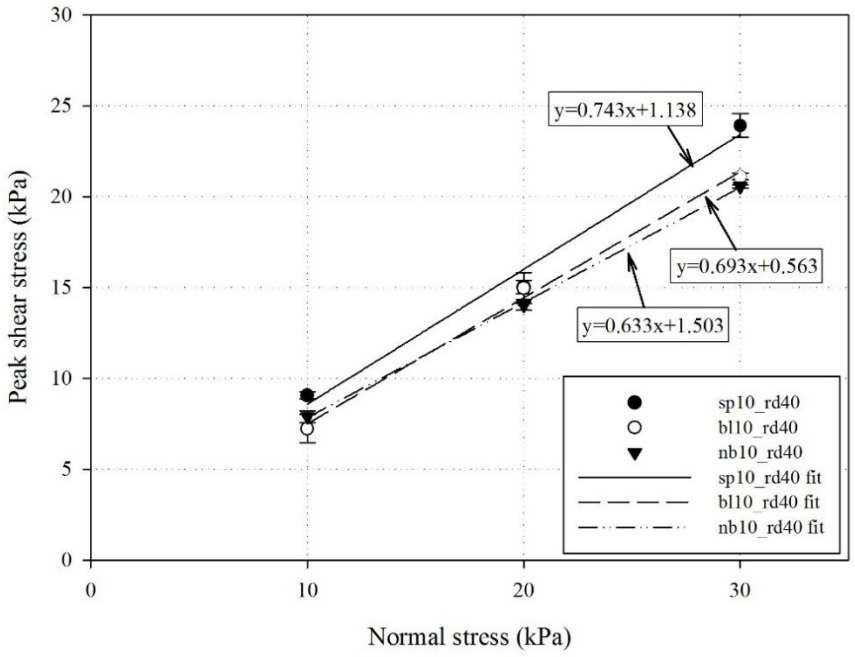


Figure 4.2. Effect of bacteria on friction angle and cohesion of sand prepared at a relative density of 40% and treated with 10 injections of cementation solution

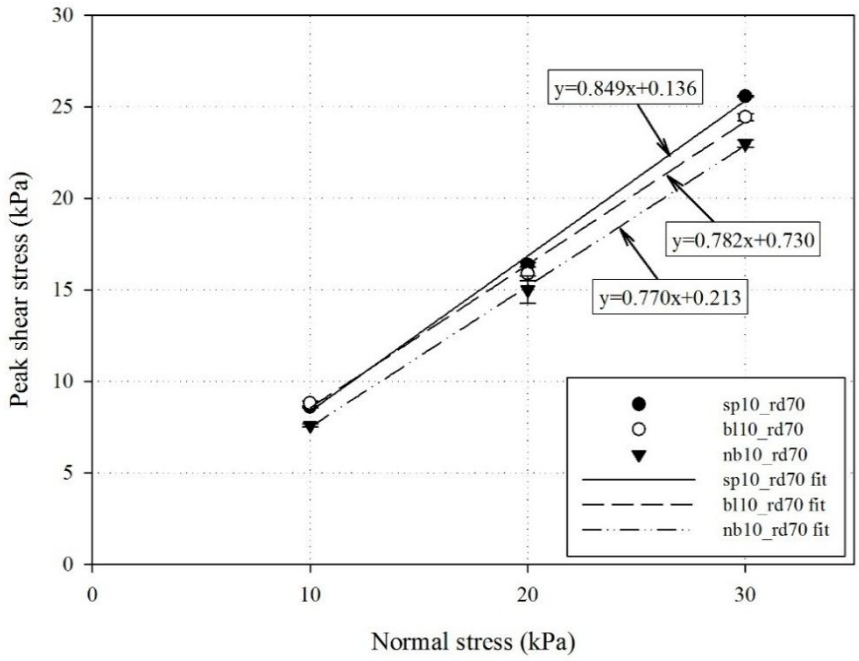


Figure 4.3. Effect of bacteria on friction angle and cohesion of sand prepared at a relative density of 70% and treated with 10 injections of cementation solution

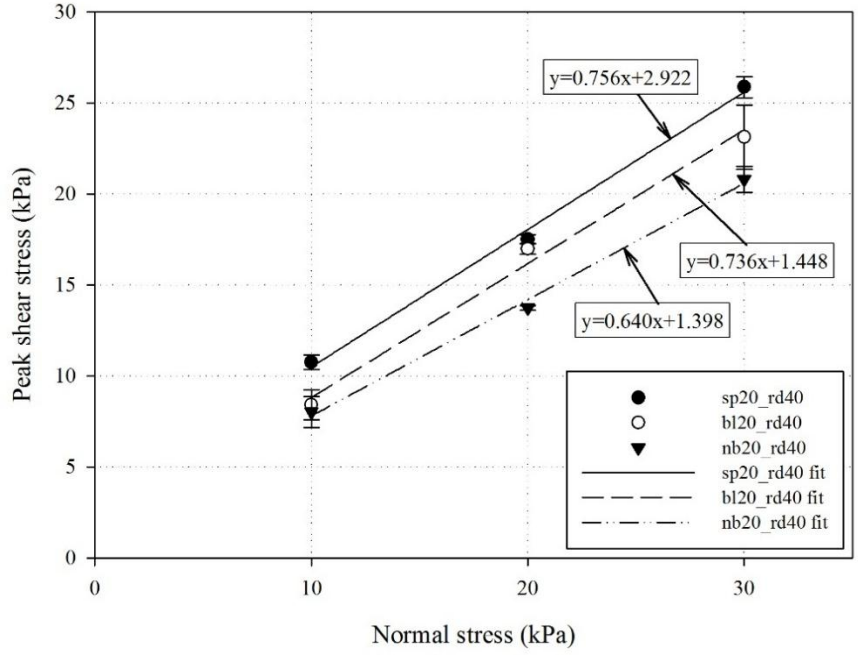


Figure 4.4. Effect of bacteria on friction angle and cohesion of sand prepared at a relative density of 40% and treated with 20 injections of cementation solution

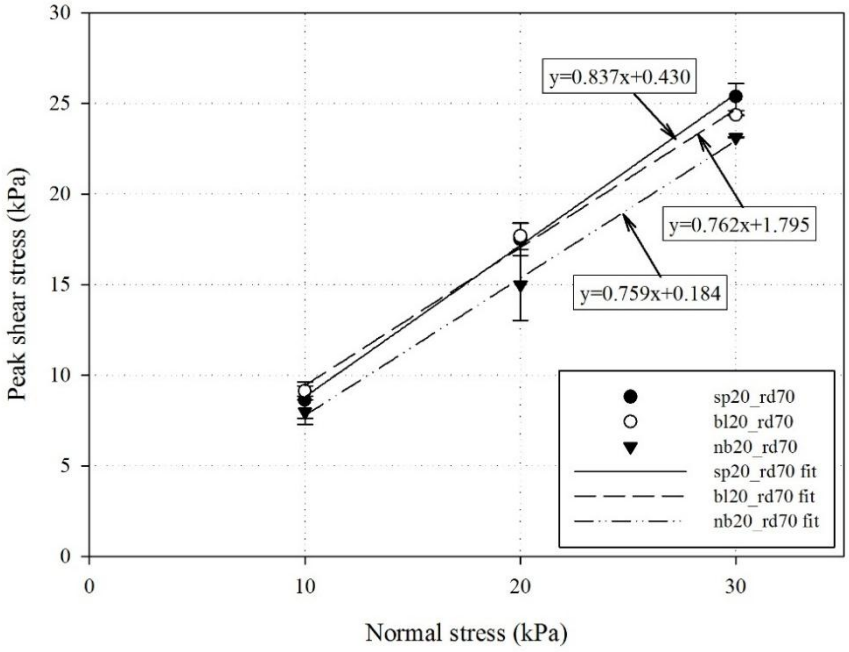


Figure 4.5. Effect of bacteria on friction angle and cohesion of sand prepared at a relative density of 70% and treated with 20 injections of cementation solution

4.3. Effect of Sand Density on Strength Improvement

The homogeneity of the distribution of the precipitated calcium carbonate is affected by the distribution of the microorganism as precipitation primarily takes place around the bacterial cells. The pore throat size directly affects the mobility of the microorganism in the soil matrix, hence the distribution. Considering the fact that the size of the bacteria is in the range of 0.5 and 3 μm (Mitchell and Santamarina, 2005), there is an applicable and effective size range of soil for bio-treatment (Rebata-Landa, 2007). Furthermore, bacteria prefer to settle around the grain contact points, which increase with increasing compaction, as it is favorable in terms of nutrients (DeJong et al., 2010). Therefore, soil compactness can either positively or negatively affect the precipitation efficiency. Figures 4.6, 4.7, 4.8 and 4.9 show the effect of compaction on the strength parameters with respect to bacteria strain and the number of injections.

For the specimens prepared at a relative density of 40%, the increase in the friction angles with respect to friction angle of the specimens not subjected to any treatment application was in the range of 12-13% and 6-11% for *S. pasteurii* and *B. licheniformis*, respectively. However, for the denser specimens, prepared at a relative density of 70%, almost no change in friction angle was obtained when *B. licheniformis* was used as microorganism. The changes for the friction angles were in the range of 6-8% and 1-2% for *S. pasteurii* and *B. licheniformis*, respectively. The less increase in the friction angle of the specimens prepared at relative density of 70% relative to 40% can be attributed to the restriction of the movement of bacteria due to the smaller pore throat size of denser specimens. It should be noticed that the improvement in friction angle was more significant for the loosely compacted sand.

The obtained peak stresses for the specimens treated with *S. pasteurii* and prepared at relative density of 40% are higher than those of specimens prepared at relative density of 70% and treated without introduction of microorganism (Figures 4.6 and 4.8). This was not the case for the specimens treated with *B. licheniformis* (Figures 4.7 and 4.9).

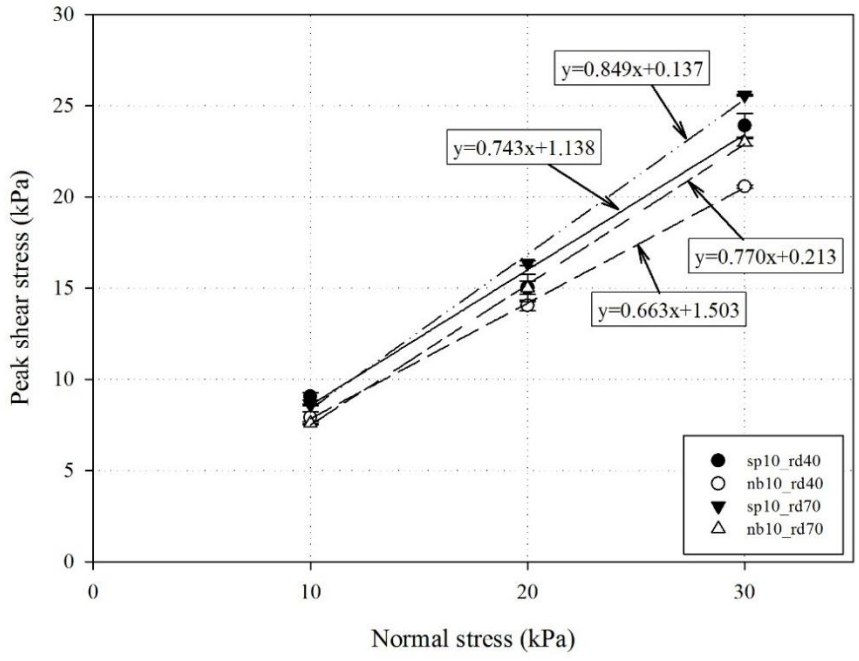


Figure 4.6. Effect of relative density on friction angle and cohesion of sand treated using *Sporosarcina pasteurii* and with the application of 10 injections of cementation solution

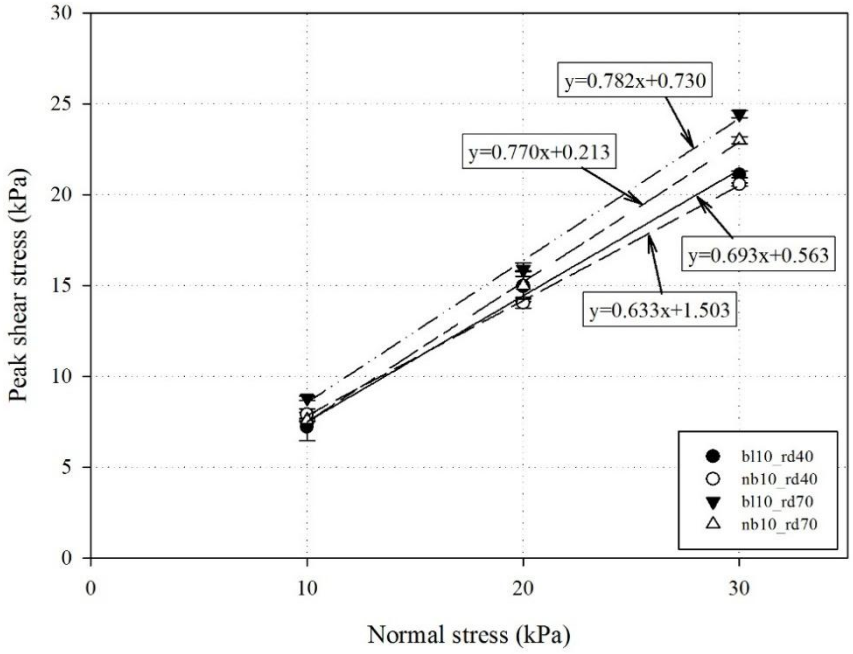


Figure 4.7. Effect of relative density on friction angle and cohesion of sand treated using *Bacillus licheniformis* and with the application of 10 injections of cementation solution

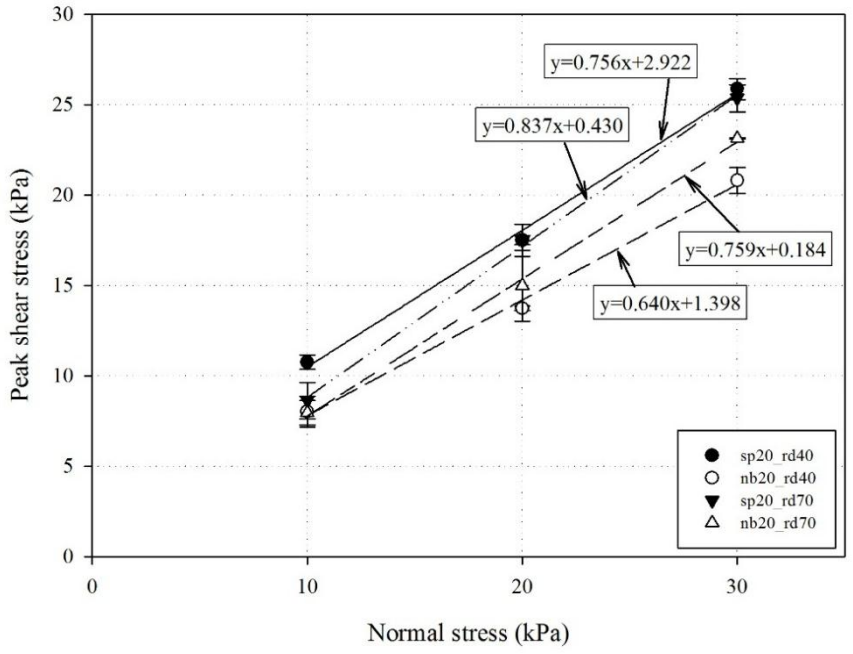


Figure 4.8. Effect of relative density on friction angle and cohesion of sand treated using *Sporosarcina pasteurii* and with the application of 20 injections of cementation solution

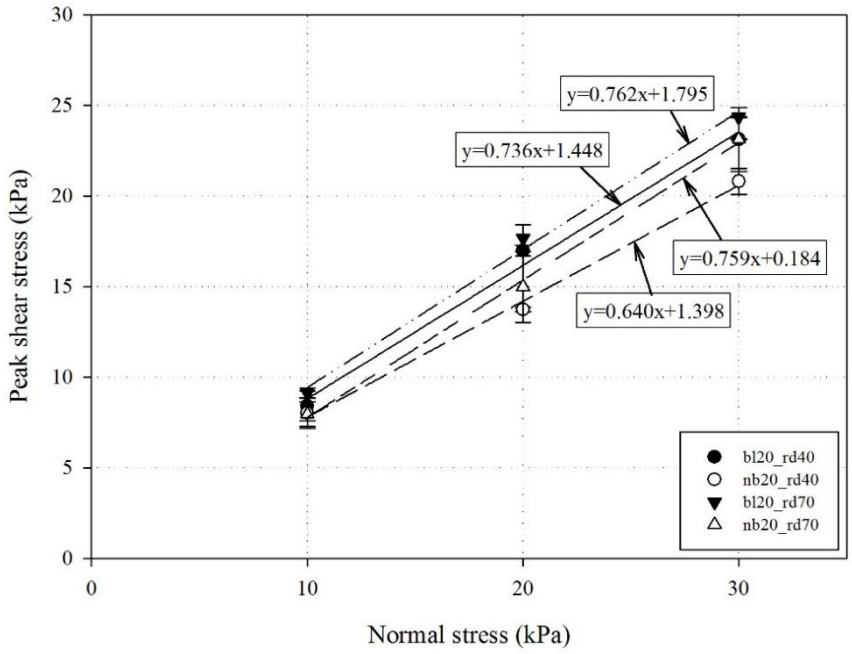


Figure 4.9. Effect of relative density on friction angle and cohesion of sand treated using *Bacillus licheniformis* and with the application of 20 injections of cementation solution

4.4. Effect of Number of Injections of Cementation Solution on Strength Improvement

Although it is understandable to expect more product after injecting more reactant, encapsulation of bacteria as $CaCO_3$ precipitates resulted in rather less improvement. In other words, there may not be a direct correlation with the injected chemical amount and the obtained precipitation. The encapsulation of bacteria can be a reason of this behavior. Due to encapsulation, access of bacteria to the nutrients prohibited so that the increase in the amount of reactants cannot be effectively used by the bacteria.

The most affected combination by the increase of number of injections is the treatment of specimens prepared at a relative density of 40% with *B. licheniformis* (Figure 4.11). The increase in the friction angle was 6 and 11% for 10 and 20 injections, respectively. For the specimens treated with *S. pasteurii*, the increase in the friction angle was almost same. The increase for the specimens having relative density of 40% was 12

and 13% for 10 and 20 injections, respectively. In case of relative density value of 70%, 8 and 6% increase were obtained for 10 and 20 injections, respectively.

The friction angle improved highly as injection number increased in case of specimen prepared at a relative density of 40%. For the specimens prepared with a relative density of 70% the improvement was below the that of specimens prepared with a relative density of 40%. Therefore, it can be concluded that for loose sand increasing the number of injections can be beneficial in terms of improvement in friction angle. For the dense sand, however, the benefits may not cover the expenses of extra injected material and injection cost. This observation is coherent with the literature (Chou, 2007).

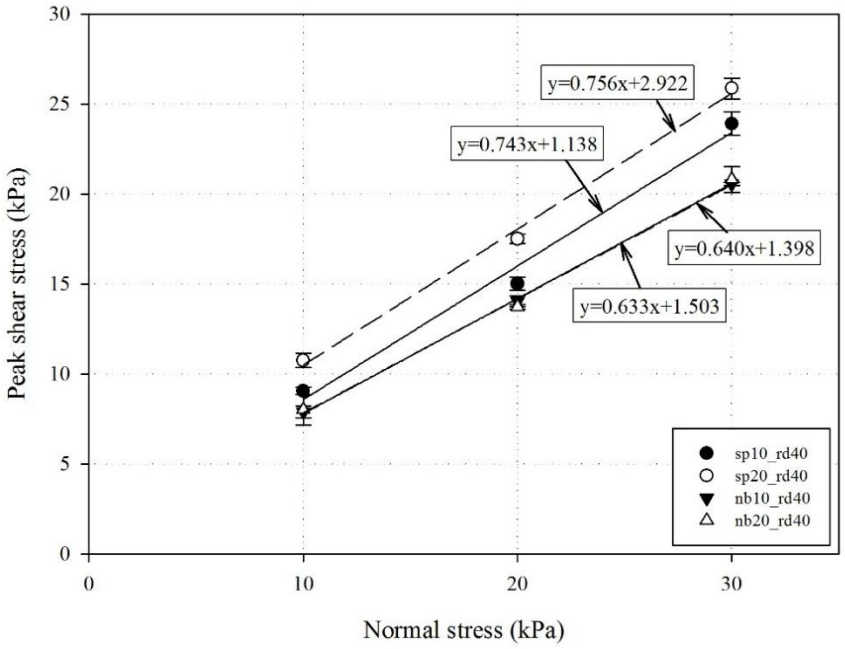


Figure 4.10. Effect of the number of injections of cementation solution of friction angle and cohesion of sand prepared at a relative density of 40% and treated using *Sporosarcina pasteurii*

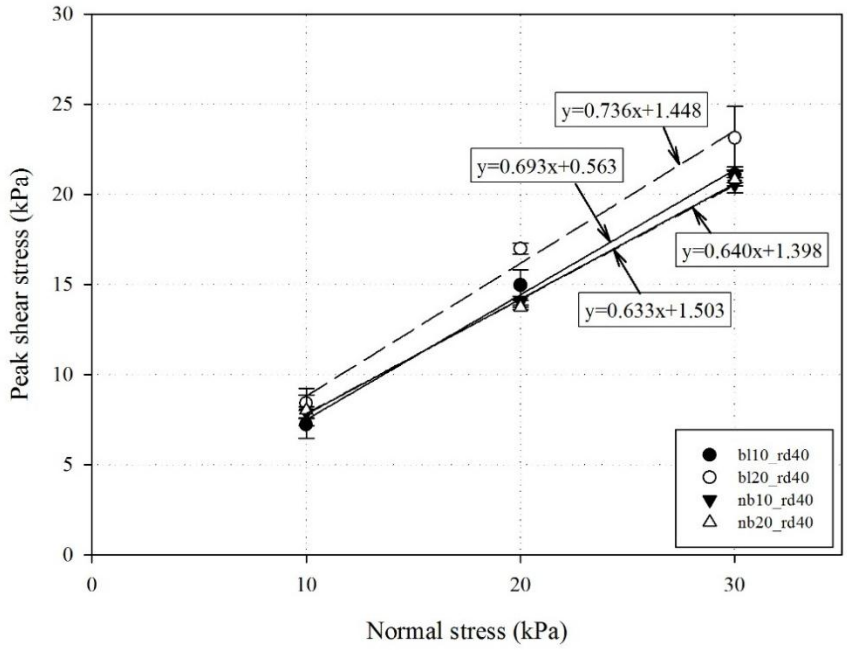


Figure 4.11. Effect of the number of injections of cementation solution of friction angle and cohesion of sand prepared at a relative density of 40% and treated using *Bacillus licheniformis*

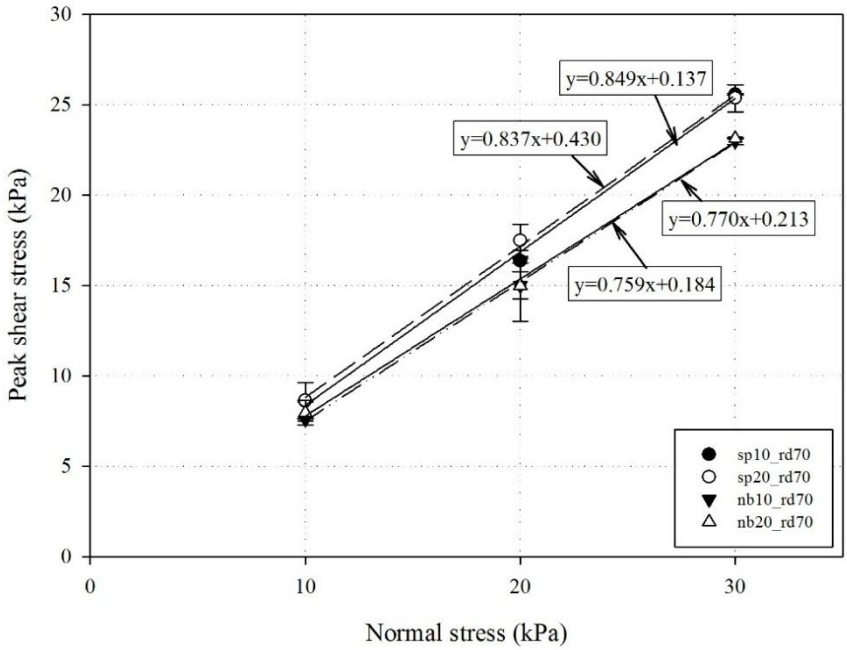


Figure 4.12. Effect of the number of injections of cementation solution of friction angle and cohesion of sand prepared at a relative density of 70% and treated using *Sporosarcina pasteurii*

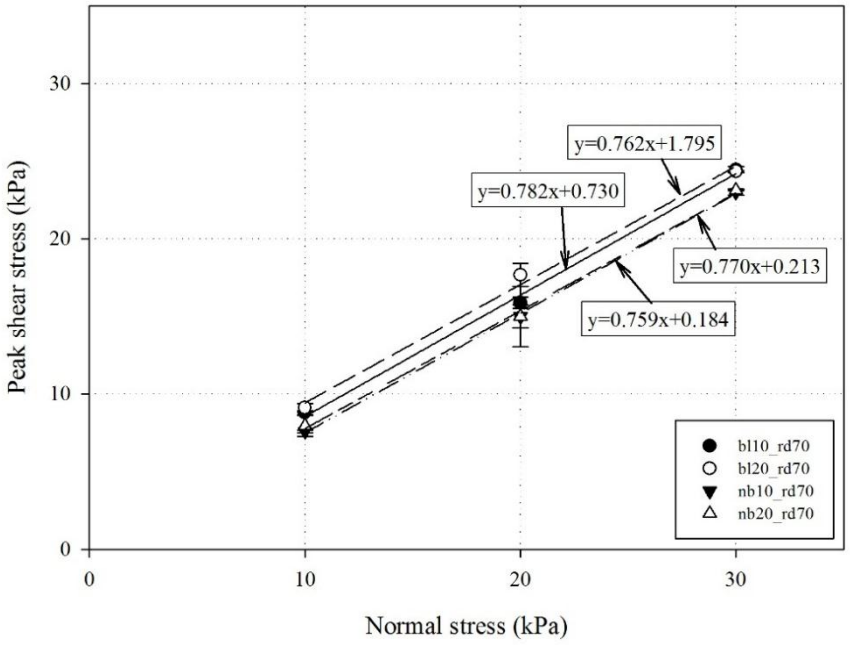


Figure 4.13. Effect of the number of injections of cementation solution of friction angle and cohesion of sand prepared at a relative density of 70% and treated using *Bacillus licheniformis*

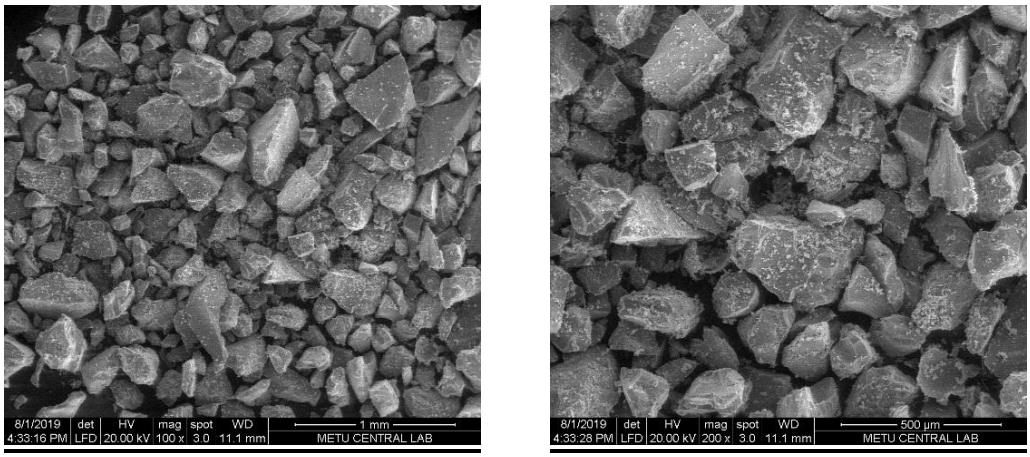
4.5. Observations in Micro-scale

To further support the findings obtained from the direct shear tests and presented above, the occurrence of $CaCO_3$ precipitation was investigated using advanced imaging tool SEM and the element composition identified by using EDX. Figures demonstrate the $CaCO_3$ formations in the soil matrix. The micrographs show that the $CaCO_3$ precipitation on sand grains when *S. pasteurii* used was more intense than the ones treated with *B. licheniformis*.

No $CaCO_3$ accumulation was observed on the specimen treated without bacteria (Figure 4.14). Figures 4.15 to 4.18 demonstrate the $CaCO_3$ accumulation on the sand grains for different combinations of relative density and number of injections for the samples treated with *S. pasteurii*. The majority of the observed accumulation can be classified as grain coating. On the other side, almost no grain contact type accumulation was observed with SEM. These observations also support the findings

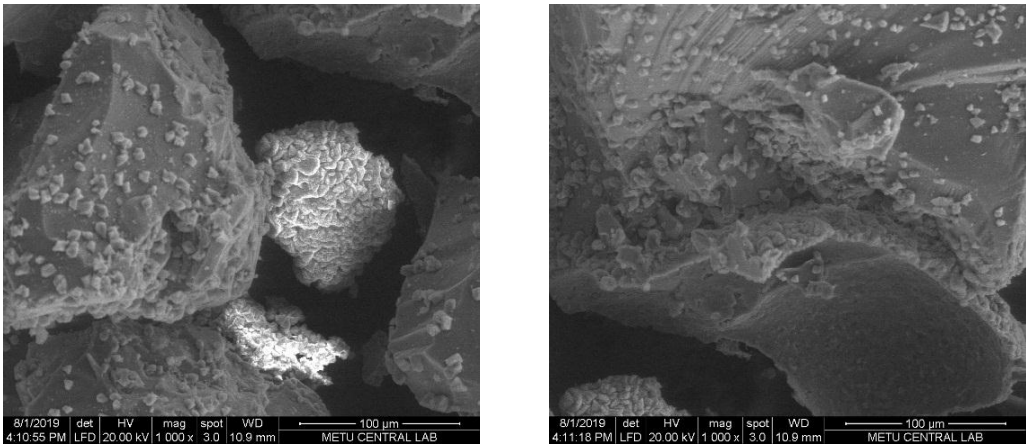
in strength tests. The observation that the strength increase is predominantly at the friction angle may be related to this finding. The roughness of the grains increased with the CaCO3 coating of the grains. Increased roughness also brought an increase in the friction angle. The proper images for the specimens treated with *B. licheniformis* could only be obtained from the specimens prepared at a relative density of 40% and treated through 20 injections (Figure 4.19) as expected from the strength test results. For the specimens prepared at relative density of 70% and treated with *B. licheniformis*, poor cementation was observed in any case of the number of injections.

The element composition profiles obtained from magnified SEM images with EDS are given in Figures 4.20 and 4.21. The profile of the sand treated without bacteria introduction shows high percentage of silica and very low percentage of calcium. The obtained calcium is most probably the residue of the cementation solution whereas the silica is the sand itself. On the other side, the profile given in Figure 4.21 shows high percentage of calcium indicating the precipitation of calcite. It should be also noted that the peak of oxygen is due to intrusion of oxygen and water during the SEM operation.



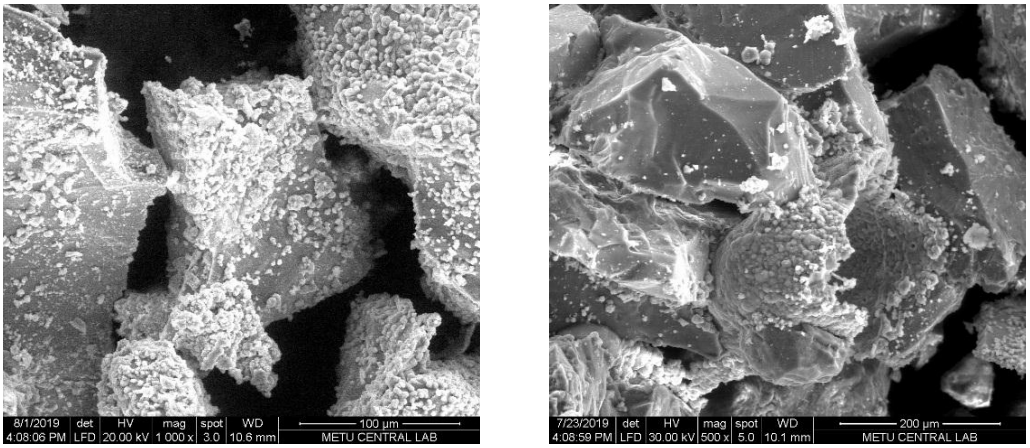
(a) (b)

Figure 4.14. Scanning electron microscopy micrographs of sand sample prepared at a relative density of 40% and treated without bacteria introduction through 10 injections (a) 100x; (b) 200x



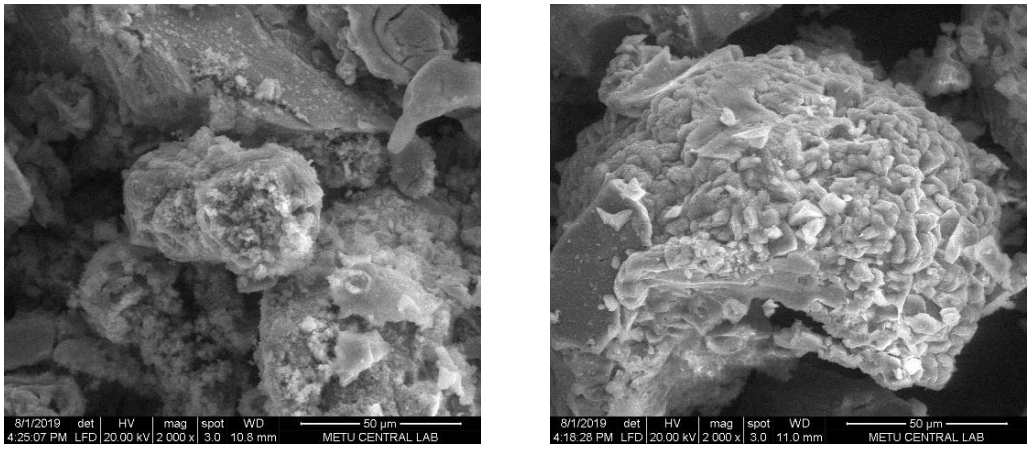
(a) (b)

Figure 4.15. Scanning electron microscopy micrographs obtained from different locations of sand sample prepared at a relative density of 40% and treated with *Sporosarcina pasteurii* through 10 injections



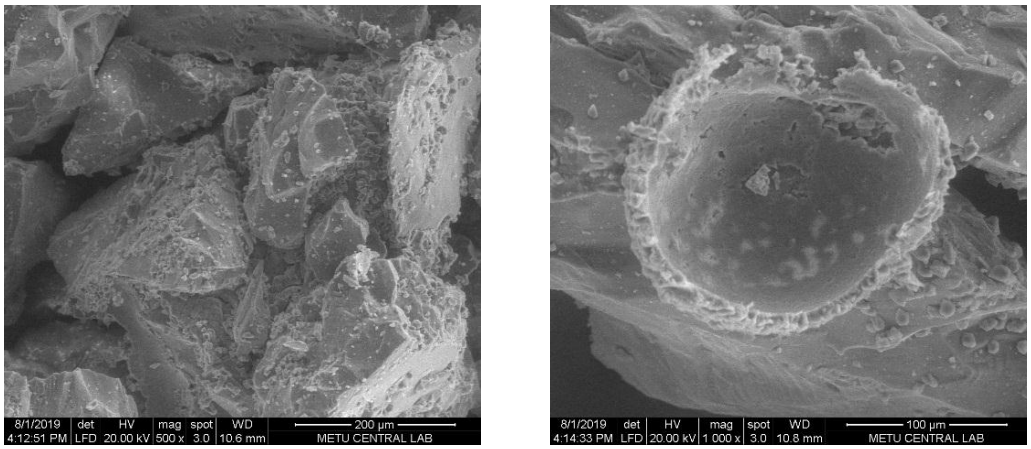
(a) (b)

Figure 4.16. Scanning electron microscopy micrographs obtained from different locations of sand sample prepared at a relative density of 40% and treated with *Sporosarcina pasteurii* through 20 injections



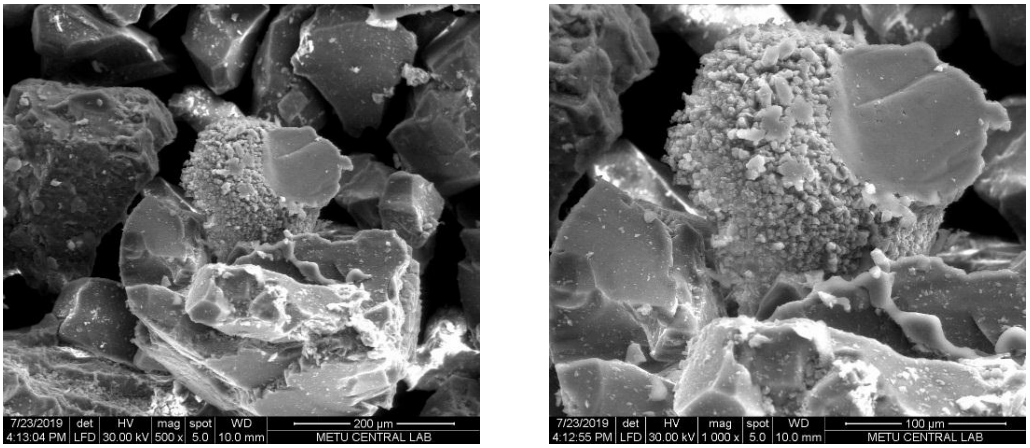
(a) (b)

Figure 4.17. Scanning electron microscopy micrographs obtained from different locations of sand sample prepared at a relative density of 70% and treated with *Sporosarcina pasteurii* through 10 injections



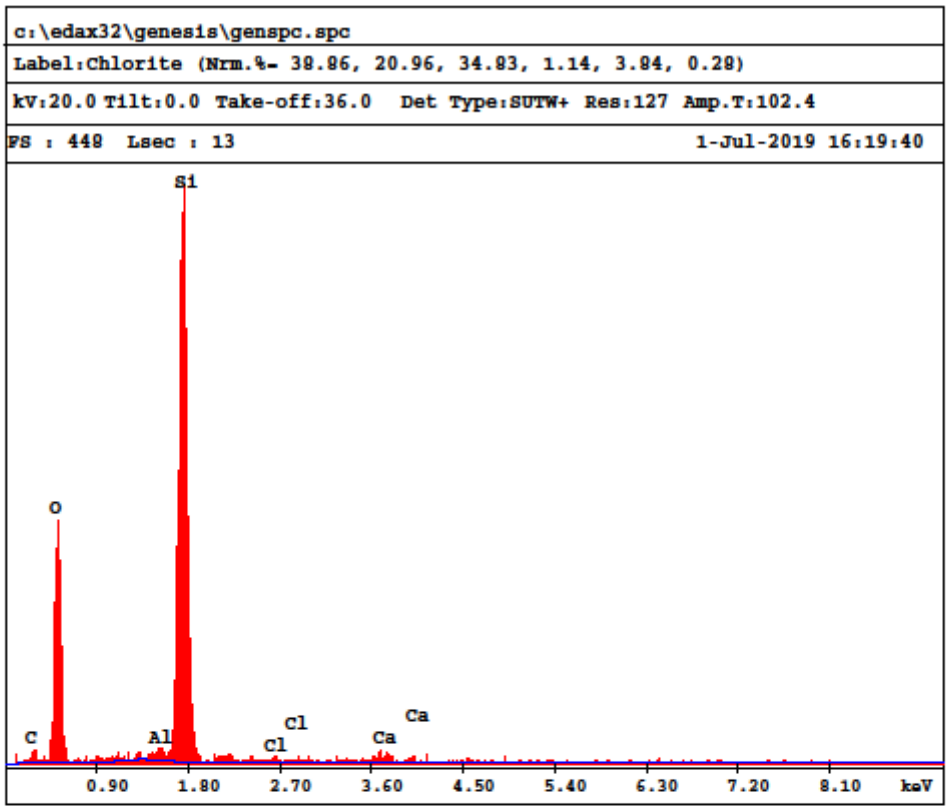
(a) (b)

Figure 4.18. Scanning electron microscopy micrographs obtained from different locations of sand sample prepared at a relative density of 70% and treated with *Sporosarcina pasteurii* through 20 injections



(a) (b)

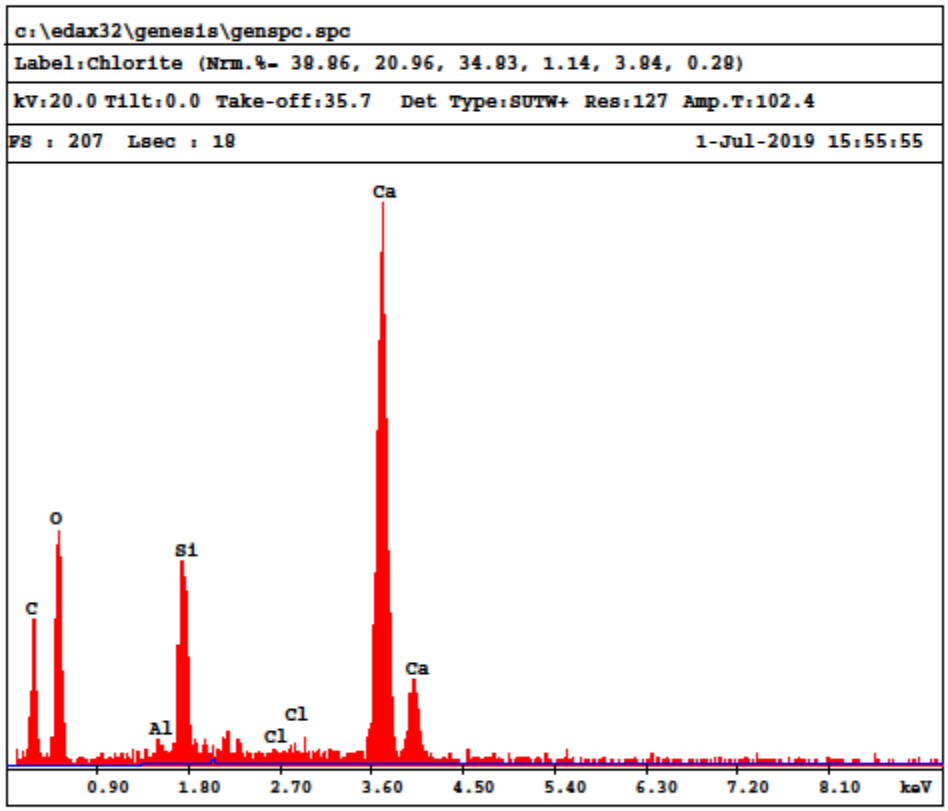
Figure 4.19. Scanning electron microscopy micrographs of sand sample prepared at a relative density of 40% and treated with *Bacillus licheniformis* through 20 injections (a) 500x; (b) 1000x



EDAX ZAF Quantification (Standardless)
Element Normalized
SEC Table : Default

Element	Wt %	At %	K-Ratio	Z	A	F
C K	8.99	14.45	0.0117	1.0356	0.1259	1.0005
O K	44.86	54.13	0.1318	1.0183	0.2883	1.0005
AlK	0.50	0.36	0.0036	0.9484	0.7386	1.0221
SiK	43.99	30.24	0.3620	0.9760	0.8429	1.0003
ClK	0.43	0.24	0.0030	0.9180	0.7579	1.0006
CaK	1.22	0.59	0.0106	0.9470	0.9186	1.0000
Total	100.00	100.00				

Figure 4.20. Element profile of sand grains taken from sample treated without bacteria introduction



EDAX ZAF Quantification (Standardless)
Element Normalized
SEC Table : Default

Element	Wt %	At %	K-Ratio	Z	A	F
C K	25.07	38.47	0.0804	1.0354	0.3098	1.0006
O K	36.73	42.31	0.0558	1.0180	0.1494	1.0001
AlK	0.62	0.42	0.0038	0.9480	0.6530	1.0056
SiK	7.58	4.97	0.0573	0.9757	0.7714	1.0050
ClK	0.36	0.19	0.0031	0.9185	0.9230	1.0276
CaK	29.66	13.64	0.2803	0.9471	0.9978	1.0000
Total	100.00	100.00				

Figure 4.21. Element profile of sand grains taken from sample treated with *Sporosarcina pasteurii*

CHAPTER 5

CONCLUSIONS

5.1. Summary

Biocalcification is one of the fundamental processes observed in natural environments when forming soil like materials. In an appropriate natural setting, the improvement of physical and engineering properties of soft soils can be achieved through utilization of the biocalcification, which may be advantageous when compared to other techniques including the nonconventional ones. In this study, geomechanical properties of sand were altered by utilizing the urease positive bacteria, through a biological process named Microbially Induced Calcium Carbonate Precipitation (MICP). With the motivation of understanding the effects of MICP process on the strength parameters of sand, direct shear tests were conducted. The experimental program consisted of three components: (1) comparison of productivity of two bacteria species, namely, *Sporosarcina pasteurii* (*S. pasteurii*) and *Bacillus licheniformis* (*B. licheniformis*), in terms of strength gain in sands prepared at two different relative densities, 40%, and 70%, and two variation of number of cementation solution injections, 10 and 20 times, (2) application of technique without introducing bacteria to evaluate the catalytic effect of bacteria, (3) verification of the mineral precipitation by using scanning electron microscopy. The choice of the bacteria species was based on the comparison of the $CaCO_3$ precipitation ability of an extensively studied bacteria, *S. pasteurii*, and comparably less recognized one in this field, *B. licheniformis*. Additionally, in the context of the study, a specimen preparation procedure was formed considering the different applications arising in the literature as no standard procedure exists for bio-treatment methods.

5.2. Conclusions

The main framework of this study was limited with the investigation of the efficiency of MICP on strength properties of poorly graded sand. Drawing sound conclusions was a challenging part of the study as it requires combined information of Microbiology, Chemistry and Civil Engineering fields. With the help of the experiments the following conclusions were drawn:

- The treatment improved the strength of the sand in the limits of applied normal stresses. The process seemed to have a more profound effect on the friction angle than it has on cohesion.
- *S. pasteurii* was more effective than *B. licheniformis* when the change in the friction angle considered. For instance, when *S. pasteurii* used as the biological agent at the initial relative density value of 40%, the changes in the friction angle were 12 and 13% for 10 and 20 treatments, respectively. For the same conditions, however, the corresponding changes were 6 and 11% when the biological agent was *B. licheniformis*.
- The efficiency of *S. pasteurii* was decreased when the soil compaction was increased. On the other hand, treatment with *B. licheniformis* resulted in a significant improvement in friction angle when the relative density was 40% whereas almost no change was obtained when relative density increased to 70%.
- The increasing number of treatments was favorable for the sand compacted to a relative density of 40% for both bacteria. For the relative density of 70%, there was no significant effect observed. Therefore, it is concluded that, for higher relative densities the expenses coming from the extra injected material may not cover the benefits obtained.
- The specimens prepared without the introduction of bacteria showed no change in the strength of the sand. This conclusion highlights the reaction accelerating effect of bacteria.

- More intense $CaCO_3$ coating on sand grains was observed for the specimens treated with *S. pasteurii* than that of *B. licheniformis*.
- Total cementation of the specimens was not achieved, instead as proved with the SEM micrographs surface roughness of the grains was increased. Thus, friction angle became the primarily affected strength parameter and almost no remarkable improvement was obtained for the cohesion values.

5.3. Future Works

The challenges and observations experienced during the study brought new questions and opened new horizons for future works. The list of those can be outlined as follows:

- Soil is one of the most important components of MICP as it geometrically (grain size distribution, surface roughness etc.) and/or chemically (pH, organic content) affect the whole process directly. In the current study, poorly graded silica sand was used. Application of the process on diverse types of soils is the next step of this work.
- The use of different types of bacteria to obtain more effective process is also considered for future works. Especially, the use of bacteria with active urease obtained from local soils would be more economical.
- A great majority of the studies have focused on treatment of sandy soils but effects of bio-treatment on silty soils and clayey soils are still not thoroughly investigated. Other bio-treatment techniques such as enzyme induced carbonate precipitation may be promising on this topic.
- The effect of changing the concentration of ingredients of the cementation solution on different bacteria need to be investigated further.
- In this study, laboratory scale specimens were used, but progress in large-scale experiments is essential to an in-depth understanding of behavior in the field. This is also necessary to reveal practical aspects of the technique.

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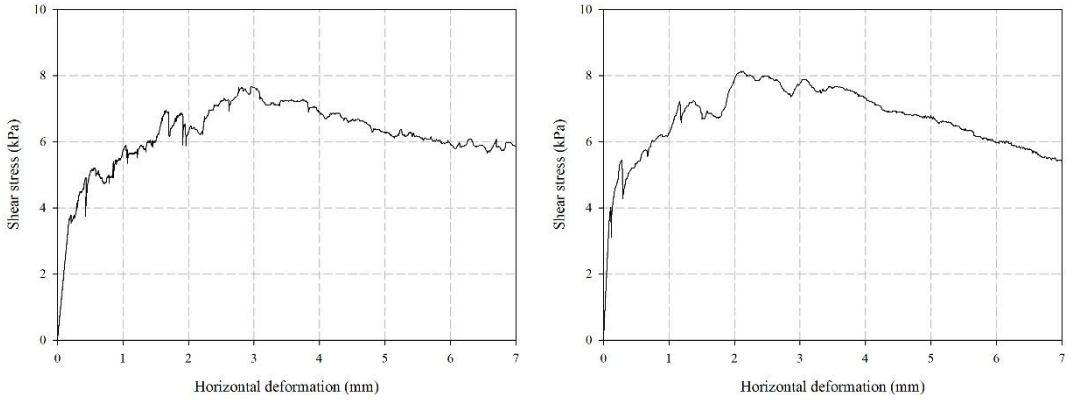
APPENDICES

A. DIRECT SHEAR TEST DATA

In this part of the thesis, direct shear test data are provided.

(a)



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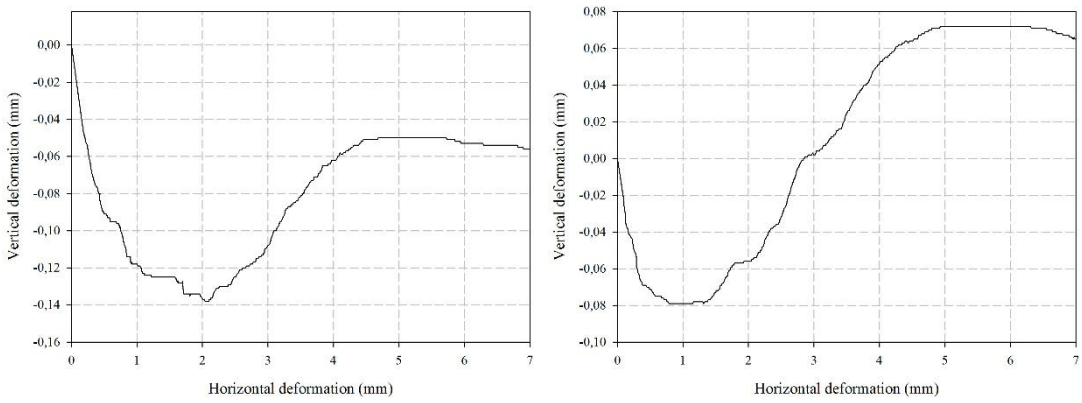
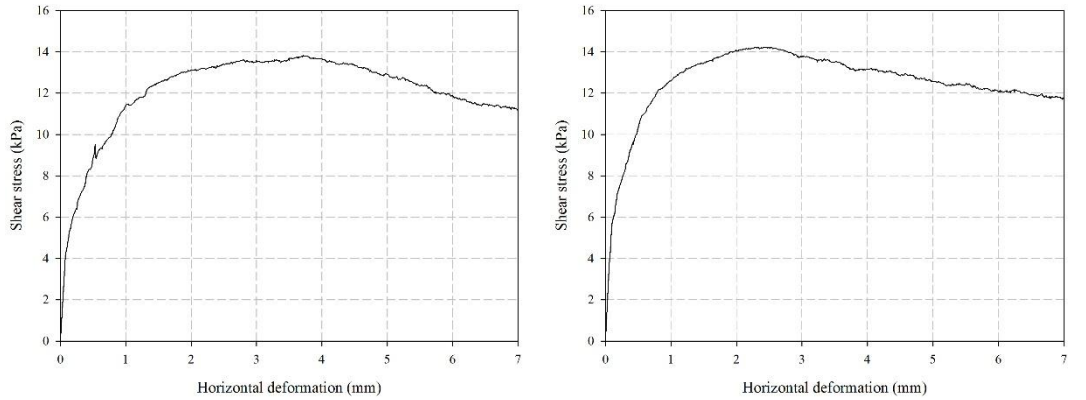


Figure A.1. (a) Horizontal deformation versus shear stress, (b) horizontal deformation versus vertical deformation relationships under 10 kPa normal stress of sand prepared at relative density of 40% and treated without bacteria introduction through 10 injections

(a)



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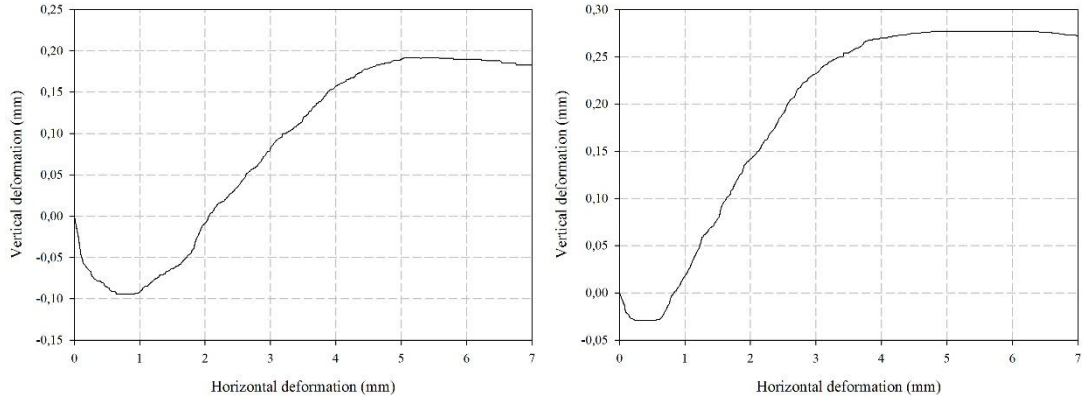
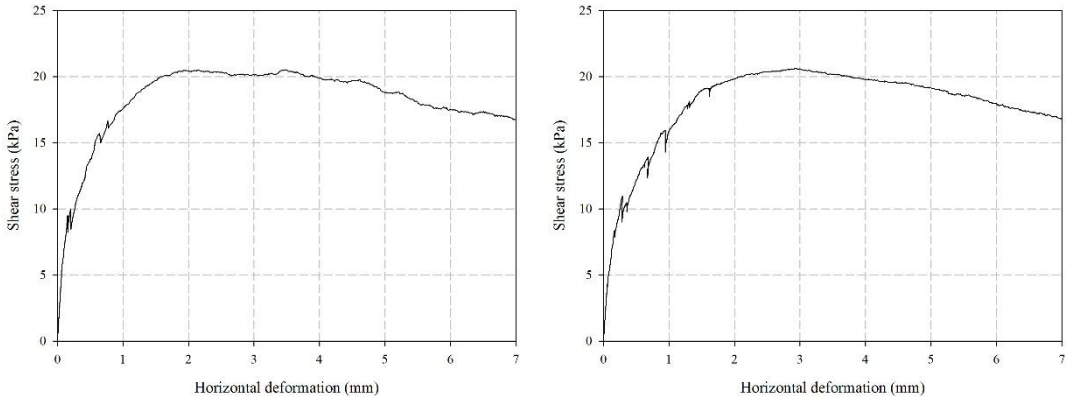


Figure A.2. (a) Horizontal deformation versus shear stress, (b) horizontal deformation versus vertical deformation relationships under 20 kPa normal stress of sand prepared at relative density of 40% and treated without bacteria introduction through 10 injections

(a)



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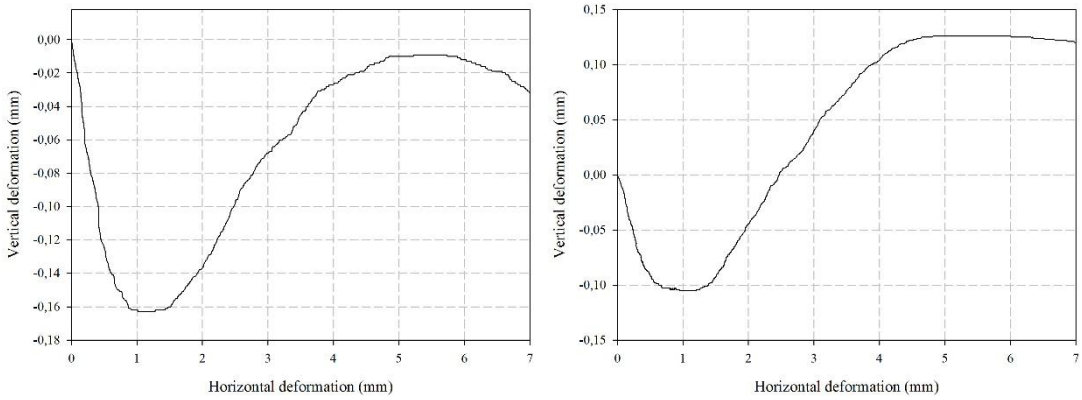
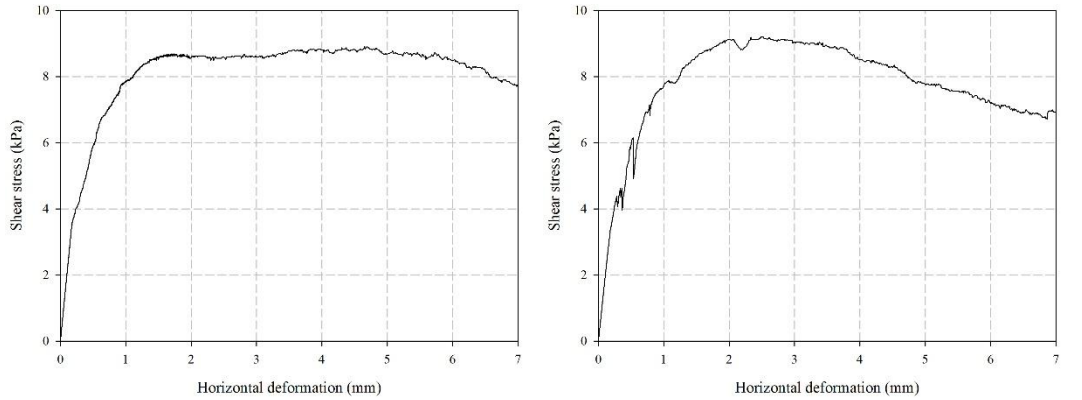


Figure A.3. (a) Horizontal deformation versus shear stress, (b) horizontal deformation versus vertical deformation relationships under 30 kPa normal stress of sand prepared at relative density of 40% and treated without bacteria introduction through 10 injections

(a)



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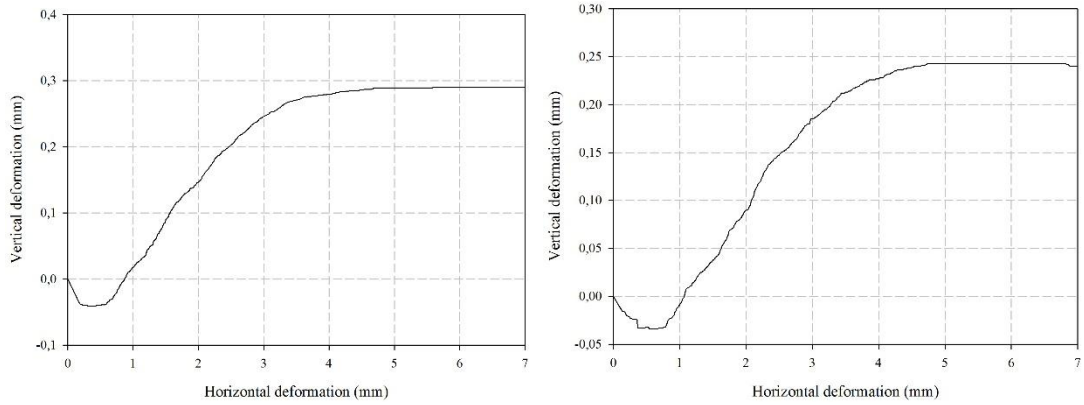
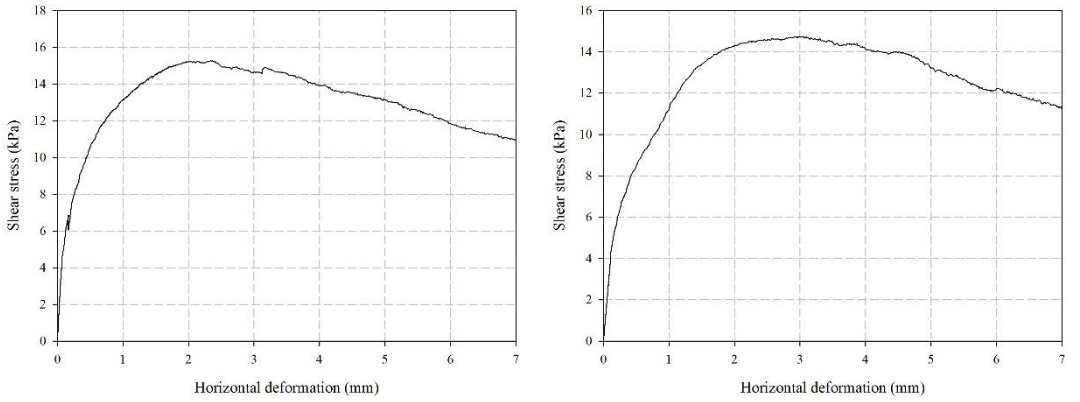


Figure A.4. (a) Horizontal deformation versus shear stress, (b) horizontal deformation versus vertical deformation relationships under 10 kPa normal stress of sand prepared at relative density of 40% and treated with *Sporosarcina pasteurii* through 10 injections

(a)



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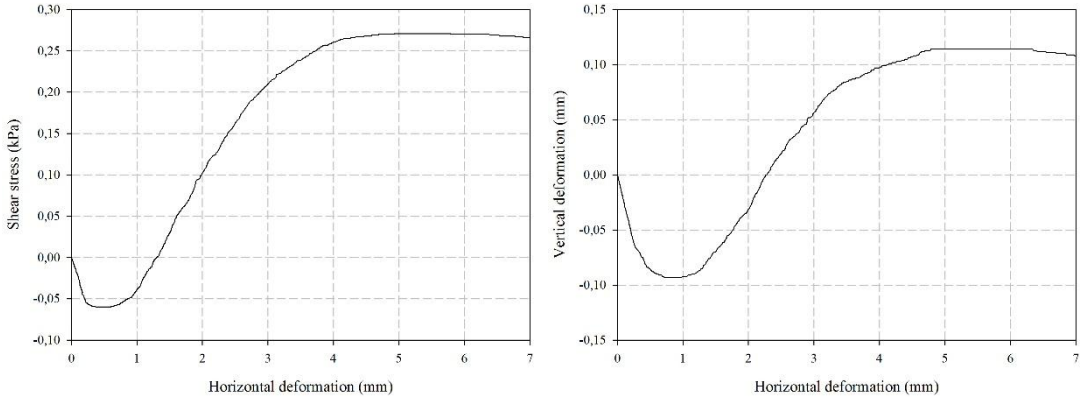
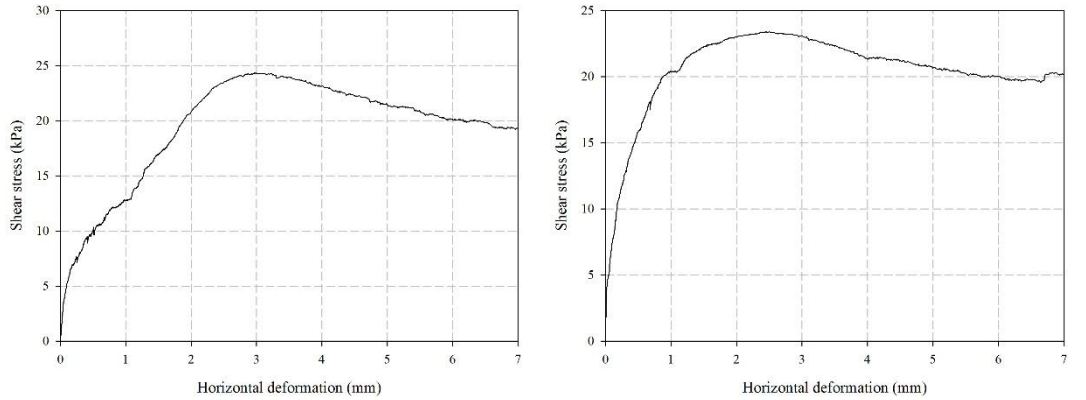


Figure A.5. (a) Horizontal deformation versus shear stress, (b) horizontal deformation versus vertical deformation relationships under 20 kPa normal stress of sand prepared at relative density of 40% and treated with *Sporosarcina pasteurii* through 10 injections

(a)



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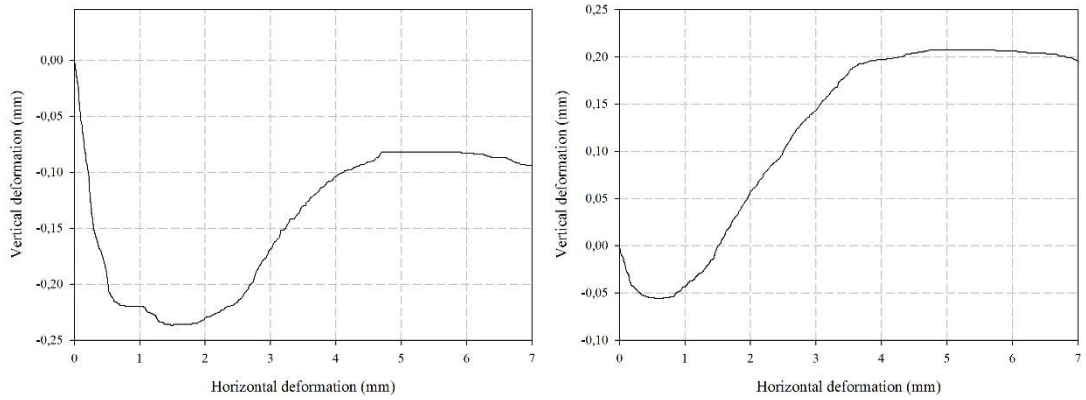
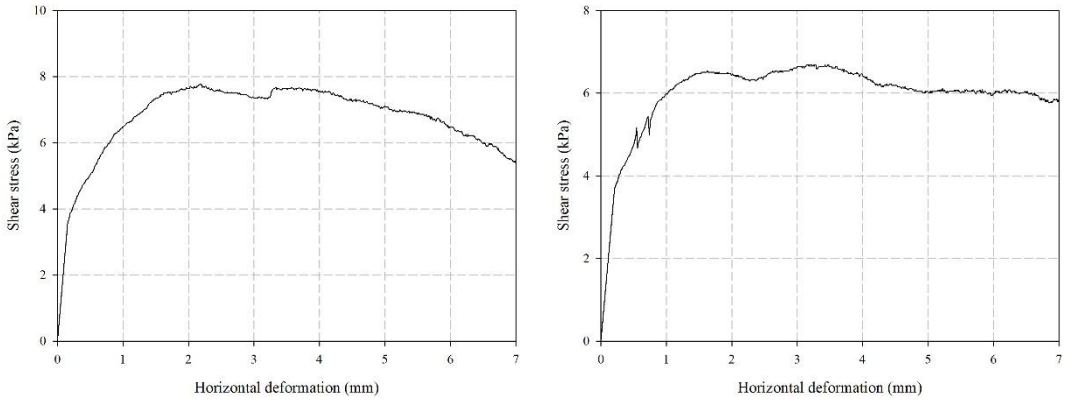


Figure A.6. (a) Horizontal deformation versus shear stress, (b) horizontal deformation versus vertical deformation relationships under 30 kPa normal stress of sand prepared at relative density of 40% and treated with *Sporosarcina pasteurii* through 10 injections

(a)



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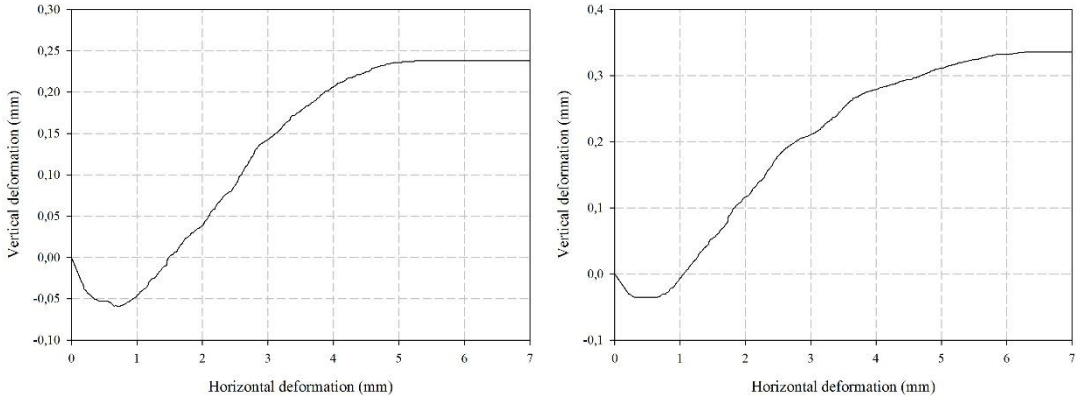
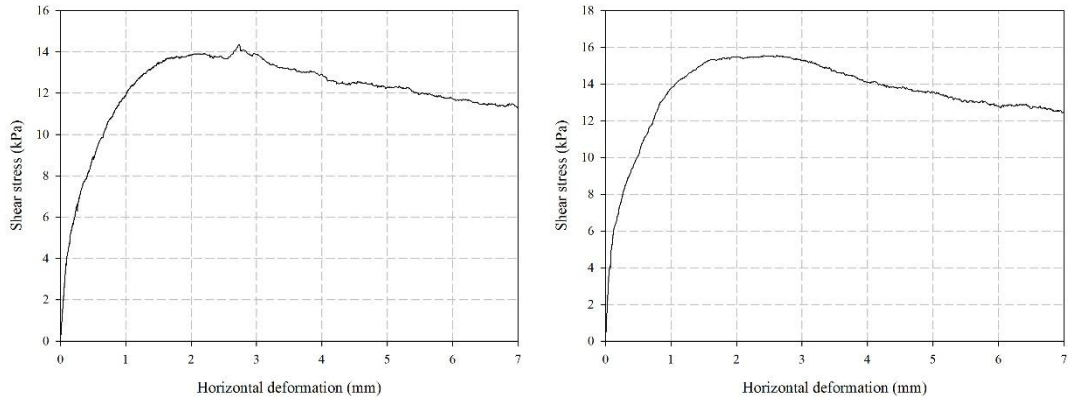


Figure A.7. (a) Horizontal deformation versus shear stress, (b) horizontal deformation versus vertical deformation relationships under 10 kPa normal stress of sand prepared at relative density of 40% and treated with *Bacillus licheniformis* through 10 injections

(a)



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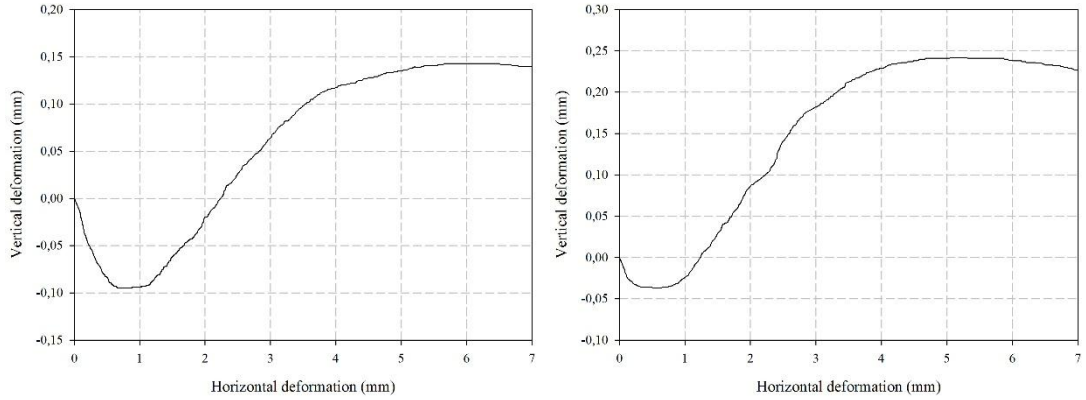
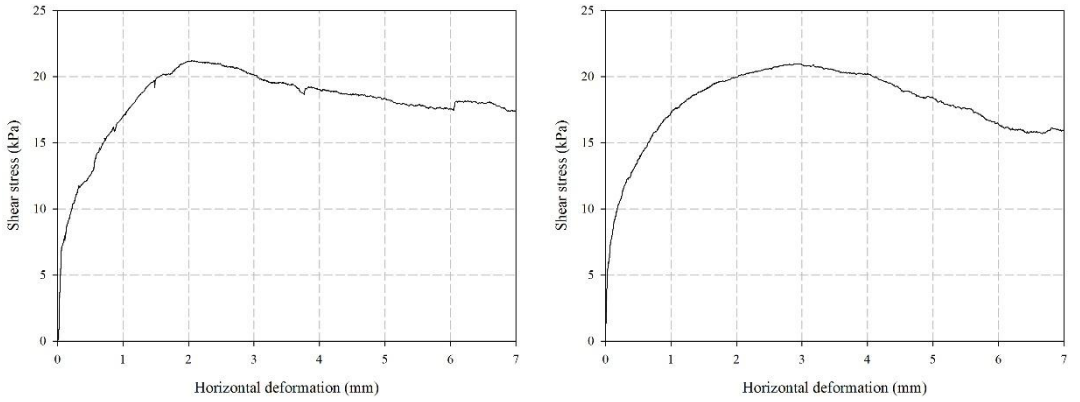


Figure A.8. (a) Horizontal deformation versus shear stress, (b) horizontal deformation versus vertical deformation relationships under 20 kPa normal stress of sand prepared at relative density of 40% and treated with *Bacillus licheniformis* through 10 injections

(a)



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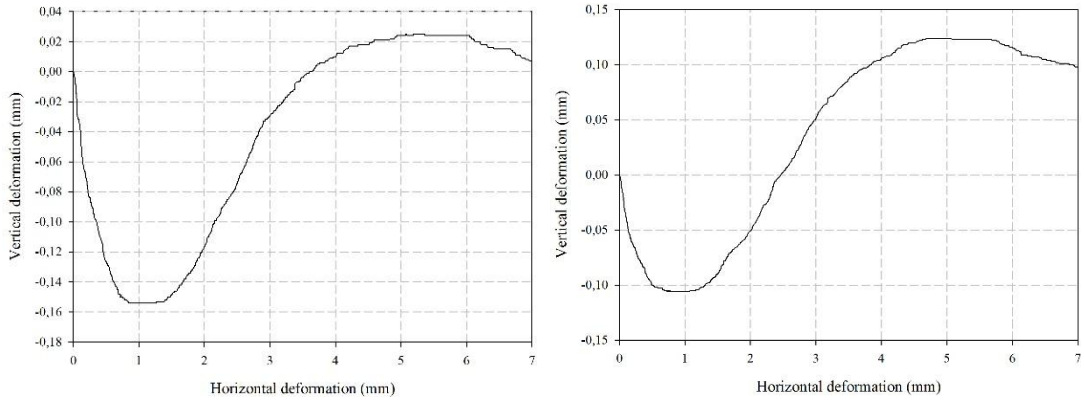
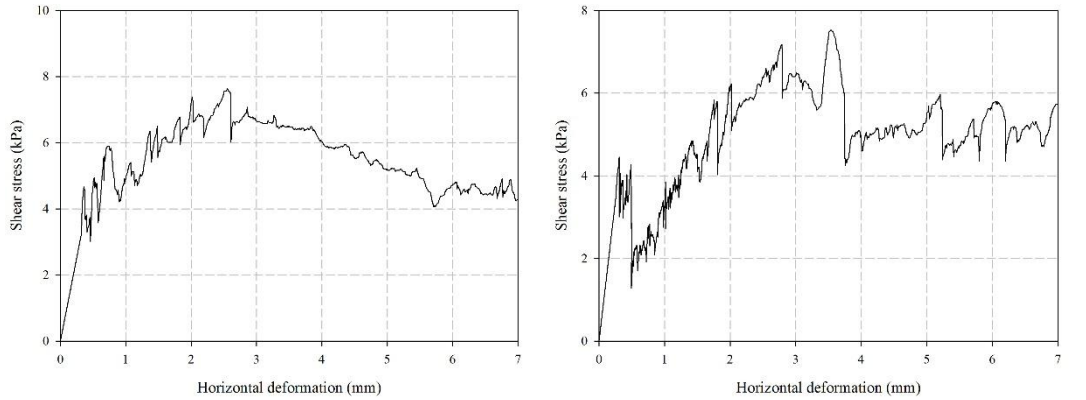


Figure A.9. (a) Horizontal deformation versus shear stress, (b) horizontal deformation versus vertical deformation relationships under 30 kPa normal stress of sand prepared at relative density of 40% and treated with *Bacillus licheniformis* through 10 injections

(a)



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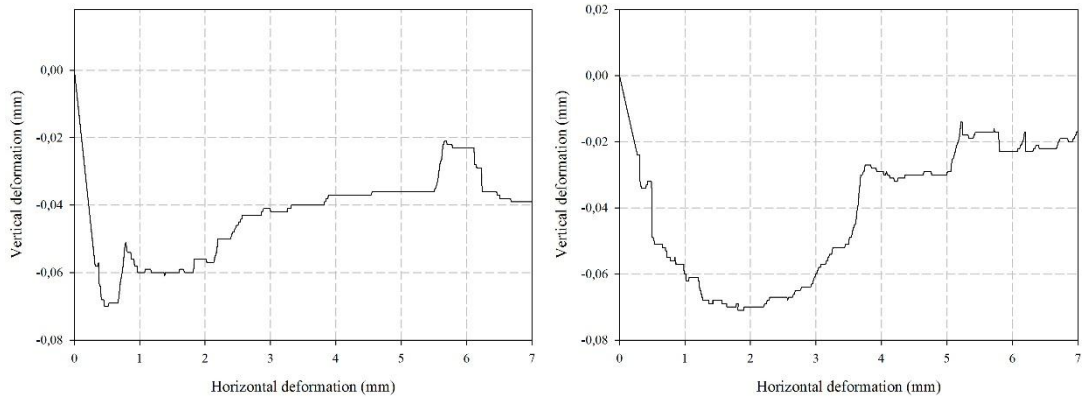
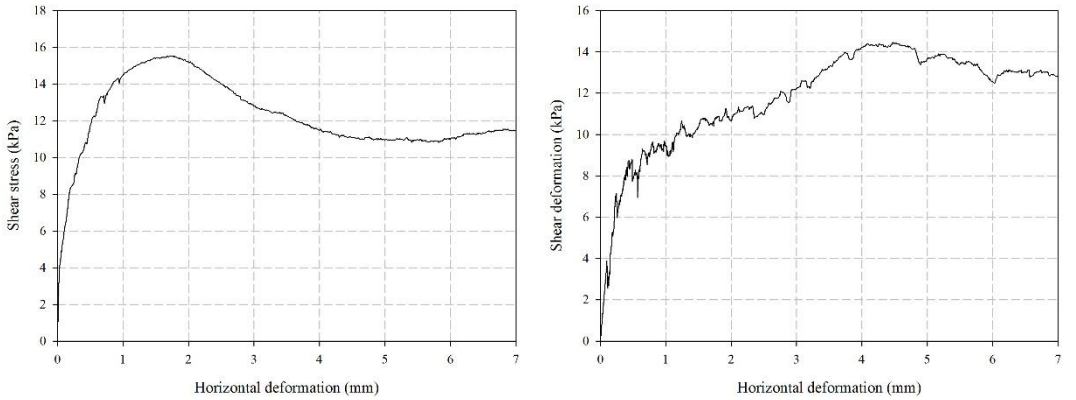


Figure A.10. (a) Horizontal deformation versus shear stress, (b) horizontal deformation versus vertical deformation relationships under 10 kPa normal stress of sand prepared at relative density of 70% and treated without bacteria introduction through 10 injections

(a)



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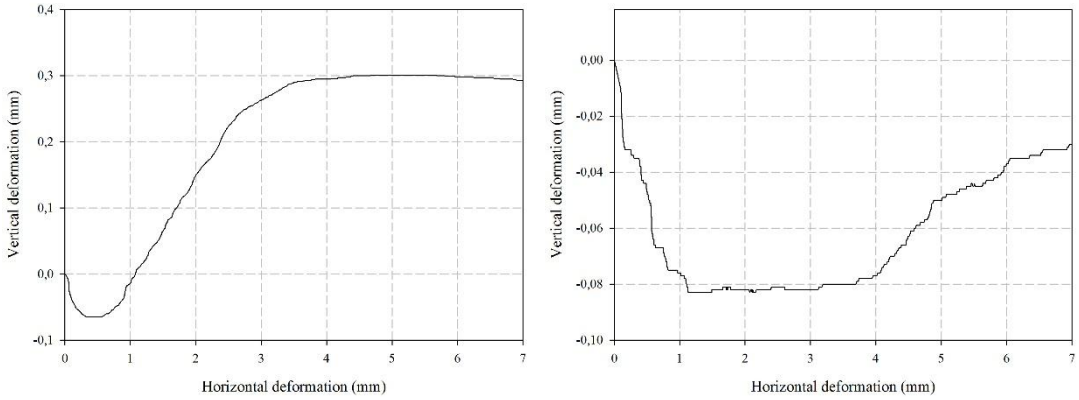
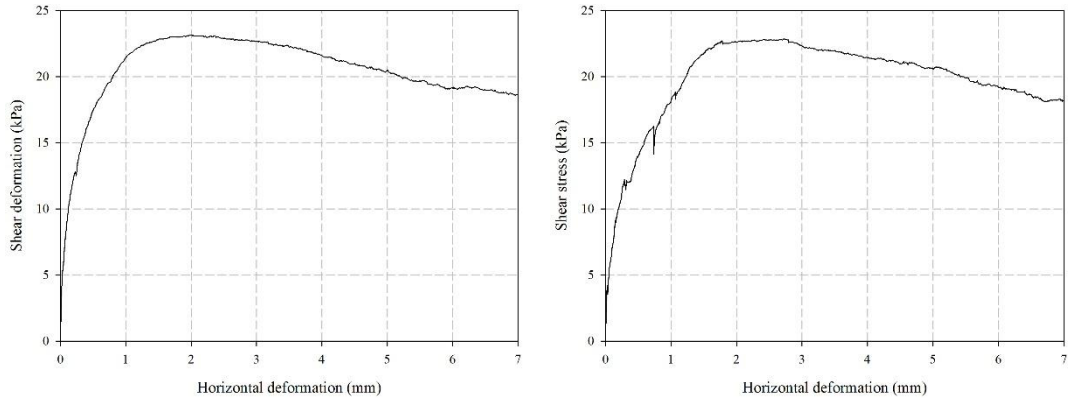


Figure A.11. (a) Horizontal deformation versus shear stress, (b) horizontal deformation versus vertical deformation relationships under 20 kPa normal stress of sand prepared at relative density of 70% and treated without bacteria introduction through 10 injections

(a)



(b)

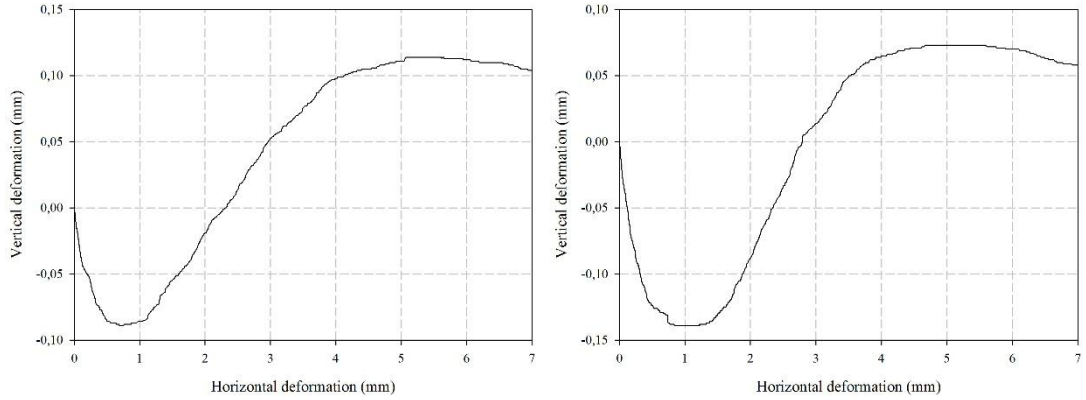
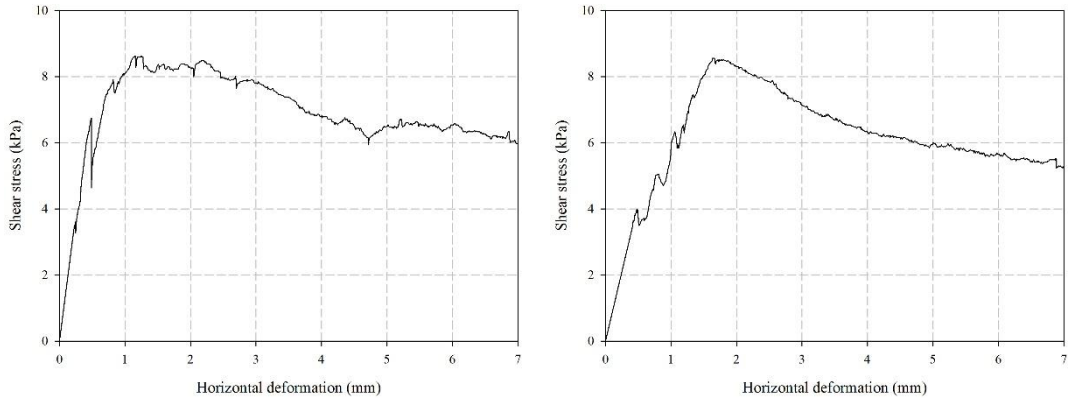


Figure 0.12. (a) Horizontal deformation versus shear stress, (b) horizontal deformation versus vertical deformation relationships under 30 kPa normal stress of sand prepared at relative density of 70% and treated without bacteria introduction through 10 injections

(a)



(b)

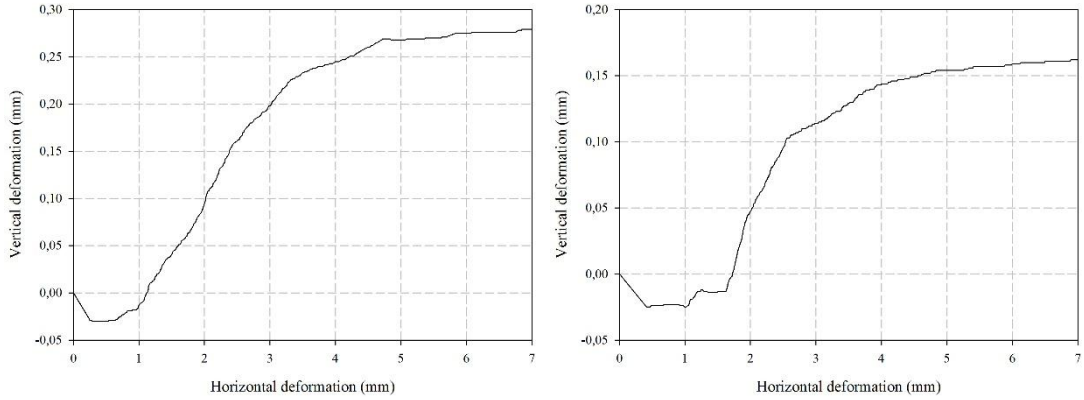
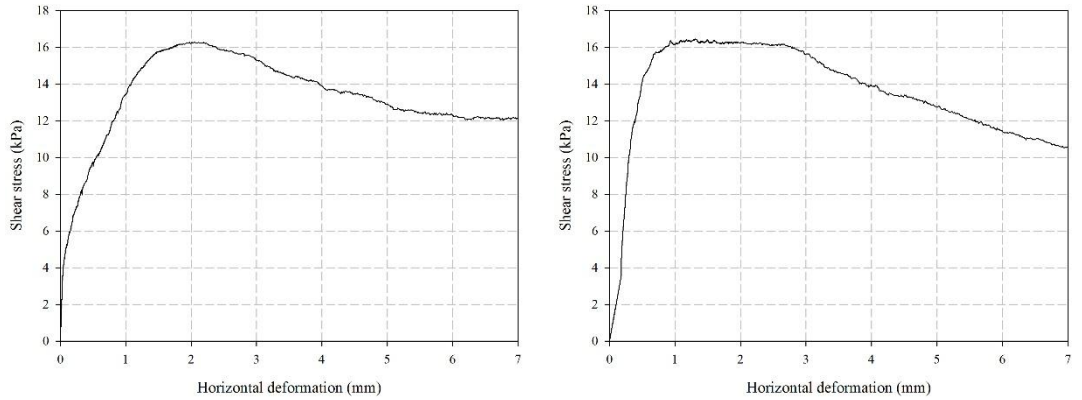


Figure A.13. (a) Horizontal deformation versus shear stress, (b) horizontal deformation versus vertical deformation relationships under 10 kPa normal stress of sand prepared at relative density of 70% and treated with *Sporosarcina pasteurii* through 10 injections

(a)



(b)

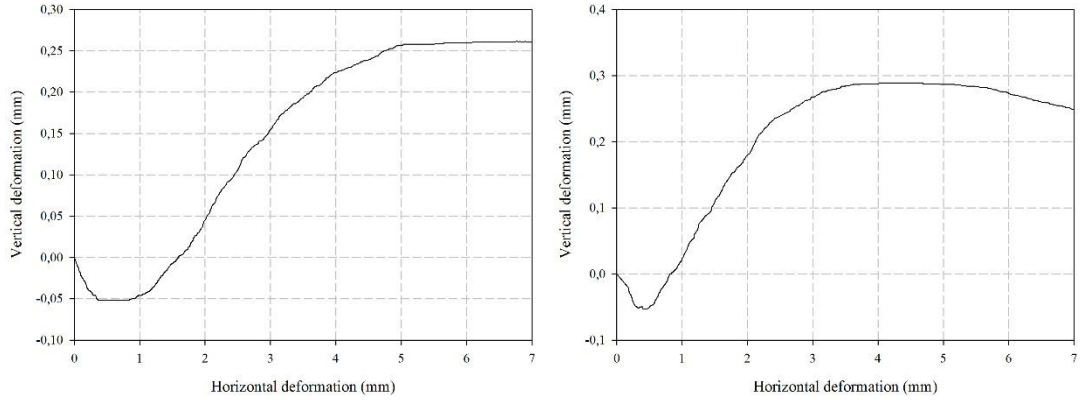
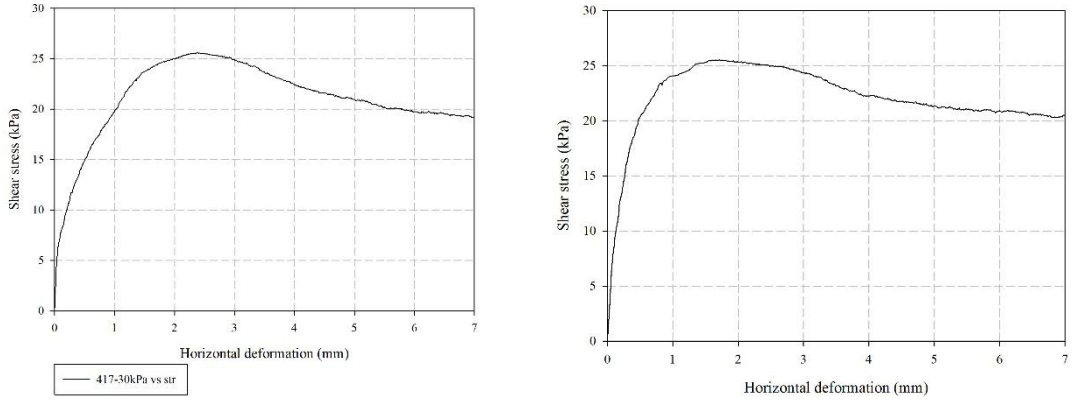


Figure A.14. (a) Horizontal deformation versus shear stress, (b) horizontal deformation versus vertical deformation relationships under 20 kPa normal stress of sand prepared at relative density of 70% and treated with *Sporosarcina pasteurii* through 10 injections

(a)



(b)

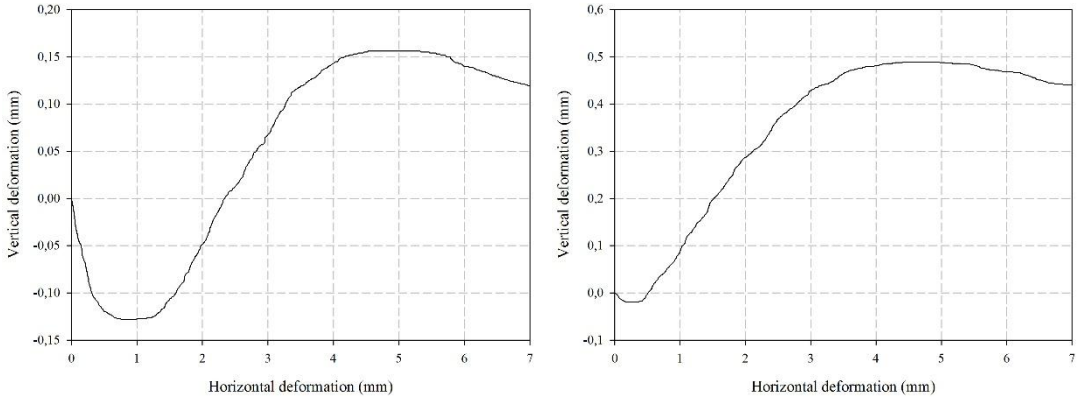
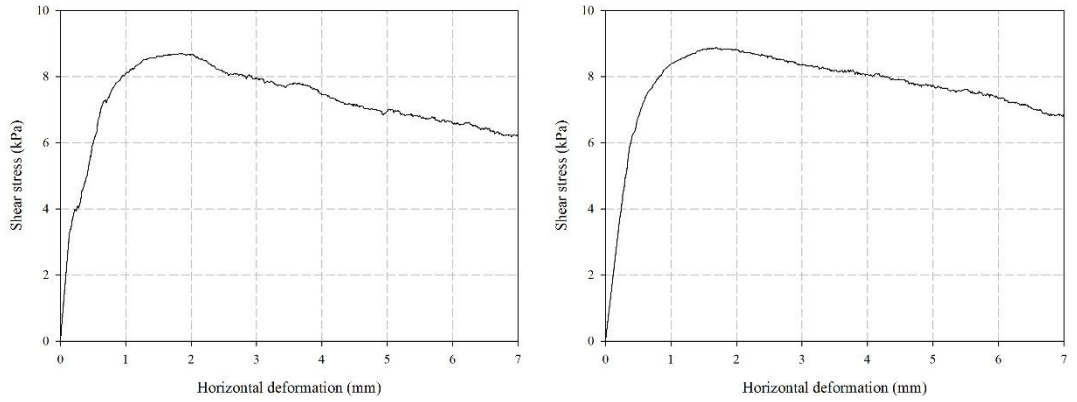


Figure A.15. (a) Horizontal deformation versus shear stress, (b) horizontal deformation versus vertical deformation relationships under 30 kPa normal stress of sand prepared at relative density of 70% and treated with *Sporosarcina pasteurii* through 10 injections

(a)



(b)

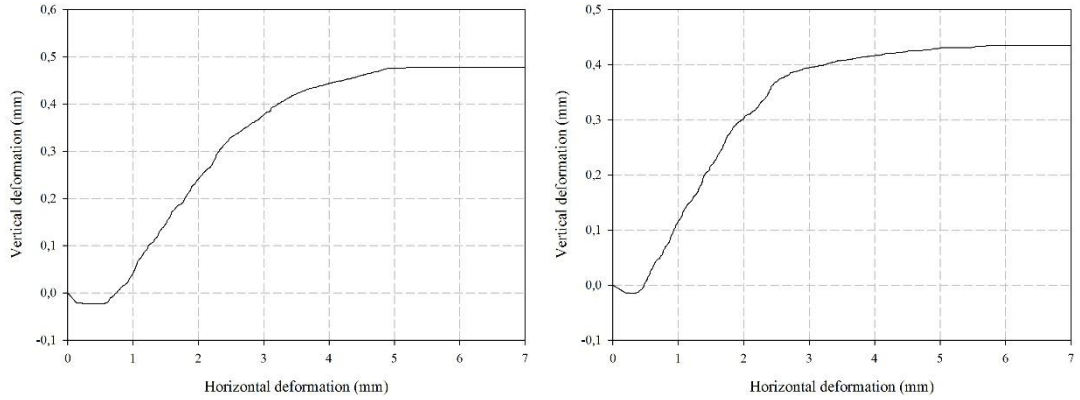
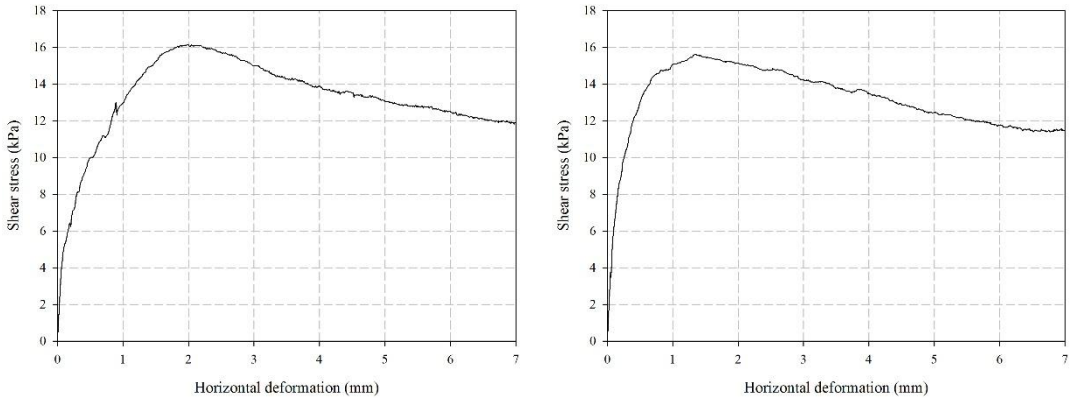


Figure A.16. (a) Horizontal deformation versus shear stress, (b) horizontal deformation versus vertical deformation relationships under 10 kPa normal stress of sand prepared at relative density of 70% and treated with *Bacillus licheniformis* through 10 injections

(a)



(b)

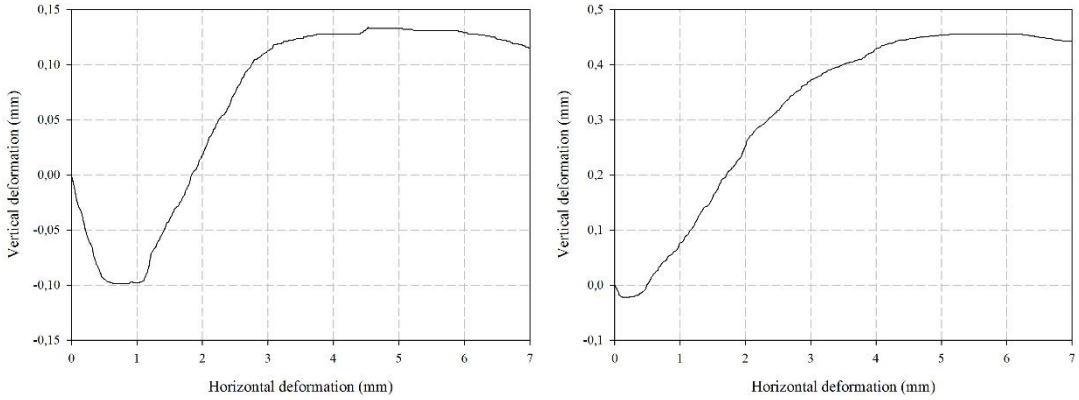
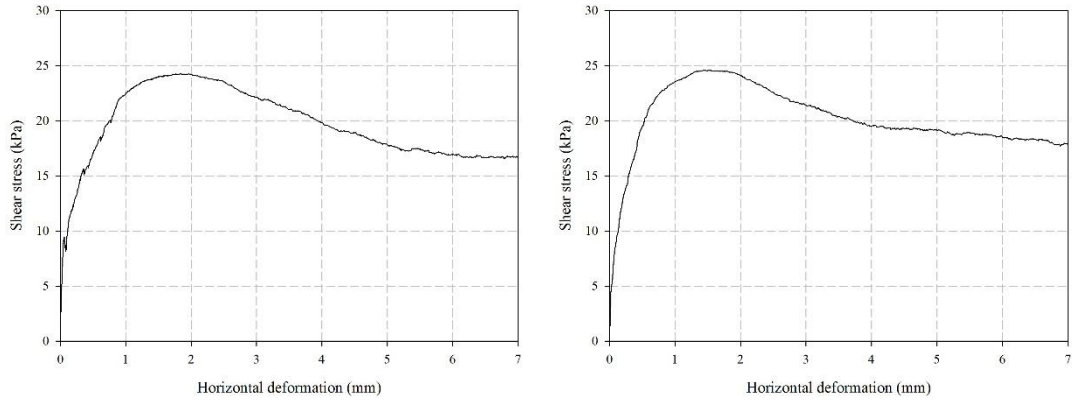


Figure A.17. (a) Horizontal deformation versus shear stress, (b) horizontal deformation versus vertical deformation relationships under 20 kPa normal stress of sand prepared at relative density of 70% and treated with *Bacillus licheniformis* through 10 injections

(a)



(b)

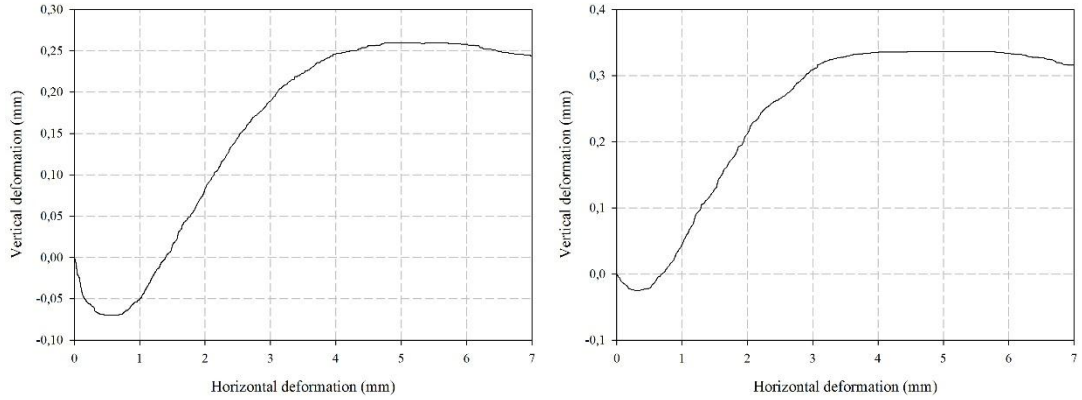
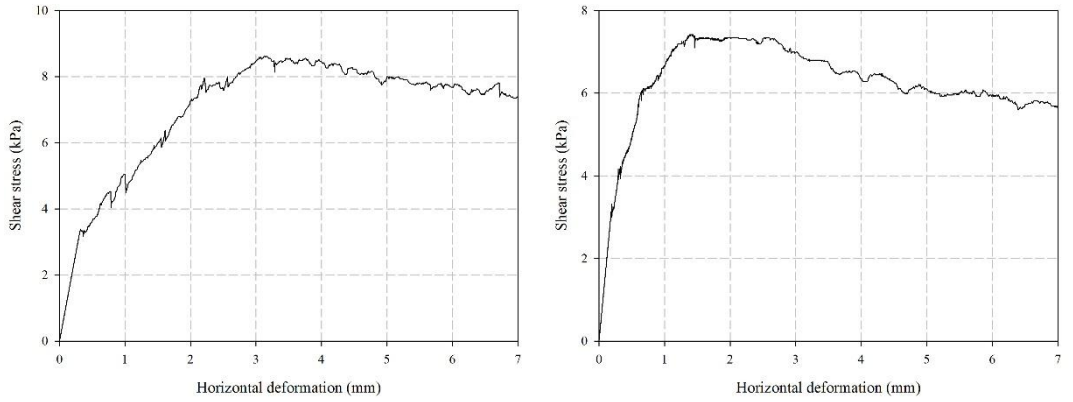


Figure A.18. (a) Horizontal deformation versus shear stress, (b) horizontal deformation versus vertical deformation relationships under 30 kPa normal stress of sand prepared at relative density of 70% and treated with *Bacillus licheniformis* through 10 injections

(a)



(b)

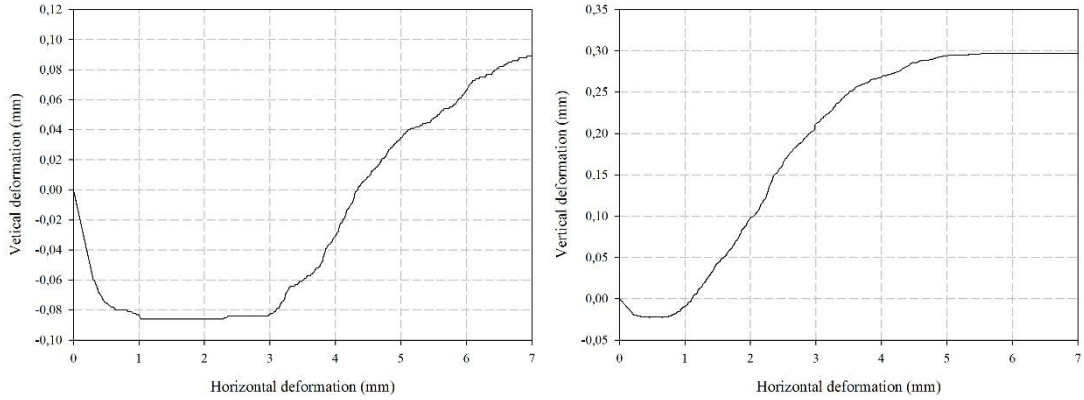
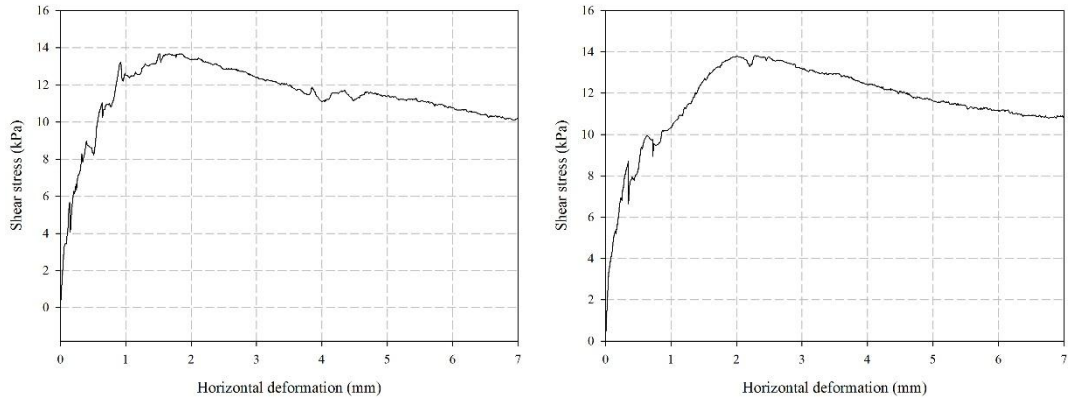


Figure A.19. (a) Horizontal deformation versus shear stress, (b) horizontal deformation versus vertical deformation relationships under 10 kPa normal stress of sand prepared at relative density of 40% and treated without bacteria introduction through 20 injections

(a)



(b)

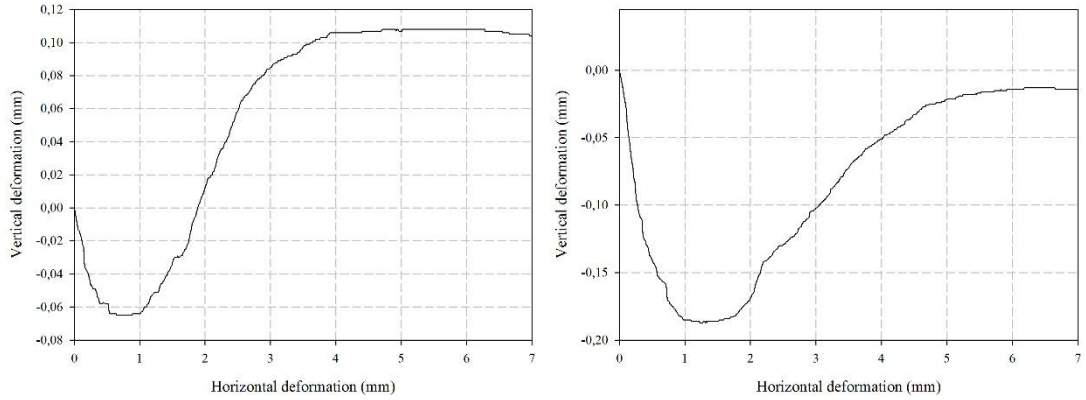
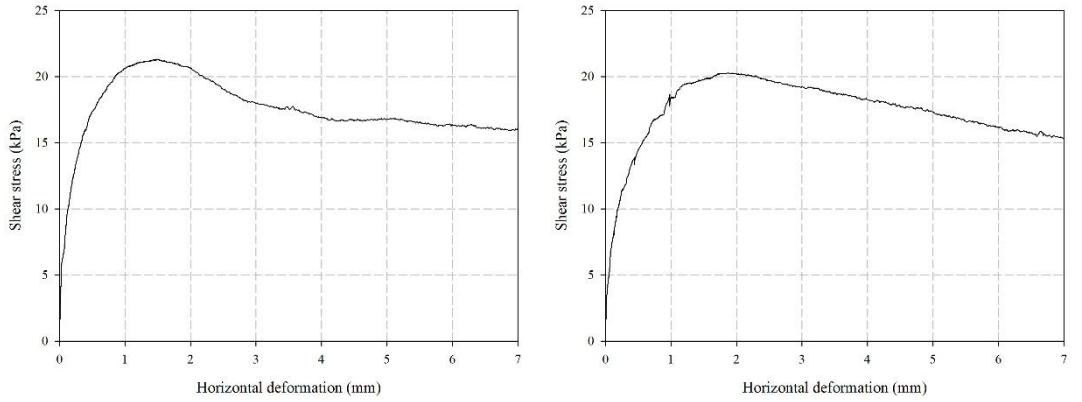


Figure A.20. (a) Horizontal deformation versus shear stress, (b) horizontal deformation versus vertical deformation relationships under 20 kPa normal stress of sand prepared at relative density of 40% and treated without bacteria introduction through 20 injections

(a)



(b)

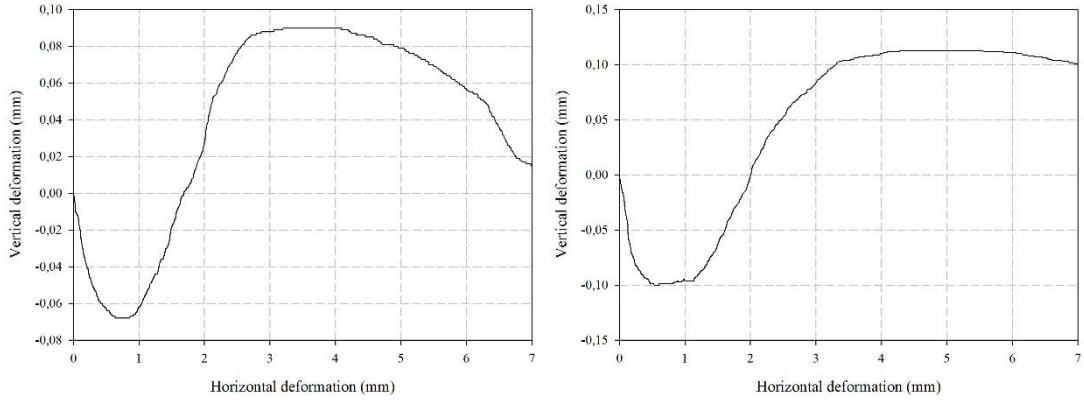
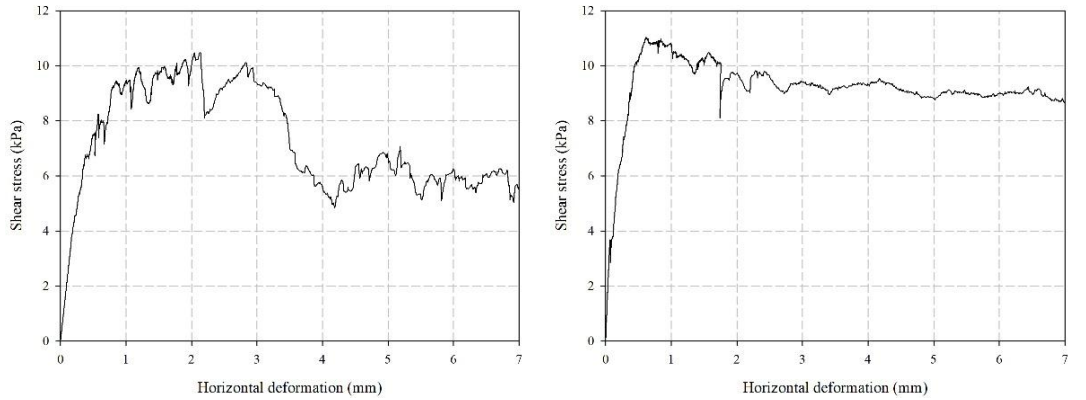


Figure A.21. (a) Horizontal deformation versus shear stress, (b) horizontal deformation versus vertical deformation relationships under 30 kPa normal stress of sand prepared at relative density of 40% and treated without bacteria introduction through 20 injections

(a)



(b)

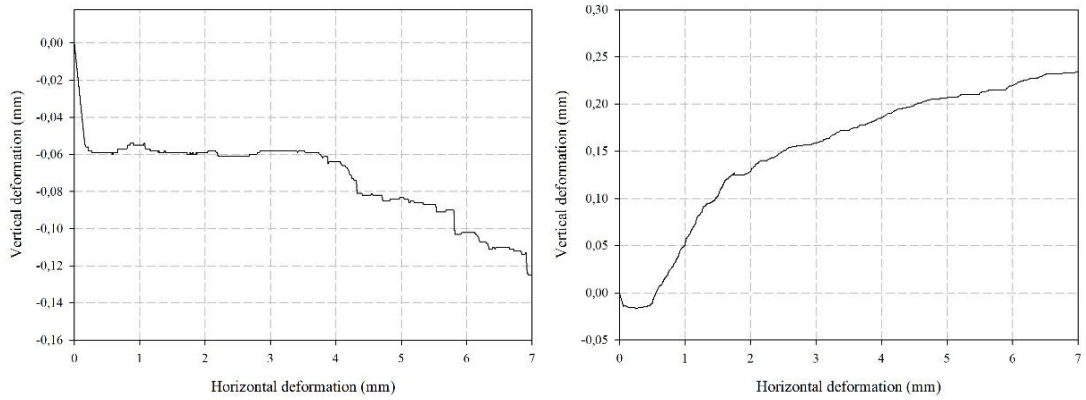
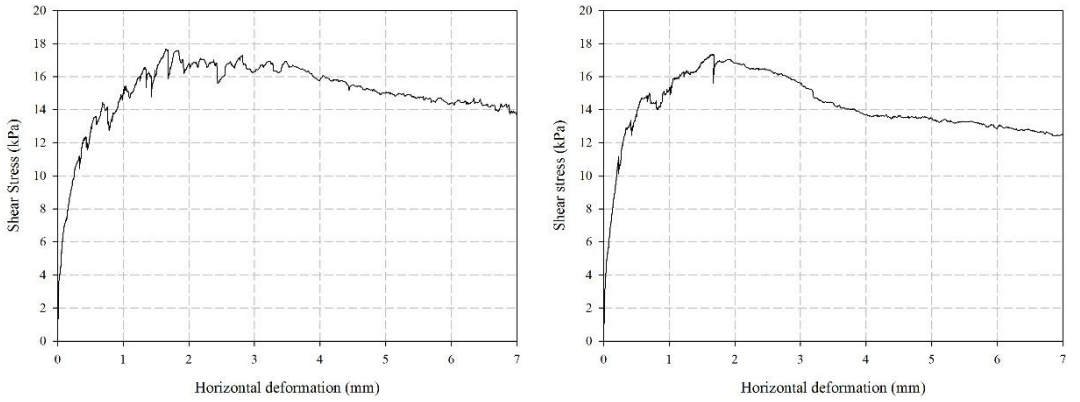


Figure A.22. (a) Horizontal deformation versus shear stress, (b) horizontal deformation versus vertical deformation relationships under 10 kPa normal stress of sand prepared at relative density of 40% and treated with *Sporosarcina pasteurii* through 20 injections

(a)



(b)

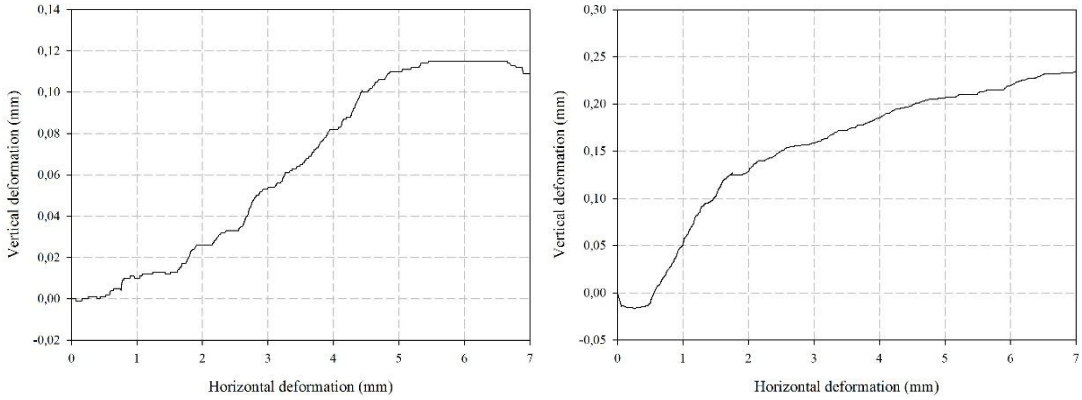
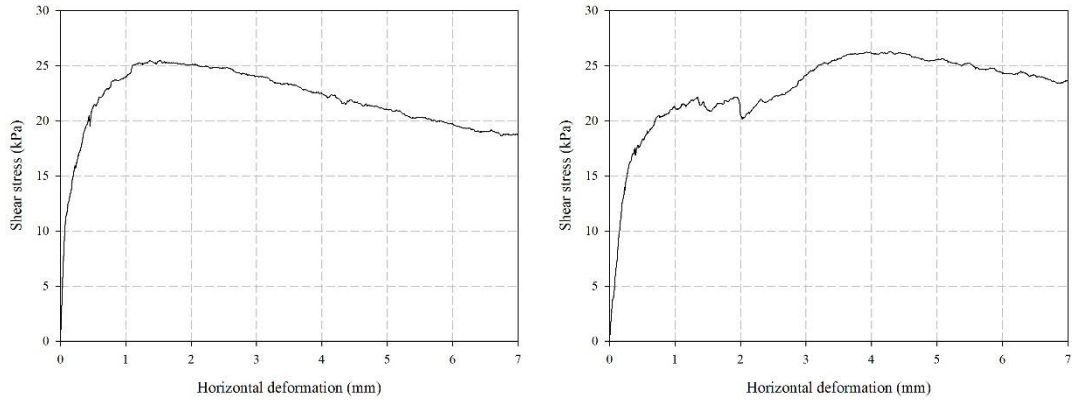


Figure A.23. (a) Horizontal deformation versus shear stress, (b) horizontal deformation versus vertical deformation relationships under 20 kPa normal stress of sand prepared at relative density of 40% and treated with *Sporosarcina pasteurii* through 20 injections

(a)



(b)

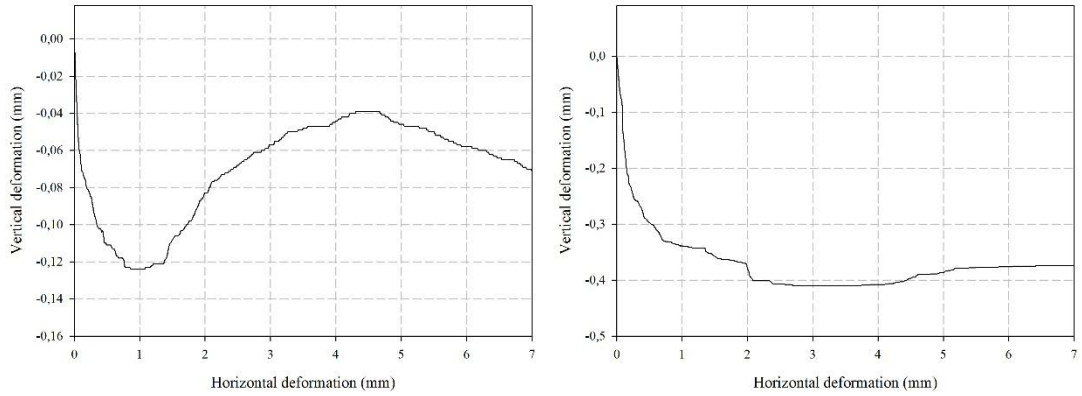
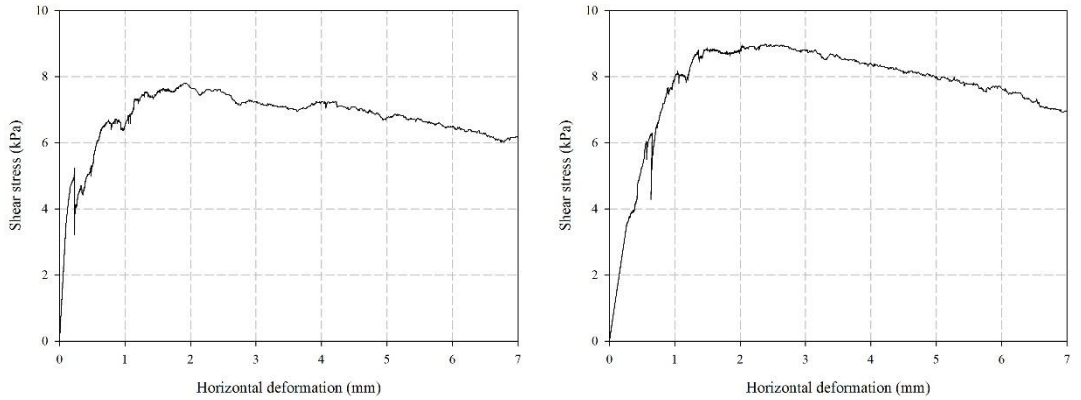


Figure A.24. (a) Horizontal deformation versus shear stress, (b) horizontal deformation versus vertical deformation relationships under 30 kPa normal stress of sand prepared at relative density of 40% and treated with *Sporosarcina pasteurii* through 20 injections

(a)



(b)

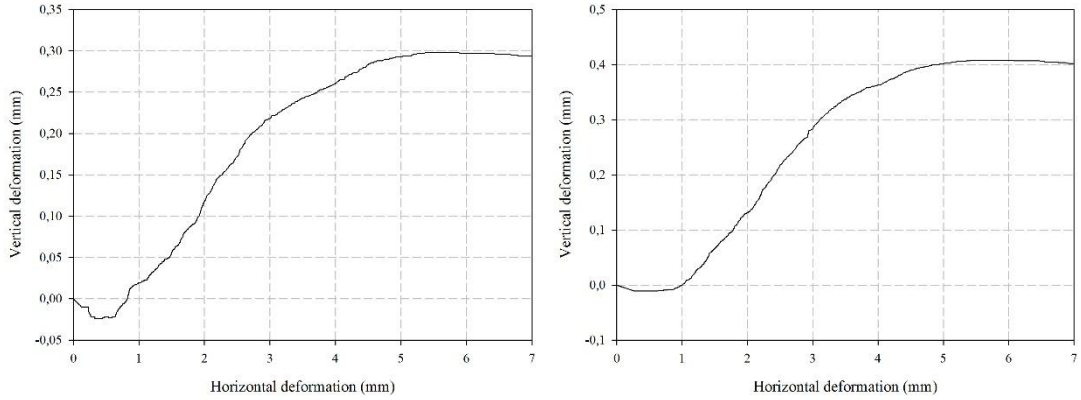
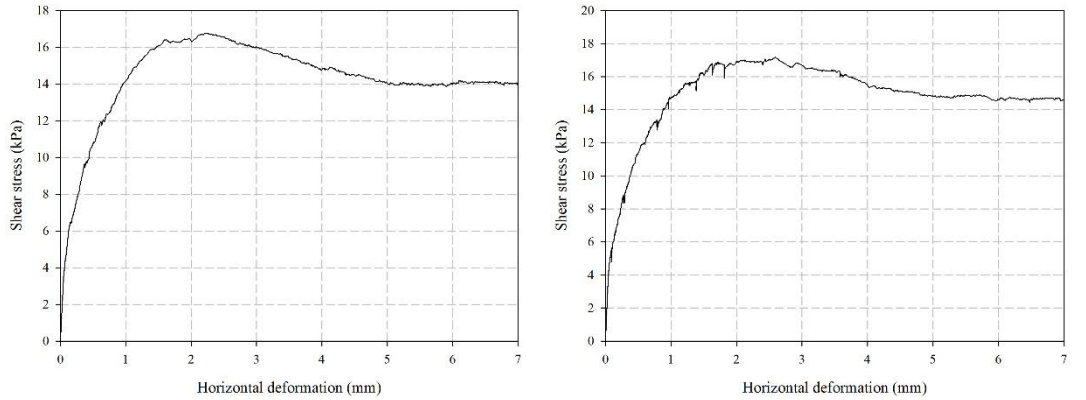


Figure A.25. (a) Horizontal deformation versus shear stress, (b) horizontal deformation versus vertical deformation relationships under 10 kPa normal stress of sand prepared at relative density of 40% and treated with *Bacillus licheniformis* through 20 injections

(a)



(b)

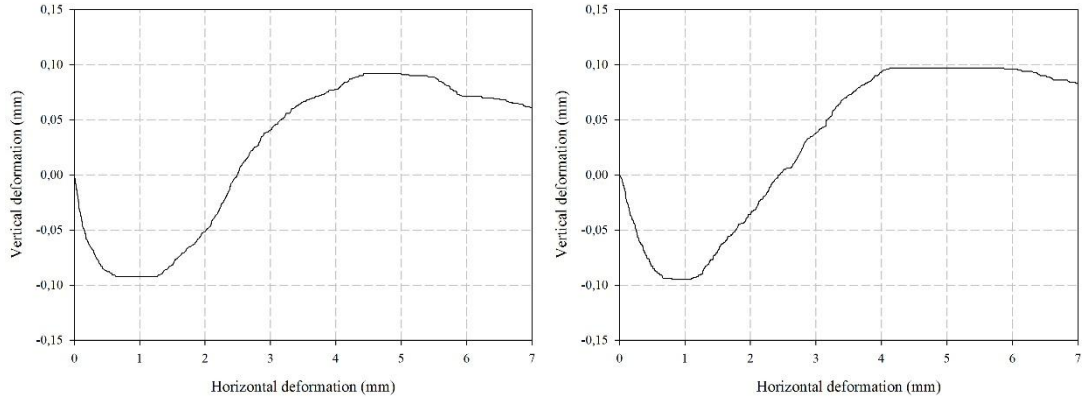
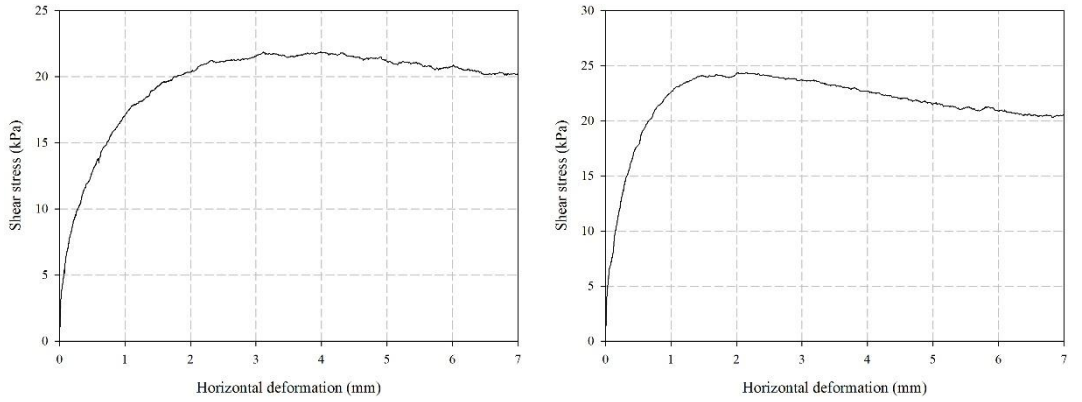


Figure A.26. (a) Horizontal deformation versus shear stress, (b) horizontal deformation versus vertical deformation relationships under 20 kPa normal stress of sand prepared at relative density of 40% and treated with *Bacillus licheniformis* through 20 injections

(a)



(b)

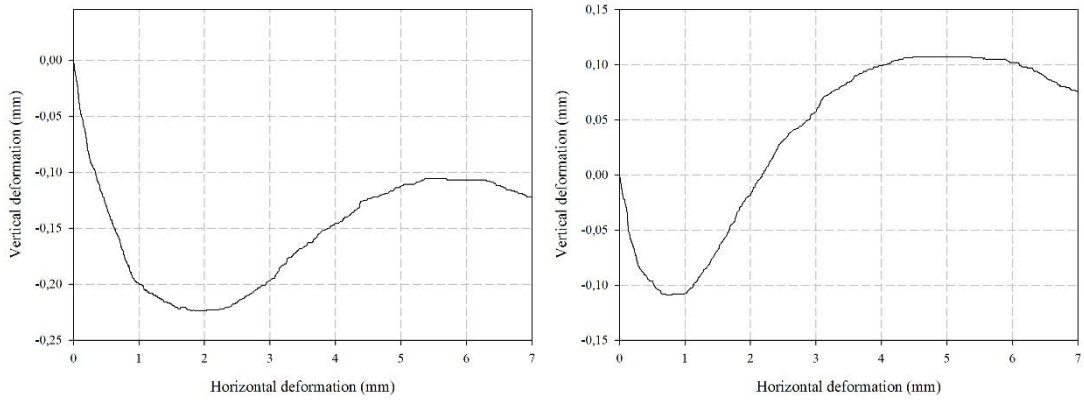
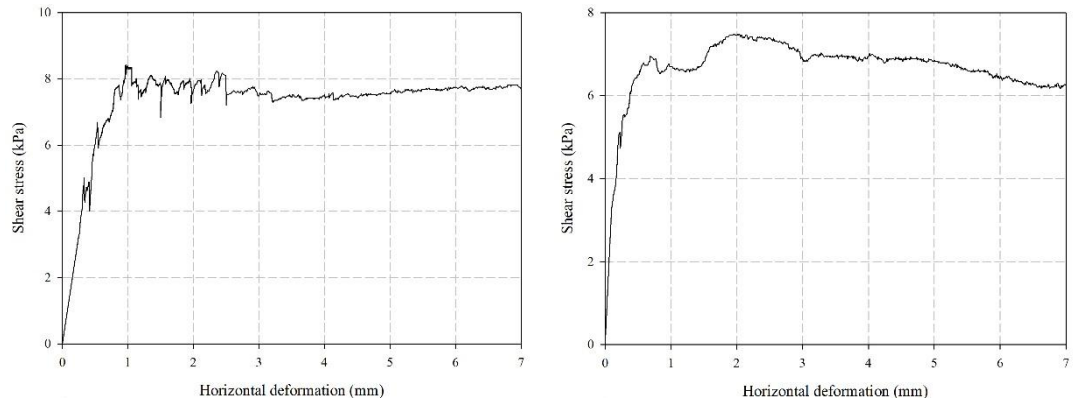


Figure A.27. (a) Horizontal deformation versus shear stress, (b) horizontal deformation versus vertical deformation relationships under 30 kPa normal stress of sand prepared at relative density of 40% and treated with *Bacillus licheniformis* through 20 injections

(a)



(b)

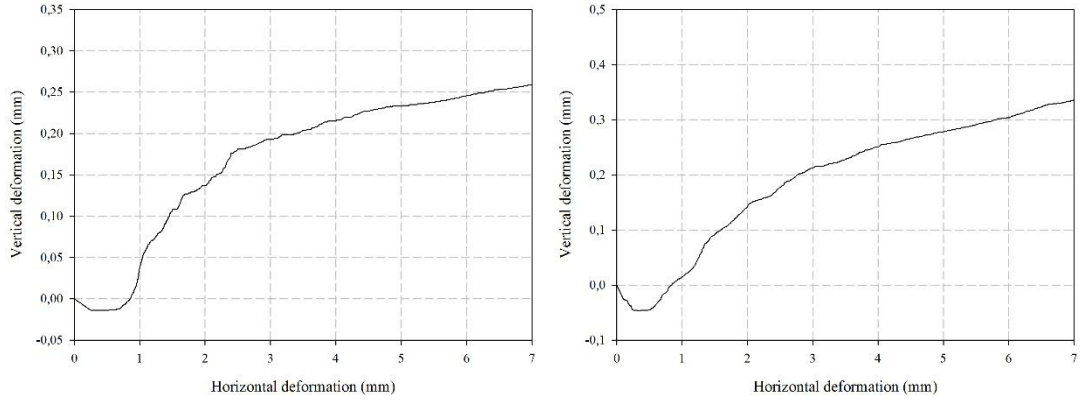
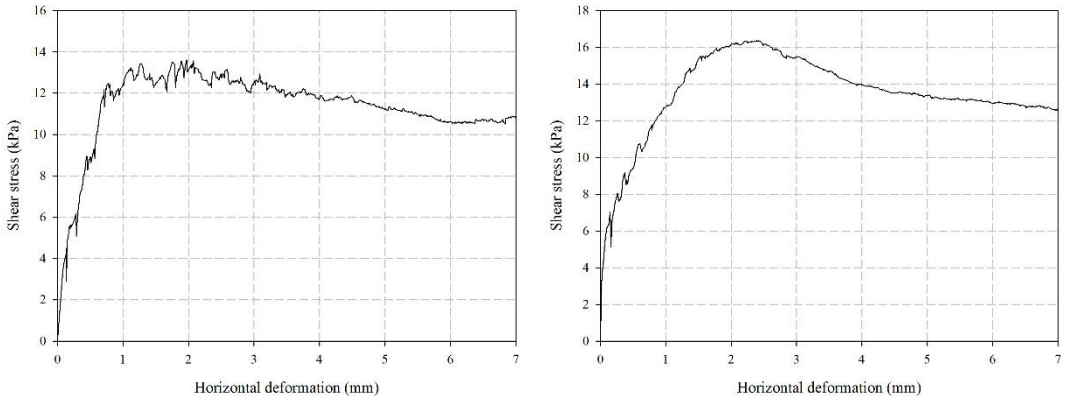


Figure A.28. (a) Horizontal deformation versus shear stress, (b) horizontal deformation versus vertical deformation relationships under 10 kPa normal stress of sand prepared at relative density of 70% and treated without bacteria introduction through 20 injections

(a)



(b)

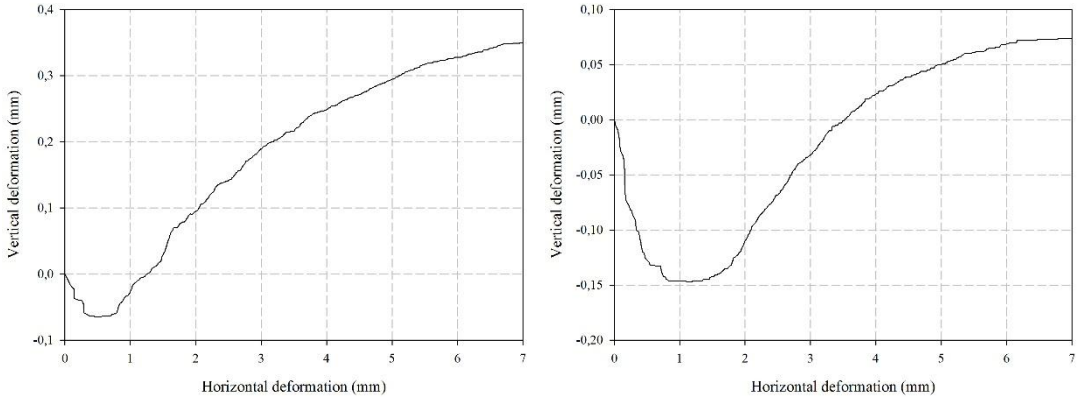
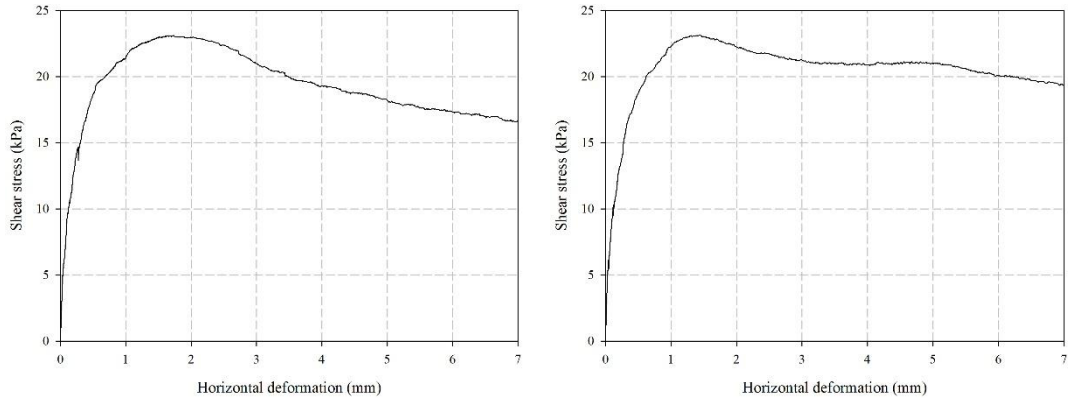


Figure A.29. (a) Horizontal deformation versus shear stress, (b) horizontal deformation versus vertical deformation relationships under 20 kPa normal stress of sand prepared at relative density of 70% and treated without bacteria introduction through 20 injections

(a)



(b)

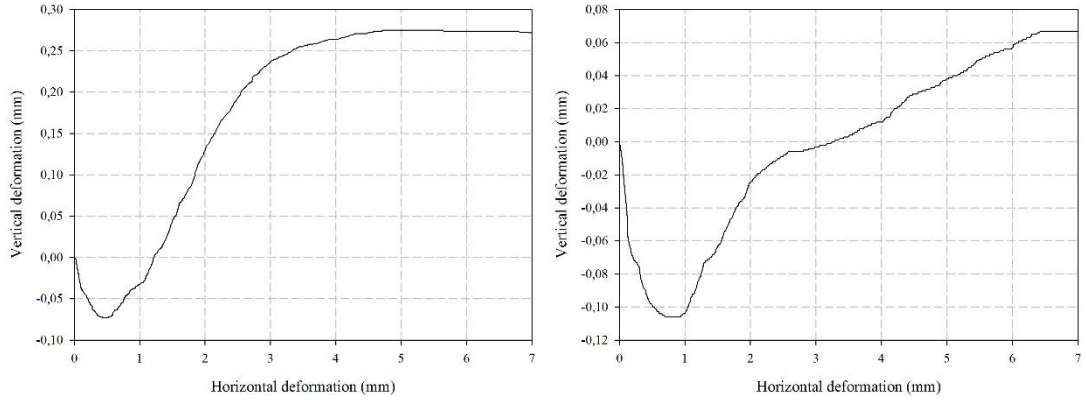
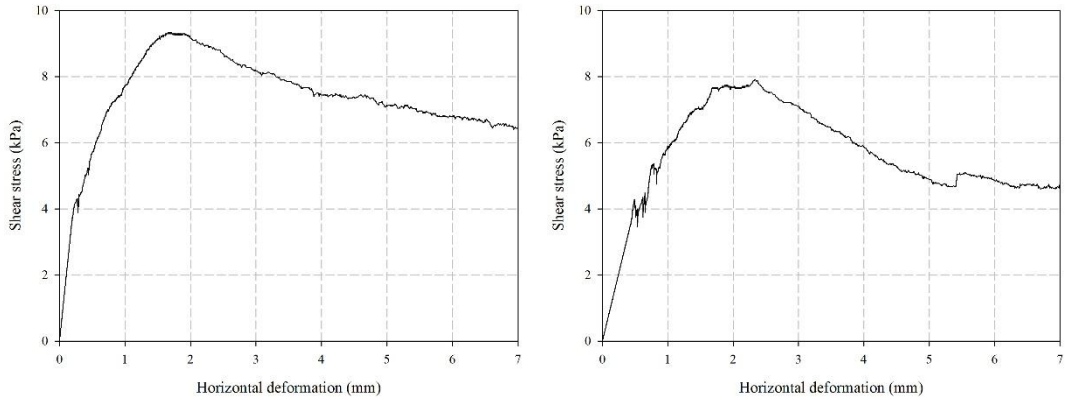


Figure A.30. (a) Horizontal deformation versus shear stress, (b) horizontal deformation versus vertical deformation relationships under 30 kPa normal stress of sand prepared at relative density of 70% and treated without bacteria introduction through 20 injections

(a)



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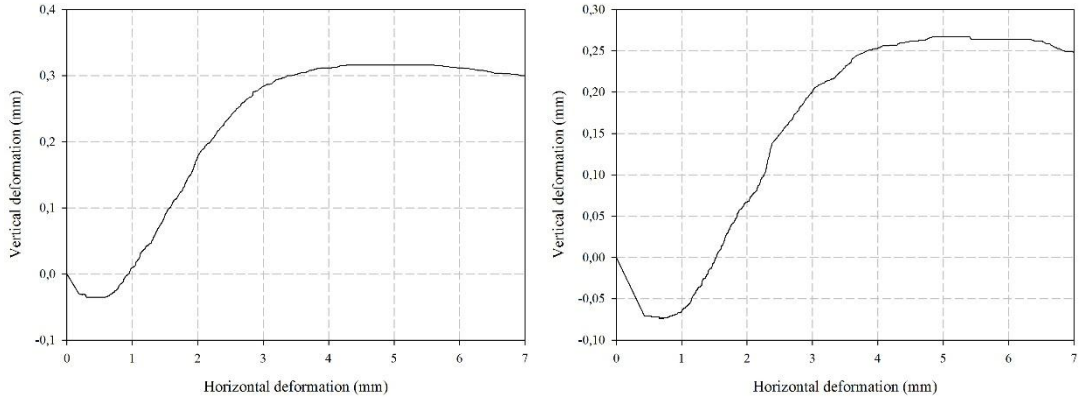
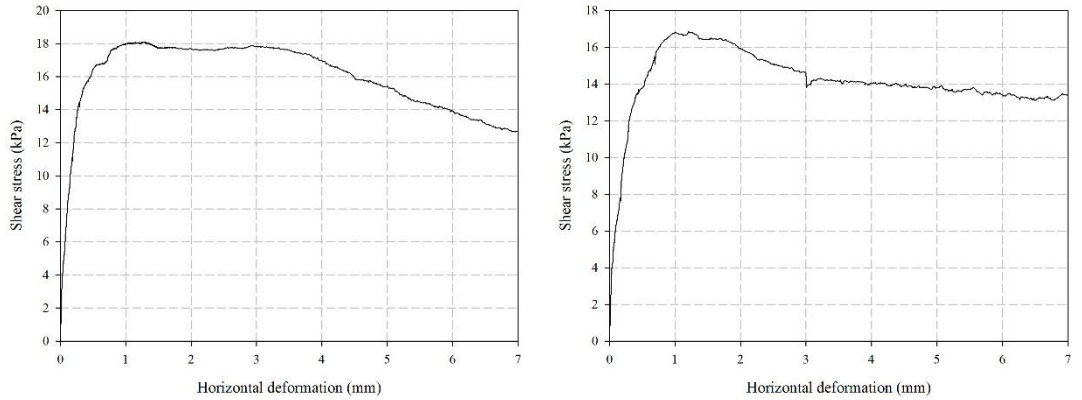


Figure A.31. (a) Horizontal deformation versus shear stress, (b) horizontal deformation versus vertical deformation relationships under 10 kPa normal stress of sand prepared at relative density of 70% and treated with *Sporosarcina pasteurii* through 20 injections

(a)



(b)

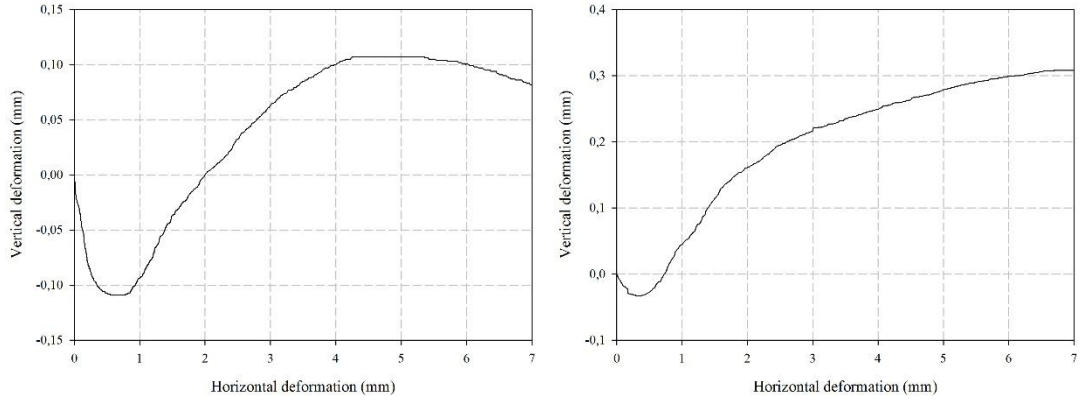
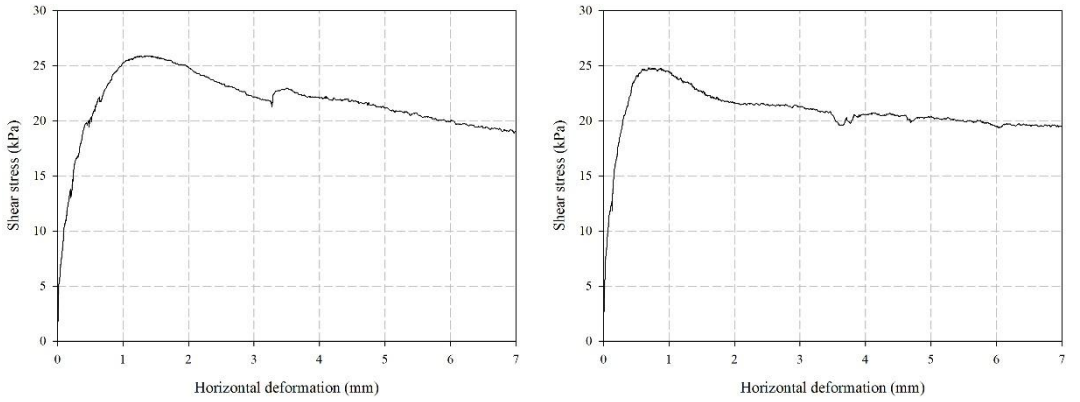


Figure A.32. (a) Horizontal deformation versus shear stress, (b) horizontal deformation versus vertical deformation relationships under 20 kPa normal stress of sand prepared at relative density of 70% and treated with *Sporosarcina pasteurii* through 20 injections

(a)



(b)

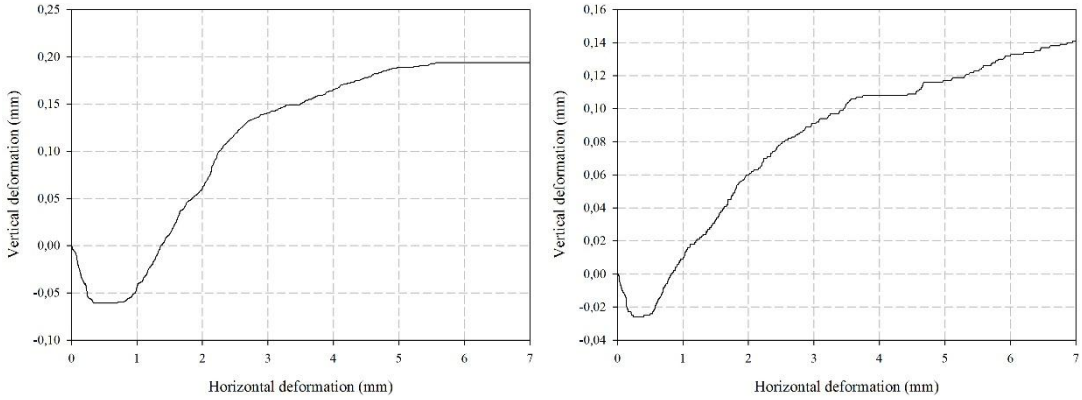
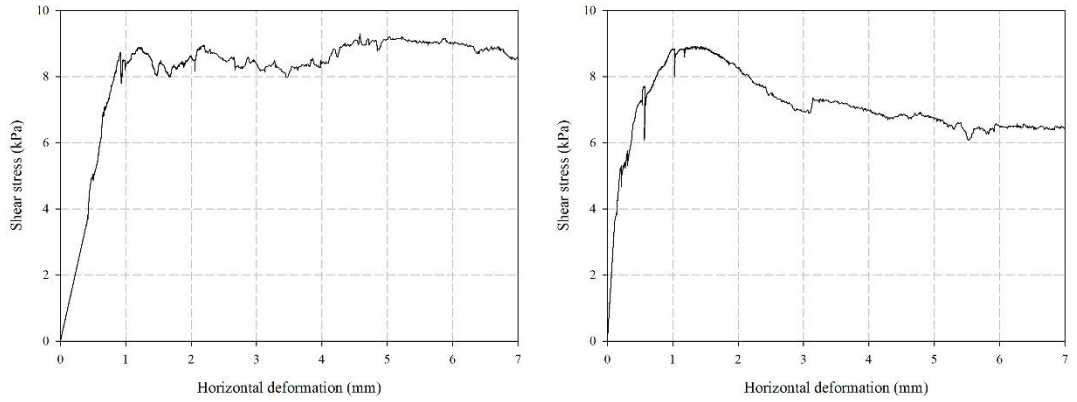


Figure A.33. (a) Horizontal deformation versus shear stress, (b) horizontal deformation versus vertical deformation relationships under 30 kPa normal stress of sand prepared at relative density of 70% and treated with *Sporosarcina pasteurii* through 20 injections

(a)



(b)

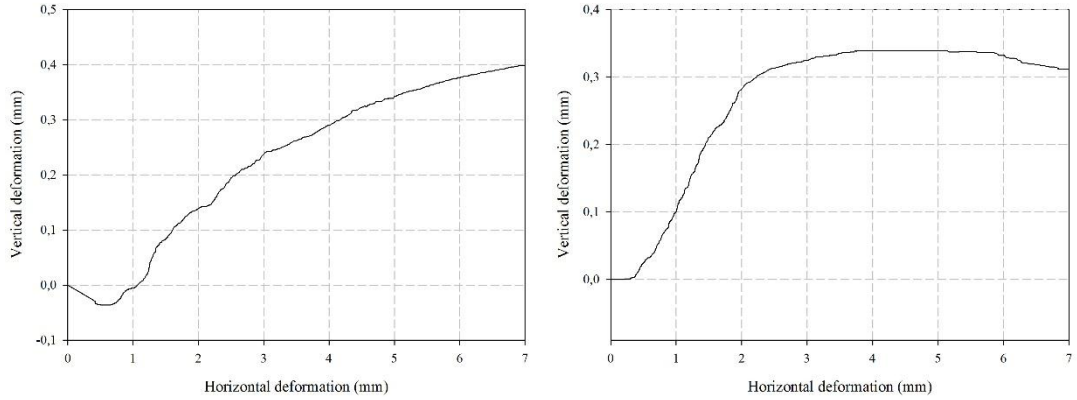
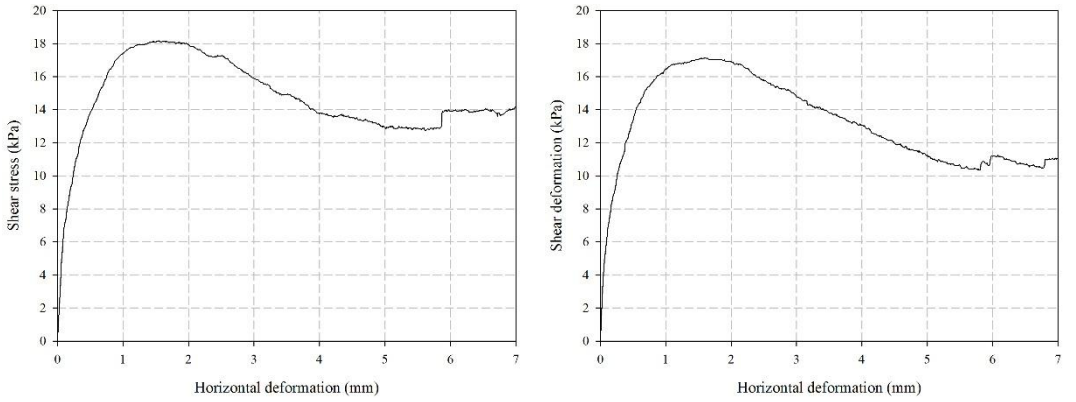


Figure A.34. (a) Horizontal deformation versus shear stress, (b) horizontal deformation versus vertical deformation relationships under 10 kPa normal stress of sand prepared at relative density of 70% and treated with *Bacillus licheniformis* through 20 injections

(a)



(b)

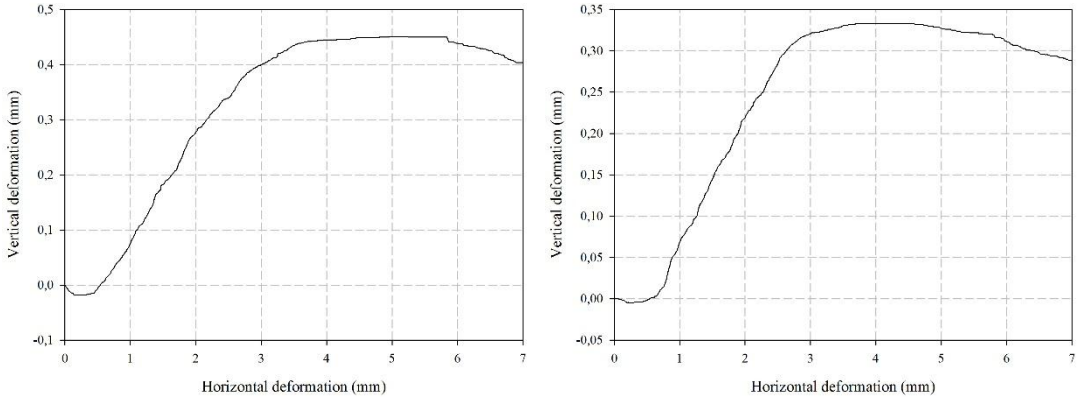
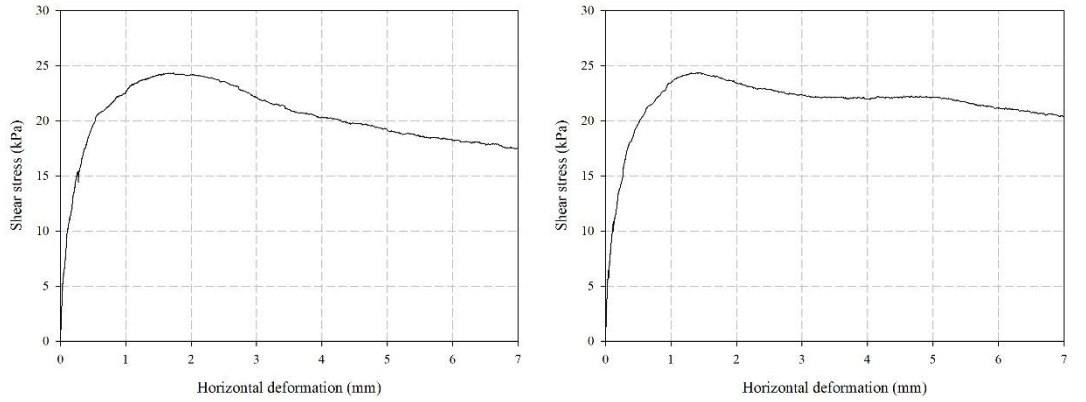


Figure A.35. (a) Horizontal deformation versus shear stress, (b) horizontal deformation versus vertical deformation relationships under 20 kPa normal stress of sand prepared at relative density of 70% and treated with *Bacillus licheniformis* through 20 injections

(a)



(b)

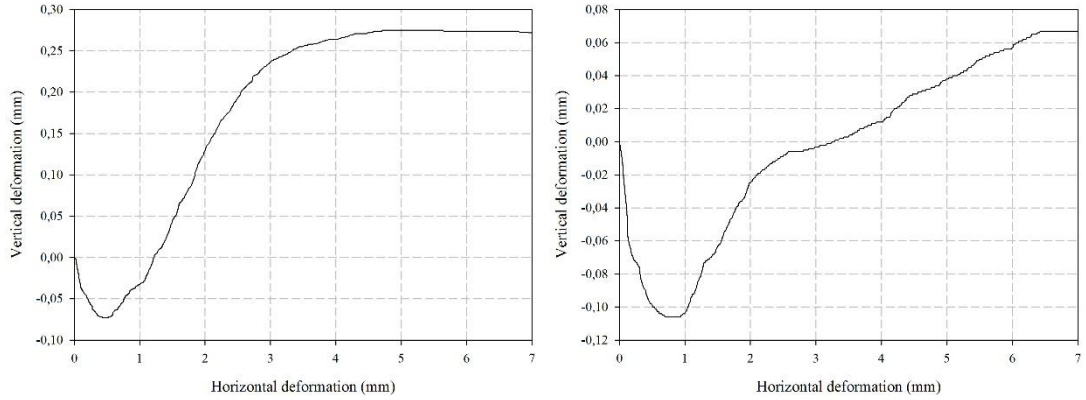


Figure A.36. (a) Horizontal deformation versus shear stress, (b) horizontal deformation versus vertical deformation relationships under 30 kPa normal stress of sand prepared at relative density of 70% and treated with *Bacillus licheniformis* through 20 injections