MICROORGANISM IN MITIGATING SEISMIC LIQUEFACTION

by

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Md. Ferdous Alam

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NOTATION

a_{max}	Peak ground acceleration
C	Ca^{2+} required
CAR	Ca required in 400 mL urea medium
CC	CO_3^{2-} required
CCL	$CaCl_2.2H_2O$ required
$CCLR$	$CaCl_2.2H_2O$ required for 400 mL urea medium
$CIPS$	Calcite in-situ precipitation system
CR	CO_3^{2-} produced in 400 mL urea medium
CRR	Cyclic resistance ratio
CSR	Cyclic stress ratio
D	Sample diameter
D_r	Relative density
FS	Factor of safety
H	Sample height
ICU	Isotropically consolidated undrained
k_o	Coefficient of lateral earth pressure at rest
LL	Liquid limit
$MICP$	Microbial induced calcite precipitation
MIU	Motility indole urease
M_L	Local magnitude
M_s	Surface wave magnitude
M_w	Moment magnitude
OCR	Over consolidation ratio
q_u	Unconfined compressive strength
SEM	Scanning electron microscope
SSR	Stock solution of $CaCl_2.2H_2O$ (140 gm/L) required for 400 mL urea medium
T	Target $CaCO_3$ (kg/m^3 of total soil volume)
TCC	Treatment cycle based on $CaCl_2.2H_2O$ required
TCU	Treatment cycle based on urea required

TP	CaCO$_3$ to be precipitated
U	Urea required
UR	Urea in 400 mL urea medium
V	Sample volume
σ_d	Single amplitude of cyclic deviator stress
σ_o'	Initial confining stress

ACKNOWLEDGEMENT

ABSTRACT

This thesis presents the results of a study in which Microbial Induced Calcite Precipitation (MICP) was used to improve the engineering behavior of the sandy soil for the purpose of mitigation of seismic liquefaction. MICP was attained using the urease positive microorganism isolated from the local garden soils. Those microorganisms were introduced to the specimens in a liquid growth medium consisting of urea and a dissolved calcium source. Two types of treatment methods were followed: method A and Method B. In method A, bacteria growth medium and additional cementation treatment solutions were applied into the specimens. In method B, bacteria and nutrient solution were mixed during the sample preparation by moist tamping method. Increase of pH and conductivity and decrease of Ca^{2+} ions of the treatment solution in method A confirmed urease activity. Increase of pressure in the pressure gauge of an experimental set up due to production of CO_2 from the bio-treated soil when reacting with HCl, suggested that the observed cement bonds were comprised of calcite. Optical Microscope images confirmed the formation of calcite bonding with the sand particles. Improvement of the soil was assessed by unconfined compression tests, needle penetration tests and cyclic triaxial tests. Unconfined compression tests and needle penetration tests showed the increase in shear strength compared to untreated specimens. Cyclic triaxial tests with different cyclic stress ratio showed the increase of number of cycles to produce 5% double amplitude axial strain and/or zero effective stress compared to untreated specimens. As such, it could be concluded that MICP technique might be a useful tool for mitigation of seismic liquefaction.

CHAPTER 1
INTRODUCTION

1.1 GENERAL

From the geotechnical engineering point of view, ground improvement may be defined as to increase shear strength, reduce compressibility and permeability of the soil depending on the specific needs for a given project at a given site. Engineering behavior and performance of the soil are effectively improved by various ground improvement techniques developed over the last century [1]. Ground improvement techniques reduce the hazards associated with an earthquake, particularly those associated with liquefaction in earthquake prone areas [2]. These soil improvement techniques utilize mechanical energy or grouting with synthetic materials like micro-fine cement, epoxy, acrylmide, phenoplasts, silicates, sodium silicate and polyurethane. All commonly used chemical grouts are toxic and/or hazardous except sodium silicate. The associated environmental risk for many cementing agents encourages the development of alternative soil improvement method that is more environmentally friendly and sustainable.

1.2 BACKGROUND AND PRESENT STATE OF THE PROBLEM

Rapid urbanization in developing countries like Bangladesh is invading the area of cities to provide basic requirements (e.g. habitation, employment opportunity etc.) of huge number of people coming from rural areas. Low lands, lakes, ponds and even rivers are being filled to develop infrastructures. In our country this filling is done with locally available river sands which are vulnerable to earthquakes as those will be liquefied when saturated. Seismic liquefaction has become a common phenomenon in sand filled areas. Most of the new structures in Dhaka city have been constructed over sand filled areas which may undergo liquefaction after a large earthquake. So it is necessary to take measures of improving the existing sand filled ground and to encourage further construction of structures over improved soil. Most of the soil improvement techniques that are utilized involve either the addition of mechanical energy and/or man-made

materials to the soil, both of which have substantial associated energy costs with their production and are not environment friendly. Together, these factors point to a need for development of new alternative soil improvement methods.

Microbial induced calcite precipitation (MICP) is a novel approach, which uses microorganism, nutrients and biological processes to improve engineering properties of the soil. This process uses nonpathogenic organisms that are indigenous to the subsurface environment. MICP is more environment friendly as compared to conventional treatment methods. This technique is also used for various applications such as restoration of calcareous stone materials, bioremediation, wastewater treatment, strengthening of concrete and selective plugging for enhanced oil recovery [3].

Microbial calcite precipitation can occur via a variety of processes. Calcium concentration, carbonate concentration, pH and the availability of nucleation sites are four key parameters that govern $CaCO_3$ precipitation. Various biological reactions produce carbonate or carbonate species. Microbial calcite precipitation by urea hydrolysis with the help of the enzyme urease in a calcium-rich environment is the most commonly studied system. Sporosarcina pasteurii (formerly known as Bacillus pasteurii), a common alkalophilic soil bacterium with a highly active urease enzyme is usually used to hydrolyze urea. This bacterium uses urea as an energy source and produces ammonia, which increases pH in the proximal environment causing precipitation of $CaCO_3$ in calcium-rich environment according to the following reactions [2].

$$NH_2 - CO - NH_2 + H_2O \xrightarrow{\text{Urease}} 2NH_3 + CO_2 \qquad (1.1)$$

$$2NH_3 + 2H_2O \rightarrow 2NH_4^+ + 2OH^- \qquad (1.2)$$

$$CO_2 + OH^- \rightarrow HCO_3^- \qquad (1.3)$$

$$HCO_3^- + H_2O + OH^- \rightarrow CO_3^{2-} + 2H_2O \qquad (1.4)$$

$$NH_2 - CO - NH_2 + 2H_2O \xrightarrow{\text{Urease}} 2NH_4^+ + CO_3^{2-} \qquad (1.5)$$

$$Ca^{2+} + CO_3^{2-} \rightarrow CaCO_3 \downarrow \qquad (1.6)$$
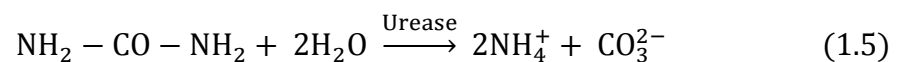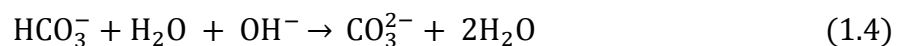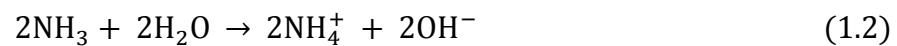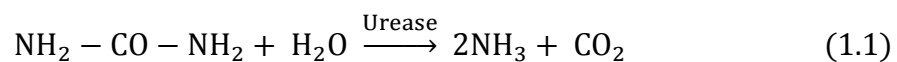
Often the microbes themselves serve as nucleation sites (for crystallization) due to local rise in pH. Calcium ions deposit on the surface of microorganisms with a net cell surface charge that is negative. The equations for the precipitation of calcite at the cell surface serving the nucleation site are as follows [1]:

$$Ca^{2+} + Cell \rightarrow Cell - Ca^{2+} \tag{1.7}$$

$$Cell - Ca^{2+} + CO_3^{2-} \rightarrow Cell - CaCO_3 \tag{1.8}$$
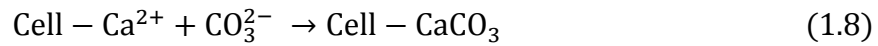
1.3   OBJECTIVES

The objectives of the study are:

- To identify suitable source of urease positive bacteria, isolate and confirm urease activity in mitigating seismic liquefaction.

- To assess the effectiveness of Microbial Induced Calcite Precipitation (MICP) in mitigating seismic liquefaction by different tests (e.g. cyclic triaxial test, unconfined compression test and needle penetration test).

- To examine microscopic structure of microbial induced calcite precipitation in order to justify the prospective of this treatment method for mitigation of seismic liquefaction.

1.4   OUTLINE OF METHODOLOGY

Soil samples as the source of microorganism were collected from a garden. Small amount of soil was used to inoculate the suitable culture medium. After incubation of several days, the culture was tested to confirm the existence of urease positive bacteria. Several trials were performed to isolate urease positive pure culture. Sand samples collected from the local river were prepared properly for all experiments. Some index properties such as maximum and minimum density, specific gravity etc. were determined for the selected samples. Specimens of 71 mm in diameter and 142 mm in height have been used which were prepared by moist tamping method. Bio-treatment was applied into the specimen by two methods, Method A and Method B. For bio-treatment method A, a setup consisting

of three sand columns was developed. Bacteria with necessary nutrients and calcium source were flown through the specimens. In each flow cycle a particular rate of flow was maintained and some chemical properties of the nutrient solution before treatment and after treatment were measured. Number of treatment cycles depends on the amount of calcite to be precipitated (it was 6 (six) for this study). However this number can easily be determined from chemical stoichiometry. After completion of all flow cycles, specimens were tested after 10 days. In method B, bacteria and nutrients were mixed with the specimen during preparation by moist tamping method. Those specimens were also tested after 10 days. Some samples were collected from the bio-treated specimens to confirm the precipitation of calcite by optical microscope and a setup developed.

1.5 ORGANIZATION OF THESIS

This thesis is arranged in the following order. Chapter 2 presents a literature review summarizing what is currently understood about the effects of natural and artificial cementation on soil behavior and previous works on microbial induced calcite precipitation. Chapter 3 discusses the materials and methods that were used in the laboratory testing stage, specifically the microorganism culturing and feeding techniques. Chapter 4 presents the results from various tests that were conducted on untreated (control) and bio-treated specimens. Also in Chapter 4, the results from optical micro scale examinations of calcite-cemented specimens are presented. Chapter 5 presents the overall conclusions from this research study, which was obtained from the tests that were conducted, along with recommendations for future research on the use of microbial calcite cementation for improvement of soil properties.

CHAPTER 2
LITERATURE REVIEW

2.1 GENERAL

During earthquake, major destruction of various types of structures occurs due to the creation of fissures, abnormal and/or unequal movement and loss of strength or stiffness of the ground. The loss of strength or stiffness of the ground results in the settlement of buildings, failure of earth dams, landslides and other hazards. The process by which loss of strength occurs in soil is called liquefaction. Various ground improvement techniques may be used as remedies of liquefaction. Bio-mediated soil improvement technique is a new and environment friendly approach. This treatment method may play a vital role in mitigating seismic liquefaction.

2.2 SEISMIC LIQUEFACTION

Kramer [4] presents a useful note on seismic liquefaction that are presented in the following sections.

Liquefaction is one of the most important, interesting, complex and controversial topics in geotechnical earthquake engineering. Its devastating effects sprang to the attention of geotechnical engineers in a three-month period in 1964 when the Good Friday earthquake (M_W = 9.2) in Alaska was followed by Niigata earthquake (M_S = 7.5) in Japan. Both earthquakes produced spectacular examples of liquefaction-induced damage, including slope failures, bridge and building foundation failures, and flotation of buried structures. In the 30 years since these earthquakes, liquefaction has been studied extensively by hundreds of researchers around the world. Much has been learned, but the road has not been smooth. Different terminologies, procedures, and methods of analysis have been proposed, and a prevailing approach has been slow to emerge. In recent years, many of these differences have been reconciled by the realization that their causes were due, in large part, to semantics.

The term liquefaction, originally coined by Mogami and Kubo, has historically been used in conjunction with a variety of phenomena that involve soil deformations caused by monotonic, transient, or repeated disturbance of saturated cohesionless soils under undrained conditions. The generation of excess pore pressure under undrained loading conditions is a hallmark of all liquefaction phenomena. The tendency for dry cohesionless soils to densify under both static and cyclic loading is well known. When cohesionless soils are saturated, however, rapid loading occurs under undrained conditions, so the tendency for densification causes excess pore pressures to increase and effective stresses to decrease. Liquefaction phenomena that result from this process can be divided into two main groups: flow liquefaction and cyclic mobility. Both flow liquefaction and cyclic mobility are very important, and any evaluation of liquefaction hazards should carefully consider both. In the field, flow liquefaction occurs much less frequently than cyclic mobility but its effects are usually far more severe. Cyclic mobility, on the other hand, can occur under a much broader range of soil and site conditions than flow liquefaction; its effects can range from insignificant to highly damaging.

2.2.1 Flow Liquefaction

Flow liquefaction produces the most dramatic effects of all the liquefaction related phenomena – tremendous instabilities known as flow failures. Flow liquefaction can occur when the shear stress required for static equilibrium of a soil mass is greater than the shear strength of the soil in its liquefied state. Once triggered the large deformations produced by flow liquefaction are actually driven by static shear stresses. The cyclic stresses may simply bring the soil to an unstable state at which its strength drops sufficiently to allow the static stresses to produce the flow failure. Flow liquefaction failures are characterized by the sudden nature of their origin, the speed with which they develop, and the large distance over which the liquefied materials often move.

2.2.2 Cyclic Mobility

Cyclic mobility is another phenomenon that can also produce unacceptably large permanent deformations during earthquake shaking. In contrast to flow liquefaction, cyclic mobility occurs when the static shear stress is less than the shear strength of the liquefied soil. The deformations produced by cyclic mobility failures develop incrementally during earthquake shaking. In contrast to flow liquefaction, the

deformations produced by cyclic mobility are driven by both cyclic and static shear stresses. These deformations, termed lateral spreading, can occur on very gently sloping ground or on virtually flat ground adjacent to bodies of water. When structures are present, lateral spreading can cause significant damage. A special case of cyclic mobility is level ground liquefaction. Because static horizontal shear stresses that could drive lateral deformations do not exist, level ground liquefaction can produce large, chaotic movement known as ground oscillation during earthquake shaking, but produces little permanent lateral soil movement. Level ground liquefaction failures are caused by the upward flow of water that occurs when seismically induced excess pore pressures dissipate. Depending on the length of time required to reach hydraulic equilibrium, level ground liquefaction failure may occur well after ground shaking has ceased. Excessive vertical settlement and consequent flooding of low-lying land and the development of sand boils (Figure 2.1) are characteristic of level ground liquefaction.

Figure 2.1: Sand Boiling

2.3 LIQUEFACTION SUSCEPTIBILITY

Not all soils are susceptible to liquefaction; consequently, the first step in a liquefaction hazard evaluation is usually the evaluation of liquefaction susceptibility. If the soil at a particular site is not susceptible, liquefaction hazards do not exist and the liquefaction hazard evaluation can be ended. If the soil is susceptible, however, the matters of liquefaction initiation and effects must be addressed. There are several criteria by which

liquefaction susceptibility can be judged, and some are different for flow liquefaction and cyclic mobility. Based on the results of laboratory tests as well as field observations and studies, the most important factors that govern liquefaction are as follows:

2.3.1 Earthquake Intensity and Duration

In order to have liquefaction of soil, there must be ground shaking. The character of the ground motion, such as acceleration and duration of shaking, determines the shear strains that cause the contraction of the soil particles and the development of excess pore water pressures leading to liquefaction. The most common cause of liquefaction is due to the seismic energy released during an earthquake. The potential for liquefaction increases as the earthquake intensity and duration of shaking increase. Those earthquakes that have the highest magnitude will produce both the largest ground acceleration and the longest duration of ground shaking. Although data are sparse, there would appear to be a shaking threshold that is needed to produce liquefaction. These threshold values are a peak ground acceleration, a_{max} of about 0.10g and local magnitude M_L of about 5. Thus, a liquefaction analysis would typically not be needed for those sites having peak ground acceleration, a_{max} less than 0.10g or local magnitude, M_L less than 5. Besides earthquakes, other conditions can cause liquefaction, such as subsurface blasting, pile driving, and vibrations from train traffic.

2.3.2 Groundwater Table

The condition most conducive to liquefaction is a near-surface groundwater table. Unsaturated soil located above the groundwater table will not liquefy. If it can be demonstrated that the soils are currently above the groundwater table and are highly unlikely to become saturated for given foreseeable changes in the hydrologic regime, then such soils generally do not need to be evaluated for liquefaction potential. At sites where the groundwater table significantly fluctuates, the liquefaction potential will also fluctuate [4]. Generally, the historic high groundwater level should be used in the liquefaction analysis unless other information indicates a higher or lower level is appropriate. Some researchers have stated that liquefaction can also occur in very large masses of sands or silts that are dry and loose and loaded so rapidly that the escape of air from the voids is restricted. Such movement of dry and loose sands is often referred to as running soil or

running ground. Although such soil may flow as liquefied soil does, it is best to consider that liquefaction only which occurs for soils that are located below the groundwater table.

2.3.3 Soil Type

The hazard associated with soil liquefaction during earthquakes has been known to be encountered in deposits consisting of fine to medium sand and sands containing low-plasticity fines. Occasionally, however, cases are reported where liquefaction apparently occurred in gravelly soils." Thus, the soil types susceptible to liquefaction are nonplastic (cohesionless) soils. An approximate listing of cohesionless soils from least to most resistant to liquefaction is clean sands, nonplastic silty sands, nonplastic silt, and gravels. There could be numerous exceptions to this sequence. For example, the rock flour in a water-saturated state does not possess significant cohesion and behaves like clean sand. This rock flour exhibits as low a resistance to liquefaction as clean sand. Some researchers stated that based on laboratory testing and field performance, the great majority of cohesive soils will not liquefy during earthquakes. In order for a cohesive soil to liquefy, it must meet all the following three criteria:

- The soil must have less than 15 percent of the particles, based on dry weight, that are finer than 0.005 mm (i.e., percent finer at 0.005 mm < 15 percent).

- The soil must have a liquid limit (LL) that is less than 35 (that is, LL < 35).

- The water content, w of the soil must be greater than 0.9 of the liquid limit [that is, $w > 0.9$ (LL)].

If the cohesive soil does not meet all three criteria, then it is generally considered to be not susceptible to liquefaction. Although the cohesive soil may not liquefy, there could still be a significant undrained shear strength loss due to the seismic shaking.

2.3.4 Soil Relative Density

Based on field studies, cohesionless soils in a loose relative density state are susceptible to liquefaction. Loose nonplastic soils will contract during the seismic shaking, which will cause the development of excess pore water pressures. Upon reaching initial liquefaction, there will be a sudden and dramatic increase in shear displacement for loose

sands. For dense sands, the state of initial liquefaction does not produce large deformations because of the dilation tendency of the sand upon reversal of the cyclic shear stress. Some researchers state that if the in stiu soil can be shown to be dilative, it need not be evaluated because it will not be susceptible to liquefaction. In essence, dilative soils are not susceptible to liquefaction because their undrained shear strength is greater than their drained shear strength.

2.3.5 Particle Size Distribution

Uniformly graded nonplastic soils tend to form more unstable particle arrangements and are more susceptible to liquefaction than well-graded soils. Well-graded soils will also have small particles that fill in the void spaces between the large particles. This tends to reduce the potential contraction of the soil, resulting in less excess pore water pressures being generated during the earthquake. Some researchers state that field evidence indicates that most liquefaction failures have involved uniformly graded granular soils.

2.3.6 Placement Conditions or Depositional Environment

Hydraulic fills (fill placed under water) tend to be more susceptible to liquefaction because of the loose and segregated soil structure created by the soil particles falling through water. Natural soil deposits formed in lakes, rivers, or the ocean also tend to form a loose and segregated soil structure and are more susceptible to liquefaction. Soils that are especially susceptible to liquefaction are formed in lacustrine, alluvial, and marine depositional environments.

2.3.7 Drainage Conditions

If the excess pore water pressure can quickly dissipate, the soil may not liquefy. Thus highly permeable gravel drains or gravel layers can reduce the liquefaction potential of adjacent soil.

2.3.8 Confining Pressures

The greater the confining pressure, the less susceptible the soil is to liquefaction. Conditions that can create a higher confining pressure are a deeper groundwater table, soil that is located at a deeper depth below ground surface, and a surcharge pressure applied at

ground surface. Case studies have shown that the possible zone of liquefaction usually extends from the ground surface to a maximum depth of about 50 ft (15 m). Deeper soils generally do not liquefy because of the higher confining pressures. This does not mean that a liquefaction analysis should not be performed for soil that is below a depth of 50 ft (15 m). In many cases, it may be appropriate to perform a liquefaction analysis for soil that is deeper than 50 ft (15 m). An example would be sloping ground, such as a sloping berm in front of a waterfront structure or the sloping shell of an earth dam. In addition, a liquefaction analysis should be performed for any soil deposit that has been loosely dumped in water (i.e., the liquefaction analysis should be performed for the entire thickness of loosely dumped fill in water, even if it exceeds 50 ft in thickness). Likewise, a site where alluvium is being rapidly deposited may also need a liquefaction investigation below a depth of 50 ft (15 m). Considerable experience and judgment are required in the determination of the proper depth to terminate a liquefaction analysis.

2.3.9 Particle Shape

The soil particle shape can also influence liquefaction potential. For example, soils having rounded particles tend to densify more easily than angular shape soil particles. Hence a soil containing rounded soil particles is more susceptible to liquefaction than a soil containing angular soil particles.

2.3.10 Aging and Cementation

Newly deposited soils tend to be more susceptible to liquefaction than older deposits of soil. It has been shown that the longer a soil is subjected to a confining pressure, the greater the liquefaction resistance. Table 2.1 presents the estimated susceptibility of sedimentary deposits to liquefaction versus the geologic age of the deposit [5]. The increase in liquefaction resistance with time could be due to the deformation or compression of soil particles into more stable arrangements. With time, there may also be the development of bonds due to cementation at particle contacts.

Table 2.1: Estimated Susceptibility of Sedimentary Deposits to Liquefaction during Strong Seismic Shaking Based on Geologic Age and Depositional Environment [5]

Type of Deposit	General Distribution of Cohesionless Sediments in Deposits	Likelihood that Cohesionless Sediments, when Saturated would be Susceptible to Liquefaction (by Age of Deposit)			
		<500 Years	Holocene	Pleistocene	Pre-Pleistocene
(a) Continental Deposits					
Alluvial Fan and Plain	Widespread	Moderate	Low	Low	Very Low
Delta and Fan Delta	Widespread	High	Moderate	Low	Very Low
Dunes	Widespread	High	Moderate	Low	Very Low
Marine Terrace/Plain	Widespread	Unknown	Low	Very Low	Very Low
Talus	Widespread	Low	Low	Very Low	Very Low
Tephra	Widespread	High	High	Unknown	Unknown
Colluviums	Variable	High	Moderate	Low	Very Low
Glacial Till	Variable	Low	Low	Very Low	Very Low
Lacustrine and Playa	Variable	High	Moderate	Low	Very Low
Loess	Variable	High	High	High	Unknown
Floodplain	Locally Variable	High	Moderate	Low	Very Low
River Channel	Locally Variable	Very High	High	Low	Very Low
Sebka	Locally Variable	High	Moderate	Low	Very Low
Residual soils	Rare	Low	Low	Very Low	Very Low
Tuff	Rare	Low	Low	Very Low	Very Low
(b) Coastal Zone					
Beach- Large Waves	Widespread	Moderate	Low	Very Low	Very Low
Beach- Large Waves	Widespread	High	Moderate	Low	Very Low
Delta	Widespread	Very High	High	Low	Very Low
Estuarine	Locally Variable	High	Moderate	Low	Very Low
Foreshore	Locally Variable	High	Moderate	Low	Very Low
Lagoonal	Locally Variable	High	Moderate	Low	Very Low
(c) Artificial					
Compacted Fill	Variable	Low	Unknown	Unknown	Unknown
Uncompacted Fill	Variable	Very High	Unknown	Unknown	Unknown
Source: Data from Youd and Hoose (1978), Reproduced from R.B. Seed (1991)					

2.3.11 Historical Environment

It has also been determined that the historical environment of the soil can affect its liquefaction potential. For example, older soil deposits that have already been subjected to seismic shaking have an increased liquefaction resistance compared to a newly formed specimen of the same soil having an identical density. Liquefaction resistance also increases with an increase in the over consolidation ratio (OCR) and the coefficient of lateral earth pressure at rest (k_0). An example would be the removal of an upper layer of soil due to erosion. Because the underlying soil has been preloaded, it will have a higher over consolidation ratio and it will have a higher coefficient of lateral earth pressure at rest (k_o). Such a soil that has been preloaded will be more resistant to liquefaction than the same soil that has not been preloaded.

2.3.12 Building Load

The construction of a heavy building on top of a sand deposit can decrease the liquefaction resistance of the soil. For example, suppose a mat supports a heavy building. The soil underlying the mat will be subjected to shear stresses caused by the building load. These shear stresses induced into the soil by the building load can make the soil more susceptible to liquefaction. The reason is that a smaller additional shear stress will be required from the earthquake in order to cause contraction and hence liquefaction of the soil. For level-ground liquefaction, the effect of the building load is ignored. Although building loads are not considered in the liquefaction analysis, the building loads must be included in all liquefaction-induced settlement, bearing capacity, and stability analyses.

2.4 EVALUATING THE LIQUEFACTION POTENTIAL

There are a number of different methods, by which the potential for liquefaction of a soil can be evaluated [5]. These methods generally compare the cyclic shear resistance of the soil with the cyclic shear stresses and strains caused by an earthquake [5]. The factor of safety against seismic liquefaction can be expressed as,

$$FS = \frac{Cyclic\ shear\ resistance\ of\ the\ soil}{Equivalent\ cyclic\ shear\ stress\ induced\ by\ an\ earthquake} \qquad (2.1)$$

There are different methods to determine both cyclic resistance of the soil and equivalent cyclic shear stress induced by an earthquake.

Characterization of liquefaction resistance based on laboratory tests has been a common practice. Because of comprehensive laboratory studies, it has been recognized reasonable and become customary to consider the combined effect of cyclic shear stress in terms of the cyclic stress ratio. Cyclic stress ratio is defined as,

$$Cyclic\ stress\ ratio\ = \frac{\sigma_d}{2\,\sigma_o'} \qquad (2.2)$$

Here,

σ_d = Single amplitude of cyclic deviator stress

σ_o' = Initial confining stress

Thus, it has become a routine practice to take cyclic stress ratio required to produce 5 % double amplitude axial strain with 20 load cycles as a factor quantifying the liquefaction resistance of sands under a given state of packing as represented by void ratio or relative density. This cyclic stress ratio is represented by,

$$CRR = (\frac{\sigma_d}{2\,\sigma_o'})_{20,5\%} \qquad (2.3)$$

Relative density has been recognized as dominant factor influencing the cyclic strength. In addition, method of preparation of samples gives different results (Figure 2.2).

In the view of the diversity of cyclic strength of sand samples reconstituted by different methods of preparation, it has been recognized that deposits of sand in the field may exhibit varying resistance to seismic application (Figure: 2.3). Therefore, it is very much necessary to collect undisturbed samples and test them under the condition representative of those in the field.
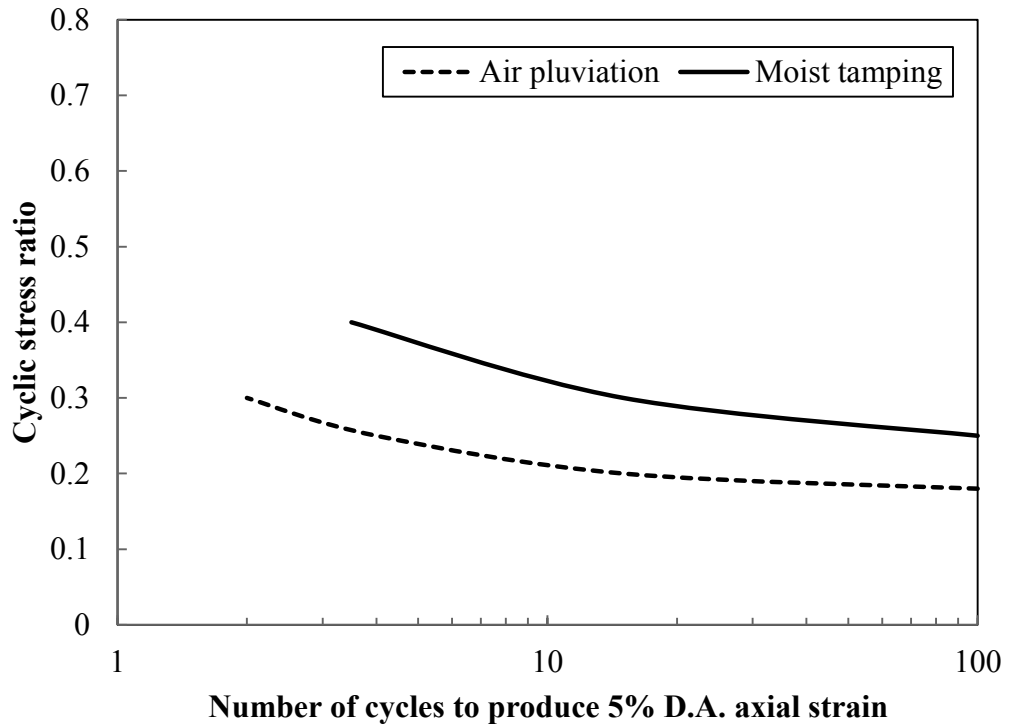
Figure 2.2: Effect of Sample Preparation on Cyclic Strength [5]
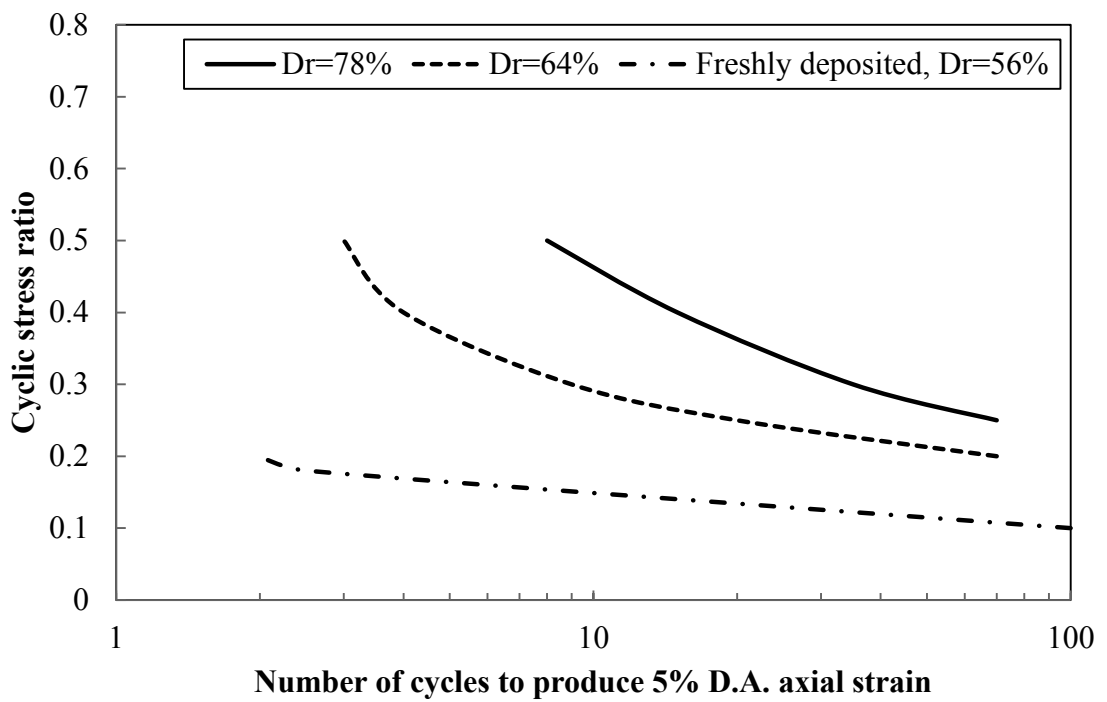
Figure 2.3: Effect of Field Condition on Cyclic Strength [5]

The type of soil most susceptible to liquefaction is one in which the resistance to deformation is mobilized by friction between particles under the influence of confining

pressure. When the soil is fine grained or contains some amount of fines, cohesion or adhesion tends to develop between fine particles, making it difficult to separate them. Consequently, sand containing some amount of fines generally shows greater amount of resistance to liquefaction. This tendency depends on the nature of the fines. If the fines comprise minerals with a dry surface texture free from adhesion, individual particles will separate readily, therefore sand containing such fines will show liquefaction potential as clean sand. On the other hand, if the fines have adhesion/cohesion property, liquefaction potential will be reduced (Figure 2.4).
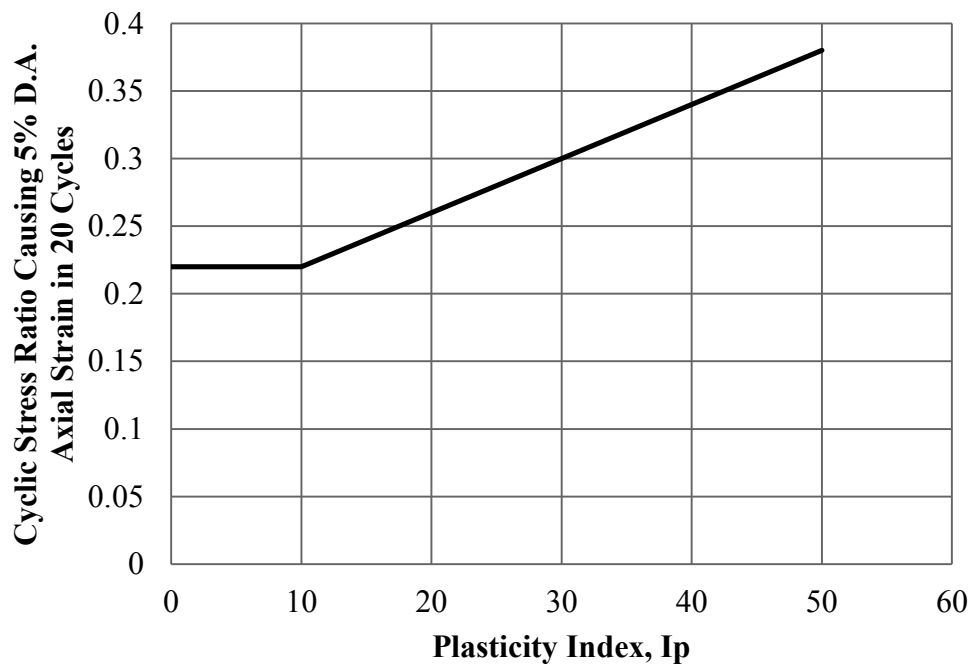
Figure 2.4: Cyclic Stress Ratio versus Plasticity Index [5]

2.5 TYPICAL EFFECTS OF LIQUEFACTION

Typical effects of liquefaction are described in the following sections.

2.5.1 Loss of Bearing Capacity

The ground can liquefy and lose its ability to support structures. It is seen in the Figure 2.5 of the overturned apartment complex buildings in Niigata in 1964 that the structure was all right but the soil failed to bear the load due to liquefaction.

Figure 2.5: Collapse/Damage of Buildings during 1964 Niigata Earthquake

2.5.2 Lateral Spreading

The ground can slide down very gentle slopes or toward stream banks, riding on a buried liquefied layer can make big cracks on the ground (Figure 2.6).

Figure 2.6: Burning Gas Main Ruptured by Lateral Movement, Balboa Blvd in Granada Hills, 1994 Northridge Earthquake

2.5.3 Sand Boiling

Sand-laden water can be ejected from a buried liquefied layer and erupt at the surface to form sand volcanoes; the surrounding ground often fractures and settles (Figure 2.7). The numerous sand boils that were observed in the earthquake, affected area provided indisputable evidence of the occurrence of liquefaction.
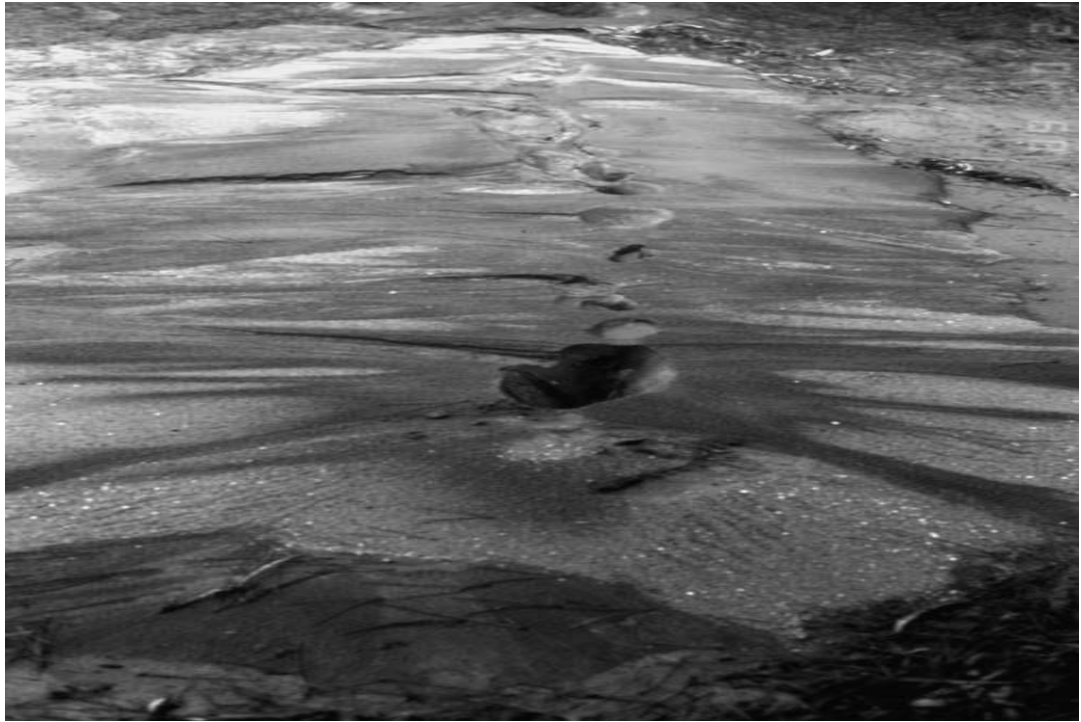
Figure 2.7: Sand Boiling along a Fissure near The Pajaro River, 1989 Loma Prieta Earthquake

2.5.4 Land Sliding

Increased water pressure can also trigger landslides and causes the collapse of dams (Figure 2.8). Liquefaction in sand layers, and in sand and silt seams in the clayey soils beneath Anchorage, caused many of the destructive landslides that occurred during the earthquake.

Figure 2.8: Lower San Fernando Dam Suffered an Underwater Slide during the San Fernando Earthquake, 1971

2.5.5 Ground Oscillation

The surface layer, riding on a buried liquefied layer, is thrown back and forth by the shaking and can be severely deformed (Figure 2.9).

Figure 2.9: Walkway and Pavement Buckled by Ground Oscillation, Marina District of San Francisco, 1989 Loma Prieta Earthquake

2.5.6 Floating of Structure

Light structures that are buried in the ground (like pipelines, sewers and nearly empty fuel tanks) can float to the surface when they are surrounded by liquefied soil (Figure 2.10).

Figure 2.10: Liquefaction in a Sewer Line (Chuetsu Earthquake)

2.5.7 Settlement

When liquefied ground re-consolidates following an earthquake, the ground surface may settle or subside as shaking decreases and the underlying liquefied soil becomes denser (Figure 2.11).

Figure 2.11: Settlement and Disruption of Ground and Pavement over Filled Ground, Dore Street, 1906 San Francisco Earthquake

2.5.8 Flow Failure

Earth moves down in steep slope with large displacement and much internal disruption of material due to liquefaction (Figure 2.12 and Figure 2.13).

Figure 2.12: A Small Flow Slide along the Shore of Lake Merced in San Francisco in 1957

Figure 2.13: Flow Failure in Highway Fill, Lake Merced, 1957 Daly City Earthquake

2.6 LIQUEFACTION IN CONTEXT OF BANGLADESH

As Bangladesh is located near a tectonically active zone, much of the country including Chittagong, Sylhet, Dhaka, Rangpur, Bogra, Mymensingh, Comilla, Rajshahi are very much vulnerable to major earthquake disaster (Figure 2.14). Although, some awareness is raised among limited groups, due to some recent earthquakes in the region, the country is still behind the minimum preparedness level to face such a disaster in any of our cities. During sustained strong shaking, poorly consolidated, water saturated sediments can liquefy and lose their ability to support loads. The foundations and supports of structures built on liquefiable sediments can fail, causing damage or destruction. However, recently some studies have been done to establish liquefaction possibility in Bangladesh by the national experts of Bangladesh. It is expected that in near future a liquefaction potential map of Bangladesh will be developed and difficulties raised in finalizing design of many important structures will be over.

2.7 MITIGATION OF LIQUEFACTION

There are three possibilities to reduce liquefaction hazards when designing and constructing new buildings or other structures as bridges, tunnels, roads etc.

2.7.1 Avoiding Liquefaction Susceptible Soils

The first possibility is to avoid construction on liquefaction susceptible soils. By characterizing the soil at a particular building site one can decide if the site is susceptible to liquefaction and therefore unsuitable for the desired structure.

2.7.2 Building Liquefaction Resistant Structures

If it is necessary to construct on liquefaction susceptible soil because of space restrictions, favorable location, or other reasons, it may be possible to make the structure liquefaction resistant by designing the foundation elements to resist the effects of liquefaction. A structure that possesses ductility, has the ability to accommodate large deformations, adjustable supports for correction of differential settlements, and having foundation design that can span soft spots can decrease the amount of damage a structure may suffer in case of liquefaction.
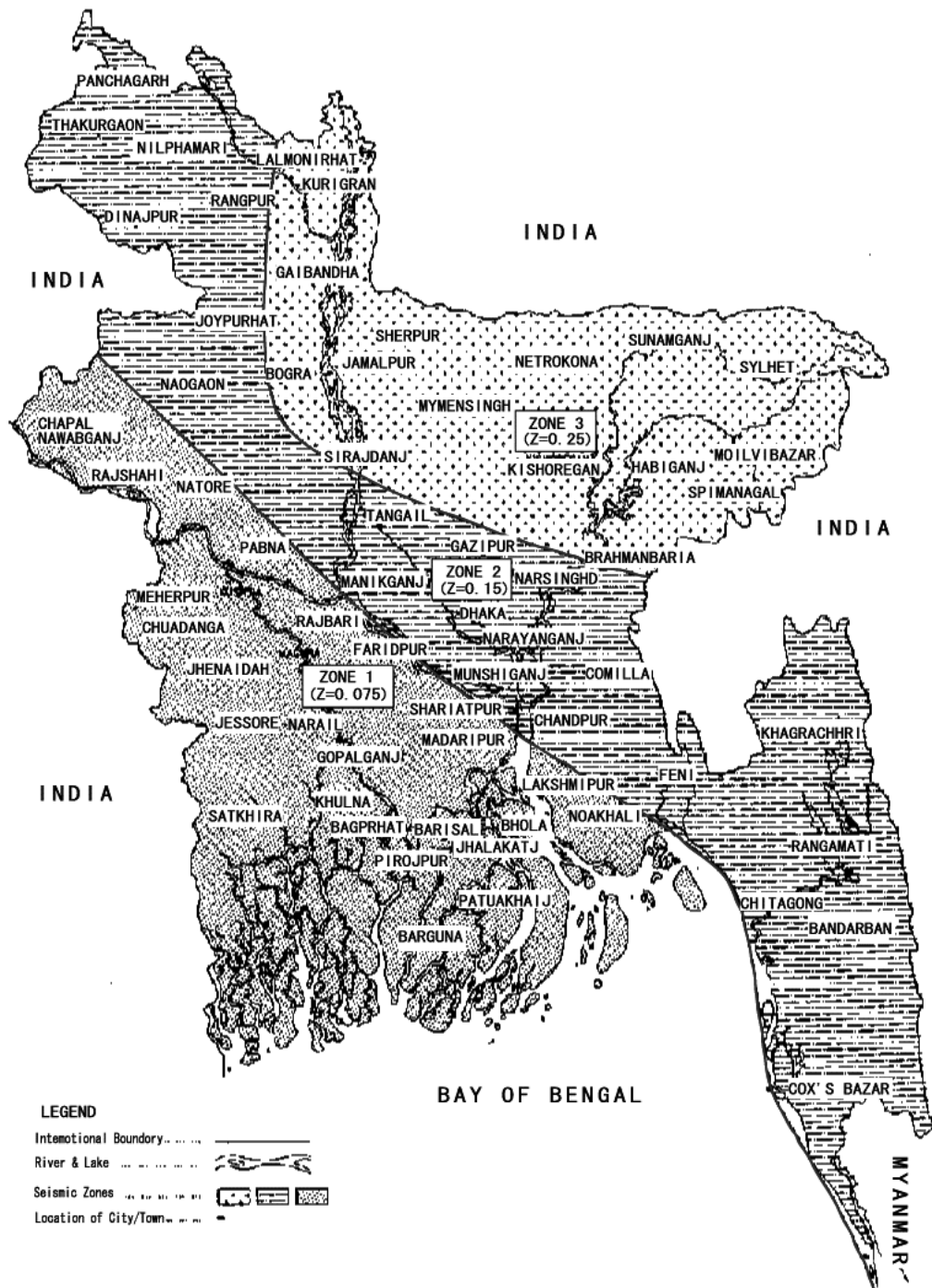
Figure 2.14: Seismic Zoning Map of Bangladesh [BNBC, 2006]

<u>Design Considerations for Shallow Foundation</u>

It is important that all foundation elements in a shallow foundation is tied together to make the foundation move or settle uniformly, thus decreasing the amount of shear forces

induced in the structural elements resting upon the foundation. A stiff foundation mat (below) is a good type of shallow foundation, which can transfer loads from locally liquefied zones to adjacent stronger ground. Buried utilities, such as sewage and water pipes, should have ductile connections to the structure to accommodate the large movements and settlements that can occur due to liquefaction.

Design Considerations for Deep Foundation

Liquefaction can cause large lateral loads on pile foundations. Sufficient resistance can be achieved by piles of larger dimensions and/or more reinforcement. It is important that the piles are connected to the cap in a ductile manner that allows some rotation to occur without a failure of the connection.

2.7.3 Improving the Soil

The third option involves mitigation of the liquefaction hazards by improving the strength, density, and/or drainage characteristics of the soil. This can be done using a variety of soil improvement techniques.

Vibroflotation

Vibroflotation involves the use of a vibrating probe that can penetrate granular soil to depths of over 100 feet. The vibrations of the probe cause the grain structure to collapse thereby densifying the soil surrounding the probe.

Dynamic Compaction

Densification by dynamic compaction is performed by dropping a heavy weight of steel or concrete in a grid pattern from heights of 30 to 100 ft.

Stone Columns

Stone columns are columns of gravel constructed in the ground.

Compaction Piles

Installation of compaction piles both densifies and reinforces the soil.

Compaction Grouting

Compaction grouting is a technique whereby a slow-flowing grout mix is injected under pressure into a granular soil. The grout forms a bulb that displaces and hence densifies the surrounding soil.

Drainage Techniques

Liquefaction hazards can be reduced by increasing the drainage ability of the soil. Drainage techniques include installation of drains of gravel, sand or synthetic materials.

2.8 GROUND IMPROVEMENT

Due to overpopulation in urban areas, and an increasing shortage of "ideal" building sites, there has been an increased need for development of ground improvement techniques within the geotechnical engineering community over the past decade [6]. The main goal of ground improvement techniques that are commonly used depends on the specific needs for a given project at a given site, but it can typically be classified into one of the following general categories:

- Increasing the shear strength of the soil to guard against catastrophic failure.

- Reducing the compressibility of the soil to prevent excessive ground movements, or reducing the permeability of the soil to reduce the rate of water seepage (common for earth dam or environmental applications).

For projects that are located in earthquake prone areas, it is also desirable to use ground improvement techniques to reduce the hazards associated with an earthquake, particularly those associated with liquefaction, which can cause catastrophic ground failures. Kamon and Bergado [7] classified commonly used ground improvement methods by soil type into four categories, as shown in Figure 2.15. While compaction and dewatering involve only work on soil, chemical admixture and reinforcement techniques require the use of additional materials as inputs into the process. These soil improvement techniques utilize either mechanical energy or synthetic materials. A common approach is to inject cementing agents into the pore space to bind soil particles together, such as micro-fine cement, epoxy, acrylate, phenoplasts, sodium silicates, and polyurethane [8]. This is

achieved using a variety of chemical, cement, jetting, and compaction grouting techniques. Except for sodium silicate, all commonly used chemical grouts are toxic and/or hazardous. The associated toxicity and potential environmental risk for many cementing agents encourages the development of alternative soil improvement methods that are more environmentally friendly and more sustainable.
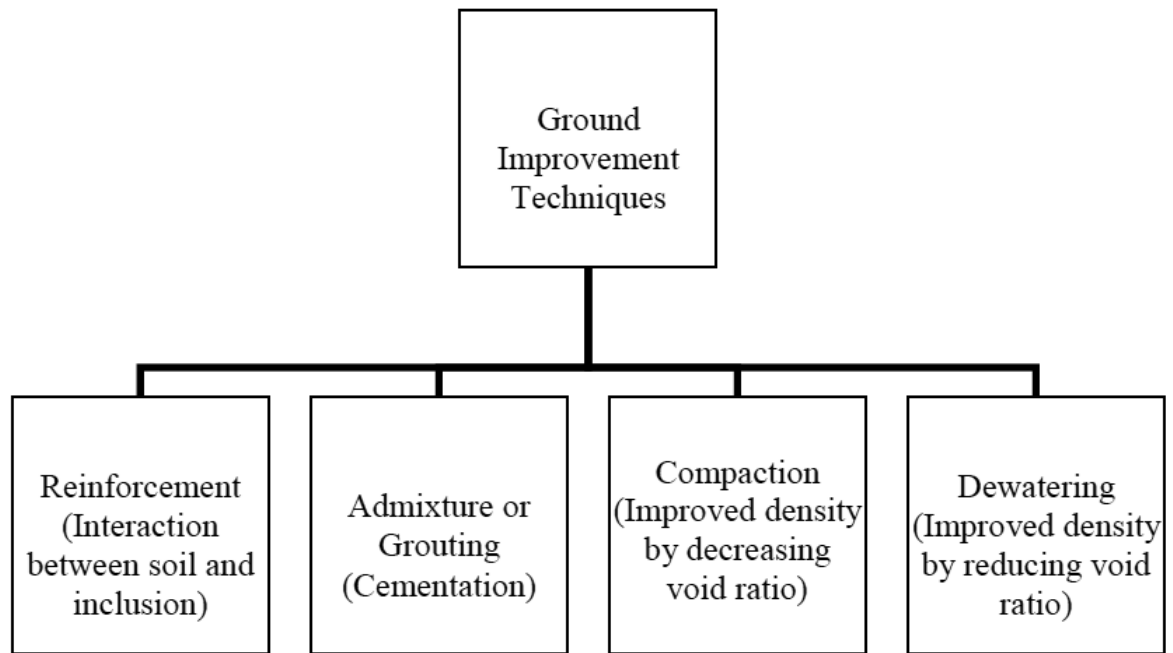
Figure 2.15: Ground Improvement Techniques [7]

2.8.1 Cementation in Soil

Various soil cementation techniques are commonly used to improve the strength and deformation behavior of soil. Lime and Portland cement are commonly used as cementing agents in many field applications, due to their widespread availability and relatively low cost [9,10]. Cement-treated soil has been frequently used for highway, railroad, and airport construction to increase the bearing capacity of soft soil subgrades [9]. Cemented soil can be classified into two categories depending on the method of cementation:

- Naturally cemented soil, which can contain carbonate, iron oxide, alumina, or organic matter, any of which may precipitate at the antiparticle contacts and act as cementing agent.

- Artificially cemented soil, in which cementing agents are added to the soil to induce cementation during experimental studies or field application. The most common cementing agents that are added to soil are lime, calcite, Portland cement, and gypsum.

Both artificial and natural cementation methods can be used in the field and the laboratory.

2.8.2 Artificially Cemented Soil

Ismail et al. [11] carried out triaxial tests on specimens treated with various cementing agents, including Portland cement, gypsum, and calcite. Their results showed that despite having the same unconfined compressive strength (q_u) and density, the effective stress paths and post-yield response of these materials might be significantly different, mainly because of the different volumetric response of the cemented granular materials during shear. Portland cement specimens showed ductile yield and strong dilation after the point of yield, while calcite and gypsum-cemented samples exhibited brittle yield, generally followed by contractive shear behavior. While the type of cement had a significant influence on the shear behavior of the soil, the density for a given level of cementation did not affect the volumetric response, especially before yield, for each of the cementing agents that were examined. These triaxial test results are shown in Figures 2.16 and 2.17. Rotta et al. [12] performed a lab-scale study to simulate the formation of cementation in a soil matrix at different depths in a sedimentary deposit. In this experiment, isotropic unconfined compression tests were carried out on artificially cemented specimen with different Portland cement contents. The test results showed that the primary yield stress in isotropic compression is a function of the curing void ratio and cement content. Depending on the cement content that was used, the isotropic compression was reduced as the void ratio decreased. Many of the investigations that are described in the available technical literature report significant difficulties when testing naturally cemented soils, primarily due to the disturbance that occurs to the soil structure during the sampling process. Some researchers attribute the loss of specimen stiffness that occurs because of sampling to breakage of the cement bonds at the inter-particle contacts. Because of its significant potential benefits as a ground improvement technique, artificial in situ soil cementation has been investigated by a number of researchers [11, 12].
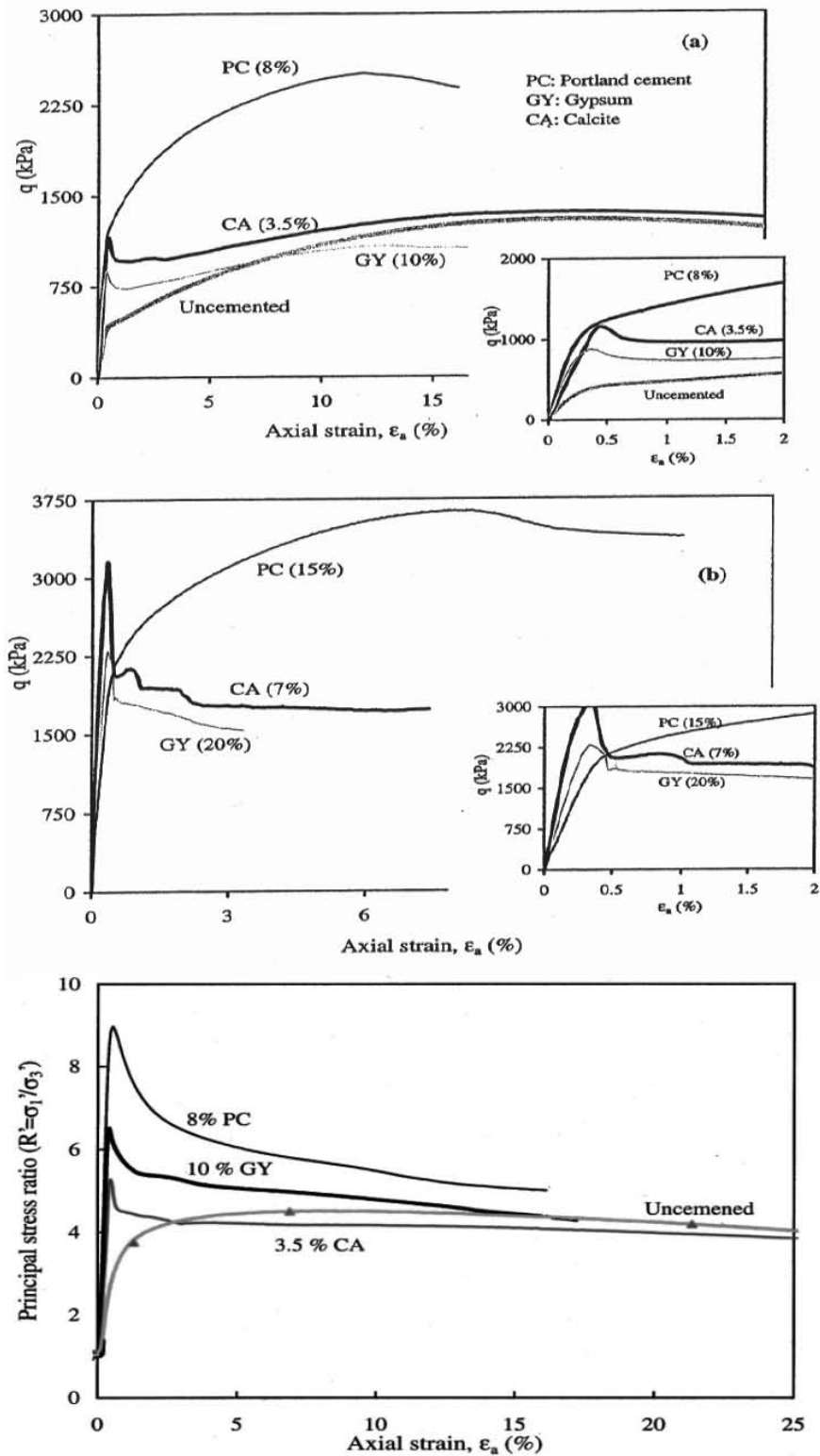
Figure 2.16: Effect of Different Cementing Agents on the Stress-Strain Behavior of Artificially Cemented Specimens; Inset Figures Show Small-Strain Shear Behavior in More Detail [11]
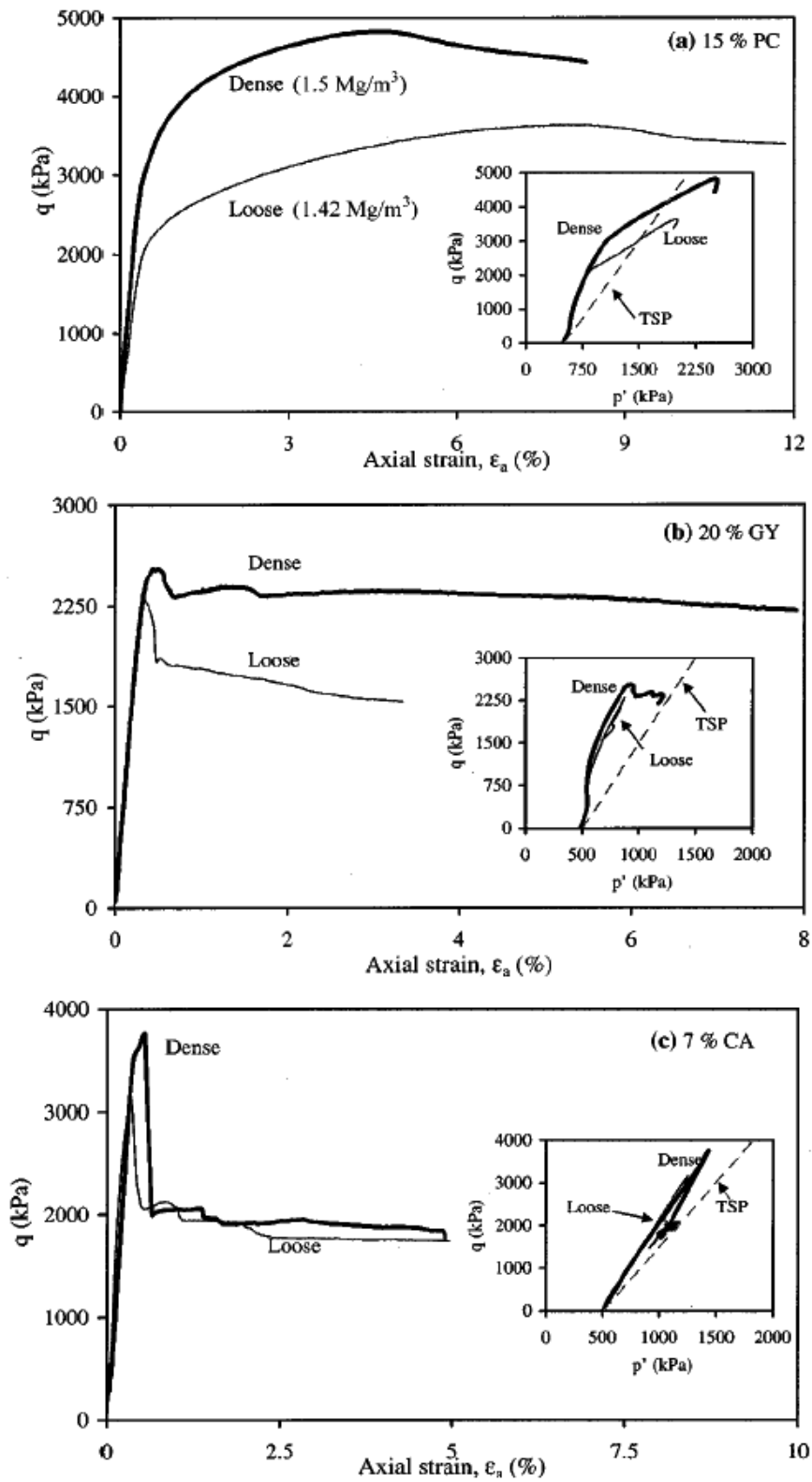
Figure 2.17: Effect of Density on Artificially Cemented Specimens; Inset Figures Show Stress-Path Response of the Specimens [11]

One of these researchers, Ismail et al. [11], used an innovative chemical cementation process called CIPS (calcite in-situ precipitation system), which was developed by CSIRO – the Division of Exploration and Mining in Australia, to better simulate naturally occurring cementing processes. CIPS is a water-based, non-particulate, low viscosity, neutral P^H and non-toxic soil cementing technique. In the CIPS technique, cementation is achieved by flushing a chemical solution through porous material, resulting in the precipitation of calcite between the soil grains, which cements the soil matrix and increases the mechanical strength of the material. A schematic diagram of the flushing process is shown in Figure 2.18. It was found that the strength of a calcite-treated material increases with the strength of individual grains, soil density, decreasing particle size of the host grains, by pre-coating of grains with calcite, and by the roundness and non-angularity of the soil grains. Scanning electron microscope (SEM) images of a sample cemented by CIPS are provided in Figure 2.19
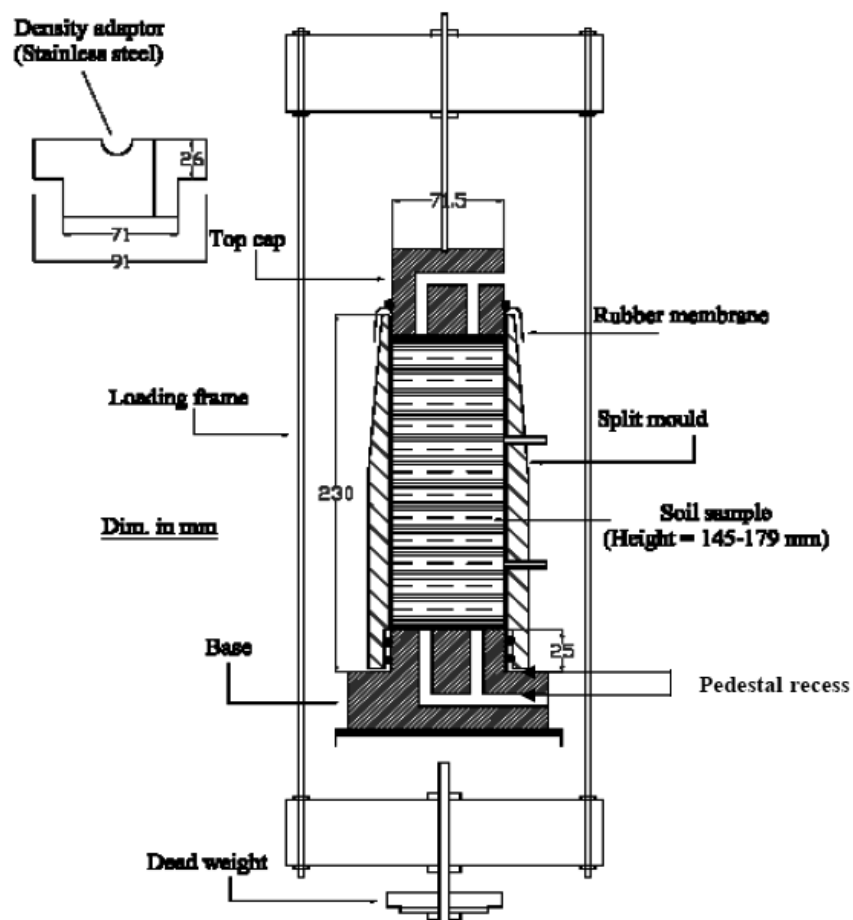
Figure 2.18: Schematic View of Set-up for Sample Flushing Technique [11]
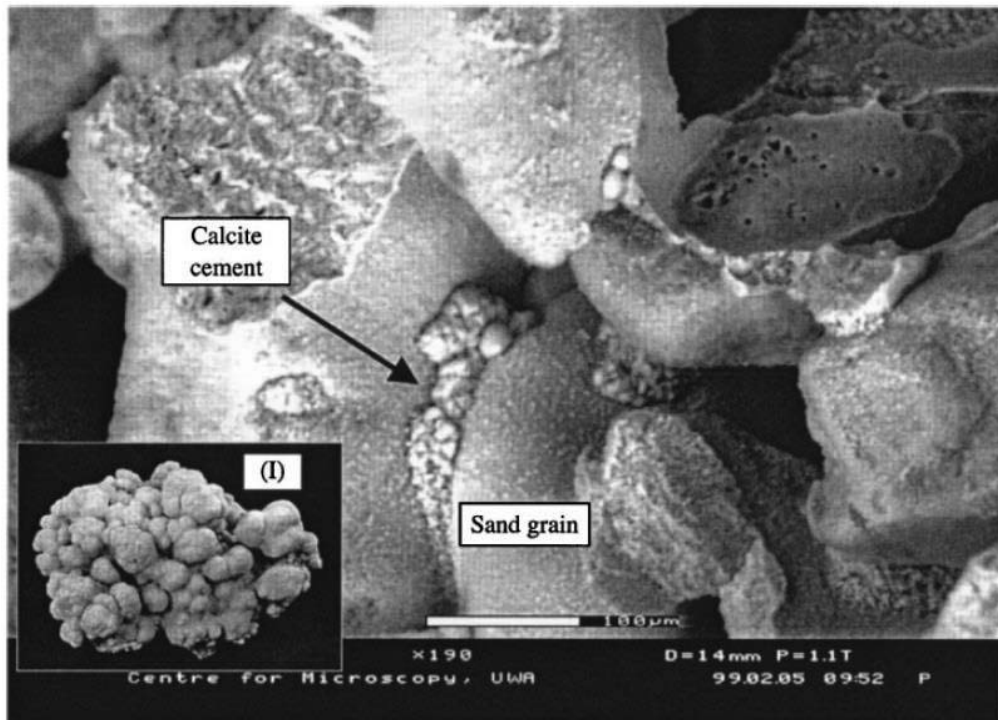
Figure 2.19: SEM images for CIPS- Cemented Calcareous Soil [11]

2.8.3 Naturally Cemented Soil

The engineering behavior of naturally cemented soil is determined by the equally significant effects of both cementation and soil structure, as well as some of the basic properties of soil mechanics, such as initial porosity and stress history. Natural cementation may occur in sands through a variety of processes. In some cases, the cementing agent has been deposited immediately after deposition of the sand when the sand was at a shallow depth. In other cases, sand grains can be transferred by streams and then deposited. In-place cementation may also occur via chemical deposition of cementing agents, cementation from weathering byproducts, a "welding" process, and/or bonding encouraged by the presence of silt or clay particles. In natural environments, cemented sands can be found at different places in the earth's crust and cementation is generally attributed to the precipitation of calcite cement [2]. In a similar fashion, the program of research described herein will focus on cementation that is caused by calcite formation aided by the presence of calcium carbonate producing bacteria.

Mechanism

There are two different processes, which can lead to in situ calcite cementation in natural environments [2]:

- First, calcite can be formed when water saturated with calcium carbonate in marine environments evaporates, a phenomenon that is commonly observed in subtropical areas.

- Second, calcite can precipitate due to a chemical reaction at the surface of soil grains close to the water-seabed boundary.

Numerous factors affect the cementation process such as chemical and physical environmental conditions, soil permeability, soil texture, composition, and stabilization of the sediment itself. The effects of structure and cementation on the mechanical behavior of soils, which are naturally cemented, are reviewed in subsequent sections.

Naturally-Cemented Sand Strength and Stress-Strain Response

Some researchers described the unique strength characteristics of naturally cemented soil, observing that this soil tends to exhibit initial yield, indicating the breakdown of cementation. Typical stress-strain behavior for naturally cemented sand tested at different confining pressures is illustrated schematically in Figure 2.20 [13]. For a relatively well-cemented soil specimen tested at a low confining pressure (e.g., Curve 3 shown in Figure 2.20), the stress-strain curves typically indicate relatively linear elastic behavior up to the point of yield, followed by a rapid drop in strength past the point of yield as the cemented bonds in the specimen are broken, to a steady state shearing condition. If similar cemented specimens are tested at higher confining pressures (e.g., Curves 1 and 2 shown in Figure 2.20) then some of the cemented bonds in the specimen will be broken, causing the initial yield point to be much lower. At very high confining pressures (e.g., Curve 1), it is possible that nearly all of the cemented bonds in the specimen will be broken, and that no elastic behavior will be observed. One significant problem with the idealized stress-strain behavior noted by Coop and Atkinson [13] in Figure 2.20 is that the post-yield, steady-state shear resistance values should not be the same, as the specimens are tested at different confining pressures. In any case, as indicated by this figure, the soil behavior of cemented sand specimens tested at different confining pressures is dependent

on the position of the initial state of the soil in the test relative to the yield locus of the bonding.
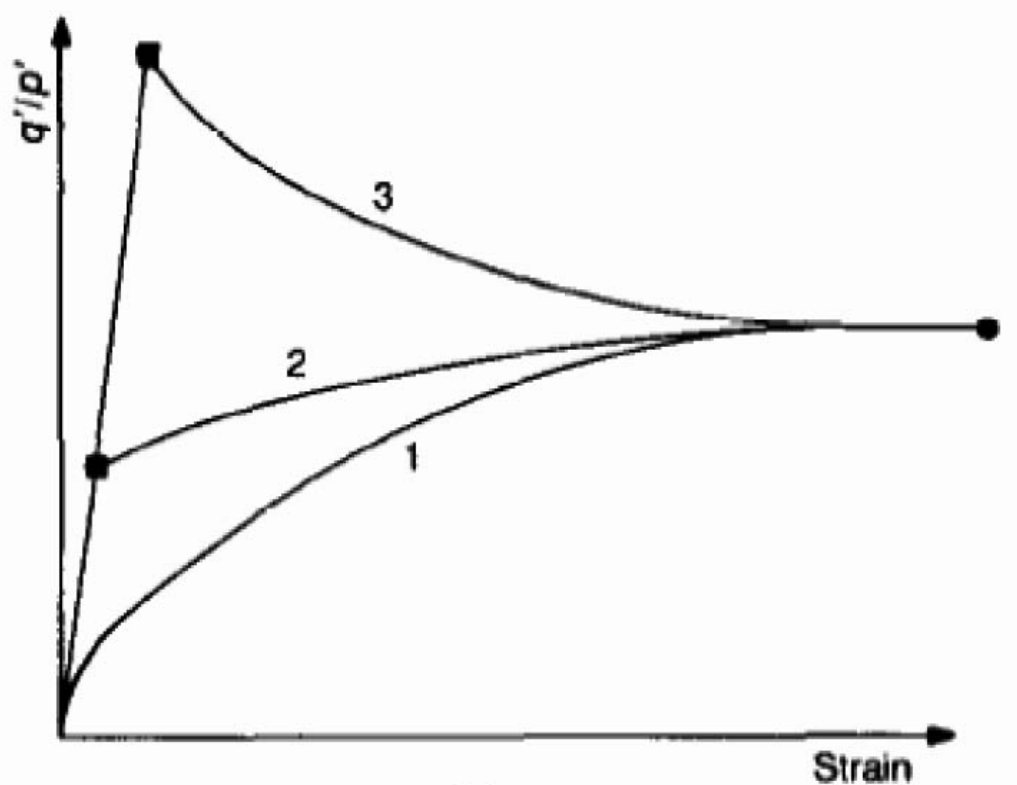
Figure 2.20: Idealized Behavior of Cemented Soils (Stress-Strain Behavior) [13]

As another way of thinking about Figure 2.20, each curve can be thought of as corresponding to a differing amount of cementation in the same soil; curves 1, 2 and 3 correspond to relatively low cementation, moderate cementation, and high cementation, respectively. If the specimens are thought about in this fashion, it can be observed that the highly cemented specimen (Curve 3) exhibits significantly higher peak strength than the low and moderately cemented specimens, and the poorly cemented specimen (Curve 1) exhibits the lowest strength. The triaxial apparatus has been the most commonly used type of experimental device in studies of the mechanical behavior of naturally cemented soils. Sangrey [14] and Mitchell [15] used it to characterize the stress-strain response and obtain estimates for the shear strength of cemented clays and Ismail [16] used it for cemented sands. Plane strain [15] and shear box devices [17] have been used to study the stress-strain response of naturally cemented soils. Results from several undrained monotonic and cyclic triaxial tests on undisturbed samples of cemented and uncemented calcareous soil have been reported by Sharma and Fahey [18]. The more heavily

cemented soils sustained higher cyclic stresses with less dependence on confining pressure.

2.9 MICROBIAL CEMENTATION IN SOIL

From a soil engineering perspective, an understanding of the microorganisms that are commonly found in soil is important for studying the influence that these microorganisms have on the mechanical properties of soil. Of particular interest is the effect that microorganisms can have on soil cementation.

2.9.1 Soil and Bacteria (Role of Microorganisms in Soil)

Bacteria are the most abundant microorganisms in soil. There are 10^8 to 10^{10} bacteria per gram of dry soil at the ground surface, with the population concentration generally decreasing with depth. The relative percentages of various types of bacteria that are commonly found in soil are provided in Table 2.2 [19]. The influence of microorganisms in mineral formation has been recognized for a wide variety of minerals, including carbonates, oxides, phosphates, sulfates, and silicates. Chemical transformation of metals and ions in soil are mediated by soil microorganisms, such as iron hydroxide transformation by iron-reducing bacteria. Precipitation of silicon dioxide, which glues soil particles together and precipitation of calcium carbonate by the microbial enzymatic hydrolysis of urea are among the most common microbial cementation processes that occur in nature. Soil bacteria can vary significantly in shape and may be nearly round, rod like, or spiral [20]. Cell diameters are usually in the range of 0.5-3 µm and spores can be as small as 0.2 µm. As shown in Figure 2.21, soil bacteria cannot go through pore throats smaller than approximately 0.4 µm, while eukaryotes, fungi, and protozoa require pore throats greater than 6 µm for entry.

Table 2.2: The Relative Percentages of Various Types of Bacteria that are Commonly Found in Soil, as Compared to the Total Bacteria Count [19]

Genus	Percentage (%)
Arthrobacter	5 to 60
Bacillus	7 to 67
Pseudomonas	3 to 15
Agrobacterium	>20
Alcaligenes	2 to 12
Flavobacterium	2 to 10
Others	<5
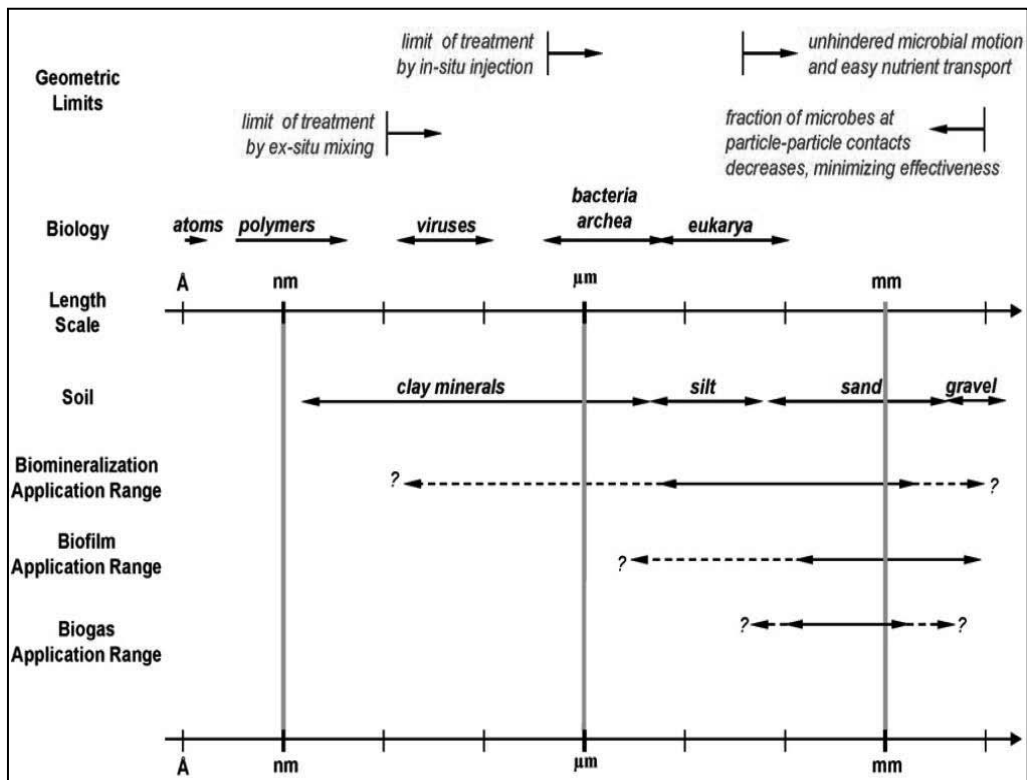
Figure 2.21: Size Comparison between Microorganisms and Various Soil Particle Sizes [6]

2.9.2 Microbial Calcite Cementation

Calcite is the most common soil carbonate, and it is typically formed either in root zones where CO_2 concentrations are high or inherited from calcareous parent materials [21]. A

series of complex biochemical reactions, such as the interaction of Bacillus pasteurii, urease, and ammonia, can result in microbial calcite cementation [21]. B. pasteurii, the most abundant alkalophilic soil microorganism, plays an important role in cementation by producing urease, which hydrolyzes urea to ammonia and carbon dioxide [21]. The ammonia increases the pH in the surrounding soil, subsequently inducing calcium carbonate ($CaCO_3$) precipitation [22]. From the study by Sarda et al. [23], it was found that B. pasteurii has high urease production. Hence, it has been used for microbial calcite cementation in many studies [21, 22, 23]. Table 2.3 shows the urease activity for different bacterial cultures.

Table 2.3: Urease Production by Different Bacterial Cultures [23]

Bacteria Culture	Urease Activity (Urea/ml)
Bacillus pasteurii NCIM 2477	17.5
Bacillus lentus NCIB 8773	10
Brevibacterium ammoniagenes ATCC 6871	12.5

In the experimental study, gram-positive, highly urease active, endospore forming bacteria Sporosarcina pasteurii is used for soil cementation. It should be noted that Bacillus pasteurii has now been reclassified as Sporosarcina pasteurii. The main nutrient solution that is commonly used to provide the necessary nutrition for the bacteria, as well as the chemical compounds that are needed for soil cementation, contains $NaHCO_3$, NH_4Cl, $CaCl_2$, urea, and a nutrient broth (e.g, bacteria nutrition broth such as those supplied by Difco, Bacto or Oxoid). Under favorable environmental conditions, S. pasteurii uses urea as an energy source, producing ammonia (NH_3) and carbon dioxide (CO_2), which tends to increase the pH in the proximal environment. This enzymatic hydrolysis of urea occurs in the bacteria cell and it is generally described using the following chemical reaction [23]:

$$NH_2 - CO - NH_2 + H_2O \xrightarrow{\text{Urease}} 2NH_3 + CO_2 \qquad (2.4)$$
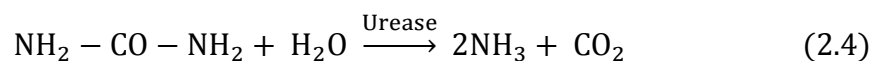
Concurrently with the enzymatic hydrolysis of urea, two reactions naturally occur in the presence of water, causing the ammonia and the carbon dioxide that are released by urea hydrolysis to be converted to ammonium (NH_4^+), carbonic acid (HCO_3^-) and CO_3^{2-}, as described in the following reactions respectively [6].

53

$$2NH_3 + H_2O \rightarrow 2NH_4^+ + 2OH^- \qquad\qquad (2.5)$$

$$CO_2 + OH^- \rightarrow HCO_3^- \qquad\qquad (2.6)$$

$$HCO_3^- + H_2O + OH^- \rightarrow CO_3^{2-} + 2H_2O \qquad\qquad (2.7)$$
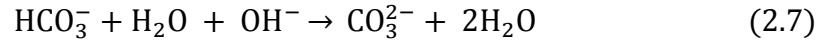
Production of NH_4^+ results in a net increase in pH, which is caused by the increase in hydroxide ions (OH^-) that occurs. This increase in pH provides an ideal environment for the bacteria to feed on the urea and precipitate calcite in the surrounding medium, because the bacteria that is used in this type of study (S. pasteurii) prefers a higher pH environment. The net increase in pH that occurs causes the calcium ions that are in the solution (which come from the dissolved $CaCl_2$) to react with CO_3^{2-} ions to form calcium carbonate ($CaCO_3$) bonds (also referred to as calcite), according to the following reaction [6].

$$Ca^{2+} + CO_3^{2-} \rightarrow CaCO_3 \qquad\qquad (2.8)$$

During this process, the bacteria cell, which has a negative charge because of the OH^- ions on the outside of its cell wall, is attracted to the soil particle's surface, which has a relatively high nutrient concentration compared to the surrounding environment [6]. Subsequently, calcite bonds tend to form at the particle-to-particle contacts in the soil matrix, which has an overall cementing effect between sand particles. The overall chemical reaction process that occurs in the sand matrix is displayed schematically in Figure 2.22.
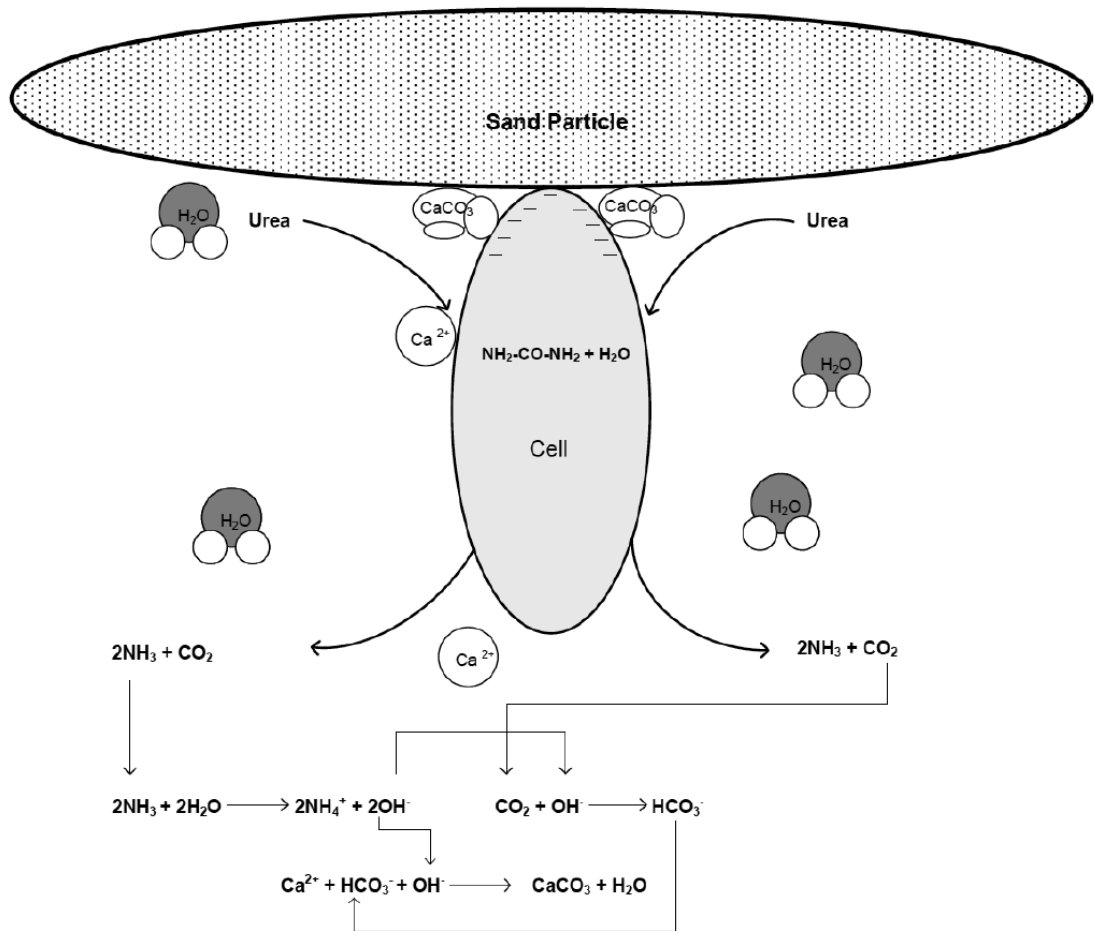
Figure 2.22: Schematic View of Biological Calcite Precipitation in Sand Matrix [2]

2.9.3 Processes Commonly Used to Introduce Bacteria to Soil

Different researchers have used a variety of methods to introduce bacteria into soil test specimens, for purposes of achieving bacterially induced cementation. One commonly used technique is to employ a peristaltic pump for introducing bacteria into the soil [1]. A number of microbiologists have also examined the physical and biochemical properties of microbial calcite cementation in soil. Table 2.4 provides an overview of the microbial calcite cementation processes that have been presented by others. Dejong et al. [1] described a method for using microbial processes to achieve "natural" calcite cementation within collapsible, loose sand using B. pasteurii and a liquid growth medium containing urea and calcium chloride. In their experiment, triaxial specimens were prepared by air pluviating Ottowa sand to achieve an approximate target relative density of 35%. Specimens were prepared for both "uncemented" (the control) and "cemented" testing, at height to diameter (72 mm) ratios of 2:1 and 1:1. For the "cemented" specimens, bacteria

were introduced into the triaxial specimens using a peristaltic pump having a flow rate of 20 ml/min, for 20 minutes. Each specimen was then allowed to rest for 4 hours, after which time additional nutrient treatment cycles were applied to "feed" the bacteria at a rate of 4 ml/min. The nutrient cycles that were applied consisted of three duration steps:

- The "injection time"

- The "set or equivalent time" and

- The "next injection time".

Once the specimens were saturated by nutrient treatment, a cementation period of 3 or 4 days was allowed, and monitored using shear wave velocity measurements applied to each specimen using bender elements. After completion of cementation, the treated 2:1 and 1:1 triaxial specimens were slowly backpressure saturated with effective confining stress of 100 kPa and 50 kPa, respectively. The specimens were then sheared at a rate of 2.5 percent per hour.

In a similar fashion as Dejong et al. [1], Whiffin et al. [24] also performed microbial calcite cementation by introducing bacteria to a soil column via fluid circulation; for this purpose, a 5 meter long PVC tube packed with sand was used. Pressure transducers were used to monitor water pressure inside the column at 0, 0.5, 1.2 and 3 meters from the top of the sand column. They also placed 10 "pore fluid sampling ports" on the side of the column along its length. The general column injection process that was followed is shown in Table 2.5.

Gollapudi et al. [25] used microbial calcite cementation to reduce the porosity of highly permeable structures. In order to introduce bacteria into the soil, they mixed a fluid urea - H_2CO_3 – $CaCl_2$ medium that contained suspended bacteria cells directly with the sand that was to be treated to make slurry. This slurry was then packed into a column (2.2 cm diameter x 48.9 cm long). To provide nutrition to the bacteria over time, an additional urea - H_2CO_3 – $CaCl_2$ nutrient broth was applied by gravity infiltration into the sand column during the curing time. Simultaneously, a similar "control" column (no bacteria included) was set up and treated with urea - H_2CO_3 – $CaCl_2$.

Table 2.4 Summary of Literature Review on Bacterial Introduction Techniques for Soil [2]

Authors	Soil Type & Microorganism	Medium	Setup	Nutrient Cycles
Gollapudi et al. [25]	Sand Bacillus pasteurii	Urea-H_2CO_3-$CaCl_2$	Soil mixed with medium and packed into a column	By gravity (4) days
Fischer et al. [26]	Sand Bacillus pasteurii	Urea-$CaCl_2$	Soil mixed with medium and packed into syringe column	By gravity (10) days
Dejong et al. [1]	Ottowa sand Bacillus pasteurii	Urea- $CaCl_2$	Sand column	Peristaltic pump (4 ml/min) (3-4 days)
Whiffin et al. [24]	Itterbeck sand Bacillus pasteurii	Urea- CaCl2	Sand column (5 m PVC tube)	Pump (0.35 L/hour)
Jonkers et al. [27]	Cement stone Bacillus pseudofirmus	N/A	Cement stone mixed with bacteria cell	No nutrient cycle 9,22,42, and 153 days (curing period)
Sarda et al. [23]	Bricks, Bacillus pasteurii	Urea- $CaCl_2$	Dried bricks immersed in bacterial medium	4 weeks

Table 2.5 A Summary of the Column Injection Process Used by Whiffin [24]

Stage	Duration (h)	Flow Rate (L/h)	Total Volume (L)
Water flush	30.7	0.35	10.75
Bacterial injection	18.1	0.35	6.34
$CaCl_2$ injection	17.1	0.35	5.99
Urea - $CaCl_2$	24.9	0.35	8.72
No injection	102	0	0
Water flush	23.7	0.35	8.30

Sarda et al. [23] reported successful bio-calcification in brick, and showed the favorable effect that microbes can have on improving the durability of bricks by reducing water absorption. They used Bacillus Pasteurii NCIM 2477 for calcification, by inoculating 50 ml of nutrient broth in a 250 ml Erlenmeyer flask with a 2% cell suspension. Oven dried bricks were then immersed in the nutrient medium that had been inoculated with bacteria cells. After a 24-hour incubation period, a urea – $CaCl_2$ solution (2% urea, 0.3% $CaCl_2$) was added to the medium. The test bricks were then cured within the medium for 4 weeks and a similar set of "control" test bricks were cured in water for 4 weeks. After curing, the bricks were removed, dried at room temperature, and weighed and tested to assess their water absorption capacity. As shown in Table 2.6, the absorption of water by bricks cured in media was lower than that by the control bricks, indicating that the pores in the bricks were blocked; this blockage was believed to be caused by a biologically aided calcite cementation process.

Table 2.6: The Results of Water Absorption for Bricks [23]

Brick Samples	% Water Absorption	% Reduction in Water Absorption
Control	25.0	---
Treated Sample	21.5	14.1

Fischer et al. [26] achieved calcium carbonate cementation using Bacillus pasteurii ATCC 6453 in a liquid medium. To prepare their test specimens, bacteria cells were suspended in a urea-$CaCl_2$ medium and mixed with 100g of sterile sand. Sand slurry containing the bacteria cells was packed into a 60 ml plastic syringe column. A "control" test column (without bacteria cells) was also packed separately. They fed these columns continuously by gravity using a urea-$CaCl_2$ medium that contained 25.2 mM of $CaCl_2$. After a 10-day curing period, the columns were air-dried and subjected to further analysis by X-ray diffraction and a scanning electron microscope. X-ray diffraction analysis of the final sand samples indicated that the most abundant compound by weight was quartz, the main component of sand. $CaCO_3$ crystals, also referred to as calcite, constituted approximately 30% of the total weight of the sand column that was treated by bacteria, while no calcite was detected in the column sample without bacteria cells. Fischer et al. [26] also observed that the rate of microbial $CaCO_3$ (calcite) cementation with cell growth was significantly faster than that of chemical precipitation.

2.9.4 Previous Research of Interest for Geotechnical Engineering Applications

The application of bacteria for microbial carbonate cementation has been used for a decade in industrial utilities for procedures such as selective mineral plugging for enhanced oil recovery, immobilizing calcium and contaminants in surface and ground water treatment, restoration of calcareous stone materials and bioremediation. It has also been used in geotechnical applications such as, remediating cracks in granite and concrete, increasing the bearing capacity of soil, bioclogging (pore filling) and biocementation (particle binding), increasing the shear strength of the soil based on the filling of pores. Filling of cracks in concrete and "bio-bricks" have been recently investigated. The general information and results from this literature review are summarized in Table 2.7, and are discussed in more detail in the following sections.

Improvement of Soil Engineering Properties

MICP improves various soil engineering properties as described below.

Shear Strength

Using a series of isotropically consolidated undrained compression (ICU) triaxial tests, Dejong et al. [1] demonstrated that microbially cemented specimens exhibited increased strength when compared to uncemented specimens, and that the shear behavior of the bacterially-cemented specimens was similar to that of gypsum-cemented soil (which was considered to be representative of typical chemically-cemented sand behavior). They used triaxial specimen height to diameter (72 mm) ratios of 2:1 and 1:1, to see the effect of cementation on different sized soil specimens, as shown in Figures 2.23 and 2.24. They also assessed the development of cementation in the soil over time via shear wave velocity measurements taken from the top to the bottom of the specimen using bender elements.

Table 2.7: Summary of Literature Review on the Effect of Bacteria for Soil Cementation

Researchers	Soil Type	Microorganism	Test Type	Results
Gollapudi et al. [25]	Sand	Bacillus pasteurii	Column test	• $CaCO_3$ deposition from microbial activity not from chemical reaction • Cementation occurred on the surface of the sand • Complete plugging resulted absence of flow • High microbial activity was observed in the highly fractured formation • 100% final permeability reduction
Canakci, H. and Cabalar, A. [28]	Leighton Buzzard sand	Biopolymer produced by Xanthomanas campestris	Direct shear test	Shear stress increased with increasing biopolymer concentration at higher calcium carbonate content
Dejong et al. [1]	Ottawa sand 50-70	Bacillus pasteurii	CIUC	Highest stress ratio same as the gypsum cemented soil
Whiffin et al. [24]	Itterbeck sand	Sporosarcina pasteurii	CD	Higher $CaCO_3$ concentration- • Decreased porosity • Increased strength Lower $CaCO_3$ concentration- • No significant effect on strength of soil

Researchers	Soil Type	Microorganism	Test Type	Results
Bianco, A and Madonia, G. [29]	Basaltic material	Bacillus pasteurii	Modified AASHTO test CBR	Bearing capacity increased 42% after only 10 days of bacterial treatment
Chunxiang et al. [30]	Cement based material	Bacillus pasteurii	X-ray diffraction (XRD) Thickness analysis SEM analysis Capillary water absorption Acid resistance test	• Value of certain Ca^{2+} concentration was related to the initial concentration of Ca^{2+} in the solution • Bacterial activity had a significant influence on water absorption of specimen surface • Deposited $CaCO_3$ layer could resist the corrosion of acid rain to a certain extent (pH 3.5-5.6)
Jonkers et al. [27]	Cement stone	Bacillus pseudofirmus	N/A	• Viable bacterial cells decreased with increasing curing time • Larger pores in young (3-7 days) specimens and smaller pores in older (28 days) specimens • Bacillus could be used as a self healing agent in concrete
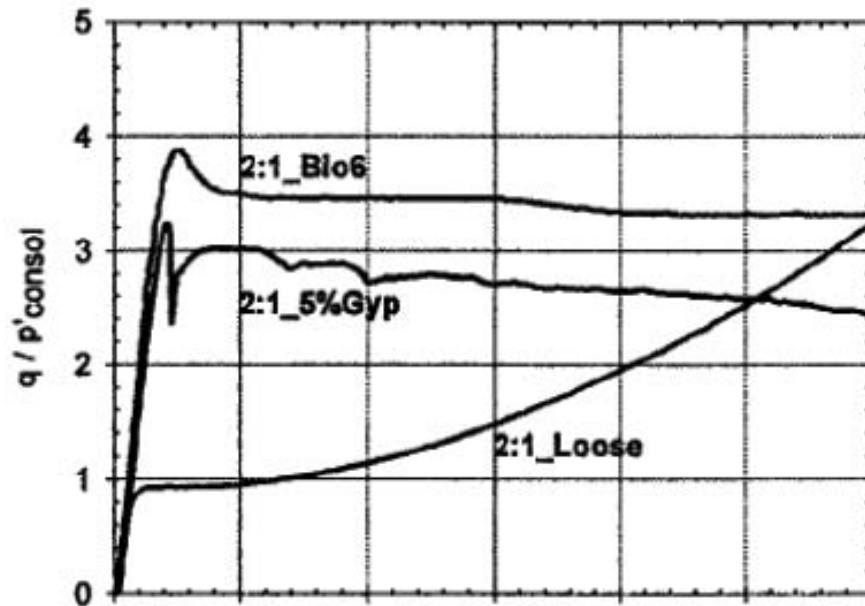
Figure 2.23: Response to Undrained Monotonic Triaxial Shear for 2:1 Specimens [1]
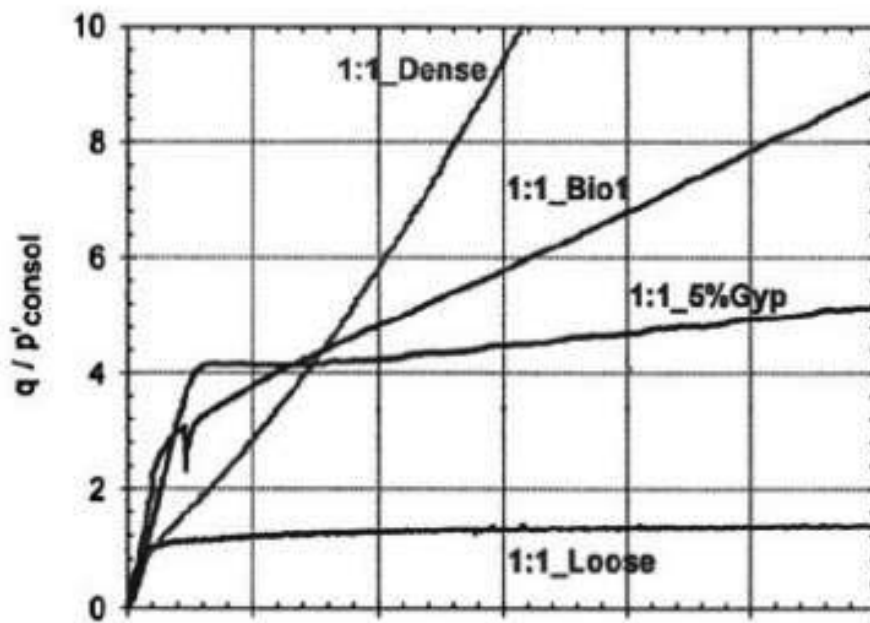
Figure 2.24: Response to Undrained Monotonic Triaxial Shear for 1:1 Specimens [1]

Porosity

Microbial cementation occurs in pores within soil particles, reducing the pore throats and subsequently preventing water flow [24]. Whiffin et al. [24] examined the phenomenon of pore throat reduction using a 5-meter sand column to simulate field conditions, as shown in Figure 2.25. They determined the primary stages of microbial cementation such as

urease activity, ammonium concentration, calcium concentration and calcium carbonate content within the soil specimen. Results from triaxial tests (Figure 2.26) conducted on specimens from the treated sand column show that the soil porosity, strength, and stiffness were all significantly affected by the calcium carbonate content. The porosities of bio-treated soil specimens were up to 90% smaller than those specimens that had not been treated, as shown in Figure 2.27.

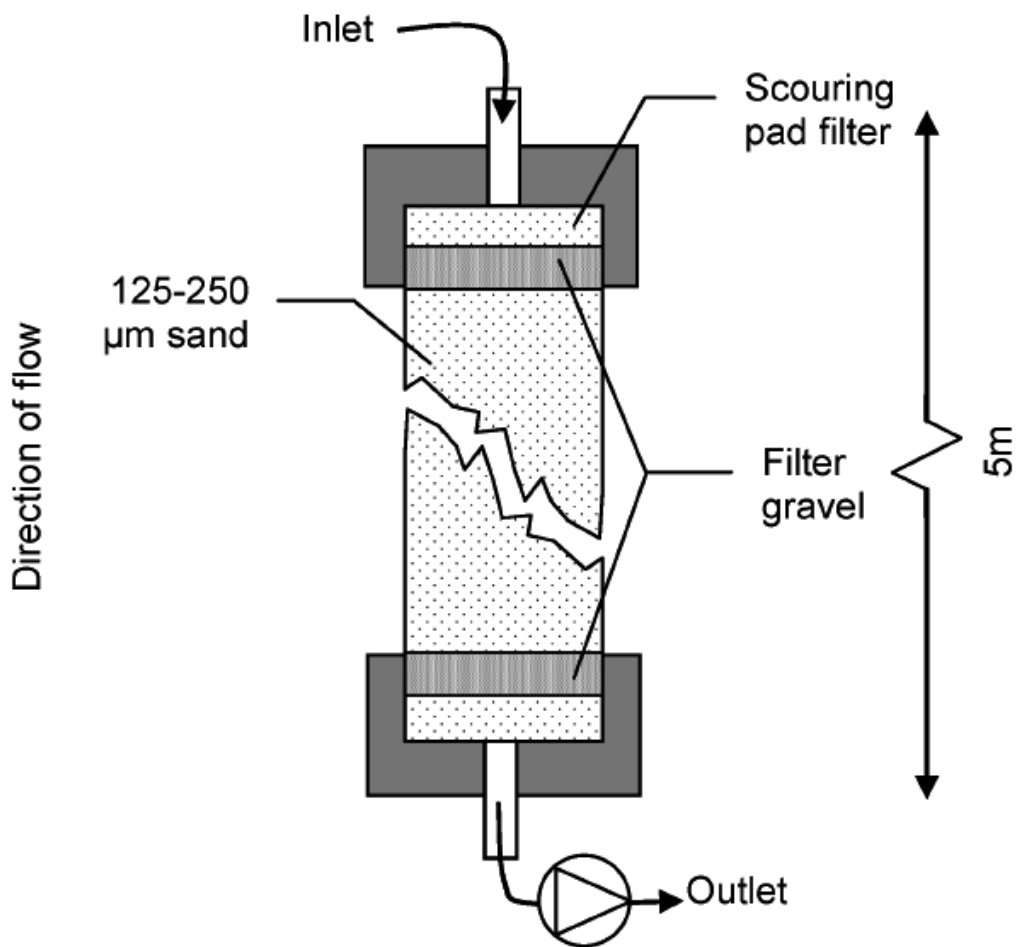**Figure 2.25:** A Schematic View of the Sand Column Used in Whiffin's Research [24]
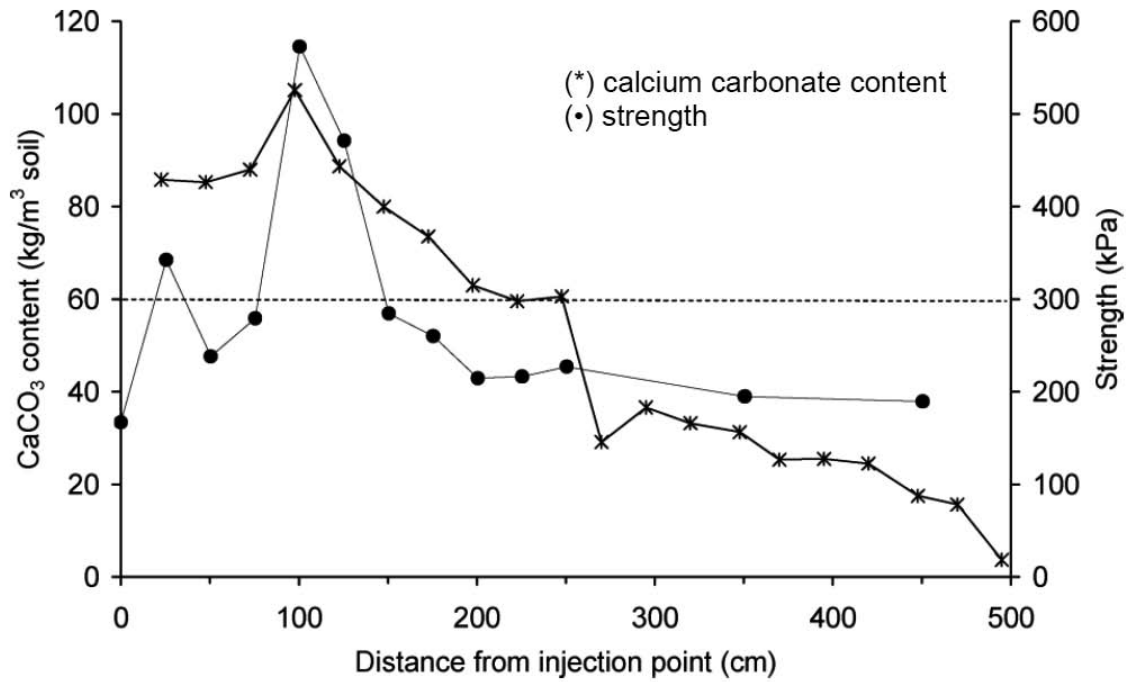
Figure 2.26: Calcium Carbonate Content and Strength vs. the Distance from the Injection Point along the Column Length. Average CaCO$_3$ Value for the Entire Column is indicated by the Dashed Line [24]
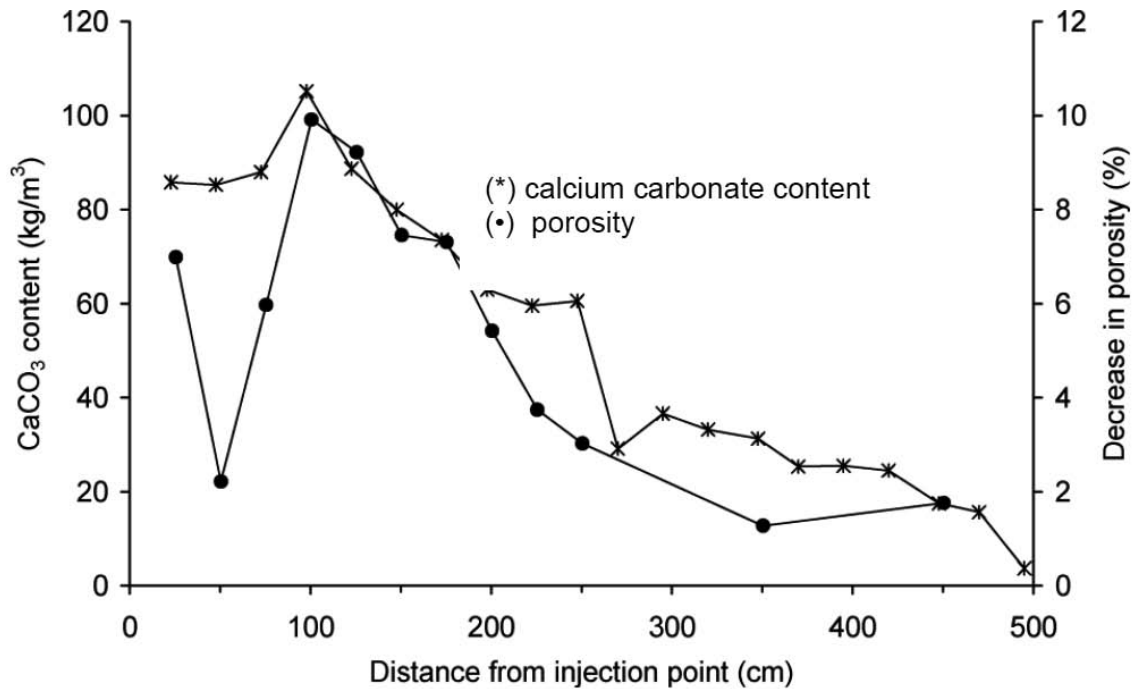

Figure 2.27: Calcium Carbonate Content and Porosity vs. the Distance from the Injection Point along the Column Length [24]

Permeability

Gollapudi et al. [25] used microbial "mineral plugging" in a highly permeable geologic formation to reduce the overall system porosity. Leaching in the rock fractures was controlled by microbial plugging. A series of column tests was conducted in order to evaluate the effectiveness of the proposed microbial technique. The flow rate into the fractures via the column was measured during the test, and absence of flow showed the completion of plugging. Their results showed that microbial plugging was highly related to bacterial concentration, pH, the flow rate in the fractures, and the presence of contaminants. The microbial process was more productive in the presence of fractures, which provided more nucleation sites for the bacteria.

Microbial Calcium Carbonate as a Protection Agent for Concrete

More recently, several studies have shown the utility of microbial calcite cementation for the protection of concrete [2]. A study conducted by Jonkers, et al. [27] investigated the use of microbial treatment as a self repairing agent for use in sustainable concrete. Concrete specimens treated by bacteria were prepared to investigate the viability of bacteria cement, the pore size distribution of aging specimens, and the effect of agent additions on the strength and self healing properties of the resulting concrete. Those concrete specimens were formed using a mix consisting of ordinary Portland cement with a bacteria suspension and then cured at different time scales. Results of compressive strength test showed that pore diameter size decreased with increasing specimen age as a result of the bacterial process. Chunxiang et al. [30] were able to protect cement-based buildings from corrosion with the aid of calcium carbonate from bacteria. They measured the permeation resistance of the treated cement-based material using capillary water absorption.

2.10   CONCLUDING REMARKS

Review of literature in the preceding sections reveals that microbial induced calcite precipitation (MICP) is a useful, more environment friendly and novel approach for the improvement of in-situ soil. All of the researchers in this field have suggested improved liquefaction behavior of bio-treated soil as compared to untreated soil. They have used bacillus pasteurii as a source of microorganisms and urea as a source of energy of the

bacteria. All of them have used a liquid medium for application of bacteria and its nutrients. Application methods are different. MICP is used not only in the soil but also in other materials, like concrete, brick etc. However, no literature was available in this context with the treatment of alluvial soil deposits of Bangladesh. As such, it was felt urgent necessity to carry out a research in an attempt to improve Bangladesh soil against liquefaction.

CHAPTER 3

EXPERIMENTAL SETUP, TEST SCHEME, METHOD AND MATERIAL

3.1 GENERAL

Several chemical and biological solutions were prepared to isolate bacteria from the soil. Trials were required to identify and isolate pure culture of urease positive bacteria. Experimental setups were developed for bio-treatment and determination of precipitated calcite in the soil. Soil specimens, prepared by moist tamping method, were treated with Bacteria along with proper nutrient solutions by two methods. During treatment, several properties of the treatment solutions were measured. After the treatment, the specimens were tested (unconfined compression test, needle penetration test and cyclic triaxial test) to compare the change in strength and stiffness with untreated specimens already tested. Precipitation of calcite was confirmed by a test performed with a setup developed and by taking optical microscope image of the dry treated sand samples.

3.2 MICROORGANISM

Highly urease positive bacteria Sporosarcina pasteurii (formerly known as Bacillus pasteurii) is used in the study of bio-mediated soil improvement technique. Sporosarcina pasteurii is gram positive, endospore forming bacteria. It has the ability to precipitate calcite and solidify sand in the presence of a calcium source and urea, through the process of biological cementation. Sporosarcina pasteurii has been proposed to be used as an ecologically sound biological construction material. However, in a developing country like Bangladesh, Sporosarcina pasteurii is not available. So it was decided to isolate urease positive bacteria from the surrounding environment with the help of Department of Microbiology, University of Dhaka.

For effective microbial calcite precipitation, the selected microorganism should be capable of producing CO_2 with an increase of pH in the surrounding environment to alkaline condition that provokes calcite precipitation [5]. Aerobic microorganisms are

good for microbial calcite precipitation because they provide two sources of CO_2: respiration by the cell and decomposition of urea [5]. In this research, a rapidly urease positive bacteria was isolated from the soil of the garden of Shahid Smrity Hall, Bangladesh University of Engineering and Technology (BUET), Dhaka-1000, Bangladesh. For the isolation, culture and long time storing of the bacteria, several solutions and media were needed to be prepared as,

   i.   Tris Buffer Solution

   ii. NH_4-YE Nutrient Broth

   iii.   Agar Plate Medium

   iv.   Motility Indole Urease (MIU) Medium

   v. T_1N_1 Medium

3.2.1 Making Tris Buffer Solution

A 0.13 Molar tris buffer solution was used to adjust the pH of the distilled water that was used as a component in the NH_4-YE nutrient broth and agar plate medium. This tris buffer solution was made using the following approach: the desired mass of tris, 15.75 gram, was dissolved into approximately 1/2 L of distilled water in a 1 L Duran bottle. Using a pH meter, the solution was titrated with 1 Molar hydrochloric acid (HCl) until a pH of 9.0 was reached. Distilled water was then added to reach the final required volume of 1 L. If the tris buffer solution was needed to be stocked for future use, it was autoclaved at 121°C and 15 psi for 15-20 minutes. Otherwise, it was mixed with the solution where it was required and autoclaved together. Before autoclaving, the opening of the bottle was closed with the cap, half circle loose or cotton plug. The autoclave machine is shown in Figure 3.1.

(a)   (b)

Figure 3.1: Autoclave Machine

3.2.2 Making NH$_4$-YE Nutrient Broth

10 gm of NH$_4$SO$_4$ and 20 gm of Yeast extract were taken into a 1 L Duran bottle. Then tris buffer solution was added to make the total volume of 1 L and it was stirred with magnetic stirrer. The opening of the bottle was closed with the cap, half circle loose or cotton plug. This broth was then autoclaved at 121°C and 15 psi pressure for about 15-20 minutes.

3.2.3 Making Agar Plate Medium

10 gm NH$_4$SO$_4$, 20 gm Yeast extract and 20 gm Agar (Bacteriological) were taken into a 1 L Duran bottle. Tris buffer solution was poured into the bottle to make the total volume of 1 L. It was stirred with magnetic stirrer. This solution was then autoclaved at 121°C and 15 psi pressure for about 15-20 minutes. After autoclaving, the laminar flow hood shown in Figure 3.2 was cleaned with 70% Ethanol (Figure 3.3). There were two Bunsen burners inside the hood. Petri dishes were kept inside the hood and autoclaved hot medium was carefully poured into each Petri dish. Before pouring the hot medium, hands

were cleaned with 70% Ethanol. Usually 20 ml of medium is required for one Petri dish. From eye inspection, it was found that, 20 ml medium filled half of the Petri dish. Therefore, each Petri dish was filled half of its capacity. Then those dishes were kept inside the hood for about 24 hours to cool and solidify the media, closed with plate covers as shown in Figure 3.4. After 24 hours, the plates were properly closed with scotch tape as shown in Figure 3.5, so that no contamination occurred. Then these plate media were incubated at 30°C for 24 hours to check contamination in the media. In case of contaminated media, growth of unknown microorganisms was found and those media were discarded. The uncontaminated media were then kept in the fridge for future use.

Figure 3.2: Laminar Flow Hood

Figure 3.3: Cleaning Laminar Flow Hood with 70% Ethanol

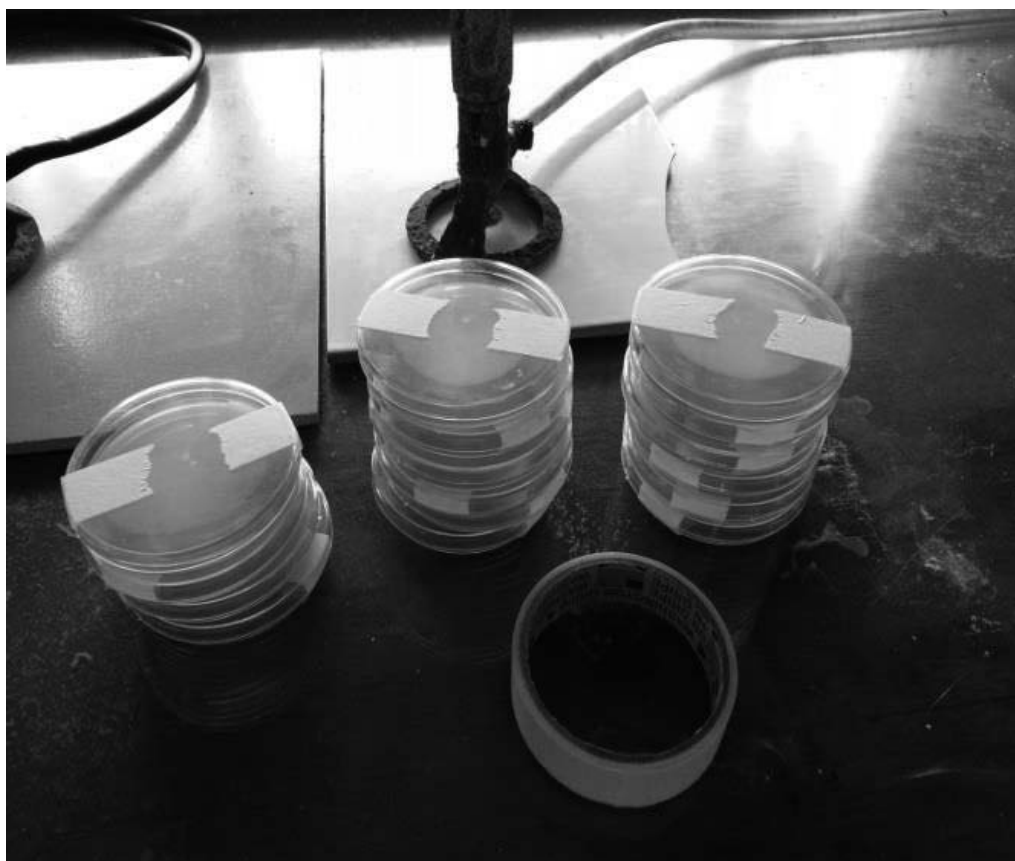**Figure 3.4:** Culture Plates Inside the Hood

Figure 3.5: Culture Plates with Scotch Tape

3.2.4 Making Motility Indole Urease (MIU) Medium

250 ml MIU medium was prepared for 50 test tubes. 4.74 gm of MIU powder was taken into a 500 ml conical flask. Distilled water was then poured into the flask so that the total volume became 250 ml. It was then properly mixed with magnetic stirrer. The opening of the flask was closed with cotton plug (Figure 3.6). After that, this solution, a 5 ml dropper wrapped with aluminum foil (Figure 3.6), a measuring cylinder wrapped with aluminum foil (Figure 3.6) and 50 test tubes (Figure 3.7) were autoclaved at 121°C and 15 psi pressure for about 15-20 minutes. The laminar flow hood was cleaned with 70% Ethanol and Bunsen burners were lighted. Then 5 gm of urea was poured into the autoclaved MIU medium very carefully inside the hood such that no contamination occurred. Before pouring urea, the cotton plug was temporarily removed and the opening of the flask was burnt for 2-3 seconds. After pouring urea into the flask, the opening was again burnt for 2-3 seconds and closed with the plug. After that, 5 ml of MIU medium was poured into

each test tube with the 50 ml dropper very carefully such that not contamination occurred. Then these test tubes were incubated at 30°C for 24 hours to check contamination (Figure 3.8). In case of contaminated tubes, pink color appeared (Figure 3.9) and those tubes were discarded. The uncontaminated tubes were then stored in the fridge.
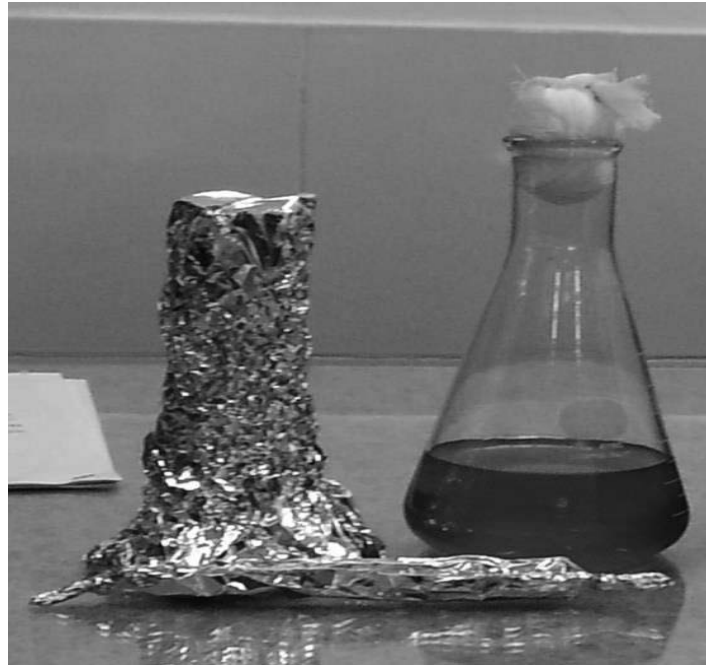

Figure 3.6: Preparing MIU Medium

Figure 3.7: Autoclaving Test Tubes Kept in Beakers
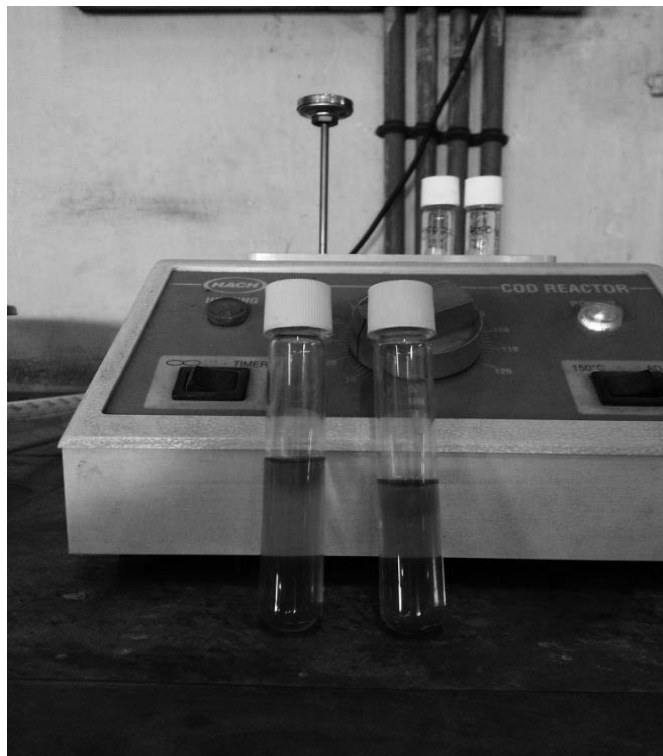
Figure 3.8: Incubating MIU Media

Figure 3.9: Contaminated MIU Media

3.2.5 Making T_1N_1 Medium

This medium was stored in small vials. Each vial was filled with 2 ml of T_1N_1 medium. 20 ml medium was prepared for 10 vials. 0.2 gm of Tryptone and 0.2 gm of NaCl were taken into a 50 ml beaker. Distilled water was poured to make the total volume of 20 ml and it was mixed properly with magnetic stirrer. pH of the solution was adjusted to 7.0 ± 0.2 with 1M HCl or NaOH. Then 0.1 gm agar (bacteriological) was mixed and the solution was boiled. 2 ml of the hot solution was poured into each vial and the cap of the vial was closed half circle loose. Then all of the filled vials were autoclaved at 121°C and 15 psi pressure for about 15-20 minutes. After cooling to the room temperature, these vials were stored in the fridge.

3.2.6 Isolation of Bacteria

Bacteria concentration in the top surface of the soil is usually higher [22] (Figure 3.10). So it was decided to isolate bacteria from the top surface of the soil. In the first cultivation, soil collected from the garden of Shahid Smrity Hall, Bangladesh University of Engineering and Technology (BUET), Dhaka-1000, Bangladesh (Figure 3.11, Figure 3.12). A 225 ml autoclaved (121°C and 15 psi for 15-20 minutes) conical flask was filled with 50 ml of autoclaved (121°C and 15 psi for 15-20 minutes) NH_4-YE broth medium inside the hood (Figure 3.13). The medium was inoculated with 5 gm of soil (Figure 3.14). After 3-4 days of incubation at 30°C (Figure 3.15), one loopful was taken from the broth and streaked into a NH_4-YE agar plate medium (Figure 3.16). This plate was incubated for one day at 30°C. Mixed culture of bacteria was grown in the plate (Figure 3.17). After that, several trial bacteria colonies were streaked into several plate media and each plate was incubated for one day at 30°C (Figure 3.18, Figure 3.19). A heavily inoculums from each plate medium was inoculated in motility indole urease (MIU) medium in the test tube for urease test (Figure 3.20). The change of color of the MIU medium from yellow to pink indicates presence of urease enzyme in the bacteria (Figure 3.21, Figure 3.22). The rapidly urease positive bacteria was determined from the possible lowest time required to change the mentioned color. This bacterium was then streaked into several vials containing T_1N_1 medium with the needle (Figure 3.23). Autoclaved paraffin oil was poured over the medium (Figure 3.24). These vials were then stored in the fridge.
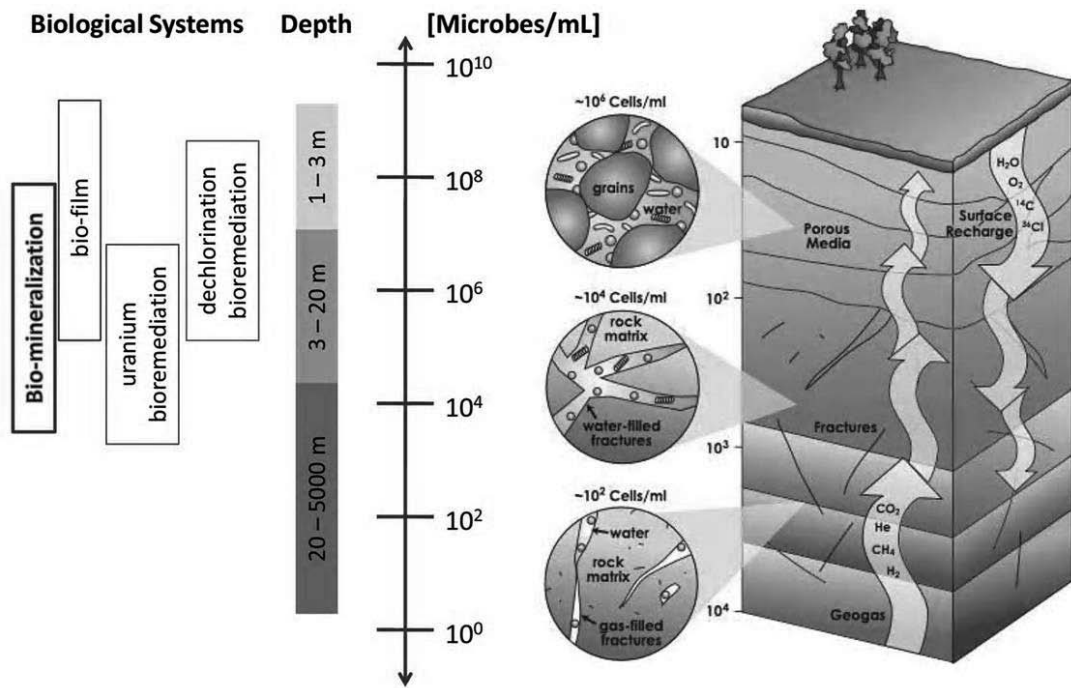
Figure 3.10: Variation of Bacteria Concentration with Depth
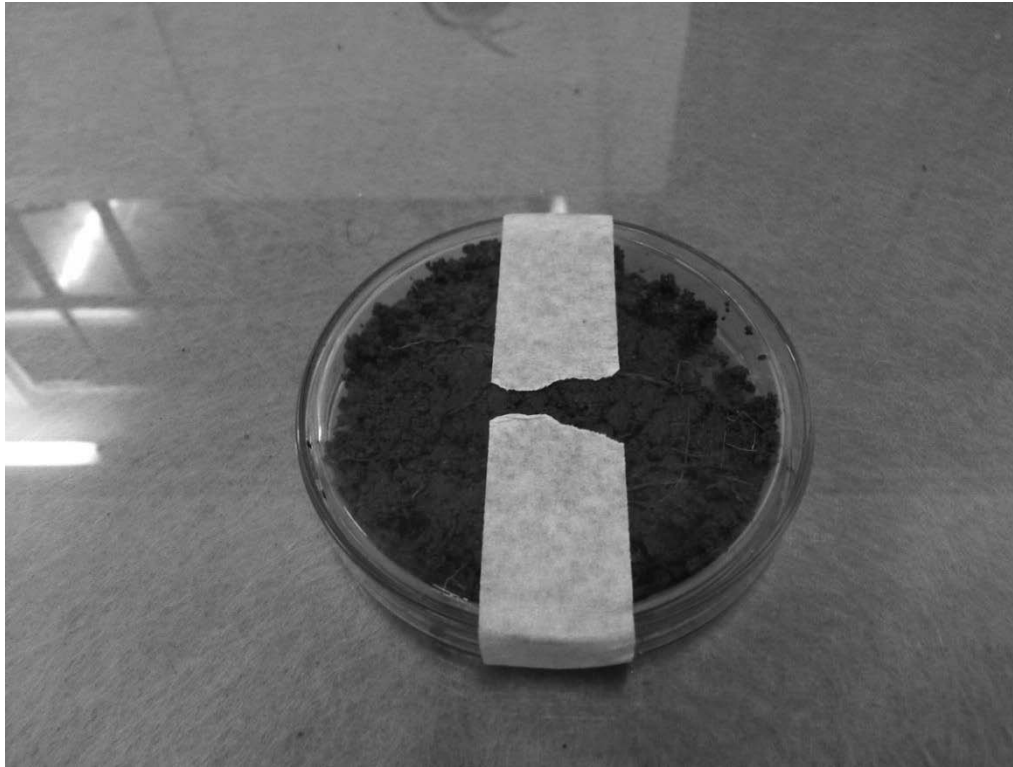

Figure 3.11: Garden of Shahid Smrity Hall, BUET Campus

Figure 3.12: Soil Collected from the Garden

Figure 3.13: Taking Soil and NH$_4$-YE Broth to Mix inside the Hood

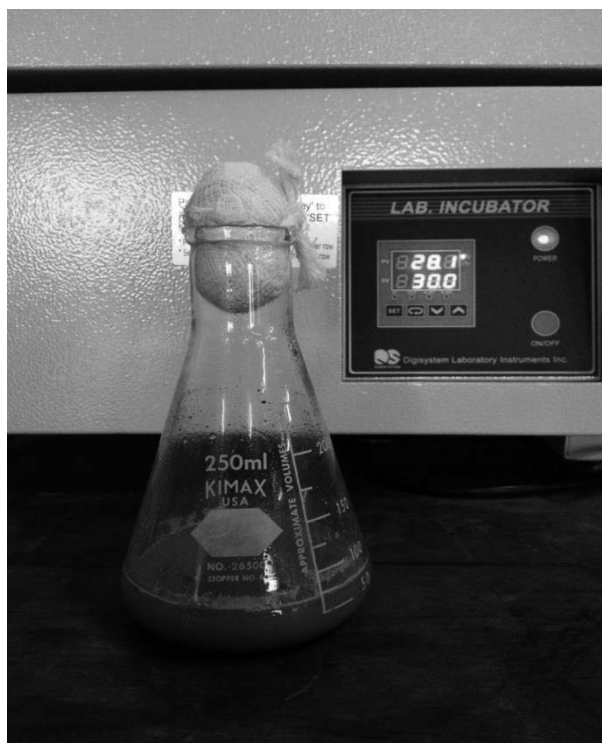**Figure 3.14:** Mixture of Soil and NH$_4$-YE Broth inside the Hood

Figure 3.15: Incubating Mixture of Soil and NH$_4$-YE Broth inside the Incubator

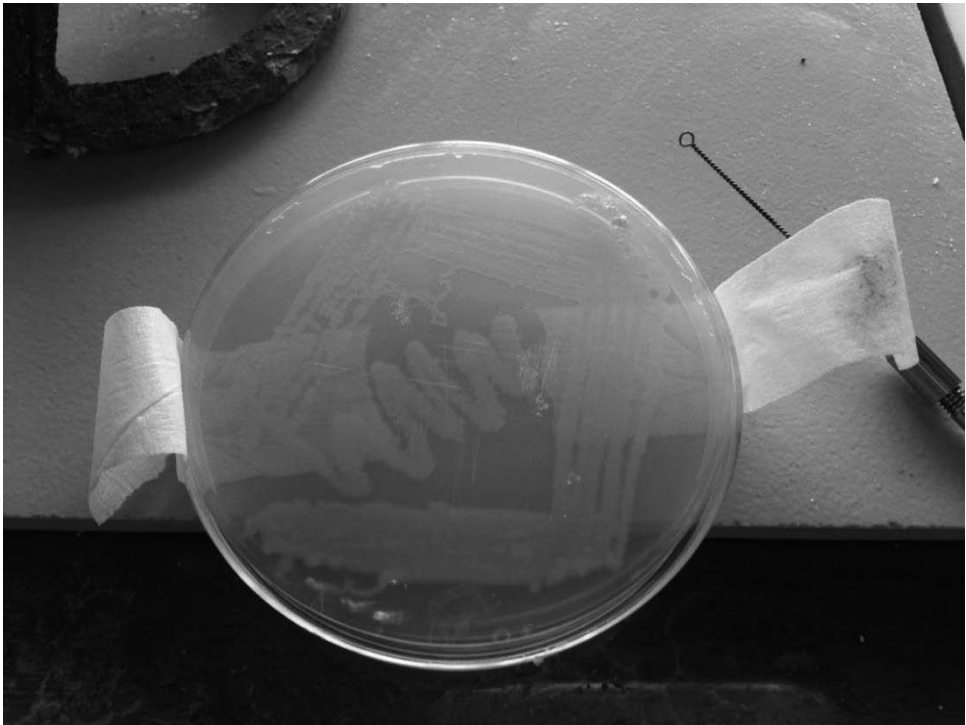**Figure 3.16:** Streaking Mixed Culture of Bacteria from NH$_4$-YE Broth to Plate Medium

Figure 3.17: Mixed Culture in the Plate

Figure 3.18: Streaking Single Colony from Mixed Culture Plate to Fresh Plate
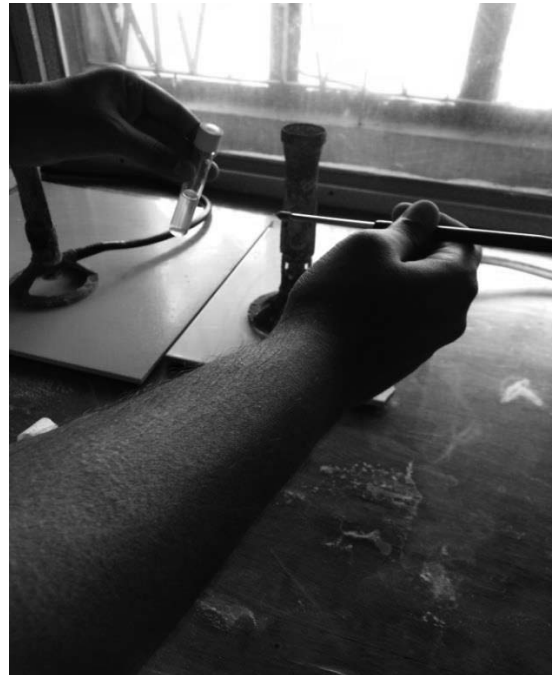

Figure 3.19: Pure Culture of Bacteria

(a)

(b)

(c)

(d)

Figure 3.20: Inoculating Pure Culture of Bacteria into the MIU Medium

Figure 3.21: Rapidly Urease Positive Bacteria (Color Change from Yellow to Pink)

Figure 3.22: Checking Urease Positive Bacteria (Color Change from Yellow to Pink)

Figure 3.23: Streaking Rapidly Urease Positive Bacteria into Vials

Figure 3.24: Pouring Paraffin Oil into the Vial

3.3 SOIL TYPE USED

Usually sandy soil is vulnerable for seismic liquefaction. Therefore, sandy soil was selected for this research. Sand was collected from the riverbed of Meghna, Bangladesh. Wash sieve, specific gravity and maximum and minimum index density tests were performed to characterize the sand. Each test was carried out at least 5 times to get representative and reliable values of different properties. These characteristic values are shown in Table-3.1.

Table 3.1: Physical Properties of the Sand Used

Property	Test Method	Value
D_{50} (mm)	ASTM D422-63, D1140-00	0.24
C_u	ASTM D422-63, D1140-00	1.93
C_c	ASTM D422-63, D1140-00	0.96
FM	ASTM D422-63, D1140-00	1.18
G_s	ASTM D0854-02	2.76
γ_{min} (gm/cc)	ASTM D4254-00	1.31
γ_{max} (gm/cc)	ASTM D4253-00	1.70
Soil Description	ASTM D2487-00	SP

Grain size distribution is shown in Figure 3.25. Bio-mediated soil improvement is suitable for sand having particle size from 0.075 mm to 4.75 mm as shown in Figure 3.26 [31]. So the selected sandy soil was suitable for this research.
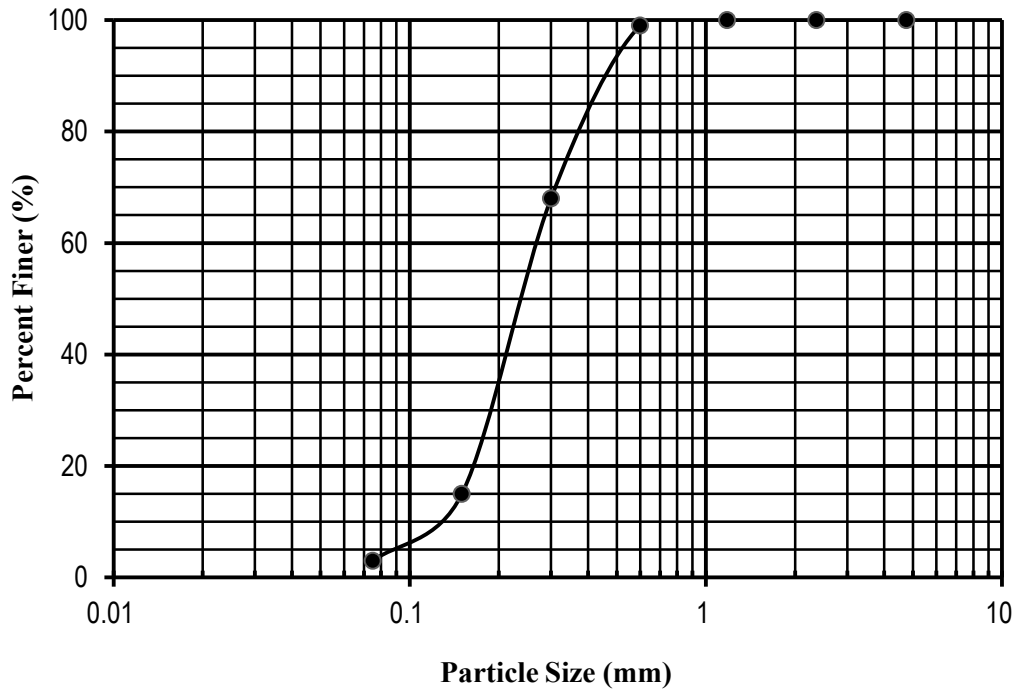
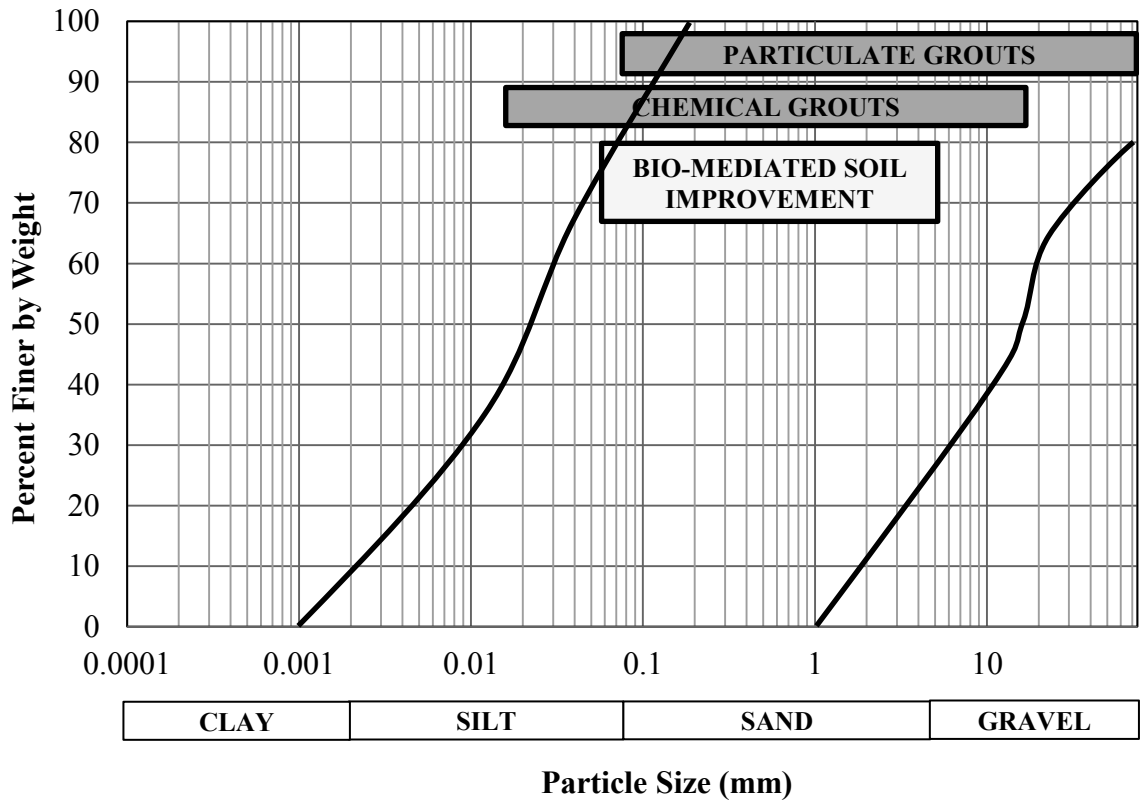**Figure 3.25:** Grain Size Distribution of the Selected Sand

Figure 3.26: Suitability of Sand for Bio-Mediated Soil Improvement Technique [31]

3.4   SPECIMEN PREPARATION

There are three kinds of procedures widely used for preparing samples of sand for laboratory testing: moist placement method (wet tamping), dry deposition method and water sedimentation method [32]. In this research, moist placement method was used to prepare all test specimens. First, the soil was sieved with #16 sieve so that rubbish materials were removed. Then the soil was oven dried at a temperature of 105°C for about 24 hours. After cooling to the room temperature, distilled water was mixed so that the water content became 10% of dry weight. 340 gm of wet soil was taken into each of three dishes. A split mold with collar was used for sample preparation. The inner diameter and height of the mold were 71 mm and 142 mm respectively so that all specimens prepared were 71 mm in diameter and 142 mm in height. The mold with the collar was filled with soil by compacting three layers of moist sand already prepared in the dishes. Each layer was compacted with a falling hammer of nearly 2.5 lb from a falling height of 6 inch. Numbers of blows for the lowest, middle and top layer were 20, 25 and 30 respectively. After tamping, the collar was removed and the extra soil was trimmed with a wire saw. Weight of mold with wet sample was taken. The weight of the mold was 1192.2 gm. So weight of the wet soil used could be determined. Relative density of each specimen was calculated. Calculated relative densities of all specimens were 48% to 50%. Various apparatus used for sample preparation are shown in Figure 3.27.

Figure 3.27: Various Apparatus Used for Sample Preparation

3.5 EXPERIMENTAL SETUP

Two experimental setups were built for this research: one for bio-treatment and the other for determination of Calcite in the treated specimens.

3.5.1 Experimental Setup for Bio-Treatment

The experimental setup for bio-treatment is shown in Figure 3.28. Three specimens can be treated at a time. The split mold of the specimen has the same dimensions as described in 3.3. These molds are removable. Each specimen can be prepared as described in 3.4. There are three inlets with inflow control valves for each specimen. Outflow can also be controlled with outflow control valve at the bottom of each specimen. Specimen mold was built with aluminum. The upper and lower cylindrical portions of the setup were built with Plexiglas.
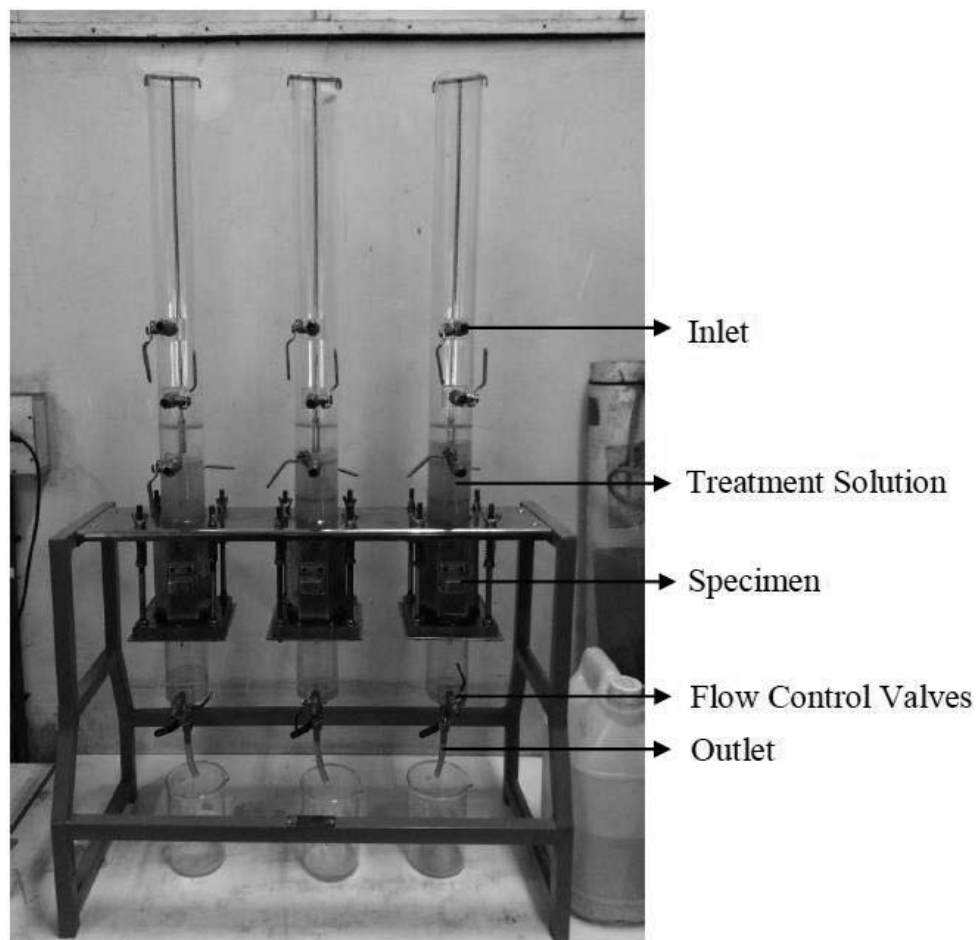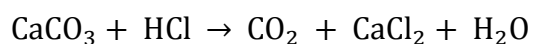

Figure 3.28: Experimental Setup for Bio-Treatment

3.5.2 Experimental Setup for Calcite Determination

The experimental setup for Calcite determination is shown in the Figure 3.29. It consists of a syringe, a silicon cork, a filtering flask, a silicon pipe, a pressure gauge and a magnetic stirrer. $CaCO_3$ produces CO_2 when reacting with HCl shown in the following reaction.

$$CaCO_3 + HCl \rightarrow CO_2 + CaCl_2 + H_2O$$

The set up was calibrated with known amount of molecular grade $CaCO_3$ from 0 gm to 0.1 gm with 1 gm to 0.9 gm of soil respectively. 5 ml of 5M HCl was pushed with the syringe in the filtering flask containing the mixture of $CaCO_3$ and soil and the pressure was measured from the pressure gauge. The calibration chart is shown in Figure 3.30. The calibration was conducted at a temperature of 25°C.
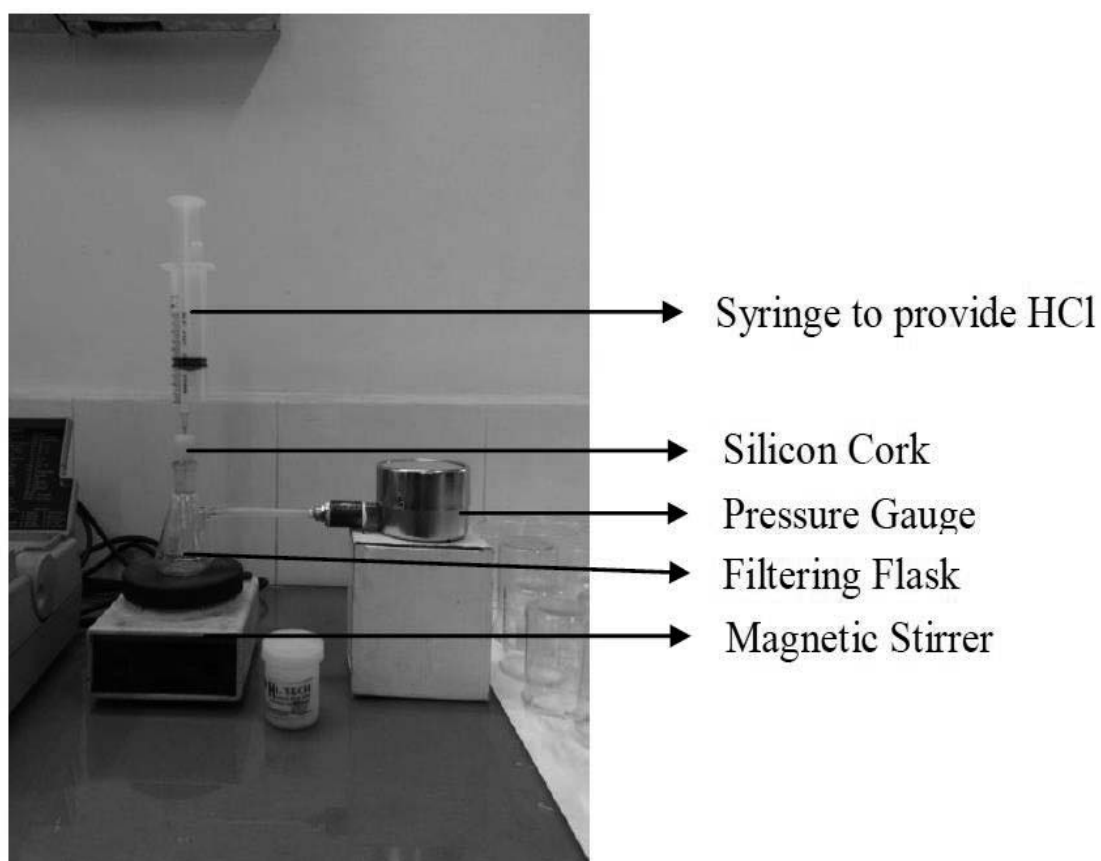

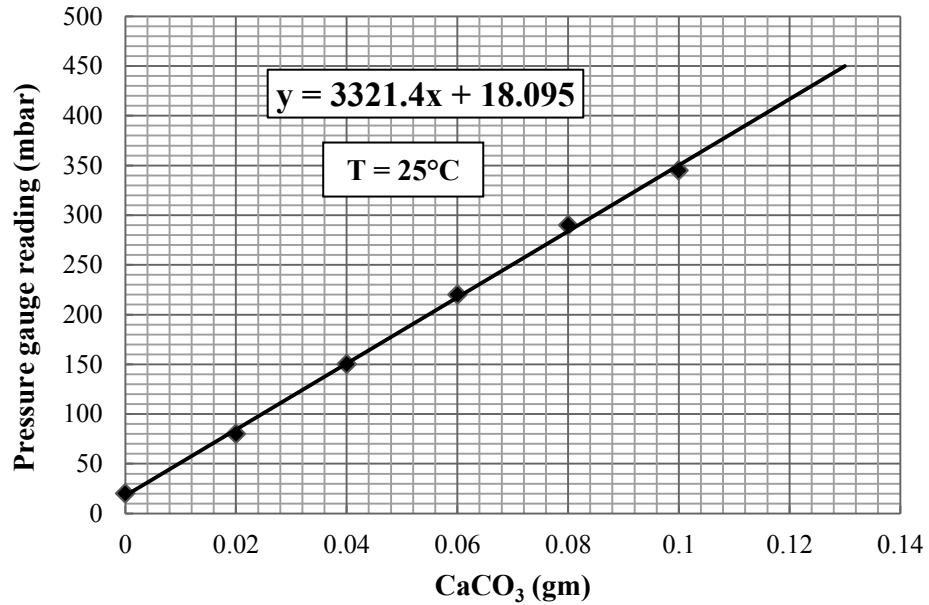**Figure 3.29:** Experimental Setup for Calcite Determination

Figure 3.30: Calibration of Experimental Setup for Calcite Determination

3.6 APPLICATION OF MICROBES AND CEMENTATION TECHNIQUE

For proper application of microorganisms and treatment solutions, two more solutions were needed to be prepared, as:

i. $CaCl_2.2H_2O$ Stock Solution and

ii. Urea Nutrient Solution

3.6.1 Making $CaCl_2.2H_2O$ Stock Solution

140 gm of $CaCl_2.2H_2O$ was taken into a 1 L Duran bottle. Distilled water was poured so that the total volume became 1 L. The mixture was properly mixed with magnetic stirrer. The opening of the bottle was closed with the cap half circle loose. Then the solution was autoclaved at 121°C and 15 psi pressure for about 15-20 minutes or used as required. Several 1 L of this solution may be prepared as shown in Figure 3.31.

3.6.2 Making Urea Nutrient Solution

20 gm urea, 3 gm nutrient powder, 10 gm NH_4Cl and 2.12 gm $NaHCO_3$ were taken into a 1 L Duran bottle. 500 ml distilled water was poured. The mixture was mixed with

magnetic stirrer and the pH of solution was adjusted to 6.0 with 5M HCl using pH meter. The remaining amount of distilled water was then poured so that the total volume became 1 L. The opening of the bottle was closed with the cap half circle loose. Then the solution was autoclaved at 121°C and 15 psi pressure for about 15-20 minutes or used as required. Several 1 L of this solution may be prepared as shown in Figure 3.31.

3.6.3 Bio-Treatment Methods

To obtain significant strength improvement in a loose granular material at least 60 kg $CaCO_3/m^3$ of soil has to be precipitated, which corresponds to approximately 2 mol $CaCO_3$ precipitate per liter of pore space [24]. In this research, 70 kg/m^3 and 100 kg/m^3 of $CaCO_3$ were targeted to precipitate using two different methods (Method A and Method B). In Method A, the application of bacteria and treatment solutions were conducted using the experimental setup described in 3.5.1 by flowing solutions from top to bottom of the specimens. In this method, the target $CaCO_3$ was 100 kg/m^3. In Method B, the bacteria and treatment ingredients were mixed at the time of sample preparation described in 3.3. The target $CaCO_3$ for this method was 70 kg/m^3. The details of these methods are described in below.

Figure 3.31: Preparing $CaCl_2.2H_2O$ Stock Solution and Urea Nutrient Solution

Bio-Treatment Method A

Three specimens were prepared as described in 3.4 using the split mold. Each specimen was recovered by splitting the mold, wrapped with very thin polythene and again encased into the mold. These molds were then set in the experimental setup described in 3.5.1. The leakage of the setup was checked by flowing distilled water through the specimens. In the mean time, the laminar flow hood was cleaned with 70% Ethanol and the Bunsen burners were burnt. Bacteria were streaked from the vial to a fresh plate medium inside the hood. The plate was then incubated at 30°C for 24 hours. For each specimen, 150 ml of NH_4-YE autoclaved broth medium was taken into a 250 ml autoclaved conical flask inside the hood. The initial portion of the opening of each flask was burnt for 2-3 seconds and it was closed with the cotton plug as shown in Figure 3.32. For each of three 150 ml of NH_4-YE broth media, one loopful of bacteria was taken from the overnight culture plate and inoculated into the medium inside the hood. These three media with bacteria were then incubated at 30°C for one day. Before inoculation of bacteria into the broth, the absorbance of the broth was measured by a HACH Spectrophotometer with a wavelength of 590 nm. The absorbance was found to be 0.1. After one day of incubation, the absorbance was again measured to have an idea about the concentration of the bacteria. The absorbance was then found as 1.0 that indicated rapid growth of bacteria in the media. 400 ml of autoclaved urea nutrient medium was taken into each of three 1 L Duran bottles inside the hood. These media were then aerated by an air aerator to increase the pH of the solution to 7.15-7.25 as shown in Figure 3.33. 8 ml of autoclaved $CaCl_2.2H_2O$ stock solution and 100 ml of NH_4-YE broth medium with bacteria (overnight culture from the incubator) were added into each aerated 400 ml urea nutrient solution using autoclaved measuring cylinders inside the hood. These solutions were then incubated over a shaker at room temperature for one day as shown in Figure 3.34. After that, these solutions were applied to the specimens at a flow rate of about 10 ml/min. This initial biological treatment was allowed to set one day to allow the microbes to attach to the sand grains of the sample. After this initial treatment, cementation treatment solution was applied. Cementation treatment solutions were prepared by mixing 400 ml autoclaved urea nutrient solution and 140 ml autoclaved $CaCl_2.2H_2O$ solution. 400 ml autoclaved urea nutrient solution was taken into each of three 1 L Duran bottles. These were then aerated by an air aerator to increase the pH to 7.15-7.25. 140 ml of autoclaved $CaCl_2.2H_2O$ stock solution was added to each of three 400 ml urea nutrient solutions

using autoclaved measuring cylinder inside the hood. About 5 ml of cementation solution was taken into a test tube from each Duran bottle. These three test tubes were properly marked to determine the concentration of Ca^{2+} ions by atomic absorption method. Then these cementation solutions were applied at a rate of about 4 ml/min. After application, again 5 ml of cementation solution, which passed through the specimen and stored in the beaker below the specimen, was taken into a test tube from each of three beakers for determination of Ca^{2+} ions by atomic absorption method. This cementation treatment was allowed to set for one day. In this way, six cementation treatments were applied to each specimen. After completion of the whole treatment process, the specimens were allowed to set for 10 days. The number of cementation treatment cycles was calculated as shown in Table 3.2. From the table, it is seen that 5 cycles of cementation treatment were enough to precipitate $CaCO_3$ of 100 kg/m^3 of soil. However, considering all kinds of uncertainties, 6 cycles of cementation treatment were applied into each specimen that corresponds to precipitated $CaCO_3$ of 143 kg/m^3 of soil.

Table 3.2: Calculation of Numbers of Treatment Cycles

Description	Value	Remarks
Target $CaCO_3$ (kg/m^3 of total soil volume), T	100	Bio-treatment method A
Sample height (mm), H	142	Inner
Sample diameter (mm), D	71	Inner
Sample volume (m^3), V	0.0005622	V= (π*D^2/4)*H/1000^3
$CaCO_3$ to be precipitated (gm), TP	56.221	TP= 100*0.0005622*1000
Ca^{2+} required (gm), C	22.488	$Ca^{2+} + CO_3^{2-} \rightarrow CaCO_3 \downarrow$ 40 gm 60 gm 100 gm
CO_3^{2-} required (gm), CC	33.732	C= 40*TP/100 CC= 60*TP/100
$CaCl_2.2H_2O$ required (gm), CCL	82.644	Molecular Weight of $CaCl_2.2H_2O$ = 147 gm

		147 gm of $CaCl_2.2H_2O$ contains 40 gm of Ca^{2+} CCL= 147*C/40
Urea required (gm), U	33.732	$NH_2 - CO - NH_2 + 2H_2O \xrightarrow{\text{Urease}} 2NH_4^+ + CO_3^{2-}$ 60 gm 36 gm 36 gm 60 gm U= 60*CC/60
Urea in 400 mL urea medium (gm), UR	8	From 3.6.2, 20 gm urea was mixed to prepare 1000 ml urea nutrient solution. UR= 20*400/1000
CO_3^{2-} produced in 400 mL urea medium (gm), CR	8	$NH_2 - CO - NH_2 + 2H_2O \xrightarrow{\text{Urease}} 2NH_4^+ + CO_3^{2-}$ 60 gm 36 gm 36 gm 60 gm CR= 60*UR/60
Ca required in 400 mL urea medium (gm), CAR	5.333	$Ca^{2+} + CO_3^{2-} \rightarrow CaCO_3 \downarrow$ 40 gm 60 gm 100 gm CAR= 40*CR/60
$CaCl_2.2H_2O$ required for 400 mL urea medium (gm), CCLR	19.6	Molecular Weight of $CaCl_2.2H_2O$ = 147 gm 147 gm of $CaCl_2.2H_2O$ contains 40 gm of Ca^{2+} CCLR= 147*CAR/40
Stock solution of $CaCl_2.2H_2O$ (140 gm/L) required for 400 mL urea medium (ml), SSR	140	Concentration of Stock Solution= 140 gm/1000 ml SSR= 1000*CCLR/140
Treatment cycle based on urea required, TCU	4.217	TCU= U/UR
Treatment cycle based on $CaCl_2.2H_2O$ required, TCC	4.217	TCC= CCL/CCLR
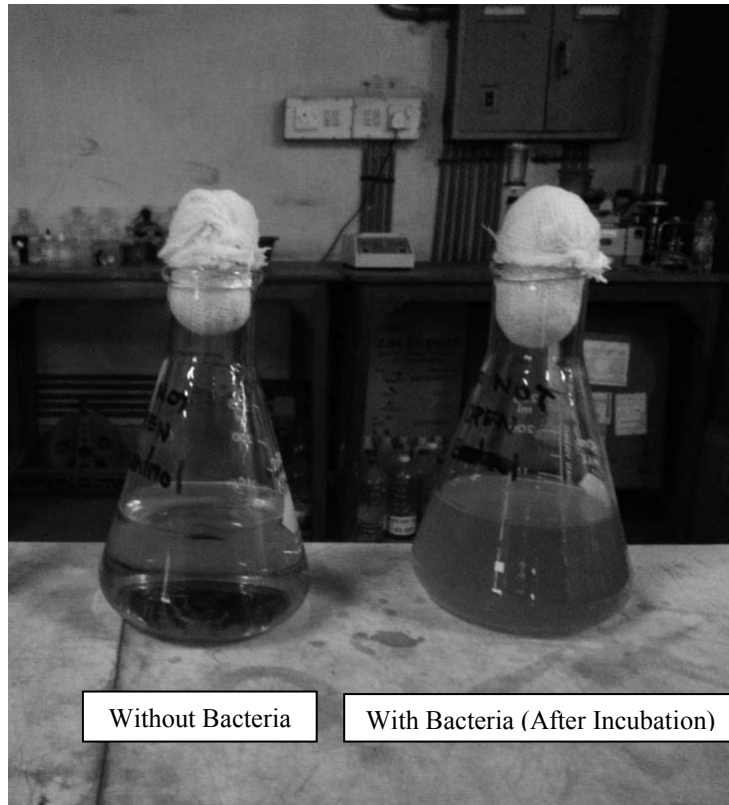
Figure 3.32: NH$_4$-YE Nutrient Broth Closed with Cotton Plug

Figure 3.33: Aeration of Urea Nutrient Solution

Figure 3.34: Urea Nutrient Solution with NH_4-YE Medium and Bacteria at Room Temperature over a Shaker

Bio-Treatment Method B

In this method, bacteria and nutrient solution were mixed with the specimens at the time of specimen preparation. 120 gm urea, 18 gm nutrient powder, 60 gm NH_4Cl and 12.72 gm $NaHCO_3$ were taken into a 1 L Duran bottle. Then distilled water was added to make the total volume of 500 ml. The mixture was mixed with magnetic stirrer and the pH of the solution was adjusted to 6.0 with 5M HCl using pH meter. The remaining amount of distilled water was then poured so that the total volume became 1 L. Then this modified urea nutrient solution was autoclaved at 121°C and 15 psi pressure for about 15-20 minutes. In the mean time, the laminar flow hood was cleaned with 70% Ethanol and the Bunsen burners were burnt. Bacteria were streaked from the vial to a fresh plate medium inside the hood. The plate was then incubated at 30°C for 24 hours. After that, 150 ml of NH_4-YE broth medium was taken into a 250 ml autoclaved conical flask inside the hood. The initial portion of the opening was burnt for 2-3 seconds and it was closed with the cotton plug. One loopful of bacteria was taken from the overnight culture plate and inoculated into the medium inside the hood. This medium with bacteria was then incubated at 30°C for one day. Before inoculation of bacteria into the broth, the absorbance of the broth was measured by a HACH Spectrophotometer with a wavelength of 590 nm. The absorbance was found to be 0.1. After one day of incubation, the absorbance was again measured to have an idea about the concentration of the bacteria.

The absorbance was then found as 1.0 that indicated rapid growth of bacteria in the media. 220.5 gm of $CaCl_2.2H_2O$ was taken into an autoclaved 1 L Duran bottle inside the hood. Modified urea nutrient solution was poured into the bottle so that the total volume became 950 ml. Then 50 ml of NH_4-YE broth with bacteria was added to the solution so that the total volume of this solution became 1 L. Now each specimen was prepared as described in 3.4 except 100 ml of this solution was used instead of distilled water. This 100 ml solution was nearly equivalent to 1.5 cycles of cementation treatment as described in 3.6.3.1 that corresponds to precipitated $CaCO_3$ of nearly 35 kg/m^3 of soil. Each of the untreated specimens was prepared with 100 ml distilled water and it was expected to compare the behavior of these untreated specimens with the behavior of treated specimens. Therefore, 100 ml of the modified nutrient solution with $CaCl_2.2H_2O$ and bacteria ought to be mixed with each specimen. Again, it was not possible to dissolve more amount of ingredients as discussed earlier in this section. So the target of precipitated $CaCO_3$ for this treatment method was accepted to be 35 kg/m^3 of soil. After preparation, each specimen was kept inside a plastic pipe in the room temperature as shown in Figure 3.35 for 20 days to continue treatment process.

Figure 3.35: Bio-Treated Samples Inside the Plastic Pipes

3.7 MONITORING AND TESTING

Following parameters were measured as a part of monitoring and testing scheme.

3.7.1 Conductivity and pH Measurement

Urea hydrolysis results increase of pH and conductivity of the surrounding medium [16]. Therefore, in bio-treatment method A, conductivity and pH of each of six nutrient solutions were measured before and after application to the specimens to check whether the applied urea was hydrolyzed or not.

3.7.2 Ca^{2+} Ions Determination

In bio-treatment method A, 5 ml solution was collected in a test tube before and after application of each cementation treatment solution to determine the concentration of Ca^{2+} ions by Atomic Absorption Method. This was done to check whether $CaCO_3$ was formed in the specimen or not. Each time of determination, the machine shown in Figure 3.36 was calibrated with the standard calcium solution and the target solution was diluted properly to adjust the concentration of calcium ions within the calibration range.

Figure 3.36: Machine for Ca^{2+} Ions Determination by Atomic Absorption Method

3.7.3 Visual Inspection

In bio-treatment method A, each treatment solution was visually inspected during treatment process and after its completion. Again, the beaker, where the treatment solution was stored after passing through the specimen, was also monitored. After completion of all treatments, the specimen and the mold were monitored during the next 10 days. Here it is mentionable that, it was not possible to extrude the specimen from the mold although the specimen was wrapped with polythene. Therefore, the specimen was split longitudinally and inspected. After removing the sample, the mold itself was inspected. In bio-treatment method B, the sample was monitored during its setting time (20 days).

3.7.4 Needle Penetration Test

As the specimen in bio-treatment method A could not be extruded, so needle penetration test was performed. In addition, to compare the behavior, this test was also performed for untreated specimen. The compression machine supplied by BUEHL+FAUBEL, A-1237, Vienna/Austria is shown in Figure 3.37. There is a calibrated proving ring with load dial gauge in the upper portion of the machine. A strain gauge was fitted in such a way that, it could measure the travel distance of the base where the specimen rested. The diameter of the needle is 8 mm. The machine was operated by the hand wheel as shown in Figure 3.37. One revolution of hand wheel corresponds to 0.063 mm rise of the ram. After placing the specimen properly on the ram, the wheel was turned at a rate of 1 cycle/2 seconds which corresponds to a displacement of 1.89 mm/min. During the test, the strain gauge readings and the load dial gauge readings were noted for analysis.

Figure 3.37: Compression Machine for Needle Penetration Test

3.7.5 Compressive Strength Test

For specimen of bio-treatment method B, compressive strength test was performed. In addition, to compare the behavior, this test was also performed for 5 untreated specimens. In this test, the same type of machine as described in 3.6.4 was used, except the ram was automatically operated by an electrical motor. The specimen was broken under axial load like concrete cylinder crushing test as shown in Figure 3.38. The specimen was compressed at a rate of 1.52 mm/min.
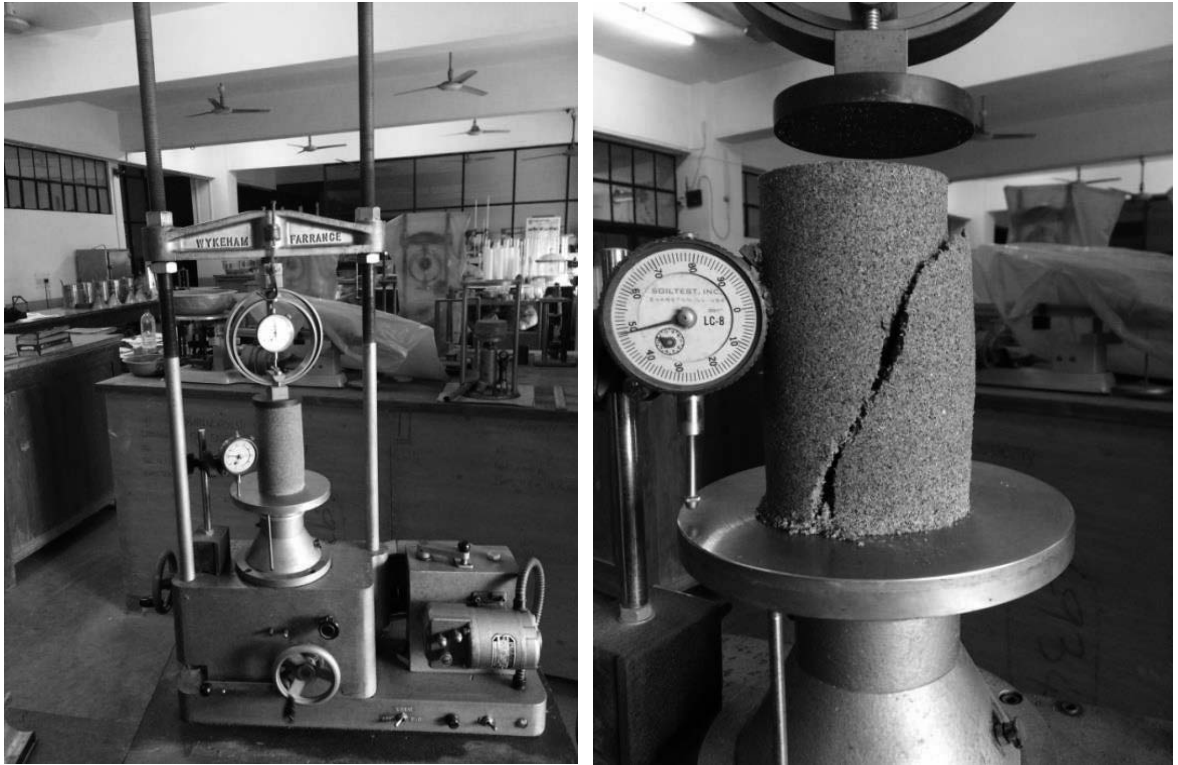
Figure 3.38: Compressive Strength Test

3.7.6 Cyclic Triaxial Test

Consolidated undrained cyclic triaxial test was performed for both untreated and bio-treated specimens of method B using cyclic triaxial machine as shown in Figure 3.39. All specimens were manually saturated with CO_2 and distilled water under a confining pressure of 25 kPa for 30 minutes and 2 hours respectively. After manual saturation, triaxial machine was started with initialization step. The initialization step continued for 15 minutes with confining pressure of 25 kPa and sample pressure of 10 kPa (effective confining pressure was 15 kPa). After initialization phase, backpressure saturation of each specimen was performed by simultaneously increasing cell and pore pressure until the saturation ratio became 0.95. During the backpressure saturation phase, the effective confining pressure was kept at a constant value of 15 kPa. After completion of backpressure saturation, each specimen was isotropically consolidated to an effective confining pressure of 50 kPa. During both saturation and consolidation, the pressure was increased very slowly at a rate of 0.167 kPa/sec. The consolidation phase was maintained for a minimum of 60 minutes and a maximum of 100 minutes. After completion of

consolidation, cyclic loading was applied. Several untreated specimens were tested with cyclic stress ratio of 0.20, 0.25 and 0.30 and cycle period of 1 second up to peak-peak axial strain of 5%. All data were stored and analyzed using the software provided by Geocomp. From the tests of untreated specimens, three sets of data were taken. Similarly three bio-treated specimens of method B were tested with the same cyclic stress ratios, cycle period and peak-peak axial strain as untreated specimens.

Figure 3.39: Cyclic Triaxial Machine

3.7.7 Precipitated Calcite Determination by Experimental setup

One (1) gm of oven dried treated sample from each of the specimens of method A and method B were used in the experimental setup as described in 3.4.2. Five (5) ml 5M HCl was pushed by the syringe as shown in Figure 3.29 in each case and the pressure gauge readings were noted. The test was performed at a temperature of 25°C.

3.7.8 Microscopic Examination of Microbial Calcite Precipitation

Direct observation of the microstructure of a soil is useful for understanding the relationship between soil structure and its mechanical behavior. In particular, previous investigations have investigated the formation of calcium carbonate ($CaCO_3$) cementation

using the scanning electron microscope (SEM). As discussed in Chapter 2: Literature Review, their results have provided evidence that any increases in the strength of the soil were primarily governed by the formation of cementing products. In a similar fashion in this study, after testing, bio-treated specimen was examined using Optical Microscope. Associated photos of interest are provided in the following chapter.

CHAPTER 4

RESULT AND DISCUSSION

4.1 GENERAL

This chapter presents the results from various tests conducted on untreated and bio-treated sand specimens. Bio-treatment has more significant benefits in loose sand than in dense sand, as loose granular soils have lower shear strengths, are more compressible, and are more susceptible to catastrophic-type liquefaction failures than dense granular soils. Consequently, all of the specimens that were tested in this research program were prepared at relative densities of approximately 50% using moist tamping method. In case of method A, pH, conductivity and Ca^{2+} ion concentration of the treatment solutions were measured before and after the application. Three specimens were treated at a time. After treatment, needle penetration test was performed for one treated specimen. In case of method B, unconfined compression test was performed for a specimen. Cyclic triaxial tests were performed for several specimens of method B. Calcite precipitation was confirmed for both method A and B using the setup developed and optical microscope image.

4.2 CONDUCTIVITY AND pH

Urea hydrolysis increases conductivity of the surrounding environment. To check whether applied urea in the specimen of treatment method A was hydrolyzed or not, conductivity of the cementation treatment solution (urea nutrient solution mixed with $CaCl_2.2H_2O$ stock solution) before application and after application was measured. The results found from these measurements are shown in Figure 4.1.
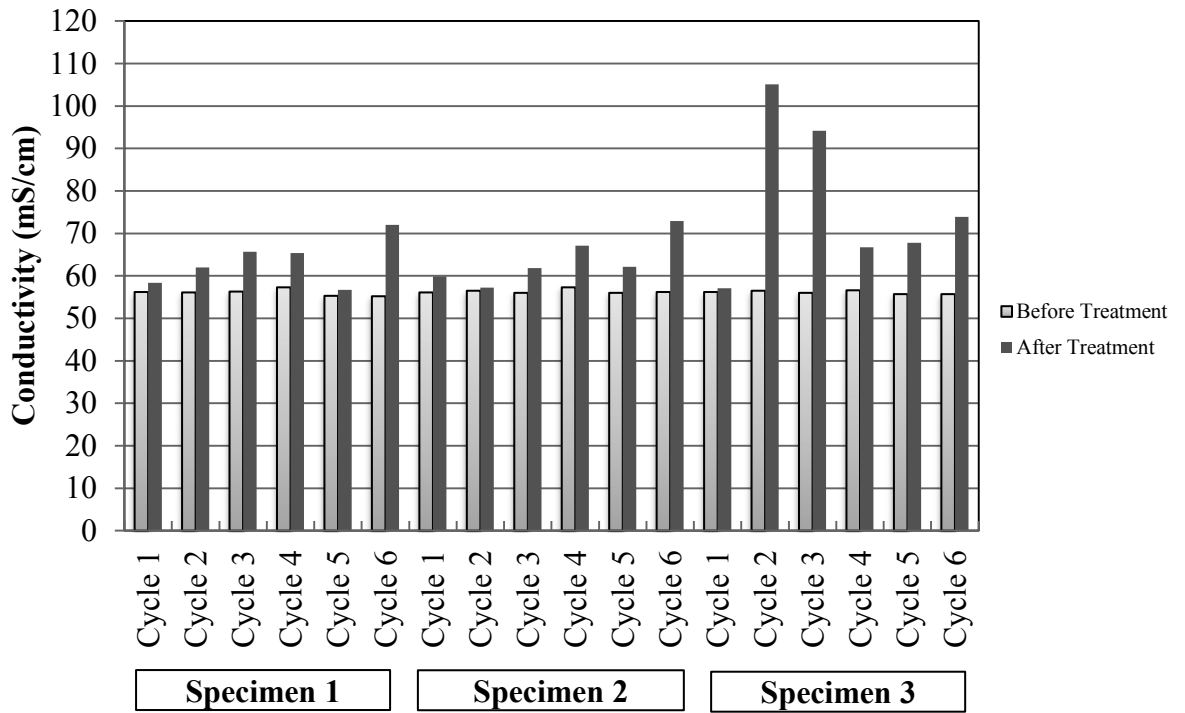
(a) Raw Data
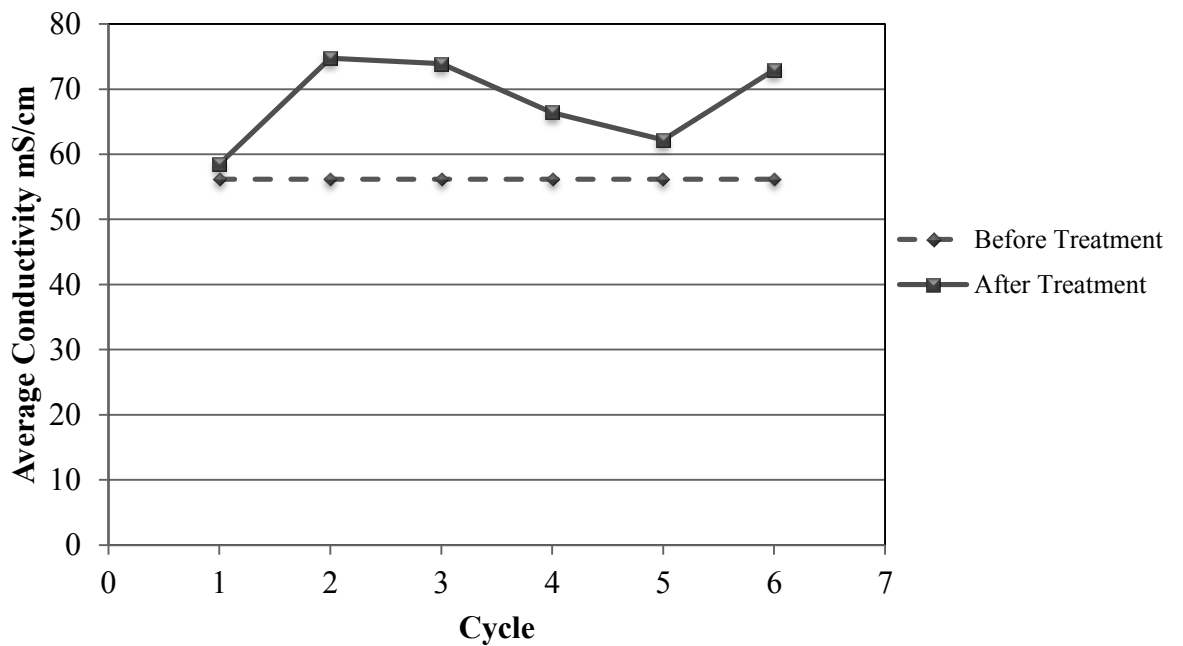
(b) Average Data

Figure 4.1: Change of Conductivity of Urea Nutrient Solutions

From the figure, it is observed that increase of conductivity is less in the early nutrient solutions. This indicates that the applied bacteria needed some times to fully utilize the applied urea. However, conductivity changes in the later nutrient solutions are relatively higher. Increase of conductivity of the nutrient solution is an indication of urea hydrolysis as described by Whiffin [3].

In the similar way as mentioned above, pH of each nutrient solution was measured before and after application. pH were also found to be increased as shown in Figure 4.2. From the figure it is observed that, pH change was not significant. But, pH might be higher inside the specimen in the micro environment. However, pH change (increase) also indicates hydrolysis of the urea as described by Whiffin [3].
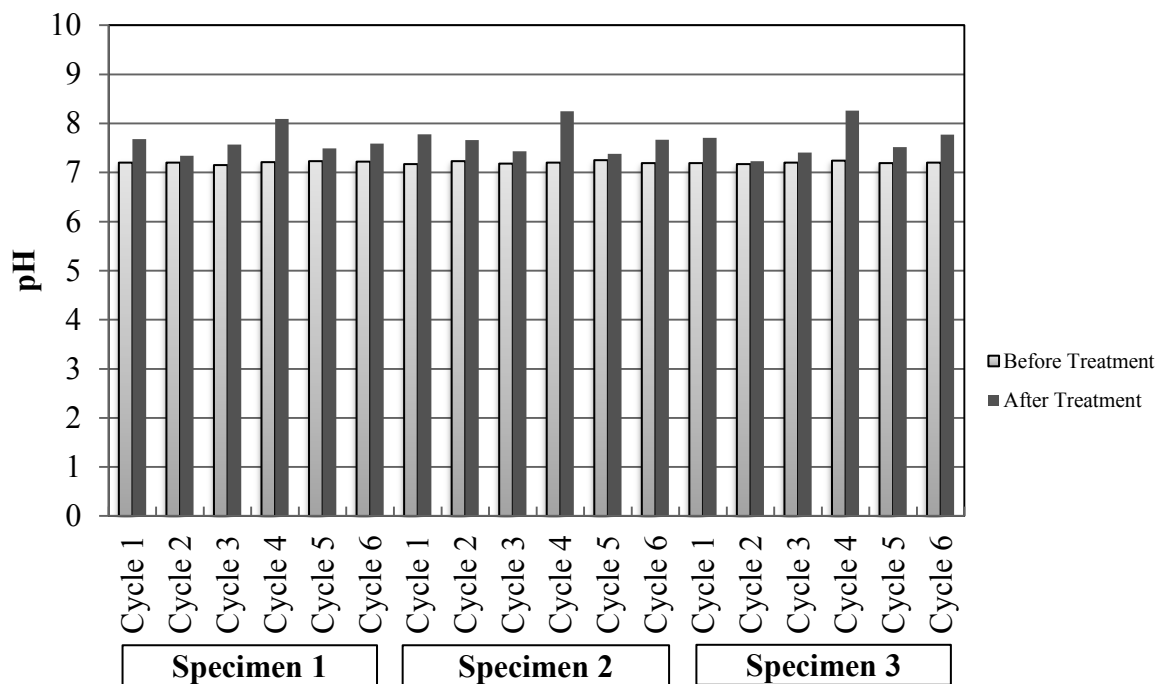
(a) Raw Data
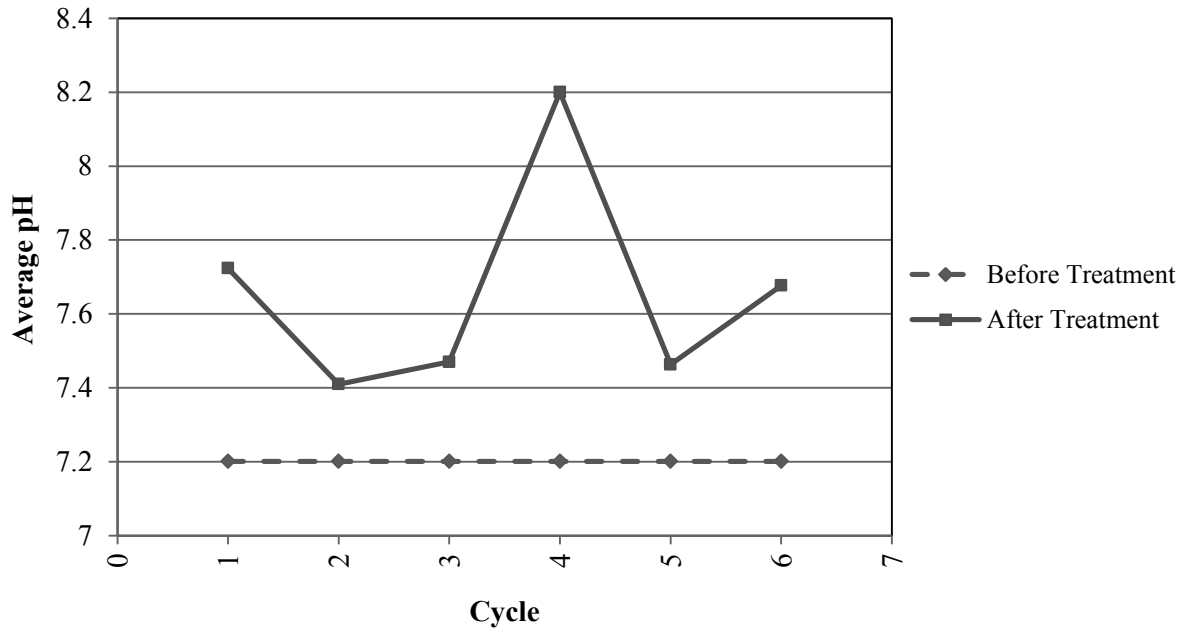
(b) Average Data

Figure 4.2: Change of pH of Urea Nutrient Solutions.

4.3 Ca^{2+} IONS CONCENTRATION

In bio-treatment method A, 5 ml solution was collected in a test tube before and after application of each cementation treatment solution to determine the concentration of Ca^{2+} ions by Atomic Absorption Method. This was done to check whether $CaCO_3$ was formed in the specimen or not. Each time of determination, the machine shown in Figure 3.36 was calibrated with the standard calcium solution and the target solution was diluted properly to adjust the concentration of calcium ions within the calibration range. Results found from these tests are shown in Figure 4.3. Theoretical concentration of Ca^{2+} ions is 9876.54 ppm if the chemicals are 100% pure which is practically not possible. Again some losses may occur during preparation of the solution. So in Figure 4.3, it is seen that the initial concentration is less than the calculated concentration. All of the concentrations after treatment were found to be less than the initial concentrations before treatment except cycle 3 and cycle 5 of specimen 1 and specimen 2 respectively. These deviations might occur due to wash out of precipitated calcite with the flowing treatment solution

and/or delayed activation of bacteria in the beaker carrying already flown solution. However, in the bar chart of average data, it is observed that in all cycles, the concentrations of calcium ions decreased after treatment.
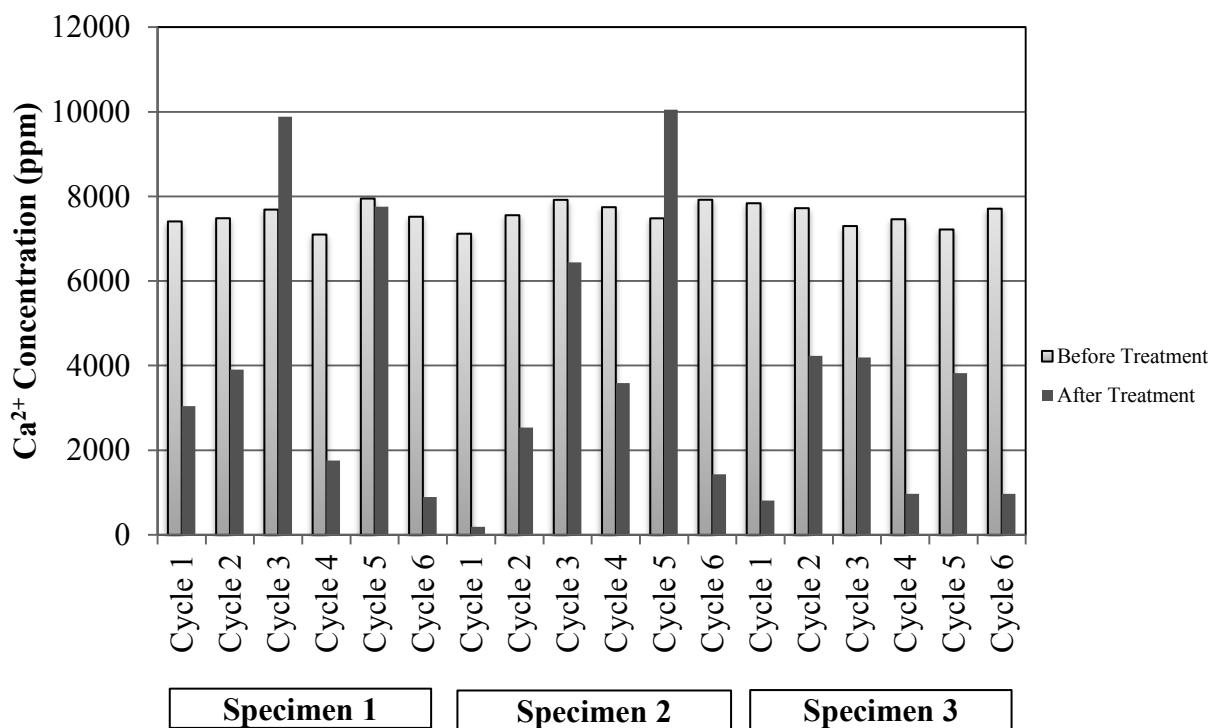
(a) Raw Data
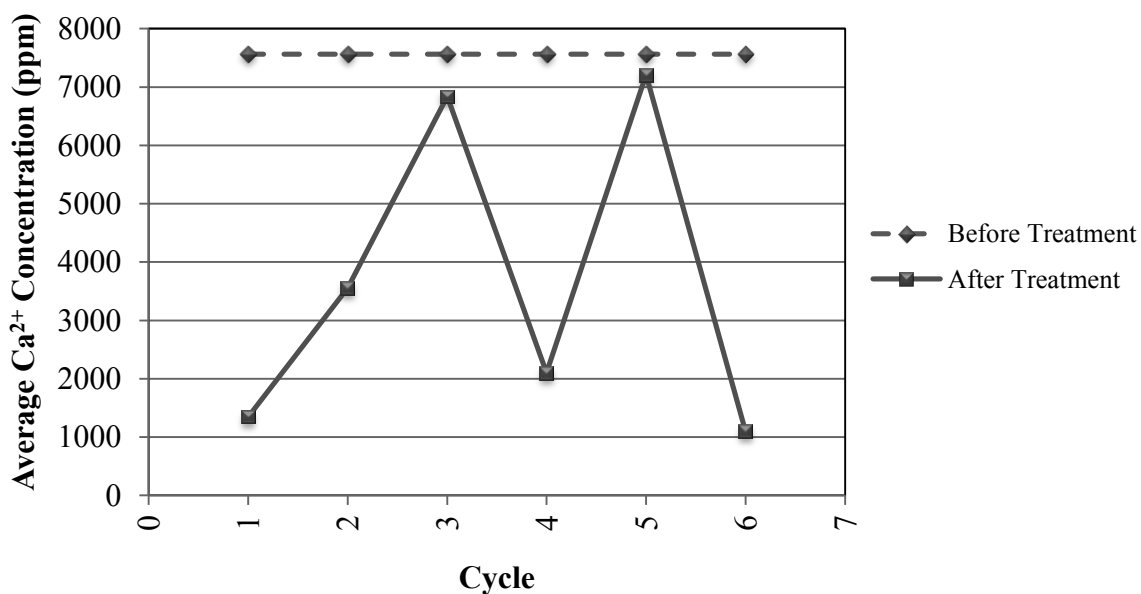
(b) Average Data

Figure 4.3: Change of Concentration of Ca^{2+} Ions of Treatment Solutions

4.4   VISUAL INSPECTION

In bio-treatment method A, each treatment solution was visually inspected during treatment process and after its completion. Again, the beaker, where the treatment solution was stored after passing through the specimen, was also monitored. Calcite was precipitated in the bottom of the beaker also as shown in Figure 4.4. After completion of all treatments, the specimen and the mold were monitored during the next 10 days. White layer of calcite was observed clearly at the top of each specimen as shown in Figure 4.5. Again, the porous plate used at the bottom of the specimen was also observed to be attached with the soil sample as shown in Figure 4.6. Here it is noteworthy that, it was not possible to extrude the specimen from the mold although the specimen was wrapped with polythene. Therefore, the specimen was split longitudinally and inspected. Calcite was found to be formed at the inside periphery of the mold as shown in Figure 4.7(a). On the other hand, the soil sample was hard enough and it was adhered to the inside wall of the mold as shown in Figure 4.7(b).
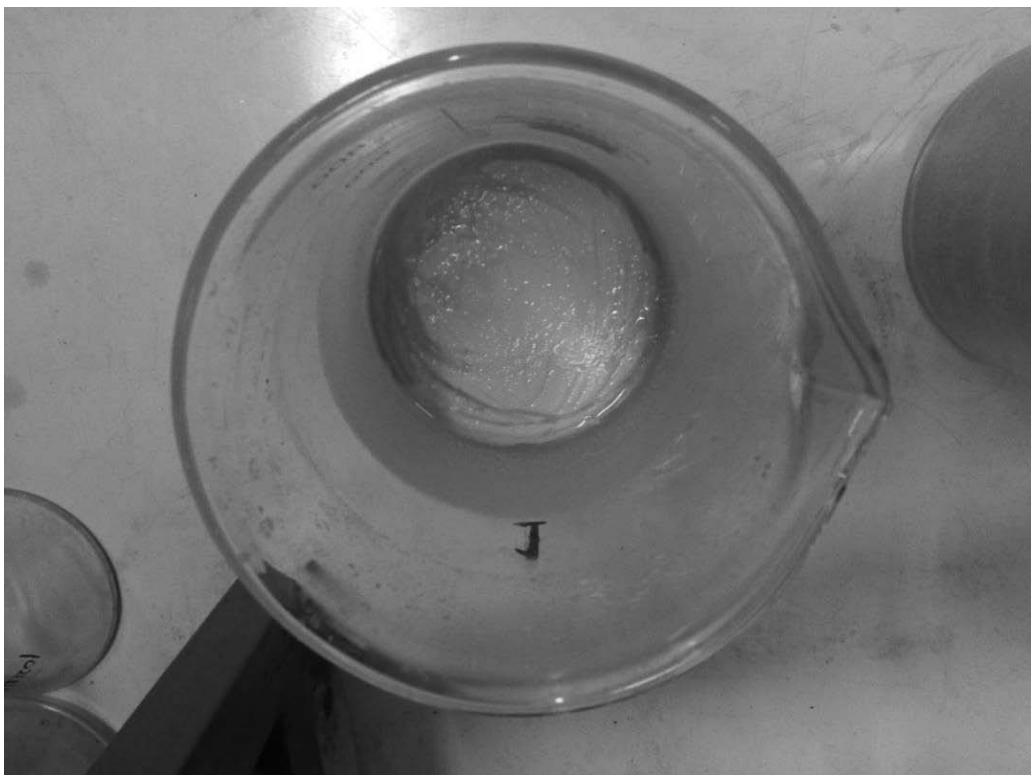
Figure 4.4: Precipitated CaCO$_3$ in the Beaker in Method A

Figure 4.5: Precipitated $CaCO_3$ at the Top of the Specimens in Method A

Figure 4.6: Presence of Precipitated $CaCO_3$ at the Bottom of the Specimens in Method A

(a) CaCO$_3$ at the Inside Wall of the Mold in Method A

(b) Hard Soil after Splitting the Mold in Method A

Figure 4.7: Presence of Calcite in the Mold in Method A

4.5   NEEDLE PENETRATION RESISTANCE

As the specimen in bio-treatment method A could not be extruded, so needle penetration test was performed as mentioned in chapter 3. In addition, to compare the behavior, this test was also performed for untreated specimen. Load vs. Penetration curves for both treated and untreated specimens are shown in Figure 4.8. From the figure it is clear that the treated specimen required more load for the same penetration of the needle compared to the untreated specimen that indicated improvement of the sample by the bio-treatment method.
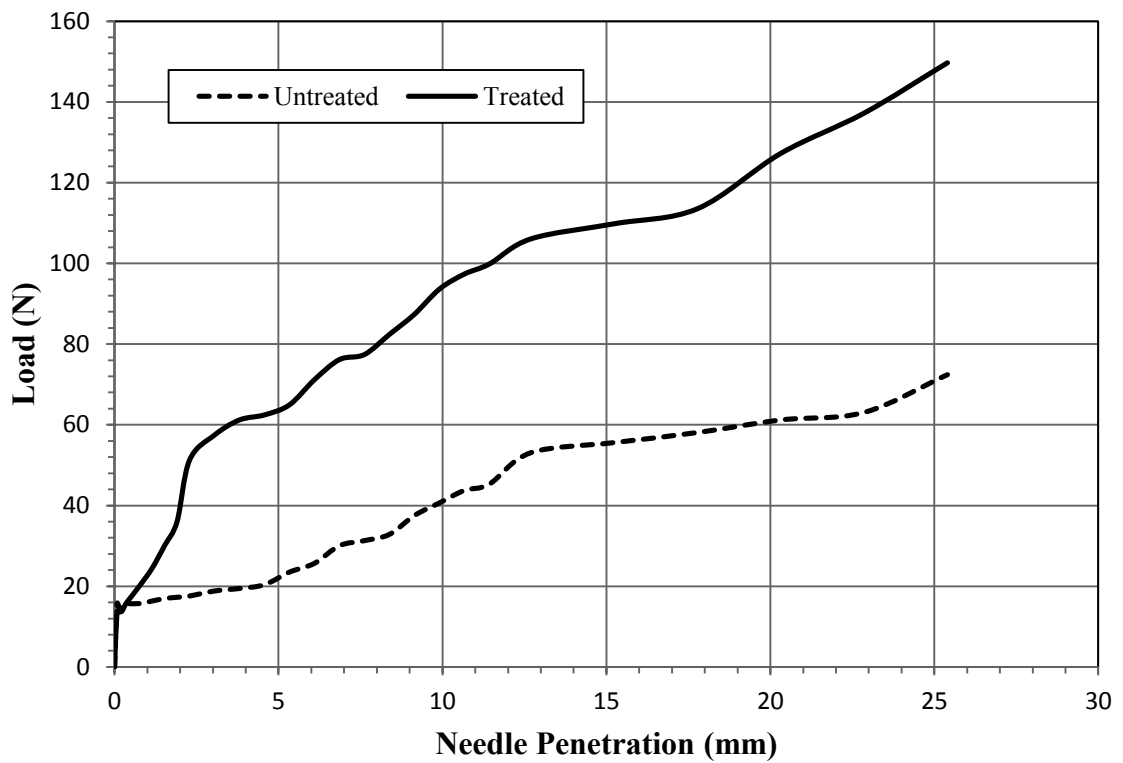
Figure 4.8: Needle Penetration Test Result for Specimen of Method A

4.6 COMPRESSIVE STRENGTH

For specimen of bio-treatment method B, compression test was performed. In addition, to compare the behavior, this test was also performed for 5 untreated specimens and the average result was taken. The strength of the treated specimen was found to be higher than the untreated specimen as shown in Figure 4.9. Again, the treated specimen showed brittle nature compared to untreated specimen as confirmed by other researchers.
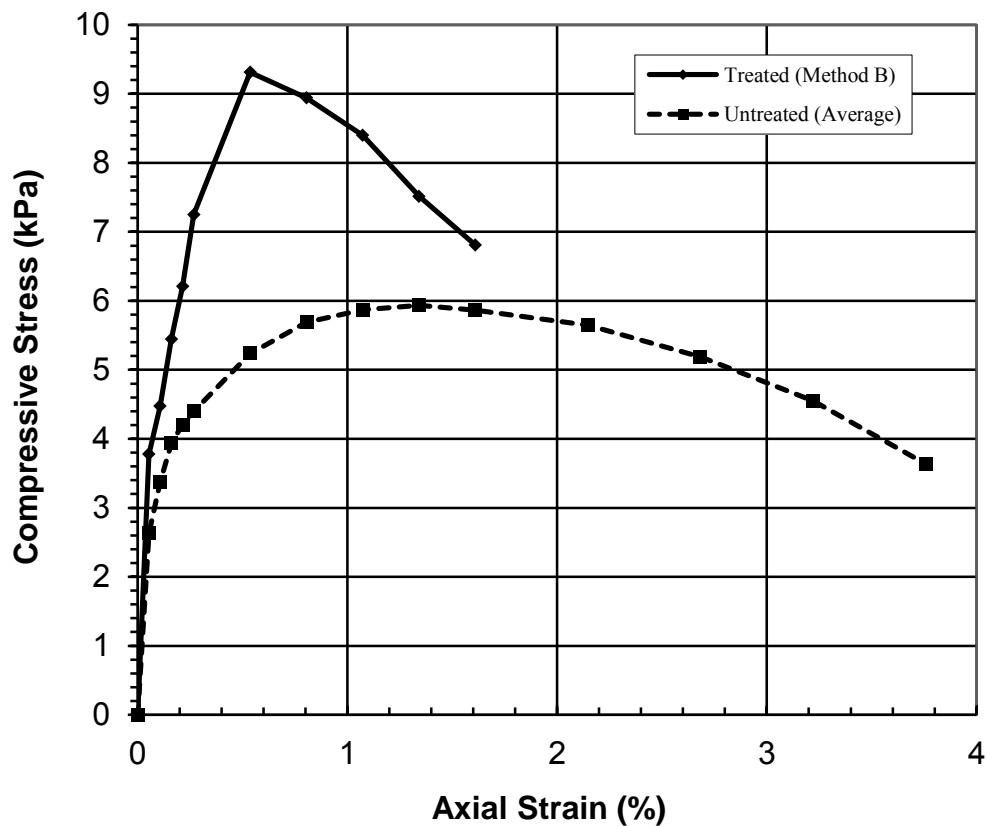
Figure 4.9: Compressive Strength Test Result for Specimen of Method B

4.7 BEHAVIOR UNDER CYCLIC LOAD

Consolidated undrained cyclic triaxial tests were performed for both untreated and bio-treated specimens of method B. All specimens were manually saturated with CO_2 and distilled water under a confining pressure of 25 kPa for 30 minutes and 2 hours respectively. After manual saturation, triaxial machine was started with initialization step.

The initialization step continued for 15 minutes with confining pressure of 25 kPa and sample pressure of 10 kPa (effective confining pressure was 15 kPa). After initialization phase, backpressure saturation of each specimen was performed by simultaneously increasing cell and pore pressure until the saturation ratio became 0.95. During the backpressure saturation phase, the effective confining pressure was kept at a constant value of 15 kPa. After completion of backpressure saturation, each specimen was isotropically consolidated to an effective confining pressure of 50 kPa. During both saturation and consolidation, the pressure was increased very slowly at a rate of 0.167 kPa/sec. The consolidation phase was maintained for a minimum of 60 minutes and a maximum of 100 minutes. After completion of consolidation, cyclic loading was applied. Several untreated specimens were tested with cyclic stress ratio of 0.20, 0.25 and 0.30 and cycle period of 1 second up to peak-peak axial strain of 5%. All data were stored and analyzed using the software provided with the machine. From the tests of untreated specimens, three sets of data were taken. Similarly three bio-treated specimens of method B were tested with the same cyclic stress ratios, cycle period and peak-peak axial strain as untreated specimens.

Figure 4.10 to 4.16 show different curves of both untreated and bio-treated specimens at a cyclic stress ratio of 0.20. In Figure 4.12, it is observed that for the treated specimen, the axial strain developed within a particular number of cycles is less than that of untreated specimen. The modulus reduction of bio-treated specimen is somewhat less than that of untreated specimen as shown in Figure 4.14. From Figure 4.15, it is seen that P-P axial strain within a particular number of cycles for untreated specimen is more than that of treated specimen. Again, excess pore water pressure also develops slowly in case of treated specimen as shown in Figure 4.16.

Results of bio-treated specimens tested at cyclic stress ratio of 0.25 are presented from Figure 4.17 to 4.22. In Figure 4.19, it is observed that for the treated specimen, the axial strain developed within a particular number of cycles is less than that of untreated specimen. As like as the bio-treated specimen tested at cyclic stress ratio of 0.20, the modulus reduction of bio-treated specimen tested at cyclic stress ratio of 0.25 is somewhat less than that of untreated specimen as shown in Figure 4.21. From Figure 4.22, it is seen that P-P axial strain within a particular number of cycles for untreated

specimen is more than that of treated specimen. Again, excess pore water pressure also develops slowly in case of treated specimen as shown in Figure 4.23.

Bio-treated specimens tested at cyclic stress ratio of 0.30 behave as like as untreated specimens. Figure 4.24 to 4.30 show different curves of both untreated and bio-treated specimens at a cyclic stress ratio of 0.30. In Figure 4.26, it is observed that for the treated specimen, the axial strain developed within a particular number of cycles is as same as that of untreated specimen. The modulus reduction of bio-treated specimen is also same as that of untreated specimen as shown in Figure 4.28. From Figure 4.29, it is seen that P-P axial strain within a particular number of cycles for untreated specimen is nearly same to that of treated specimen. Again, excess pore water pressure in the treated specimen also develops in the same manner as that of untreated specimen as shown in Figure 4.30. The behavior of untreated and treated specimens at cyclic stress ratio of 0.30 is same because at this high cyclic stress ratio the bond between the sand grains by calcite is broken at the very initial stage of cyclic loading. Higher precipitation of calcite will require more CSR (greater than 0.30) to break the bond.

However, from Figure 4.31, the cyclic resistance ratio (CSR to cause liquefaction in 20 cycles) for untreated specimen is found as 0.243. On the other hand, the cyclic resistance ratio for bio-treated specimen has been found as 0.261 which indicates improvement of the specimen by bio-treatment method. So it can be concluded that bio-mediated soil is more liquefaction resistant than untreated soil.

(a) Untreated
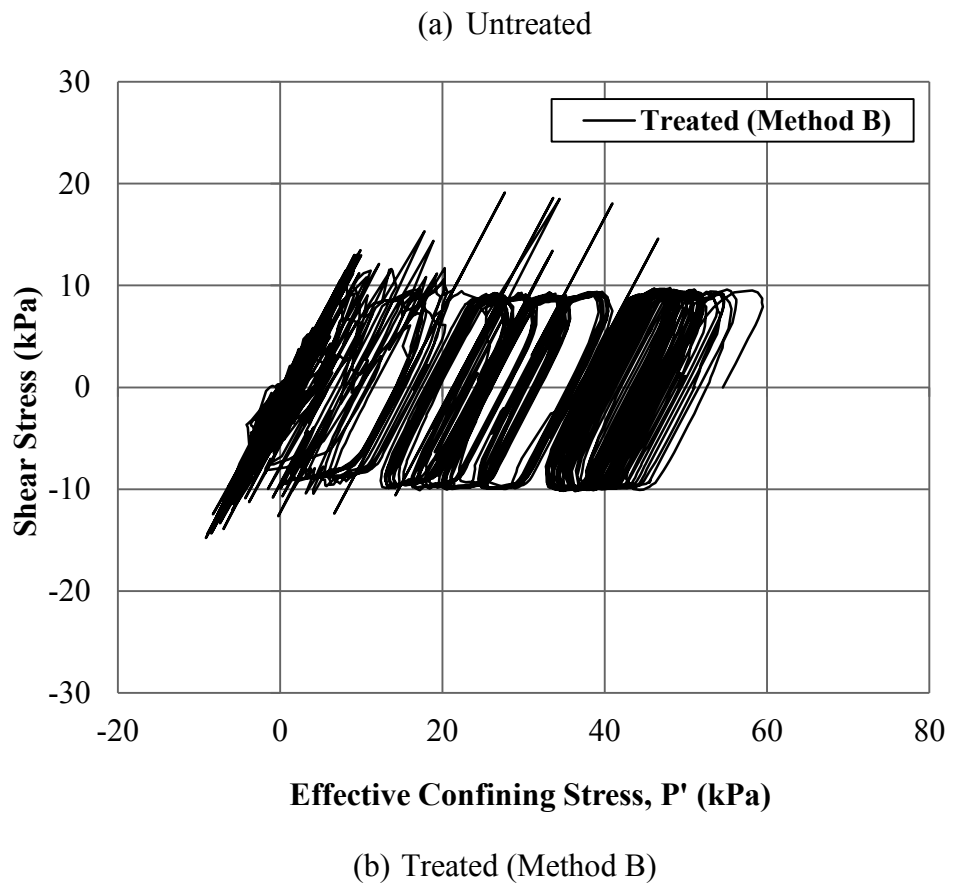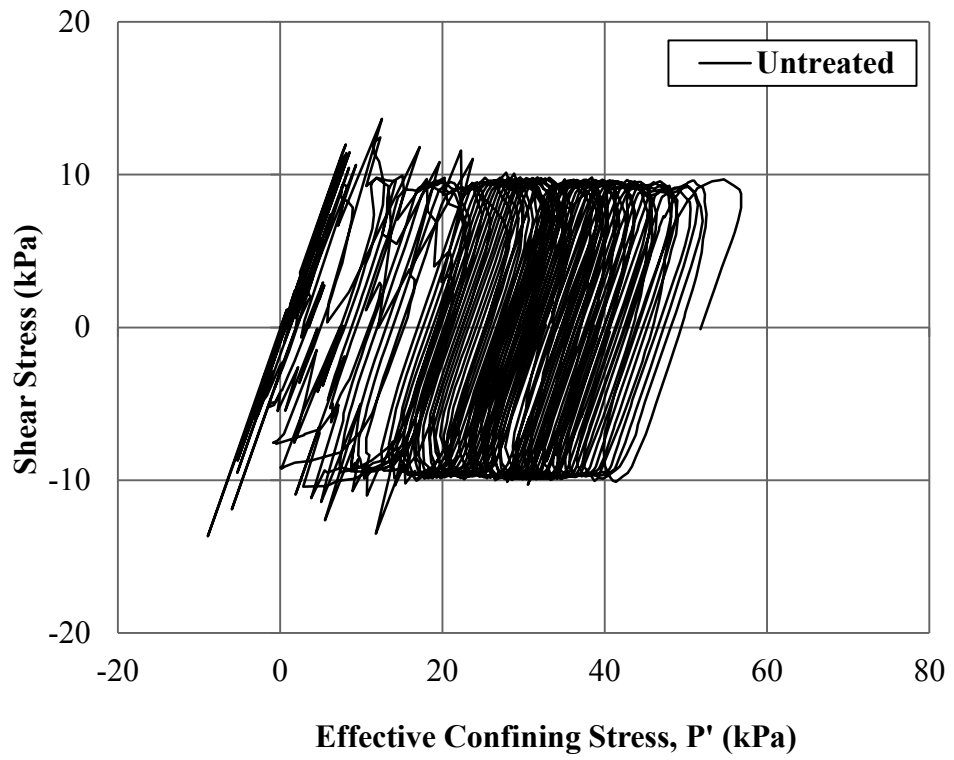
(b) Treated (Method B)

Figure 4.10: Shear Stress vs. Effective Confining Stress Plot for CSR: 0.20

(a) Untreated
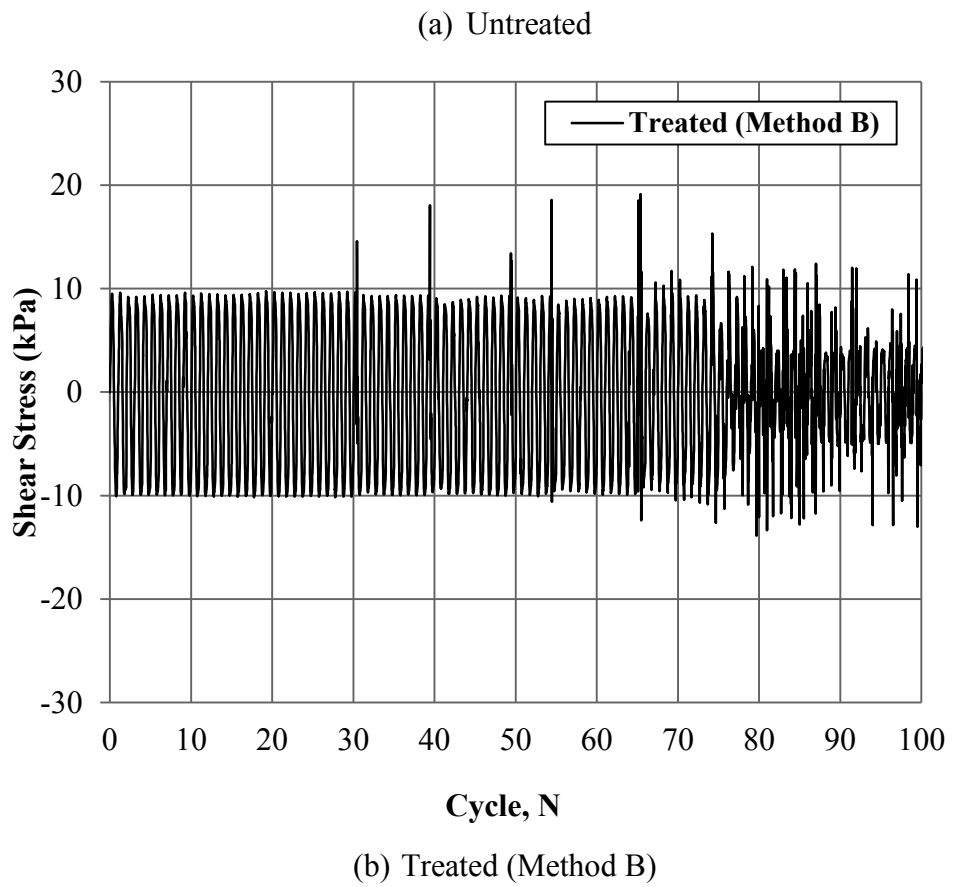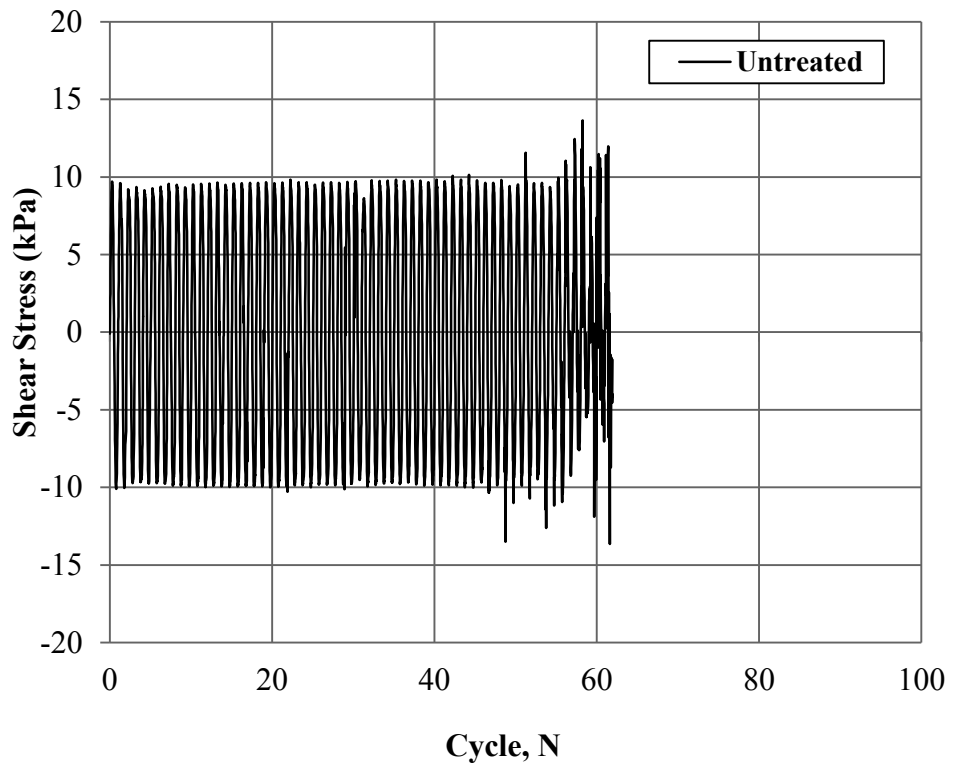
(b) Treated (Method B)

Figure 4.11: Shear Stress vs. Cycle Plot for CSR: 0.20
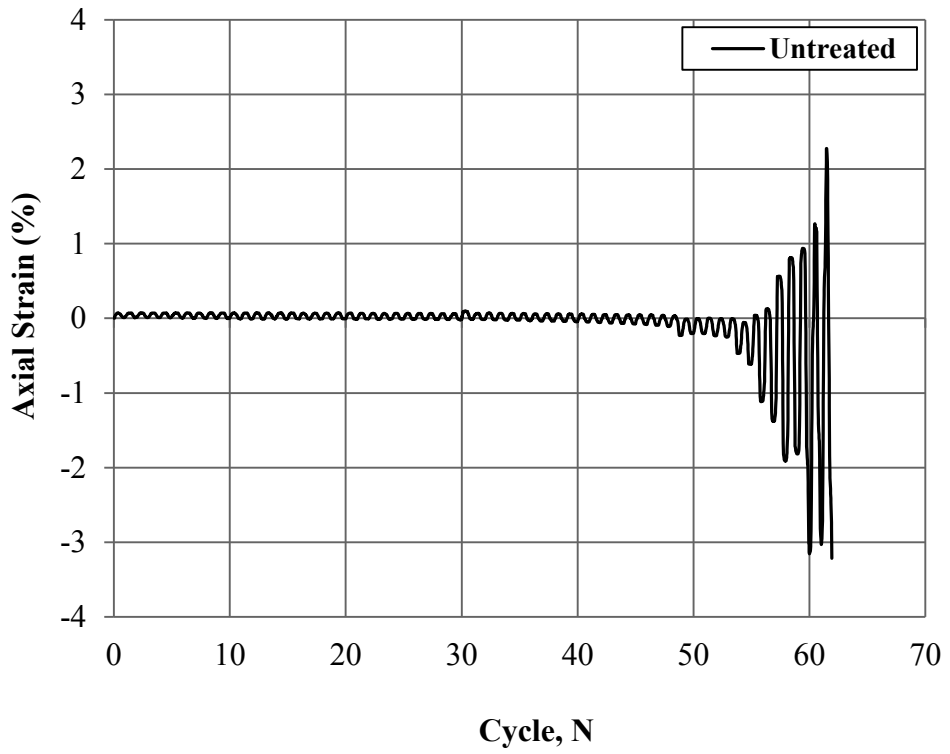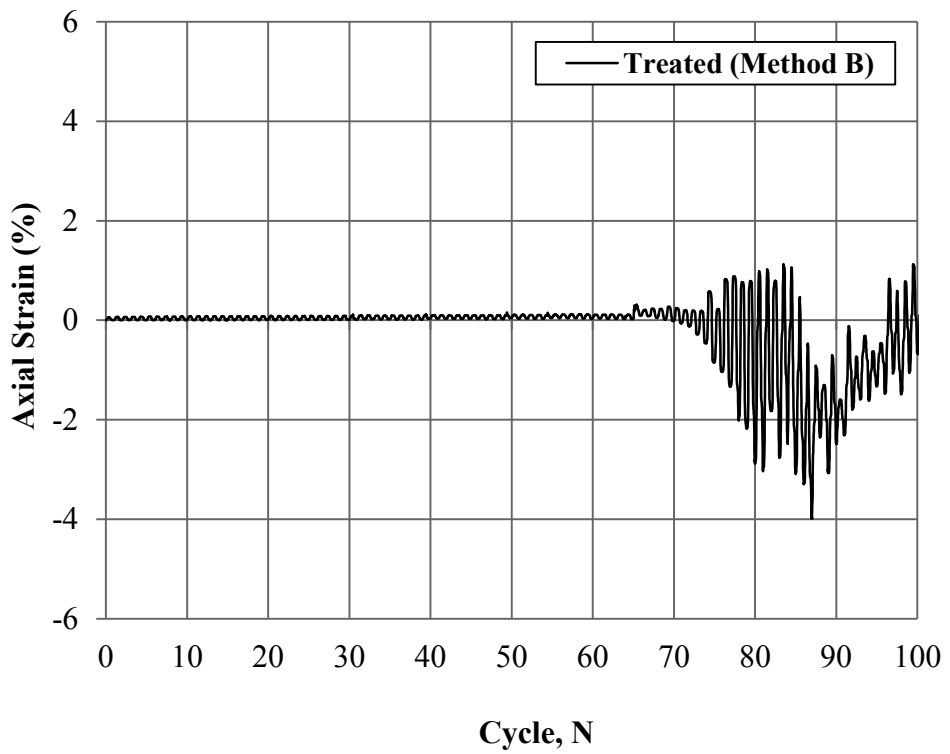
(a) Untreated

(b) Treated (Method B)

Figure 4.12: Axial Strain vs. Cycle Plot for CSR: 0.20
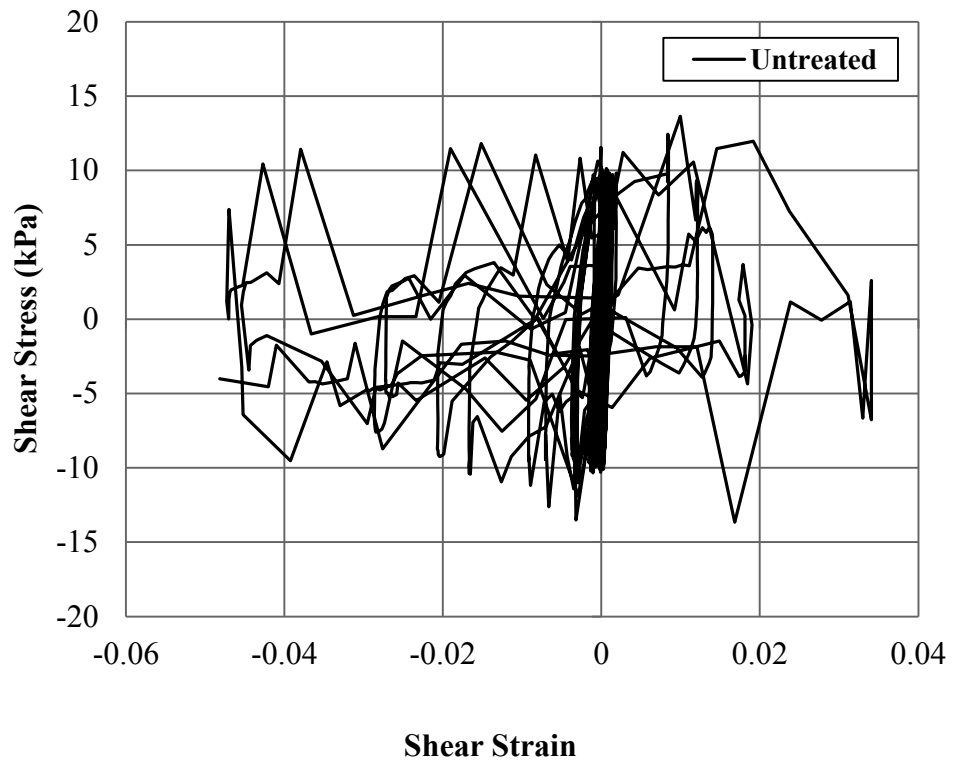
(a) Untreated
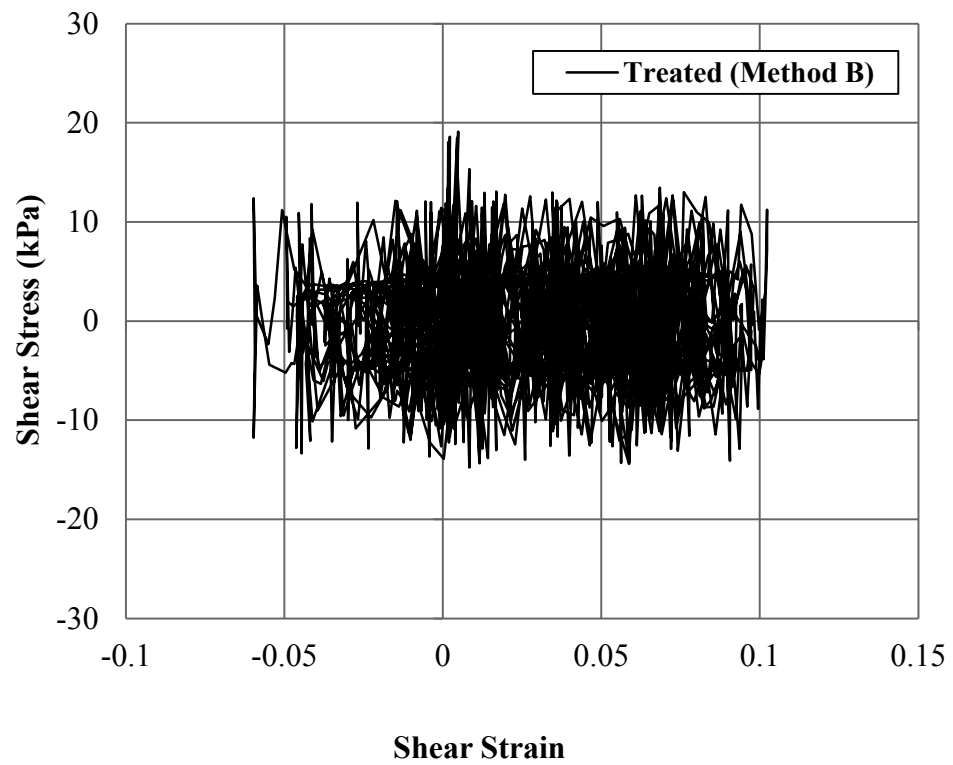
(b) Treated (Method B)

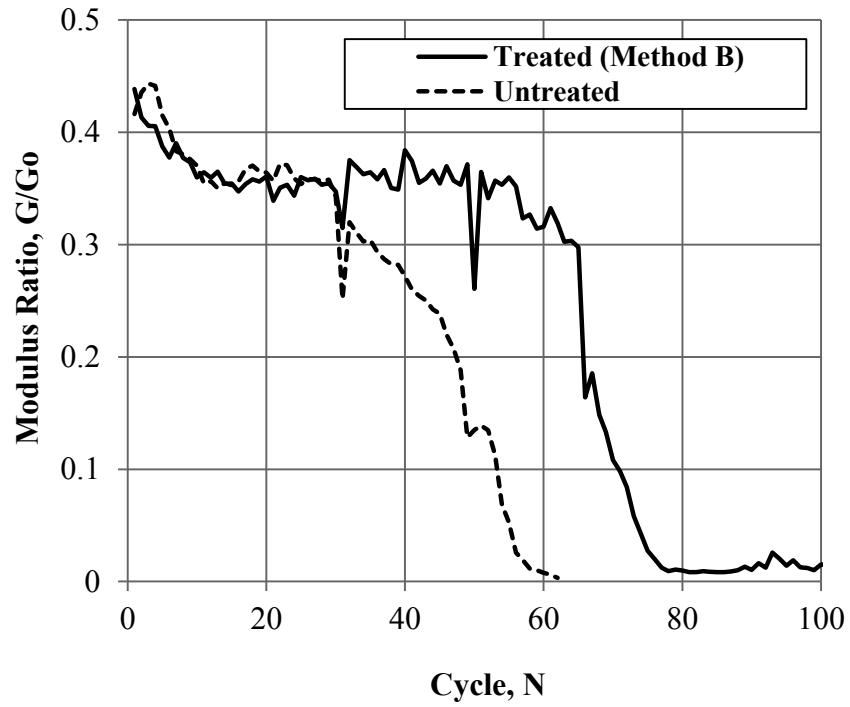**Figure 4.13:** Shear Stress vs. Shear Strain Plot for CSR: 0.20
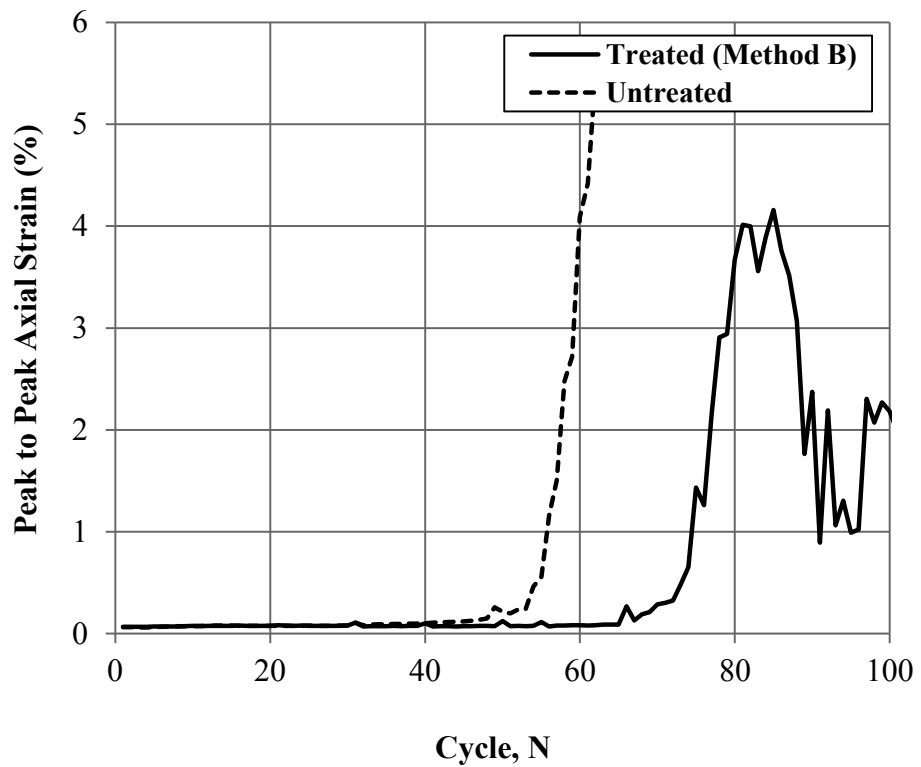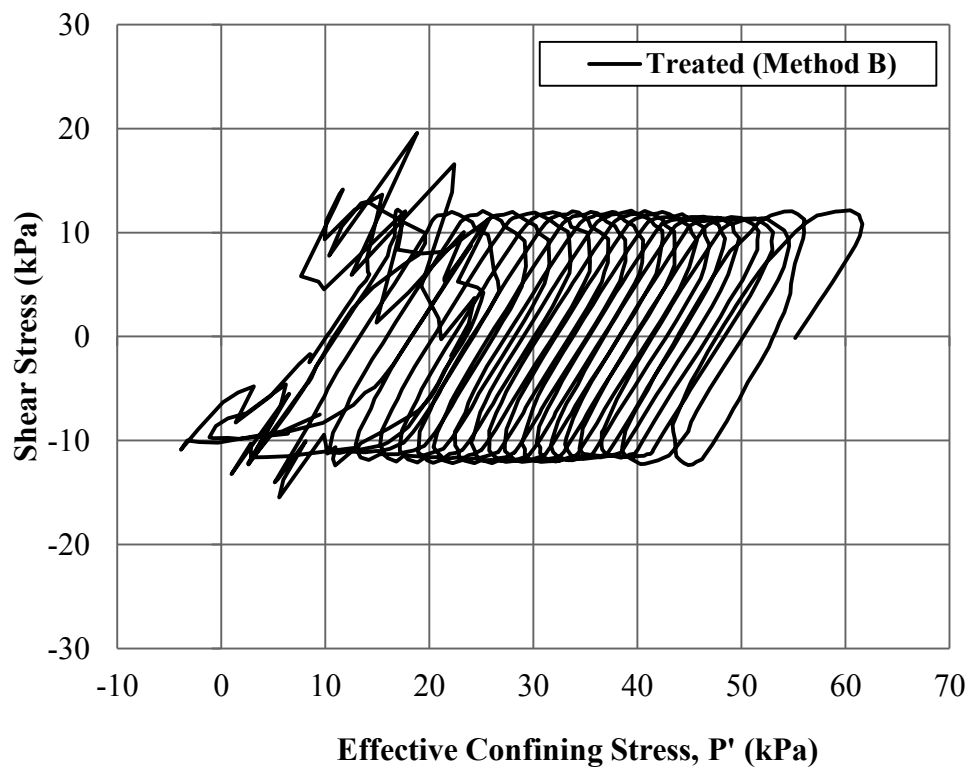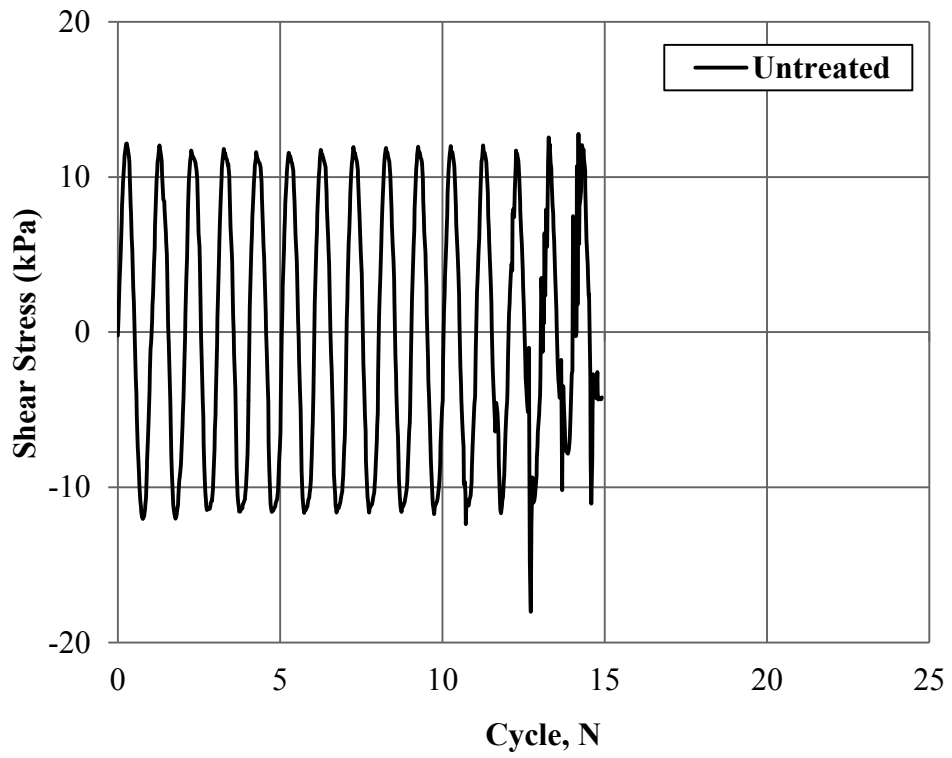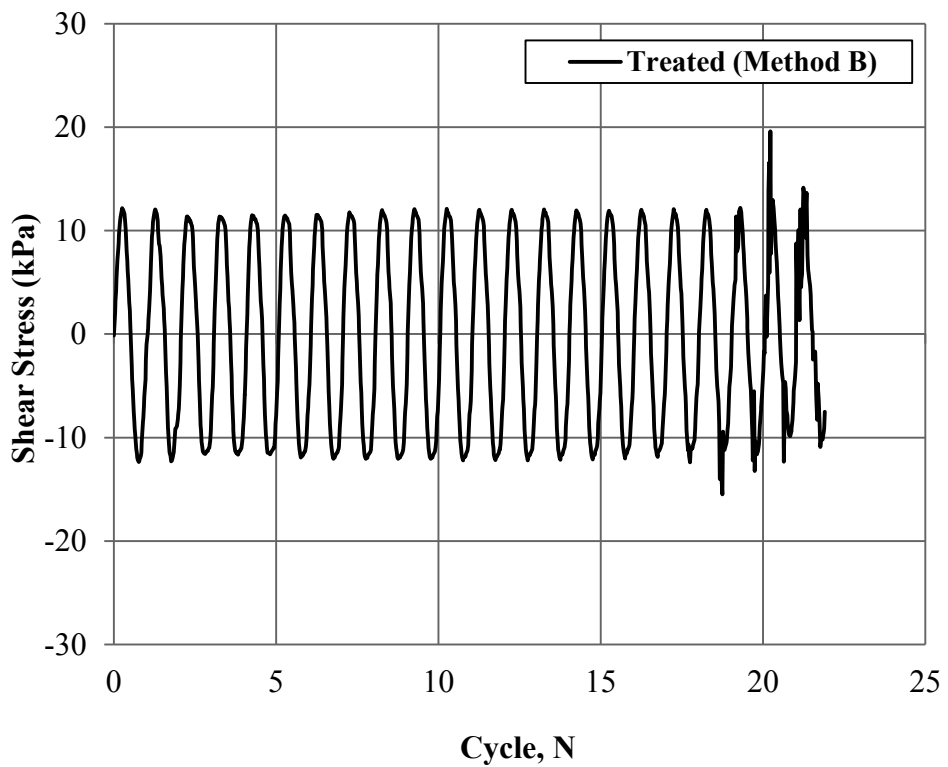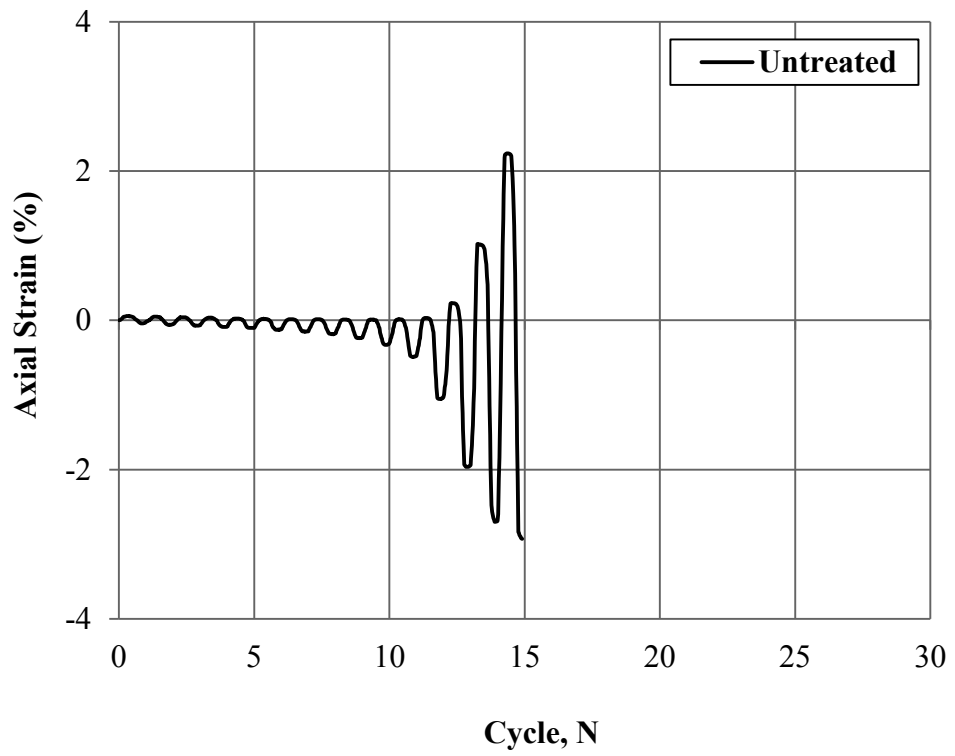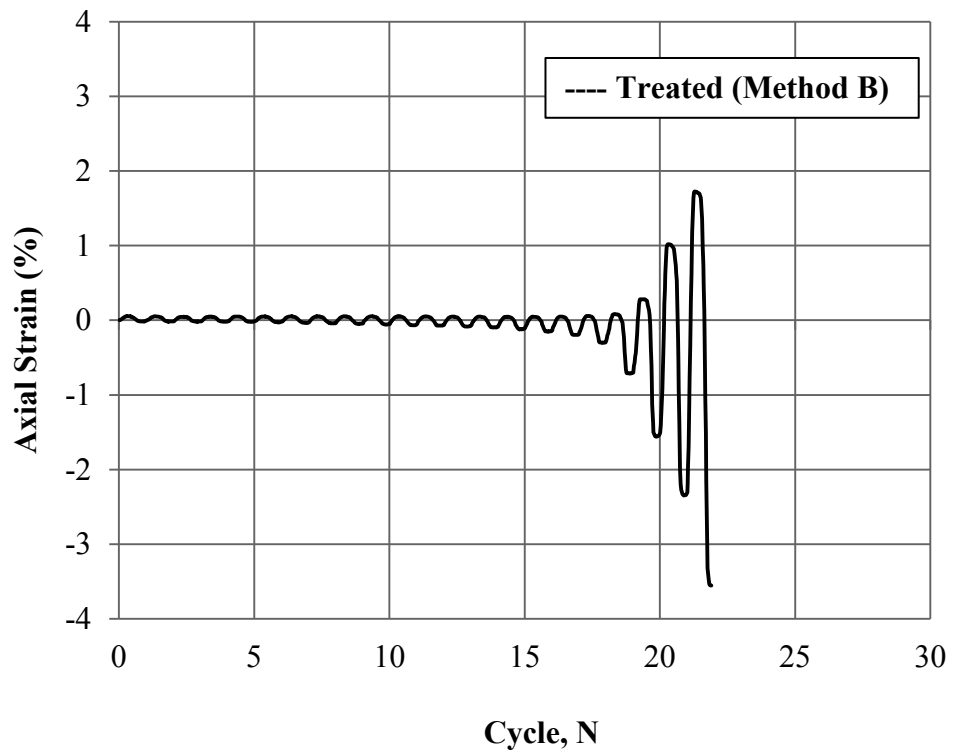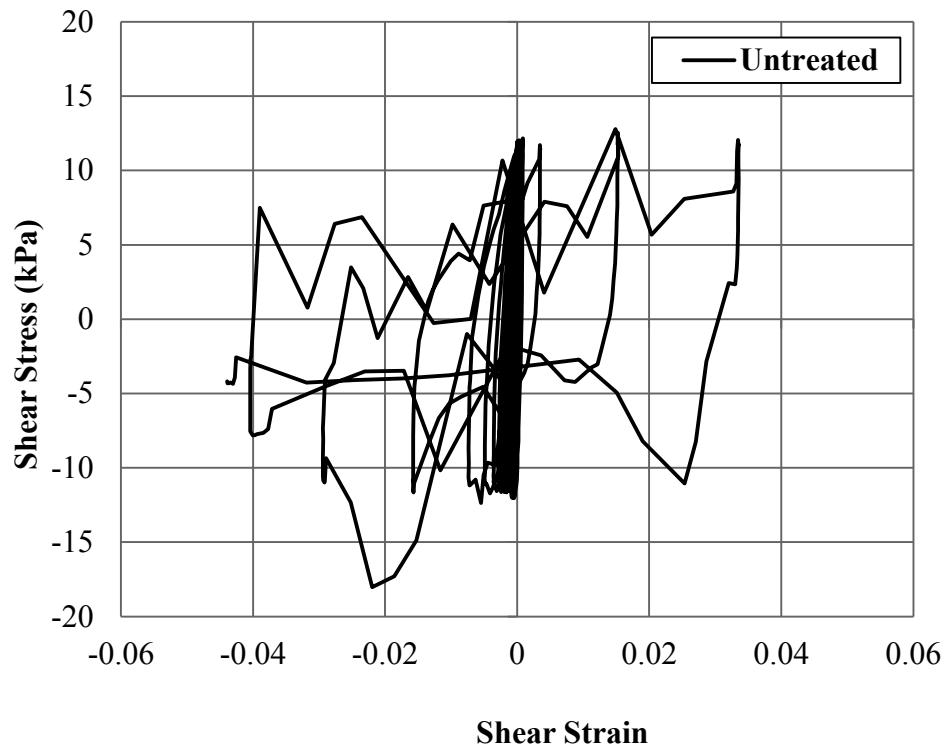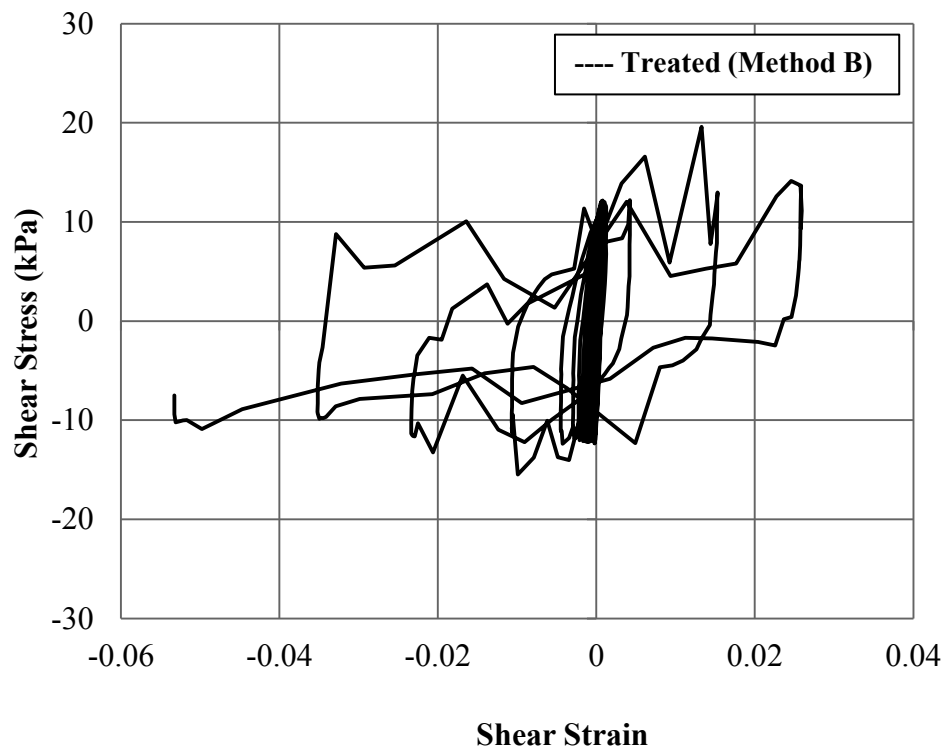
Figure 4.14: Modulus Ratio vs. Cycle Plots for CSR: 0.20

Figure 4.15: Peak to Peak Axial Strain vs. Cycle Plots for CSR: 0.20
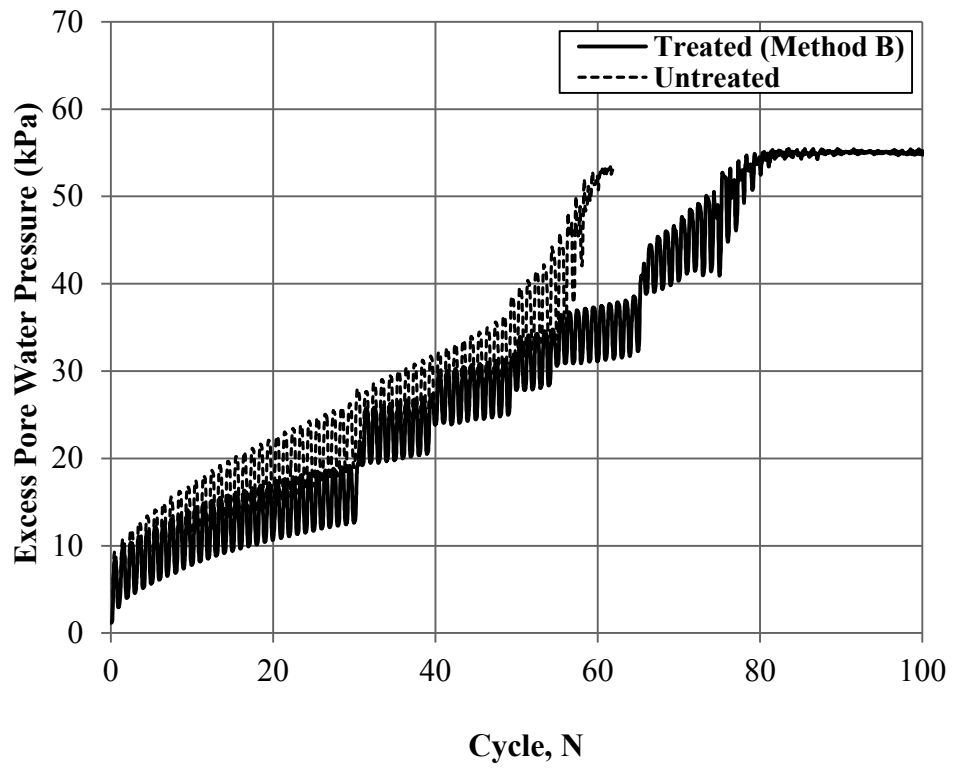
Figure 4.16: Excess Pore Water Pressure vs. Cycle Plots for CSR: 0.20
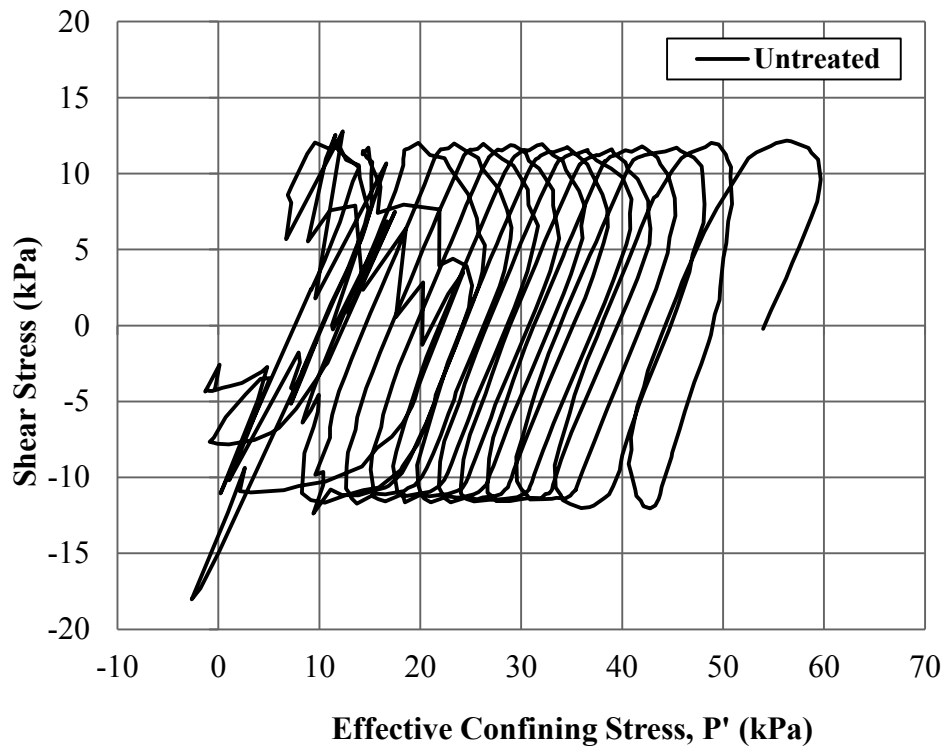
(a) Untreated

(b) Treated (Method B)

Figure 4.17: Shear Stress vs. Effective Confining Stress Plot for CSR: 0.25

(a) Untreated

(b) Treated (Method B)

Figure 4.18: Shear Strain vs. Cycle Plot for CSR: 0.25

(a) Untreated

(b) Treated (Method B)

Figure 4.19: Axial Strain vs. Cycle Plot for CSR: 0.25

(a) Untreated

(b) Treated (Method B)

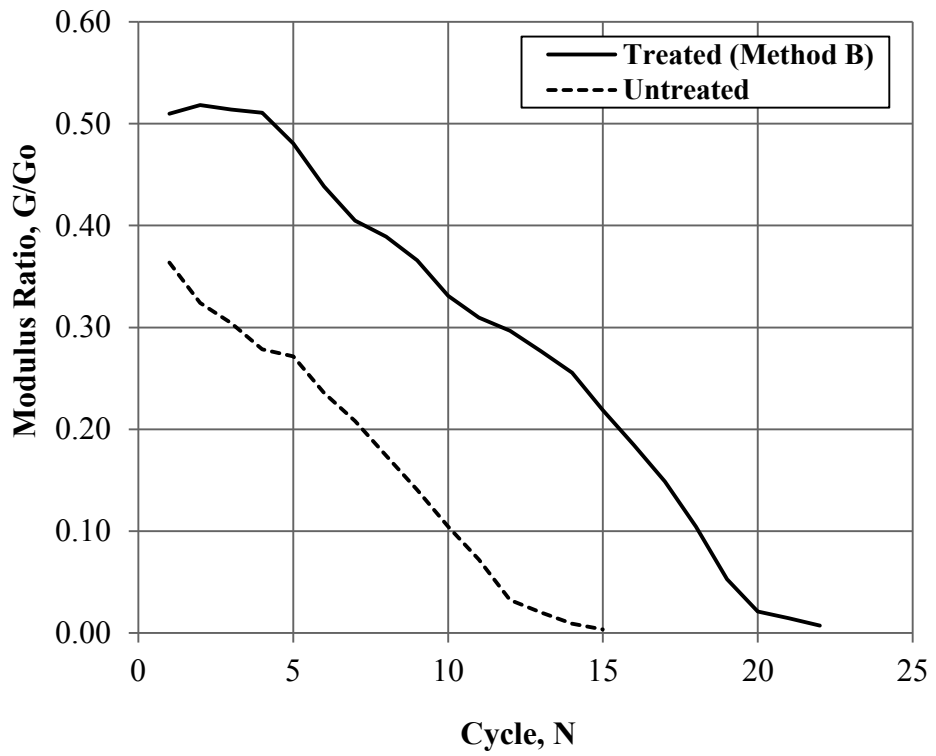**Figure 4.20:** Shear Strain vs. Shear Stress Plot for CSR: 0.25

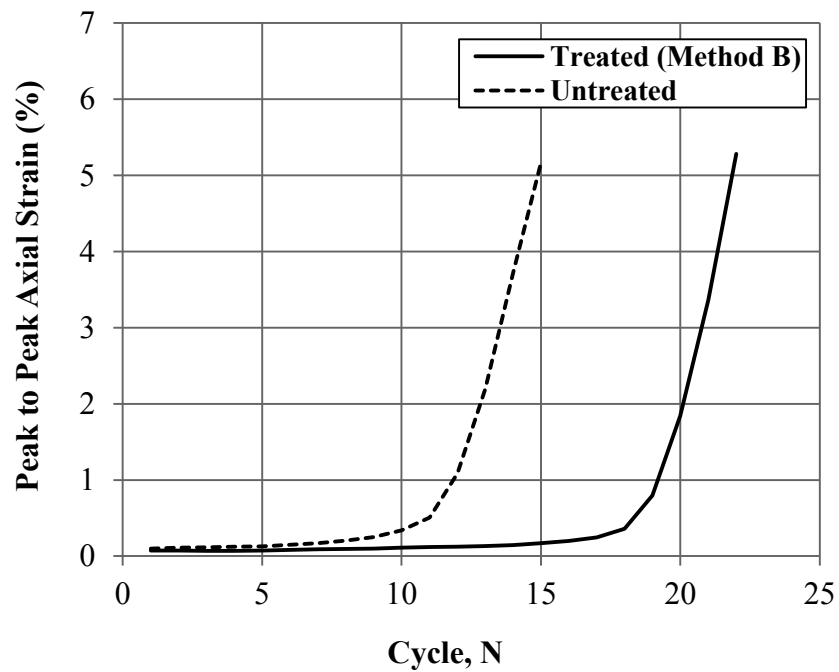**Figure 4.21:** Modulus Ratio vs. Cycle Plots for CSR: 0.25

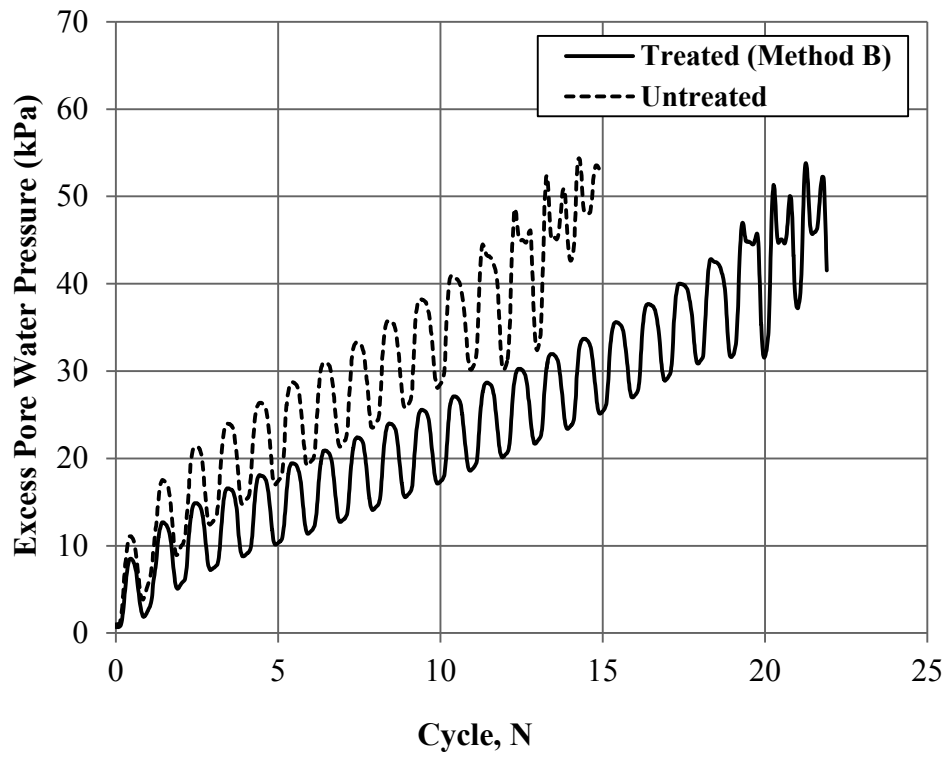**Figure 4.22:** Peak to Peak Axial Strain vs. Cycle Plots for CSR: 0.25
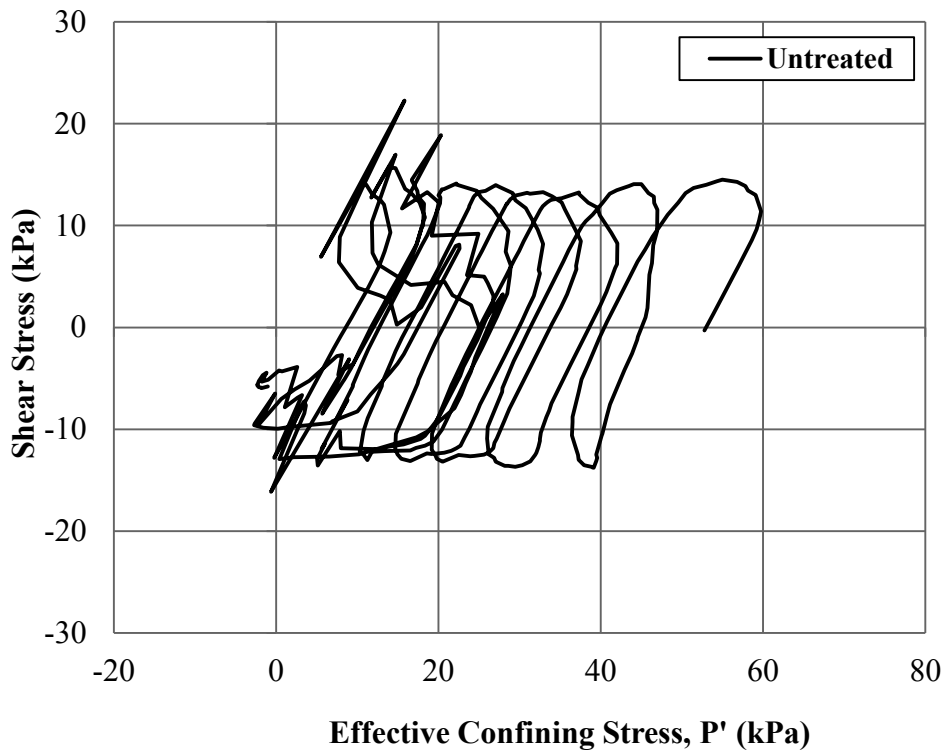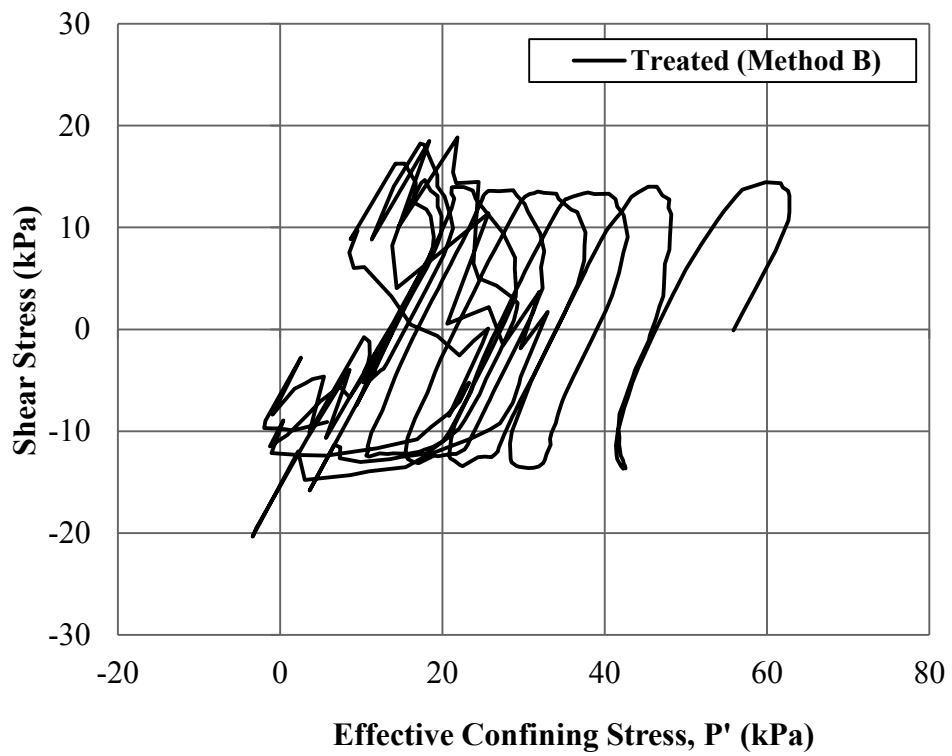
Figure 4.23: Excess Pore Water Pressure vs. Cycle Plots for CSR: 0.25
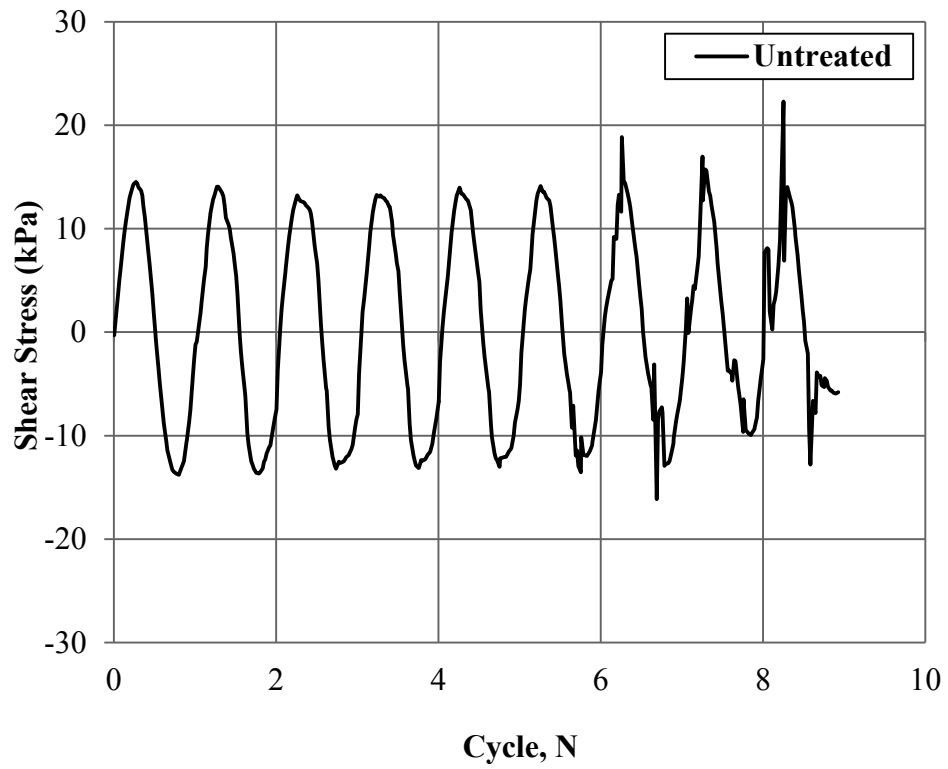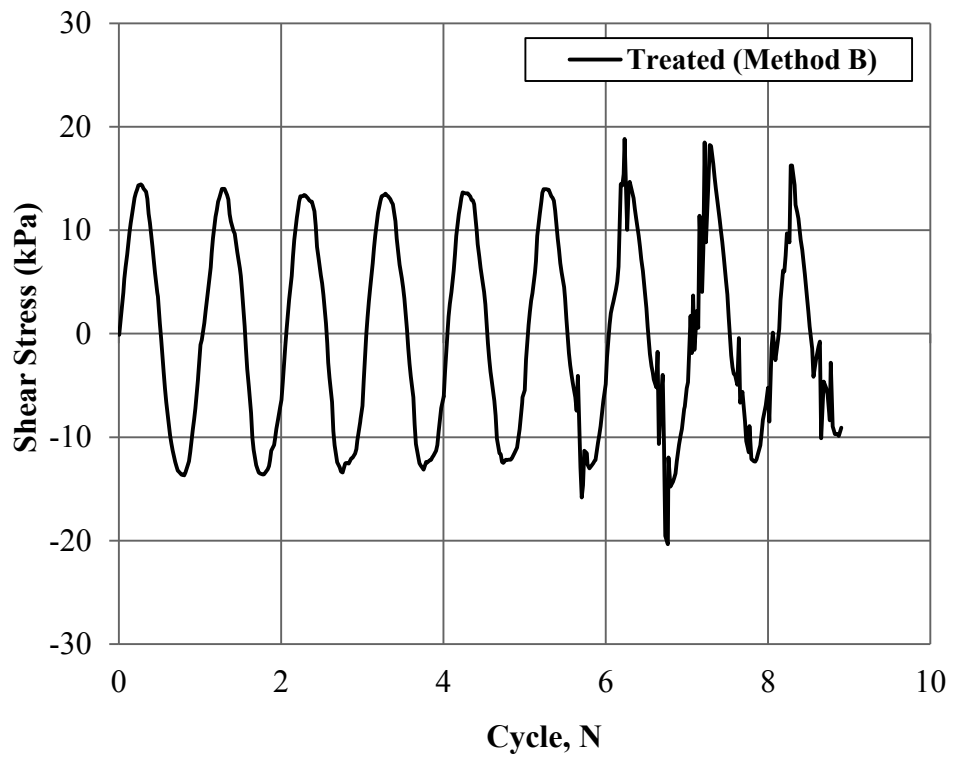
(a) Untreated

(b) Treated (Method B)

Figure 4.24: Shear Stress vs. Effective Confining Stress Plot for CSR: 0.30

(a) Untreated

(b) Treated (Method B)

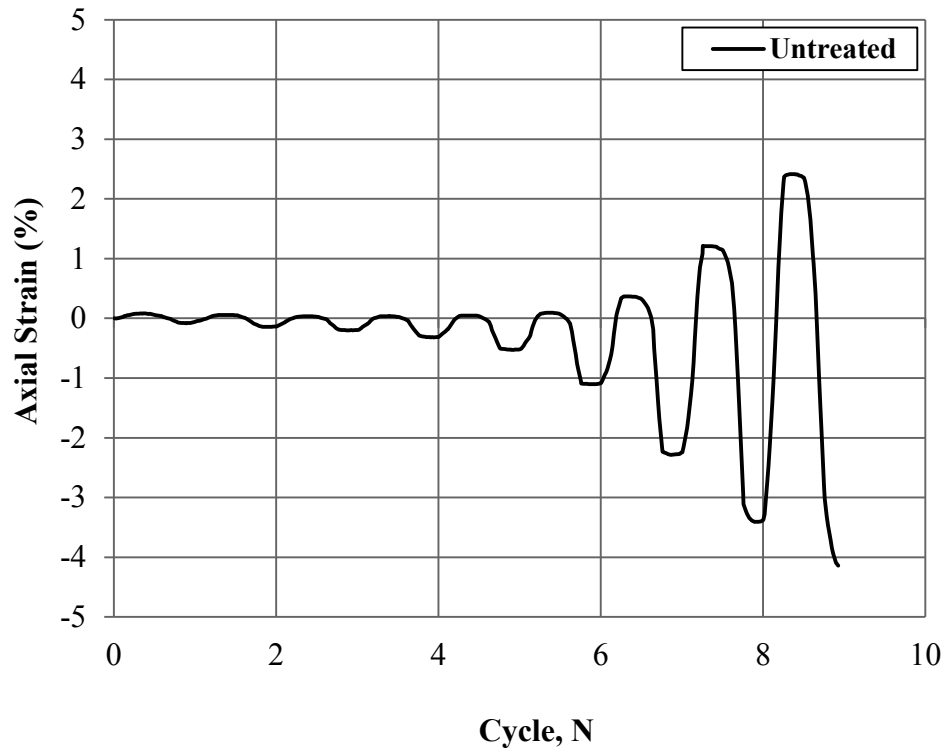**Figure 4.25:** Shear Stress vs. Cycle Plot for CSR: 0.30
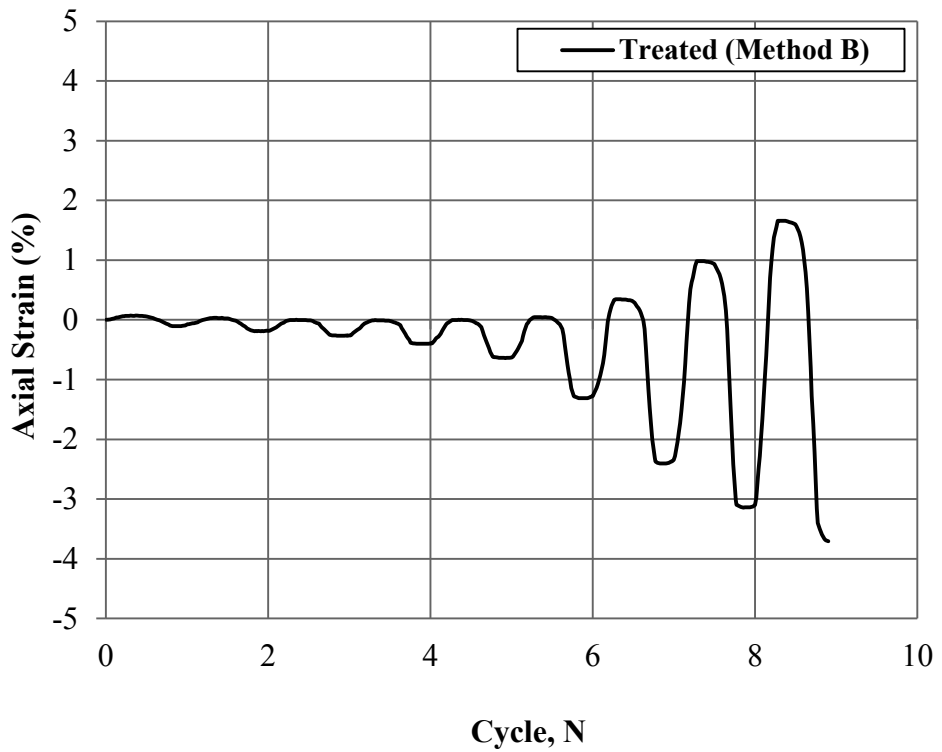
(a) Untreated

(b) Treated (Method B)

Figure 4.26: Axial Strain vs. Cycle Plot for CSR: 0.30
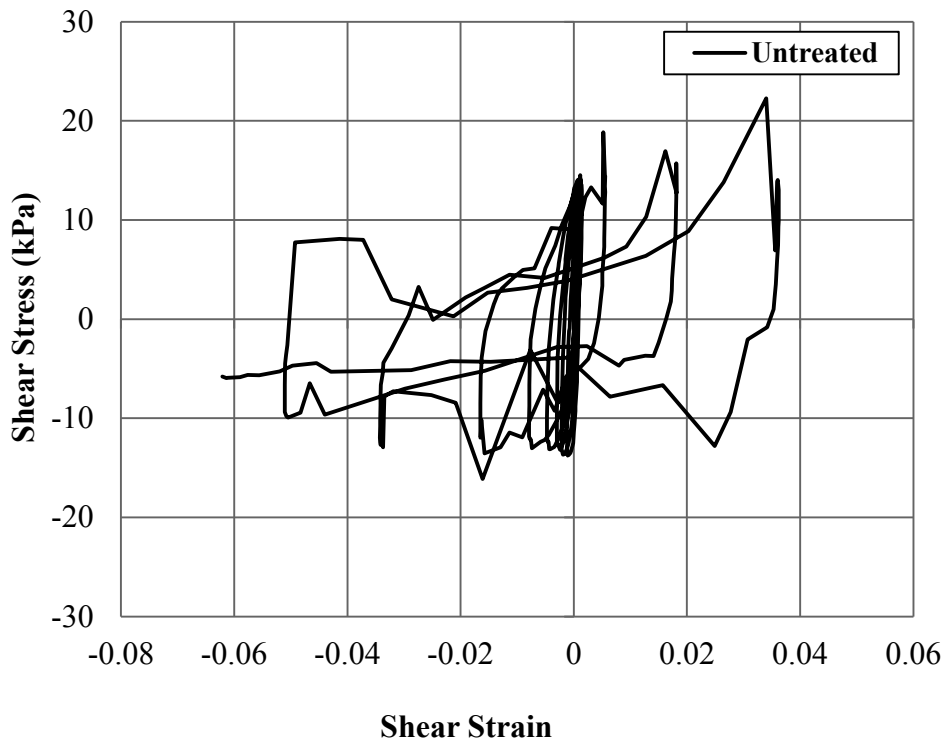
(a) Untreated
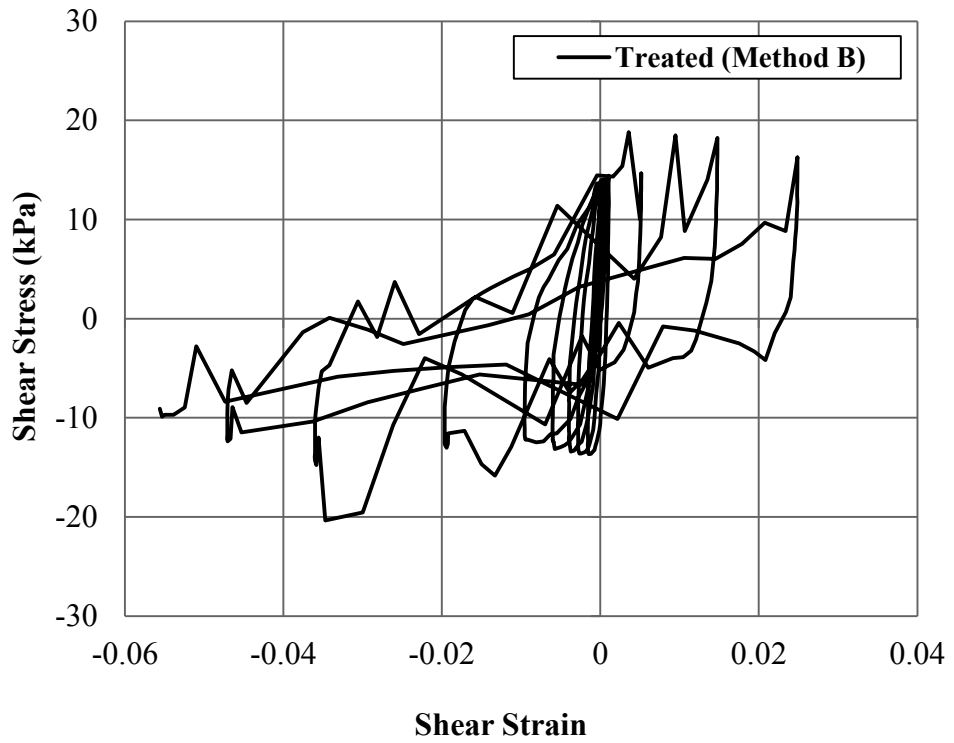
(b) Treated (Method B)

Figure 4.27: Shear Stress vs. Shear Strain Plot for CSR: 0.30
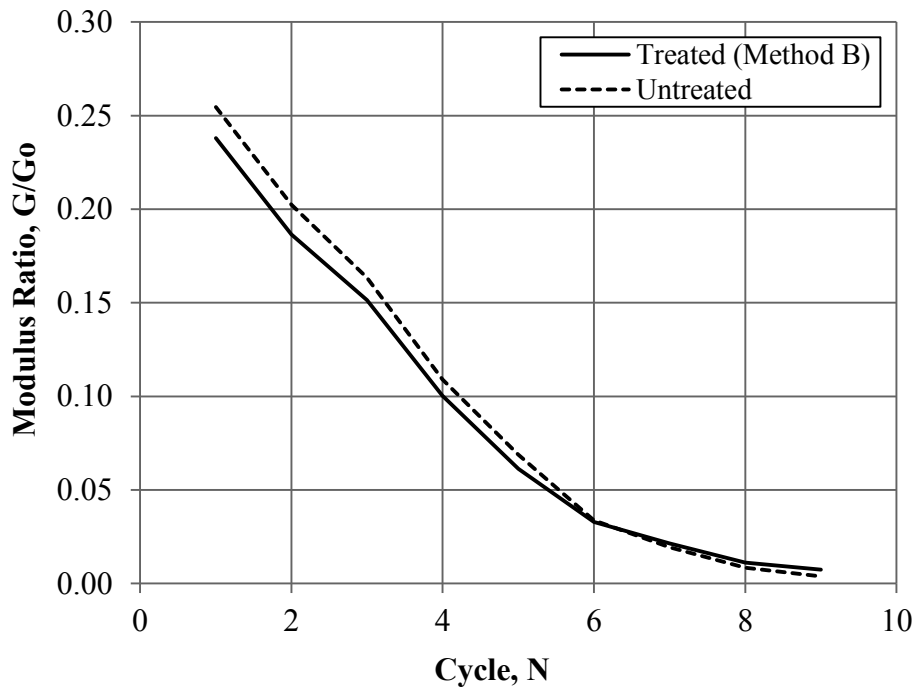
Figure 4.28: Modulus Ratio vs. Cycle Plots for CSR: 0.25
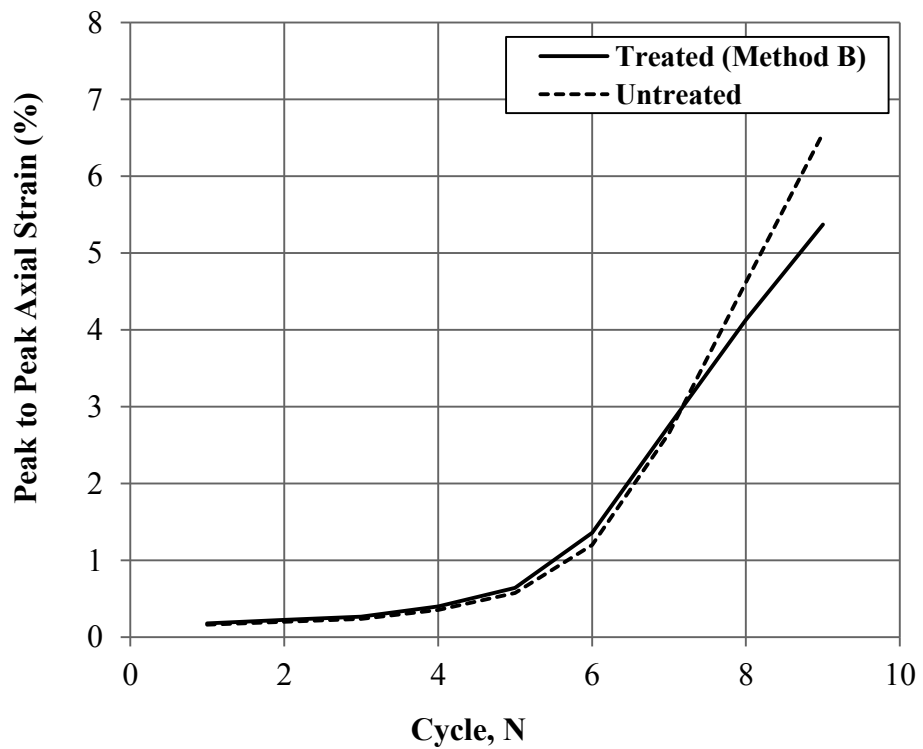
Figure 4.29: Peak to Peak Axial Strain vs. Cycle Plots for CSR: 0.30

Figure 4.30: Excess Pore Water Pressure vs. Cycle Plots for CSR: 0.30

Figure 4.31: Cyclic Stress Ratio vs. Number of Cycles Plots for Liquefaction to be occurred

4.8 EXPERIMENTAL RESULTS OF CALCITE PRECIPITATION

1 gm of oven dried sample from the specimens of each of method A and method B was used with 5 ml 5M HCl as discussed in 3.6.7. The average pressure gauge readings for the samples of method A and method B were found to be 112 mbar and 48 mbar respectively. The average oven dried sample used to prepare a test specimen of 142 mm height and 71 mm diameter was 906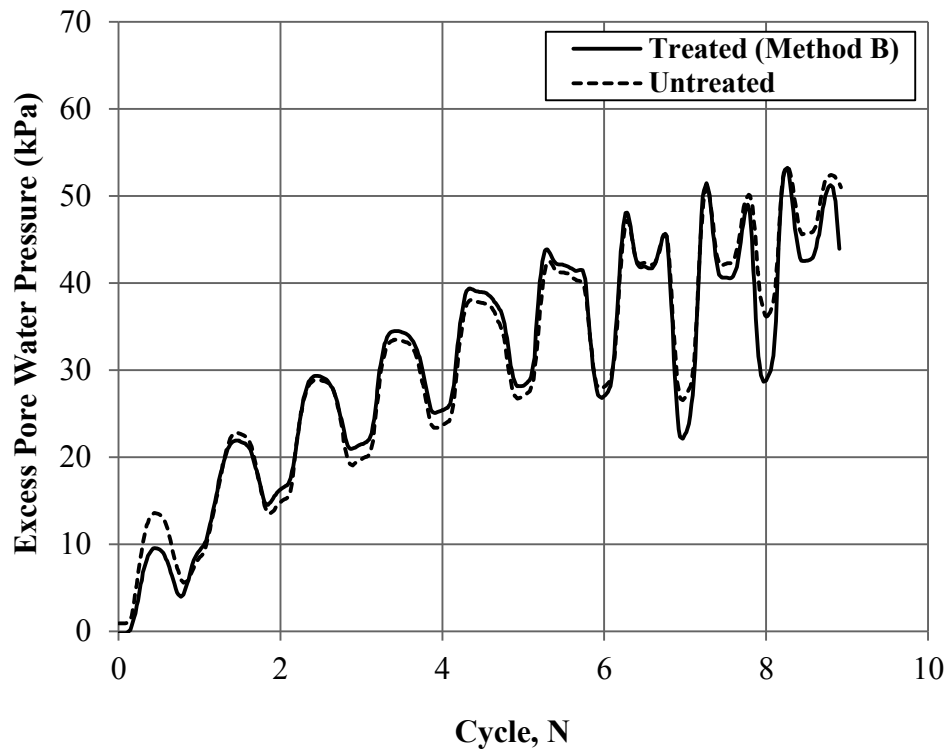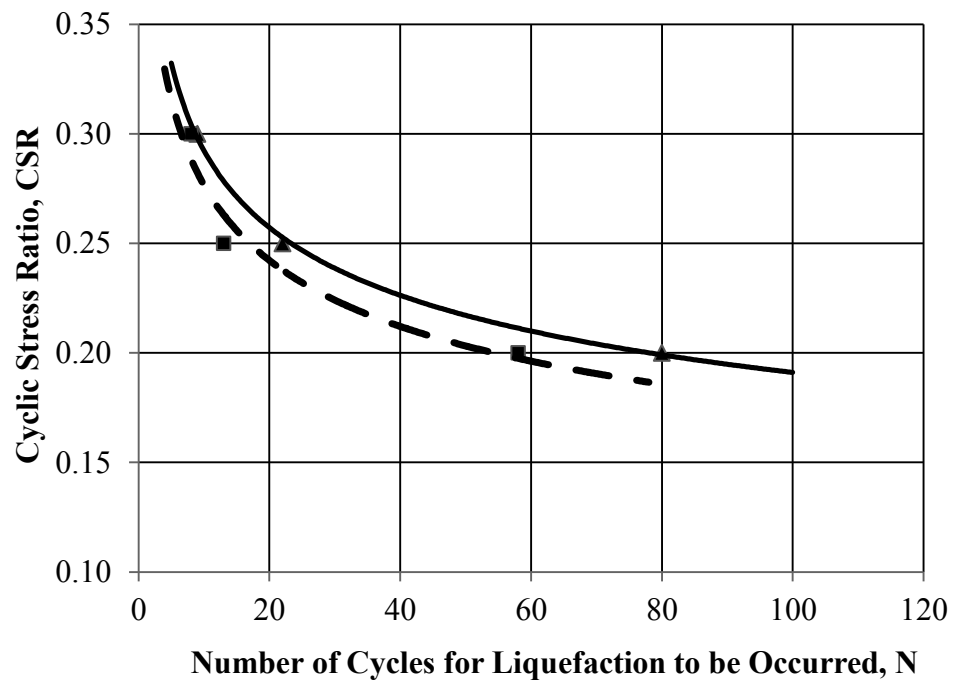 gm. According to equation of Figure 3.30, a pressure gauge reading of 112 mbar indicates 0.02828 gm of calcite in 1 gm of oven dried soil. So for 906 gm of oven dried soil it should be 25.62 gm which indicates about 45.57 kg $CaCO_3/m^3$ (bulk) of soil. From this, the efficiency of the treatment method A was found to be 31.87%. Again, according to equation of Figure 3.30, a pressure gauge reading of 48 mbar indicates 0.00901 gm of calcite in 1 gm of oven dried soil. So for 906 gm of oven dried soil it should be 8.16 gm which indicates about 14.5 kg $CaCO_3/m^3$ (bulk) of soil. From this, the efficiency of the treatment method B was found to be 41.43%. It is observed that the treatment method B is more efficient than method A because there is no chance of loss of precipitated calcite as occurred in method A. However, for both methods the efficiencies were less than the desired results. This might occur due to insufficient activity of the bacteria used. The bacteria used for the study might not the most rapidly urease positive.

4.9 MICROSCOPIC EXAMINATION RESULTS OF MICROBIAL CALCITE PRECIPITATION

Previous investigators have investigated the formation of calcium carbonate ($CaCO_3$) cementation using the scanning electron microscope (SEM). Their results have provided evidence that any increases in the strength of the soil were primarily governed by the formation of cementing products as shown in Figure 4.32. In a similar fashion in this study, after testing, bio-treated specimen was examined using Optical Microscope. Associated photo of interest is shown in Figure 4.33. Figure 4.33 confirmed the formation of calcite around the sand particles. The image was taken at a magnification of 200X.
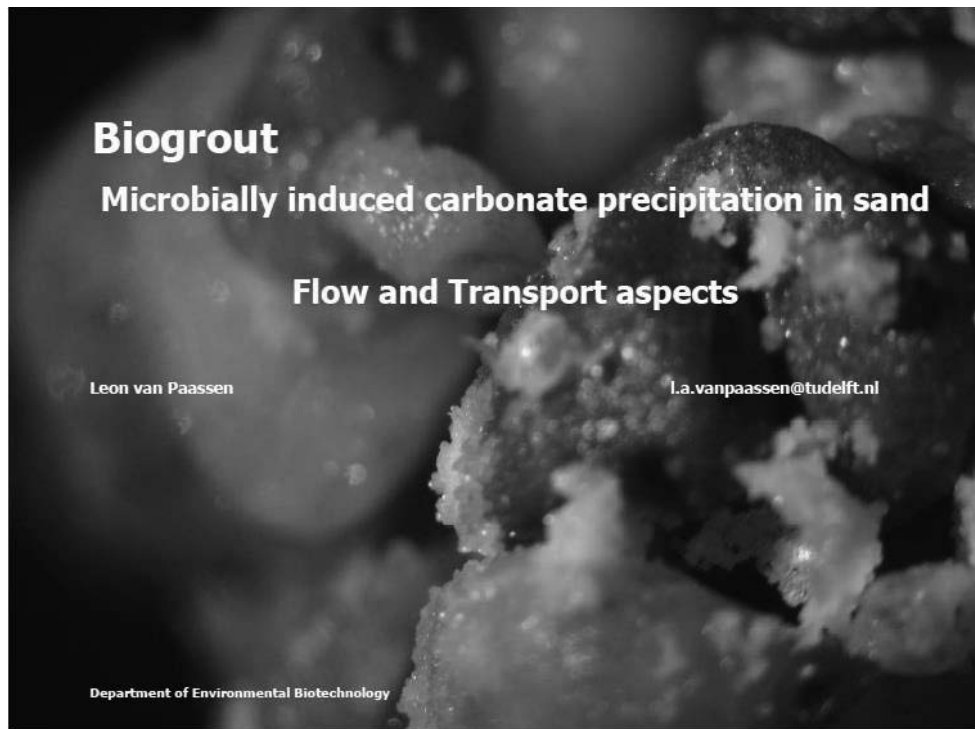
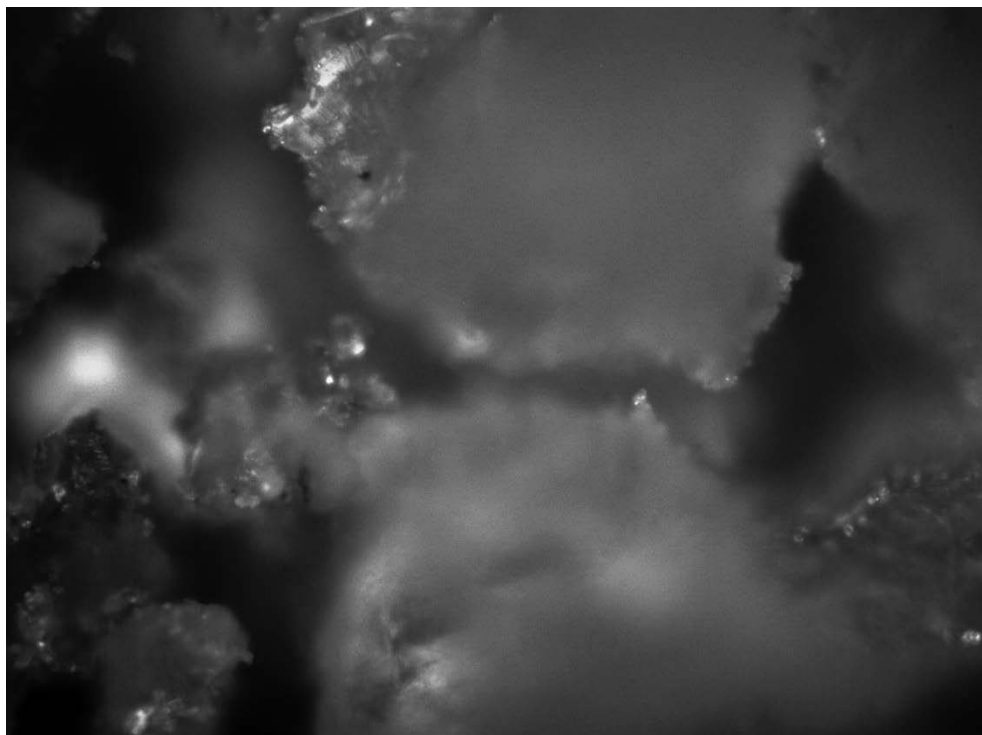**Figure 4.32:** CaCO$_3$ Formation around the Sand Grains (SEM)

Figure 4.33: CaCO$_3$ Formation Around the Sand Grains (Optical Microscope)

CHAPTER 5
CONCLUSION

5.1 GENERAL

To assess the effectiveness of microbial induced calcite precipitation in mitigating seismic liquefaction, urease positive bacteria was isolated from the environment and cultured successfully. Test specimens of 71 mm in diameter and 142 mm in height were treated with the bacteria along with nutrient solutions. Precipitation of calcite was monitored by measuring some chemical parameters, such as pH, conductivity and Ca^{2+} ion concentration in the solution. Improved behavior of the treated specimens was confirmed by needle penetration test, unconfined compression test and cyclic triaxial test. Needle penetration test and unconfined compression test showed improved strength of the bio-treated specimen. Cyclic triaxial test showed increase of CRR of the treated specimen compared to untreated specimen. Optical microscopic image of the treated sample confirmed presence of calcite around the sand grains.

5.2 CONCLUSIONS

The study mainly focused on the potential of bio-treated specimen in mitigating seismic liquefaction. The findings from the study are given below. From the first point described, it is confirmed that MICP may be a useful tool for improving the soil in mitigating seismic liquefaction.

(i)   Axial strain development, modulus ratio reduction and excess pore water pressure development were slower for bio-treated specimen compared to untreated specimen in case of CSR 0.20 and 0.25. The behavior of bio-treated and untreated specimen was same in case of CSR 0.30 because of breakage of the cementing bond due to higher cyclic deviator stress. However, the cycles required for 5% DA axial strain or zero effective stress for bio-treated specimen were 80, 22 and 9 which were greater than 58, 13 and 8 of untreated specimen. The cyclic resistance ratio of bio-treated specimen was 0.261 which was greater than 0.243 of untreated specimen indicating that the bio-treated specimen was more resistant to liquefaction.

(ii)   Urease positive bacteria was successfully isolated from the soil and cultured properly for using in the bio-treatment technique.

(iii)   Urease activity of the isolated bacteria was confirmed with the increase of pH and conductivity of the nutrient solution, decrease of concentration of calcium ion of the nutrient solution and whitish deposit formation into the beaker, inside the wall of the mold and into the soil specimen.

(iv)   Average 2.5 times increase of load for a particular penetration in needle penetration test was found.

(v)   The compressive strength of bio-treated specimen of method B was found to be 56% higher than the untreated specimen.

(vi)   The calcite precipitation of method A was 45.57 kg/m^3 of soil. The efficiency was 31.87%. Again, the calcite precipitation of method B was found to be 14.50 kg/m^3 of soil. The efficiency of method B was 41.43%.

(vii)   Optical microscope image at a magnification of 200X of bio-treated specimen clearly showed the formation of calcite around the sand grain.

5.3   RECOMMENDATION FOR FURTHER STUDY

This experimental study has been done first time in Bangladesh. It was a challenging task to end the study with successful findings. Isolating and culturing bacteria were really difficult works. However, followings are the recommendation for further study in the context of this research:

a.   Other reliable sources of urease positive bacteria may be studied.

b.   Better experimental setup may be developed to treat the specimens by method A so that those can be easily extruded from molds after completion of the treatment.

c.   Study on the reasons of low efficiency of treatment methods used in this research may be done.

d.   Low cost sources of urea may be studied.

e.   Better process of moist tamping method with bacterial nutrients may be studied so that high amount of nutrients can be mixed with the specimen.

f.   SEM image of the bio-treated sample may be taken.

g.   Other calcite confirmation test like XRD technique may be done.

REFERENCES

[1] DeJong, J. T., Fritzges, M. B. and Nüsslein, K., Microbially Induced Cementation to Control Sand Response to Undrained Shear. Journal of Geotechnical and Geoenvironmental Engineering, 10.1061/(ASCE)1090-0241(2006)132:11(1381), 2006.

[2] Ozdogan, A., A Study on the Triaxial Shear Behavior and Microstructure of Biologically Treated Sand Specimens. M.Sc. Thesis, The University of Delaware, 2010.

[3] Whiffin, V. S., Microbial $CaCO_3$ Precipitation for the Production of Biocement. PhD Thesis, School of Biological sciences & Biotechnology, Murdoch University, Western Australia, 2004.

[4] Kramer, S. L., Geotechnical Earthquake Engineering. Prentice-Hall International Series in Civil Engineering and Engineering Mechanics, Upper Saddle River, New Jersey 07458, Prentice Hall, 1996.

[5] Naeim, F., The Seismic Design Handbook. 2nd Edition, Los Angeles, California, 2000.

[6] Dejong, J. T., Mortensen, B. M., Martinez, B. C., & Nelson, D. C., Biomediated soil improvement. Ecological Engineering 36(2), 197-210, 2010.

[7] Kamon, M. and Bergado, D. T., Ground Improvement Techniques. Proc., 9th Asian Regional Conf. on Soil Mech. and Found., 2, 526-546, 1992.

[8] Karol, R. H., Chemical Grouting and Soil Stabilization. New York: Marcel Dekker, 558, 2003.

[9] Bergado, D. T., Anderson, L. R., Miura, N. and Balasubramaniam, A. S., Soft Ground Improvement in Lowland and Other Environments. American Society of Civil Engineers, ASCE, 1996.

[10] Consoli, N. C., Rotta, G. V., & Prietto, P. M., The Influence of Curing under Stress on the Triaxial Responce of Cemented soils. Geotechnique 50, 99-105, 2000.

[11] Ismail, M. A., Joer, H. A., Sim, W. H., & Randolph, M. F., Effect of Cement Type on Shear Behavior of Cemented Calcreous Soil. Journal of Geotechnical and Geoenvironmental, Vol.128, 520-529, 2002.

[12] Rotta, G. V., Consoli, N. C., Prietto, P. M., Coop, M. R., & Graham, J., Isotropic Yielding in an Artificially Cemented Soil Cured under Stress. Geotechnique, Vol 53 , 493-501, 2003.

[13] Coop, M. R. and Atkinson, J. H., The Mechanics of Cemented Carbonate Sands. Géotechnique, Vol.43, 53-67, 1993.

[14] Sangrey, D. A., Naturally Cemented Sensitive Soils. Geotechnique, Vol. 22 , 139-152, 1972.

[15] Mitchell, J. K., The Properties of Cement-Stabilized Soils. Residential Workshop on Materials and Methods for Low Cost Road, Rail and Reclamation Works, Australia, 365 404, 1975.

[16] Ismail, M. A., Joer, H. A., Randolph, M. F., & Meritt, A., Cementation of Porous Materials using Calcite. Geotechnique, Vol.52, 313-324, 2001.

[17] Wallace, K. B., Structural Behaviour of Residual Soils of the Continually Wet Highlands of Papua New Guinea. Geotechnique , 23(2), 203-218, 1973.

[18] Sharma, S. S. and Fahey, M., Deformation Characteristics of Two Cemented Calcareous Soils. Canadian Geotechnical Journal 41, 1139-1151, 2004.

[19] Alexander, M., Introduction to Soil Microbiology. 2nd Edition, New York: Cornell University, 1977.

[20] Fritzges, M. B., Biologically Induced Improvement of the Response of Sands. Master's thesis, University of Massachusetts, Amherst, MA, 111, 2005.

[21] Bang, S. S., Galinat, J. K., & Ramakrishnan, V., Calcite Precipitation Induced by Polyurethane-Immobilized Bacillus Pasteurii. Enzyme and Microbial Technology,28 , 404-409, 2001.

[22] Bang, S. S., & Pazirandeh, M., Physical Properties and Heavy Metal Uptake of Encapsulated Eshcerichia Coli Expressing a Metal Binding Gene. J Microencapsulation , 16, 489-99, 1999.

[23] Sarda, D., Choonia, S., Sarode, D. D., & Lele, S. S., Biocalcification by Bacillus Pasteurii Urease: a Novel Application. Jeo Industrial Microbiol Biotechnol , 36, 1111-1115, 2009.

[24] Whiffin, V. S., Paassen, L. A., & Harkes, M. P., Microbial Carbonate Precipitation as a Soil Improvement Technique. Geomicrobiology Journal, 24:417-423, 2007.

[25] Gollapudi, U. K., Knutson, C. L., Bang, S. S., & Islam, M. R., A New Method for Controlling Leaching Through Permeable Channels. Chemosphere, Vol. 30, 695-705, 1995.

[26] Fischer, S. S., Galinat, J. K., & Bang, S. S., Microbiological precipitation of $CaCO_3$. Soil Biology and Biochemistry, 31, 1563-1571, 1999.

[27] Jonkers, H. M., Thijssen, A., Muyzer, G., Copuroglu, O., & Schlangen, E., Application of Bacteria as Self-Healing Agent for the Development of Sustainable Concrete. Ecological Engineering, doi:10.1016/j.ecoleng.2008.12.036, 3-6, 2009.

[28] Canakci, H. and Cabalar, A. F., Improvement of a Sand Matrice Using Biopolymer-Forming Bacteria. Proc. of International Conference on New Developments in Soil Mechanics and Geotechnical Engineering, Lefkose, N. Cyprus, 2003.

[29] Bianco, A. and Madonia, G., B.U.L.M. Technique for Increase of the Bearing Capacity in the Pavement Layers Subjected to Biological Treatment. University of Palermo, 4th International Siiv Congress, Palermo (Italy),16, 2007.

[30] Chunxiang, Q., Jianyun, W., Ruixing, W., & Liang, C., Corrosion Protection of Cement-Based Building Materials by Surface Deposition of $CaCO_3$ by Bacillus Pasteurii. Materials Science and Engineering, 29, 1273-1280, 2009.

[31] DeJong, J., Bio-Mediated Ground Improvement: Part of the Future?, Presentation, 2010.

[32] Ishihara, K., Soil Behaviour in Earthquake Geotechnics. Clarendon Press, Oxford, 1996.