CAIT-UTC-032

Evaluation of Biotechnologies for Flexible Pavement Applications

Final Report
December 2016

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State of New Jersey
Department of Transportation
And
U.S. Department of Transportation
Federal Highway Administration

Disclaimer Statement

TECHNICAL REPORT STANDARD TITLE PAGE

1. Report No.	2. Government Accession No.	3. Recipient's Catalog No.
CAIT-UTC-032		

4. Title and Subtitle	5. Report Date
Evaluation of Biotechnologies for Flexible Pavement Applications	December 2016
	6. Performing Organization Code CAIT/Rutgers

7. Author(s)	8. Performing Organization Report No.
Christopher Ericson, Thomas Bennert, Ph.D., Vivek Tandon, Nii Attoh-Okine, Eileen Sheehy	CAIT-UTC-032

9. Performing Organization, Name and Address	10. Work Unit No.
University of Delaware, Newark, DE 19716 Center for Transportation Infrastructure System, The University of Texas at El Paso, 500 W. University Avenue, El Paso, TX 79968-0516	11. Contract or Grant No. DTRT12-G-UTC16

12. Sponsoring Agency Name and Address	13. Type of Report and Period Covered
Center for Advanced Infrastructure and Transportation Rutgers, The State University of New Jersey 100 Brett Road Piscataway, NJ 08854	Final Report 6/01/13 - 12/1/2016
	14. Sponsoring Agency Code

15. Supplementary Notes

U.S Department of Transportation/Research and Innovative Technology Administration
1200 New Jersey Avenue, SE
Washington, DC 20590-0001

16. Abstract

With solid data from environmental scientists supporting climate change there has been a strong push in the industry to look for alternative "green" or environmentally friendly methods to keep building and maintaining our infrastructure. This collaborative report looks into microbial processes to stabilize soil subgrade for roadways and asphalt like liquids extracted from algae. Not only are new bio technologies looked into for building but also microbial deterioration is looked into and needs to be addressed when evaluating the life cycle of a capital project.

17. Key Words	18 Distributional Statement
Acidulated Soy Soapstock, Lipids, Microalgae, Asphalt Mixtures, Binder, Bio Additives, Cement, Calcium Carbonate	

19. Security Classification	20. Security Classification (of this page)	21. No. of Pages	22. Price
Unclassified	Unclassified	133	

Form DOT F 1700.7 (8-09)

Acknowledgments

The research report herein was performed under CAIT-UTC 032 by the Center for Advanced Infrastructure and Transportation (CAIT) at Rutgers University, University of Texas at El Paso, and University of Delaware.

Dr. Thomas Bennert, Program Director, Pavement Resource Program (PRP) at CAIT, was the principal investigator with the work being done under his general supervision.

CAIT would like to extend their gratitude to all the individuals who worked on the project including Wen Zhang, Ph.D. from NJIT for supplying the microalgae. Special thanks are extended to Dorothy Libring, undergraduate researcher and Christopher Ericson, senior laboratory engineer, for their work throughout the project.

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CHAPTER 1

1.1 INTRODUCTION

As of recent there has been a strong push for our society as a whole to be more sustainable. However, our society today has become so dependent on reliable roads and infrastructure that a failure causes economical stress in the surrounding communities. As a result of making sure to reduce failures, the designers and builders of our infrastructure have more or less left the environmental impacts associated with the projects to the way side. This paper looks into some viable organic/renewable technologies to help our infrastructure while helping reduce the impact on our environment.

This collaborate research includes research from Rutgers University: Center for Advanced Infrastructure and Transportation, Center for Transportation Infrastructure System
The University of Texas at El Paso, and the University of Delaware. This paper is divided into chapters which are the papers submitted from all the universities involved with the research.

1.2 RESEARCH NEED STATEMENT

The use of biotechnology has many benefits in construction applications, in this case, the construction and performance of flexible pavements. From a materials standpoint. the potential use of biomaterials can reduce the dependency on petroleum products required for asphalt materials, as well as helping to reduce greenhouse emissions during production and construction. If adaptable, biomaterials may also be able to help increase the general life of the pavement while reducing the cost of construction. Biotechnologies may also be able to help in the

stabilization of subgrade soils prior to constructing roadways over top of them. Researchers have found that the use of microbial activity allows for a level of stabilization in liquefiable soils. Including the use of biomaterials to help stabilize these problematic soils is a cost effective and environmentally sensitive solution. Although biomaterials has shown to help improve pavement and soil performance, there is also evidence to show that some pavement biodeterioration does occur and may affect the general roughness of the pavement. To conclude the research study, an assessment of paved road deterioration due to biodeterioration and how it influences roughness progression will also be conducted.

CHAPTER 2

Report # provided by Rutgers

Soil Stabilization Using Microbial Activity: A Feasibility Study

FINAL REPORT

November 2014

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U.S. Department of Transportation

Federal Highway Administration

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1. Report No.	2. Government Accession No.	3. Recipient's Catalog No.
Report # provided by Rutgers		

TECHNICAL REPORT STANDARD TITLE PAGE

4. Title and Subtitle	5. Report Date
Soil Stabilization Using Microbial Activity: A Feasibility Study	November 2014
	6. Performing Organization Code CAIT/CTIS (UTEP)
7. Author(s) Sundeep Inti, Megha Sharma, and Vivek Tandon	8. Performing Organization Report No. Report # provided by Rutgers
9. Performing Organization Name and Address Center for Transportation Infrastructure System The University of Texas at El Paso 500 W. University Avenue El Paso, Texas 79968-0516	10. Work Unit No. 11. Contract or Grant No. 4648
12. Sponsoring Agency Name and Address Center for Advanced Infrastructure and Transportation Rutgers, The State University of New Jersey 100 Brett Road Piscataway, NJ 08854	13. Type of Report and Period Covered Final Report 6/1/13 – 10/31/14 14. Sponsoring Agency Code
15. Supplementary Notes U.S. Department of Transportation/Research and Innovative Technology Administration 1200 New Jersey Avenue, SE Washington, DC 20590-0001	

16. Abstract

It is quite common that bearing capacity of the soil is enhanced through treatment to provide a stable supporting layer (subgrade) for construction. These treatments can be classified as soil modification or soil stabilization. The purpose of subgrade modification is to create a working platform for construction equipment while the purpose of subgrade stabilization is to enhance the strength of the subgrade. The additional benefit of stabilization is that the increased strength can be used in the design methodology for adjusting the thickness of base layer. Traditional methods of subgrade stabilization include chemical processes such as mixing with cement, fly ash, lime, lime byproducts, and blends of any one of these materials. In recent years, a new method of stabilization has been proposed and is commonly known as microbial geo-technology, which uses bacteria to stabilize soils and is the focus of this study. The preliminary evaluation suggests that bacteria-treatment is a viable alternative as microbial precipitation enhanced strength and reduced permanent deformation due to traffic loading. The influence of bacteria-treatment was most pronounced for sandy soils and least for clayey soil. In addition, the micro level evaluation verified deposition of calcite on soils. However, further research is needed before it can be implemented in the field.

17. Key Words	18. Distribution Statement
Bio-cementation, Mutated Bacteria, Calcite, Precipitation, and Strength	

19. Security Classification (of this report)	20. Security Classification (of this page)	21. No. of Pages	22. Price
Unclassified	Unclassified	53	

Form DOT F 1700.7 (8-69)

Acknowledgments

The authors like to gratefully acknowledge financial support for this project received from the Center for Advanced Infrastructure and Transportation of Rutgers, The State University of New Jersey. In addition, authors gratefully acknowledge both financial and laboratory support for this project from the College of Engineering, Office of Sponsored Research, the Civil Engineering Department, Biomolecule Analysis Core Facility, the Center for Transportation Infrastructure Systems, and the Nano Material Integration Laboratory at The University of Texas at El Paso.

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1. INTRODUCTION

1.1 Problem Statement

State highway agencies often construct highways on lower quality subgrade (in-situ soil) materials. To meet the bearing capacity requirements, the lower quality material is typically modified either through mechanical modification or through chemical stabilization. In recent years, researchers have proposed a sustainable approach of using bacteria to stabilize soils. Dejong et al. (2010) proposed to take advantage of a natural soil bacterium (Bacillus pasteurii). This microbe causes calcite (calcium carbonate) to be deposited around sand, cementing them together. By injecting bacterial cultures, additional nutrients and oxygen, DeJong et al. found that they could turn loose, liquefiable sand into a solid cylinder. Similarly, Hamden et al. (2011) used Microbially induced calcium carbonate precipitation (MICP) to mitigate earthquake potential of liquefiable soils, enhancement of the bearing capacity of shallow foundations, control of groundwater flow, facilitation of excavation and tunneling in running and flowing sands, and reduction of erosion and scour potential, etc. Thus, MICP offers the promise of a sustainable, non-disruptive and energy efficient engineering solution to stabilize soils and is the focus of this study.

1.2 Research Objective, Approach and Scope

The main objective of this study is to evaluate feasibility of enhancing quality of soils through MICP. To achieve objectives of this study, the following approach is proposed:

- Develop a methodology for bacterial growth for soil stabilization.
- Develop a methodology for mixing of bacteria in the soil.
- Identify presence of MICP using micro-level tests and evaluate influence of MICP on soil strength.

To achieve objectives of this study within time constrained, the study was performed with one bacteria type and three soil types.

2. REVIEW OF INFORMATION

State Highway Agencies (SHA) often construct on roadbeds that do not possess sufficient strength to support traffic loads imposed upon them during construction or the service life of the highway. It is quite common that bearing capacity of the soil is enhanced through treatment to provide a stable subgrade or a working platform for the construction of the pavement. These treatments can be classified as soil modification or soil stabilization. The purpose of subgrade modification is to create a working platform for construction equipment while the purpose of subgrade stabilization is to enhance the strength of the subgrade. The additional benefit of stabilization is that the increased strength can be used in the design methodology for adjusting base layer thickness. The methods of subgrade modification include physical processes such as soil densification, blends with granular material, use of reinforcements (Geogrids), undercutting and replacement, and stabilization includes chemical processes such as mixing with cement, fly ash, lime, lime byproducts, and blends of any one of these materials. Soil properties such as strength, compressibility, hydraulic conductivity, workability, swelling potential, and volume change tendencies may be altered by various soil modification or stabilization methods. In recent years, a new method of stabilization has been proposed and is commonly known as microbial geo-technology, which uses bacteria to stabilize soils.

2.1 Microbial Geo-Technology

The microbial geo-technology deals with enhancing the mechanical properties of geological materials through microbial activities. According to Volodymyr and Cheu (2008), the most suitable microorganisms are facultative anaerobic and micro aerophilic bacteria. Two notable applications of microbial geo-technology are bio-clogging and bio-cementation. Bio-clogging is the production of pore filling materials, through microbial means, such that the porosity and hydraulic conductivity of soil can be reduced. Bio-cementation is the generation of particle binding materials through microbial processes such that the shear strength of soil can be increased. Additionally, the bio-cementation can also lead to bio-clogging due to deposition of binding material.

2.1.1 Bio-Cementation

2.1.1.1 Introduction

Bio-cementation or bio-mineralization is a widespread complex phenomenon that binds materials through microbial activities to increase the strength and durability. In this process, micro-organisms or bacteria form minerals like calcium carbonate ($CaCO_3$) in various geothermal systems. The process creates heterogeneous materials composed of biologic (or organic) and inorganic compounds like carbonate, phosphate, oxalate, silica, iron, or sulfur-containing minerals, with heterogeneous distributions that reflect the environment in which they form (Skinner et al. 2003). Biologically induced mineralization is also an important geological process that helps in the formation of microfossil, hot spring deposition and transfer of chemical elements (Merz 1992, Jones et al. 1997, Konhauser et al. 1996). Although bacterial

cells are very minuscule, they have the largest surface to volume ratio of any life form. Therefore, they provide a large contact area that can interact with the surrounding environments and are responsible for the transformation of at least one third of the elements in the periodic table (Belkova 2005). The unique properties and functions of bio-mineralization have inspired innovative high-performance composites for construction applications, as well as other new materials (Bright 1994, Newnham 1997, and Travis 1997). Moreover, bio-mineralization have advantage of low investment and maintenance cost. It also offers benefits to environments and aesthetics (Karol 2003). For example: a potential use of this technology is carbon sequestration, which involves carbon dioxide (CO_2) capturing and converts it to calcium carbonate ($CaCO_3$).

2.1.1.2 Bacteria

Bacteria are single-celled (unicellular) micro-organisms, spherical, rod-shaped, spiral and appearing singly or in a chain that undergo metabolism, reproduction and growth, differentiation, communication, movement and evaluation. Activity and growth of bacteria depends on several growth limiting factors. These are a source of carbon for cell mass, a source of energy to sustain life activity, water, other nutrients and a favorable environment (including temperature, pH, salinity, and sufficient space).

The bacterial growth curve depends on inoculation of viable cells into a sterile broth (bacterial growth medium) and incubation of the culture under adequate temperature, pH and gaseous conditions. Under growth promoting conditions, the cells will reproduce rapidly and the dynamics of microbial growth can be plotted in population growth curve. A typical growth curve under these conditions is shown in Figure 2.1 and various growth phases are as follows:

- Lag Phase: During this stage the cells are adjusting to the new environment. A cellular metabolism is accelerated, resulting in rapid biosynthesis of cellular macromolecules, primarily enzymes. Although the cells are moderately increasing in size, there is limited cell division and therefore only a moderate increase in cell numbers.
- Starting Phase: In this phase, bacteria just start to grow after getting nourishment in a favorable environment.
- Logarithmic (log or exponential) phase: In the logarithmic phase, the physiologically robust cells reproduce at a uniform and rapid rate by binary fission. Thus, there is a rapid exponential increase in population, which doubles regularly until a maximum number of cells are reached. The length of the log phase depends on the organisms and the composition of the medium and varies significantly depending on bacteria type.
- Stationary Phase: During this phase, the number of cells undergoing division is equal to the number of cells that are dying. Therefore, there is no further increase in cell number and the population is maintained at is maximum level for a period of time. The primary factors responsible for this phase are the depletion of some

essential nutrients and the accumulation of toxic acidic or alkaline waste products in the medium.

- Decline or Death Phase: Because of the continuing depletion of nutrients and buildup of metabolic wastes, the microorganisms die rapidly at a uniform rate during this phase. The decrease in bacteria population closely parallels to its increase during the log phase. Theoretically, the entire population should die during a time interval equal to that of the log phase. Since a small number of highly resistant organisms persist for an indeterminate length of time, this does not happen.
- Slow-down Phase: In this phase, bacteria just start dying due to lack of nutrients.

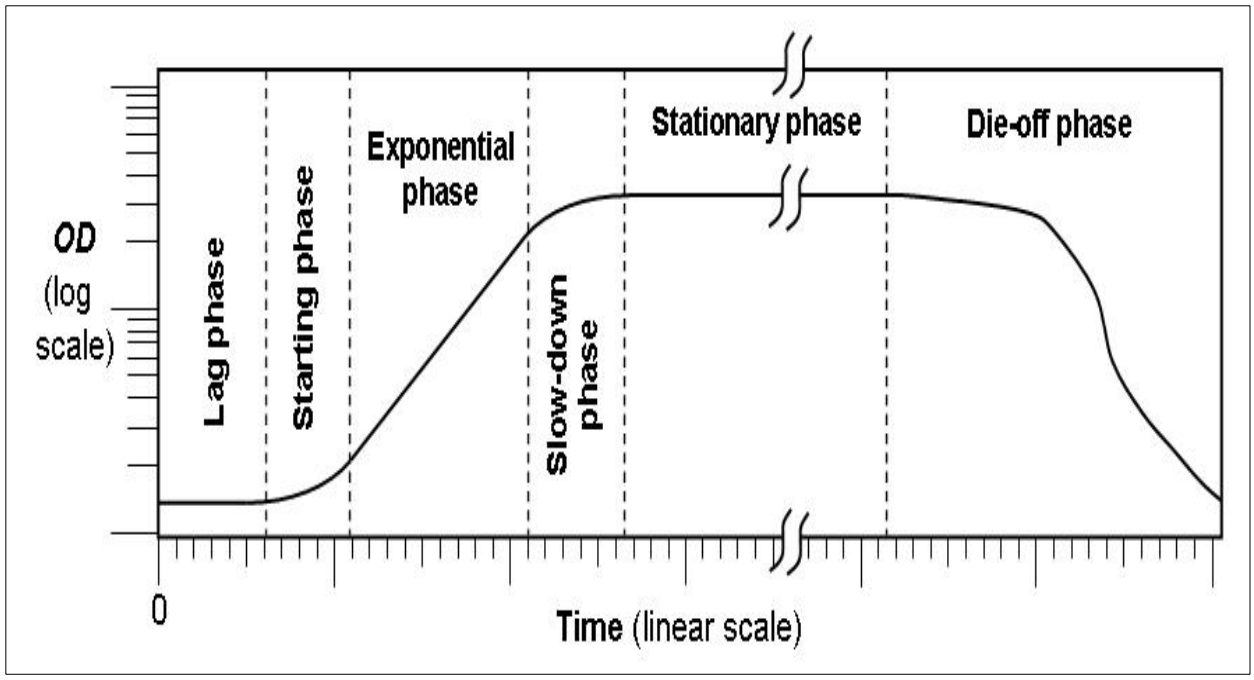


Figure 2.1 The Bacterial Growth (Friedrich, 2010)

2.1.1.3 Microbiologically Induced Carbonate Precipitation (MICP)

Carbonate precipitation is a common natural phenomenon found in the environment. According to Boquet et al. (1973), calcium carbonate is a general phenomenon in the bacterial world, and under suitable conditions, most bacteria are able to precipitate calcite crystals. Precipitation of $CaCO_3$ can occur in two ways: abiotic and biotic pathways. Abiotic precipitation occurs in supersaturated solutions through evaporation while pressure decreases and temperature increases (Castanier et al. 1999). Biotic precipitation can be either biotically controlled or biotically induced. When an organism exerts some sort of control over the location, size, and composition of the minerals formed, like skeletons and shells, the process is

said to be biotically controlled (Frankel et al. 2003). If the precipitation arises as a result of the metabolic activity of an organism, and the organism has little or no control over the mineralization, the process is biotically induced (Frankel et al. 2003). Carbonate precipitation has great importance in many environmental and civil engineering (material) applications. Abiotic precipitation has been used for purposes as wide ranging as permeability reduction in unconsolidated soils (Bird et al. 2008) to methods for carbon dioxide disposal (Lackner et al. 1995). Biologically induced carbonate precipitation by bacteria has been proposed for several biotechnological applications.

Carbonate mineralization by bacteria can occur in two ways: active or passive pathways. Active precipitation occurs as a by-product of common microbial processes such as photosynthesis, urea hydrolysis, sulfate reduction, and iron reduction (Knorre et al. 2000). Actually these processes increase the pH in the environment surrounding the bacteria that alters the saturation state of carbonate and other ions, such as calcium and iron. These new saturation states allows carbonate to precipitate out of solution as calcium carbonate (calcite, aragonite, or vaterite), magnesite, siderite, dolomite, or any number of carbonate minerals. One engineering application for active carbonate precipitation is the use of iron (III) reducing bacteria to stabilize fly ash, a residue generated by the combustion of coal (Roh et al. 2001).

In passive carbonate precipitation, heterogeneous nucleation on negatively charged points of bacteria attracts positively charged ions, allowing for the precipitation of carbonate (Bazylinski et al. 2003). Calcium carbonate is one of the most common products of carbonate precipitation, as both calcium and carbonate ions are abundant in natural environments.

Sporosarcina pasteurii formerly known as "*Bacillus pasteurii*", is a bacterium with the ability to precipitate calcite and solidify sand given a calcium source and urea, through the process of biological cementation. *S. pasteurii* has been proposed to be used as an ecologically sound biological construction material. Carbonate mineralization of these bacteria follows active pathways. The process is fairly straightforward. In the first step, bacteria get nutrition from culture medium and secrete urease enzyme (Urea-amino-hydrolease), this enzyme hydrolyzed urea ($CO(NH_2)_2$) to ammonia (NH_3) and carbonic acid (H_2CO_3) in the series of reactions outlined bin equations 2-4 through 2-6 (Burne et al. 2000). Then this ammonia and carbonic acid equilibrate in water to form bicarbonate (HCO_3^-), ammonium (NH_4^+), and hydroxide ion (OH^-) (Equations 2-7 & 8). After this, the pH increase due to formation of NH_4^+ which is essential for creation of calcite. This rise in pH shifts the bicarbonate equilibrium to form carbonate ions (Equation 2-9) which, in the presence of soluble calcium (Ca^{2+}), precipitates out of solution as calcium carbonate (Burne et al. 2000; Castanier et al. 1999). The overall reaction from the hydrolysis of urea in the presence of calcium is listed in the following equations.

$$CO(NH_2)_2 + H_2O \rightarrow NH_2COOH + NH_3$$ 2-1

Urease enzyme (catalyst)

$$NH_2COOH + H_2O \rightarrow NH_3 + H_2CO_3$$ 2-2

$$CO(NH_2)_2 + 2H_2O \rightarrow 2NH_3 + H_2CO_3$$ 2-3

(Overall, step 1)

$$H_2CO_3 \leftrightarrow HCO_3^- + H^+$$ 2-4

$$2NH_3 + 2H_2O \leftrightarrow 2NH_4^+ + 2OH^-$$ 2-1

$$HCO_3^- + H^+ + 2OH^- \leftrightarrow CO_3^{2-} + 2H_2O$$ 2-2

$$CO_3^{2-} + Ca^{2+} \leftrightarrow CaCO_3$$ 2-3

$$CO(NH_2)_2 + 2H_2O + Ca^{2+} \leftrightarrow 2NH^{4+} + CaCO_3$$ 2-4

(Overall process)

These reactions occur under the influence of natural environmental factors. These factors are the type of bacteria; bacteria cell concentration, temperature, urea concentration, calcium concentration, ionic strength, and the pH of the media. Since the activity of the urease enzyme is controlled by these factors, it may have a significant impact on MICP. The bacteria should possess high ureolytic efficiency, alkalophilic (optimum growth rate occurs at pH around 9, and no growth at all around pH 6.5), non-pathogenic, and possess the ability to deposit calcite homogeneously on the substratum (George et al. 2010). The bacteria should also have a high negative zeta-potential (Dick et al. 2006) to promote adhesion and surface colonization, and produce enormous amounts of urease enzyme in the presence of high concentrations of ammonium (Kaltwasser et al. 1972) to enhance both the rate of ureolysis and microbial carbonate precipitation (Nemati et al. 2003).

Urease-catalyzed ureolysis is also influenced by temperature like any other enzymatic reaction. The optimum temperature ranges from 20 to 37 °C depending on environmental conditions and concentrations of other reactants in the system (George, et al. 2010). It has been reported that the rate of ureolysis increases with temperature, when temperature rises from 15 to 20 °C, the

rate of ureolysis k_{urea} is 5 to 10 times greater from 10°C (Mitchell, et al. 2005). Thus, the rate of ureolysis can be enhanced by increasing the temperature within the optimum range.

Nemati and Voordouw (2003) verified that increasing urea and Ca^{2+} beyond 36 and 90g/L, respectively, does not have any significant effect on bacterial calcium carbonate precipitation. Since Ca^{2+} is not utilized by microbial metabolic processes, it would accumulate outside the cell where it would be readily available for MICP (Silver et al. 1975).

Ionic strength is also an important factor which influences enzymatic reactions like temperature and concentration. In bacteria transport of porous media, the total interaction energy needed by microbial particles to adhere and attach themselves to solid surfaces as explained by the classical Derjaguin– Landau–Verwey–Overbeek theory which is composed of the repulsive electrostatic forces and the attractive Van Der Waals forces (George et al. 2010). High ionic strength increases electrical double layer (EDL) compression by decreasing EDL repulsive forces leaving attractive Van Der Waals forces to dominate, and in the process promotes bacterial adhesion and attachment to the substratum (Faibish et al. 1998). Martell and Smith (1974) showed that the equilibrium constant for ammonia speciation increase from 9.3 to 9.4 by raising ionic strength from 0.1 to 1.0.

A pH increase is an indication of urea hydrolysis, and is an important property of alkalophiles (optimum growth at pH 9 and no growth below pH 6.5). At any pH levels, NH_3 gas and dissolved NH_4^+ exist at different concentrations. A higher concentration of NH3 creates favorable conditions for MICP (George, et al. 2010).

The phenomenon of MICP is not very well understood (Douglas et al. 1998). Knorre and Krumbien (2000) elucidated that MICP occurs as a result of common microbial metabolic processes such as photosynthesis, urea hydrolysis, and sulfate reduction. According to Ramachandran (2001), use of bacteria in PCC construction industry is considered unorthodox. But MICP is pollution free and natural activity and improves the performance of PCC or mortar (Ghosh et al. 2005). This recent research on bio-mineralization is leading use of microorganism as potential new material in construction industry. Some calcite forming bacteria strains, as example *Arthrobacter crystallopoietes* (ATCC 15481), *Sporosarcina pasteurii* (ATCC 11859), *Bacillus sphaericus* (ATCC 14577), and *Lysinibacillus fusiformis* (ATCC 7055) etc., have enough potentiality to precipitate calcium carbonate in optimum condition to improve the strength of PCC (Park, et al, 2009).

2.1.1.4 Bio-Cementation of Soils

The roles of microorganisms as explained by Van Paassen (2009) are

1. Producing carbonate (hydrolysis)
2. Producing alkalinity (increasing the pH locally, which causes the dissolved inorganic carbon which is mainly present as bicarbonate to dissociate causing an increase in carbonate concentration).
3. Acting as nucleation sites in an already oversaturated solution.

Bachmeier (2002) studied the urease activity MICP. *Bacillus pasteurii* and *Escherichia coli*, microorganisms were used for the experiments. These two microorganisms have ability to precipitate calcite. They used *Bacillus pasteurii* which was immobilized in polyurethane (PU) foam to compare the efficiency of calcite precipitation between the free and immobilized enzymes. After the process of MICP, Scanning Electron Microscope (SEM) was used. SEM images identified calcite precipitation throughout the matrices of PU. In comparison, SEM images of calcite precipitation induced by the PU-immobilized urease showed smaller and less organized crystals on the surface, and PU foam has well organized crystals within the matrices.

DeJong et al. (2006) used microorganism *Bacillus pasteurii* for achieving MICP, an aerobic bacterium pervasive in natural soil deposits. The microbes were introduced to the sand specimens in a liquid growth medium amended with urea and a dissolved calcium source. To increase the cementation level of the sand particle matrix, cementation treatments were done on the specimen. The results of both MICP and gypsum-cemented specimens were assessed non-destructively by measuring the shear wave velocity. SEM microscopy verified formation of a cemented sand matrix with a concentration of precipitated calcite forming bonds at particle-particle contacts. X-ray compositional mapping confirmed that the observed cement bonds were comprised of calcite.

DeJong et al. (2006) recommended the use of Bacillus Pasteurii as the stabilization microorganism applied in soil improvement. These bacteria used urea as the nutrient and grow at 30±2°C with sufficient oxygen. To ensure the growing of bacteria and effective chemical reaction, nutrients and chemicals supplements are necessary.

Whiffin et al. (2007) used MICP as a soil improvement technique. For evaluating MICP as a strengthening process, a five meter sand column was treated with bacteria and reagents under conditions that were realistic for field applications. The injection and reaction parameters were monitored during the process and both bacteria and process reagents could be injected over the full column length at low pressures (hydraulic gradient < 1; a flow rate of approximately 7 m/day) without resulting in clogging of the material. After treatment, the column was subjected to mechanical testing, which indicated a significant improvement of strength and stiffness over several meters. Calcium carbonate was precipitated over the entire five meter treatment length.

Table 2.1 shows the summary of various researches done on different types of soils. The review of information indicated that bio-cementation has been successfully used in strengthening of sand columns. In addition, the review also indicated that MICP can be used for healing as well as strengthening of the concrete. The other applications include slope stability, bioremediation of piping erosion in sands. However, the use bio-cementation has been limited to sands and its application for stabilization of clay or silt has not been well documented. In addition, there is no documented application of mutated bacteria for soil remediation. In a study, Achal et.al (2009) investigated that ultra violet (UV) irradiation of BP not only increased the efficiency of this

bacterium to grow at high pH but also increased urease activity for more calcite formation. The influence of mutation on calcite precipitation needs to be evaluated as well.

Therefore, the main focus of the study is to develop a methodology for mixing mutated bacteria (MB) in the soil and evaluate its influence on strength of clayey, as well as sandy soil.

TABLE 2.1 SUMMARY OF BIO-CEMENTATION BY VARIOUS RESEARCHERS

No.	Bacteria Type	Material	Authors	Title	Summary
1	*Bacillus pasteurii*	Sand	JT DeJong et al	Bio mediated soil improvement (2010)	• This paper presents an overview of bio-mediated improvement systems (bio-mediated calcite precipitation of sands). • Alternative biological processes for inducing calcite precipitation were identified (denitrification) and various microscopy techniques (SEM) were used to assess how the pore space volume is altered by calcite precipitation. • Non-destructive geophysical process monitoring techniques (Shear wave velocity, compression wave velocity, and resistivity) are described and their utility explored. • Microscopy technique established calcite precipitation reduced the pore spaces, thus, effectively resulting in densification of the soil. • The geophysical method allowed monitoring of site during treatment and throughout the service life. • Results indicate that the permeability is reduced by 10^{-3}, stiffness is increased by 10^{2}, compressibility is deceased by 10^{-2}, shear strength increased by 10^{2} and the volumetric response to be changed from being contractive to being dilative.
2	*Bacillus pasteurii*	SW (Well graded sand)	Meyer F.D et al	Microbiologically-Induced Soil Stabilization: Application of Sporosarcina pasteuri for Fugitive Dust Control (2011)	• The objective was to introduce a biological dust control technique utilizing a naturally occurring soil microorganism. • To evaluate the dust suppressive potential of this microbial calcite, *S. pasteurii* was suspended in culture medium and applied to locally available sand. • The treated soil samples were tested via a wind tunnel at specified intervals and mass losses were measured. In order to identify the optimum conditions of microbial dust suppression, the effects of concentration of *S. pasteurii*, temperature and humidity, and the soil preparation method (washed or unwashed) were examined. • Under 20% humidity, 45°C environments, dust control achieved was optimum. The effect of dust control reached to maximum when the concentration of microorganism was 1 x 10^{6} cells/ml in liquid medium. • After wind tunnel testing, the mass loss is limited to 1% or less compared to mass loss with no treatment.

No.	Bacteria Type	Material	Authors	Title	Summary
3	*Bacillus pasteurii*	Silica Sand, Surface material from unpaved road and concrete coarse aggregate	Li, S.	A laboratory study of the effects of biostabilization on on geomaterials (2013)	• The objective was to introduce biological additives into geomaterial specimens to test the strength and other geotechnical properties of soil and analyse the micro structure of untreated and bio-treated specimens. • Eight tests were conducted on the bio-treated specimens, which are, compression test, micro analysis (SEM and EDS), mercury porosimetry, Iowa pore index, freezing and thawing, soil index test, abrasion and impact. • Bio-treatment increased the average UCS of silica sand samples by 3–6 times. • Bio-stabilization effect is slightly better when NH_4Cl liquid medium is used. It reduces the porosity of concrete pavement coarse aggregate by microbially induced precipitation. After 6 cycles of bio-treatments, mercury intrusion volume was decreased from 0.0697mL/g to 0.0199mL/g. The pores were seemingly plugged by bio-precipitants. • The soundness of aggregate test showed that the bio-treated aggregate has 20% less mass loss compared to the untreated aggregate.
4	*Bacillus pasteurii*	Sand	Whiffing et al	Microbial Carbonate Precipitation as a soil improvement technique (2007)	• In order to evaluate MICP as a soil strengthening process, a five meter sand column was treated with bacteria. • The injection and reaction parameters were monitored during the process and both bacteria and process reagents could be injected over the full column length at low pressures (hydraulic gradient < 1; a flow rate of approximately 7 m/day). • After treatment, the column was subjected to mechanical testing, which indicated a significant improvement of strength and stiffness over several meters. Calcium carbonate was precipitated over the entire five meter treatment length. • The average permeability over the column after treatment was 9×10^{-6} m/s compared to the original material permeability of 2×10^{-5} m/s.

No.	Bacteria Type	Material	Authors	Title	Summary
5	*Castellaniella denitrificans*	Garden soil or sludge from a waste water treatment	Van Paassen et al.	Potential soil reinforcement by biological denitrification (2010)	• In this paper, an alternative for MICP method is used, i.e., microbial denitrification of calcium nitrate, using calcium salts of fatty acids as electron donor and carbon source. • In this process, organic compounds like acetate can be oxidized to produce carbonate ions and alkalinity, which are required for the precipitation of calcite, while nitrate is reduced to nitrogen gas. • Using calcium salts of both the electron donor and acceptor results in a high CaCO3 yield. • The occurrence of inhibitive intermediates (nitrite and nitrous oxide) at high concentrations and the heterogeneous distribution of calcium carbonate in the sand column still negatively affect the potential of denitrification as soil reinforcement method. • The rate of calcium carbonate formation by denitrification is far lower than for the urease process.
6	*Pseudomonas denitrificans*	Granular soils	Hamdan Nasser et al	Carbonate Mineral Precipitation for Soil Improvement through Microbial Denitrification (2011)	• This paper discuss about the use of microbial denitrification as a preferred method for MICP. • Bench scale bioreactor and column tests using *Pseudomonas denitrificans* have shown that calcite can be precipitated from calcium rich pore water using denitrification. • As denitrifying bacteria are ubiquitous in the subsurface, denitrification offers the potential for bio-stimulation of indigenous microorganisms. • Denitrification does not produce toxic end products, may be cost effective since nearly 100% utilization of electron donor is possible, and does not require the addition of potentially harmful exogenous organic materials such as urea. • In denitrification, the total dissolved solids in the reactors and columns are reduced by addressing the loss of free calcium in the form of calcium phosphate

					precipitate from the pore fluid.

No.	Bacteria Type	Material	Authors	Title	Summary
7	*Bacillus pasteurii*	Sand	Van der Ruyt et al.	Biological in situ reinforcement of sand in near-shore areas (2009)	• In this study, BioGrout (MICP) process is adopted for increasing the strength of the sand and its potential applications are mentioned • BioGrout can be applied in existing dunes that are not wide enough to withstand design conditions, or in situations where, for example, buildings need some extra protection • To ensure a stable slope with sufficient resistance against liquefaction, BioGrout solutions can be applied to create a cemented zone that is no longer be able to liquefy • The main advantage of BioGrout is that soil (or sand) can be strengthened without interfering with the hydraulics of the treated soil. Therefore natural aquifers are not interrupted by the construction, and maintain their function, even at a local level. Also, the soil can be strengthened without excavation or replacement.
8	Organic soil (compost-green waste and biosolids)mixed with sand	Sand	Adams, and Xiao	Bioremediation of Piping Erosion in Sand (2011)	• This study aims to explore a remediation method in which organic soil is mixed with sand to increase the sand's resistance to piping erosion. • In this study, a mixture of green and manure compost, referred to as co-compost, is used as the source of organic soil. Hole-erosion tests are performed to quantify the erosion of a silty sand, the co-compost, and various ratios of sand–cocompost mixtures. • The potential increase in consolidation settlement and reduction in shear strength and permeability due to the addition of organic matter were also investigated. • Greater proportions of organic soil mixed with typical construction sand result in increased resistances to piping erosion. • With the addition of organic matter to sand, consolidation settlement increases, undrained compression strength decreases, and permeability was reduced by two orders of magnitude.

No.	Bacteria Type	Material	Authors	Title	Summary
9	*Bacillus*	Concrete	Jonkers et al.	Application of bacteria as self-healing agent for the development of sustainable (2010)	• This study investigated the potential of bacteria to act as self-healing agent in concrete, i.e. their ability to repair occurring cracks. • Ordinary Portland cement was mixed with tap water in a water-to-cement weight ratio of 0.4 or 0.5. Bacteria containing specimens were prepared by addition of washed spore suspensions replacing part of the makeup water. • Liquid paste was poured in moulds (with dimensions of 4 cm $\times$ 4cm $\times$ 4 cm), gastight sealed and cured at room temperature. Bacterial specimens contained $1\text{-}10 \times 10^8$ spores cm^{-3} cement stone. • ESEM analysis indicates that copious and robust minerals are produced • Bacteria plus calcium lactate, representing a two-component healing agent, produce 20–80 micrometres sized mineral-like precipitates on crack surfaces of young, 7 days cured, cement stone specimens • Bacterial cement stone specimens appeared to produce substantially more crack-plugging minerals than control specimens
10	*Bacillus Pasteruii*	Concrete	Ramakrishnan et al.	Improvement of concrete durability by bacterial mineral precipitation (2005)	• In this paper, a technique in remediating cracks and fissures in concrete by utilizing microbiologically induced calcite ($CaCo_3$) precipitation is discussed. • They conducted a durability study of concrete beams that were treated with bacteria grown in three mediums: water, urea, and phosphate buffer. • The beams were exposed to sulphate, alkaline, and freeze thaw environments. Scanning electron microscope (SEM) and X-ray diffraction (XRD) were used to analyse the quantity and shape of MICP. They found the durability of concrete beams treated with bacteria was much higher than the control group. • The authors concluded phosphate-buffer was the most effective bacteria medium and at the end of 28 curing days beams with bacterial concentration of 1 x 10^6 cells/ml, 1 x 10^7 cells/ml, and 1 x 10^8 cells/ml had 13%, 20%, 34% less shrinkage deformations respectively than that of the control beams.

No.	Bacteria Type	Material	Authors	Title	Summary
11	*Bacillus sphaericus*	Limestone	Muynck et al.	Influence of urea and calcium dosage on the effectiveness of bacterially induced Carbonate precipitation on limestone (2010)	• In this study, the influence of the chemical parameters, i.e. concentration of calcium salts and urea, on the effectiveness of the bio-deposition treatment has been examined. • The amount of calcium carbonate that can be precipitated in the stone is conditioned both by the amount of cells retained in the stone and the concentration of urea and calcium used. • From sonication experiments, a good consolidation was observed for limestone prisms treated with a calcium dosage of 17 g $Ca^{2+}m^{-2}$ with no improvement at higher concentrations. • For limestone prisms of 4 cm $\times$ 2cm $\times$ 1 cm, the bio-deposition treatment resulted in a 63% lower weight loss upon sonication compared to untreated specimens. • The waterproofing effect was observed to increase with increasing calcium dosages. While for a calcium dosage of 17 g $Ca^{2+}m^{-2}$ the water absorption was similar to that of untreated specimens, concentrations of 67 g $Ca^{2+}m^{-2}$ resulted in a 50% decrease of the rate of water absorption. • The optimum calcium dosage can be tentatively estimated to about 13.4 mg Ca^{2+} cm^{-2}.Bio deposition treated specimens showed a similarly decreased water absorption and resistance towards sonication as specimens treated with ethyl-silicates

3. DESIGN OF EXPERIMENT AND BACTERIA CULTURE

To study the influence of bacteria-treatment, an experiment design was developed and soil properties were evaluated using standard test methods and verified using micro level tests. In this chapter, the proposed experiment design, test methods for performance evaluation, and developed bacteria-treatment techniques are discussed. A brief discussion on bacteria mutation and growth is also presented.

3.1 Experiment Design

Three different types of soils (sand, silt and clay) were considered for this study. Table 3.1 shows the test conducted on the soil specimens (before and after bacteria-treatment. The purpose of selected test methods was to identify influence of bio-cementation on strength increase as well as to identify influence of calcite deposition on properties that can influence soil classification. The laboratory tests were conducted on specimens treated with mutated bacteria (*Bacillus Pasteurii*). In addition to the above tests micro analysis of the soils was also done using X-ray diffraction (XRD) and SEM (Scanning Electron Microscope).

Table 3.1 Various Test for Evaluating Soil Properties

Tests	Reference
Moisture Dry Density & Optimum Moisture Content	ASTM D698
Unconfined Compressive Strength	ASTM D2166
Resilient Modulus	AASHTO T 307
Free- Free Resonant Column	ASTM C215
Atterberg Limits	ASTM D4318-10
Sieve Analysis	ASTM D6913-04

3.1.1 Soils

Silty soil (AASHTO Classification A-2-4) was obtained from Strahan Road (close to Rio Grande River), El Paso, Texas and its gradation is shown in Figure 3.1. Sandy soil was prepared in the laboratory by mixing the silty soil and sand in a ratio of 1:1, the gradation is shown in Figure 3.2 (AASHTO Classification A-1-b). The sand was manufactured sand obtained from Jobe Materials (El Paso, Texas). Clayey soil (AASHTO Classification A-2-6) used in this study was obtained from Horizon cotton fields, El Paso, Texas and its gradation is shown in Figure 3.3.

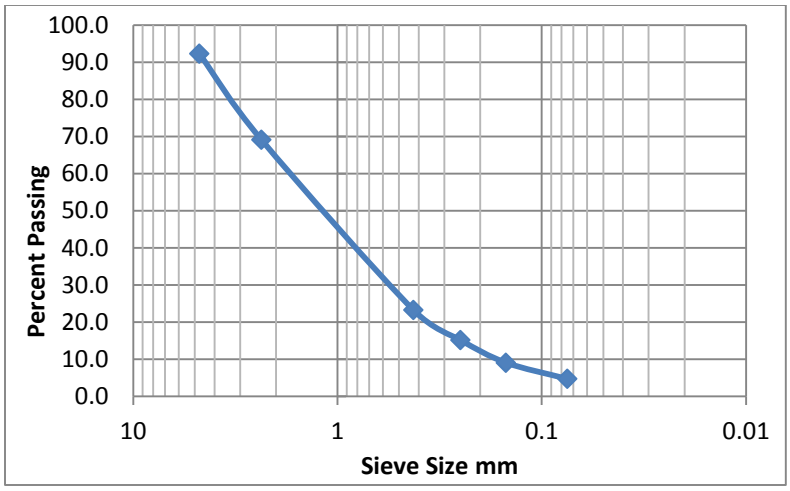


Figure 3.1 Gradation of Silty Soil

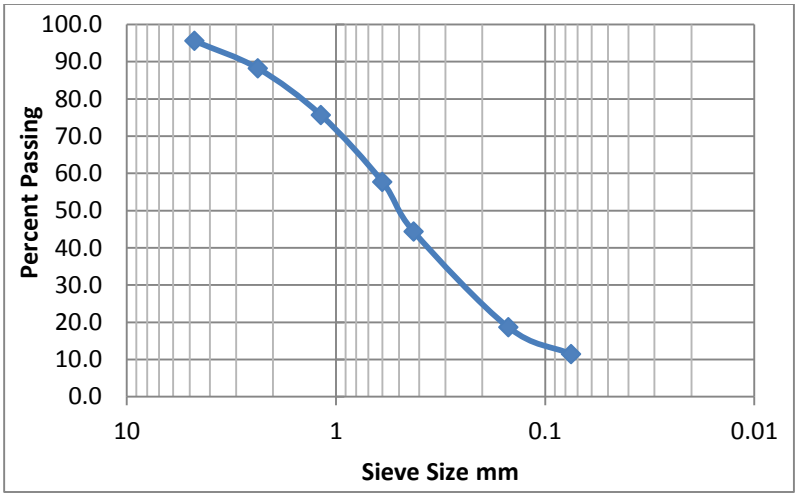


Figure 3.2 Gradation of Sandy Soil

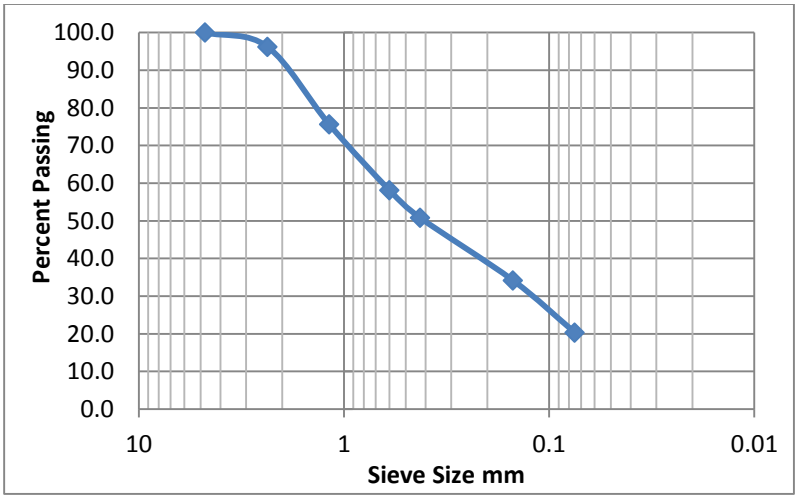


Figure 3.3 Gradation of Clayey Soil

3.2 Bacteria Culture and Mutation

Bacterial growth requires specific nutrients as well as procedure to minimize bacterial death during growth and stabilization. Therefore, optimal growth medium and a procedure was developed. Since bacteria mutation depends on the type of bacteria as well as a specific procedure, a procedure was also developed for mutation and to make sure that reverse mutation does not occur. The following sections describe bacterial growth and mutation process.

3.2.1 Introduction

For this research, a vial of Sporosarcina pasteurii or Bacillus pasteurii (BP) was procured from American Type Culture Collection (ATCC-11859). This vial of BP was then cultured to multiply the organisms by letting them reproduce in a conducive medium under controlled laboratory conditions. However, it is very difficult for micro-organisms to survive in a high pH environment (around 12), which causes bacterial death or formation of endospores. Thus, the micro-organisms were mutated such that they can survive in a high pH environment.

3.2.2 Constituents required for Bacterial Growth

Various chemicals were used to culture Bacillus pasteurii bacteria and to provide nutrients for bacterial growth during soil stabilization. The constituents and their compositions were maintained constant throughout the study to minimize the influence of changes in chemicals on the growth of bacteria. For mutated bacteria (MB), a higher pH was maintained to improve the survivability and minimize mutation reversal. In the following sections chemicals required for bacterial growth and bacteria mutation process are discussed.

3.2.2.1 Chemicals for Growth of Bacteria and Curing of Soils

Various chemicals were used to the prepare medium for growth and isolation of bacteria. The culture medium for growth was prepared by mixing Yeast extract (Sigma Aldrich; product no.Y1001), Tris Buffer (Sigma Aldrich; product no. T6066), and Ammonium Sulfate ($(NH_4)_2SO_4^-$, Sigma Aldrich; product no.A2939). The amounts of ingredients required for optimal growth are shown in Table 3.2. After growth of the bacteria, the culture medium was washed (using 50 mM sodium phosphate buffer) to remove the chemicals used for bacterial growth. It was then adjusted for the required optical density of bacteria. The buffer was prepared by mixing 8.2 grams of Sodium Phosphate (Na_3PO_4, Sigma Aldrich; product no. 342483) in 1 liter of water. The washed growth of bacteria was used throughout the specimen preparation.

A growth medium (Tris-YE) was used for stock and pilot cultures of B. pasteurii. This medium was prepared following ATCC Medium 1376 protocol. Individual ingredients were autoclaved aseptically (free of micro-organisms) in a cell culture hood (Figure 3.4) and combined afterwards to avoid precipitation of ingredients. Each ingredient was mixed in 0.01059 ft^3 of deionized water and autoclaved. In the autoclave, a high pressure stream of 2.9 psi/min was applied at 249.8°F for roughly 90 minutes, depending on the size of the loads and the contents. Then, the autoclaved ingredients were cooled at 122-131°F and aseptically combined. The pH

of the culture medium was approximately 9.0 at this stage. The culture medium obtained from this process is termed as Tris-Yeast medium. A small amount of BP was taken from the vial and cultured in 0.0035 ft^3 of Tris-Yeast medium at 80°F under aerobic conditions and incubated in a shaker operated at 200 rpm for 24 h (Figure 3.5). The bacteria and the culture medium were either frozen for future use or washed using 50 millimolar sodium phosphate buffer (pH 7.5) to prepare specimens. To prepare the frozen stock of BP strain for future use, $1.71x10^{-5}$ ft^3 of culture medium (which includes bacteria) were aliquoted and mixed with $1.71x10^{-5}$ ft^3 of 15% glycerol. This mixture was frozen at -94°F to be used for future growth of bacteria.

Table 3.2 Ingredients of Tris –YE bacteria Culture Medium

Ingredient	Amount per liter
Yeast Extract	20 g
Ammonium Sulfa	10 g
0.13 M Tris Buf	.75

Figure 3.4 Cellculture Hood (Labgard, Class-II, type A2)

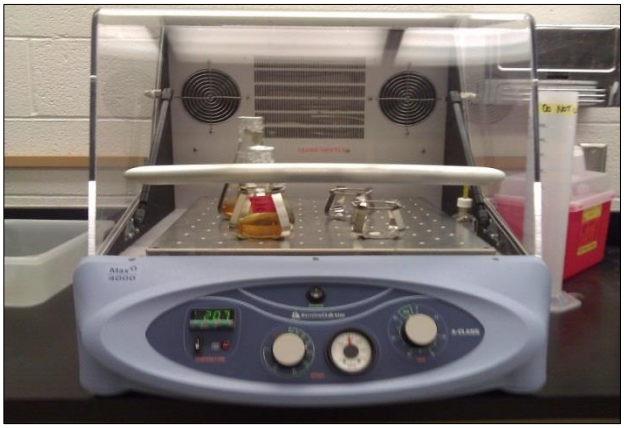


Figure 3.5 Shake Incubator

The culture medium obtained after 24 hours of shaking was poured into a container and centrifuged at 4,000 rpm for 15 minutes to harvest bacterial cell pellets. These cell pellets were then washed twice with a 50 millimolar sodium phosphate buffer solution of pH 7.5 containing 8.2 g (0.289 oz.) of sodium phosphate per 0.001 m^3 (0.035 ft^3) of water in order to eradicate the effect of culture media ingredients on soil samples. The final cell pellets were then suspended in phosphate buffer and adjusted to obtain the desired optical density using a spectrophotometer (Figure 3.6). This bacterial suspension was used for preparing specimens. Optical density 0.6 is considered in the present study.

Figure 3.6 Spectrophotometer

1.1.1.1 Mutation of Bacteria

B. Pasteurii grows well at an optimum pH of 9.0 and also has the capability to produce endospore. An endospore is a dormant form of the cell that endures extreme environmental conditions. However, a high pH increases osmotic pressure of, which reduces the survivability of bacteria and minimizes urease activity. Achal et. al. (2009) investigated and found that ultra violet (UV) irradiation of BP not only increased the efficiency of this bacterium to grow at high pH, but also increased urease activity for more calcite formation.

It has been found that UV light is lethal and mutagenic in a variety of organisms including bacteria (Auerbach 1976, Witkin 1976). It is generally accepted that exposing bacteria to UV irradiation results in DNA mutation by changing its DNA structure, which allows bacteria to survive under adverse conditions (Miguel & Tyrrell 1983).

To obtain mutated bacteria, the BP was cultured in the regular growth medium as described previously. After 24 h of incubation, this bacterial culture was exposed to UV irradiation using a Philips 20W germicidal lamp for 20 min. According to Achal et al. (2009), a typical survival rate is less than 10%. A small portion (1 mL) of irradiated bacteria culture was randomly selected and transferred to a 100 mL of Tris-YE media to grow the MB for 48 h in a shaker operated at 200 rpm and 86 °F under aerobic conditions. To minimize reverse mutation, higher pH is required. Therefore, 4 molar sodium hydroxide (NaOH) was added to Tris-YE medium to

increase pH from 9.0 to 10.5. This process was repeated three times and each time samples were randomly selected from immediate previous culture of MB. The purpose of culturing MB several times was to make sure that MB is incapable of mutation reversal. Similar to BP, MB were frozen for future use or washed with phosphate buffer for immediate sample preparation.

3.3 Sample Preparation with Bacteria

Traditionally, the water content is varied to develop dry density and moisture content relationship for determining optimum moisture content. The strength and resilient modulus tests are performed by preparing specimens at the determined optimum moisture content. Since specimen preparation process at optimum moisture content has been standardized, the sample preparation process for soils without bacteria will not be discussed herein. The process of introducing bacteria, bacteria curing medium, and drying of soil is further discussed in the following sections.

3.3.1 Sodium Phosphate Buffer

Since bacteria cannot survive in water, due to difference in osmotic pressure, a sodium phosphate buffer is proposed to be used for introducing bacteria in the soil. Sodium phosphate buffer (SPB) of pH 7.5 was used in the specimens. This buffer contains 8.2 g of Sodium Phosphate (Na_3PO_4) per liter of distilled water. The sodium phosphate buffer is similar to the one used for washing of bacteria after growth or mutation.

3.3.2 Urea Calcium Chloride Medium

Since bacteria needs nutrients for survival that in turn leads to calcite precipitation, nutrients for bacterial survival are provided through urea calcium chloride medium (Halder, 2012). Table 3.3 contains ingredients for Urea-$CaCl_2$ medium per liter of distilled water. Chemicals used for Urea-$CaCl_2$ medium are Urea (NH_2CONH_2, Sigma Aldrich; product no.U5378), Calcium Chloride ($CaCl_2.2H_2O$, Sigma Aldrich; product no.C5080), Nutrient broth (Sigma Aldrich; product no.S4681), Ammonium Chloride (NH_4Cl, Sigma Aldrich; product no.A0171), and Sodium bi-carbonate ($NaHCO_3$, Sigma Aldrich; product no.S5761).

3.3.3 Sample Preparation

The laboratory evaluation of soil samples prepared with and without bacteria required a standard sample size; therefore, 4x8 in. cylindrical size was selected for evaluating the influence of bacteria-treatment on strength and modulus. The specimen preparation process for bacteria-treated samples required use of sodium phosphate buffer as well as urea calcium chloride medium, therefore, a procedure was developed and is discussed in the following paragraphs.

Table 3.3 Composition of Urea Calcium Chloride Medium

Chemicals	Amount per liter
Nutrient Broth	3 g
Urea	20 g
Sodium Bicarbonate	2.12 g

Ammonium Chloride	10 g
Calcium Chloride	3.7 g

To minimize lysing of bacteria during mixing, the bacteria is mixed with sodium phosphate buffer solution. The mutated bacteria (MB) sample, obtained in section 3.2.2.2, is placed in an autoclaved container. The sodium phosphate buffer is then added to the sample and optical density (OD) of the solution is determined with the help of spectrophotometer. The buffer quantity is increased until OD of 0.6 is reached (Halder, 2012). This sodium phosphate buffer bacterium solution (SPBS) is then mixed with the soil (Figure 3.7a) using standard laboratory trowel. The amount SPBS selected was 1 to 2% less than the optimum moisture content as suggested by Li (2013). The purpose of using less than optimum moisture content was to make sure soil is not fully saturated as it will reduce oxygen availability for the bacterial survival while ensuring enough bacterial solution is present to cover the soil sample.

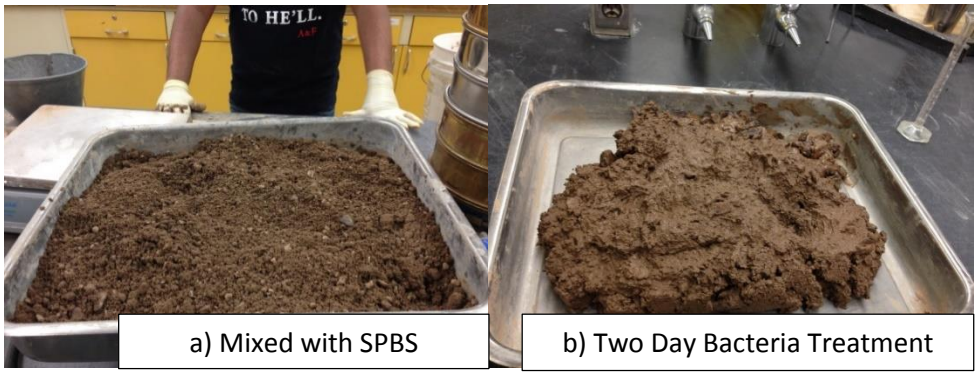
The soil mixed with SPBS was then placed inside a 6x12 in. cylindrical mold with holes at the bottom. A 2 in. aggregate layer was placed at the bottom as well as at the top of bacteria mixed soil. The purpose of the holes as well as the aggregate layer was to make sure that the urea calcium chloride medium can be easily provided to the soil as well as can be drained out of the soil. After placement of soil and aggregate layer, the urea calcium chloride medium was allowed to flow at a fixed rate using pipette. The rate of flow of 120 mL/hour was maintained as proposed by Montoya (2012). A rate of flow less than the mentioned amount will not be enough for bacterial survival while a higher rate will increase moisture content of the soil and reduce oxygen needed for bacterial survival. Additionally, bacteria lyse within two days (Lee et al. 2013) after mixing, therefore, there was not an additional benefit of providing nutrient to the soil for more than two days. Thus, urea calcium chloride medium was provided for two days at the rate of 120 mL/hour. After two days of bacteria-treatment, the soil is taken out of the cylindrical mold and is shown in Figure 3.7b. A comparison between Figures 3.7a and 3.7b suggests that the moisture content of bacteria-treated sample is higher. After bacteria-treatment, the samples were taken out of the mold and placed inside an oven maintained at 212 °F for a day (Li 2013). The drying process allowed identification of calcite deposition and also to make sure bacteria has lysed (Figure 3.8). The dried samples were crushed and again tested, as specified in Table 3.1.

3.4 Macro Level Laboratory Tests

Since most of the tests listed in Table 3.1 are standard, they are not discussed herein. Even though standardized, the free-free resonant column (FFRC) test is not frequently used, therefore, a brief description is included in Appendix A. In FFRC testing, the specimen is impacted with a hammer, and the resonant frequency associated with the standing waves within the specimen is measured. The resonant frequency along with the length of the specimen can be used to determine the seismic modulus (ASTM C215). For this test to be effective, a specimen with a length-to-diameter ratio of at about 1.5 to 2 is required.

3.5 Micro Level Laboratory Tests

To verify results of macro test results and to identify presence of calcite micro level tests were performed. Investigation of different micro-structure characteristics such as elemental analysis of materials were performed using X-ray diffractometer (XRD) and scanning electron microscopy (SEM).



a) Mixed with SPBS | b) Two Day Bacteria Treatment

Figure 3.7 Bactria-Treatment of Soil

Figure 3.8 Calcite Deposition (Oven Dried Soil)

3.5.1 XRD Analysis

X-rays are electromagnetic radiation of wavelength varying from 0.1 Å to 100 Å. For diffraction application, short wavelength X-rays, also called hard X-rays (0.1 Å to few Å), are used (Introduction to X-ray diffraction 2011). X-ray diffraction is a non-destructive technique for characterizing the crystalline material. An X-ray beam, produced by the X-ray tube is directed towards the sample. When the beam strikes the sample, diffraction occurs (Figure 3.9) according to Bragg law (ASM Handbook) which is as follows:

$$\lambda = 2d \sin\theta \qquad (3.1)$$

Where d is the inter-planar spacing, θ is the scattering angle, λ is the wavelength of X-ray and n is an integer. Bragg condition is also the condition for constructive interference. Bragg law is

satisfied by varying the angle θ. The diffraction pattern is usually recorded as intensity versus diffraction angle 2θ. The peak positions in the diffraction pattern are directly related to d and the peak intensity depends on the atom in the diffracting plane.

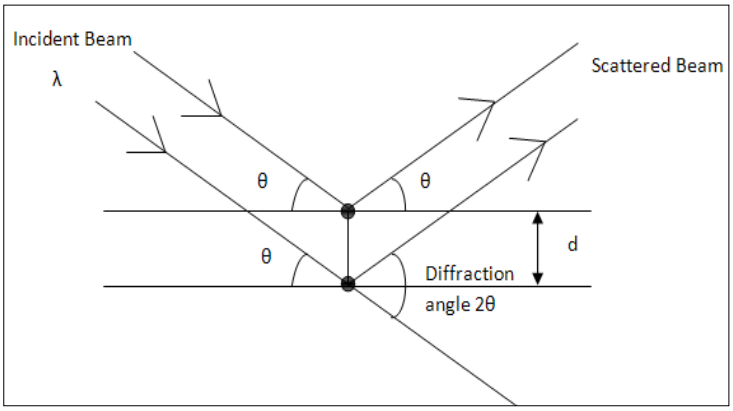


Figure 3.9 Schematic of X-ray Diffraction

Micro-structural characterization of the bacteria treated soil samples were investigated using Bruker D8 Discover advance XRD (Figure 3.10). Cu Kα radiation of wavelength λ=1.5406 Å was used to analyze soil using 0.2 mm slit in a scanning range of 10-90° in 2θ scale at a rate of 5°/min.

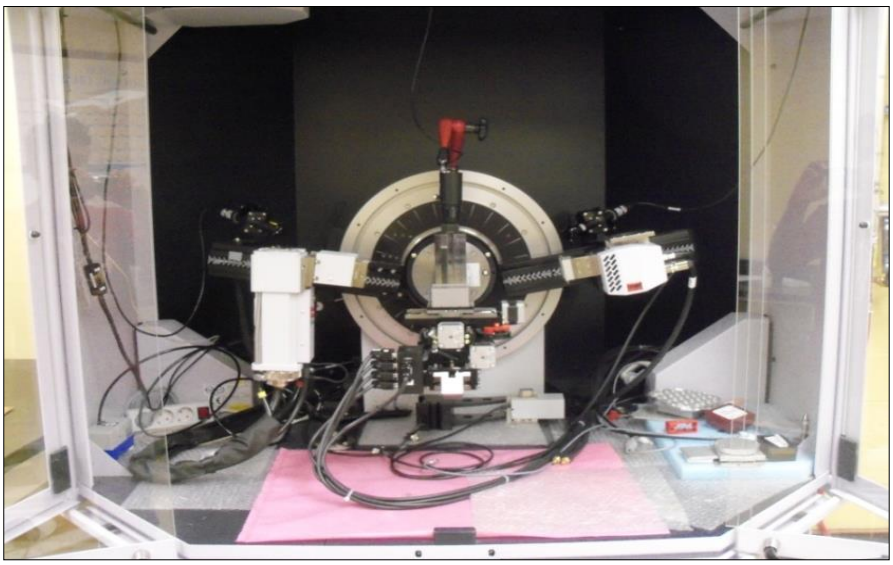


Figure 3.10 Bruker D8 X-ray Diffractometer

3.5.2 Scanning Electron Microscopy

SEM uses electrons instead of light to produce an image. The schematic of SEM is shown in Figure 3.11. An electron beam is produced by the electron gun. The beam travels through the microscope which is kept in a vacuum. Electromagnetic lenses (condenser and objective) are used to focus the beam onto the sample surface. The interaction of the electron beam and the sample surface causes emission of electrons (secondary, back-scatter and auger) and X-rays.

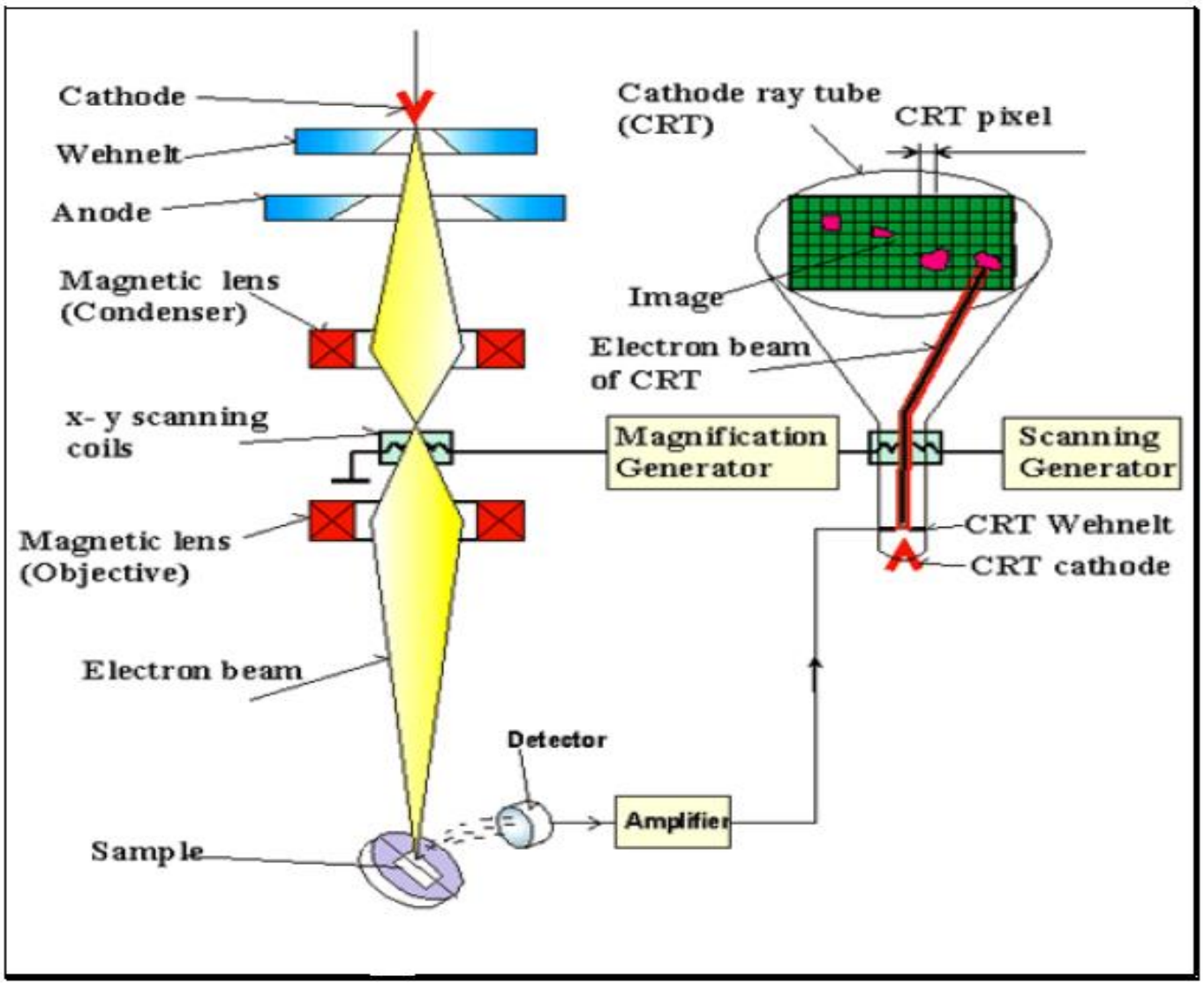


Figure 3.11 Schematic of Scanning Electron Microscope

Secondary electrons are detected by the detector and converted to a signal which produces the final image. The resolution of SEM image is very high (1-20 nm) compared to optical microscope because the wavelength of electron beam is extremely small (Dmitri 2011). Backscattered electron can be detected to produce backscattering image which is useful for contrasting sample area having different chemical compositions.

Microstructure of fragment of the different soil samples was investigated using Hitachi Table Top SEM TM-1000 (Figure 3.12). Images were taken at 3kV or 7 kV to reduce the effect of charging and Energy Dispersive X-ray Spectroscopy (EDS) analysis was performed using Genesis Spectrum Version-6.04 software at 20 kV.

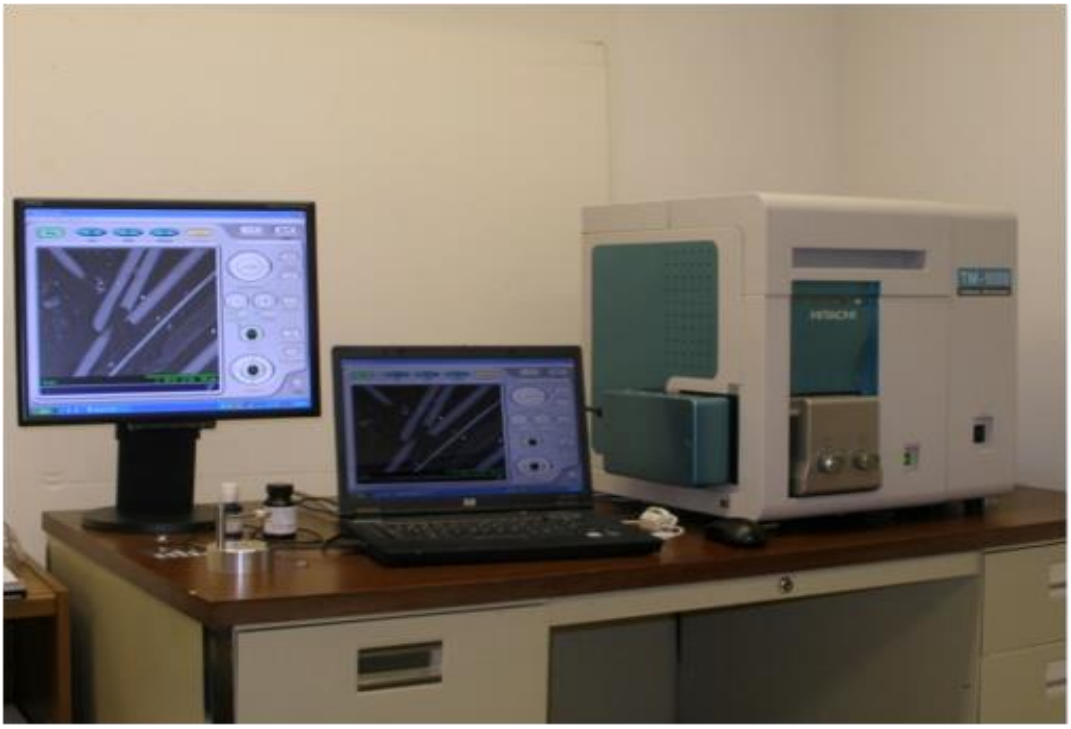


Figure 3.12 Hitachi TM-1000 Scanning Electron Microscope

4. RESULTS AND DISCUSSION

The bacteria-treatment procedure developed in the previous chapter was used for preparing soil samples for evaluating influence of bacteria-treatment on mechanical properties. In addition, a portion of samples were collected to perform micro level testing using XRD and SEM.

4.1 Macro Level: Laboratory Tests

The three soil types selected for evaluation were first classified as per AASHTO soil classification system. Additionally, their mechanical properties like unconfined compressive strength, resilient modulus, and seismic modulus (FFRC testing) were evaluated. Since maximum grain size of selected soils was less than 4 mm, it was decided to use 4by8 in. cylindrical specimens for evaluation of mechanical property in order to minimize amount of bacteria needed for the treatment. Similar tests were performed on soil specimens after bacteria-treatment. To quantify the influence of bacteria-treatment on desired parameter, three specimens were tested to evaluate mechanical properties and obtained average as well as standard deviation are shown in Table 4.1 while standard deviations are shown in bracket. The bacteria-treated samples were oven dried and crushed before performing classification or mechanical property evaluation.

Table 4.1 Soil Classification and Mechanical Property Test Results

Tests	Soil 1 (Silt)		Soil 2 (Sand)		Soil 3 (Clay)	
	Before Treatment	After Treatment	Before Treatment	After Treatment	Before Treatment	After Treatment
Liquid Limit, %	23.4	NP	14.5	NP	40.0	32.7
Plastic Limit, %	13.6	NP	NP	NP	18.8	21.0
Plasticity Index, %	9.8	NP	NP	NP	15.2	11.7
Maximum Dry Density, lb/ft^3	125.0	124.5	137.5	138.0	121.4	87.0
Soil Classification (AASHTO)	A-2-4	A-2-4	A-1-b	A-1-b	A-2-6	A-2-6
Optimum Moisture Content, %	16.4	15.2	9.8	9.2	20.0	18.5
Unconfined Compressive Strength, psi	14.8	15.4	24.8	26.3	22.0	21.6
	4.110	3.431	3.296	4.759	4.069	3.637
Resilient Modulus, psi	11.6	12.8	14.6	25.0	7.5	11.5
	3.724	4.211	5.854	8.475	1.403	2.691

Seismic Modulus, ksi	7.5	8.6	19.4	23.5	13.6	10.3
	0.071	0.077	0.072	0.054	0.066	0.072

The sieve analysis and measured Atterberg limits suggested that evaluated soil types are sandy, clayey, and silty. As per AASHTO soil classification system, the soils can be classified as A-1-b, A-2-6, and A-2-4, respectively. Although gradation (Figure 4.1) and plasticity of soils slightly changed after treatment, the change was not significant enough to influence soil classification. Although the plasticity index of silty soil was 9.8 before treatment, the soil became non plastic after treatment. Although plasticity index of clay reduced slightly (from 15.2 to 11.7), it was not enough to make soil non-plastic.

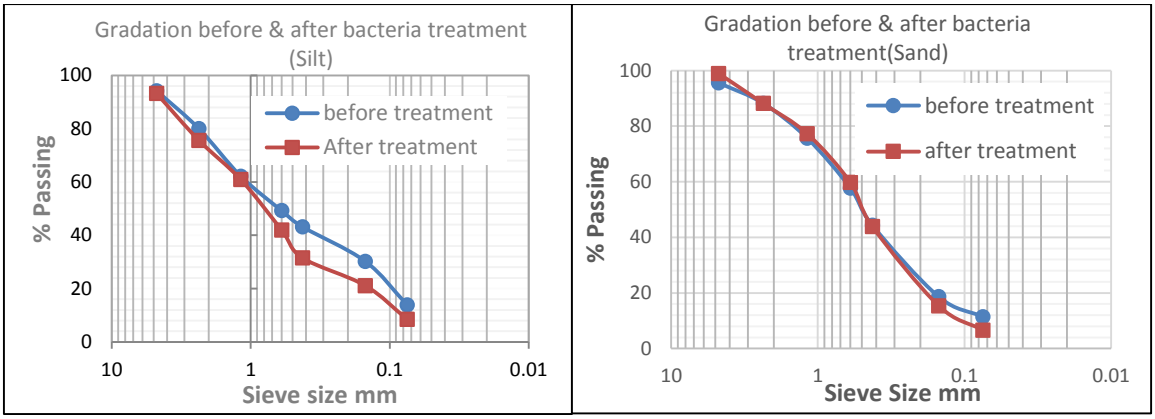


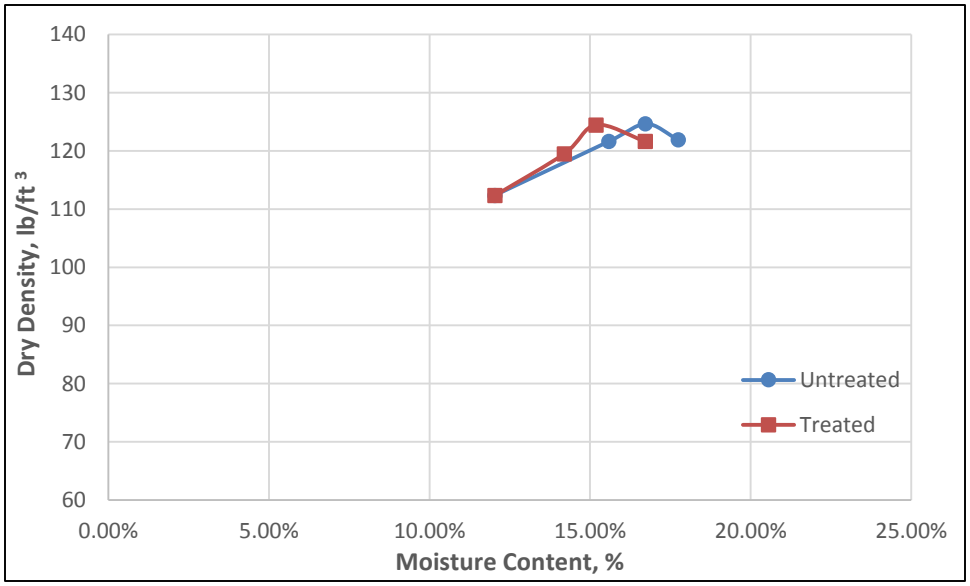
Figure 4.1 Influence of Bacteria-Treatment on Gradation

The influence of bacteria-treatment on moisture density curve (Figures 4.2 through 4.4) was minimal for sandy and silty soils while it was significant for clayey soil. The maximum dry density of sandy and silty soils were similar before and after treatment, however, maximum dry density of clayey soil changed from 121.4 to 87 lb/ft^3 after treatment. In terms of optimum moisture content, all three soils required less moisture to attain maximum dry density. An explanation could not be found for significant drop in dry density of clayey soil and needs to be further explored in future research.

The unconfined compressive strength (UCS) results are shown in Figures 4.5 through 4.8. The test results suggest a slight increase in UCS of sandy soils but no influence on silty or clayey soils strength (Figure 4.5). The standard deviation of measured UCS was within 3.25 to 4.75 psi. The stress strain curve of UCS test for each soil is shown in Figures 4.6 through 4.8. The figures also show a slight increase in strength of sandy soil. Additionally, the brittleness of clayey soil increased after bacteria-treatment (Figure 4.8) while sandy as well as silty soils brittleness reduced or remained the same after bacteria-treatment. Although dry density of clayey soil reduced significantly, the drop in UCS of soil was not that apparent.

The resilient modulus test results are shown in Figure 4.9. The results indicate that resilient modulus of all three soils increased after bacteria-treatment. The increase was significant (more

than 50%) for sandy and clayey soil while it was less significant for silty soil (less than 5%). The standard deviation of measured resilient modulus was within in the range of 1.4 to 4.2 psi for silty and clayey soil while it was higher for sandy soil (5.8 to 8.5 psi). The increase in resilient modulus suggest that the soil will exhibit less of permanent deformation during service life of pavement under repeated traffic loading. Also, the influence of lower dry density of clay on resilient modulus was insignificant.



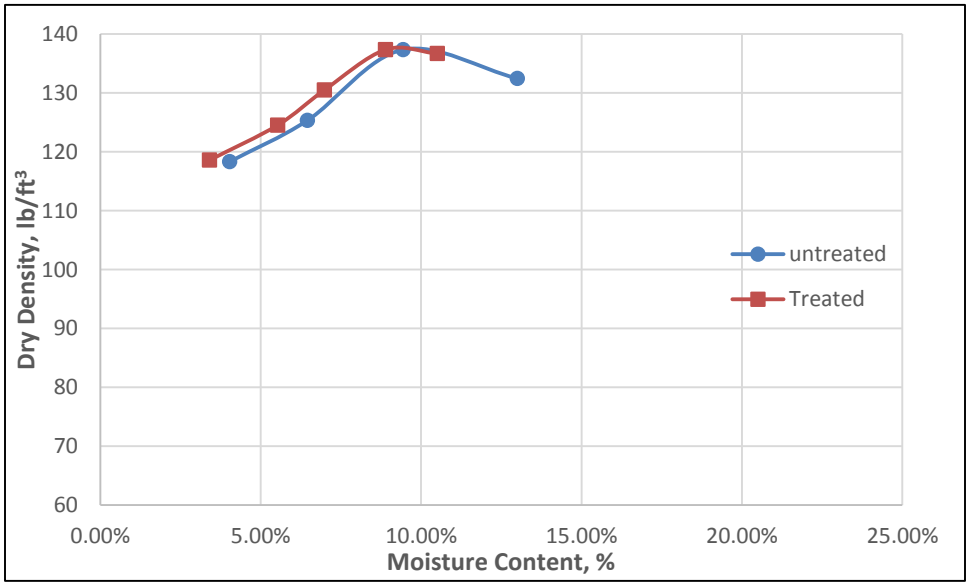


Figure 4.2 Moistue Density Curve of Silty Soil

Figure 4.3 Moistue Density Curve of Sandy Soil

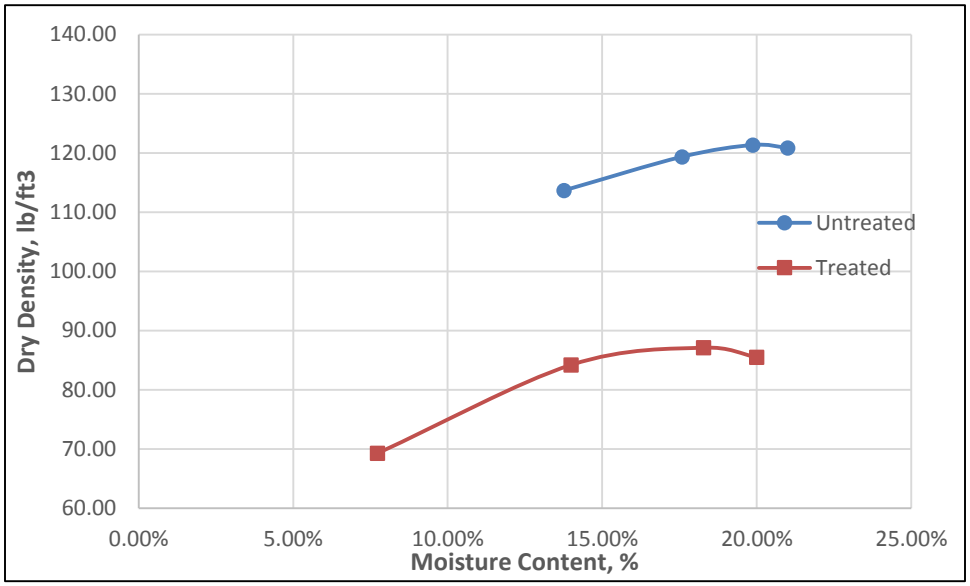


Figure 4.4 Moistue Density Curve of Clayey Soil

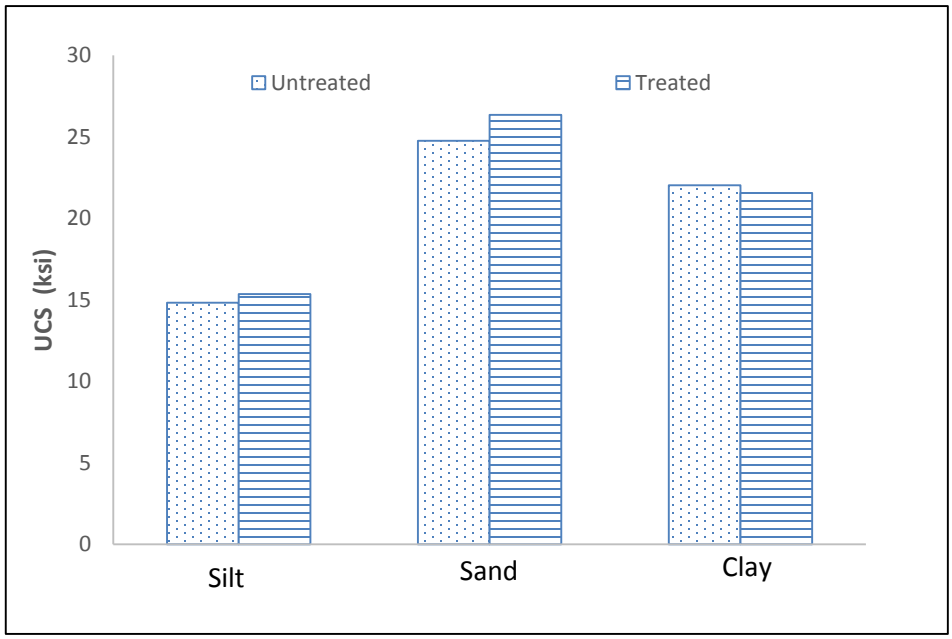


Figure 4.5 Influence of Bacteria-Treatment on Unconfined Compressive Strength

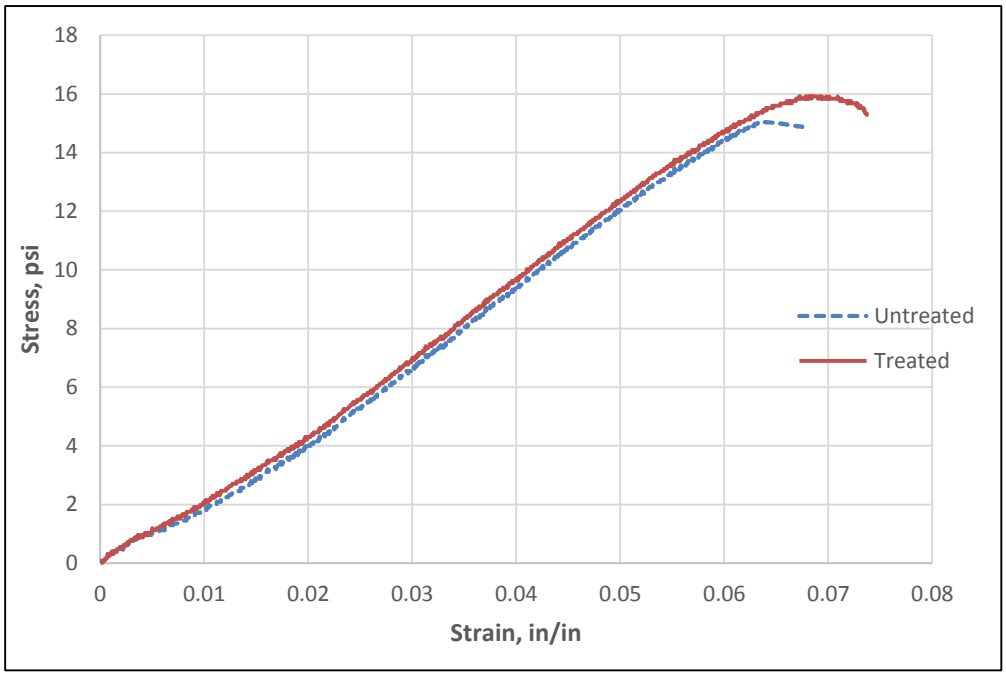


Figure 4.6 Influence of Bacteria-Treatment on Stress-Strain Relationship for Silty Soil

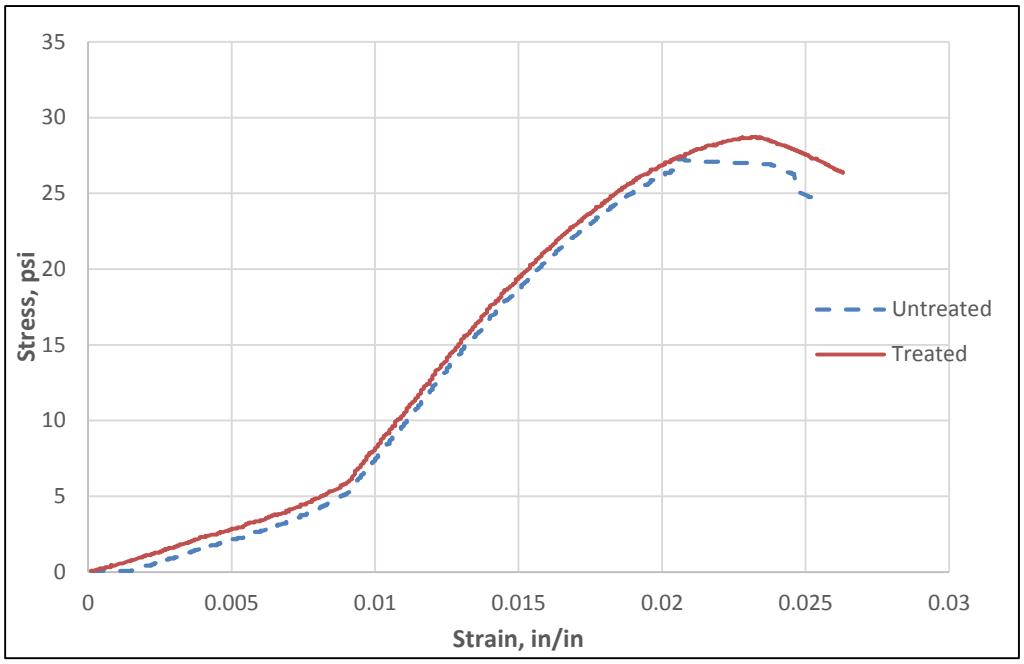


Figure 4.7 Influence of Bacteria-Treatment on Stress-Strain Relationship for Sandy Soil

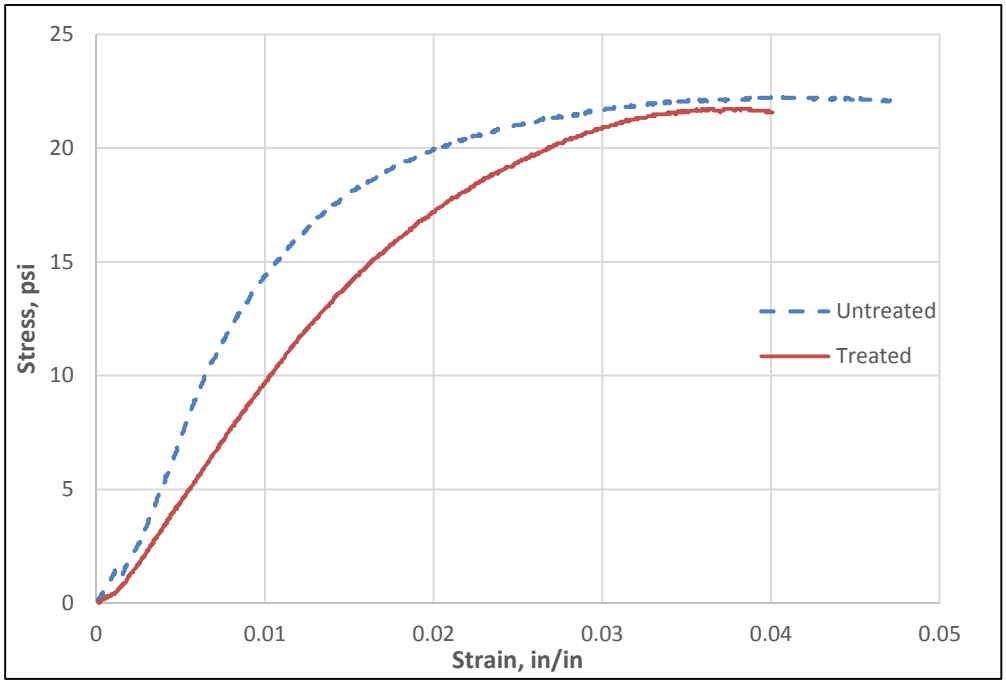


Figure 4.8 Influence of Bacteria-Treatment on Stress-Strain Relationship for Clayey Soil

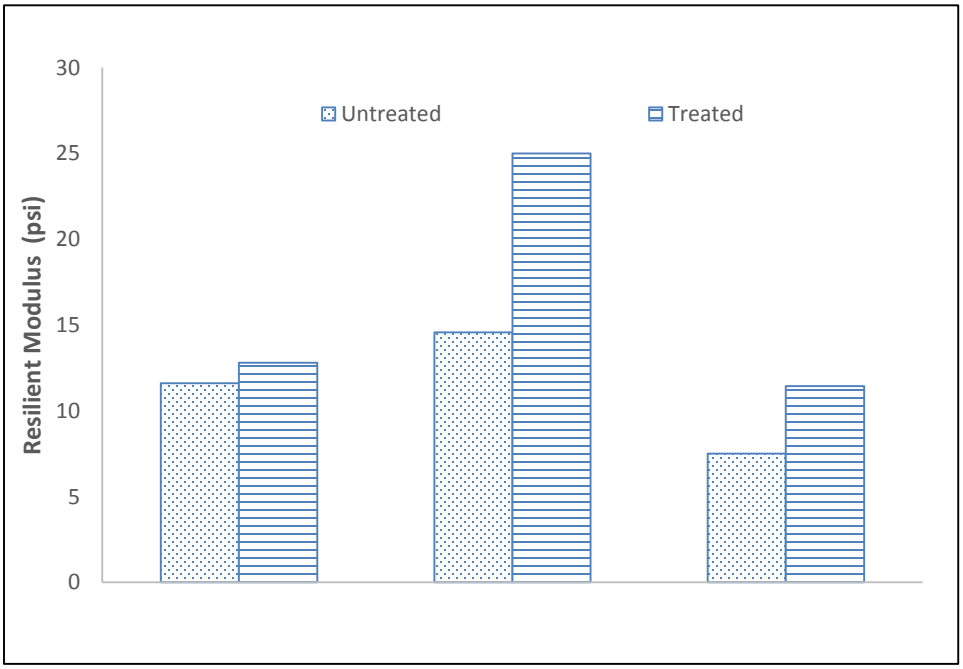


Figure 4.9 Influence of Bacteria-Treatment on Resilient Modulus

The FFRC test results are shown in Figure 4.10. The seismic modulus of sandy soil increased significantly from 19.4 to 23.5 ksi after bacteria-treatment. Additionally, the seismic modulus of silty soil slightly increased from 7.5 to 8.6 ksi after bacteria treatment. However, seismic modulus of clayey soil decreased from 13.6 to 10.3 ksi due to bacteria-treatment. The standard deviation of measured resilient modulus was less than 1 ksi for all the soil types.

Overall, the test results suggest that the bacteria-treatment improved sandy soil mechanical properties significantly while its influence on silty and clayey soil was mixed. One of the factor leading to mixed behavior can be attributed to the process of preparing soil samples. The bacteria-treated soils were crushed and oven-dried before molding specimens for testing. The main benefit of bacteria-treatment is that it fills up pores within the treated sample. The crushing and drying of soil minimizes the benefit of pore clogging. Therefore, test setup needs to be developed that treats soil on large scale which allows in-situ testing like California Bearing Ratio (CBR) testing. The other alternative could be that cores can be extracted from large scale sample and tested in the laboratory to identify influence of bacteria-treatment.

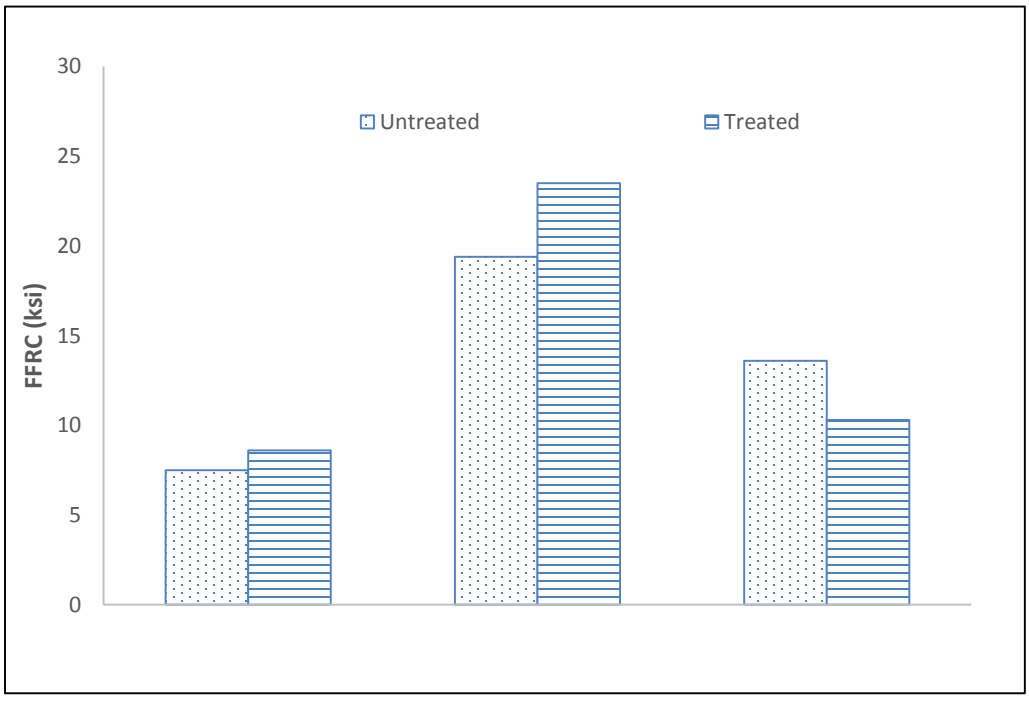


Figure 4.10 Influence of Bacteria-Treatment on Seismic Modulus

4.2 Micro Level: XRD Analysis

Small amount of crushed soil sample after bacteria-treatment was collected and dried at 100°C and then analyzed using XRD. Cu Kα radiation of wavelength λ=1.5406 Å was used to examine the fragment of soil in a scanning range 10-75° in 2θ scale at a scanning speed 5°/min in continuous scan mode. XRD patterns obtained from two trials are presented in Figures 4.11 and 4.12. Calcite present in the sample is shown as a calcite peaks. Further evaluation and comparison could not be performed due to equipment breakage.

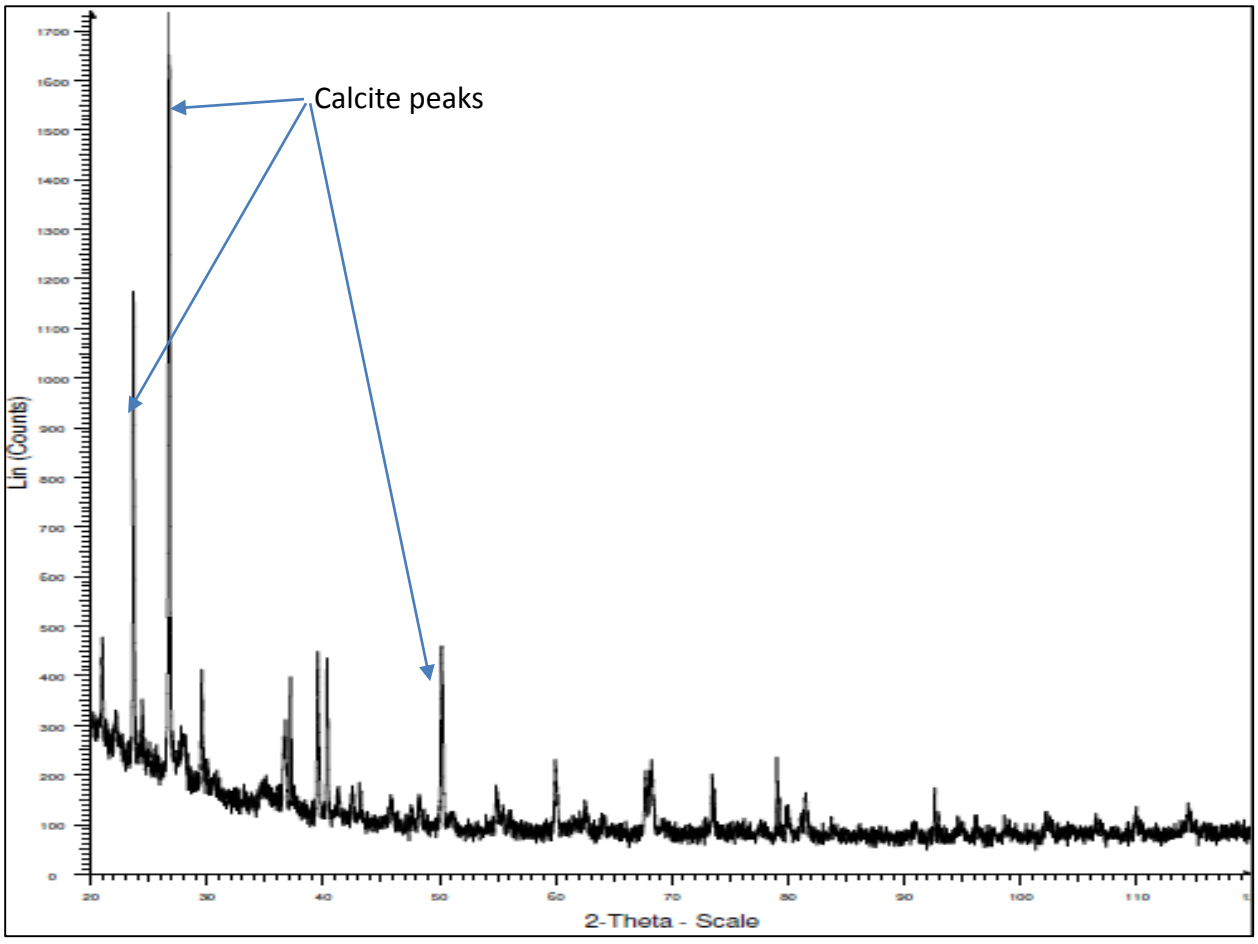


Figure 4.11 X-Ray Diffraction Pattern of Sandy Soil Treated with Bacteria (Trial 1)

4.3 Micro Level: SEM Analysis

Micro-structure analysis of untreated and treated bacteria soils was performed using Hitachi TM-1000 SEM. SEM images were obtained using 3kV of energy and at different magnification level. The advantage of using high-resolution low voltage imaging is that it allows observation of compositional differences as well as precise location of nanoparticles within the individual pores.

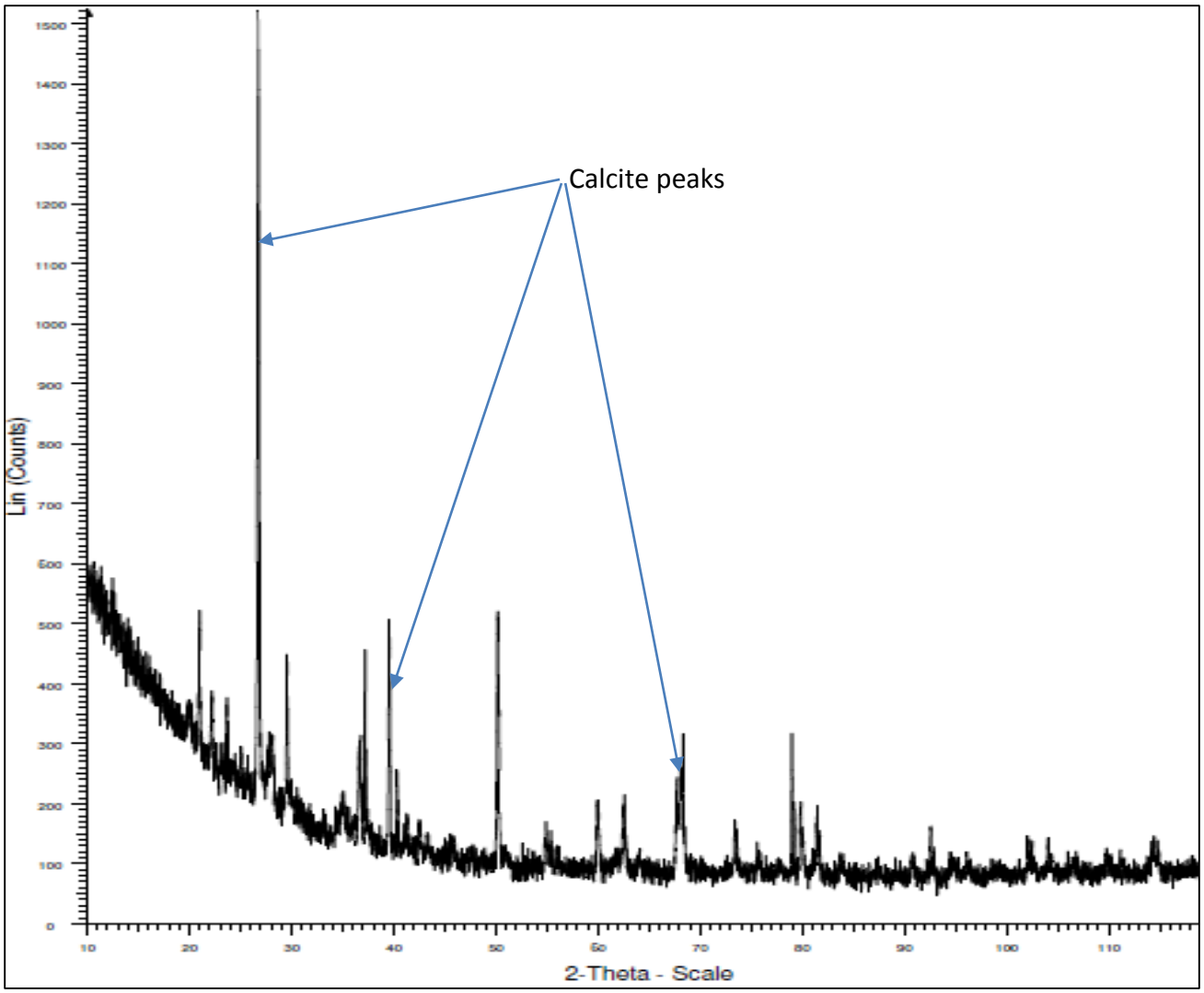


Figure 4.12 X-Ray Diffraction Pattern of Sandy Soil Treated with Bacteria (Trial 2)

The SEM images (Figure 4.13 – 4.18) indicate precipitation of calcite crystals over the soil surface and from these images shape and distribution of the particles can be understood. Low voltage analysis helps in reducing the charging effect of the scanned area and also reduces local radiation damage of the sample induced by energetic electrons. The images were obtained at

specific magnification and at different locations. From the SEM images, the uniform distribution of the calcite crystals over the soil surface is confirmed.

SEM images for untreated sandy soil are shown in Figure 4-13 while for bacteria treated sandy soil is shown in Figure 4-14. Both the images were obtained at similar magnification levels. Comparing the images of treated and untreated soils, it is observed that, there is no formation of any crystal like structure in untreated soil even when the magnification level was increased from 3,000 to 5,000. While for treated soil, at 3,000 magnification level a layer of calcite crystals was observed over the soil surface while an increase in magnification level to 5,000 suggested a a rhombohedral calcite structure. An increase in magnification level revealed the distribution of calcite over the soil surface.

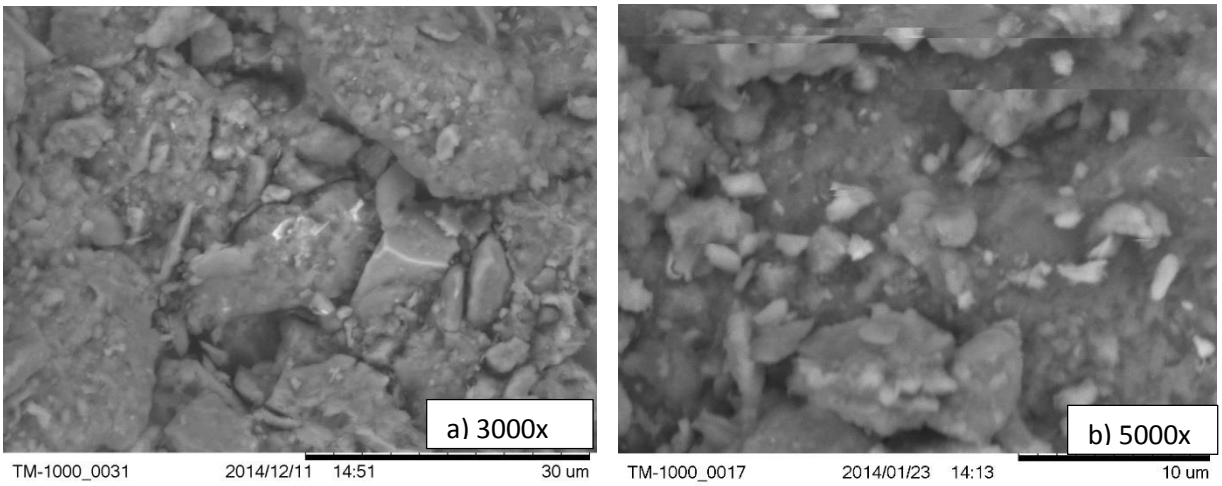


Figure 4-13 SEM Images of Untreated Sandy soil

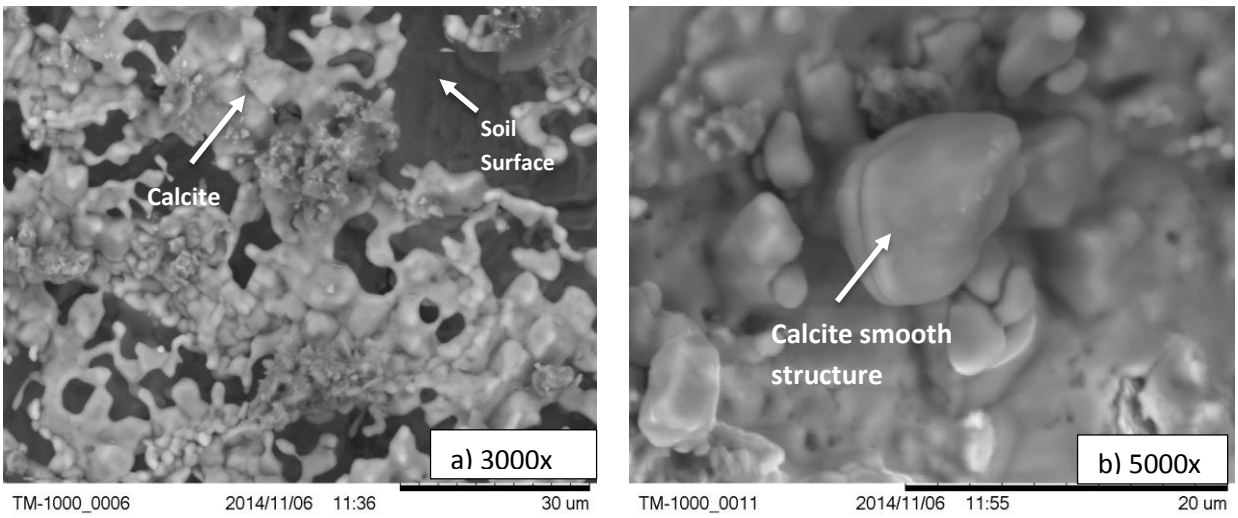


Figure 4-14 SEM Images of Treated Sandy Soil

The SEM images for untreated silty soil (Figure 4-15) and treated silty soil (Figure 4-16) also exhibited presence of calcite precipitation similar to the sandy soil.

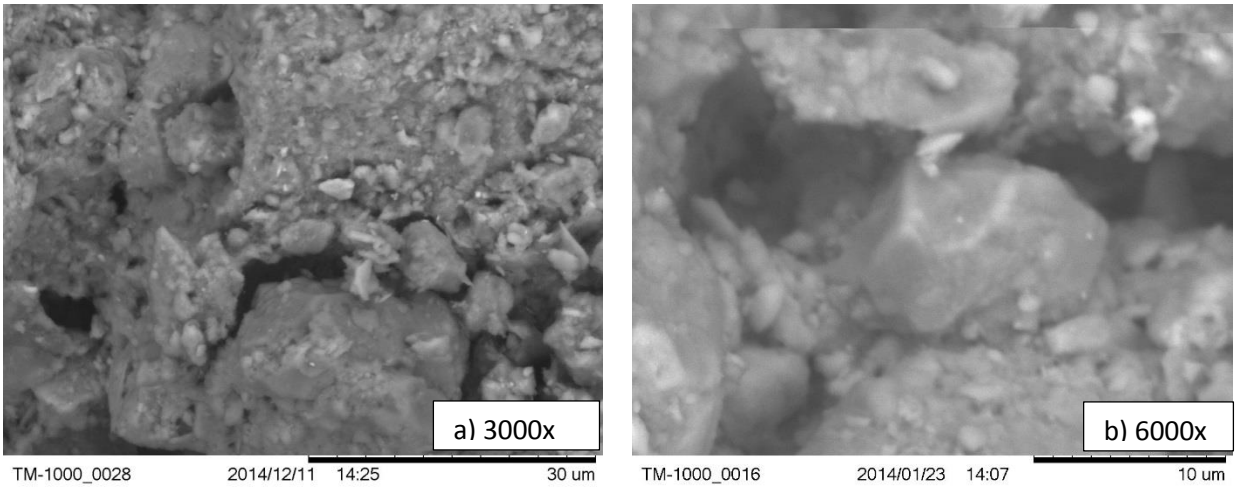


Figure 4-15 SEM Images of Untreated Silty soil

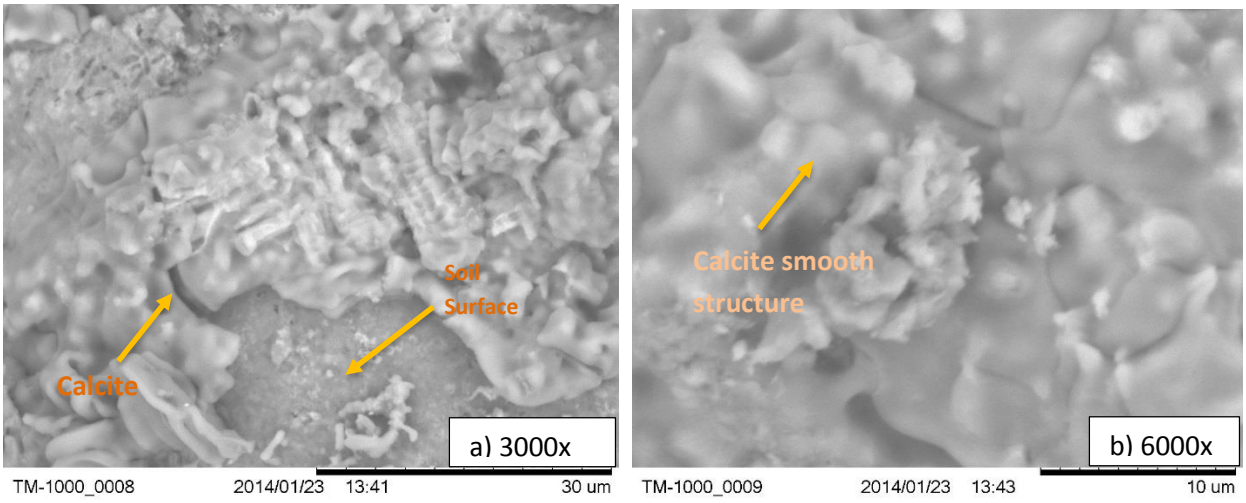


Figure 4-16 SEM Images of Treated Silty Soil

SEM images of untreated clay (Figure 4-17) and treated clay (Figure 4-18), did not show much difference. This may have occurred due to less formation of calcite in clayey soil. From SEM images, we can see the formation of calcite but the distribution is not uniform and also at higher magnification the crystal structure is not clear.

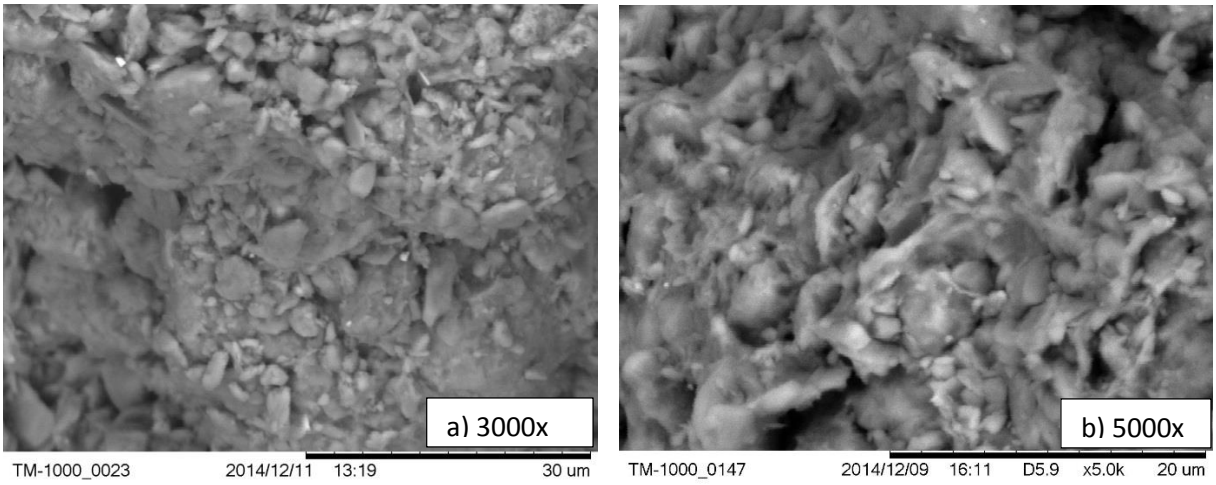


Figure 4-17 SEM Images of Untreated Clayey Soil

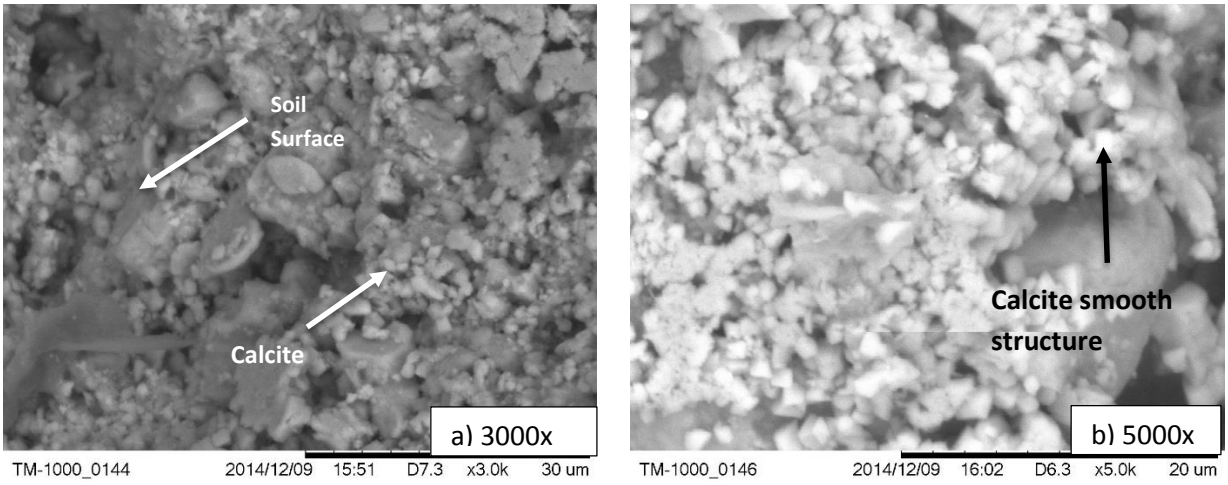


Figure 4-18 SEM Images of Treated Clayey Soil

The micro level analysis confirmed the results obtained from macro level analysis indicating that the calcite precipitation is more dominant in sandy and clayey soils while minimal precipitation could be observed in clayey soils. One of the explanation could be that porosity of soil is important for providing nutrient to the bacteria for better precipitation of calcite.

5. SUMMARY, CONCLUSIONS AND RECOMENDATION

The main purpose of this project was to evaluate the feasibility of soil stabilization through bacteria-treatment of the soils. The aerobic bacteria was used to increase the compressive strength and stabilize the soil through calcite precipitation. Since, the whole process of urease activity increases pH of the environment, the survivability of bacteria becomes an issue. To survive in a high pH (around 12), the bacteria were mutated by exposing bacteria to ultraviolet rays. The advantage of mutation is that the bacteria can withstand higher pH as well as forms more calcite than normal bacteria. The soil with and without bacteria-treatment was tested to evaluate the influence of treatment.

Based on the analysis of test results, the following conclusions can be drawn:

1. The bacteria induced calcite precipitation was observed and verified using micro level analysis.

2. Bacteria treatment of sandy and clayey soil resulted in enhancing compressive strength as well as modulus. However, clayey soils did not exhibit significant improvement in mechanical properties and could be attributed to the lower porosity of clay which hinders in providing nutrient, thus, lower calcite precipitation.

3. XRD analysis of bacteria-treated samples displayed a larger calcite peak suggesting calcite precipitation.

4. SEM investigation indicated full growth of calcite crystal in bacteria-treated samples and presence of more calcium in those samples.

Although the research was able to identify increase in strength due to MICP, the following needs to be studied further before field implementation:

- In addition to strength, the soil properties such as compressibility, hydraulic conductivity, workability, swelling potential, durability, and volume change tendencies may be altered due to bio-cementation and needs to further evaluated.
- Even though sandy and silty soils exhibited enhancement in strength and modulus, the clayey soil exhibited wither loss or minimal enhancement in measured mechanical properties and needs to be investigated.
- The soil stabilization at large scale needs to be evaluated to identify practicality of bio-cementation.
- Cost benefit analysis needs to be performed to document benefits of bio-cementation.

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7. APPENDIX A

The Free-free Resonant Column (FFRC) tests had been developed for measuring the seismic modulus of Portland cement concrete but have been found suitable for asphalt concrete specimens as well. The FFRC test is performed by generating and measuring the velocity of wave propagation in a medium. When a cylindrical specimen is subjected to an impulse load at one end, seismic energy over a large range frequency will propagate within the specimen. Depending on the dimensions and the density of the specimen, energy associated with one or more frequencies are trapped and resonate as they propagate within the specimen. The goal with this test is to determine these resonant frequencies. Since the dimensions of the specimen are known, if one can determine the resonant frequencies, one can readily determine the modulus of the specimen using principles of wave propagation in a solid rod.

In general, the impulse load generates two types of wave energy: longitudinal and shear. Once the longitudinal resonant frequency, f_L, and the length of the specimen, L, are known, the laboratory Young's modulus, E_{lab}, can be found from the following relation:

$$E_{lab} = \rho (2 f_L L)^2 \quad \text{(A.1)}$$

where ρ is mass density. The mass density is calculated from:

$$\rho = M / (L A_s) \quad \text{(A.2)}$$

where A_s is the cross-sectional area of the specimen. Poisson's ratio, ν, is determined from

$$\nu = \frac{0.5\alpha - 1}{\alpha - 1} \quad \text{(A.3)}$$

where

$$\alpha = \left(\frac{f_L}{f_S} \right)^2 C_{L/D} \tag{A.4}$$

with $C_{L/D}$ being a correction factor when the length-to-diameter ratio differs from 2 and f_s=shear (or torsional) resonant frequency.

One of the advantages of the two methods is that they provide properties that can also be directly measured in the field with a nondestructive testing device called the Portable Seismic Property Analyzer (PSPA).

The free-free resonant column setup was also used in the laboratory for determining the seismic modulus of asphalt concrete and synthetic specimens. A simplified setup has been developed at UTEP, as shown in Figure A.1 so that a test can be performed in less than 1 minute. The FFRC consists of a data acquisition system, an accelerometer, and an instrumented hammer. The accelerometer is securely placed on one end of the specimen, and the other end is impacted with the instrumented hammer. The signals from the load cell and accelerometer are used to determine the resonant frequencies. The frequencies of the primary (compression) wave and secondary (shear) wave are found with the help of the developed software that could interpret the data from the sensors and presents it graphically (Figure A.2). The modulus of the specimen is determined using principles of wave propagation by measuring the dimensions and weight of the specimen.

To perform the FFRC test, the diameter, length and mass of the specimen are measured before the specimen is placed on test stand. An accelerometer is attached to one end of the specimen. A convenient way of attaching the accelerometer to the specimen is to use a magnet. The specimen is struck mildly and rapidly with the instrument hammer at the opposite end of the specimen. The test is repeated three times to obtain an average resonant frequency.

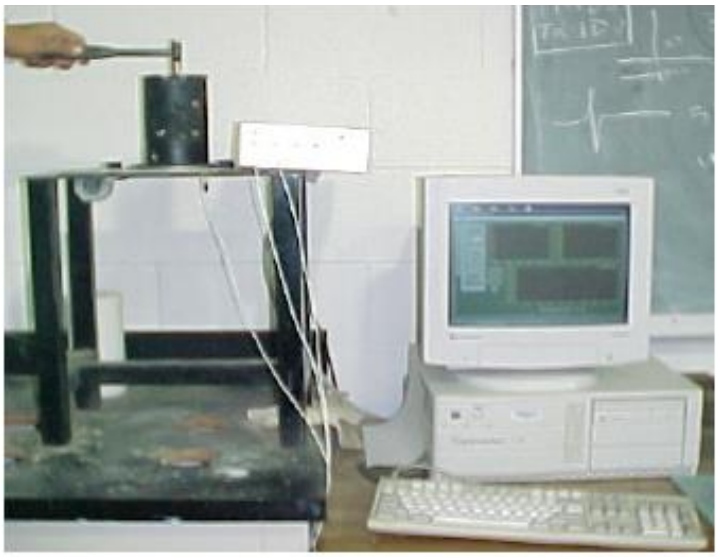

Figure A.1 – Free-free Resonant Column Test Setup

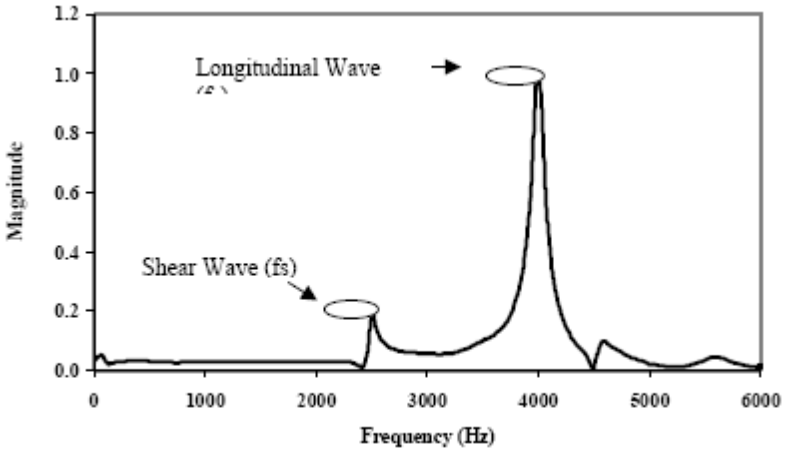

Figure A.2 – P-wave and S-wave Generated by Using FFRC

CHAPTER 3

Biodeterioration of Construction Materials –

A General Overview

FINAL REPORT
February 2016

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TECHNICAL REPORT STANDARD TITLE PAGE

1. Report No.	2. Government Accession No.	3. Recipient's Catalog No.
Report # provided by Rutgers		

4. Title and Subtitle	5. Report Date
Biodeterioration of Construction Materials – A General Overview	February 2016
	6. Performing Organization Code CAIT/University of Delaware
7. Author(s) Farzana Atique Nii Attoh-Okine	8. Performing Organization Report No. Report # provided by Rutgers
9. Performing Organization Name and Address University of Delaware Newark, DE 19716	10. Work Unit No.
	11. Contract or Grant No. DCTR432139
12. Sponsoring Agency Name and Address Center for Advanced Infrastructure and Transportation Rutgers, The State University of New Jersey 100 Brett Road Piscataway, NJ 08854	13. Type of Report and Period Covered Final Report January 2014 - December 2015
	14. Sponsoring Agency Code

15. Supplementary Notes
U.S. Department of Transportation/Research and Innovative Technology Administration
1200 New Jersey Avenue, SE
Washington, DC 20590-0001

16. Abstract

Developing a well balanced budget and expenditure program for any infrastructure network requires predicting the rate of deterioration of the infrastructure and nature of the changes in its physical, chemical and biological characteristics. The correct timing, type and cost of maintenance and rehabilitation needs can therefore be estimated. For the model to be useful for evaluating the primary options available in maintenance and rehabilitation, it must show explicitly the primary effects of loads, age, distress and biological effects on the structure. This will enable better trade-offs between the intervention options of minimal maintenance at different periods and condition levels to be made. During the service life of the infrastructure, natural aging, loads, and damage of structure are caused by different physical, chemical and biological processes. This report attempts to introduce the missing component of infrastructure biodeterioration. The report presents a general overview and definitions only.

17. Key Words	18. Distribution Statement
Construction Materials; Bio deterioration; Infrastructure performance	

19. Security Classification (of this report)	20. Security Classification (of this page)	21. No. of Pages	22. Price
Unclassified	Unclassified	14	

Form DOT F 1700.7 (8-69)

Acknowledgments

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1. DESCRIPTION OF THE PROBLEM

Civil infrastructure systems are critical assets for a country's socioeconomic development. Designing and maintaining infrastructure systems have become important issues due to the fact that many infrastructures are aging and nearing the end of their useful life time frame. A comprehensive life-cycle cost analysis should consider factors that can cause a structural system to perform unacceptably at any point in its lifetime. This includes extreme events, such as earthquakes and hurricanes, or the progressive and sustained loss of capacity caused by operational or environmental factors. Biodeterioration of structural materials is an important component in causing continued loss of capacity in structures. Even though the effects of biodeterioration on wood in an aggressive environment are widely known, not much is known about the effects of biodeterioration on reinforced concrete and steel components of infrastructure systems.

As with other civil infrastructure systems, developing a well-balanced budget and expenditure program for a highway network requires predicting the rate of deterioration of the pavement and the nature of the changes in its physical, chemical, and biological characteristics. For the model to be useful, it must evaluate the primary effects of traffic, pavement strength, age, distress, and biological elements on the structure.

For the model to be useful for evaluating the primary options available in maintenance and rehabilitation, it must show explicitly the primary effects of traffic, pavement strength, age, distress, and biological effects on the structure. This will provide better trade-offs between the intervention options of minimal maintenance, patching, recycling, resurfacing, and other maintenance tasks.

1.1 DEFINITION OF BIODETERIORATION

A widely accepted definition of biodeterioration was proposed by Hueck (1968) as: "any undesirable change in the properties of a material caused by the vital activities of organisms." Similarly, Rose (1981) defines biodeterioration as the process by which "biological agents (i.e., live organisms) are the cause of the [*structural*] lowering in quality or value."

1.2 CLASSIFICATION OF BIODETERIORATION

According to Gaylarde et al. (2003) biodeterioration can be classified into (Sanchez-Silva and Rosowsky, 2008):

1. Physical or mechanical: This encompasses all physical actions of organisms that contribute to a physical deterioration of the material. It is often referred to as the process by which live organisms disrupt the material structure by growth or by movement but do not use the material as food source.
2. Fouling or soiling (aesthetic): This is caused by the presence of organisms, their dead bodies, excreta, or metabolic products forming a microbial layer known as biofilm on a surface material. It is primarily associated with the microorganism causing unacceptable appearance but not affecting the material performance.
3. Chemical: (1) assimilatory and (2) dissimilatory. Assimilatory occurs when the organisms use the structural component as a source of food (i.e., carbon and/or energy source), thus modifying the properties of the material (e.g., degradation of fuels, metals). Dissimilatory occurs when the live organisms' excreted waste products or other substances (e.g., H_2S, FeS) adversely affect the material.

The common live organisms associated with biodeterioration of construction materials are as follows:

1. Marine borers (e.g., gribble and shipworms);
2. Insects (e.g., termites and wood-boring beetles);
3. Fungi (soft rots, white and brown rots), primary and secondary molds, stainers algae, and lichens; and
4. Microorganisms (e.g., bacteria).

1.3 ENVIRONMENTAL CONDITIONS FOR ORGANISM GROWTH

The existence and growth of microorganisms require environmental conditions such as the availability of water, light, oxygen, and nitrogen within an environment with appropriate temperature and pH.

The water requirement for microorganisms is expressed by the so-called *water activity* of the environment (a_w). The water activity is defined as: $\ln(a_w)=-(\nu \cdot m \cdot \theta)/55.5$; where ν =number of ions formed by each solute molecule; m =molar concentration of solute; and θ =molar osmotic coefficient (Rose 1981). Pure water has a value of a_w =1.0, and this value decreases when solutes are dissolved in it. Microorganisms can grow in media with $0.63<a_w<0.99$. It is known that bacteria require values of $0.93<a_w<0.99$, while yeast or molds grow in lower values of $0.88<a_w<0.91$.

Table 1 by Viitanen and Salonvaara (2001) shows how the humidity and moisture range and the temperature range affect building components.

Table 2. Organisms involved in damages and defects of building components (Viitanen and Salonvaara, 2001)

Organism	Damage/problem type	Humidity or moisture range (RH or MC %)	Temperature range (℃)
Bacteria	Biocorrosion of many different materials, smell, health problems	Wet materials RH>97%	ca -5 to +60
Mold fungi	Surface growth on different materials and health problems	Ambient RH>75%, depends on duration, temperature, and species	ca -5 to +45
Blue stain fungi	Blue stain of wood, permeability change of wood	Wood moisture content > 25-120%, RH >95%	ca -5 to +45
Decay fungi	Different types of decay in wood (soft rot, brown rot, or white rot), deterioration of other materials, strength loss of materials	Ambient RH>95%, MC >25-120%, depends upon duration, temperature, fungus and materials	ca 0 to +45
Algae and lichen	Surface growth of different materials outside or weathered material	Wet material, also nitrogen and low pH are needed	ca 0 to +45
Insects	Different type of damages in organic materials, surface failures or strength loss	Ambient RH >65% depends on duration, temperature, species, and environment	ca 5 to +50

1.4 EFFECTS OF BIODETERIORATION ON CONSTRUCTION MATERIALS

1.4.1 WOOD

Wood is the primary material used in residential construction in the US. The low durability of wood construction is mainly caused by the lack of attention given to construction details, which can cause the growth of microorganisms. Wood deterioration depends significantly on the presence of water. Laboratory testing has shown that the optimal wood moisture levels for most decay fungi are between 40 and 80% (Scheffer, 1973). A oxygen concentration also increases the possibility of biodeterioration. In addition to water and oxygen, nutrients are required for biological activity; this is closely related to the ratio of carbon to nitrogen in the wood. When this ratio is very high, the low availability of nitrogen reduces the chance of fungi attack. Other favorable conditions for increased chance of microorganism growth are pH values between 3 and 6 and temperature in the range of 0-45 ^{0}C. Wood deterioration results from the destruction of 1) cellulose, 2) hemicellulose, or 3) lignin. Figure 1 shows how different types of organisms damage the wood structure.

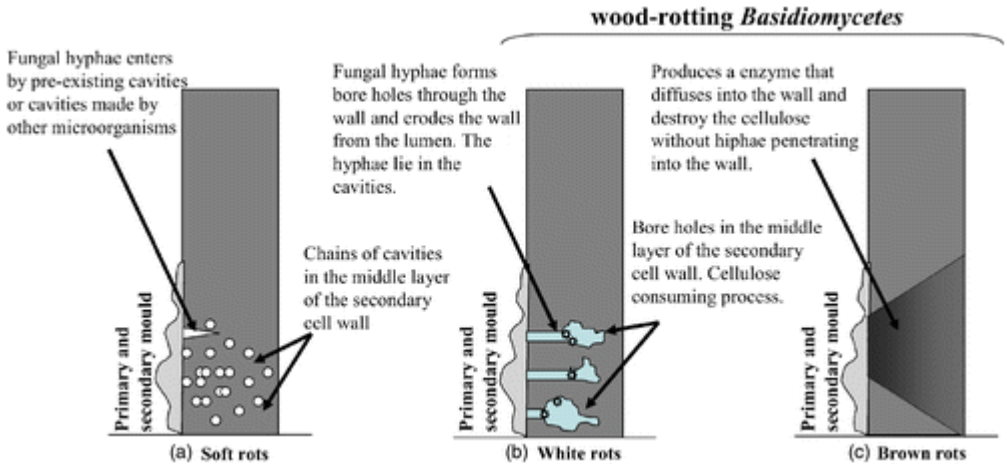

Figure 19. Damage effects of soft, white, and brown rot on wood structure (Sanchez-Silva and Rosowsky, 2008)

The structural degradation of wood depends on the relationship between the structure, the characteristics of the organism, and the environment. Microorganisms use the wood as a food source, thereby reducing the weight of the wood. Figure 2 shows the phenomenological decay of wood resistance as a result of fungi. The weight loss (biomass loss) is a measure of decay and is expressed as:

$$W_L = \frac{W_0 - W_{decayed}}{W_o^d} \qquad (1)$$

Where W_o= original weight, $W_{decayed}$=decayed weight, and W_o^d= origin oven dry weight.

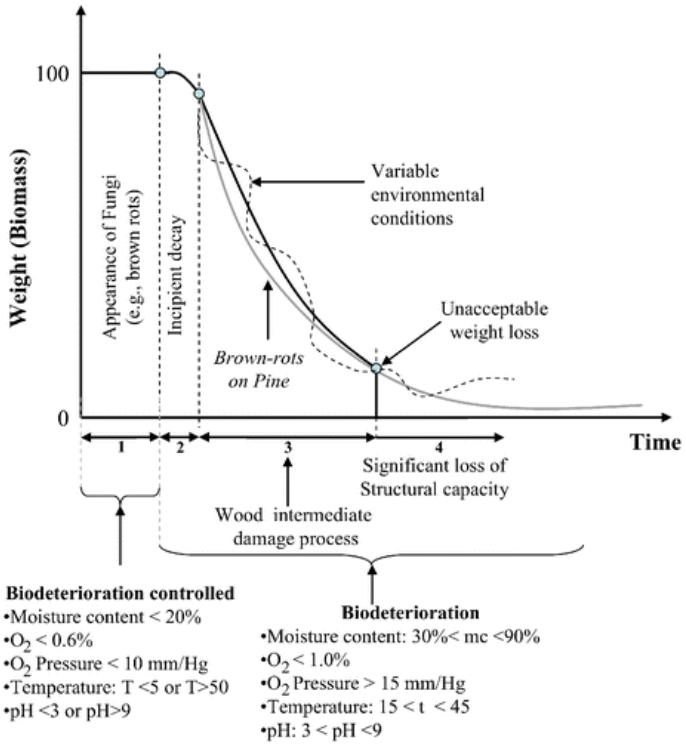

Figure 20. Phenomenological decay of wood resistance as a result of fungi (Sanchez-Silva and Rosowsky, 2008)

1.4.2 METAL

Biodeterioration of metals occurs from the associated processes that accelerate corrosion rather than from the direct action of microorganisms on the material. Microorganism activity related to metal corrosion is classified in terms of:

1. Oxygen users.
2. Acid producers.
3. Slime formers.

The corrosion process is electrochemical in nature and can occur under these three circumstances:

1. Attack by concentration cells.
2. Action of sulfides (S^{-2}).
3. Effect of organic and inorganic acids.

The process of corrosion caused by biofilm is shown in Figure 3.

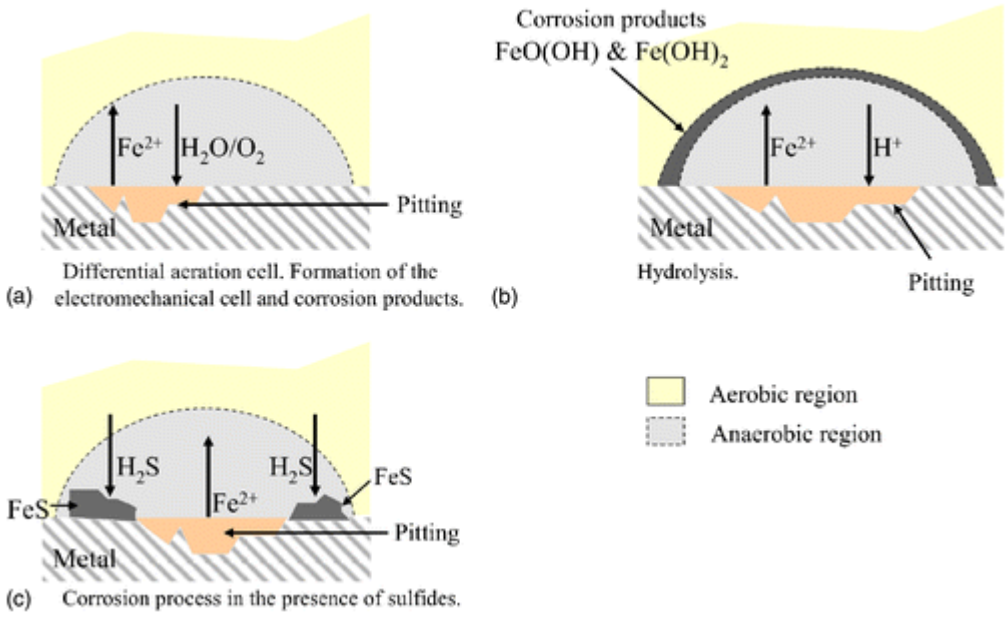

Figure 21. Corrosion caused by concentration cell formation (Sanchez-Silva and Rosowsky, 2008)

1.4.3 STONE

Evaluation of the biological contribution of stone decay begins with the description of the type of stone material and exposure with the conditions for the object/building and the presence of water and nutrients. Microbial colonization of stones depends on environmental factors, such as water availability, pH, climatic exposure, and nutrient sources, and on petrologic parameters. The parameters are mineral composition, the type of stone, and the porosity and permeability of the rock material (Warscheid and Braams, 2000).

1.4.4 REINFORCED CONCRETE (RC)

Reinforced concrete structures are highly durable; however, deterioration of RC is often found in structures subjected to an aggressive environment such as sulfate attack and chloride ion penetration. The main organisms involved in RC biodeterioration are: 1) bacteria, 2) fungi, and 3) algae and lichens. All of those organisms erode and perforate the concrete (Gaylarde et al., 2003). The favorable environmental conditions for organism growth on concrete surfaces are (Sanchez-Silva and Rosowsky, 2008):

1. Elevated relative humidity (i.e. between 60 and 98%.
2. Long cycles of humidification and drying or freezing and defrosting.

3. High carbon dioxide concentrations.
4. High concentration of chloride ions or other salts (e.g. marine environments).
5. High concentration of sulfates and small amount of acids. (e.g. sewer pipes or residual water treatment plants).

Table 2 shows the characteristics of the main bacteria that cause biodeterioration in concrete materials.

Table 3. Effects of bacteria on RC structures (Bastidas-Arteaga et al., 2008)

Bacteria type	Lifestyle	Temperature and pH range	Consequences on concrete
1. Cyanobacteria	Autotrophic, aerobic or anaerobic	−60 to 85 °C (wide pH range)	Generate tensile stresses leading to an increment in the size of cracks.
2. Nitrobacteria	Heterotrophic and anaerobic	18–25 °C, pH < 7.5	Nitrifying bacteria (Nitrosonoma and Nitrobacter) produce calcium nitrate by solubilizing some of cement components.
3. Sulfur-reducing bacteria	Heterotrophic and anaerobic	25–44 °C, 5.5 < pH < 9	Produce H_2S that is used for the *sulfur-oxidizing bacteria* to produce sulfuric acid. This process is commonly called *concrete corrosion*.
4. Sulfur-oxidizing bacteria	Heterotrophic and aerobic	25–44 °C, 2 < pH < 9	Produce sulfuric acid, acetic acid, sulfates, sulfur, sulfites, and polythionates that affect the concrete chemically.
4.1 *Neutrophilic sulfur oxidizing* (NSOM)		pH ≈ 7	
4.2 *Acidophilic sulfur oxidizing* (ASOM)		pH < 3.0	

1.4.5 CONCRETE

Mechanisms of biodeterioration can be summarized as shown in Table 3 (Silva and Naik, 2013). Organisms can grow on concrete surfaces under these conditions: elevated relative humidity, long cycles of humidification and drying or freezing and thawing, high carbon dioxide concentrations, high concentration of chlorides or other salts, and high concentration of sulphates and small amounts of acids. Attacks of organisms on concrete can be 1) physical or mechanical, 2) fouling or soiling, or 3) chemical attack by using a structural component as a food source or by excreting waste products that

affect the material. Biodeterioration of concrete structures can also be caused by the settlement of macroscopic organisms, such as zebra mussels.

Table 4. Mechanisms of biodeterioration (Silva and Naik 2013)

Mechanisms	Examples
Physical or mechanical attack	Can be from fungi, cyanobacteria, algae, mussels
Fouling or soiling	Discoloration, biofilm enables growth of other organisms, traps moisture
Chemical attack	Microbes utilize ions present in cement, metabolites solubilize minerals, enzymes break down mortar

2. APPROACH

There have been a large number of performance models developed in the past decades; however, a coupled performance model of roughness and biological components is not yet available. To develop the model, discrete values of pavement distress data such as cracking (traverse, edge cracking, and other types), raveling, rut depth, and pavement roughness will be collected. Also, three primary variables, pavement strength, traffic loading, and regional location in terms of m-factor, will also be collected.

Microbes have shown to consume and/or degrade asphaltene and polyaromatic compounds from both natural (Raggi et al., 2013, Mahmoudi et al., 2013) and human-made sources. Ultimately, the goal of this research is to quantify the contributions of these microbes to asphalt binder stability and degradation. Microbial colonization within the binder and production of extracellular polysaccharides (EPS) can strengthen the binder. However, consumption of these polysaccharides or of the polymers mixed with the binder to improve its performance can reduce the strength and flexibility of the asphalt. To be able to predict the conditions under which these processes will occur, we must first isolate microbial species capable of growth in the dry, organic-rich environment of asphalt, identify their requirements for growth, and quantify the rate of EPS production and asphaltene degradation under both ideal (laboratory) and non-ideal (field) conditions. The mathematical models built on these data will allow more accurate prediction of performance under field conditions of a variety of structures.

A summarized algorithm for predicting pavement roughness coupled with biodeterioration can be universally applicable and serve as a primary performance model for pavement management forecasting and pavement design. Given the uncertainties in both the parameters and the model, the probabilistic approach appears to be more appropriate than the deterministic approach. In modeling the

performance over the pavement life cycle, the important features to capture are the two phases of the deterioration rate, before and after cracking, and the different mechanisms causing roughness.

3. METHODOLOGY

Bastidas-Arteaga et. al (2008) proposed a model for the variation of concrete products with time as:

$$W_m(t) = \begin{bmatrix} 0 & \tau_{ini} \geq t \\ f(t, DO_2, A, g) & \tau_w \geq t \geq \tau_{ini} \\ h(t, DO_2, A, g) & t \geq \tau_w \end{bmatrix} \quad (2)$$

Where τ_{ini}, τ_w= time to corrosion initiation and time to corrosion of the total bar surface, respectively. DO_2= diffusion coefficient of O_2, and g describes the geometry of the problem.

3.1 COUPLED DETERIORATION MODEL

The coupled deterioration model suggested by Bastidas-Arteaga et al. (2008) took into account three effects: 1) corrosion induced by chloride ingress, 2) cracking as a result of corrosion products, and 3) reduction of concrete cover caused by biodeterioration. Figure 5 represents the conceptual description of the coupled model.

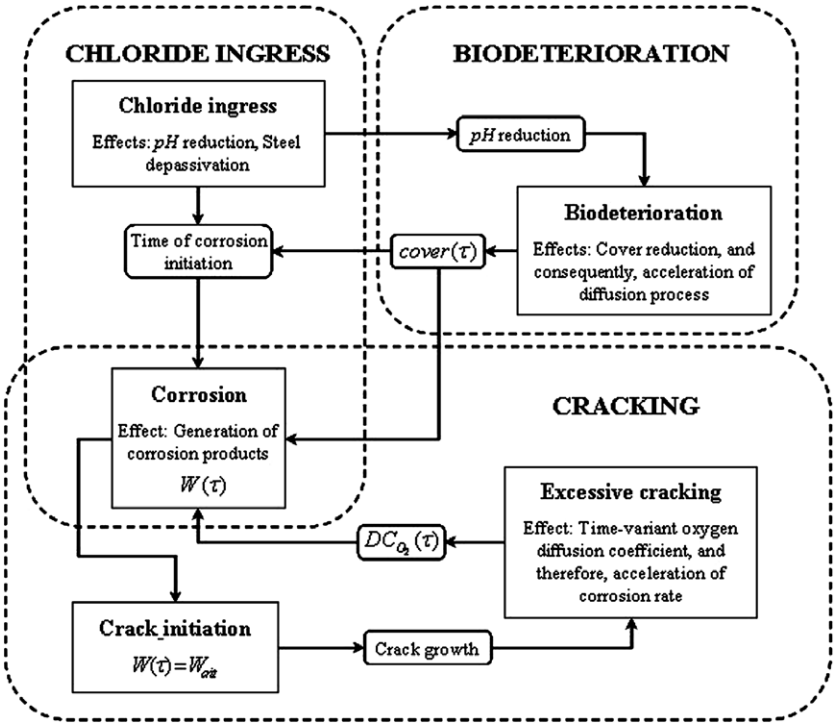

Figure 22. Conceptual description of the coupled model for biodeterioration, chloride ingress, and cracking of concrete structures (Bastidas-Arteaga et. al., 2008)

Where $DC_{O_2}(\tau)$ is the diffusion coefficient of oxygen in the concrete, $W(\tau)$ is the amount of corrosion product, and the amount of critical rust products W_{crit} (g) is defined as the amount at which all free spaces between the steel bar and the concrete are filled and the cracking begins.

Due to live organism action, the thickness of the concrete cover is a time-dependent function. The thickness at time τ, cover (τ), is calculated as:

$$cover(\tau) = c - \int_0^\tau \gamma(\tau) d\tau \qquad (3)$$

Where c in the cover at τ =0 and $\gamma(\tau)$ is the biodeterioration rate function as discussed below.

3.2 TIME-DEPENDENT BIODETERIORATION RATE FUNCTION

Biodeterioration is a complex process in which many classes of organisms, environmental variables, and other processes interact. Computing the RC time-dependent biodeterioration rate is an important aspect for the accuracy of the numerical model. Bastidas-Arteaga et al. (2008) remarked that there is a

lack of availability of numerical models and no sufficient data available to perform appropriate statistical analysis. The method proposed by the authors is based on fuzzy inference, which is a valuable tool for managing situations where information about a process can only be described conceptually or if there is not enough data to build a robust statistical model. Based on fuzzy logic, Bastidas-Arteaga et al. (2008) proposed a model to obtain a time-dependent function of the biodeterioration rate. The process can be divided into three stages as shown in Figure 5.

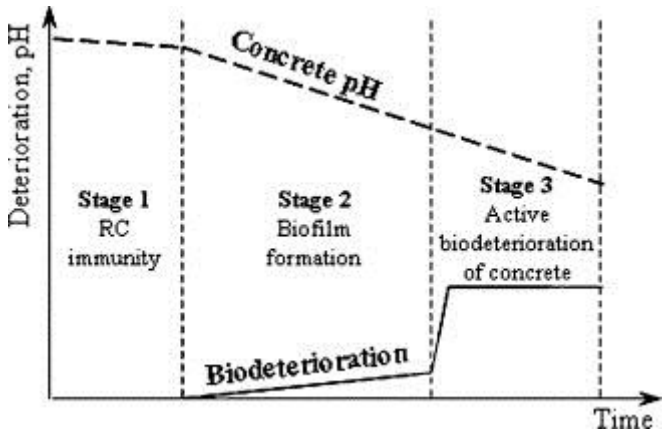

Figure 23. Conceptual model of biodeterioration (Bastidas-Arteaga et. al, 2008)

The process is divided into three stages:

1. RC immunity: In this stage, due to high concrete alkalinity (pH between 11 and 13), the organisms cannot survive or adhere to the concrete; therefore, reinforced concrete is immune to biological damage.

2. Biofilm formation: The presence of CO_2 in the atmosphere causes carbonation, which in turn leads to a reduction of the pH of the RC surface (until approximately 9). In addition, erosive action of the water and/or friction with other materials generate a certain roughness on the concrete surface that allows microorganisms to adhere, forming the biofilm.

3. Active biodeterioration of concrete: In this stage, the concrete pH continues to decrease by the joint action of carbonation and organisms until it reaches a value of pH < 5. The RC surface is highly deteriorated, and the cracks have a critical size. All those conditions make it possible for other organisms to stick as well to the biofilm on the surface, contributing to RC chemical deterioration. When the concrete is cracked, some organisms, like the endolithic cells, algae, and fungi, ingress into cracks, generating tensile stresses that deteriorate the concrete by increasing the crack size. Crack formation can also be promoted as a result of the weakened concrete microstructure caused by fungi and other microorganisms that might have already

diffused into the concrete matrix.

Based on the conceptual model, the membership functions for the age of the structure (i.e., in time units (years) and the biodeterioration rate c (mm/year) can be defined. Then, the structure life can be divided into two stages:

1. Initial age: At this age, microorganisms can hardly live on the surface of the concrete because it is even and the pH of the concrete is high.
2. Active biodeterioration age: At this stage, the biodeterioration is active because the surface offers optimal conditions for colonization by microorganisms.

These stages are represented by membership functions of sigmoidal form (see Figure 6a):

$$\mu_{age}(\tau, a, b) = \frac{1}{1+\exp(-a(\tau-b))} \quad (4)$$

Where τ is the time in years, and a, b are constants that define the shape of the function. In addition, it is necessary to define two membership functions for the corresponding biodeterioration rate, γ. The first corresponds to the initiation of the biodeterioration process for which the maximum membership value assigned is γ_{ini} = 0 mm/year. Then, in this case, the modified Gaussian function that describes the initial biodeterioration rate membership is given by (as shown in Figure 6b):

$$\mu_{\gamma 1}(\gamma, \sigma) = \begin{bmatrix} \exp\left(-\frac{1}{2}\left[\frac{\gamma}{\sigma}\right]^2\right) if\ \gamma \gg 0 \\ 0 \qquad\qquad otherwise \end{bmatrix} \quad (5)$$

Where σ is a shape parameter. The second function corresponds to the time within which biodeterioration is active; thus, based on the values of the biodeterioration rate caused by the acidophilic sulfur oxidizing microorganisms (ASOM) and the average active biodeterioration rate, γ_{av}, is assigned to the maximum membership value. In this case, the membership function that describes the corrosion rate is:

$$\mu_{\gamma 2}(\gamma, \gamma_{av}, \sigma) = \exp\left(-\frac{1}{2}\left[\frac{\gamma-\gamma_{av}}{\sigma}\right]^2\right) \quad (6)$$

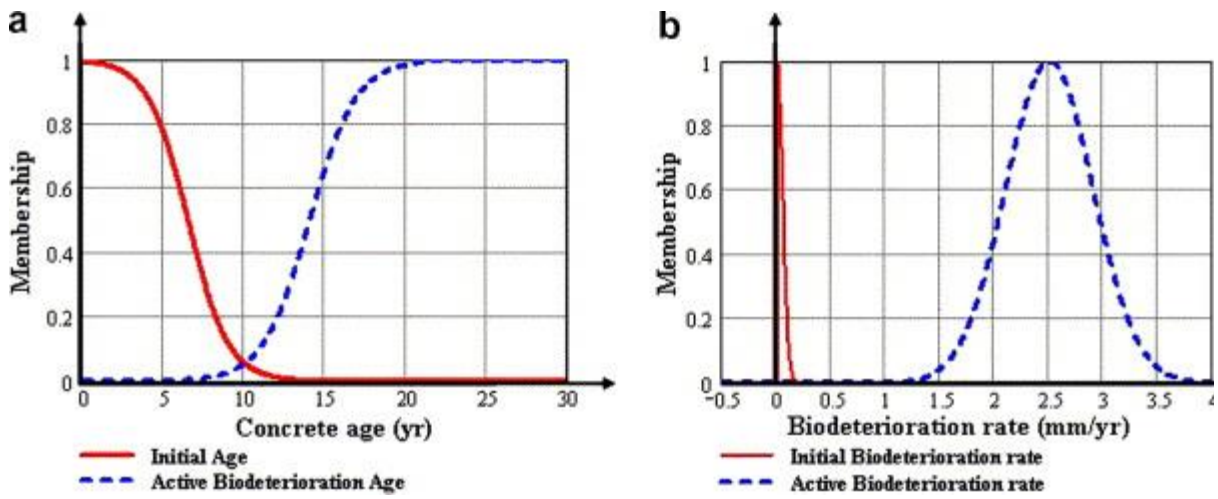

Figure 24. (a) Age membership functions, (b) biodeterioration rate membership functions (Bastidas-Arteaga et. al, 2008)

Figure 6 illustrates these functions for the following parameters: $a_{\tau_{ini}} = -0.8$ and $b_{\tau_{ini}} = 6.5$ for the initial age; $a_{\tau_{act}} = 0.7$ and $b_{\tau_{act}} = 12$ for the active biodeterioration age; $\sigma_{\gamma_{ini}} = 0.1$for the initial biodeterioration rate; and $\gamma_{av} = 2.5\ mm/y$ and $\sigma_{\gamma_{act}} = 0.4$ for the active biodeterioration rate.

The rules used for the fuzzy inference system are:

1. If the structure is in the initial age, then the biodeterioration rate is the initial.
2. If the structure is in the active biodeterioration age, then the biodeterioration rate is active.

Based on these rules, a response surface can be determined as follows:

$$S(\tau,\gamma) = \max\begin{pmatrix} (\mu_{age1}(\tau, a_{\tau_{ini}}, b_{\tau_{ini}})\mu_{\gamma_1}(\gamma, \sigma_{\gamma_{ini}}) \\ (\mu_{age2}(\tau, a_{\tau_{act}}, b_{\tau_{act}})\mu_{\gamma_2}(\gamma, \gamma_{av}, \sigma_{\gamma_{act}}) \end{pmatrix} \quad (7)$$

Where, $S(\tau,\gamma)$ is the surface generated by the maximum of products composition, $(\mu_{age1}(\tau, a_{\tau_{ini}}, b_{\tau_{ini}}))$ is the membership function corresponding to the concrete initial age, $(\mu_{age2}(\tau, a_{\tau_{iniact}}, b_{\tau_{act}})$ corresponds to the active biodeterioration age, $\mu_{\gamma_1}(\gamma, \sigma_{\gamma_{ini}})$ corresponds to the initial biodeterioration rate, and $\mu_{\gamma_2}(\gamma, \gamma_{av}, \sigma_{\gamma_{act}})$ corresponds to the active biodeterioration rate. The surface generated by Eq. (7) is shown in Figure 7a. By using the strategy of the center of gravity for the surface defuzzification, it is possible to obtain a time-dependent biodeterioration rate function, $\gamma(\tau)$, as shown in Figure 7b:

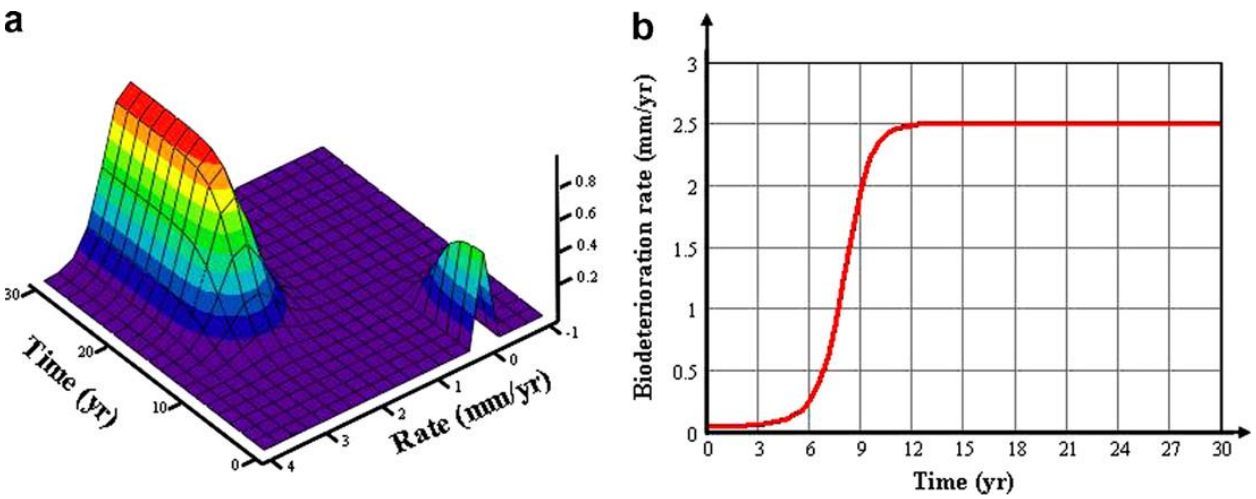

Figure 25. (a) Surface generated in FIS type, (b) time-dependent biodeterioration rate function (Bastidas-Arteaga et. al., 2008).

Advantages: Bastidas-Arteaga et al. (2008) stated that the advantage of this solution is that it provides an estimation of the time-dependent change of the biodeterioration rate depending on the expert's knowledge. The expert determines the form of the membership functions and has to assign values to the variables a, b, γ_{av}, and σ based on his/her expertise. An example is given where an expert can define the membership functions of the biodeterioration rate considering the environmental factors and nutrient availability of the determinate place and the age membership functions, taking into account the type of cement or the condition of the structure at a given time. It is also stated that the biodeterioration rate function can be updated permanently by modifying the membership functions based on field measurements and experimental data.

4. FINDINGS

There has been substantial experimental work on biodeterioration of various construction materials. Unfortunately, the effect of microbes on asphalt binder stability and degradation is missing. Therefore, there is a need to investigate how various microbes can affect asphalt binders, which is a major component of asphaltic concrete.

5. CONCLUSION

Generally, there is the need to develop approaches to prevent biodeterioration of construction materials. Although there have been major advances in protecting synthetic materials, the area of construction materials has been lagging.

Furthermore, it is very clear that physical and chemical methods may not be adequate in determining

the resistance of construction materials, including asphalt, to biological attacks. Protection of materials from bio-deterioration can begin at the surface.

6. RECOMMENDATIONS

- There is a need to develop coupled models of infrastructure deterioration based on biodeterioration and other deterioration parameters. For example, a coupled model between pavement roughness and biodeterioration.

- A summary algorithm for predicting pavement roughness coupled with biodeterioration can be universally applicable and serve as a primary performance model for pavement management and design.

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CHAPTER 4

Evaluation of Biotechnologies for Flexible Pavement Applications

FINAL REPORT
May 2016

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U.S. Department of Transportation
Federal Highway Administration

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The Center for Advanced Infrastructure and Transportation (CAIT) is a Tier I UTC Consortium led by Rutgers, The State University. Members of the consortium are the University of Delaware, Utah State University, Columbia University, New Jersey Institute of Technology, Princeton University, University of Texas at El Paso, University of Virginia and Virginia Polytechnic Institute. The Center is funded by the U.S. Department of Transportation.

TECHNICAL REPORT STANDARD TITLE PAGE

1. Report No.	2. Government Accession No.	3. Recipient's Catalog No.
CAIT-UTC 032		

4. Title and Subtitle	5. Report Date
Evaluation of Biotechnologies for Flexible Pavement Applications	April 2016
	6. Performing Organization Code CAIT/Rutgers University

7. Author(s)	8. Performing Organization Report No.
Christopher Ericson, Thomas Bennert, Ph.D.	CAIT-UTC 032

9. Performing Organization Name and Address	10. Work Unit No.
Center for Advanced Infrastructure and Transportation Rutgers, The State University of New Jersey 100 Brett Road Piscataway, NJ 08854	11. Contract or Grant No. 4-35811

12. Sponsoring Agency Name and Address	13. Type of Report and Period Covered
Center for Advanced Infrastructure and Transportation Rutgers, The State University of New Jersey 100 Brett Road Piscataway, NJ 08854	Final Report 6/1/2013 – 5/1/2016
	14. Sponsoring Agency Code

15. Supplementary Notes
U.S. Department of Transportation/Research and Innovative Technology Administration
1200 New Jersey Avenue, SE
Washington, DC 20590-0001

16. Abstract

Acidulated soy soapstock and lipids extracted from microalgae were looked at in this study as viable options for additives in asphalt binder to provide anti-oxidant and rejuvenating properties. Unfortunately it was to difficult to acquire in any means enough processed high lipid microalgae for this study but we were able to thoroughly examine acidulated soy soapstock to see if it had any rheological redeeming qualities as an additive. This study looked at asphalt binder mixed with 1% and 5% by weight acidulated soy soapstock and were tested using the AASHTO-specified Performance Grade test, along with Direct Tension Testing, Separation Testing, and MasterCurve analysis. These results were compared to the control sample which had no additives in it. The binders which contained bio-additives were softer and easier to work with than the control binder, which is promising for the asphalt industry as it may make recycled asphalts easier and more cost-effective to work with.

17. Key Words	18. Distribution Statement
Acidulated Soy Soapstock, Lipids, Microalgae, Asphalt Mixtures, Binder, Bio Additives	

19. Security Classification (of this report)	20. Security Classification (of this page)	21. No. of Pages	22. Price
Unclassified	Unclassified	36	

Acknowledgments

The research report herein was performed under CAIT-UTC 032 by the Center for Advanced Infrastructure and Transportation (CAIT) at Rutgers University, University of Texas at El Paso, and University of Delaware.

Dr. Thomas Bennert, Program Director, Pavement Resource Program (PRP) at CAIT, was the principal investigator with the work being done under his general supervision.

CAIT would like to extend their gratitude to all the individuals who worked on the project including Wen Zhang, Ph.D. from NJIT for supplying the microalgae. Special thanks are extended to Dorothy Libring, undergraduate researcher and Christopher Ericson, senior laboratory engineer, for their work throughout the project.

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CHAPTER 1

1.1 INTRODUCTION

Asphalt is a necessary product for our expected standard of living today. In the interest of environmental responsibility, companies are adding more recycled content to their binders than ever before. This makes it more sustainable and decreases the cost of materials to the contractor. Problems arise when using recycled asphalt, however, which detract from these benefits. Recycled content makes binder stiffer and more difficult to work with. Machines must heat the material to much higher temperatures to yield a workable product. Maintaining this high heat is more expensive and consumes more fuel than used for traditional binders. Therefore, finding a substance that can be added to binders to lower their workable temperature range is a current goal in the civil engineering industry. Rutgers University aims to prove through this paper that adding bio-additives to asphalt will soften the binder and make it more workable, without negatively impacting its performance. A successful first analysis will spawn more specific research into the feasibility of using these materials on an industrial scale.

1.2 RESEARCH NEED STATEMENT

The use of biotechnology has many benefits in construction applications, in this case, the construction and performance of flexible pavements. From a materials standpoint. the potential use of biomaterials can reduce the dependency on petroleum products required for asphalt materials, as well as helping to reduce greenhouse emissions during production and construction. If adaptable, biomaterials may also be able to help increase the general life of the pavement while reducing the cost of construction. Biotechnologies may also be able to help in the stabilization of subgrade soils prior to constructing roadways over top of them. Researchers have

found that the use of microbial activity allows for a level of stabilization in liquefiable soils. Including the use of biomaterials to help stabilize these problematic soils is a cost effective and environmentally sensitive solution. Although biomaterials has shown to help improve pavement and soil performance, there is also evidence to show that some pavement biodeterioration does occur and may affect the general roughness of the pavement. To conclude the research study, an assessment of paved road deterioration due to biodeterioration and how it influences roughness progression will also be conducted.

CHAPTER 2

2.1 INTRODUCTION

A tremendous amount of literature was identified related to bio additives being added to asphalt and its influence on HMA mechanical properties. There have also been studies on 100% replacement of petroleum based asphalt binder in HMA however this study only looked at bio-additives.

2.2 LITERATURE REVIEW

1. **Seidel, J. C., & Haddock, J. E. (2014). Rheological characterization of asphalt binders modified with soybean fatty acids. *Construction and Building Materials*,*53*, 324-332.**

Seidel and Haddock (2004) conducted a study on the rheological properties of asphalts blended with soybean acidulated soapstock (SAS). SAS is a relatively low-cost and highly concentrated source of soybean fatty acids. The study found that as SAS is added to binders, they become softer and their high temperature viscosities are reduced. However the study showed that the addition of 1% SAS reduces thermal stress accumulation. These findings suggest that SAS could be used as a fluxing agent (i.e., consistency reducer) for stiff, hard and viscous asphalt binders, increasing their workability. SAS may also improve low temperature performance of an asphalt binder by reducing thermal stress development.

2. **Tang, S. (2010). Asphalt modification by utilizing bio-oil ESP and tall oil additive.**

Tang, S. (2010) studied the effects of adding corn stover, oak wood, and switch grass derived bio-oil to asphalt binder. Also as part of the study tall oil was also added to asphalt binder to see its effects. The study concluded that there is some indication that these additives can increase the high temperature performance of asphalt binders. However, the increase in high temperature performance greatly affects the low temperature binder properties. This would not be good for regions that have severe freeze thaw cycles. However, the tall oil does provide significant rehabilitation effects to the bio-oil modified binders at low temperatures. In the study the dynamic modulus test results did not really correlate with the asphalt binder test results. This would suggest a potential for greater mix performance improvement than that which can be seen by just using asphalt binder testing.

3. **Fini, E. H., Kalberer, E. W., Shahbazi, A., Basti, M., You, Z., Ozer, H., & Aurangzeb, Q. (2011). Chemical characterization of biobinder from swine manure: Sustainable modifier for asphalt binder. Journal of Materials in Civil Engineering, 23(11), 1506-1513.**

Fini et al. (2011) studied the production, modification, and characterization of biobinder from swine manure. They used a hydrothermal process to convert the swine manure to a bio-oil. The bio-oil was then fractionated to extract water, solid residue, and some of the organic compounds. The sticky residue after fractionation was then used as a replacement for asphalt binder. They found biobinder from swine manure to be a promising candidate for partial replacement for petroleum-asphalt binder instead of being a complete replacement. The use of biobinder was shown to improve petroleum-asphalt binder's low temperature properties while reducing asphalt pavement construction costs.

4. **Raouf, M. A., & Williams, R. C. (2010). Temperature and shear susceptibility of a nonpetroleum binder as a pavement material. Transportation Research Record: Journal of the Transportation Research Board, 2180(1), 9-18.**

Raouf and Williams (2010) found that temperature is the main contributor to the viscosity of the bio-oils rather than shear rate. Therefore the effect of temperature in changing the viscosity of the bio-oils was more significant than the effect of shear rate. This means that the bio-oils act similarly to conventional petroleum based asphalt binders. The findings from this study are in compliance with conclusions reported previously by other researchers. Raouf and Williams also found that the addition of polymer modifiers to oakwood-derived bio-oil led to a shift in the temperature range and made it very close to that of bitumen binders modified with bio-binders.

5. **Chailleux, E., Mariane, A. U. D. O., Bujoli, B., Queffelec, C., Legrand, J., & Lepine, O. (2012, January). Alternative Binder from microalgae: Algoroute project. In Workshop Alternative Binders for Sustainable Asphalt Pavements (pp. pp-7).**

Chailleux et al. (2012) looked at using the lipids extracted from microalgae as a 100% asphalt binder replacement due to its rheological similarity to asphalt binder. This is a very neat concept however it is not economically feasibility or environmentally friendly yet for a total replacement. The energy consumed to process the algae is to high to call it environmentally friendly.

CHAPTER 3

3.1 OVERVIEW

We mainly looked at the viability of 2 bio-additives in asphalt binder. The goal was to look at the performance properties of asphalt binder with the additive in it at different percentages. In the following sections the work plan of both additives are discussed.

3.2 ACIDULATED SOY SOAPSTOCK

Acidulated soy soapstock is a by-product of the refining process of producing soybean oil. It is a highly concentrated solution of plant-based fatty acids and exhibits a low viscosity liquid at room temperature. The acidulated soy soapstock was added into virgin asphalt binder with a Performance Grade (PG grade) of 64-22. Two mixes were produced containing 1% and 5% of ASS by weight. The asphalt binders were mixed using a low shear Glas-Col Precision Stirrer, which ran for a half hour at 145 °C to achieve a homogenous mixture.

Binder tests were performed according to AASHTO specifications on three bio-binder mixtures to ascertain their physical properties; the two bio-additive mixtures and the virgin 64-22 were tested. The testing consisted of a full PG grade according to AASHTO R-29, Multiple Stress Creep Recovery analysis, Direct Tension Test, Separation Test, and a Mastercurve analysis. The instruments needed to perform these tests are listed below. All tests were performed to AASHTO specifications with the exception of the Mastercurve testing and analysis.

3.3 ALGAE

Today, there are many companies that grow algae for the food and drug industry. However, the algae they grow are rich in proteins and vitamins but they are very low in lipids. The algae used in this study was Scenedesmus Dimorphus that is high in lipids and a perfect candidate for this study. The algae was grown by Wen Zhang at his laboratory at NJIT, sonicated, dried, semi crushed, and mailed to us in small quantities. The production of the algae was a slow process and we were only able to get roughly 8-10 grams of dried algae roughly every 2 weeks. The algae was expected to yield around 10% by weight of the dried algae of lipids when extracted. Therefore we needed around 3000g of dried algae in order to obtain 300g lipids in order to use as an additive at different percentages in asphalt binder to conduct testing as a viable additive. Below is a brief description of how the algae was processed before it was sent to us.

3.3.1 ALGAE PROCESSING BEFORE SENDING TO RUTGERS

Process of Algae done by Dr. Wen Zhang at NJIT:

Step 1: Centrifuge algae at 5000 x gravity for 10 minutes of more to ensure separation of algal cells from the medium. Then discard the supernatant

Step 2: Ultrasound the cells at 2kW for 15 minutes cycling on for 30 seconds and then pausing for 30 seconds for a total of 30 cycles. This is cooled by ice and then maintained at room temperature.

Step 3: Dry the concentrated biomass in the oven at 60C until weight of the biomass is constant over time.

3.3.2 ALGAE PROCESSING AT RUTGERS TO EXTRACT LIPIDS

Figure 1 shows the soxhlet extractor setup. The condenser is on top of where the sample is held. As the hexane is heated up in the mantle it will boil at approximately 68°C. The hexane vapor will then travel up through the whole apparatus and up to the condenser. The hexane will condense in the condenser and then drip down onto the algal biomass. The cotton which is below the biomass is to prevent large algal particles from clogging the apparatus and from getting into the reservoir.

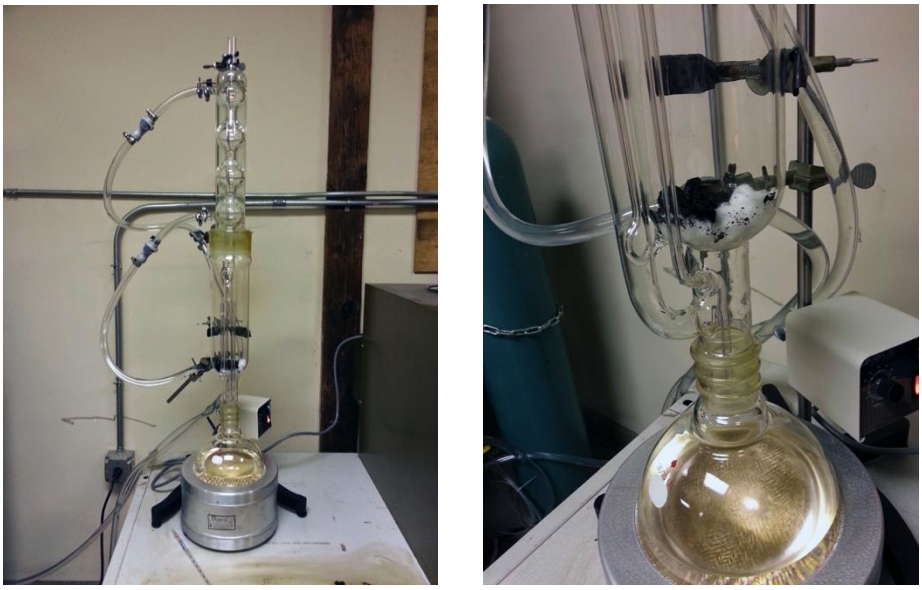

Figure 39: Sohlet Extractor Setup. Algal Biomass sits on top of Cotton

Figure 40: Sohxlet Filling up With Hexane. Algal Biomass is Reacting

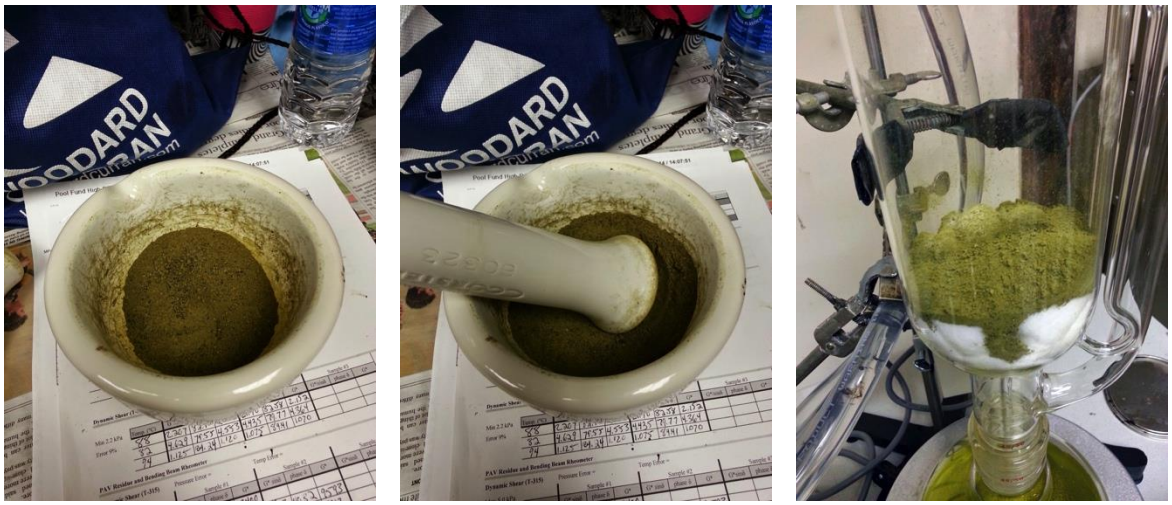

Figure 3: Algal Biomass Ground up using Mortar & Pestle and Put Back into Soxhlet

Figure 2 is showing the soxhlet extractor is filling up with condensed hexane and the photos on the left is showing the algal biomass is reacting with the hexane and releasing some sort of gas. After running this setup for approximately 8 hours we were left with only a slightly green hexane and algal solution in the collection flask. We then decided to take out the algal biomass and physically crush it up with a mortar and pestle and put back into the soxhlet extractor with new cotton as shown in figure 3.

The algal biomass was surprisingly very difficult to crush by hand. It was more difficult to crush than ice cubes. In order to crush it we had to wrap the algal biomass in a towel and smash the material with a hammer. After a few blows we were able to put it in the mortar and pestle to grind it into a fine powder. Using a coffee bean grinder probably would have been a better solution however we did not have one to use. Figure 4 is showing the soxhlet extractor running with the ground algal biomass powder.

Figure 4: Soxhlet Extractor Running With Ground up Algal Biomass

3.3.3 Recovery of Algal Biomass Lipids from Hexane Solution

Once the soxhlet extractor has run for 8 hours we are left with a solution of lipids in the hexane and the solution has a very dark green color to it. We then take the solution and distill the hexane from the lipids using our Buchi Rotovap. We use CO2 as the sweep gas to aid in the distillation process along with applying a vacuum to the system. Figure 5 shows the setup of the Rotovap in the process of distillation.

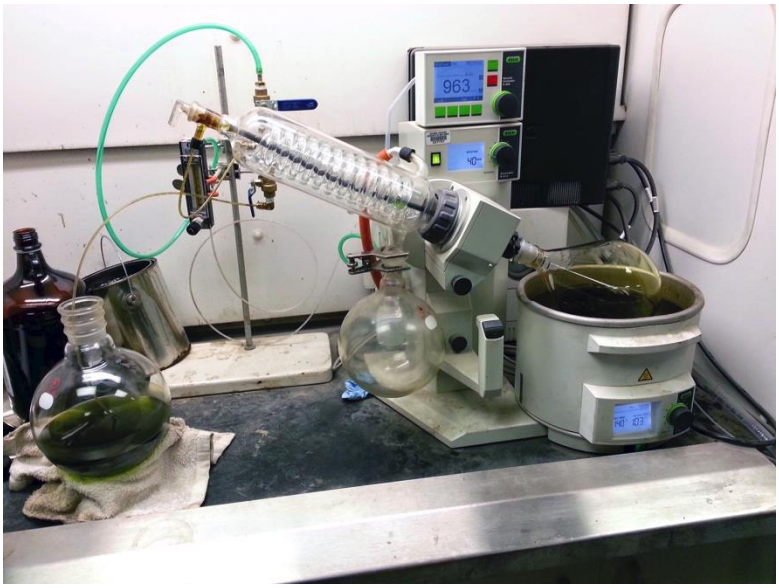

Figure 5: Buchi Rotovap Setup Distilling Hexane From Solution

Figure 6: Algal Biomass Lipids.

Figure 6 is showing the end product of the algal biomass extraction & recovery process. The yield was just under 10% by weight of the dried weight of the algal biomass. Specifically we started with 30.55g of dried algal biomass and we extracted 2.8g of lipids from the biomass.

CHAPTER 4

Performing the tests listed above gives an understanding of the physical characteristics of asphalt binder. These values represent how a binder will perform under certain conditions, including temperature and loading, over the life of the material. Important values to understand are the Performance Grade of the binder and the Critical Cracking temperatures. A broad understanding of the material can be gained from a MasterCurve analysis. Finally, in binders with additives, a Separation Test is used to understand how the homogeny of the mixture is maintained when being stored for long periods of time in heated silos.

4.1 PERFORMANCE GRADE RESULTS

A PG grade is a specification which denotes the performance of a binder under different loading applications. It is denoted as two numbers, representing the maximum and minimum temperatures which produce acceptable performance during these tests. For example, the virgin binder used in this study has an industry standard PG grade of 64-22. This conveys that it will perform well at temperatures between 64 and -22 degrees Celsius. As stated, this rating is an industry standard, which means that the binder falls between 64-22 and the next standard grade, 70-22. These standards are typically listed in 6 degree intervals. The results of the laboratory PG

grade of the same material, as performed in this study, show that the specific PG grade of the binder is 66.3-28.8.

In order to find these values, un-aged binder is tested as well as binder that has been aged artificially. The Rolling Thin Film Oven (RTFO) and Pressure Aging Vessel (PAV) are used to create binder representative of that which has been newly mixed and compacted, and after it has been oxidizing in the field for an extended period of time, respectively. The full results of these tests can be found in Appendix A. A summary is listed below.

Binder	Performance Grade	RTFO Mass Loss Percent
64-22 Control Sample	66.3 – 28.8	0.052
64-22 + 1% ASS	65 - 29.5	0.072
64-22 + 5% ASS	58.4 - 32.6	0.159

Table 1: PG Grades of Asphalt Binders

These results show that acidulated soy soapstock affects the PG grade of asphalt binder. Both binders which contain the bio-additive have entered a lower range of acceptable temperatures. The maximum and minimum temperature designations have both decreased, meaning that the binders will give the same performance at progressively lower temperatures. This change in temperature occurs in a linear fashion depending on the percentage of ASS added to the binder.

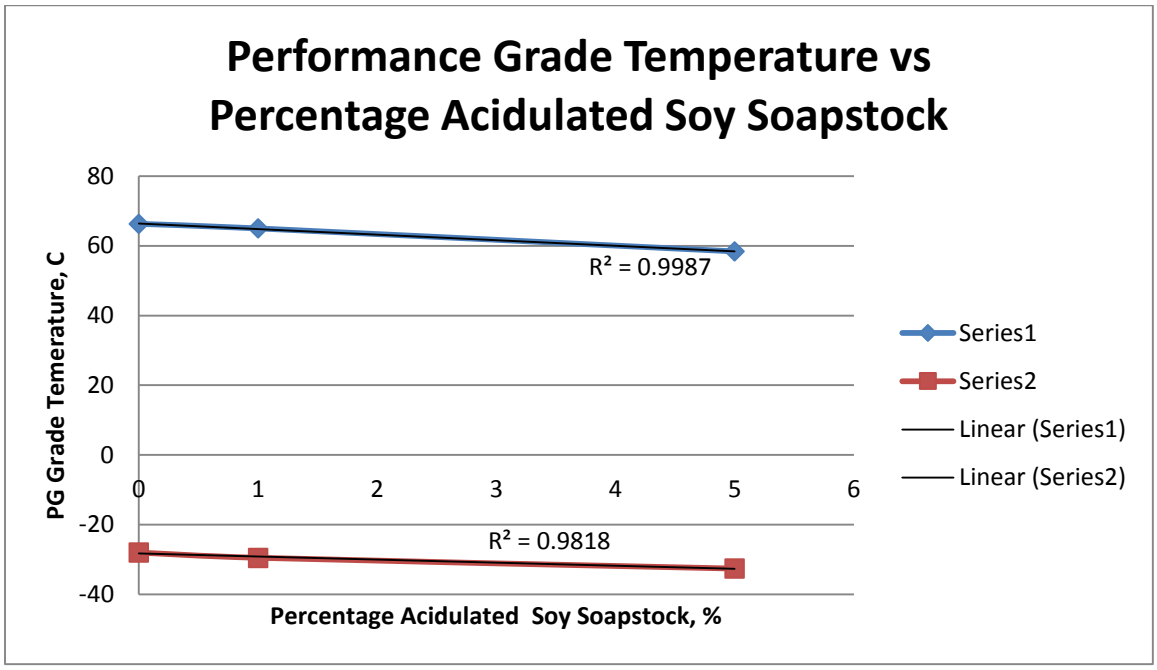

Figure 7: Performance Grade Temperature vs. Percentage Acidulated Soy Soapstock

Another important factor to consider is the loss of mass which occurs due to heating and oxidation when the binder is in the RTFO. Due to the organic nature of the bio-additive, the binders which contained ASS lost more mass than the control. All 3 binders are well below the maximum accepted mass loss percentage of 1% loss.

4.2 CRITICAL CRACKING RESULTS

The Critical Cracking value represents the temperature at which a binder will fail to relax while cooling and fail. The temperature varies depending on the cooling rate of the binder. This means that, if asphalt is being worked in temperatures near its critical cracking point, it can be protected by allowing it to cool more slowly. The critical cracking values are found by inputting the results

of the BBR and DTT tests into a program called TsarPlus. The results can be found below in the tables.

TCMODEL Critical Cracking Temperature (°C)				
Starting Temperature of cooling event	Cooling Rate	Cooling Rate	Cooling Rate	Cooling Rate
	C/hr	C/hr	C/hr	C/hr
	1	2	5.6	10
10C	-27.6	-26.2	-23.8	-22.5
5C	-27.6	-26.2	-23.8	-22.5
0C	-27.7	-26.2	-23.9	-22.5
-5C	-27.7	-26.2	-23.9	-22.6

Table 2: 64-22 Bio-Binders Control Critical Cracking Temperature

TCMODEL Critical Cracking Temperature (°C)				
Starting Temperature of cooling event	Cooling Rate	Cooling Rate	Cooling Rate	Cooling Rate
	C/hr	C/hr	C/hr	C/hr
	1	2	5.6	10
10C	-28.3	-27.0	-24.7	-23.4
5C	-28.3	-27.0	-24.7	-23.4
0C	-28.3	-27.0	-24.7	-23.4
-5C	-28.3	-27.0	-24.8	-23.5

Table 3: 64-22 + 1% Acidulated Soy Soapstock

TCMODEL Critical Cracking Temperature (°C)				
Starting Temperature of cooling event	Cooling Rate	Cooling Rate	Cooling Rate	Cooling Rate
	C/hr	C/hr	C/hr	C/hr
	1	2	5.6	10
10C	-32.1	-30.3	-27.5	-26.1
5C	-32.1	-30.3	-27.5	-26.1
0C	-32.1	-30.3	-27.5	-26.2
-5C	-32.2	-30.3	-27.5	-26.2

Table 4: 64-22 + 5% Acidulated Soy Soapstock

The binders which contain acidulated soy soapstock have lower critical cracking temperatures at all cooling rates compared to the control.

4.3 MASTERCURVE ANALYSIS RESULTS

As explained in the methodology section, a MasterCurve analysis represents the stiffness of a binder at a broad range of temperatures and loading frequencies. Asphalt binder is stiffer at cooler temperatures. It also acts stiffer when being loaded at a high frequency. A MasterCurve test applies loads on a sample of asphalt at varying temperatures and frequencies, and from this data can extrapolate a smooth curve which represents a wide range of situations. The Rutgers University sequence tests the binder at 0 – 60 degrees Celsius, in 15 degree intervals. The data collected by the DSR is then entered into RHEA software, which converts the raw data into points which compare the complex modulus of the binder at different frequencies. When these points are plotted on a graph on a logarithmic scale, they create a MasterCurve. When multiple binders are compared, the superpositioning of these curves on one graph shows the relative stiffness of each binder. Figure 8 shows the results of the MasterCurve analysis for the three binders in this experiment.

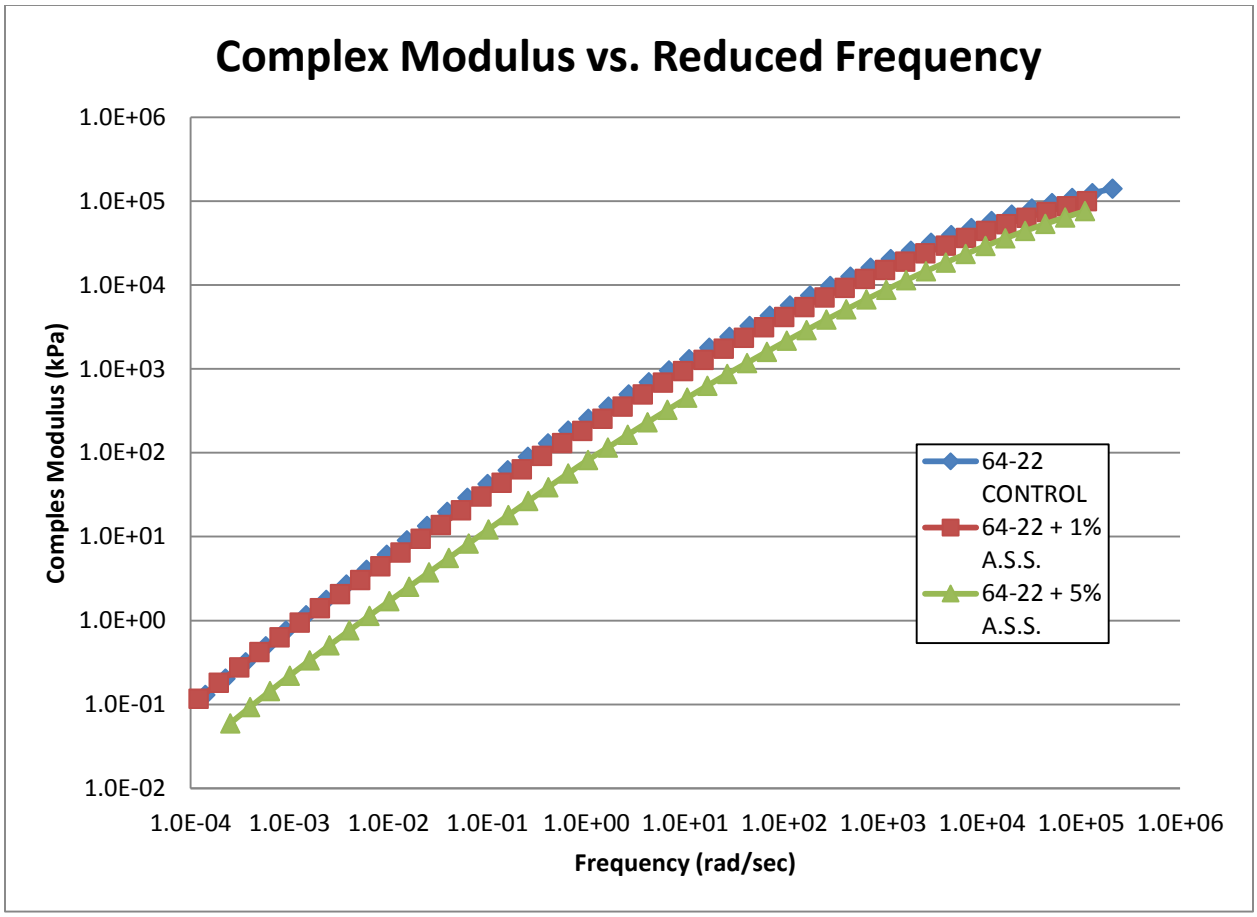

Figure 8: Complex Modulus vs. Reduced Frequency

The virgin 64-22 binder is stiffer than the binders which contain the bio-additive. The more acidulated soy soapstock the sample contains, the less viscous it becomes. This difference becomes less pronounced at high frequencies, as the binders approach the glassy region and begin to exhibit the characteristics of a solid rather than a liquid.

The MasterCurve analysis also produces values which are known in the industry as Black Space Values. These values are found by taking the complex dynamic shear modulus (G*) and phase angle of the binder at two sets of temperature and frequency conditions. These conditions are considered to be equivalent in terms of the change in physical properties that occur in the binder.

Theoretically, the G* and phase angle should be the same at both of these loading conditions. Two more important values are the crossover frequency and the rheological index, or R-value. The crossover frequency represents the frequency at a given temperature where the tangent of the phase angle, tan(δ) = 1. In general, the crossover frequency represents the stiffness of the binder. A higher frequency represents a softer binder, as binder stiffens at increased loading speeds. The rheological index is the difference between the glassy modulus and the dynamic complex modulus (G*) at the crossover frequency. The R-value is a parameter which represents the ability of the binder to relax. Typical values for the R-value are between 1.2 and 2. Table 6 shows the Black Space results for this experiment.

	44.7C & 10 rad/s		15C & .005 rad/s			
	G*, Pa	Phase Angle, °	G*, Pa	Phase Angle, °	Crossover Freq. rad/s	R-Value
64-22 Control	6.64E+01	71.5	2.83E+01	73.3	805.01	1.888
64-22 +1% Acidulated Soy Soapstock	5.54E+01	70.9	2.17E+01	73.1	943.75	1.959
64-22 +5% Acidulated Soy Soapstock	2.64E+01	73.2	6.68E+00	76.1	5125.29	2.019

Table 5: Glover-Rowe Black Space Parameters

The comparison of the two loading conditions do not yield similar G* values, but the phase angles are close enough to be statistically significant. The crossover frequency results confirm that binders containing more bio-additive are softer than their virgin counterparts. The R-values of all three binders are in the expected range.

4.4 SEPARATION TEST RESULTS

When an additive is combined with virgin binder, it is thoroughly stirred to ensure a homogenous mixture. Differences in the densities of the two materials, however, can sometimes produce separation of the binder from the additive during storage. A separation test is performed to ensure that the modified binder does not separate enough to cause quality control issues. The separation test was performed to SHRP Plus specifications. The full results can be seen in the Appendix.

Due to unknown human errors during the performance of the Separation Test, the results are inconclusive. It appears that the binder containing 1% acidulated soy soapstock aged dramatically, and became stiffer than the virgin binder. Discarding the results of the 1% bio-binder due to this discrepancy, however, the separation test results were actually improved by the addition of the acidulated soy soapstock. The percent difference between the test results of the top and bottom of the separation tube for the virgin 64-22 binder was around 11%. The average separation of the 5% acidulated soy soapstock mix was around 7%. This result is unexpected, and further testing should be done to investigate further.

CHAPTER 5

5.1 RECOMMENDATIONS

In conclusion, it can be seen that adding acidulated soy soapstock to virgin binder softens the mixture. This result supports the goals of the research project. Adding soy soapstock to recycled asphalt would lower the workable temperature, making it a more environmentally friendly alternative. The soy soapstock does not negatively affect the performance of the binder in terms of critical cracking or load performance, as demonstrated by the BBR, DTT and MasterCurve analyses.

5.2 FURTHER RESEARCH

Due to the successful nature of this first experiment with bio-additives, Rutgers University plans to pursue more in-depth research on the topic. A comparison of the performance of soapstock with other additives is the next step. Rutgers has already collected a sample of algae from the labs at NJIT, with the intention of producing a microalgae lipid extraction. Preliminary extractions, performed with a Soxhlet Extractor, yield the following results:

Algae – scenedesmus dimorphus – 21.37 g
chlorella zofingiensis – 9.18 g
Total – 30.55 g
Lipid Extraction – 9% yield, 2.8 grams

An immediate observation for the algae bio-additive is that pure algae samples are far more difficult to obtain than soy soapstock. This could limit the usefulness of such an additive, as the main goal of this research is to decrease the total cost of producing and working with recycled asphalt binder. This research still holds merit in the civil engineering community. If the algae

lipids produce favorable results over other bio-additives, cheaper sources of bulk algae may come into existence due to the rise in demand.

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APPENDIX

Standard Practice for

Grading or Verifying the Performance Grade (PG) of an Asphalt Binder

AASHTO Designation: R 29-08

Project ID: 64-22 Bio Binders **Sample ID:** Control Sample
Technician: Dorothy Libring **Mix Type:**
Date: 2/10/14

Summary of Results

Continuous Grade (°C)	**66.3-28.8 (18.2)**		**Specific Gravity**	
Asphalt Content (%)	**#DIV/0!**		**RTFO Mass Loss %**	0.052
Critical Cracking Temp (°C)			**Softening Point (°C)**	
			Flash Point Temperature (°C)	
Rotational Viscosity (Pa·s)	**135 °C**	0.49		
	165 °C	0.13		

Original DSR

Temp (°C)	64			70		
	Sample 1	Sample 2	**Average**	Sample 1	Sample 2	**Average**
G*/sinδ (kPa)	1.298	1.345	**1.322**	0.6317	0.6551	**0.643**
δ (°)	85.71	85.72	**85.72**	87.23	87.26	**87.25**
G* (kPa)	1.294	1.341	**1.318**	0.631	0.654	**0.643**

RTFO DSR

Temp (°C)	64			70		
	Sample 1	Sample 2	**Average**	Sample 1	Sample 2	**Average**
G*/sinδ (kPa)	3.569	3.461	**3.515**	1.708	1.651	**1.680**
δ (°)	80.02	80.28	**80.15**	82.06	82.37	**82.22**
G* (kPa)	3.515	3.412	**3.464**	1.692	1.636	**1.664**

Pressure Aging Vessel

Temp (°C)	16			19		
DSR	Sample 1	Sample 2	**Average**	Sample 1	Sample 2	**Average**
G*sinδ (Pa)	6532	6067	**6300**	4766	4377	**4572**
δ (°)	44.88	44.84	**44.86**	46.72	46.77	**46.75**
G* (Pa)	9257	8605	**8931**	6547	6007	**6277**

Temp (°C)	-18		-24	
BBR	S (Mpa)	m-value	S (Mpa)	m-value
Sample 1	276	0.334	569	0.262
Sample 2	269	0.334	582	0.265
Average	**272.5**	**0.334**	**575.5**	**0.264**

Standard Method of Test for

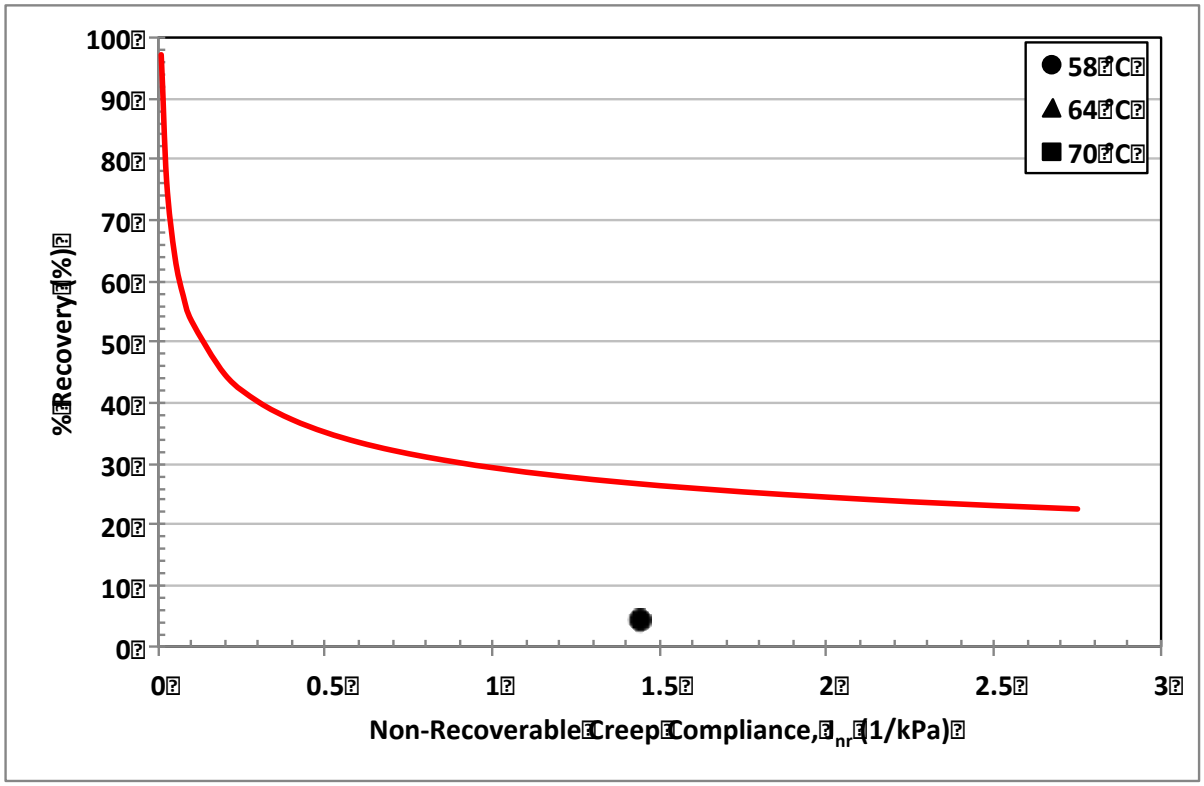
Multiple Stress Creep Recovery (MSCR) Test of Asphalt Binder Using a Dynamic Shear Rheometer (DSR)

AASHTO Designation: TP 70-12

Project ID: 64-22 Bio Binders
Technician: Dorothy Libring
Date: 2/10/14

Sample ID: Control Sample
Mix Type:

MSCR	Temp	58°C	64°C	70°C
	PASS/FAIL	**FAIL**	**FAIL**	**FAIL**
Average J_{nr} (kPa)⁻¹ @ 0.1 kPa		1.267	3.044	6.94
Average J_{nr} (kPa)⁻¹ @ 3.2 kPa		1.441	3.545	8.057
Non-Recoverable Strain @ 0.1 kPa (%)		89.21	94.01	97.45
Non-Recoverable Strain @ 3.2 kPa (%)		95.72	98.89	100.08
Recoverable Strain @ 0.1 kPa (%)		10.79	5.99	2.55
Recoverable Strain @ 3.2 kPa (%)		4.28	1.11	-0.08



Standard Practice for

Grading or Verifying the Performance Grade (PG) of an Asphalt Binder

AASHTO Designation: R 29-08

Project ID: 64-22 Bio Binders
Technician: Dorothy Libring
Date: 2/10/14

Sample ID: w/ 1% Acidulated Soy Soapstock
Mix Type:

Summary of Results

Continuous Grade (°C)	**65-29.5 (18.4)**	**Specific Gravity**	
Asphalt Content (%)	**#DIV/0!**	**RTFO Mass Loss %**	0.072
Critical Cracking Temp (°C)		**Softening Point (°C)**	
		Flash Point Temperature (°C)	
Rotational Viscosity (Pa·s)	**135°C** 0.58		
	165°C 0.15		

Original DSR

Temp (°C)	64			Temp (°C)	70	
	Sample 1	Sample 2	**Average**	Sample 1	Sample 2	**Average**
G*/sinδ (kPa)	1.126	1.127	**1.127**	0.562	0.568	**0.565**
δ (°)	84.7	85.38	**85.04**	85.96	86.95	**86.46**
G* (kPa)	1.122	1.123	**1.123**	0.5606	0.567	**0.564**

RTFO DSR

Temp (°C)	64			Temp (°C)	70	
	Sample 1	Sample 2	**Average**	Sample 1	Sample 2	**Average**
G*/sinδ (kPa)	2.723	2.851	**2.787**	1.297	1.344	**1.321**
δ (°)	81.69	81.45	**81.57**	83.73	83.62	**83.68**
G* (kPa)	2.694	2.82	**2.757**	1.29	1.336	**1.313**

Pressure Aging Vessel

Temp (°C)	16			Temp (°C)	19	
DSR	Sample 1	Sample 2	**Average**	Sample 1	Sample 2	**Average**
G*sinδ (Pa)	6625	6672	**6649**	4525	4825	**4675**
δ (°)	45.31	46.02	**45.67**	47.78	47.94	**47.86**
G* (Pa)	9318	9273	**9296**	6110	6499	**6305**

Temp (°C)	-18		Temp (°C)	-24	
BBR	S (Mpa)	m-value	S (Mpa)	m-value	
Sample 1	243	0.347	539	0.271	
Sample 2	247	0.344	540	0.270	
Average	**245**	**0.346**	**539.5**	**0.271**	

Standard Method of Test for

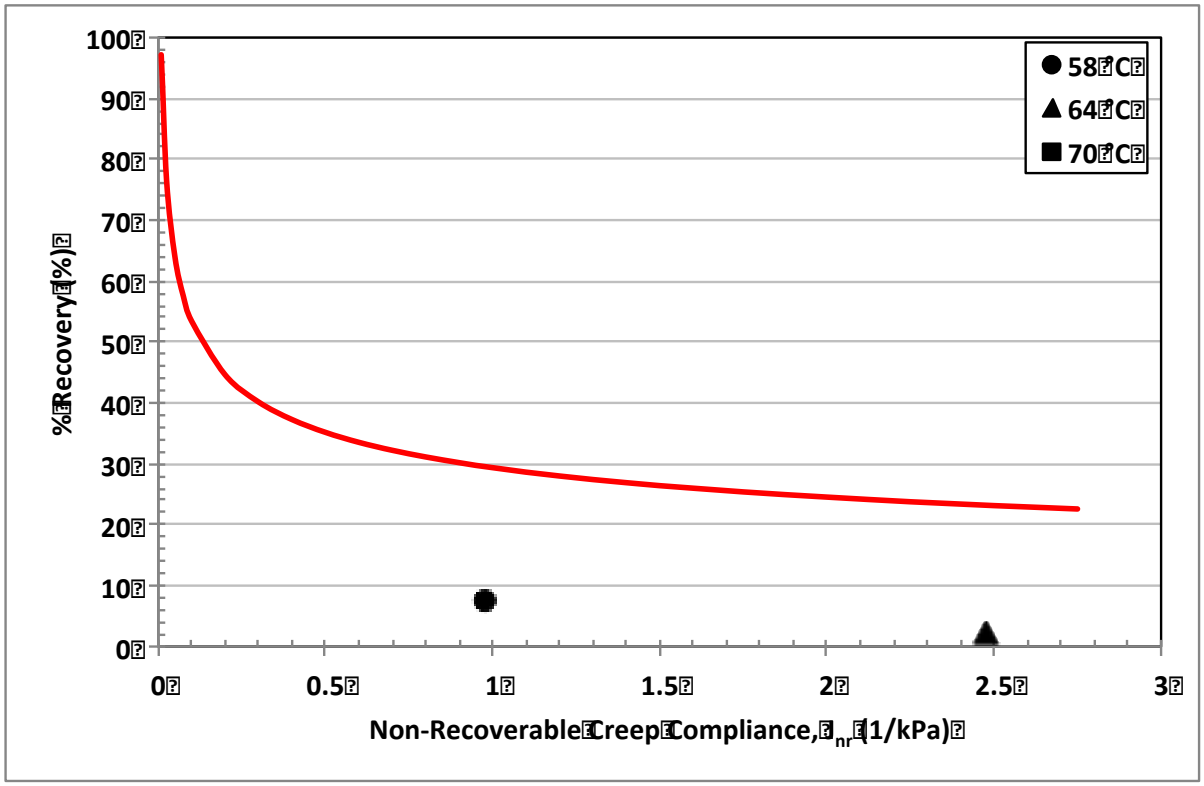
Multiple Stress Creep Recovery (MSCR) Test of Asphalt Binder Using a Dynamic Shear Rheometer (DSR)

AASHTO Designation: TP 70-12

Project ID: 64-22 Bio Binders
Technician: Dorothy Libring
Date: 2/10/14

Sample ID: w/ 1% Acidulated Soy Soapstock
Mix Type:

MSCR	Temp	58°C	64°C	70°C
	PASS/FAIL	**FAIL**	**FAIL**	**FAIL**
Average J_{nr} (Pa)@0.1kPa		0.8437	2.067	4.821
Average J_{nr} (Pa)@3.2kPa		0.9734	2.477	5.738
Non-Recoverable Strain@0.1kPa (%)		84.41	91.03	95.33
Non-Recoverable Strain@3.2kPa (%)		92.22	97.63	99.6
Recoverable Strain@0.1kPa (%)		15.59	8.97	4.67
Recoverable Strain@3.2kPa (%)		7.78	2.37	0.4



Standard Practice for

Grading or Verifying the Performance Grade (PG) of an Asphalt Binder

AASHTO Designation: R 29-08

Project ID: 64-22 Bio Binders
Technician: Dorothy Libring
Date: 2/10/14
Sample ID: w/ 5% Acidulated Soy Soapstock
Mix Type:

Summary of Results

Continuous Grade (°C)	**58.4-32.6 (15.1)**	**Specific Gravity**	
Asphalt Content (%)	**#DIV/0!**	**RTFO Mass Loss %**	0.159
Critical Cracking Temp (°C)		**Softening Point (°C)**	
		Flash Point Temperature (°C)	

Rotational Viscosity (Pa·s)	**135°C**	0.35
	165°C	0.11

Original DSR

Temp (°C)	58			64		
	Sample 1	Sample 2	**Average**	Sample 1	Sample 2	**Average**
G*/sinδ (kPa)	1.006	1.092	**1.049**	0.5172	0.5366	**0.527**
δ (°)	85.75	85.73	**85.74**	87.33	87.27	**87.30**
G* (kPa)	1.003	1.089	**1.046**	0.5167	0.536	**0.526**

RTFO DSR

Temp (°C)	58			64		
	Sample 1	Sample 2	**Average**	Sample 1	Sample 2	**Average**
G*/sinδ (kPa)	3.203	3.209	**3.206**	1.508	1.508	**1.508**
δ (°)	80.53	80.53	**80.53**	82.77	82.82	**82.80**
G* (kPa)	3.16	3.165	**3.163**	1.497	1.497	**1.497**

Pressure Aging Vessel

Temp (°C)	13			16		
DSR	Sample 1	Sample 2	**Average**	Sample 1	Sample 2	**Average**
G*sinδ (Pa)	6737	6213	**6475**	4403	4472	**4438**
δ (°)	45.37	46.09	**45.73**	48.15	47.83	**47.99**
G* (Pa)	9466	8624	**9045**	5910	6034	**5972**

	Temp (°C)	-18	Temp (°C)	-24
BBR	S (Mpa)	m-value	S (Mpa)	m-value
Sample 1	159	0.373	354	0.308
Sample 2	161	0.363	374	0.304
Average	**160**	**0.368**	**364**	**0.306**

Standard Method of Test for

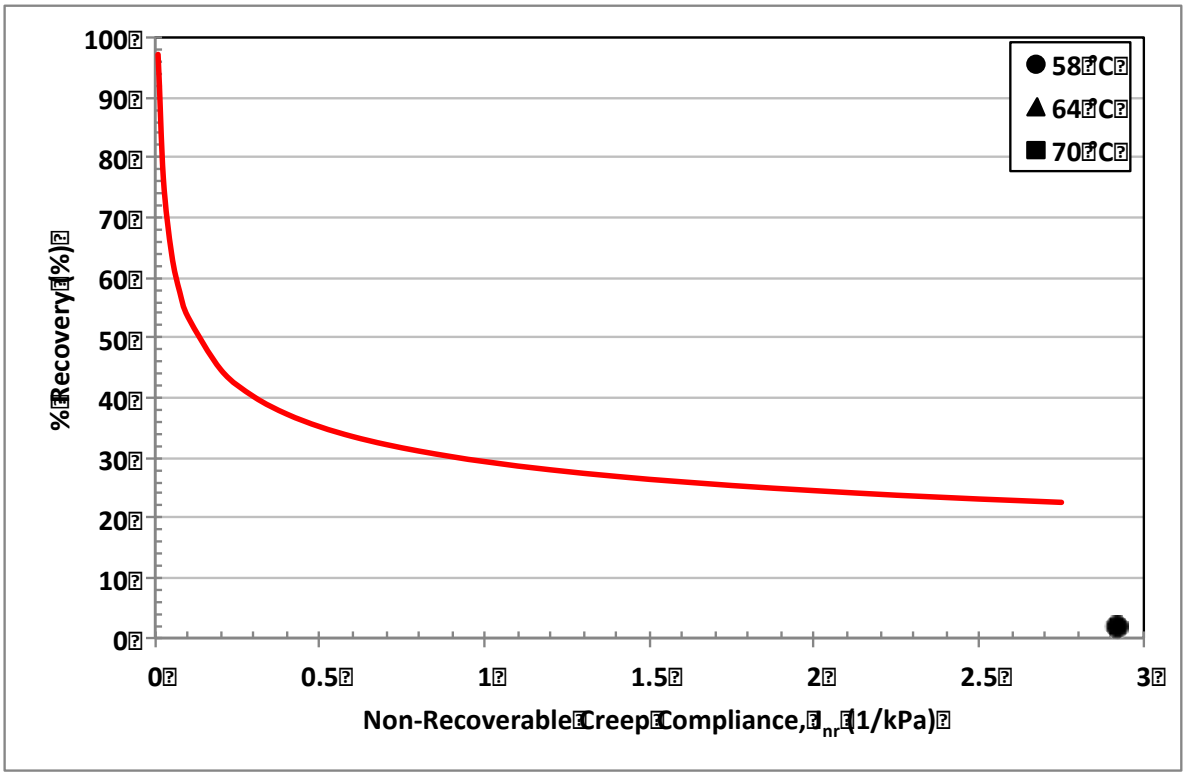
Multiple Stress Creep Recovery (MSCR) Test of Asphalt Binder Using a Dynamic Shear Rheometer (DSR)

AASHTO Designation: TP 70-12

Project ID: 64-22 Bio Binders
Technician: Dorothy Libring
Date: 2/10/14

Sample ID: w/ 5% Acidulated Soy Soapstock
Mix Type:

MSCR	Temp	58°C	64°C	70°C
	PASS/FAIL	**FAIL**	**FAIL**	**FAIL**
Average J_{nr} (kPa)@0.1kPa		2.466	5.611	12.06
Average J_{nr} (kPa)@3.2kPa		2.921	6.726	14.25
Non-Recoverable Strain@0.1kPa (%)		91.99	95.9	98.44
Non-Recoverable Strain@3.2kPa (%)		98.16	100.48	100.61
Recoverable Strain@0.1kPa (%)		8.01	4.1	1.56
Recoverable Strain@3.2kPa (%)		1.84	-0.48	-0.61



Separation Test

64-22 Control Sample

Top

Temp (°C)	64			Temp (°C)	70		
	Sample 1	Sample 2	**Average**		Sample 1	Sample 2	**Average**
G*/sinδ (kPa)	1.831	1.777	**1.804**		0.8826	0.8484	**0.866**
δ (°)	84.32	84.41	**84.37**		86.07	86.19	**86.13**
G* (kPa)	1.822	1.769	**1.796**		0.8805	0.847	**0.864**

Bottom

Temp (°C)	64			Temp (°C)	70		
	Sample 1	Sample 2	**Average**		Sample 1	Sample 2	**Average**
G*/sinδ (kPa)	2.029	2.016	**2.023**		0.9721	0.9652	**0.969**
δ (°)	83.81	83.8	**83.81**		85.63	85.66	**85.65**
G* (kPa)	2.018	2.004	**2.011**		0.9693	0.962	**0.966**

	Average Difference		**Percent Difference**	
	64	**70**	**64**	**70**
G*/sinδ (kPa)	1.91325	0.917075	11.4%	11.2%
δ (°)	84.085	85.8875	0.7%	0.6%
G* (kPa)	1.90325	0.914675	11.3%	11.2%

Separation Test

64-22 + 1% Acidulated Soy Soapstock

Top

Temp (°C)	70			Temp (°C)	76	
	Sample 1	Sample 2	**Average**	Sample 1	Sample 2	**Average**
G*/sinδ (kPa)	1.343	1.377	**1.360**	0.6842	0.6986	**0.691**
δ (°)	83.48	83.37	**83.43**	85.45	85.32	**85.39**
G* (kPa)	1.335	1.368	**1.352**	0.6821	0.696	**0.689**

Bottom

Temp (°C)	70			Temp (°C)	76	
	Sample 1	Sample 2	**Average**	Sample 1	Sample 2	**Average**
G*/sinδ (kPa)	1.63	1.657	**1.644**	0.8151	0.8237	**0.819**
δ (°)	82.32	82.41	**82.37**	84.41	84.47	**84.44**
G* (kPa)	1.616	1.642	**1.629**	0.8113	0.820	**0.816**

	Average Difference		**Percent Difference**	
	70	**76**	**70**	**76**
G*/sinδ (kPa)	1.50175	0.7554	18.9%	16.9%
δ (°)	82.895	84.9125	1.3%	1.1%
G* (kPa)	1.49025	0.7524	18.6%	16.8%

Separation Test

64-22 + 5% Acidulated Soy Soapstock

Top

Temp (°C)	64			Temp (°C)	70	
	Sample 1	Sample 2	**Average**	Sample 1	Sample 2	**Average**
G*/sinδ (kPa)	1.112	1.236	**1.174**	0.5695	0.6185	**0.594**
δ (°)	84.31	84.09	**84.20**	86.17	85.94	**86.06**
G* (kPa)	1.107	1.229	**1.168**	0.5682	0.617	**0.593**

Bottom

Temp (°C)	64			Temp (°C)	70	
	Sample 1	Sample 2	**Average**	Sample 1	Sample 2	**Average**
G*/sinδ (kPa)	1.085	1.112	**1.099**	0.547	0.559	**0.553**
δ (°)	84.65	84.53	**84.59**	86.38	86.38	**86.38**
G* (kPa)	1.081	1.107	**1.094**	0.546	0.558	**0.552**

	Average Difference		**Percent Difference**	
	64	**70**	**64**	**70**
G*/sinδ (kPa)	1.13625	0.5735	6.6%	7.1%
δ (°)	84.395	86.2175	0.5%	0.4%
G* (kPa)	1.131	0.572275	6.5%	7.1%