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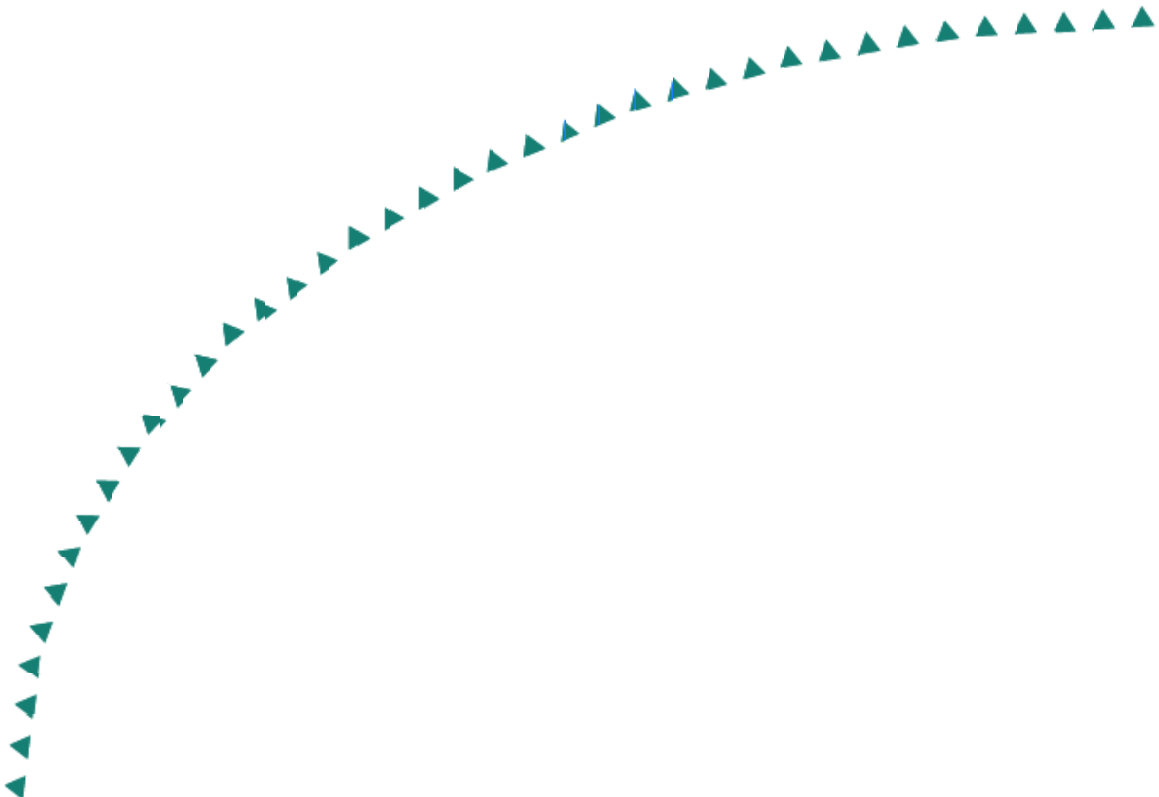
Final Report

PRELIMINARY LABORATORY INVESTIGATION OF ENZYME SOLUTIONS AS A SOIL STABILIZER



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This research studied the effect of two enzymes as soil stabilizers on two soil types to determine how and under what conditions they function. Researchers evaluated the chemical composition, mode of action, resilient modulus, and shear strength to determine the effects of the enzymes A and B on the soils I and II. The enzymes produced a high concentration of protein and observations suggest the enzymes behave like a surfactant, which effects its stabilization performance. The specimens were subjected to testing of varying lengths of time to determine their performance. Researchers observed an increase in the resilient modulus as the curing time increased but that an increase in application rate, as suggested by manufacturers, did not improve the performance of the enzymes. The study also suggests noticeable differences between the two enzymes and their effects on the soils in terms of resilient modulus and the stiffness of the soil.

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EXECUTIVE SUMMARY

Enzymes as soil stabilizers have been used to improve the strength of subgrades due to low cost and relatively wide applicability compared to standard stabilizers.

The use of enzymes as stabilizer has not been subjected to any technical development and is presently carried out using empirical guidelines based on previous experience. It is not clear how and under what conditions these products work. Therefore, it becomes an important priority to study and determine the effects of the enzymes on the strength of different soils.

The chemical composition and mode of action of two commercial soil stabilizers were evaluated using standard and innovative analytical techniques. The enzyme stabilizer product studied shows a high concentration of protein, but did not appear to contain active enzymes based on standard enzymatic activity tests. Results from quantitative surface tension testing and qualitative observations suggest that the enzymes behave like a surfactant, which may play a role in its soil stabilization performance.

Two types of soils (soil I and II) and two enzyme products (A and B) were studied in this research. The "three kneading feet tool" was used as a laboratory compaction device for the specimen preparation; the target density was 95% of the maximum dry density obtained in laboratory conditions using T99 procedure. The target moisture was the optimum water content; the enzyme was considered part of the water needed to obtain the optimum moisture content. The enzyme application rate was 1 cc of enzyme per 5 liters of water. The specimens were subject to resilient modulus testing and shear strength testing.

The resilient modulus testing was performed according to the specification described in NCHRP (National Cooperative Highway Research Program) report 1-28A. The effect of time on the performance was also evaluated by running tests on specimens cured for various times. A program developed in Visual BASIC, which is based on the recommendations for the analysis of resilient modulus data as part of NCHRP 1-28A

protocol, was used to analyze the resilient modulus data. The limited data obtained in this project showed that the addition of enzyme A did not improve significantly the resilient modulus of soil I, but increased the resilient modulus of soil II in average by 54%. On the other hand, the addition of enzyme B to soil I and II had a pronounced effect on the resilient modulus. The stiffness of soil I was increased in average by 69% and by 77% for soil II. The type of soil affected significantly the effectiveness of the treatments. Chemical composition and percent of fines are properties that affect the stabilization mechanism. It was found that the resilient modulus increased as the curing time increases for all mixtures of soils and enzymes. It was also noticed that an increment in the application rate suggested by the manufacturers did not improve the effectiveness of the stabilization process.

Shear strength tests were also performed on 26 specimens following the NCHRP 1-28A protocol. Two different confining pressures were used; 4 and 8 psi. The limited number of specimens tested show that at least 4 months of curing time are needed to observe improvement in the shear strength. It was observed that enzyme A increased in average the shear strength of soil I by 9%, and by 23% for soil II. On the other hand, enzyme B increased in average the shear strength by 31% for soil I and 39% for soil II.

Recommendations for further study include testing more combinations of soils and enzymes to encompass a wider range of materials and validating the laboratory results with field performance.

CHAPTER 1

INTRODUCTION

Background

In recent years, more attention has been given to the use of enzymes as soil stabilizers due to expansion in manufacturing capacity, low cost, and relatively wide applicability compared to standard stabilizers (hydrated lime, portland cement, and bitumen) which require large amounts of stabilizers to stabilize soils (high costs). Although enzyme-based soil stabilizers appear to have many advantages compared to conventional chemical stabilizers, it is unclear how these products work and under what conditions. The process has not been subjected to a rigorous technical investigation and is presently carried out using empirical guidelines based on experience. It becomes therefore important to perform a research study that can give objective scientific support to the use of enzymes as a soil stabilizer.

A review on the literature on the stabilization mechanism, the product information available from the manufacturers, and on field performance is first conducted. Next, chemical analysis of two commercially available products is performed to better understand the stabilization mechanism. Two subgrade soils are then stabilized and resilient modulus and shear tests are performed to study the effect of the enzyme modification on the mechanical properties of the control materials.

Objectives

The main objective is to investigate the stabilization mechanism of some of the commercially available enzyme-based products to better understand their potential value for road construction. Limited laboratory experiments are performed to determine if these products improve the material properties of subgrade soils and if they offer superior mechanical properties compared to other types of stabilization for which comprehensive laboratory and field performance already exists.

Research Approach

In order to achieve the objectives of this study the following approach is taken:

- A literature search on unconventional stabilization mechanisms is conducted.
- A chemical analysis of the stabilizing solutions is performed to obtain information relevant to understanding the stabilization process. The analysis includes determining the solution pH, the protein content (enzyme content), metals concentration, total organic carbon concentration and inorganic anion concentration.
- The enzyme activity is investigated by adding various probe compounds to the solution that are known to react in certain ways (e.g. oxidation) and determine if the reactions proceed faster in the presence of the enzyme.
- Resilient modulus tests and shear tests are performed for two types of soils with and without two different enzyme products to study their effects on the mechanical properties of the control materials.
- A statistical analysis is performed on the experimental results to determine if the addition of the enzyme improves the mechanical properties of the subgrade soils.

Report Organization

This report contains seven chapters: Introduction, Literature Review, Chemical Analysis, Mechanical Testing, Resilient Modulus Testing, Shear Strength Testing, and Conclusions and Recommendations. The Literature Review provides a background of non-standard stabilizers and the enzyme stabilization mechanism. Also, a review of previous studies on enzyme based soil stabilization is presented. The Chemical Analysis describes and presents the results from the standard and analytical techniques used to evaluate the chemical composition and the activity of the enzymes. Mechanical Testing describes the materials, specimen preparation technique and the details of the planned mechanical testing. Resilient Modulus Testing discusses the experimental work including the statistical analysis. Shear Strength Testing presents the tests results and data analysis for shear strength. The report closes with final conclusions and recommendations and an appendix that contains the experimental data for resilient modulus.

CHAPTER 2
LITERATURE REVIEW

Introduction

The non-standard stabilizers, when applied to the appropriate soil and aggregates using the right construction techniques, can produce dramatic improvement on these materials. These non-standard stabilizers are by-products of unrelated processes, modified specifically for use as stabilizers [1].

Unlike the standard stabilizers such as Portland cement, lime and bitumen, these stabilizers have no laboratory tests that can be used to predict their field performance. Because of the lack of communication between the manufacturers (unfamiliar with the road design process) and the engineers, the considerable benefits of the non-standard stabilizers remain undiscovered or not clear.

Soils are not an inert material; in fact they are chemical substances and will react with other chemicals if certain conditions are present. These reactions result from the attraction of positive and negative charges in the components of the soil and the chemical substances. If something happens to alter these charges, the reactions are changed and furthermore the properties of the materials are changed. To better understand the stabilizing mechanism of the non-standard stabilizers, Scholen [1] introduced the concepts of soil electrolyte systems, osmotic gradient pressure and colloid activity; a brief summary of these concepts is presented below.

Soil Electrolyte Systems

Many subgrades, aggregates and mixtures of crushed rock and soils are known to behave as electrolyte systems where ion exchanges occur within the material. Knowledge of the layered lattice structure of clay materials, and of colloid transport and osmotic pressure gradients is critical in understanding the behavior of these electrolytes soils . Most clays have a molecular structure with a net negative charge. To maintain the electrical neutrality, cations (positively charged) are attracted to and held on the edges and surfaces of clay particles. These cations are called “exchangeable cations” because in

most cases cations of one type may be exchanged with cations of another type. When the cation charge in the clay structure is weak, the remaining negative charge attracts polarized water molecules, filling the spaces of the clays structure with ionized water.

Osmotic Pressure Gradients

Individual cations are unable to disperse freely in the soil structure because of the attractions of the negatively charged surface of the clay particles. This inability to disperse evenly throughout the solution creates an osmotic pressure gradient, which tries to equalize the cation concentration. As a consequence, a movement of moisture from areas of low cation concentration to areas of high cation concentration is produced to achieve the equilibrium of the cation concentration.

Colloid Activity

Colloids are amorphous molecules without crystalline structure with a size of less than a micron. Particles of this size are strongly influenced by Brownian motion caused by random thermal motion. Colloids are present in high concentrations when clay soils are present. Colloids have a net negative charge that enables them to attract and transport free cations in the soil electrolyte solution, subsequently losing the cation when passing close to the more strongly charged clay particle, leaving as a consequence the colloid free to seek more free cations. Both electrochemical and physical effects influence this mechanism.

The physical phenomena are related to Brownian motion, laminar shear velocity and pore-size distribution. Brownian motion overcomes the effects of gravitational force and prevents deposition; the laminar shear velocity affects the rate of cation exchange with the clay structure; and the pore-size distribution determines the shear velocity and how close the clay lattice is to the passing colloids and cations.

The electrochemical effects are related to the forces between positive and negative particles (Van der Waals forces), and to the repulsion forces between ions of the same charge. If a solution with cations is introduced into the clay structure, a microenvironment is created in which the cations are prevented from dispersing by their adjacent clay lattice. If the soil is not completely saturated, the liquid phase will move in

laminar flow through the soil pores by capillary forces, leaving the higher concentration of cations close to the surface.

This creates an osmotic gradient pressure, which draws colloidal particles from zones of lower cation concentration. These colloidal particles take some of the free cations, reducing the ion concentration and the osmotic gradient pressure. This results in a hydraulic gradient pressure in the opposite directions which takes the cation transporting colloids outward from the original zone of cation concentration to another zone where another clay lattice is present, resulting in a new zone of osmotic pressure and cation concentration.

Mechanism of the non-standard stabilizers

The flow of cations through the clay deposits gives the shrinking and swelling properties of the soils; when a stabilizer solution is added to the soil, the magnitude of the effect depends on the characteristics of the particular cation. In general there are two main characteristics, the valence of the cation, i.e., the number of positive charges, and the size of the cation [1].

The size determines the mobility of the cation: smaller ones will travel a greater distance throughout the soil structure (the hydrogen ion is the smallest one). With respect to the valence, the hydrogen ion is doubly effective affecting the clay structure because even though it has only a single charge, the hydrogen ion produces an effect of valence of two due to its high ionization energy. These hydrogen cations exert a stronger pull on the clay layers pulling the structure of the soil together and removing the trapped moisture permitted by the single sodium and potassium cations [1].

This loss of moisture results in a strengthening of the molecular structure of the clay and also in a reduction of the particle size and plasticity. Thus changes in the environment of the clay from a basic to acidic type of environment can result in the change of the molecular structure of the soil for a long period of time.

Organic cations created by the growth of vegetation also have the capacity to exchange charges with other ions in the clay lattice. Some of the organic cations are huge in size equaling the size of the smaller clay particles. These larger organic cations can blanket an entire clay molecule, neutralizing its negative charges, and thus reducing

its sensitivity to moisture. Soil bacteria make use of this process to stabilize their environment, producing enzymes that catalyze the reactions between clays and organic cations to produce stable soil [1].

The Non-Standard stabilizers can be classified in two groups: Chemical stabilizers and Pozzolan stabilizers. The chemical stabilizers are also subdivided into five groups: Sulfonated Oils, Ammonium Chloride, Enzymes, Mineral Pitches and Acrylic Polymers. A brief summary of the description introduced by Scholen [1] of each type of stabilizers is presented below.

Chemical Stabilizers

These are chemical substances that can enter into the natural reactions of the soil and control the moisture getting to the clay particles, therefore converting the clay fraction to permanent cement that holds the mass of aggregate together. The chemical stabilizer in order to perform well must provide strong and soluble cations that can exchange with the weaker clay cations to remove the water from the clay lattice, resulting in a soil mass with higher density and permanent structural change.

The sulfonated naphthalene and D-limonene produce powerful hydrogen ions, which penetrate into the clay lattice, producing the breakdown of the structure and the further release of moisture resulting in a dense soil structure.

The ammonium chloride produces NH_4+ ions that adhere strongly to the edge of the clay, releasing the surface water and altering the surface structure to reduce capillarity.

The mineral pitches are hard resinous pitch that comes from the distillation of pulp waste. This type of stabilizer performs similarly to emulsified asphalt but is capable of developing five times the strength of an asphalt cement; it can be used for dust control and surface treatments.

The acrylic polymers are prepared in emulsions formed with forty-to-sixty percent solids; they are non-toxic and non-flammable. On drying they form a glass like thermoplastic coating, which will form a weather resistant web between the soil grains.

Pozzolan Stabilizers

The pozzolans come from coal-burning power plants. These non-standard stabilizers differ from other chemical stabilizers because they are a waste or byproduct from other industrial processes and lack the quality control of chemical commercially produced stabilizers**.**

One of the main products is lime. When lime is introduced into a soil with trapped moisture, it ionizes and produces calcium cations that can exchange with the clay lattice. The calcium cation exchanges with the sodium and potassium in the clay structure in the same way that the chemical stabilizers exchange ions. Because the calcium cation is large it cannot move far into the clay structure; adequate mixing is therefore required to obtain the benefits of this type of stabilization. The stronger ionization energy of calcium pulls together the structure of the clay, releasing the water in excess and breaking down the clay lattice.

The presence of lime increases the pH of the soil. The high pH releases alumina and silica from the pozzolans and from the clay structure. These free alumina and silica react irreversible with the calcium ions to form calcium aluminum silicates that are similar to the components of portland cement. These calcium silicates have net negative charges, which attract ionized water (molecules that act as dipoles) to create a network of hydration bonds that cement the particles of the soil together.

Enzymes as a Soil Stabilizer

The enzymes are adsorbed by the clay lattice, and then released upon exchange with metals cations. They have an important effect on the clay lattice, initially causing them to expand and then to tighten. The enzymes can be absorbed also by colloids enabling them to be transported through the soil electrolyte media. The enzymes also help the soil bacteria to release hydrogen ions, resulting in pH gradients at the surfaces of the clay particles, which assist in breaking up the structure of the clay.

An enzyme is by definition an organic catalyst that speeds up a chemical reaction, that otherwise would happen at a slower rate, without becoming a part of the end product. The enzyme combines with the large organic molecules to form a reactant intermediary, which exchanges ions with the clay structure, breaking down the lattice and causing the

cover-up effect, which prevents further absorption of water and the loss of density. The enzyme is regenerated by the reaction and goes to react again. Because the ions are large, little osmotic migration takes place and a good mixing process is required. Compaction of aggregates near the optimum moisture content by construction equipment produces the desired high densities characteristic of shale. The resulting surface has the properties of durable "shale" produced in a fraction of the time (millions of years) required by nature.

The idea of using enzyme stabilization for roads was developed from the application of enzyme products used to treat soil in order to improve horticultural applications. A modification to the process produced a material, which was suitable for stabilization of poor ground for road traffic. When added to a soil, the enzymes increase the wetting and bonding capacity of the soil particles. The enzyme allows soil materials to become more easily wet and more densely compacted. Also, it improves the chemical bonding that helps to fuse the soil particles together, creating a more permanent structure that is more resistant to weathering, wear and water penetration.

The Concept of Enzyme Stabilization

Enzyme stabilization is commonly demonstrated by termites and ants in Latin America, Africa and Asia. Ant saliva, full of enzymes, is used to build soil structures, which are rock hard and meters high. These structures are known to stand firm despite heavy tropical rain seasons [2].

Review of Previous Studies on Enzyme Based Soil Stabilization

Wright-Fox (1993) carried out a study to assess the stabilization potential of enzymes [3]. Standard soil tests were used for the study as no specific standards are available for enzyme-stabilized materials. Results from strength and index tests (e.g. liquid and plastic limit) conducted by Wright-Fox showed an increase in the unconfined compressive strength of the stabilized material as compared to control specimens. There was a 15% increase in the undrained shear strength of the stabilized material. The soil used was silty clay with a liquid limit of 66% and plasticity index of 42%. The index tests performed did not show any variation from the control specimen. Thus the enzymes might not offer waterproofing qualities using the recommended rate of application. Wright-Fox (1993) concluded that enzymes may provide some additional shear strength

for some soils and that the soil stabilization with enzymes should be considered for various applications but only on a case-by-case basis [3].

Brown and Zoorob (2003) carried out research on the stabilization of aggregate-clay mixes with enzymes [4]. Standard tests such as liquid limit and compressive strength were used for this study. A summary of the findings of that investigation is shown in Table 2.1. It can be seen that there is a possibility of achieving stabilization with soils containing Keuper Marl type of clay.

Table 2.1 Brown and Zoorob [4] Study of Enzyme Stabilization on Soils.

Type of Soil	Liquid Limit	Moisture Evaporation Rate	Compressive Strength
China Clay	Increases	Lower than control specimen	Decreases
Gault Clay	Increases	Lower than control specimen	Inconclusive
Keuper Marl	Decreases	Similar to control specimen	Inconclusive

The tests performed during this research have shown inconclusive improvements on the control properties. The authors recommended that further investigation should consider the importance of running tests to determine the soil's organic content, or, even better, run to perform a full chemical analysis on the compounds contained in the soil prior to stabilization. This investigation did not take into account several important factors such as curing temperatures and times, durability tests and enzyme concentration.

Field performance

The enzyme products have been used in more than 40 countries in the construction of structures from rural roads to highways for the past 30 years. According

to the manufacturers in the overwhelming majority of the cases enzyme stabilization provided a tool that enhanced the life-cycle and quality of the resulting product. A short review of some of the projects where enzymes were used as a road stabilizer is presented below.

A World Bank study on soil stabilization using enzymes in Paraguay reported consistent road improvements and better performance from soil stabilizer treated roads compared to untreated roads. The conclusions were drawn based on data gathered on a large-scale study from multiple sites using commercial enzymes and documentation of road performance for up to 33 months [5].

Stabilization with enzymes has been used in India. Good performance of these roads despite the heavy traffic and the high rainfall has been found. Besides an increase in the strength and durability of the roads, a reduction in project cost has also been achieved [6]

Enzymes have been used successfully to stabilize roads in Malaysia, China and the Western USA at low cost [2].

In Mendocino County, California Department of Transportation has conducted several tests of a compaction additive based on enzymes. This natural product helped the road base to set very tightly, reducing dust and improving chip-seal applications. With air quality and water quality agencies requiring dust reduction, this is a potentially effective new product, cheaper than asphalt [7].

Emery County in Utah has more than 40 miles of surface-dressed roads treated with the product that have been in use for several years. The climate is extremely arid and the 15 to 20% clay content in the aggregates has a very low Plasticity Index (PI) (<3%). A practical procedure for application of the treatment has been evolved. Jerome County in Idaho is nearby and reported a similar experience [8].

Two city streets in Stillwater, Oklahoma were also treated with enzyme products. The clay had a plastic index of 20% and good performance was reported [8].

A number of projects have been completed in Panaji (India) with the use of enzymes. A rural road and a city road in Maharasthra have lasted for more than two years without any damage [2].

Road sections placed in western Pennsylvania in the fall of 1992 passed sub-freezing winters and over forty freeze-thaw cycles and required no maintenance for ruts, potholes or wash boarding during three years. The road sections then received chip-seal coats and asphalt surfaces with no requirement for repairs to the stabilized base [2].
Enzymes have been used to stabilize more than 160 miles of subgrades and road surfacing in sites located across the National Forest land of the United States Department of Agriculture, where intense rainfall, highly erosive aggregate surfacing and expansive clay are found. The performance of the test sections shows improvement over non-stabilized control sections and historical performances of these sections before stabilization. Failures in the test sections have been related with the misuse of the enzymes, such as application over the wrong type of soil and gradation [1].

A brief summary of some projects (e.g. location, size and year of the project) around the world that used enzyme as a soil stabilizer is presented in table 2.2.

Description of the Products Investigated

Two commercially available enzyme-based products were evaluated in this study, product A and B. The manufacturer's information available for these two products is presented below.

Product A and B are organic non-biological enzyme formulations supplied as a liquids. Enzymes are natural organic compounds which act as catalysts. Their large molecular structures have active sites, which assist bonding and interactions [9]. Product A is also blended with a biodegradable surfactant to reduce the surface tension and promote enzymatic reactions, which has a wetting action that improves compactibility, allowing higher dry densities to be achieved. It is claimed that the treatment with this product is permanent and that the treated layer becomes impermeable [10].

Table 2.2 Projects where Enzyme Stabilization Treatments were Used [2]

Country	Location	Commissioner	Works	Meter	m²	Year
Kenya	Nairobi	City council	Trunk Access Roads	5.000	35.000	1995/6
	Limuru	Tropiflora Farm	Infarm Roads	600	3.000	1995
	Limuru	City council	Feeder Road	2.400	12.000	1996
	Thika	Delmote	Industrial Road	1.200	12.000	1996
	Kiambu	Valentine Growers	Feeder Road	650	3.300	1996
	Naivasha	Oserain Growers	Main Feeder Road	550	1.200	1996
	Naivasha	Green Park Resort	Main Traffic Road	1.500	7.500	1997
	Kericho	African Highlands	Main Feeder Road	860	6.500	1997
	Sotic	Sotic Tea Growers	Factory Road		12.000	1998
Uganda	Rwebisenggo	County Council	Rural Feeder Road	5.000	45.000	1998
	Salaama	Kampala city council	City Trunk Road	870	9.000	1997/8
	Kisoga	Min of Works	Rural Traffic Road	3.000	21.000	1998/9
	Rakai	City Council	City Trunk Road	1.400	9.000	1998
	Luweero	Ministers of Works	Main Traffic Road	68.000	700.000	1998/0
Tanzania	Mombo	Tembo Mill	Main Feeder Road	3.500	15.000	1998/9
U.S.A	Virginia	Federal Highway	Country Road	6.000	30.000	1999
	Texas	City Council	Rural Feeder Roads	5.000	20.000	1999
Canada	Winnipeg	Nat Park Authorities	Access Roads	12.000	40.000	1998/9
Mexico	Colima	Nueva Tierra Farm	2 Water Reservoirs	9.000	25.000m3	1999
Holland	Volkel	Min Defensie	Patrol Roads Airforce	13.000	34.000	2000/1
	Peel		Patrol Roads Airforce	6.000	20.000	2000/1
	Eindhoven		Patrol Roads Airforce	3.000	9.700	2001
	Vught	LDG Blijendijk	Main Acces Road	1.500	8.000	2000
	Doodewaard	Mauritz Tree Farm	Trail Feeder Road	150	600	1996
	Utrecht	recreation Resort	Park Roads	600	2..500	1997
	Breda	LDG Ijzer Hek	Main Acces Roads	800	3.400	1997
			Forest Walk	70	200	1997
	St. Oedenrode	Tree Farm	Feeder Roads	1.400	4.500	2000
	Otterloo	Hoge veluwe	Acces/Feeder Roads	24.000	50.000	2001
Belgium	Beerle	Moriz S.A	Feerder Road	450	900	1999
Poland	Krakow	Min of Works	Rural Main Roads	12.000	80.000	Ongoing
Malaysia	Sarawak	Porim Palm Oil	Main Feeder Roads	8.000	12.000	1998
P.N.G.	West New - Britain	Hargy Palm Oil Bialla	Airstrip	600	12.000	1998
Switzerland	Neundorf	Flueckiger	Building Foundation		4.000	2000

The enzyme is made from fermenting sugar beets a process similar to beer brewing, but the process continues until everything is fermented. The enzymes increase the wetting action, allowing higher compaction. The enzyme cements the soil by forming weak ionic bonds between negative and positive ions present in the soil structure.

Enzymes can be used to stabilize a wide variety of soils. The manufacturer reports the following advantages of using their products for soil stabilization: low cost, easy application, wide applicability, and environmentally friendly [6, 9]. In addition, it results in a soil with a high resistance to frost heaving.

The Civil Engineering Research Foundation (CERF) funded by the Federal Highway Administration made an evaluation of the environmental impact of the use of enzymes as soil stabilizer. The study found that there are seven chemicals in the enzyme-soil solution [6]. The chemical concentrations in soil were compared with Risk-Based Concentrations (RBC) in residential soil, which was developed by the Environmental Protection Agency (EPA) as a screen level for contaminants on a concerned site. It was found that the enzymes did not increase risk-based concentrations (RBC) levels of soils and it was practically nontoxic in all the toxicological analyses.

The enzyme is a natural organic compound derived from crop-plant biomass. It is similar to proteins and acts as a catalyst. The large molecular structures contain active sites that assist molecular bonding and interactions. Enzymes accelerate the cohesive bonding of soil particles and create a tight permanent layer. Unlike inorganic or petroleum-based products that have a temporary action, enzymes create a dense and permanent base and subgrade that resists water penetration, weathering and wear [2].

In normal road construction methods, compaction levels in the range of 90-95 percent are usually obtained, while with enzyme compaction densities of up to 100-105 percent may be reached. The enzyme stabilization can be applied to most soils, which contain a minimum of eight to eleven percent of cohesive fines [2].

The basic effects of the action of the enzyme into the structure of the soil can be summarized as follows [11]. Initially, the film of absorbed water is greatly reduced and in fact entirely broken, as shown schematically in Figures 2.1 and 2.2.

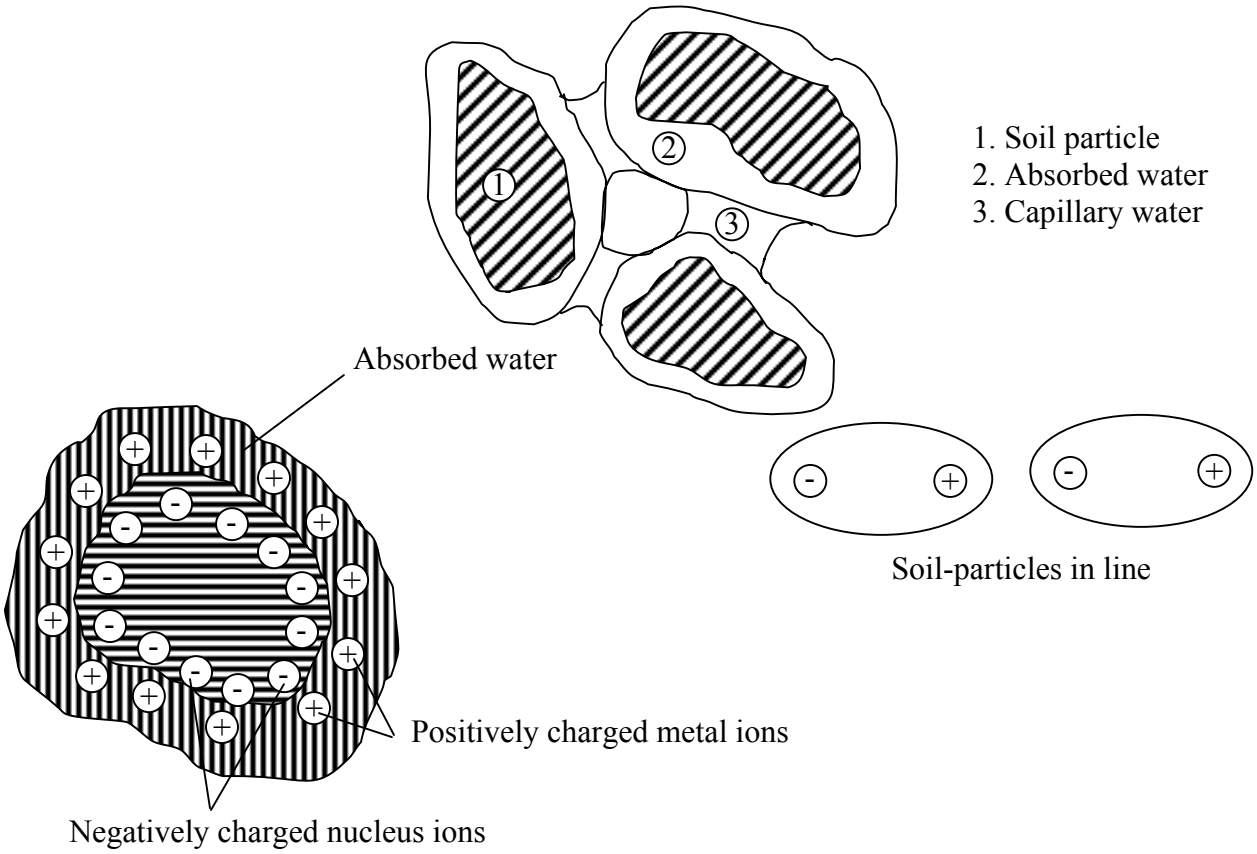


Figure 2.1 Absorbed Water in the Structure of the Soil [11].

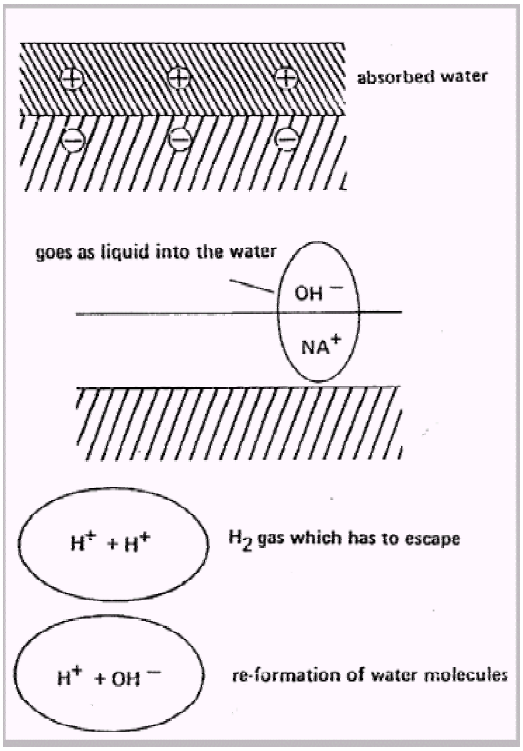


Figure 2.2 Elimination of the Absorbed Water in the Soil [11].

The most difficult problem is raised by the presence of absorbed water in the soil that adheres to the entire surface of each soil particle. This film of water enveloping the particles, which ultimately governs the expansion and shrinkage of colloidal soil constituents, cannot be completely eliminated by purely mechanical methods. However, by means of temperature effects, addition or removal of water with mechanical pressure, it is possible to vary the amount of water held in this manner. Such variations are attended by swelling or shrinkage. This provides an ideal point of operation for the enzyme [11].

The electrostatic characteristics of soil particles will also have to be considered to understand the mechanism of soil-enzyme interaction. As a result of lowering the dipole moment of the water molecule by the enzyme, dissociation occurs in a hydroxyl (-) and a hydrogen (+) ion. The hydroxyl ion in turn dissociates into oxygen and hydrogen, while the hydrogen atom of the hydroxyl is transformed into a hydronium ion. The latter can accept or reject positive or negative charges, according to circumstances. Normally the finest colloidal particles of soil are negatively charged. The enveloping film of absorbed water contains a sufficient number of positive charged metal ions - such as sodium, potassium, aluminum and magnesium - which ensure charge equalization with respect to the electrically negative soil ion [11].

In bringing about this phenomenon, the positive charges of the hydronium ion or of the negatively charged hydroxyl ion will normally combine with the positively charged metal ions in the water adhering to the surface of the particles. Because of the effect of the enzyme formulation in reducing the electric charge of the water molecule, there is sufficient negative charge to exert adequate pressure on the positively charged metal ions in the absorbed water film. As a result of this, the existing electrostatic potential barrier is broken. When this reaction occurs, the metal ions migrate into the free water, which can be washed out or removed by evaporation. Thus the film of absorbed water enveloping the particles is reduced. The particles thereby lose their swelling capacity and the soil as a whole acquires a friable structure [11].

The hydrogen ions, which are liberated in the dissociation of the water molecules, can once again react with free hydroxyl ions and form water along the gaseous hydrogen. It is important to note that the moisture content of the soil affects the surface tension and

is thus a factor affecting compaction. The enzyme reduces surface tension making the soil compaction easier to perform.

After the absorbed water is reduced, the soil particles tend to agglomerate and as a result of the relative movement between particles, the surface area is reduced and less absorbed water can be held, which in turn reduces the swelling capacity.

Some of the properties modified by the stabilization process according to the manufacturers are listed below:

- Increased compressive strength: the enzyme acts as a catalyst to accelerate and strengthen road material bonding. The enzyme creates a denser, more cohesive and stable soil.
- Reduced compaction effort and improved soil workability: lubricates the soil particles. This makes the soil easier to grade and allows the compactor to achieve targeted soil density with fewer passes.
- Increased soil density: helps reduce voids between soil particles by altering electro-chemical attraction in soil particles and releasing bound water. The result is a tighter, dryer, denser road foundation.
- Lowered water permeability: a tighter soil configuration reduces the migration of water that normally occurs in the voids between particles. It produces a greater resistance to water penetration deterioration.

Some of the advantages of using enzyme-based stabilizers instead of the traditional stabilizers are listed below:

- Environmentally safe: enzymes are natural, safe (organic) materials. These materials are nontoxic and will cause no harm or danger to humans, animals, fish or vegetation.
- Cost effective: all-weather, low-maintenance soils for road construction can be achieved for a small fraction of bituminous paving or other resurfacing costs.
- Simple to use: the enzyme is added to water, applied with a sprayer truck and mixed into the material. Normally the enzyme comes in liquid concentrate. This benefit eases handling and preparation procedures and adds to the cost effectiveness.

CHAPTER 3
CHEMICAL ANALYSIS

Introduction

The composition and activity of two commercial soil stabilizers, product A and Base-1, were evaluated using both standard and innovative analytical techniques. The goal of these analyses was to determine how the soil stabilizers work (what is the mechanism of stabilization). In addition, surface tension testing was done to study if the two enzyme base products (A and B) analyzed in this study showed surfactant-like behavior as claimed by the manufacturers.

Experimental Methods

Basic Chemical Analyses

At the beginning of the project, two manufacturers agreed to have their products investigated. Even though several manufacturers have expressed their support for the experimental work included in the present study, most of them were concerned that a chemical analysis of their product would violate their proprietary rights over the product formulation. For the chemical analysis products A and Base-1 were obtained from the manufacturers. Later on another enzyme product was made available by its manufacturer for mechanical testing (identified as product B) and for the surface tension experiment but not for chemical analysis.

Full-strength sub-samples or diluted solutions of the soil stabilizers were used in the analyses. Dilutions were prepared using high-purity deionized (DI) water or tap water (for surface tension tests only) and the resulting solutions were analyzed for pH, metals concentrations (e.g., Ca, Fe, Al), total organic carbon concentration, and inorganic anion concentrations (e.g., Cl^-, NO_3^-, SO_4^{2-}) as described in table 3.1.

Protein Content and Enzymatic activity

The protein content (a measure of enzyme content) and enzymatic activity of the product A were evaluated. Probe compounds were used to analyze for the presence of

active aminopeptidase (protein degrading), lipase (lipid degrading), or glucosidase (sugar degrading) enzymes. The objectives of these analyses were to:

1. Determine if active enzymes are present in the product A and
2. Attempt to determine how product A stabilizes the soil.

Table 3.1 Basic Chemical Analyses and Testing Methods Used

Analysis	Method
pH	pH meter
Dissolved metals	ICP-MS[1]
Protein content	Lowry method[2]
Inorganic anions	Ion chromatography[3]

[1] ICP-MS = inductively coupled plasma – mass spectrometry
[2] Lowry et al. (1951) [12]
[3] 761 Compact IC with 766 IC Autosampler, Metrohm-Peak, Houston, TX

Three fluorogenic model substrates containing either 4 methylumbelliferone (MUF) or 7-amino-4-methyl coumarin (AMC) were used as probe compounds: leucine-AMC (tests for aminopeptidase activity), MUF-heptanoate (tests for lipase activity), and MUF-α-glucoside (tests for glucosidase activity). Product A was added to buffered (Tris-HCl, pH 7.5) solutions containing one of the probe compounds. This approach is described in more detail in LaPara et al. [13]. In these experiments, the degradation of the probe compound results in an increase in fluorescence as measured by a fluorometer. The response of the test solution is compared with the response of a simple buffered water solution (negative control). If the reaction proceeds faster (i.e. greater slope of fluorescence reading versus time) in the presence of the enzyme solution than in the control, then the test solution has catalyzed the degradation of the probe compound.

Surface Tension

The surfactant-like behavior of product A was assessed by measuring the surface tension of product A solutions over a range of concentrations. Proteins are large macromolecules that resemble surfactants in chemical structure and behavior (*e.g.*, protein solutions exhibit foaming when shaken). The experiment was repeated for

product B once this product was made available. The surface tensions of the test solutions were measured with a tensiometer (Fisher Surface Tensiomat, Model 21, Fisher Scientific, Pittsburgh, PA) as shown in Figure 3.1. The results from the analyses of the product A and B solutions were compared with those obtained from the analysis of solutions of a common surfactant (sodium dodecyl sulfate or SDS).

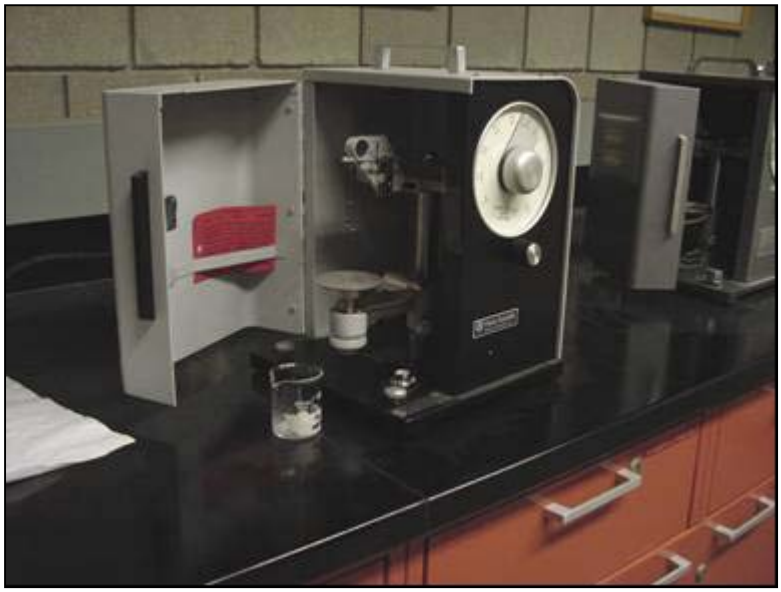


Figure 3.1 Photograph of Tensiometer

Results

Basic Chemical Analyses

The pH of product A was 4.77 while the pH of Base-1 was 11.34. Thus, product A is acidic and the Base 1 is basic. The concentrations of metals and common inorganic anions (Cl^- and SO_4^{2-}) in the two soil stabilizers are provided in Table 3.2 and Table 3.3, respectively. The main conclusions from these data are that the product A has a very high concentration of potassium (K), and moderate-to-high concentrations of calcium (Ca), magnesium (Mg), and sodium (Na). These results seem to indicate that these metals do not play a significant role in the soil-stabilizing activity. On the other hand, the extremely high concentrations of Na and silicon (Si) in the Base-1 solution suggest that this product primarily contains sodium silicates. In the presence of sufficient calcium

(Ca) and water, the silicates should form a calcium silicate hydrate or cement-like material similar to that formed in concrete.

Table 3.2 Comparison of Metal Concentrations in Products A and Base-1

Metal	**Concentration, mg/L**	
	A	**Base-1**
Al	2.74	60.4
Ca	719	420
Fe	24.1	3.19
K	7800	1.55
Mg	337	2.13
Mn	2.11	< 1.0
Na	169	31,000
P	< 1.0	2.94
Rb	11.0	< 1.0
Si	318	63,000
Zn	3.05	< 1.0

Table 3.3 Comparison of Common Inorganic Anions in products A and Base 1

Metal	**Concentration, mg/L**	
	A	**Base-1**
Cl^-	1150	14.5
NO_3^-	ND*	ND
SO_4^{2-}	664	27.8

* ND = not detected.

Protein Concentration and Enzyme Activity

The protein concentration in the undiluted product A was 9230 mg/L. Proteins are biomolecules comprising of amino acids that may or may not exhibit enzymatic activity. Enzymatic activity would be indicated by the ability to catalyze a reaction, such as the breakdown of glucose. Thus, the presence of protein alone does not indicate that the solution will exhibit enzymatic activity.

In the enzyme activity tests, the fluorescence readings of the product A test solutions were typically less than those in the negative controls, which suggests quenching of the fluorescence by substances in product A (data not shown). In any event, the presence of product A did not result in an increase in the slope of the fluorescence versus time curve for any of the substrates. Thus, it was concluded that the product A exhibited no detectable enzymatic activity for the aforementioned substrates. The three substrates used in these experiments test for the activity of three major classes of enzymes. The inability of product A to catalyze the degradation of these compounds does not definitively preclude the presence of active enzymes in the samples as there are thousands of enzymes that catalyze the breakdown of virtually all organic compounds. Nevertheless, the absence of enzymatic activity in these experiments is curious, and suggests that either:

1. Product A is a highly purified enzyme solution that contains only a single enzyme or group of enzymes that catalyze reactions not tested for in our experiments or
2. Product A may not stabilize soil via enzymatic activity but rather via some other mechanism, possibly due to their surfactant-like characteristics.

Surface Tension

The results of the surface tension experimental results are shown in Figure 3.2. Product A is more effective at reducing the surface tension of water than a common surfactant (SDS). Thus, it appears that the proteins in product A cause this product to behave like a surfactant. In addition, qualitative observations of foam production during agitation of diluted product A solutions also confirm its surfactant-like behavior.

It is therefore hypothesized that the surfactant-like character of the product A may be responsible for its soil stabilizing performance, by enhancing the ability to compact

the soil and remove water. More work is needed, including soil testing, to confirm this hypothesis. On the other hand, product B did not reduce the surface tension of water and no foam production during mixing was observed, therefore, product B does not behave like a surfactant.

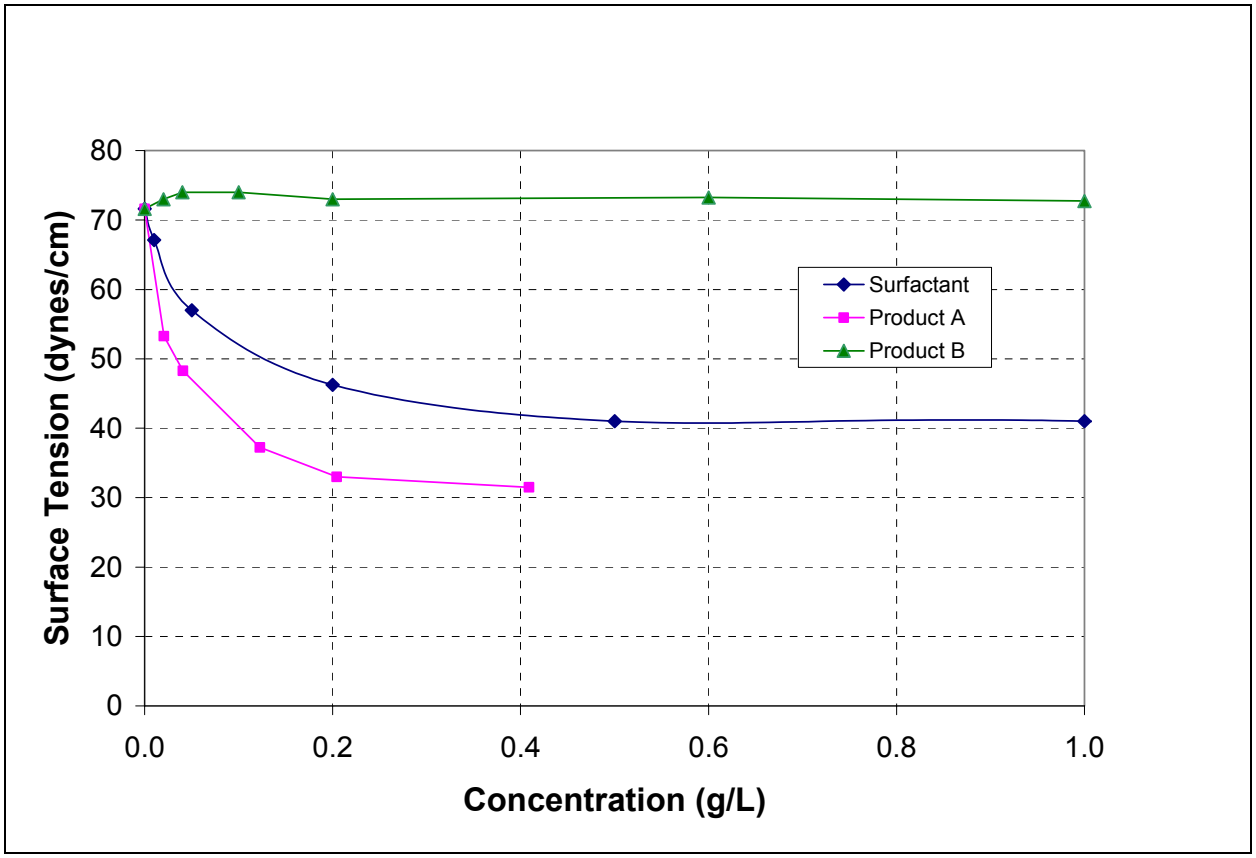


Figure 3.2 Results Surface Tension Test of Product A, B and SDS (surfactant).

Summary

Two soil stabilization products, product A and Base-1, were first tested to determine their chemical composition and mode of action. The product A contains a high concentration of protein, but did not appear to contain active enzymes based on standard enzymatic activity assays. The results from quantitative surface tension testing and qualitative observations on product A and on an additional product B made available later in the study suggest that product A behaves like a surfactant and product B does not behaves like a surfactant; this behavior may play a role in its soil stabilization

performance. Base-1, on the other hand, contains high concentrations of sodium and silicon, which suggests that it acts like cement by forming hydrated calcium silicate when added to soil.

CHAPTER 4
MECHANICAL TESTING

Introduction

The mechanical testing plan was developed based on information from the literature review and recommendations from Mn/DOT staff. The next paragraphs provide a description of the controls materials and specimen preparation technique used. A detailed description of the testing procedures used in this study is also presented.

Control Materials

Two types of soils were used to evaluate the stabilization properties of two enzyme products based on recommendation made by MnDOT research project engineer John Siekmeier. Soil I and II are natural Minnesota subgrades from Duluth and MnROAD respectively. Soil I has 96% of fines (75% of clay) a SPG of 2.73 and plasticity index of 52%. Soil II has 60% of fines (14.5% of clay) and plasticity index of 9.4%. The properties are listed in table 4.1. Two types of commercially available enzymes were used to test these two types of subgrades:

- Product A.
- Product B.

Specimen Preparation

Laboratory compaction methods that reproduce the same effects as those produced by compaction equipment in the field are required for specimen preparation. Static compaction for clayey soils seems to poorly represent field compaction [14]. Kneading compaction procedure instead represents a better way to reproduce the effects of field compaction (tamping feet) [14].

The “three kneading feet tool” was used as a laboratory compaction device for the specimen preparation. Work done by Koaussi [14] using this technique shows that more homogeneous specimens are obtained with the three kneading compaction procedure.

Dry densities of the samples are close to the in situ densities if the kneading compaction technique is used with five layers and a pressure of 1.25 MPa [14].

Table 4.1 Properties of Soils I and II

	Soil I **Soil ID = TH 23** **Field ID = PH2DUA1**	**Soil II** **Soil ID = MR1VNP1** **Field ID = MR1VNP1**
% Passing 2″	100	100
% Passing 1″	100	100
% Passing ¾″	100	99.7
% Passing 3/8″	99.9	98.2
% Passing #4	99.5	96
% Passing #10	98.8	93.8
% Passing #20	98.4	89.7
% Passing #40	98	85
% Passing #60	97.5	78.2
% Passing #100	96.9	69.2
% Passing #200	96.4	59.7
Liquid Limit (%)	84.9	25.8
Plastic Limit (%)	32.9	16.4
Plasticity Index (%)	52	9.4
% Silt	21.2	45.3
% Clay	75.2	14.5
Textural Class	C	L
AASHTO Group	A-7-6	A-4
Group Index	60.3	2.9
Opt Moisture (%)	26.5	16.1
Max Density (lb/ft^3)	90.4	107.4
SPG	2.728	-

The three kneading feet tool was made of a wood disk (100 mm diameter) under which three wood kneading tampers of 30 mm diameter are fixed (see Figure 4.1). The dimensions of the tampers were set to have the same percentage of the surface covered in the field by a typical caterpillar tamping roller [14]. The position of the three kneading feet is such that they have to be applied eight times to compact the whole surface of the specimen (45 degrees rotation between two successive loadings). This also corresponds to a normal field practice of eight passes [14].

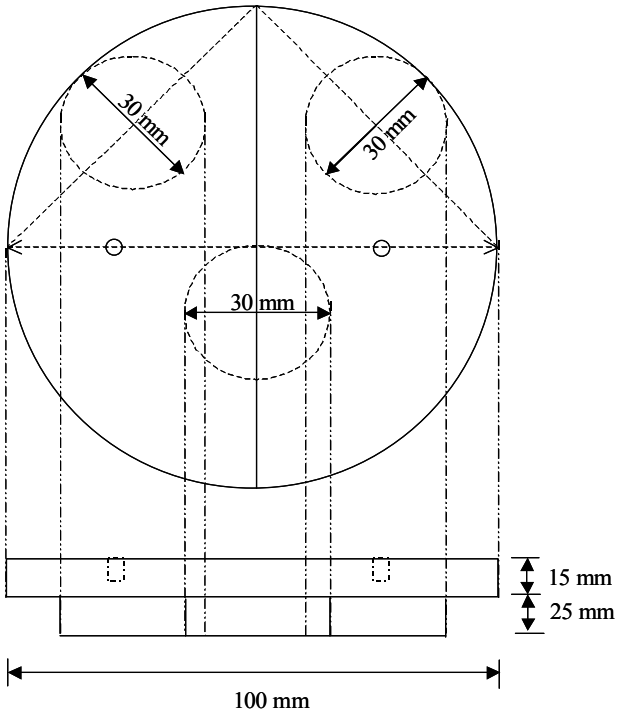


Figure 4.1 Kneading Compaction Platen

The target density was 95% of the maximum dry density obtained in laboratory conditions using T99 procedure [15], and the target moisture was the optimum water content. The addition of the enzyme was done according to the manufacturer instructions. The enzyme was considered part of the water needed to obtain the optimum moisture content.

According to the manufacturers, the rate of application is 1 cc of enzyme per 5 liters of water used to obtain the optimum moisture content. The following steps were performed to prepare the samples:

- First the soil was dried for 24 hrs at a temperature of 140°F.
- Then the soil was chopped into small pieces (see Figure 4.2) and pushed through the sieve No 4 (see Figure 4.3).

Figure 4.2 Soil II Preparation

Figure 4.3 Sieve No 4

- The soil and the additive were mixed using the target density and optimum moisture content (enzyme is part of the water added to obtain 95% of the maximum dry density). A blender was used to mix the soil with the enzyme, see Figure 4.4.

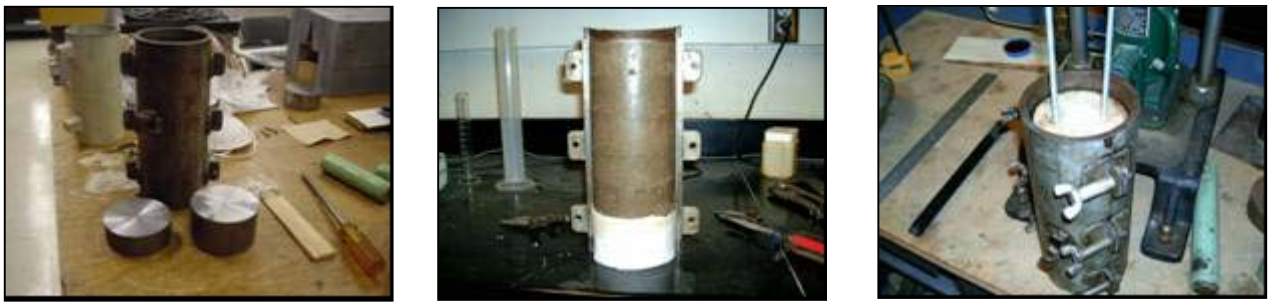
a **b** **c**

Figure 4.4 a) Blender Used for Mixing, b) Enzyme Mixed with Water, c) Soil Mixed with Enzyme

- The mixture (or blend) was placed in five layers in the 4" mold for compaction using a static load frame, see Figure 4.5.

Figure 4.5 Static Load Frame Used for Compaction

- Each layer was compacted using the kneading compactor platen eight times to cover the surface of the sample, see Figure 4.6.



a **b** **c**

Figure 4.6 a) 4" Mold and Platens, b) Sample of Soil II after Compaction, c) 4" Mold and Kneading Compaction Platen.

After compaction the specimens were stored in a humid room to cure for different length of time.

Resilient Modulus Test

A common parameter used to define the stiffness of the soil is the resilient modulus (M_R). The resilient modulus is calculated based on the recoverable strain under cyclic axial stress [16]. Two M_R test protocols are commonly used for soils. They are described in the Long Term Pavement Performance Program (LTPP) report P46 [17] and the National Cooperative Highway Research Program NCHRP (NCHR) report 1-28A [18]. In this study the resilient modulus testing was performed according to specifications

described in NCHRP 1-28A [18]. The effect of time on the performance was also evaluated by running tests on specimens cured (stored) for various times.

In the resilient modulus test, repeated load compression cycles are applied to test specimens of 4" diameter and 8" height (see Figure 4.7). Each cycle is 1s duration, which consists of 0.2s of haversine pulse loading and 0.8s of rest period (see Figure 4.8). For each test, this one-second cycle is repeated 1000 times at a confining pressure of 4 psi (27.6 kPa) and a deviatoric stress of 7.8 psi (53.8 kPa) to condition the specimen.

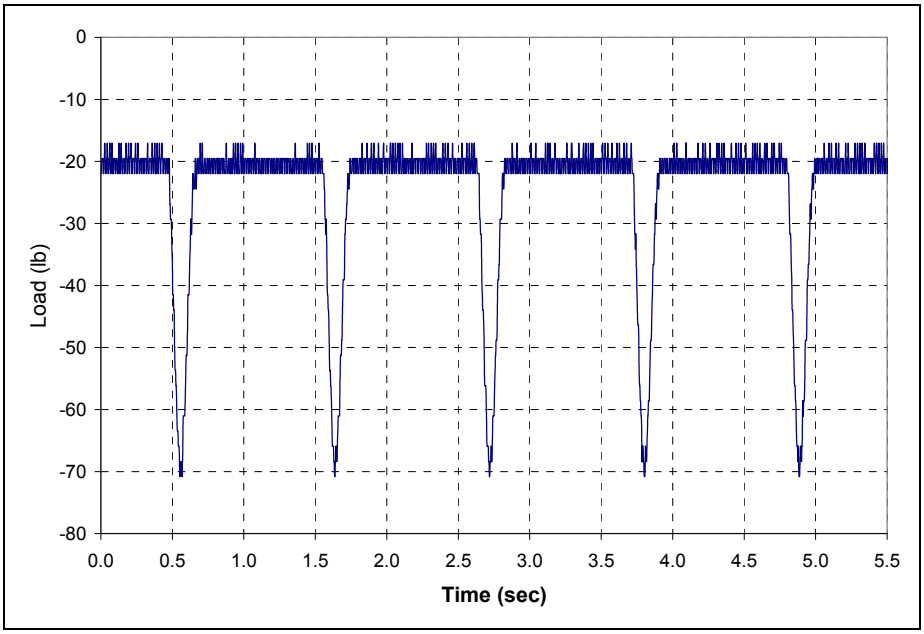


Figure 4.7 Loading Cycles for Resilient Modulus Test

Then the one-second cycle is repeated 100 times for each of the loading sequences presented in Table 4.2. The stress conditions used in each sequence represent the range of stress states likely to be developed beneath flexible pavements subjected to moving wheel loads [19]. During the test, the axial force and displacement is measured and the resilient modulus is calculated from:

$$M_R = \frac{\Delta\sigma_d}{\Delta\varepsilon_r} \tag{1}$$

where

$$\Delta\sigma_d = \frac{F_{AXIAL}}{A} \tag{2}$$

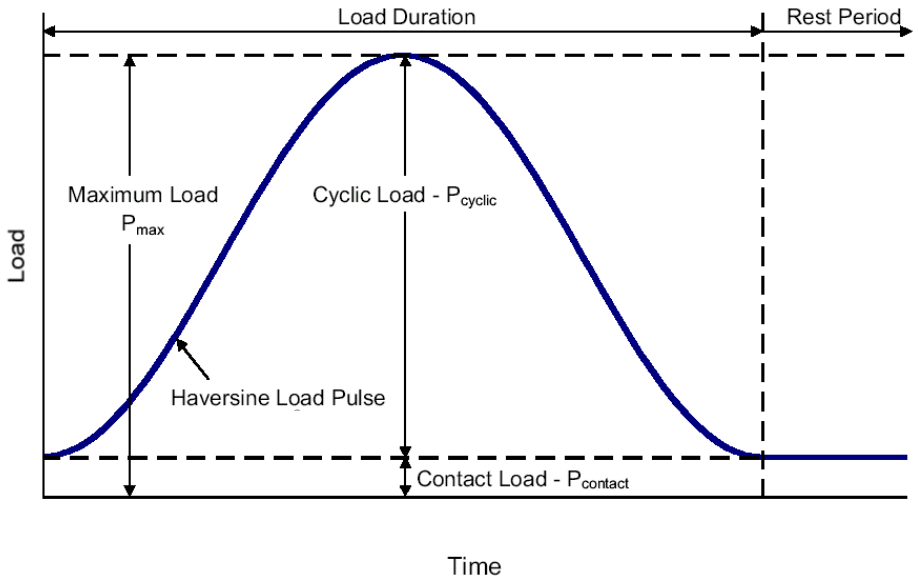


Figure 4.8 Cyclic Load Applied in Resilient Modulus Test [19]

Table 4.2 Test Sequence for Subgrade Soils (NCHRP 1-28A) [18]

Sequence	Confinement Pressure		Contact Stress			Cyclic Stress		Maximum Stress			Nrep
	kPa	psi	kPa	psi	kN	kPa	psi	kPa	psi	kN	
0	27.6	4	5.5	0.8	0.0446	48.3	7	53.8	7.8	0.436	1000
1	55.2	8	11	1.6	0.0892	27.6	4	38.6	5.6	0.313	100
2	41.4	6	8.3	1.2	0.0673	27.6	4	35.9	5.2	0.291	100
3	27.6	4	5.5	0.8	0.0446	27.6	4	33.1	4.8	0.268	100
4	13.8	2	2.8	0.4	0.0227	27.6	4	30.4	4.4	0.246	100
5	55.2	8	11	1.6	0.0892	48.3	7	59.3	8.6	0.481	100
6	41.4	6	8.3	1.2	0.0673	48.3	7	56.6	8.2	0.459	100
7	27.6	4	5.5	0.8	0.0446	48.3	7	53.8	7.8	0.436	100
8	13.8	2	2.8	0.4	0.0227	48.3	7	51.1	7.4	0.414	100
9	55.2	8	11	1.6	0.0892	69	10	80	11.6	0.649	100
10	41.4	6	8.3	1.2	0.0673	69	10	77.3	11.2	0.627	100
11	27.6	4	5.5	0.8	0.0446	69	10	74.5	10.8	0.604	100
12	13.8	2	2.8	0.4	0.0227	69	10	71.8	10.4	0.582	100
13	55.2	8	11	1.6	0.0892	96.6	14	107.6	15.6	0.872	100
14	41.4	6	8.3	1.2	0.0673	96.6	14	104.9	15.2	0.850	100
15	27.6	4	5.5	0.8	0.0446	96.6	14	102.1	14.8	0.828	100
16	13.8	2	2.8	0.4	0.0227	96.6	14	99.4	14.4	0.806	100

$$\Delta\varepsilon_r = \frac{\delta_{average}}{l_o} \quad (3)$$

and where

F_{AXIAL} = axial force [lb]

A = the cross sectional area of the specimen [in^2]

l_o = 4"

$\delta_{average}$ = is the average of the recoverable axial displacement measured with three LVDTs (Linear Variable Differential Transformers) [in] (see Figure 4.9).

Testing Equipment

All tests were performed on an MTS servo-hydraulic testing system with a maximum capacity of 5 kips and a maximum stroke of 4". A triaxial cell that meets the specifications of NCHRP 1-28A was used [18]. The interior of the cell is 19.5" in height and 9.5" diameter; a brass port in the front of the base plate of the triaxial cell serves as the connection for the air supply used to control the pressure within the specimen (Figure 4.9). The triaxial cell contains two types of instrumentation: a load cell and three LVDTs. The load cell used to measure the axial force applied to the specimen has a capacity of 5 kips.

Figure 4.9 Resilient Modulus Test Setup [7]

The three LVDTs used to measure the vertical deformation have 0.5" strokes and spring-loaded tips (Figure 4.10). The LVDTs are located at equal distances around an aluminum collar, which attaches to the specimen's membrane (Figure 4.10 and 4.11). A second collar with three columns mounted attaches to the specimen 4" below the first collar, these columns work as contacts for the spring-loaded tips of LVDTs. The setup allows the two collars to move independently of each other. Therefore, the displacement measured by the three LVDTs is the displacement of the specimen over the 4" gage length.

Figure 4.10 LVDT's and Spacers [20]

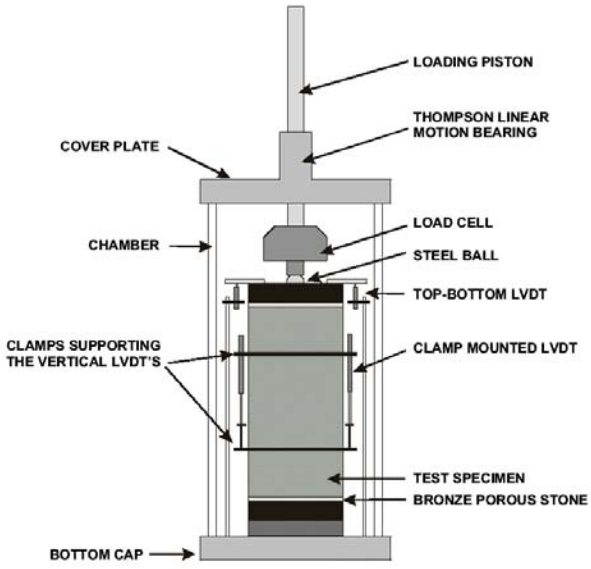


Figure 4.11 Resilient Modulus Test Setup[6]

A data collection program named MR Data Acquisition [20] was used to acquire the signals from the instruments. This program was created using LabVIEW (National Instruments, Austin, TX) by Davich [20]. The program records data at a rate of 400 points per second from the load cell, and the three LVDTs attached to the specimen.

To produce the load paths described in the NCHRP 1-28A report for each loading sequence, a system control routine named "MR Test - Final External-4in" was developed in TestWare-SX. TestWare is a software package used to custom-design experimental testing setups and to collect the raw data from the test. A summary of the procedure is presented in the flowchart shown in Figure 4.12.

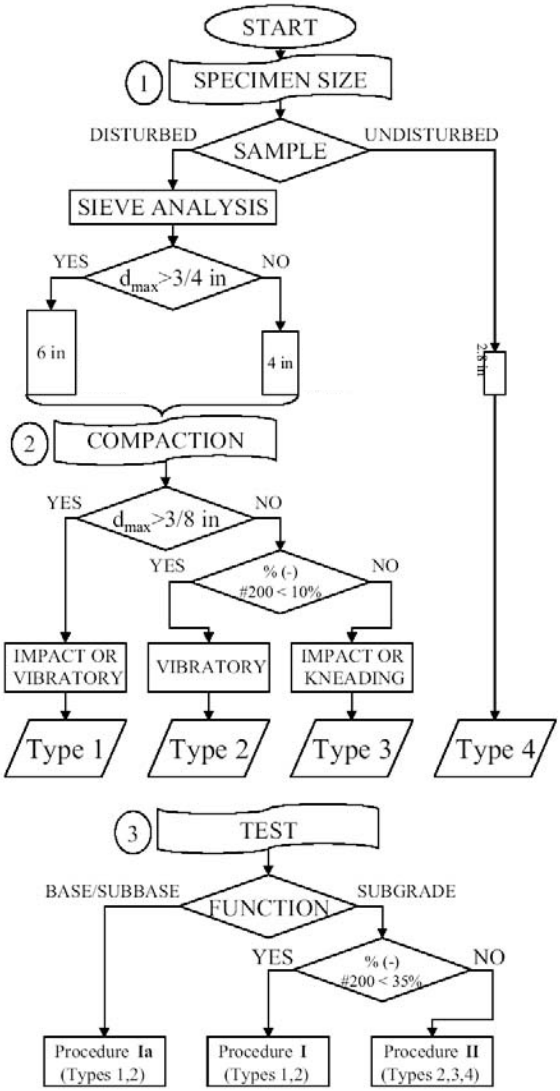


Figure 4.12 Resilient Modulus Test Flow Chart [19]

Shear Strength Test (Triaxial compression test)

Triaxial compression testing was performed according to the specifications described in NCHRP 1-28A [18] to evaluate the improvement in shear strength of the control materials. Two different confining pressures of 4 psi and 8 psi, respectively, were used. The triaxial cell and LVDT's setup for the resilient modulus test was also used in the shear strength test. Monotonic load was applied to the specimens until failure and the axial load and displacement was measured and saved for analysis.

CHAPTER 5
RESILIENT MODULUS TESTING

Introduction

Cylindrical specimens 4-in diameter by 8-in height were prepared according to the procedure explained in chapter 4. A total of 35 specimens were prepared from two types of soils (Soil I and II) and two enzymes (A and B). Table 5.1 shows the parameters obtained during sample preparation, including moisture content and density. The specimens were named according to the soil and enzyme type and enzyme concentration. For example, "S-1-2-1-B" was the first specimen made from soil 2 using 1cc of enzyme B per 5 liters of the water used to obtain the optimum moisture content. "S-1-1-05-B" was the first specimen made from soil 1 using 0.5 cc of enzyme B per 5 liters of the water.

Results

A total of 47 resilient modulus tests were performed following the NCHRP 1-28A protocol [18] to analyze the effect of the enzyme stabilization on the stiffness of two different soils. Figure 5.1 shows the test matrix for the resilient modulus.

Twenty-two specimens were prepared using soil II and thirteen using soil I due to the lack of availability of this material during the project (another research project was using the same soil). At least four specimens were tested using the same soil type and enzyme concentration recommended by the manufacturer (1cc per 5 liters of water) and at least three specimens were tested for each soil type without application of enzymes. A limited number of tests (six) were run at different concentrations (0.5cc per 5 liters of water and 1.5cc per 5 liters of water) to study the effect of the variation of the enzyme concentration on the resilient modulus. The specimens were tested at different curing times, from 21 days to 154 days. Most of the specimens were tested after four weeks of curing time. The resilient modulus test results as a function of the age of the specimen are presented in Appendix A.

Table 5.1 Sample Preparation Data

Sample ID	Soil Type	Enzyme	Concentration (cc per 5 liters of water)	Moisture Content (%)	Density (lb/ft^3)
S-1-1-0	1	-	-	25.87	114.96
S-1-1-0-2	1	-	-	23.75	115.13
S-2-1-0	1	-	-	25.62	115.57
S-3-1-0	1	-	-	24.95	116.64
S-1-1-1-A	1	A	1	25.80	114.84
S-2-1-1-A	1	A	1	26.00	114.19
S-3-1-1-A	1	A	1	26.82	114.67
S-4-1-1-A	1	A	1	27.32	115.59
S-5-1-1-A	1	A	1	26.02	116.15
S-1-1-1-B	1	B	1	29.34	120.33
S-2-1-1-B	1	B	1	25.45	119.08
S-1-1-05-B	1	B	0.5	26.20	117.24
S-1-1-15-B	1	B	1.5	30.81	119.10
S-1-2-0	2	-	-	16.08	124.49
S-2-2-0	2	-	-	16.81	128.60
S-3-2-0	2	-	-	17.20	130.53
S-5-2-0	2	-	-	16.50	129.70
S-1-2-1-A	2	A	1	15.93	126.26
S-2-2-1-A	2	A	1	15.90	124.54
S-3-2-1-A	2	A	1	17.04	130.20
S-4-2-1-A	2	A	1	15.50	127.71
S-5-2-1-A	2	A	1	15.71	127.93
S-6-2-1-A	2	A	1	15.81	127.48
S-7-2-1-A	2	A	1	16.21	127.21
S-1-2-1-B	2	B	1	17.12	127.55
S-2-2-1-B	2	B	1	17.46	128.60
S-3-2-1-B	2	B	1	17.01	124.57
S-4-2-1-B	2	B	1	16.96	124.67
S-5-2-1-B	2	B	1	17.10	125.40
S-6-2-1-B	2	B	1	17.96	125.39
S-7-2-1-B	2	B	1	16.96	126.56
S-1-2-05-A	2	A	0.5	17.50	129.79
S-1-2-15-A	2	A	1.5	16.82	129.84
S-1-2-05-B	2	B	0.5	18.70	130.54
S-1-2-15-B	2	B	1.5	19.11	130.57

Matrix of Resilient Modulus test

Enzyme A

	SOIL 1	SOIL 2
Control test	4	3
1cc / 5 L of enzyme	6	9
0.5 cc/ 5 L of enzyme	-	1
1.5 cc/ 5 L of enzyme	-	1

Enzyme B

	SOIL 1	SOIL 2
Control test	4	3
1cc / 5 L of enzyme	4	8
0.5 cc/ 5 L of enzyme	1	1
1.5 cc/ 5 L of enzyme	1	1

Figure 5.1 Test Matrix

Evaluation of the uniformity of the deformation

Non-uniformity between displacements measurements using the three LVDT's in the resilient modulus setup is inevitable. This is because in most of the cases a specimen that was originally cylindrical in shape does not remain cylinder after testing, which means that the loading plates rotate during testing, producing bending and rotation of the specimen. The following phenomena can contribute to differences between the three LVDT readings [21]:

- Slippage between LVDTs and the membrane.
- Bending produced during testing when the specimen ends are not completely parallel to the platens. Bending could also happen when the axis of the specimen is not aligned with the center of the platen.
- Heterogeneity of the test specimen.

To quantify the degree of non-uniformity for a resilient modulus test the following uniformity coefficient α is defined [21]:

$$\alpha = \frac{\sqrt{\Delta\delta_1^2 + \Delta\delta_2^2 + \Delta\delta_3^2}}{\delta_{average}} \quad (1)$$

$$\Delta\delta_i = \delta_{average} - \delta_i \quad (2)$$

where $\delta_{average}$ is the average of the displacements when the maximum load is applied and $\Delta\delta_i$ is the difference between $\delta_{average}$ and the displacement from the corresponding LVDT.

The results from Table 5.2 shows that only six resilient modulus tests have α value greater than 1 ($\alpha>1$) indicating the non-uniformity deformations between LVDT's were minimized during testing. Although the coefficient α indicates the degree of non-uniformity in the displacements from the LVDTs, there are other ways to calculate how accurate and uniform the displacement readings are. Kim [21] worked with the uniformity ratio (γ) to calculate the uniformity in the displacements from the LVDTs for the same resilient modulus setup. Kim [21] shows that if rotation (bending, eccentricity between the middle of the platen and the axis of the load) occurs, the measured displacement consists of a component due to axial force and a component due to bending [21]. To evaluate the influence of rotation on the displacements obtained from three LVDTs, the uniformity ratio (γ) is used. However, if rotation occurs, for equi-angular placement of the LVDTs Kim [21] shows that the mean of the three LVDTs readings is equal to the displacement from the axial load and rotation does not affect the average value of axial deformation.

To reduce the effects on the non-uniformity of the LVDTs displacement readings in the calculation of the resilient modulus, the following steps were made prior to testing:

- Leveled the top surface of the specimen with sand.
- Used double o-rings to make sure slippage between the LVDT holder and specimen would not occur.
- Aligned the specimen's center with axis of load using a pin guide in the bottom platen.

Table 5.2 α-Values for Resilient Modulus Tests

Sample ID	Uniformity Coefficient α
S-1-1-0	0.74
S-1-1-0-2	1.25
S-2-1-0	1.01
S-3-1-0	1.03
S-1-1-1-A	0.71
S-2-1-1-A	0.46
S-3-1-1-A	0.56
S-4-1-1-A	1.35
S-5-1-1-A	0.77
S-1-1-1-B	0.71
S-2-1-1-B	0.50
S-1-1-05-B	1.06
S-1-1-15-B	1.24
S-1-2-0	0.61
S-2-2-0	0.02
S-3-2-0	0.73
S-5-2-0	0.86
S-1-2-1-A	0.73
S-2-2-1-A	0.22
S-3-2-1-A	0.12
S-4-2-1-A	0.14
S-5-2-1-A	0.08
S-6-2-1-A	0.07
S-7-2-1-A	0.12
S-1-2-1-B	0.37
S-2-2-1-B	0.30
S-3-2-1-B	0.07
S-4-2-1-B	0.01
S-5-2-1-B	0.29
S-6-2-1-B	0.45
S-7-2-1-B	0.26
S-1-2-05-A	0.60
S-1-2-15-A	0.56
S-1-2-05-B	0.45
S-1-2-15-B	0.37

General Resilient Modulus Test Results

To analyze the resilient modulus data, a program developed in Visual BASIC was used. This program is based on the recommendations for the analysis of resilient modulus data in the National Cooperative Highway Research Program NCHRP 1-28A protocol [18].

Figures 5.2 to 5.5 show the resilient modulus for soils I and II as a function of the mean stress. The mean stress is defined as follows:

$$\theta = \frac{\sigma_1 + \sigma_2 + \sigma_3}{3} \tag{3}$$

where σ_1, σ_2, σ_3 are the principal stresses for the resilient modulus test; σ_2 and σ_3 are equal. Equation (3) becomes:

$$\theta = \frac{\Delta\sigma + 3\sigma_C}{3} \tag{4}$$

where $\Delta\sigma$ is the deviatoric cyclic stress and σ_c is the confining pressure for the corresponding sequence.

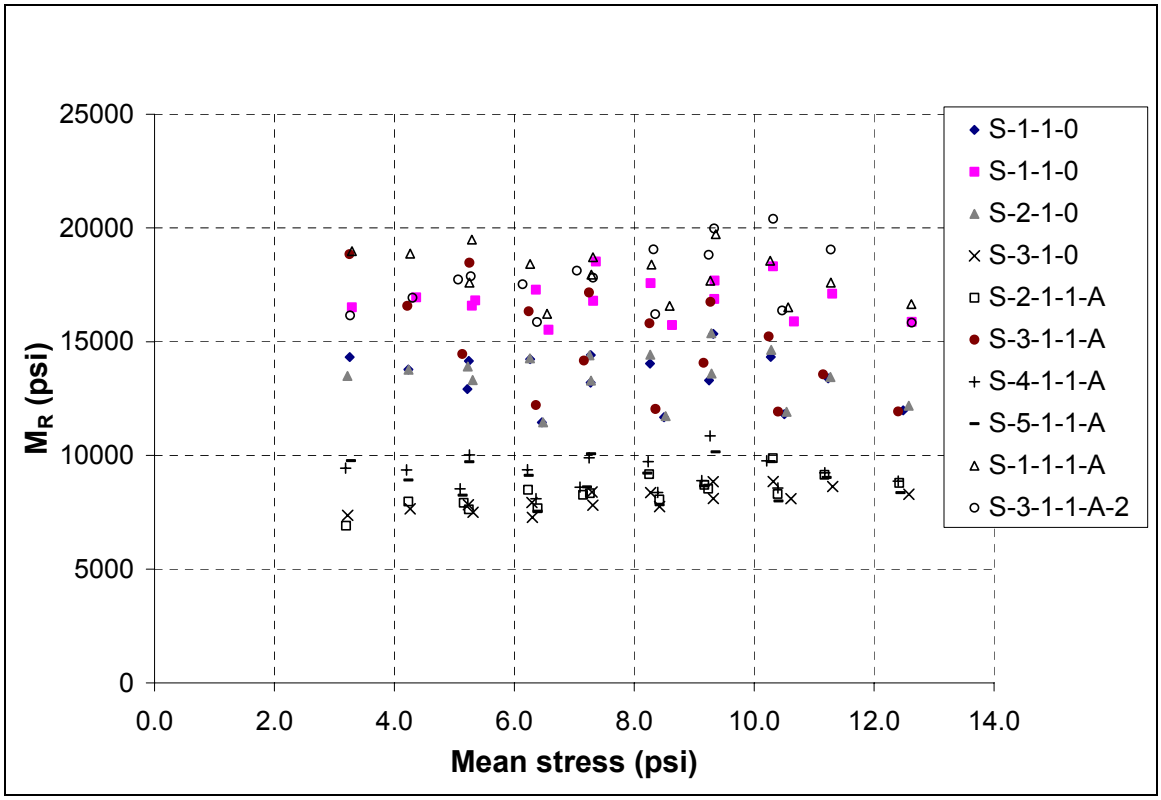


Figure 5.2 M_R vs Mean Stress for Soil I with Enzyme A

Figures 5.2 to 5.5 show that the M_R values for soils I and II (cohesive soils) decreases with the increment of the deviatoric cyclic stress and increases with the increment of the confining pressure.

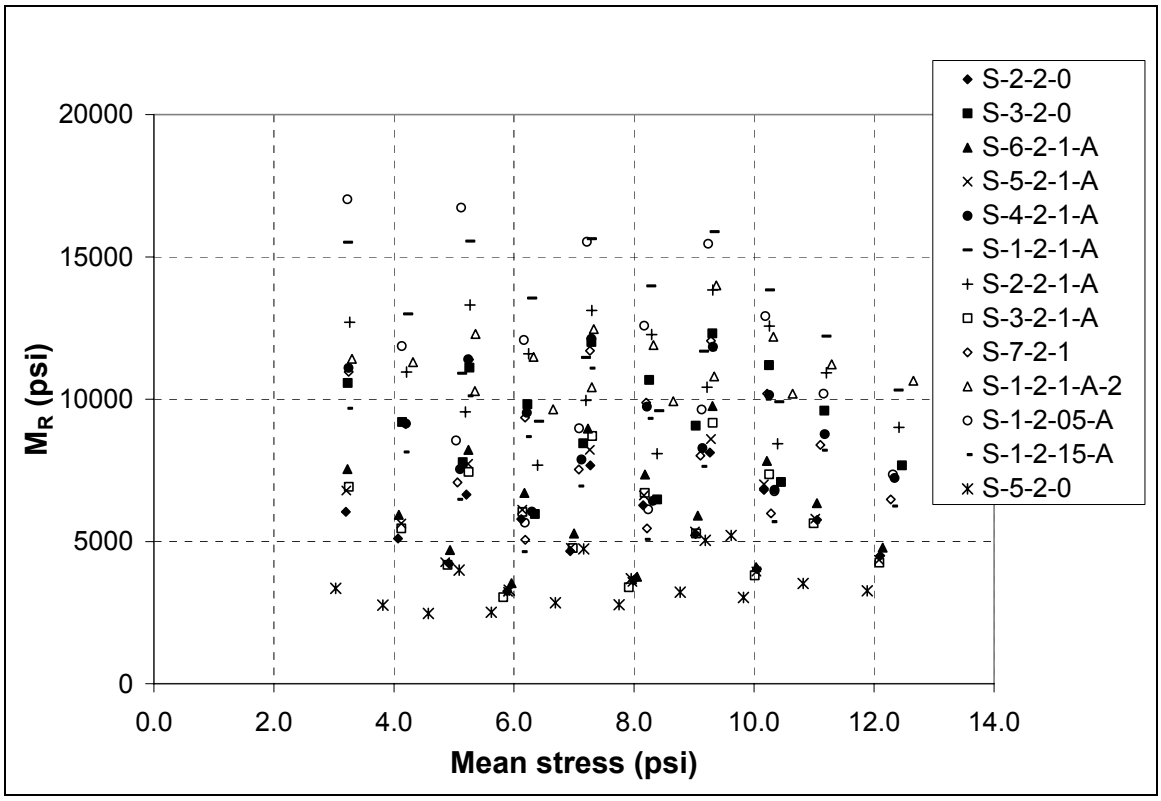


Figure 5.3 M_R vs Mean Stress for Soil II with Enzyme A

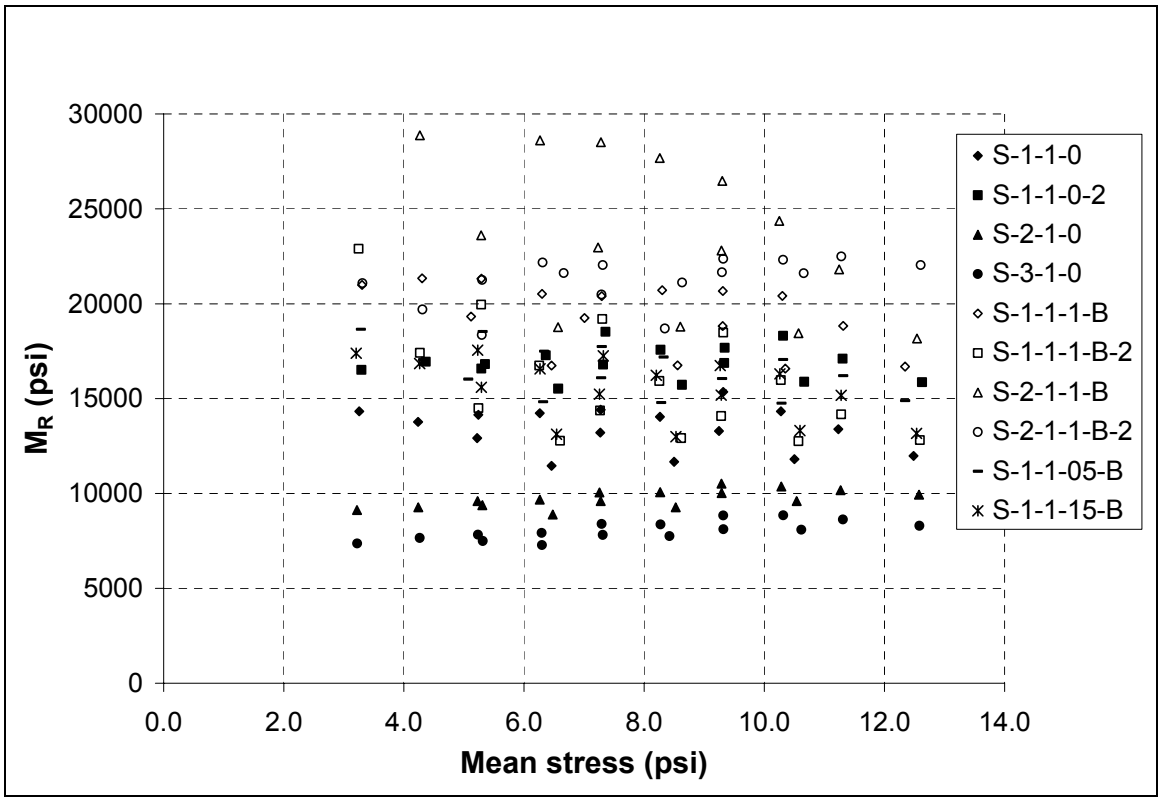


Figure 5.4 M_R vs Mean Stress for Soil I with Enzyme B

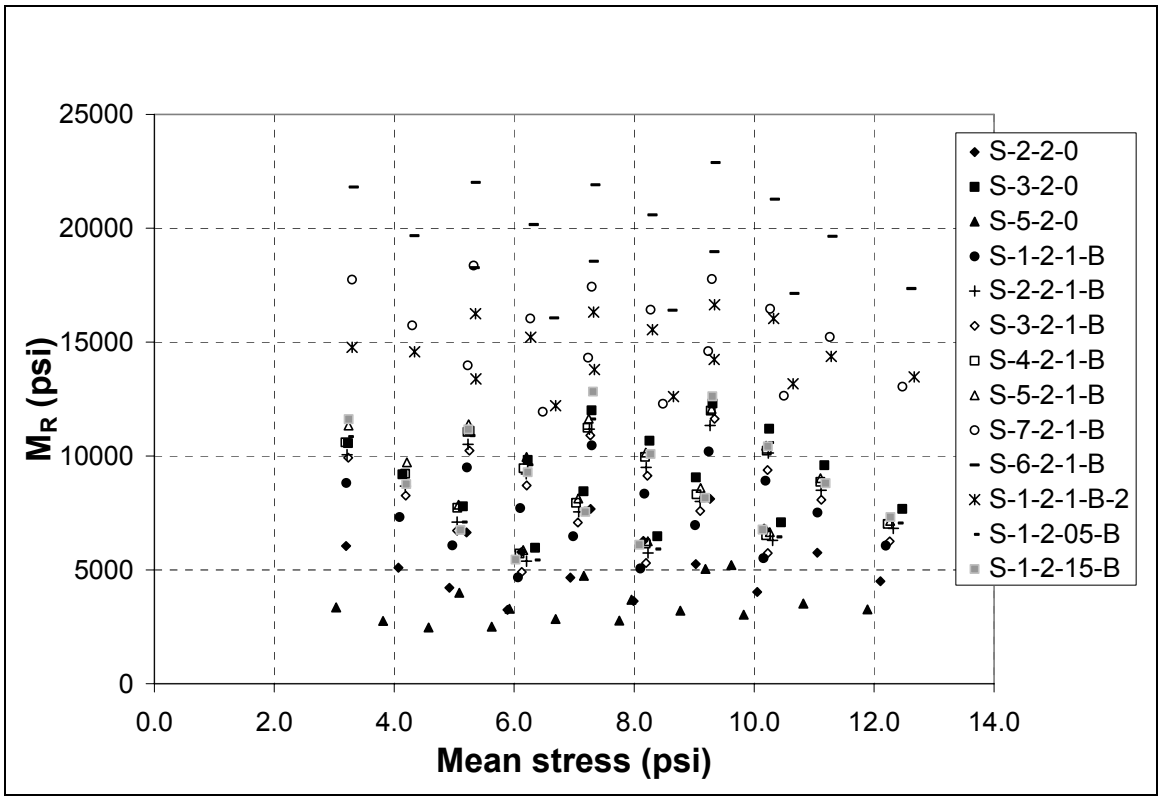


Figure 5.5 M_R vs Mean Stress for Soil II with Enzyme B

Figure 5.2 shows the results from the resilient modulus tests for soil I and enzyme A; a total of four untreated specimens and six treated specimens using the manufactures application rate were tested.

Figure 5.3 shows the results for soil II and enzyme A. Eight specimens were tested with the manufacturer's suggested application rate. Two treated specimens using different enzyme concentrations and three untreated specimens were also tested.

The resilient modulus values for soil I and enzyme B is shown in Figure 5.4, four tests were run using the manufacturer's suggested application rate. One test was done using 0.5cc of enzyme B per 5 liters of water, another test with 1.5cc of enzyme B per 5 liters of water and three tests were run on untreated specimens.

Figure 5.5 shows the M_R values for soil II and enzyme B. Eight treated specimens were tested with the manufacturer's suggested concentration, two treated specimens were tested using different enzyme concentrations and three tests were run on untreated specimens.

A comparison between the resilient modulus of treated (enzyme A and B) and untreated specimens for different combinations of deviatoric cyclic stress and confining pressure are shown in Figures 5.6 to 5.13.

Figure 5.6 shows the average results for the resilient modulus of soil I (treated and untreated) at different confining pressures (8psi, 6psi, 4psi, 2psi) and deviatoric stress of 4 psi. The results indicated that treatment with enzyme B increases the resilient modulus by 85% and the treatment of soil I with enzyme A increases the resilient modulus on average by 10%.

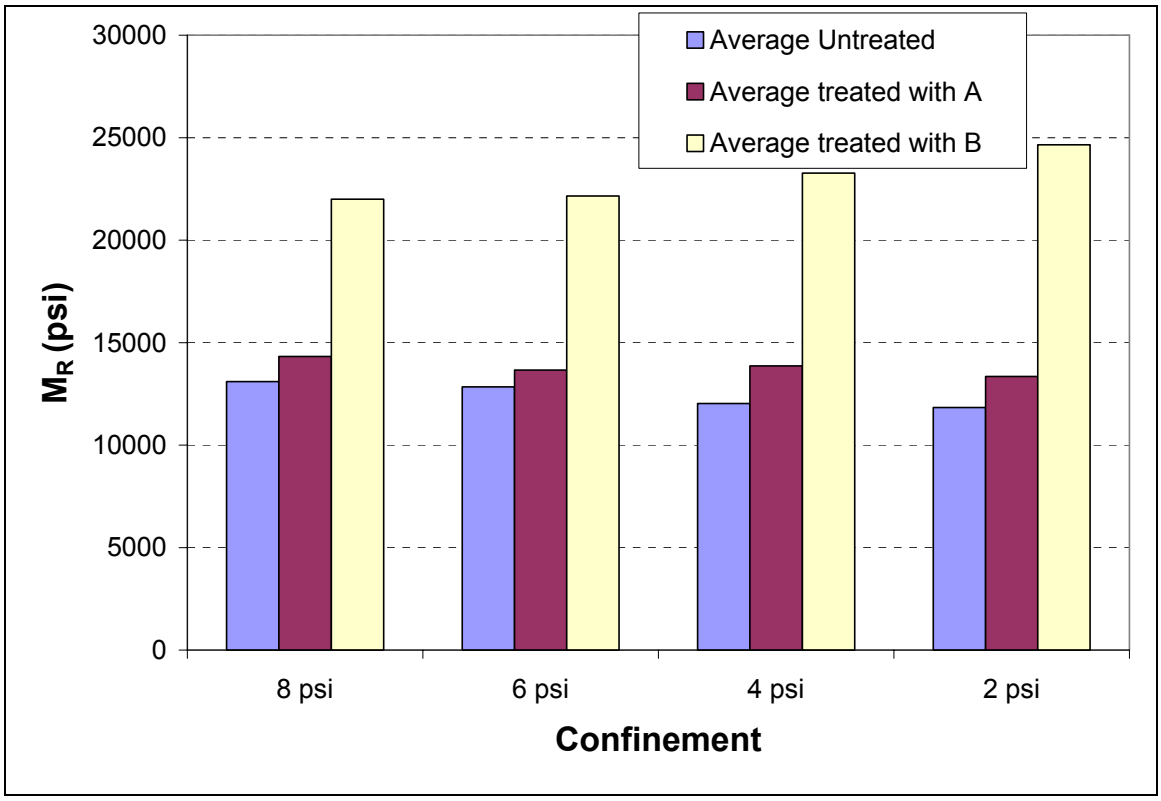


Figure 5.6 M_R Results for Soil I Deviatoric = 4 psi

For a deviatoric stress of 7 psi, the treatment with enzyme A increases the resilient modulus by 8% and treatment with enzyme B increases the M_R values by 72% (see Figure 5.7).

Figure 5.8 shows that an increase of 6% in the M_R of soil I for a deviatoric stress of 10 psi was obtained when enzyme A was used, and an increase of 63% when enzyme B was used.

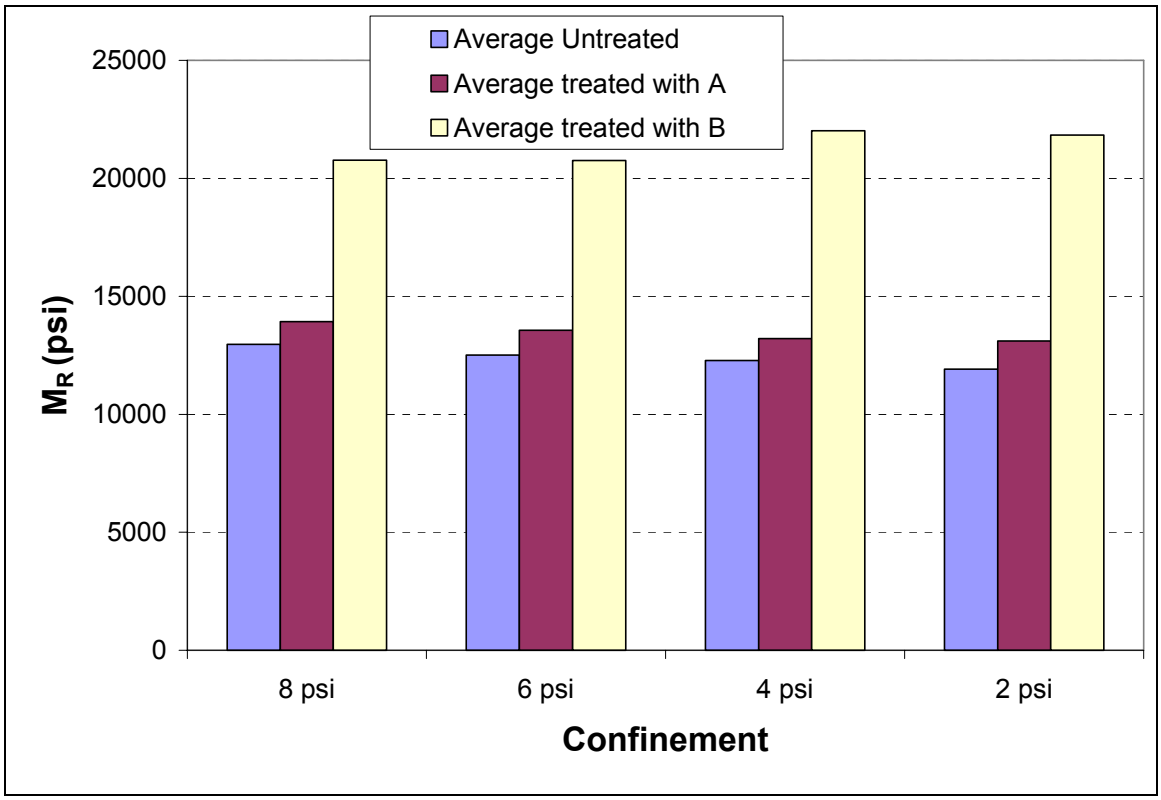


Figure 5.7 M_R Results for Soil I Deviatoric = 7 psi

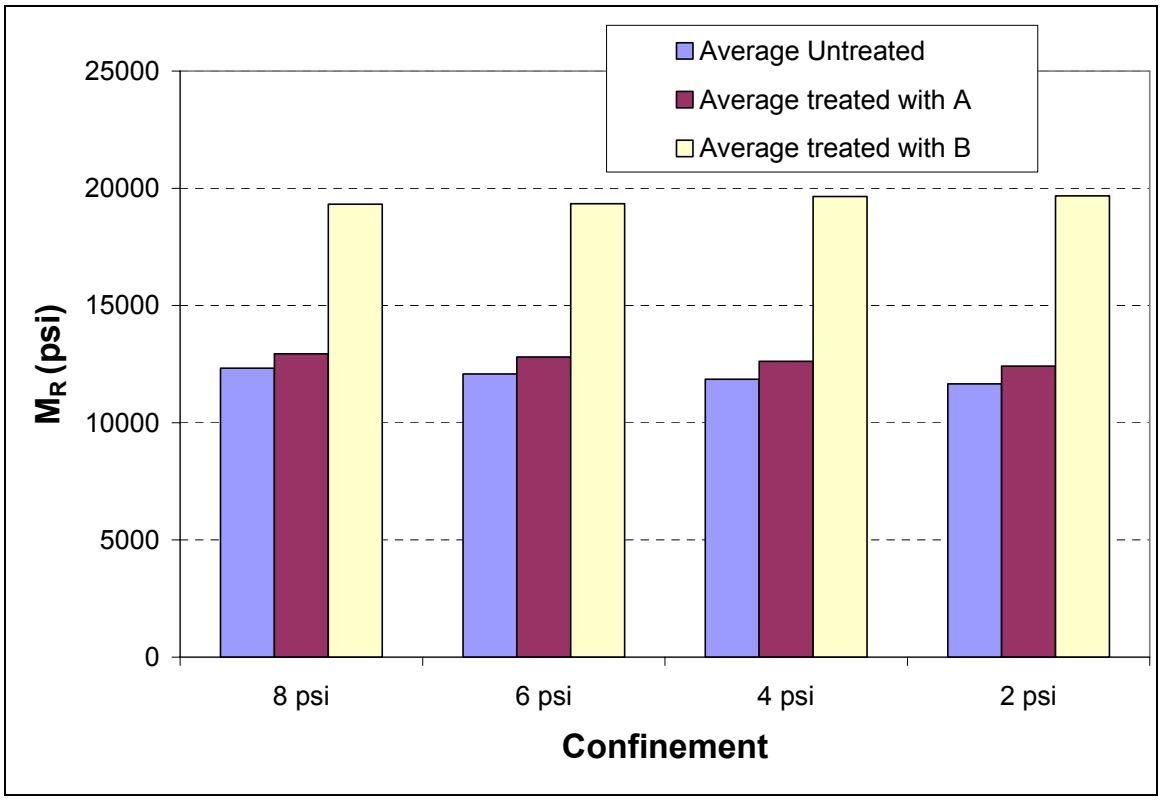


Figure 5.8 M_R Results for Soil I Deviatoric = 10 psi

An increment of 3% in the resilient modulus of soil I for a deviatoric stress of 14 psi was obtained when enzyme A was used and 55% increment for treatment with enzyme B (see Figure 5.9).

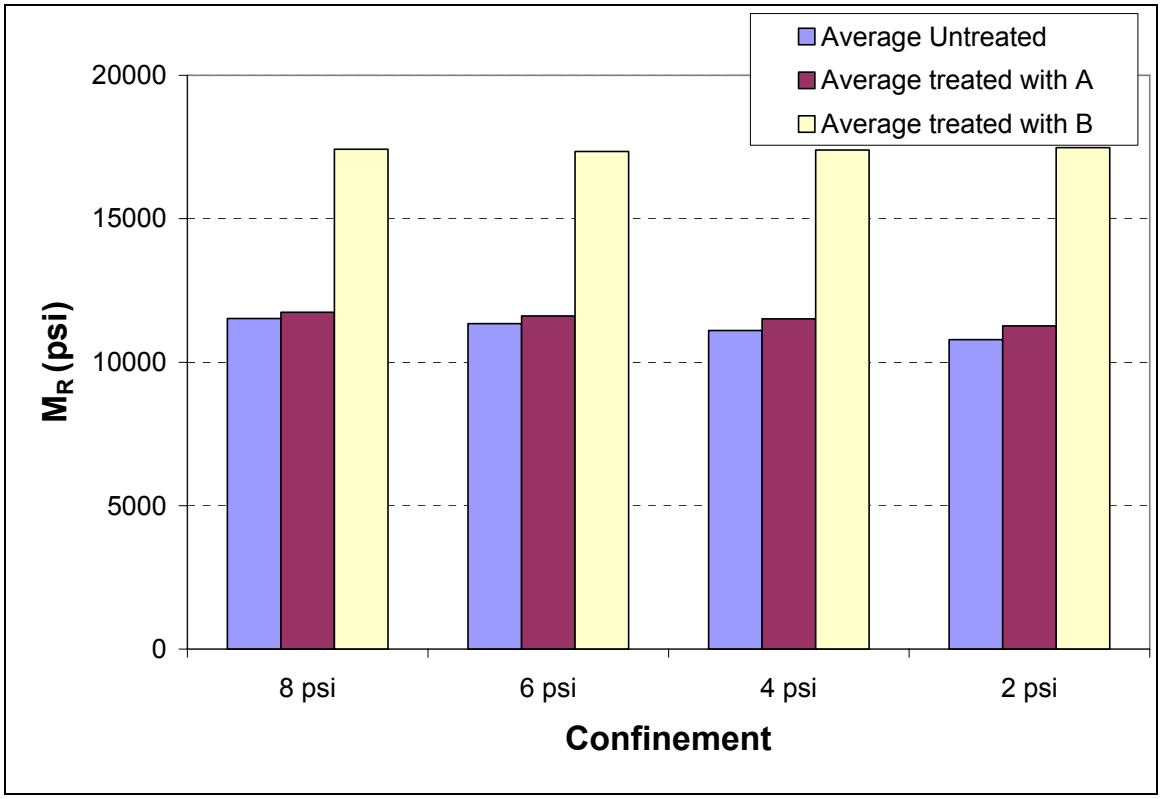


Figure 5.9 M_R Results for Soil I Deviatoric = 14 psi

The results from soil II shows that for a deviatoric stress of 4 psi an average increase of 51% was obtained when using enzyme A and 57% average increment for the treatment with enzyme B (see Figure 5.10).

Figure 5.11 shows that the M_R values for soil II increases by 51% for a deviatoric stress of 7 psi for the treatment with enzyme A and by 73% for the treatment with enzyme B.

The treatment of soil II with enzyme A increases the M_R values on average by 55% for a deviatoric stress of 10 psi and an average increment of 100% when treatment with enzyme was used (see Figure 5.12).

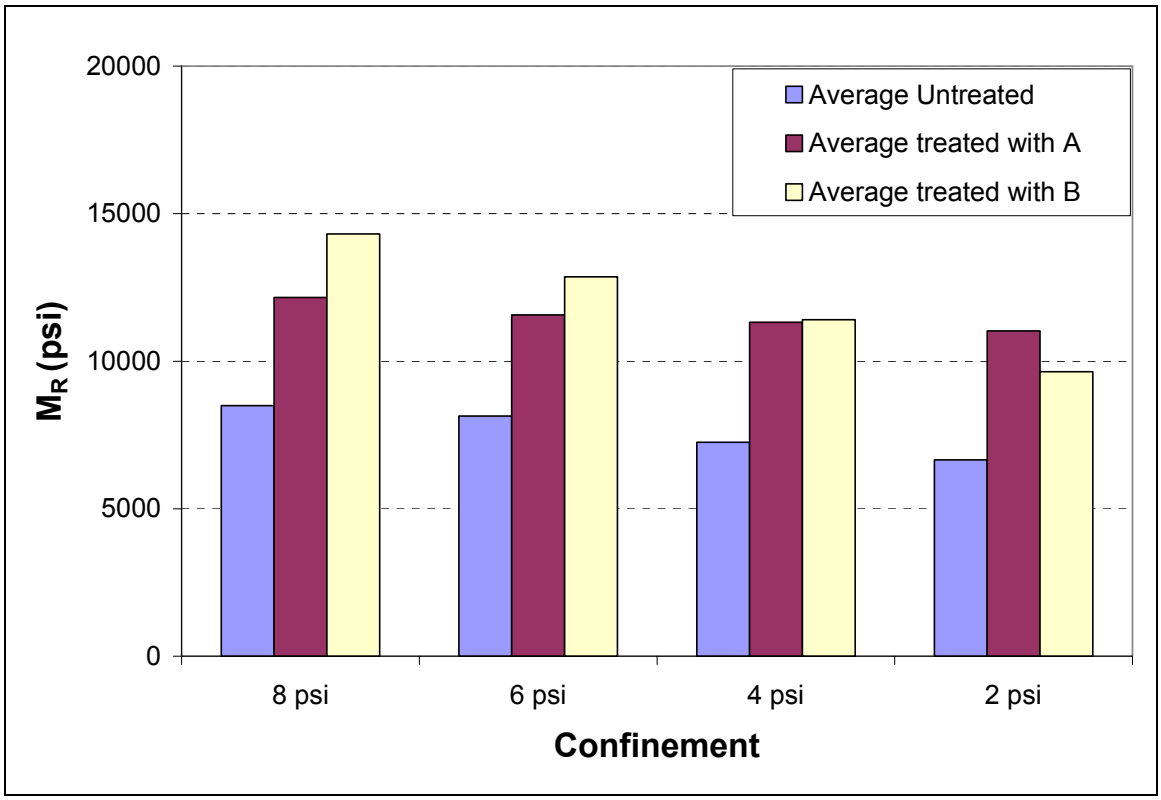


Figure 5.10 M_R Results for Soil II Deviatoric = 4 psi

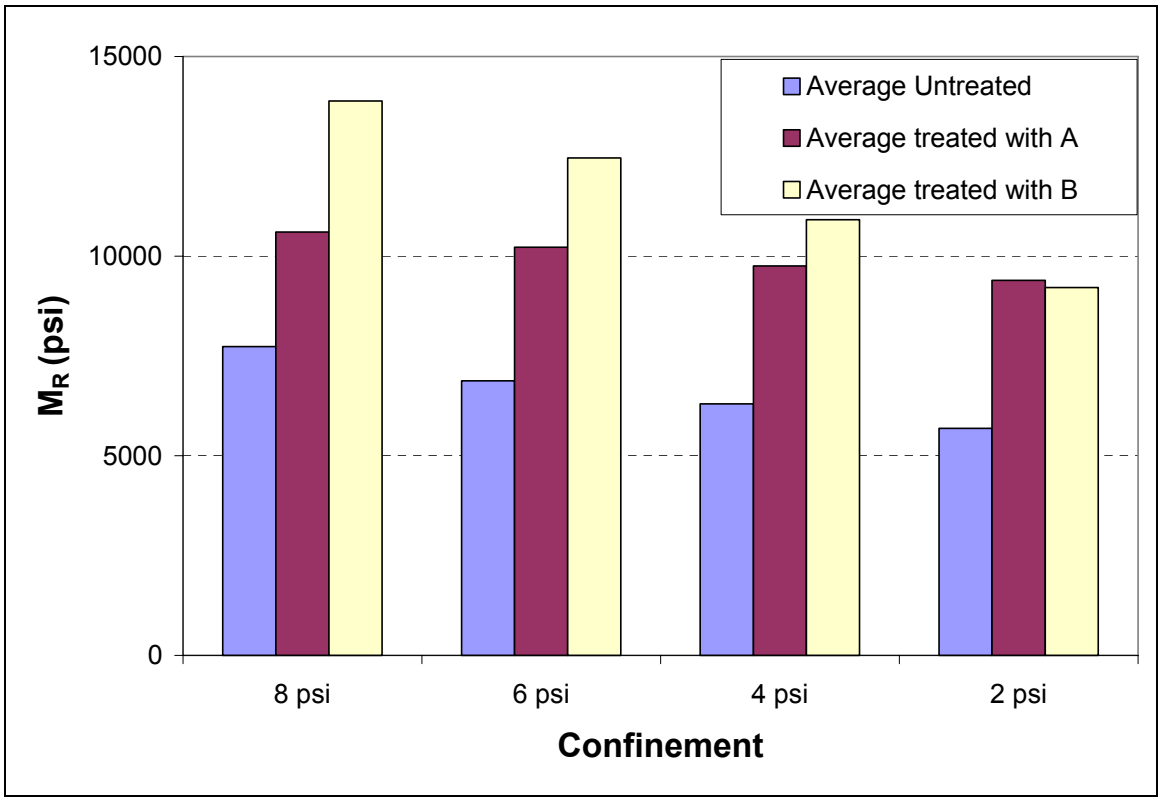


Figure 5.11 M_R Results for Soil II Deviatoric = 7 psi

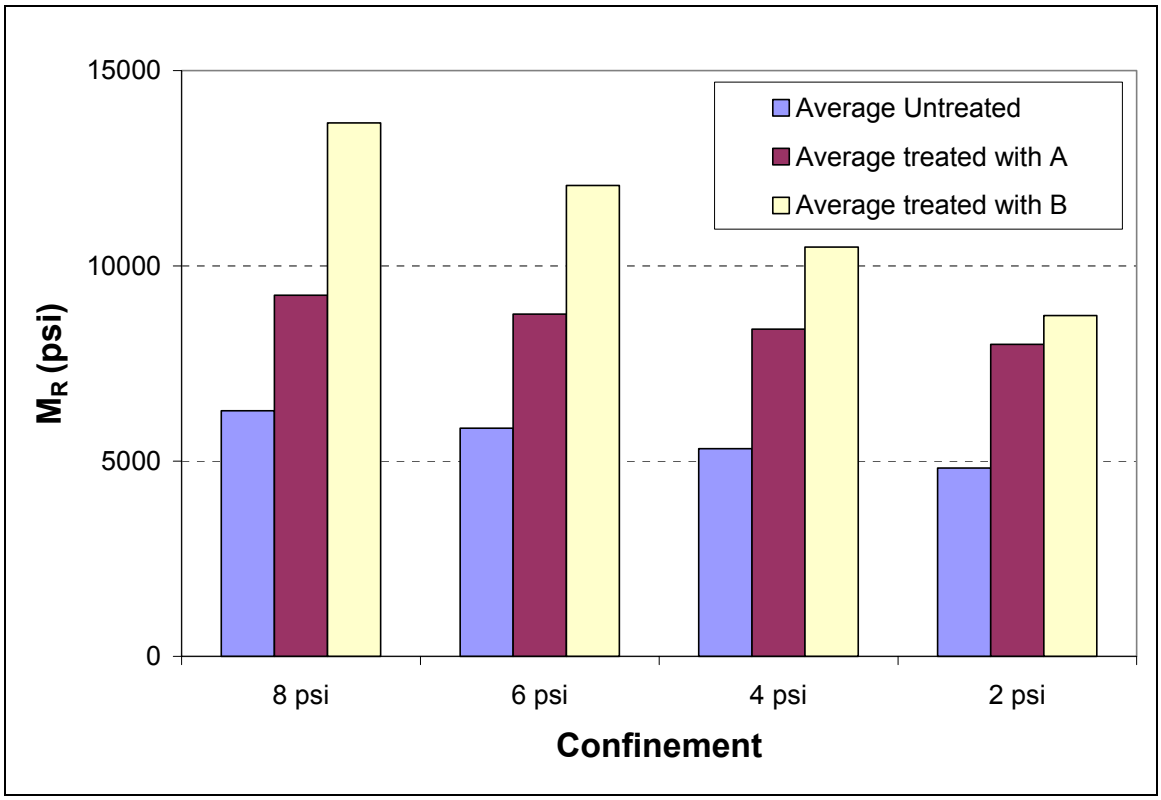


Figure 5.12 M_R Results for Soil II Deviatoric = 10 psi

Figure 5.13 shows that for soil II and deviatoric stress of 14 psi when enzyme A is used an average increment of 60% was obtained and when using enzyme B an average increment of 137% was found.

Statistical Analysis

The same procedures used to estimate and test hypotheses about a single parameter can be modified to be used for inferences about two parameters (e.g. comparison between two population's means) [22].

When the sample sizes of the two populations are small, the Central Limit Theorem cannot be applied, therefore, the z statistic cannot longer be used in the analysis [22]. However the Student's t-distribution can be used for the statistical analysis when the number of samples are less than 30 (n_1<30 and n_2<30). Due to the limited number of resilient modulus tests for the two treatments (Enzyme A and Enzyme B), the Student's t-

distribution was used to compare the population means of treated and untreated soil I and the population means of treated and untreated soil II.

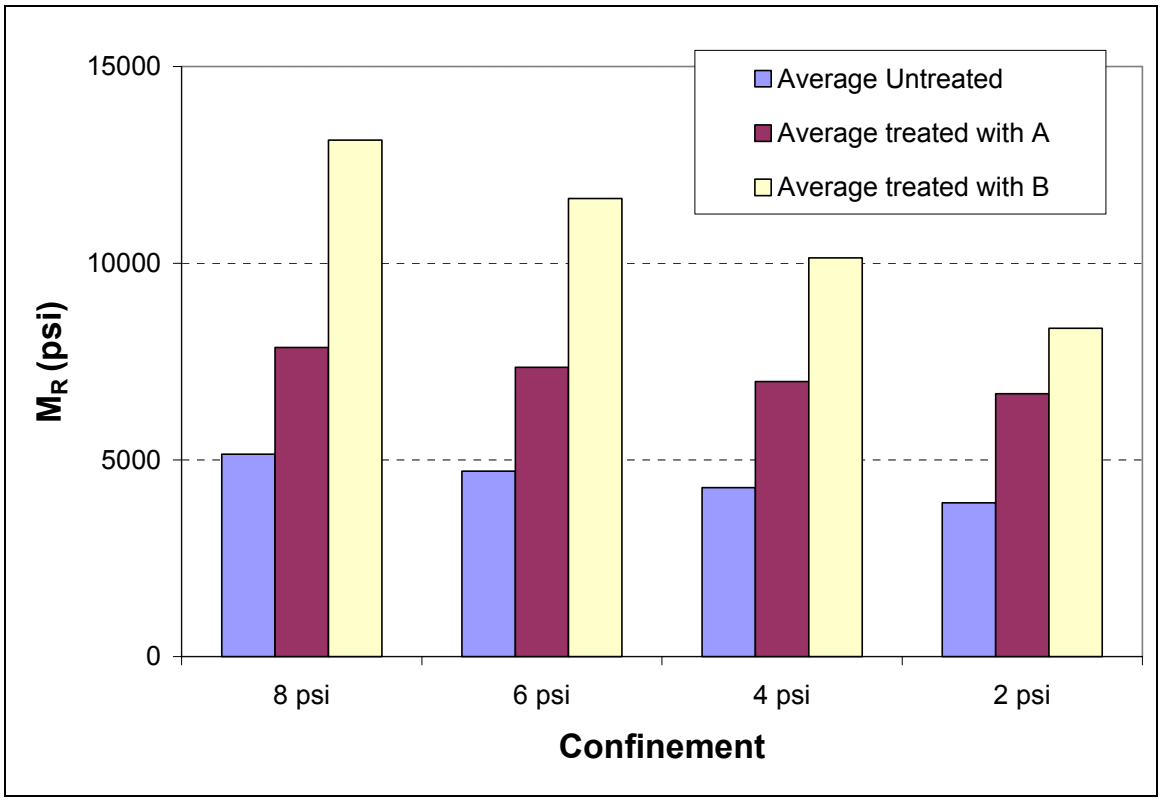
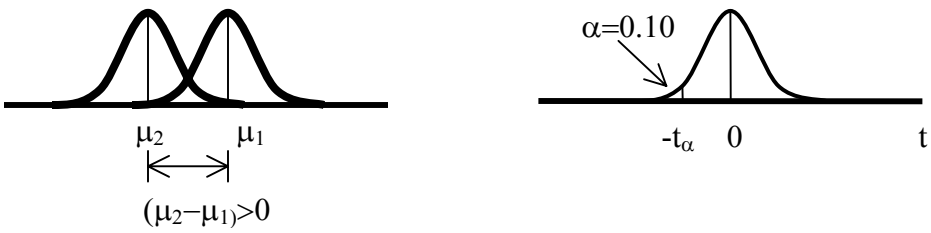


Figure 5.13 M_R Results for Soil II Deviatoric = 14 psi

When the t-distribution is used, both sampled populations have to be approximately normal distributed with equal standard deviation (see Figure 5.14a), also the samples must be selected independently [22]. The assumptions of normality and equal variances imply that the relative frequency distributions for the two populations would look as shown in Figure 5.14a [22].



(a) **(b)**

Figure 5.14 (a) Assumptions for Two-Sample Test. (b) Rejection Region for Test of Hypotheses

Both assumptions can be made from the populations of untreated and treated specimens. The variance can be assumed to be approximately equal because the same material, procedure, preparation machine, testing machine and operator were used to obtain the resilient modulus for the treated and untreated populations. Therefore the human error and material's intrinsic variance was the same for both populations.

If the two populations are assumed to have equal variances ($\sigma^2_1=\sigma^2_2$), then it is reasonable to use the information of both samples to calculate a pooled sample estimator of σ^2 to be used in the calculation of confidence intervals and tests of hypotheses [22]. The following formula was used to estimate the variance of the population:

$$S_p^2 = \frac{(n_1 - 1)s_1^2 + (n_2 - 1)s_2^2}{n_1 + n_2 - 2} \tag{5}$$

where,

S^2_p= variance pooled estimator.

s^2_1= calculated variance for population 1 (untreated tests).

s^2_2= calculated variance for population 2 (treated tests).

n_1= number of samples in population 1 (untreated tests).

n_2= number of samples in population 2 (treated tests).

The confidence interval and test of hypotheses formulas used to compare the populations means are shown in equation (6) and (7), respectively.

$$\bar{X}_1 - \bar{X}_2 \pm t_{\alpha/2}\sqrt{S_p^2\left(\frac{1}{n_1} + \frac{1}{n_2}\right)} \tag{6}$$

where,

X_1= mean estimator for population 1 (average for untreated tests).

$\overline{X_2}$= mean estimator for population 2 (average for treated tests).

n_1= number of samples in population 1 (untreated tests).

n_2= number of samples in population 2 (treated tests).

$t_{\alpha/2}$= t-student value based on α (type I error) and $n_1+ n_2$-2 degrees of freedom.

S^2_p= variance pooled estimator.

For the test of hypotheses a one-tailed test was used (see Figure 5.14b). The following null and alternative hypotheses were used with α=0.10 (type I error, reject H_o when H_o is true):

$$H_0 : \mu_1 - \mu_2 = 0$$
$$H_a : \mu_1 - \mu_2 < 0 \quad (7)$$

with the test statistic:

$$t = \frac{\bar{X}_1 - \bar{X}_2}{\sqrt{S_p^2\left(\frac{1}{n_1} + \frac{1}{n_2}\right)}} \quad (8)$$

and rejection region (values of the test statistic for which the null hypotheses is rejected):

$t < -t_\alpha$

where t_α are based on $n_1 + n_2 - 2$ degrees of freedom and the selected type I error (α).

A formal statistic comparison using the procedures explained before was performed for the results of the resilient modulus test for untreated and treated soils I and II. Tables 5.3 to 5.10 show the results of the statistical analysis of the resilient modulus data. Both 95% confidence intervals and tests of hypotheses with α=0.10 is presented.

Table 5.3 shows the 95% confidence intervals for the true mean difference (μ_1-μ_2) between resilient modulus of untreated and treated (with enzyme A) specimens of soil I. using the same estimation procedure over and over again for different samples, 95% of the confidence intervals formed in this way will enclose the true difference in population means (μ_1-μ_2), therefore it is hard to conclude from the intervals shown in Table 5.3 that the treatment with enzyme A on soil I improves the resilient modulus. For example, the confidence interval for the sequence 1 (deviatoric stress=4 psi and confining pressure= 8 psi) is (-6821, 6778). This confidence interval indicates that the mean μ_1 (the mean of untreated specimens) could be 6821 psi smaller than the mean μ_2 (the mean of treated specimens with A) and could be also 6778 psi larger than the mean μ_2. Therefore it is difficult to conclude the effectiveness of the enzyme A on soil I.

The results shown in Table 5.4 at the end of this chapter support the conclusions obtained from the results of the confidence intervals presented in Table 5.3. For all the sixteen sequences, failure to reject the null hypotheses was found. This means that we

cannot conclude that the difference between the population means of untreated and treated (with enzyme A) specimens of soil I is negative (μ_1-μ_2 <0). Therefore, it is difficult to conclude the effectiveness of treatment with enzyme A for soil I.

Table 5.5 shows the 95% confidence intervals for untreated and treated (with enzyme A) specimens of soil II. The confidence interval for the sequence 1 is (-7831, 500). This confidence interval indicates that the mean μ_1 could be 7831 psi smaller than the mean μ_2 and could also be 500 psi larger than the mean μ_2. Therefore there is a better chance to obtain a true negative mean difference and thus conclude that the resilient modulus increases when enzyme A is mixed with soil II.

Table 5.6 shows the test of hypotheses results for the treatment with enzyme A in soil II. Fail to reject the null hypotheses was found just for four out of the 16 sequences. This result supports the conclusion found with the confidence intervals in Table 5.5. There is an improvement in the resilient modulus when enzyme A is used in soil II.

Enzyme B was found to be effective in improving the resilient modulus of soil I (see Table 5.7 and Table 5.8). For example, the confidence interval for the sequence 16 (deviatoric stress=14 psi and confining pressure= 2 psi) is (-12539, -842). This means, that the mean μ_2 of the resilient modulus of treated (with enzyme B) specimens is between 12539 and 842 psi more than the mean μ_1 of resilient modulus of untreated specimens. Furthermore, Table 5.8 shows that for all combinations of deviatoric stresses and confinement pressures rejection of the null hypotheses was found, thus, there was support for the conclusion of effectiveness of enzyme B in the resilient modulus of soil I.

Table 5.9 shows the 95% confidence intervals for soil II treated with enzyme B. The confidence interval for the sequence 1 is (-10573, 2374), indicating that μ_1 (population mean of untreated specimens of soil II) could be 10573 psi smaller than the mean μ_2 (population mean of treated specimens of soil II with enzyme B). It can be seen also that μ_1 could be 2374 psi larger than the mean μ_2. Therefore, it is more likely to obtain a true negative mean difference (μ_1-μ_2<0) and thus conclude that the resilient modulus increases when enzyme B is used with soil II.

Table 5.10 shows the results for the test of the hypothesis for the treatment of soil II with enzyme B. Only 6 out of the 16 sequences were found to fail to reject H_o, thus supporting the results found before for the enzyme B and soil II combination.

From the statistical analysis of the data presented the following results can be observed:

(1) Treatment of soil I with enzyme A is not effective.
(2) Treatment of soil II with enzyme A increases the resilient modulus.
(3) Enzyme B increases the stiffness of soil I.
(4) Resilient modulus values were increased when enzyme B was used in soil II.
(5) The effectiveness of the enzyme stabilization is highly dependent on the soil type (e.g. chemical composition, clay content).
(6) The most effective treatment was the use of enzyme B in soil I (high clay content).

Analysis and Discussion

The resilient modulus represents the stiffness of the material tested. Testing was performed in order to evaluate:

(1) The change in resilient modulus with the addition of enzymes;
(2) Enzyme concentration effect on resilient modulus;
(3) Enzyme curing-time effect on the resilient modulus.

Figure 5.15 illustrates the effect of enzyme A on the resilient modulus of soil I. On average, the mechanical properties of soil I are not affected by the enzyme A application. On the other hand, the stiffness of soil I increases considerably when enzyme B is used (see Figure 5.16). Figures 5.17 and 5.18 shows that on average the resilient modulus increases for treatments of soil II with enzyme A and enzyme B.

General Observations and Comments

Several conclusions can be drawn from the test data and the above plots. Generally, the resilient modulus for the specimens treated with enzyme is higher than the untreated specimen's modulus. However, for soil I the resilient modulus does not increase with the addition of enzyme A. For example, Figure 5.15 shows the average resilient modulus values for soil I when is treated with enzyme A, it is clear that for all the combinations of deviatoric cyclic stress and confining pressures there is no improvement in the M_R values of the treated specimens.

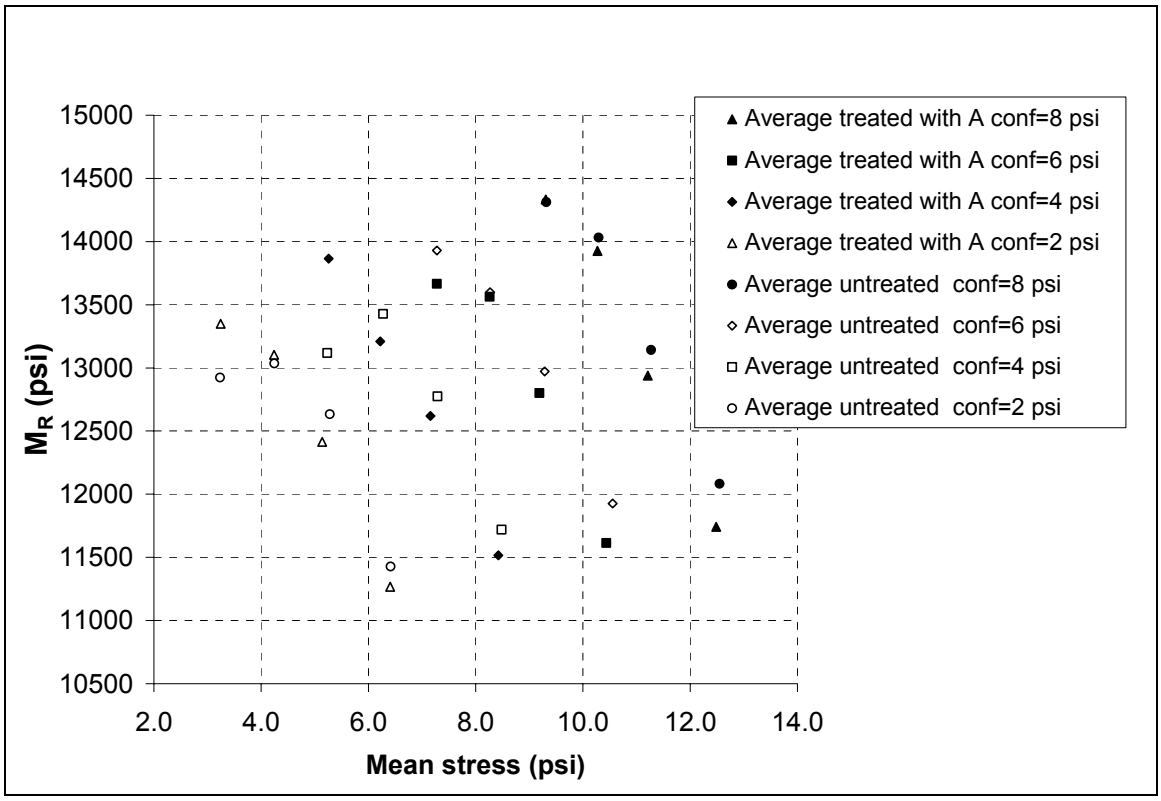


Figure 5.15 Average M_R vs Mean Stress for Soil I with Enzyme A

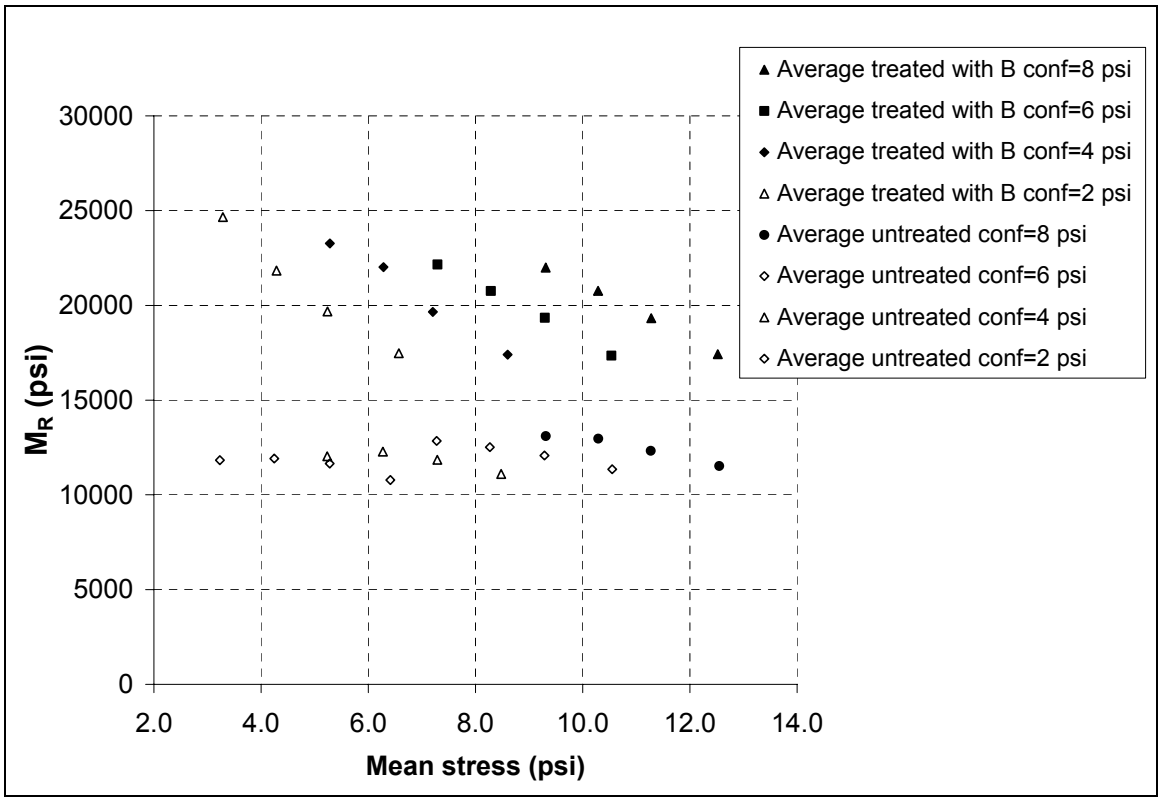


Figure 5.16 Average M_R vs Mean Stress for Soil I with Enzyme B

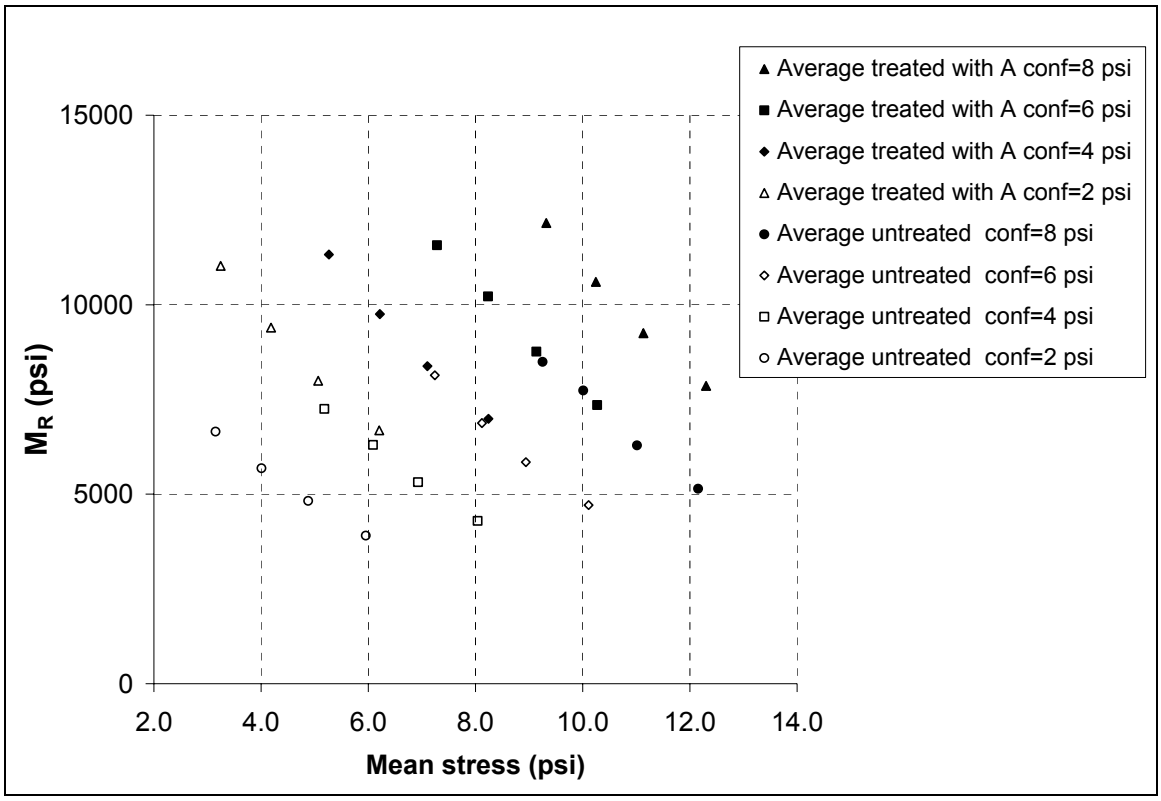


Figure 5.17 Average M_R vs Mean Stress for Soil II with Enzyme A

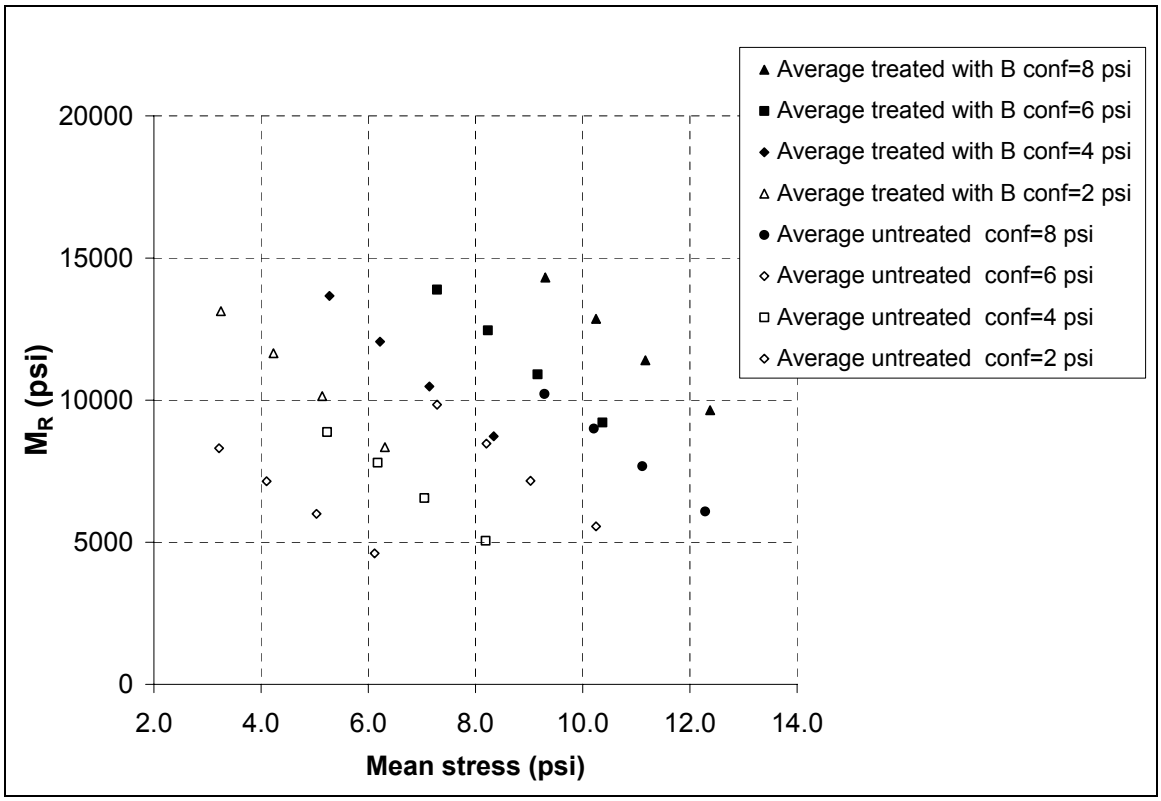


Figure 5.18 Average M_R vs Mean Stress for Soil II with Enzyme B

As described previously, the enzyme effect on the resilient modulus of the material depends on many characteristics of the soil such as the fines content, water content, chemical composition, gradation and other characteristics. Thus it is important to select the proper treatment to be applied in the subgrade of the pavement structure. Like in the case of this research, not all the combinations of enzyme and soil type improved the mechanical properties.

Deviations from the use of the manufacturer's suggested concentration rate would reduce the resilient modulus values (see Figures 5.19 to 5.23) that can be obtained when the optimum rate is used (1cc per 5 liters of water).

The results shown in Figures 5.24 and 5.25 demonstrate that the enzyme stabilization activity inside the soil's structure continues with time. The resilient modulus for all combinations of soil (I and II) and enzyme type (A, B) increases with curing time.

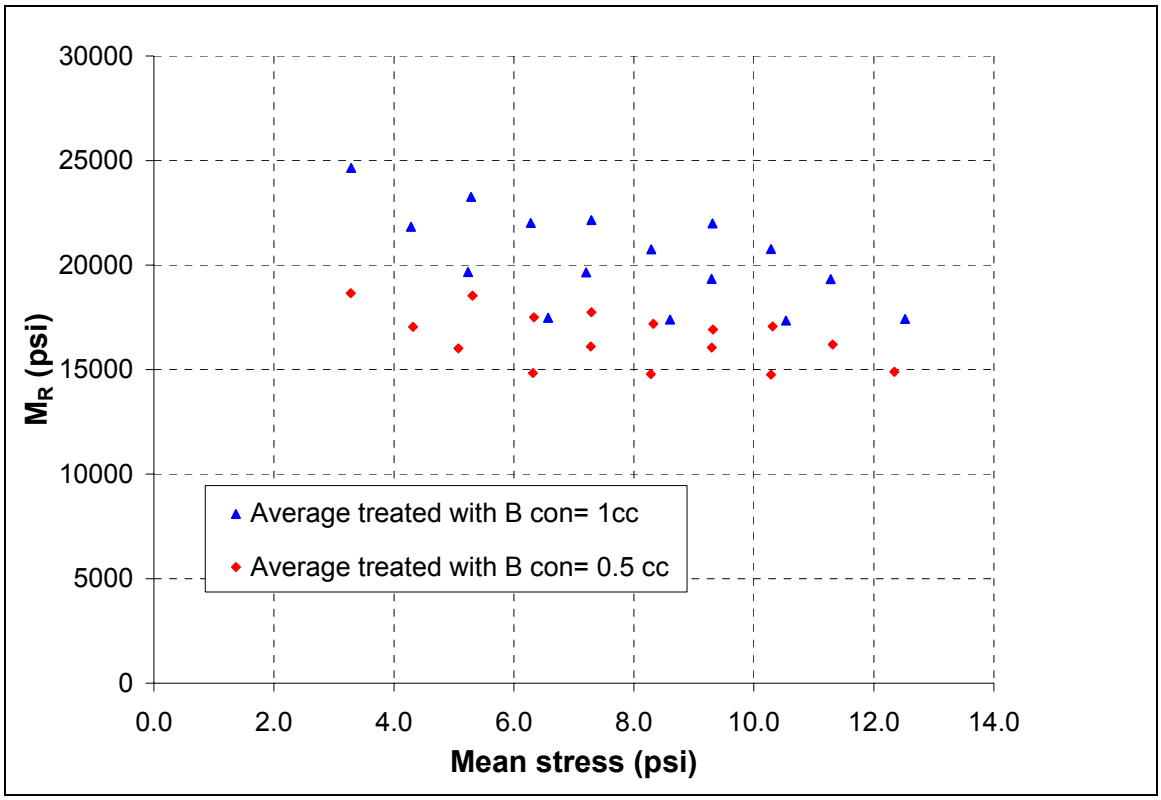


Figure 5.19 Effect of Enzyme B Concentration for Soil I

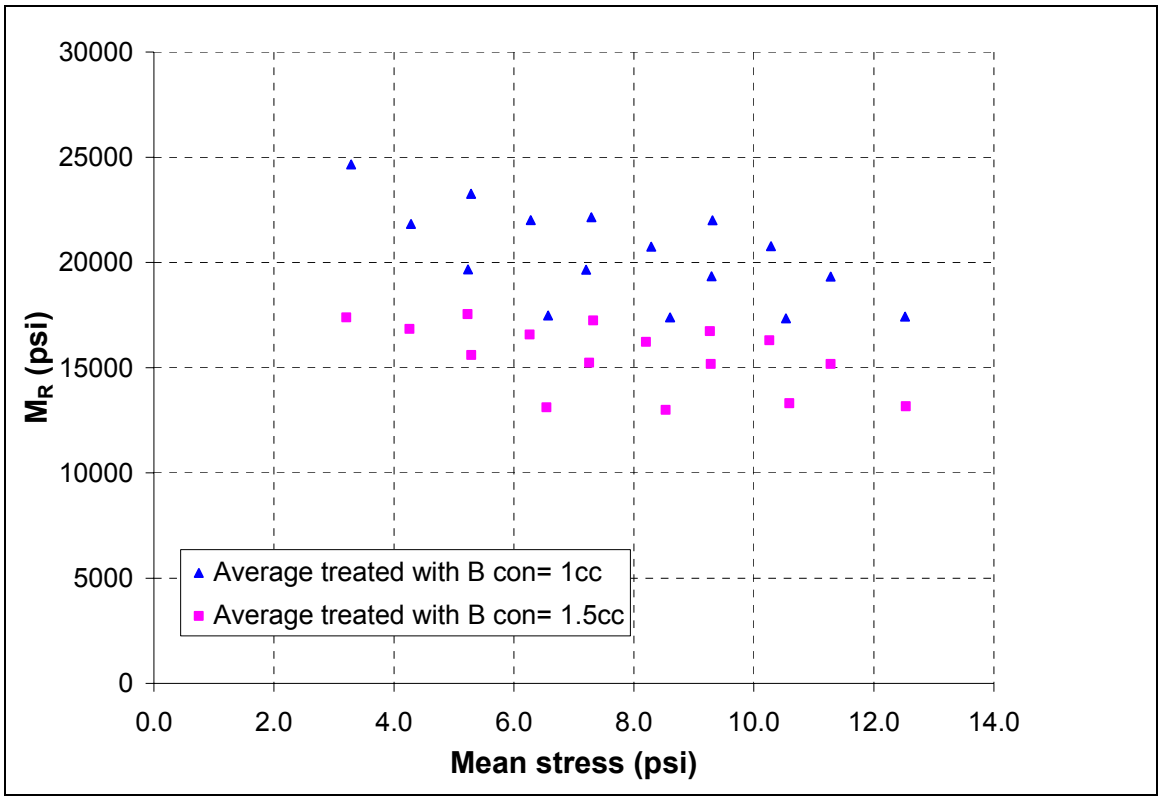


Figure 5.20 Effect of Enzyme B Concentration for Soil I

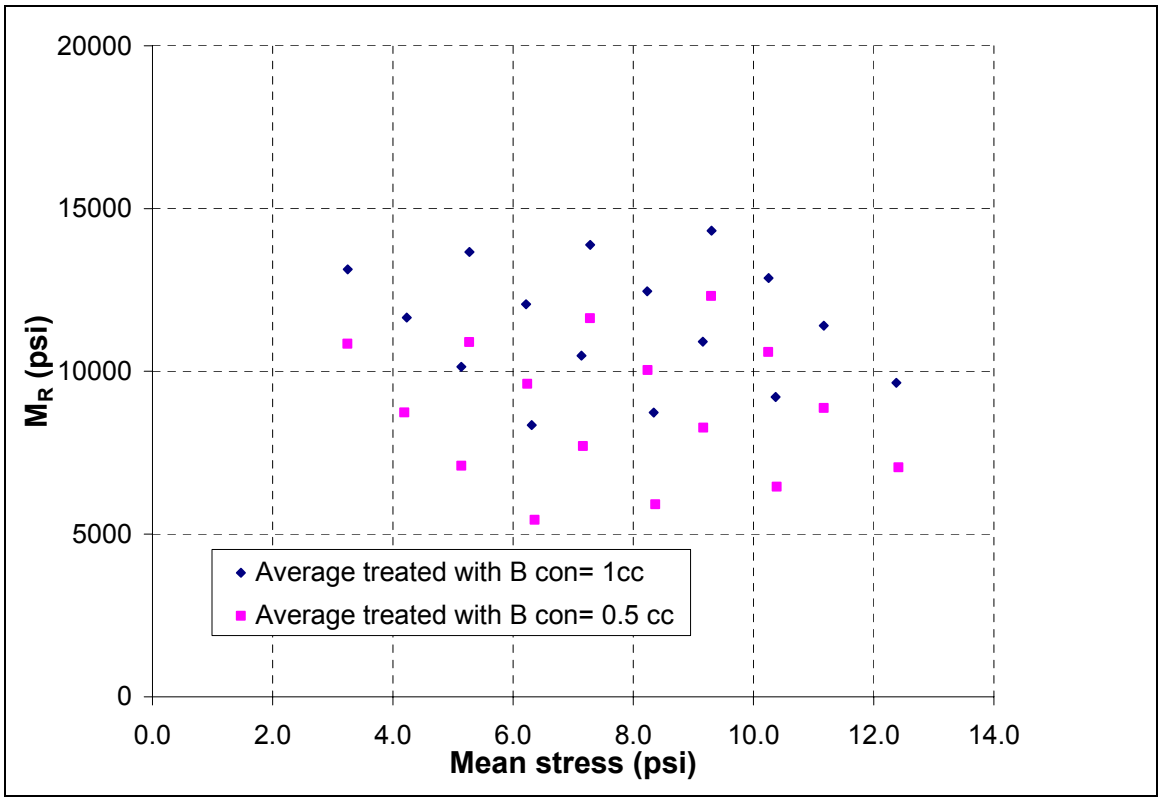


Figure 5.21 Effect of Enzyme B Concentration for Soil II

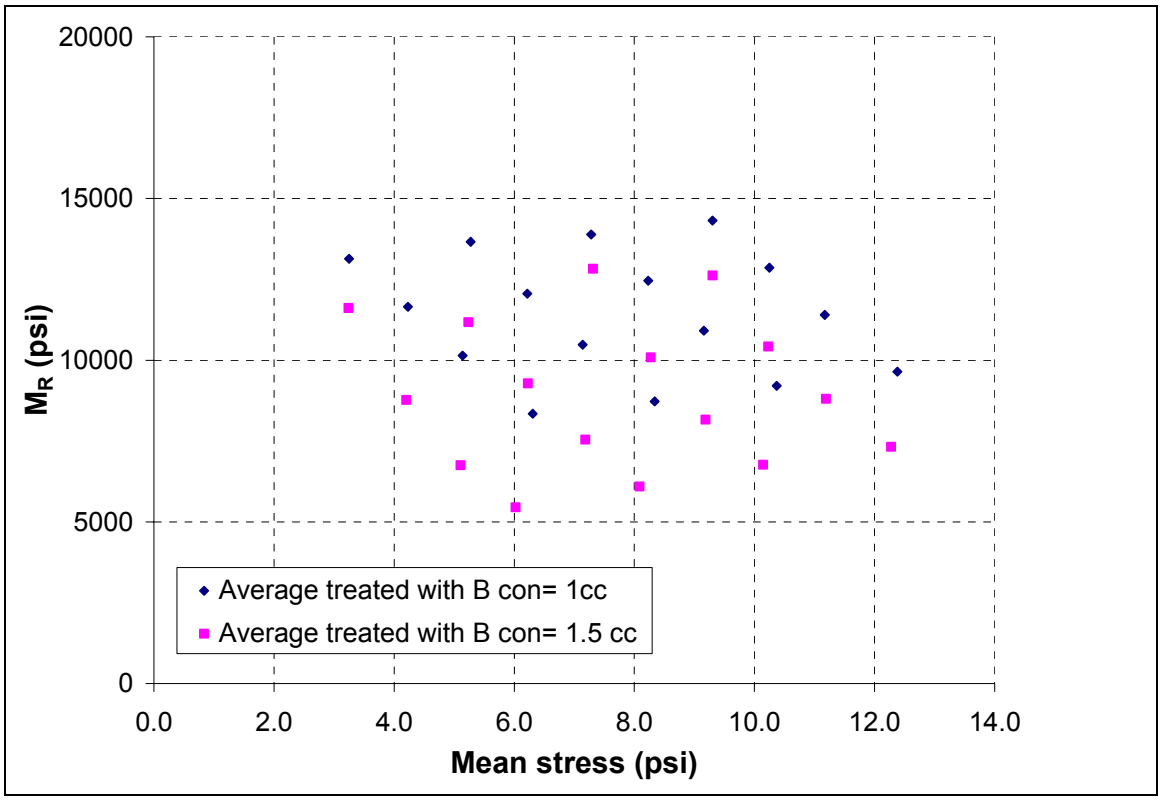


Figure 5.22 Effect of Enzyme B Concentration for Soil II

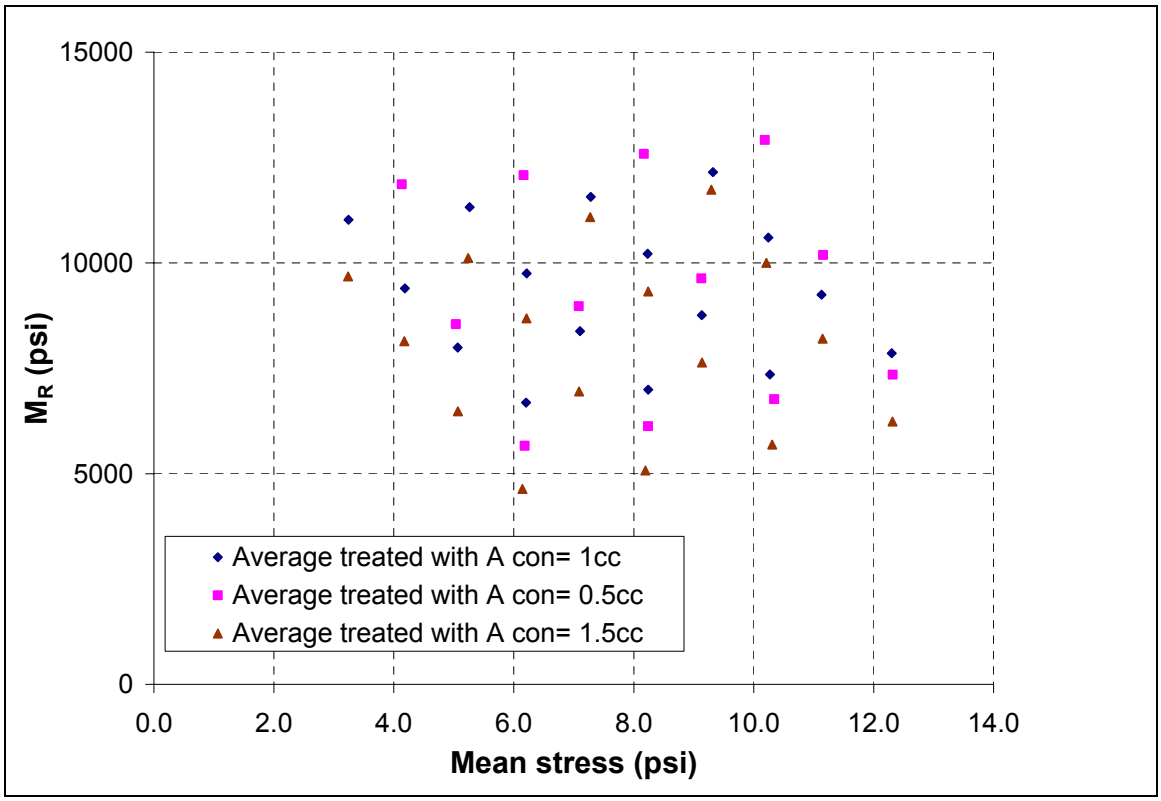


Figure 5.23 Effect of Enzyme A Concentration for Soil II

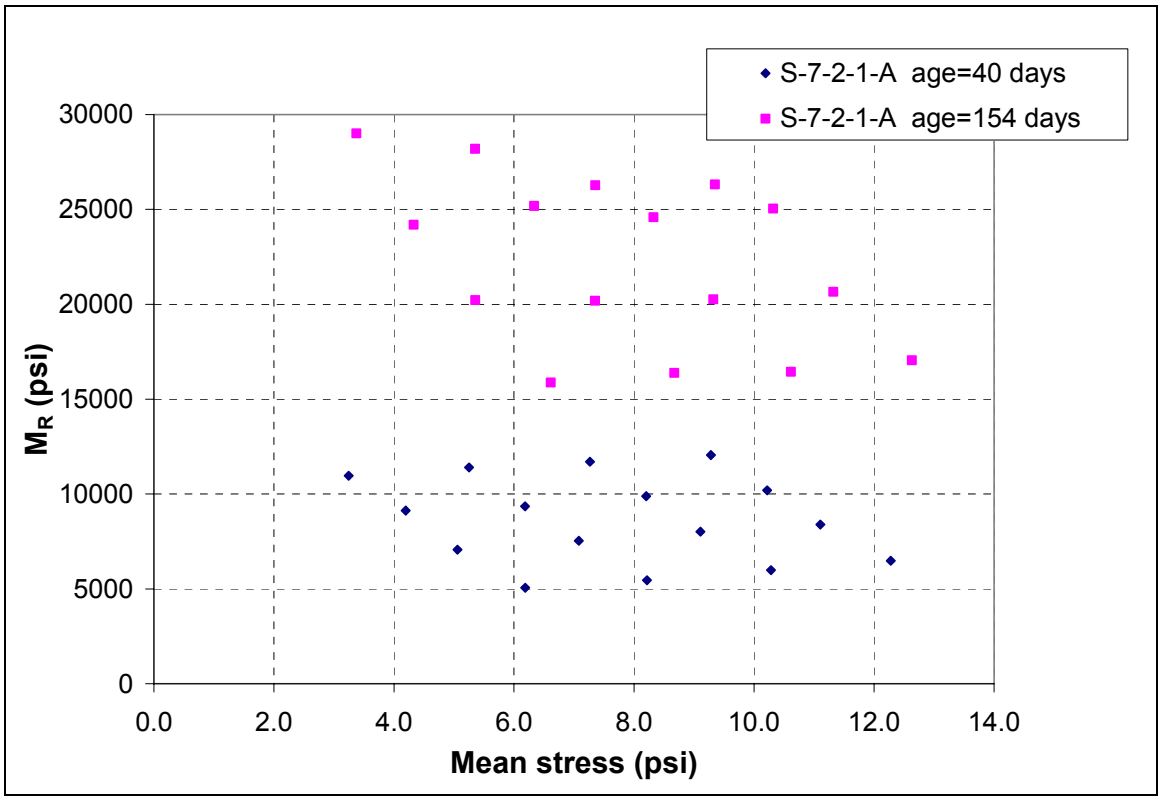


Figure 5.24 Effect of Curing Time Enzyme A

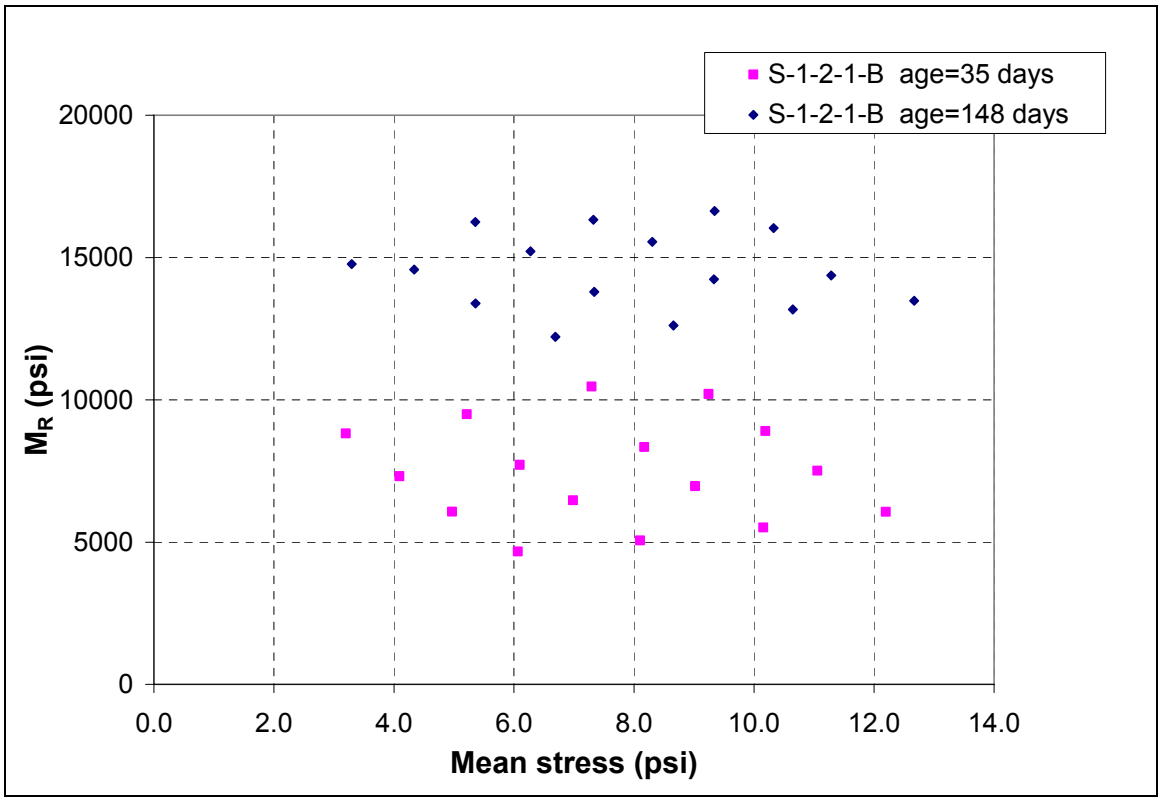


Figure 5.25 Effect of Curing Time Enzyme B

Table 5.3 95% Confidence Intervals for the True Mean Difference between Resilient Modulus Test Values for Soil I without and with Enzyme A

Conf (psi)	Devia (psi)	Untreated			Treated with A			S_p^2	$t_{\alpha/2}$	Confidence Interval	
		n_1	$\overline{x}_1$	Std. Dev.	n_2	$\overline{x}_2$	Std. Dev.			Lower limit	Upper Limit
8	4	4	14311	3807	6	14333	5100	21686900	2.262	-6821	6778
8	7	4	14032	3899	6	13926	4828	20270664	2.262	-6468	6680
8	10	4	13141	3474	6	12938	4535	17379231	2.262	-5884	6290
8	14	4	12083	3095	6	11742	3724	12259658	2.262	-4771	5453
6	4	4	13930	4173	6	13667	4700	20338367	2.262	-6322	6848
6	7	4	13600	3831	6	13564	4723	19448246	2.262	-6403	6475
6	10	4	12972	3626	6	12801	4711	18799554	2.262	-6160	6502
6	14	4	11927	3184	6	11614	4000	13803455	2.262	-5112	5738
4	4	4	13118	3727	6	13865	5288	22689800	2.262	-7702	6208
4	7	4	13427	3938	6	13210	4677	19486569	2.262	-6229	6662
4	10	4	12775	3707	6	12619	4738	19184061	2.262	-6239	6551
4	14	4	11720	3257	6	11516	4083	14396027	2.262	-5336	5744
2	4	4	12924	3918	6	13348	5278	23171381	2.262	-7453	6604
2	7	4	13035	3887	6	13103	4856	20401435	2.262	-6663	6527
2	10	4	12635	3848	6	12415	4732	19549973	2.262	-6236	6676
2	14	4	11429	3366	6	11268	4084	14673518	2.262	-5432	5754

Table 5.4 Small-Sample Test of Hypotheses for ($\mu_1-\mu_2$) Soil I and Enzyme A

Conf (psi)	Devia (psi)	Untreated			Treated with A			S_p^2	$-t_\alpha$	$t_{statistic}$	Reject Region
		n_1	$\bar{x}_1$	Std. Dev.	n_2	$\bar{x}_2$	Std. Dev.				$t<-t_\alpha$
8	4	4	14311	3807	6	14333	5100	21686900	-1.383	-0.007	Fail to reject Ho
8	7	4	14032	3899	6	13926	4828	20270664	-1.383	0.037	Fail to reject Ho
8	10	4	13141	3474	6	12938	4535	17379231	-1.383	0.075	Fail to reject Ho
8	14	4	12083	3095	6	11742	3724	12259658	-1.383	0.151	Fail to reject Ho
6	4	4	13930	4173	6	13667	4700	20338367	-1.383	0.090	Fail to reject Ho
6	7	4	13600	3831	6	13564	4723	19448246	-1.383	0.013	Fail to reject Ho
6	10	4	12972	3626	6	12801	4711	18799554	-1.383	0.061	Fail to reject Ho
6	14	4	11927	3184	6	11614	4000	13803455	-1.383	0.131	Fail to reject Ho
4	4	4	13118	3727	6	13865	5288	22689800	-1.383	-0.243	Fail to reject Ho
4	7	4	13427	3938	6	13210	4677	19486569	-1.383	0.076	Fail to reject Ho
4	10	4	12775	3707	6	12619	4738	19184061	-1.383	0.055	Fail to reject Ho
4	14	4	11720	3257	6	11516	4083	14396027	-1.383	0.083	Fail to reject Ho
2	4	4	12924	3918	6	13348	5278	23171381	-1.383	-0.137	Fail to reject Ho
2	7	4	13035	3887	6	13103	4856	20401435	-1.383	-0.023	Fail to reject Ho
2	10	4	12635	3848	6	12415	4732	19549973	-1.383	0.077	Fail to reject Ho
2	14	4	11429	3366	6	11268	4084	14673518	-1.383	0.065	Fail to reject Ho

Table 5.5 95% Confidence Intervals for the True Mean Difference between Resilient Modulus Test Values for Soil II without and with Enzyme A

Conf (psi)	Devia (psi)	Untreated			Treated with A			S_p^2	$t_{\alpha/2}$	Confidence Interval	
		n_1	$\overline{x}_1$	Std. Dev.	n_2	$\overline{x}_2$	Std. Dev.			Lower limit	Upper Limit
8	4	3	8492	3648	9	12157	2550	7865286	2.228	-7831	500
8	7	3	7738	3098	9	10602	2781	8107770	2.228	-7093	1366
8	10	3	6291	3070	9	9249	2994	9058567	2.228	-7428	1513
8	14	3	5147	2274	9	7858	3275	9613562	2.228	-7317	1894
6	4	3	8140	3657	9	11568	2471	7558008	2.228	-7511	656
6	7	3	6880	3532	9	10220	2872	9095014	2.228	-7820	1139
6	10	3	5847	2970	9	8763	2899	8487461	2.228	-7244	1411
6	14	3	4715	2113	9	7352	3249	9337621	2.228	-7176	1901
4	4	3	7250	3597	9	11325	2977	9676282	2.228	-8695	546
4	7	3	6300	3298	9	9752	2975	9255365	2.228	-7971	1066
4	10	3	5319	2858	9	8380	3109	9366840	2.228	-7607	1485
4	14	3	4294	1938	9	6992	3299	9460296	2.228	-7266	1871
2	4	3	6655	3648	9	11026	3496	12441424	2.228	-9611	868
2	7	3	5684	3255	9	9394	3201	10318637	2.228	-8481	1061
2	10	3	4825	2709	9	7993	3282	10085796	2.228	-7886	1548
2	14	3	3908	1818	9	6686	3366	9726459	2.228	-7410	1854

Table 5.6 Small-Sample Test of Hypotheses for ($\mu_1-\mu_2$) Soil II and Enzyme A

Conf (psi)	Devia (psi)	Untreated			Treated with A			S_p^2	$-t_\alpha$	$t_{statistic}$	Reject Region
		n_1	$\overline{x}_1$	Std. Dev.	n_2	$\overline{x}_2$	Std. Dev.				$t<-t_\alpha$
8	4	3	8492	3648	9	12157	2550	7865286	-1.372	-1.960	Reject Ho
8	7	3	7738	3098	9	10602	2781	8107770	-1.372	-1.509	Reject Ho
8	10	3	6291	3070	9	9249	2994	9058567	-1.372	-1.474	Reject Ho
8	14	3	5147	2274	9	7858	3275	9613562	-1.372	-1.312	Fail to reject Ho
6	4	3	8140	3657	9	11568	2471	7558008	-1.372	-1.870	Reject Ho
6	7	3	6880	3532	9	10220	2872	9095014	-1.372	-1.661	Reject Ho
6	10	3	5847	2970	9	8763	2899	8487461	-1.372	-1.502	Reject Ho
6	14	3	4715	2113	9	7352	3249	9337621	-1.372	-1.295	Fail to reject Ho
4	4	3	7250	3597	9	11325	2977	9676282	-1.372	-1.965	Reject Ho
4	7	3	6300	3298	9	9752	2975	9255365	-1.372	-1.702	Reject Ho
4	10	3	5319	2858	9	8380	3109	9366840	-1.372	-1.500	Reject Ho
4	14	3	4294	1938	9	6992	3299	9460296	-1.372	-1.315	Fail to reject Ho
2	4	3	6655	3648	9	11026	3496	12441424	-1.372	-1.859	Reject Ho
2	7	3	5684	3255	9	9394	3201	10318637	-1.372	-1.732	Reject Ho
2	10	3	4825	2709	9	7993	3282	10085796	-1.372	-1.497	Reject Ho
2	14	3	3908	1818	9	6686	3366	9726459	-1.372	-1.336	Fail to reject Ho

Table 5.7 95% Confidence Intervals for the True Mean Difference between Resilient Modulus Test Values for Soil I without and with Enzyme B

Conf (psi)	Devia (psi)	Untreated			Treated with B			S_p^2	$t_{\alpha/2}$	Confidence Interval	
		n_1	$\overline{x}_1$	Std. Dev.	n_2	$\overline{x}_2$	Std. Dev.			Lower limit	Upper Limit
8	4	4	13098	4118	4	22000	3383	14200343	2.262	-14929	-2874
8	7	4	12964	4246	4	20766	3579	15415935	2.262	-14082	-1522
8	10	4	12323	3753	4	19327	3786	14209922	2.262	-13033	-974
8	14	4	11521	3268	4	17426	3813	12610626	2.262	-11585	-225
6	4	4	12843	4558	4	22150	4283	19559069	2.262	-16381	-2233
6	7	4	12510	4126	4	20755	5021	21115669	2.262	-15595	-895
6	10	4	12077	3853	4	19343	3885	14969041	2.262	-13454	-1077
6	14	4	11347	3391	4	17348	3696	12579601	2.262	-11673	-327
4	4	4	12039	4032	4	23267	6896	31907148	2.262	-20263	-2193
4	7	4	12278	4268	4	22012	4956	21386585	2.262	-17131	-2337
4	10	4	11852	3987	4	19654	3861	15401864	2.262	-14080	-1525
4	14	4	11106	3479	4	17394	3478	12098654	2.262	-11852	-725
2	4	4	11833	4296	4	24654	6063	27609893	2.262	-21225	-4416
2	7	4	11912	4239	4	21832	4966	21313510	2.262	-17304	-2536
2	10	4	11654	4109	4	19675	3868	15923520	2.262	-14403	-1638
2	14	4	10787	3595	4	17478	3717	13371719	2.262	-12539	-842

Table 5.8 Small-Sample Test of Hypotheses for ($\mu_1-\mu_2$) Soil I and Enzyme B

Conf (psi)	Devia (psi)	Untreated			Treated with B			S_p^2	$-t_\alpha$	$t_{statistic}$	Reject Region
		n_1	$\overline{x}_1$	Std. Dev.	n_2	$\overline{x}_2$	Std. Dev.				$t<-t_\alpha$
8	4	4	13098	4118	4	22000	3383	14200343	-1.383	-3.341	Reject Ho
8	7	4	12964	4246	4	20766	3579	15415935	-1.383	-2.810	Reject Ho
8	10	4	12323	3753	4	19327	3786	14209922	-1.383	-2.628	Reject Ho
8	14	4	11521	3268	4	17426	3813	12610626	-1.383	-2.352	Reject Ho
6	4	4	12843	4558	4	22150	4283	19559069	-1.383	-2.976	Reject Ho
6	7	4	12510	4126	4	20755	5021	21115669	-1.383	-2.537	Reject Ho
6	10	4	12077	3853	4	19343	3885	14969041	-1.383	-2.656	Reject Ho
6	14	4	11347	3391	4	17348	3696	12579601	-1.383	-2.392	Reject Ho
4	4	4	12039	4032	4	23267	6896	31907148	-1.383	-2.811	Reject Ho
4	7	4	12278	4268	4	22012	4956	21386585	-1.383	-2.977	Reject Ho
4	10	4	11852	3987	4	19654	3861	15401864	-1.383	-2.812	Reject Ho
4	14	4	11106	3479	4	17394	3478	12098654	-1.383	-2.557	Reject Ho
2	4	4	11833	4296	4	24654	6063	27609893	-1.383	-3.450	Reject Ho
2	7	4	11912	4239	4	21832	4966	21313510	-1.383	-3.039	Reject Ho
2	10	4	11654	4109	4	19675	3868	15923520	-1.383	-2.843	Reject Ho
2	14	4	10787	3595	4	17478	3717	13371719	-1.383	-2.587	Reject Ho

Table 5.9 95% Confidence Intervals for the True Mean Difference between Resilient Modulus Test Values for Soil II without and with Enzyme B

Conf (psi)	Devia (psi)	Untreated			Treated with B			S_p^2	$t_{\alpha/2}$	Confidence Interval	
		n_1	$\overline{x}_1$	Std. Dev.	n_2	$\overline{x}_2$	Std. Dev.			Lower limit	Upper Limit
8	4	3	10217	3648	8	14316	4379	17869926	2.262	-10573	2374
8	7	3	9002	3098	8	12860	4501	17887362	2.262	-10334	2619
8	10	3	7675	3070	8	11402	4442	17440828	2.262	-10122	2668
8	14	3	6087	2274	8	9647	4329	15725795	2.262	-9632	2513
6	4	3	9841	3657	8	13886	4186	16601223	2.262	-10284	2195
6	7	3	8474	3532	8	12457	4469	18306914	2.262	-10535	2570
6	10	3	7163	2970	8	10910	4421	17164219	2.262	-10092	2597
6	14	3	5556	2113	8	9211	4446	16366770	2.262	-9850	2541
4	4	3	8879	3597	8	13664	4623	19493825	2.262	-11547	1976
4	7	3	7804	3298	8	12058	4485	18061882	2.262	-10762	2254
4	10	3	6556	2858	8	10480	4457	17267177	2.262	-10288	2439
4	14	3	5054	1938	8	8728	4368	15670862	2.262	-9736	2388
2	4	3	8307	3648	8	13133	4585	19310808	2.262	-11555	1904
2	7	3	7146	3255	8	11648	4444	17712670	2.262	-10947	1943
2	10	3	6001	2709	8	10140	4469	17166977	2.262	-10484	2206
2	14	3	4607	1818	8	8346	4384	15684680	2.262	-9804	2326

Table 5.10 Small-Sample Test of Hypotheses for ($\mu_1-\mu_2$) Soil II and Enzyme B

Conf (psi)	Devia (psi)	Untreated			Treated with B			S_p^2	$-t_\alpha$	$t_{statistic}$	Reject Region
		n_1	$\overline{x}_1$	Std. Dev.	n_2	$\overline{x}_2$	Std. Dev.				$t<-t_\alpha$
8	4	3	10217	3648	8	14316	4379	17869926	-1.383	-1.432	Reject Ho
8	7	3	9002	3098	8	12860	4501	17887362	-1.383	-1.347	Fail to reject Ho
8	10	3	7675	3070	8	11402	4442	17440828	-1.383	-1.318	Fail to reject Ho
8	14	3	6087	2274	8	9647	4329	15725795	-1.383	-1.326	Fail to reject Ho
6	4	3	9841	3657	8	13886	4186	16601223	-1.383	-1.466	Reject Ho
6	7	3	8474	3532	8	12457	4469	18306914	-1.383	-1.375	Fail to reject Ho
6	10	3	7163	2970	8	10910	4421	17164219	-1.383	-1.336	Fail to reject Ho
6	14	3	5556	2113	8	9211	4446	16366770	-1.383	-1.334	Fail to reject Ho
4	4	3	8879	3597	8	13664	4623	19493825	-1.383	-1.601	Reject Ho
4	7	3	7804	3298	8	12058	4485	18061882	-1.383	-1.479	Reject Ho
4	10	3	6556	2858	8	10480	4457	17267177	-1.383	-1.395	Reject Ho
4	14	3	5054	1938	8	8728	4368	15670862	-1.383	-1.371	Reject Ho
2	4	3	8307	3648	8	13133	4585	19310808	-1.383	-1.622	Reject Ho
2	7	3	7146	3255	8	11648	4444	17712670	-1.383	-1.580	Reject Ho
2	10	3	6001	2709	8	10140	4469	17166977	-1.383	-1.476	Reject Ho
2	14	3	4607	1818	8	8346	4384	15684680	-1.383	-1.395	Reject Ho

CHAPTER 6
SHEAR STRENGTH TESTING

Results

A total of 26 specimens were tested for shear strength following the NCHRP 1-28A protocol [18]. Two different confining pressures were used; 4 and 8 psi, respectively. The influence of the two treatments (enzyme A and B) on the soil's shear strength (I and II) was studied. Also the effect of curing time in the shear strength of the control materials was observed.

Analysis and Discussion

Table 6.1 shows the summary of the shear strength test for soil I. Moisture content, density, age of the specimen, confinement pressure, and maximum deviatoric stress at failure are presented.

The first conclusion that can be drawn is that for the range of pressures tested (4 and 8 psi), the specimens of soil I behave as non-pressure dependent, the maximum deviatoric stress obtained for the specimens of soil I varies slightly if it is tested at 4 psi or 8 psi of confining pressure.

The results in Table 6.1 shows that if the samples are cured for just four weeks the shear strength of the soil will be not affected; on average the shear strength of the treated specimens was the same as the untreated specimens after approximately four weeks of curing time.

According to the enzyme manufacturer the enzyme stabilization mechanism process takes between three and seven days after mixing with the soil. However, the limited tests results shown in table 6.1 and 6.2 demonstrate that at least four months of curing time are needed to observe improvement in the shear strength of the material.

Table 6.2 shows the summary of the shear-strength test for soil II. Similar conclusions obtained with soil I can be drawn for this material. Non-pressure dependence behavior is observed and higher curing time than those recommended by manufacturer is needed to obtain an increase in the shear strength.

Table 6.1 Shear Strength Test for Soil I

Soil I					
Sample name	**MC (%)**	**Density (lb/ft^3)**	**Age (days)**	**Confinement (psi)**	**Max deviatoric (psi)**
S-1-1-0	23.90	114.71	27	4	51.10
S-1-1-0-2	21.02	113.65	54	4	77.52
S-2-1-0	23.26	115.29	26	8	49.35
S-3-1-0	23.27	115.36	109	8	72.86
S-2-1-1-A	23.75	113.57	28	4	50.51
S-3-1-1-A	20.60	109.73	172	4	128.42
S-4-1-1-A	22.81	115.46	21	4	46.82
S-5-1-1-A	24.30	115.92	25	8	48.38
S-1-1-1-B	23.25	118.41	46	8	76.35
S-2-1-1-B	25.45	116.16	46	4	88.01

Table 6.2 Shear Strength Test for Soil II

Soil II					
Sample name	**MC (%)**	**Density (lb/ft^3)**	**Age (days)**	**Confinement (psi)**	**Max deviatoric (psi)**
S-2-2-0	17.39	128.47	35	4	37.30
S-3-2-0	15.53	129.47	35	4	46.43
S-5-2-0	16.50	129.70	15	8	33.42
S-2-2-1-A	10.69	119.08	207	4	90.92
S-3-2-1-A	15.26	130.04	36	4	34.39
S4-2-1-A	15.45	127.20	36	8	39.24
S-5-2-1-A	15.33	127.73	40	4	34.78
S-6-2-1-A	15.94	127.36	40	8	34.39
S-7-2-1-A	15.68	125.81	195	8	54.79
S-1-2-1-B	12.84	122.95	189	4	124.53
S-2-2-1-B	17.06	128.45	35	4	32.44
S-3-2-1-B	15.23	124.43	35	8	33.42
S-4-2-1-B	15.06	124.60	30	8	32.25
S-5-2-1-B	13.91	125.24	30	4	34.00
S-6-2-1-B	14.49	122.11	184	8	83.35
S-7-2-1-B	16.68	125.30	55	4	42.35

Figures 6.1 to 6.4 show the stress-strain curves from the shear strength test for treated (enzyme A and B) and untreated soils I and II at two different confining pressures. It can be seen from the figures that for the specimens with long curing period time, the maximum deviatoric stresses obtained were higher than the deviatoric stresses for untreated specimens. It is also observed that the slope of the curve for small strains of

treated specimens with a long curing period is higher than the slope of the curve of untreated specimens. This observation confirms the results obtained in the resilient modulus data analysis.

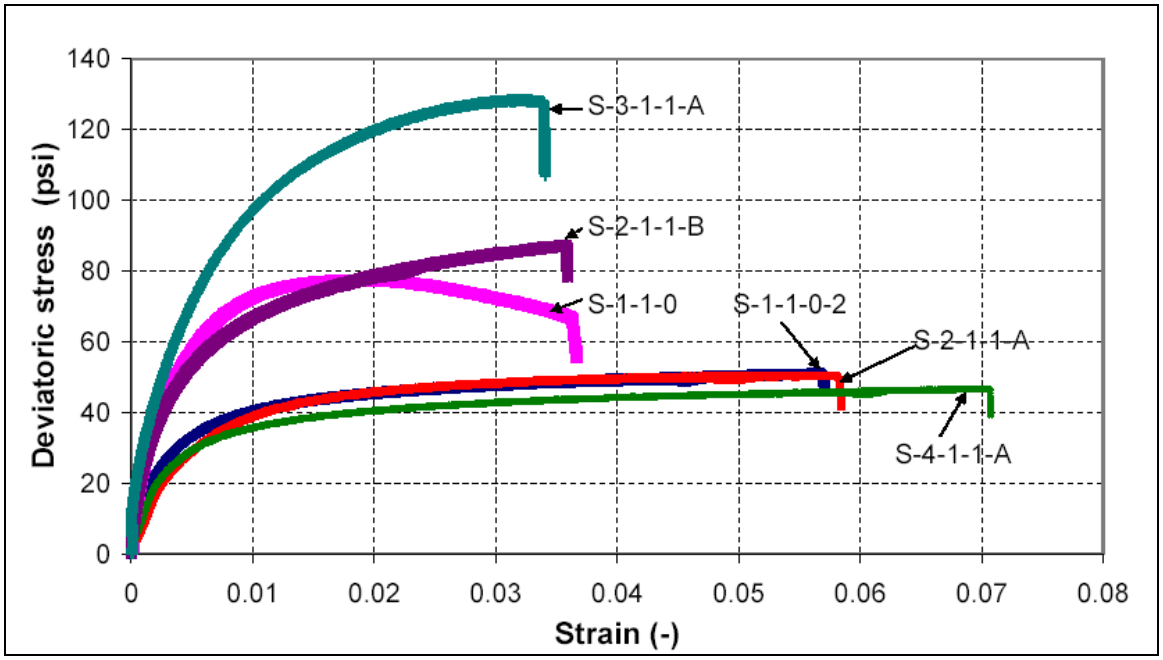


Figure 6.1 Results from Shear Strength Test σ_3=4 psi for Soil I

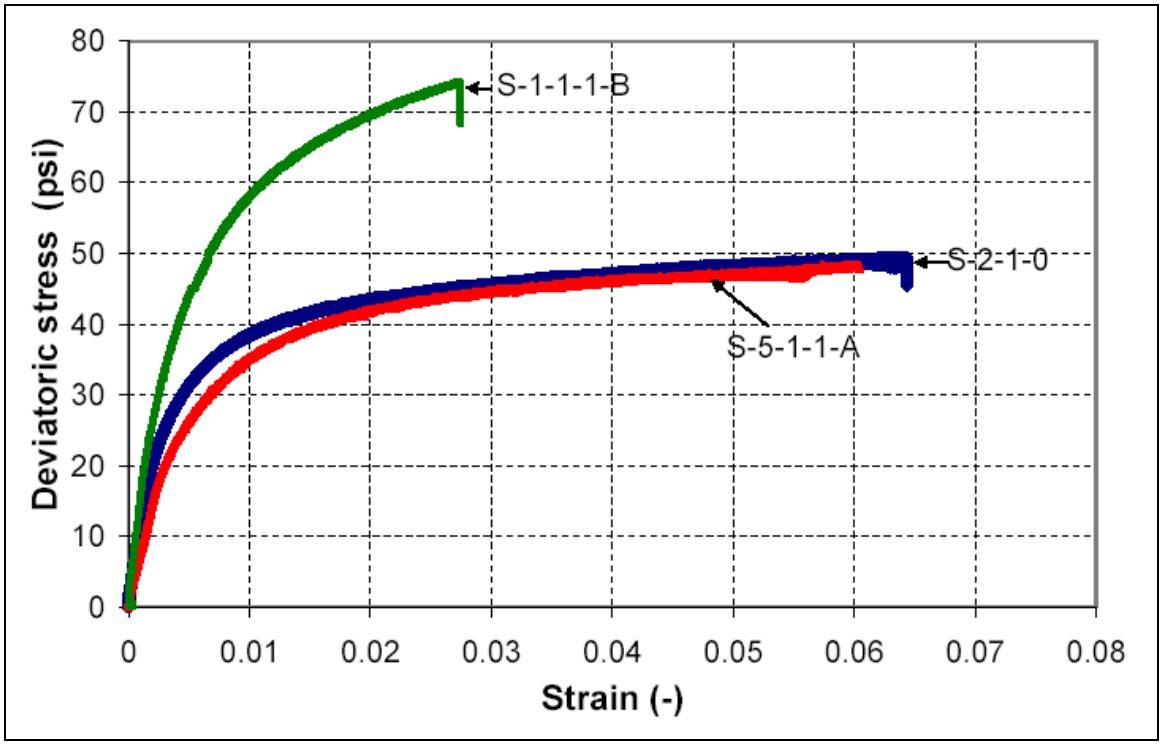


Figure 6.2 Results from Shear Strength Test σ_3=8 psi for Soil I

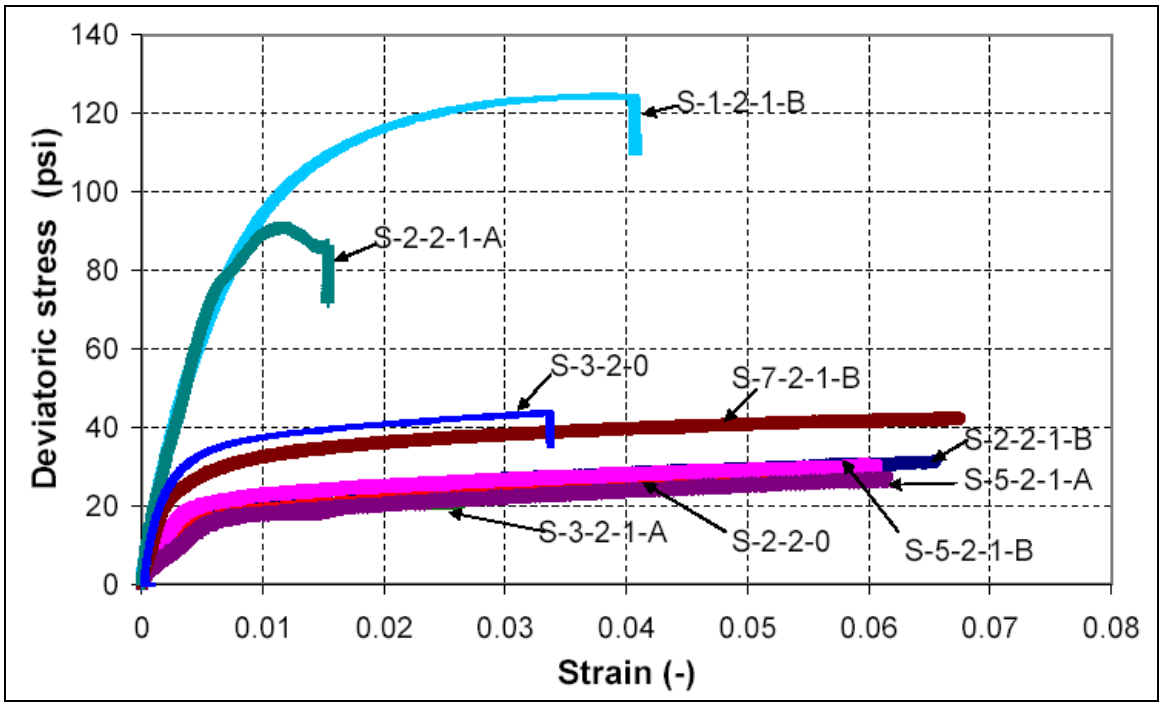


Figure 6.3 Results from Shear Strength Test σ_3=4 psi for Soil II

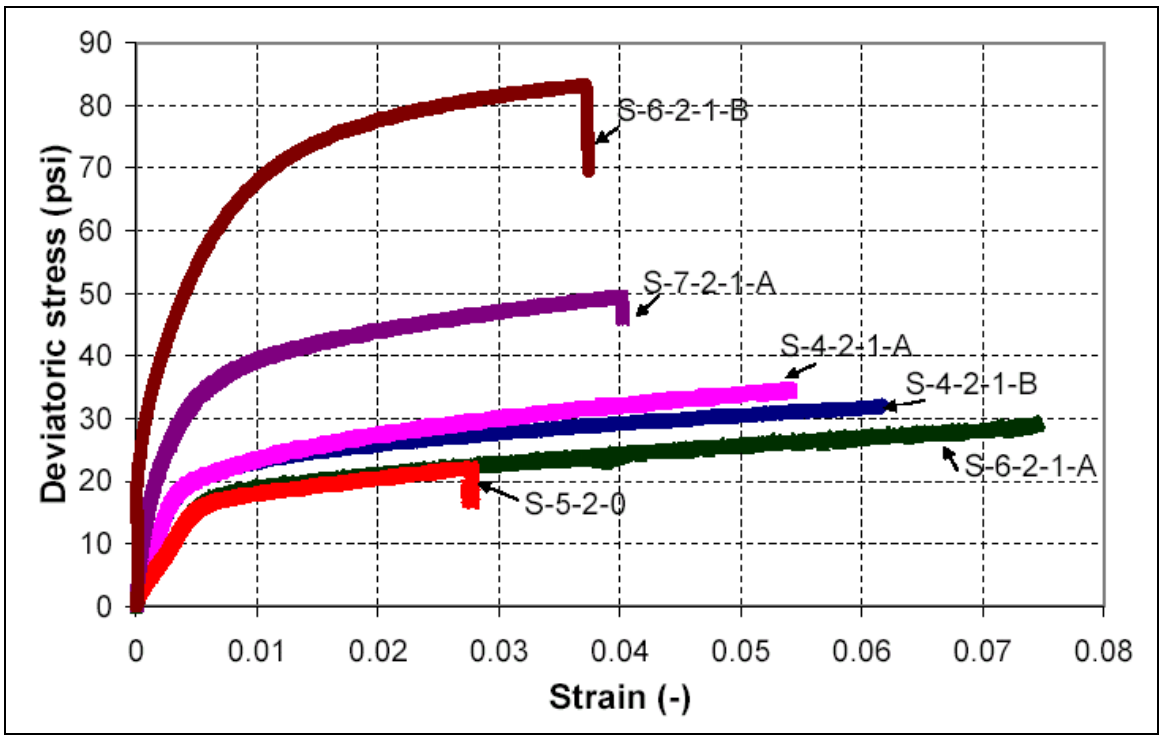


Figure 6.4 Results from Shear Strength Test σ_3=8 psi for Soil II

Figure 6.5 shows the average effect of each treatment in the shear strength of soil I and II. Enzyme A increases the shear strength of soil I by 9%, and by 23% the shear strength of soil II. On the other hand, enzyme B increases the maximum deviatoric stress obtained by 31% for soil I and 39% for soil II.

Figure 6.6 shows a typical shear failure of soil I and II found during testing. The typical 45-degree plane of failure was observed for almost all specimens (barrel failure was observed in few specimens).

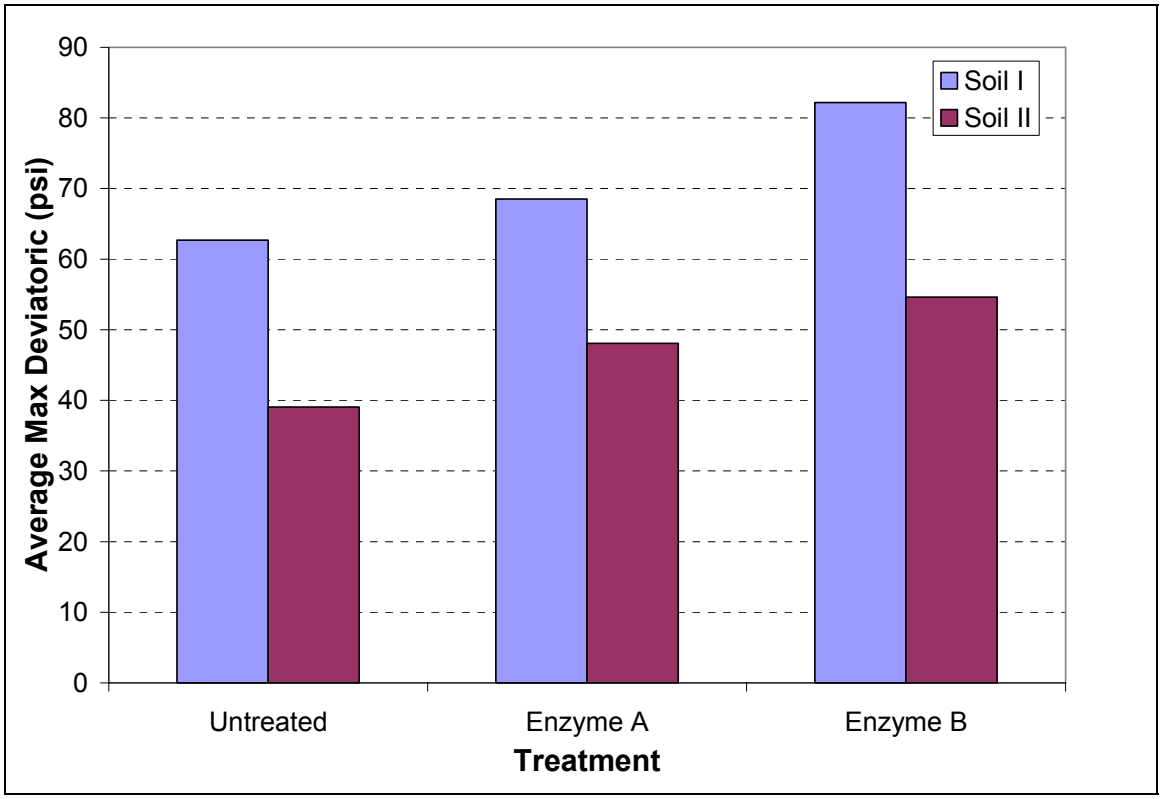


Figure 6.5 Results from Shear Strength Test for Soil I and II

Figure 6.6 Soil II and I Specimens after Shear Strength Test

CHAPTER 7
CONCLUSIONS AND RECOMENDATIONS

Based on the analysis performed on the experimental data obtained in this study the following conclusions can be drawn:

1. The specimen preparation process showed that both product A and B reduced the compaction effort and improved soil workability. Thus, less pressure was used to obtain the target density of the treated specimens compare to the untreated specimens.
2. Enzyme A contains a high concentration of protein, but does not appear to contain active enzymes based on standard enzymatic activity tests. No chemical analysis was performed on enzyme B in this project.
3. The results from surface-tension testing and qualitative observations suggest that enzyme A behaves like a surfactant (reduction of the surface tension of water with the increase of enzyme concentration), contrary to the behavior observed in enzyme B.
4. The resilient modulus for soil I and II (cohesive soils) follow the trend found in the literature: decreases with the increase of the deviatoric cyclic stress and increases with the increase of the confining pressure.
5. The addition of enzyme A did not improve the resilient modulus of soil I but increased in average by 54% the resilient modulus of soil II.
6. The addition of enzyme B to soils I and II had a pronounced effect on the resilient modulus. The stiffness of soil I increased in average by 69% and for soil II by 77%.
7. The type of soil affected significantly the effectiveness of the treatments. The percent of fines and the chemical composition are properties that affect the stabilization mechanism. Therefore, special attention should be paid to select the proper treatment to be used for different soils.
8. The resilient modulus increased as the curing time increased for all the combination of soils and enzymes.
9. Increasing the application rate suggested by the manufacturer did not improve the effectiveness of the stabilization process.

10. Limited number of tests showed that at least four months of curing time are needed to observe an improvement on the shear strength of both soils.
11. Enzyme A increased in average the shear strength of soil I by 9%, and the shear strength of soil II by 23%.
12. Enzyme B increased in average the shear strength of soil I by 31% and of soil II by 39%.

The conclusions presented above refer to a limited number of soil and enzyme stabilizers combinations tested in laboratory conditions and should not be extrapolated to other combinations of materials. These results should be validated with field experiments that involve the same combination of materials used in this study. At this time, it is not known if the results obtained in this study accurately predict field performance.

Assuming that the laboratory work reasonably predicts the field behavior, the following steps are recommended for practical applications:

- Obtain representative soil samples from the construction site and prepare enzyme modified soil specimens for laboratory testing following the manufacturer guidelines and the method proposed in this study
- Perform the tests described in this study on the enzyme modified specimens
- If the laboratory results show a significant improvement in the soil properties, use the product for field operations as indicated by the manufacturer.

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APPENDIX A

Resilient Modulus Test Results for Untreated Soil I

	S-1-1-0 (27)		S-1-1-0-2 (20)		S-2-1-0 (26)		S-3-1-0 (82)		AVERAGE UNTREATED		Std. Dev	
Conf (psi)	Mean stress	MR (psi)	Mean stress	MR (psi)	Mean stress	MR (psi)	Mean stress	MR (psi)	Mean stress	MR (psi)	Mean stress	MR (psi)
8	9.32	15348.99	9.34	17684.93	9.29	15368.33	9.32	8843.69	9.31	14311.48	0.02	3806.58
8	10.28	14328.66	10.31	18307.63	10.28	14644.35	10.32	8849.06	10.29	14032.42	0.02	3899.01
8	11.23	13384.57	11.30	17104.74	11.27	13445.38	11.31	8630.09	11.27	13141.20	0.04	3474.26
8	12.49	11983.19	12.63	15868.95	12.58	12187.49	12.58	8290.94	12.55	12082.64	0.06	3094.84
6	7.28	14407.73	7.36	18521.89	7.25	14401.97	7.29	8387.82	7.27	13929.85	0.04	4173.41
6	8.26	14034.19	8.27	17572.09	8.27	14425.60	8.27	8366.38	8.27	13599.56	0.01	3831.38
6	9.25	13295.93	9.33	16881.80	9.29	13601.65	9.32	8108.97	9.29	12972.09	0.04	3625.69
6	10.50	11809.35	10.66	15890.00	10.54	11913.68	10.61	8093.82	10.55	11926.71	0.07	3183.96
4	5.24	14149.60	5.29	16579.21	5.22	13914.96	5.23	7826.51	5.23	13117.57	0.03	3727.34
4	6.26	14229.87	6.36	17285.55	6.26	14268.74	6.29	7923.82	6.27	13427.00	0.05	3938.13
4	7.27	13202.06	7.32	16797.20	7.28	13286.70	7.31	7813.74	7.29	12774.92	0.02	3707.49
4	8.50	11673.13	8.63	15727.72	8.52	11730.53	8.42	7749.91	8.48	11720.32	0.09	3257.08
2	3.26	14324.45	3.29	16513.55	3.22	13491.94	3.22	7365.93	3.23	12923.97	0.03	3918.37
2	4.23	13776.31	4.36	16945.16	4.24	13764.76	4.26	7654.40	4.25	13035.16	0.06	3886.83
2	5.22	12916.76	5.35	16814.56	5.31	13311.57	5.31	7495.23	5.28	12634.53	0.06	3848.08
2	6.46	11456.69	6.57	15523.13	6.48	11454.66	6.30	7279.65	6.41	11428.53	0.11	3365.54

Resilient Modulus Test Results for Soil I and Enzyme A

	S-2-1-1-A (28)		S-3-1-1-A (28)		S-4-1-1-A (21)		S-5-1-1-A (25)		S-1-1-1-A (130)		S-3-1-1-A-2 (133)	
Conf (psi)	Mean stress	MR (psi)	Mean stress	MR (psi)	Mean stress	MR (psi)	Mean stress	MR (psi)	Mean stress	MR (psi)	Mean stress	MR (psi)
8	9.24	8531.68	9.27	16749.84	9.27	10855.86	9.36	10153.12	9.36	19731.72	9.33	19973.97
8	10.31	9876.35	10.24	15231.45	10.21	9758.05	10.28	9726.36	10.27	18563.72	10.32	20401.90
8	11.17	9149.66	11.15	13565.90	11.18	9232.65	11.21	9032.45	11.28	17600.25	11.28	19050.04
8	12.42	8792.26	12.40	11931.73	12.40	8870.48	12.44	8361.25	12.62	16658.81	12.63	15835.43
6	7.26	8333.87	7.25	17164.95	7.25	9897.06	7.28	10076.76	7.31	18713.49	7.31	17815.20
6	8.25	9175.51	8.25	15809.69	8.23	9722.82	8.21	9215.20	8.29	18395.46	8.32	19062.70
6	9.17	8699.04	9.16	14070.45	9.13	8885.76	9.16	8653.89	9.27	17677.23	9.24	18819.03
6	10.39	8299.46	10.40	11929.46	10.39	8568.91	10.41	8001.51	10.57	16510.96	10.47	16370.71
4	5.24	7624.09	5.25	18470.11	5.25	10017.13	5.25	9716.76	5.29	19484.00	5.27	17877.01
4	6.23	8486.10	6.24	16334.84	6.22	9366.00	6.24	9123.27	6.26	18421.39	6.14	17528.56
4	7.15	8261.38	7.16	14166.29	7.10	8604.15	7.21	8612.70	7.29	17942.44	7.04	18127.60
4	8.42	8064.40	8.35	12042.06	8.39	8374.87	8.42	7826.05	8.59	16576.69	8.35	16212.72
2	3.19	6909.58	3.25	18844.91	3.19	9437.93	3.27	9768.54	3.29	18975.13	3.26	16154.28
2	4.23	7969.99	4.21	16570.88	4.20	9359.61	4.23	8912.97	4.26	18872.21	4.30	16932.12
2	5.16	7916.02	5.13	14457.08	5.10	8536.32	5.14	8246.66	5.25	17599.69	5.06	17731.55
2	6.39	7678.15	6.36	12209.70	6.36	8093.45	6.39	7540.17	6.55	16226.07	6.38	15858.96

Resilient Modulus Test Results for Untreated Soil II

	S-2-2-0 (35)		S-3-2-0 (15)		S-5-2-0 (15)		AVERAGE UNTREATED		Std. Dev	
Conf (psi)	Mean stress (psi)	MR (psi)	Mean stress (psi)	MR (psi)	Mean stress (psi)	MR (psi)	Mean stress(psi)	MR (psi)	Mean stress (psi)	MR (psi)
8	9.27	8122.36	9.31	12310.77	9.19	5042.39	9.25	8491.84	0.06	3648.25
8	10.16	6811.15	10.25	11193.29	9.62	5209.76	10.01	7738.07	0.34	3097.59
8	11.05	5758.04	11.17	9592.29	10.82	3521.83	11.01	6290.72	0.18	3070.09
8	12.10	4500.59	12.47	7674.35	11.89	3265.26	12.15	5146.73	0.29	2274.46
6	7.27	7674.72	7.29	12008.02	7.16	4738.69	7.24	8140.48	0.07	3656.98
6	8.15	6271.05	8.25	10676.69	7.95	3691.67	8.12	6879.80	0.15	3532.07
6	9.03	5260.12	9.03	9066.28	8.77	3213.97	8.94	5846.79	0.15	2969.93
6	10.05	4027.14	10.45	7085.80	9.82	3030.83	10.11	4714.59	0.32	2113.09
4	5.21	6647.52	5.26	11110.18	5.09	3993.13	5.18	7250.28	0.09	3596.61
4	6.12	5783.43	6.22	9824.88	5.92	3290.67	6.09	6299.66	0.15	3297.55
4	6.94	4664.01	7.16	8447.97	6.69	2845.47	6.93	5319.15	0.23	2858.13
4	7.99	3631.79	8.39	6476.42	7.75	2774.80	8.04	4294.34	0.32	1937.71
2	3.20	6046.13	3.23	10568.58	3.03	3349.62	3.15	6654.78	0.11	3647.76
2	4.07	5099.74	4.14	9192.33	3.81	2760.96	4.01	5684.34	0.17	3255.30
2	4.92	4215.49	5.15	7786.43	4.57	2471.78	4.88	4824.57	0.29	2709.17
2	5.89	3250.01	6.35	5963.41	5.62	2510.28	5.95	3907.90	0.37	1818.14

Resilient Modulus Test Results for Soil II and Enzyme A

	S-6-2-1-A (40)		S-5-2-1-A (40)		S-4-2-1-A (36)		S-1-2-1-A (42)		S-2-2-1-A (42)	
Conf (psi)	Mean stress (psi)	MR (psi)	Mean stress (psi)	MR (psi)	Mean stress (psi)	MR (psi)	Mean stress (psi)	MR (psi)	Mean stress (psi)	MR (psi)
8	9.31	9760.02	9.28	8599.80	9.32	11839.75	9.34	15883.45	9.31	13835.19
8	10.22	7827.13	10.17	7010.09	10.25	10152.11	10.27	13833.40	10.25	12571.88
8	11.05	6347.03	11.02	5799.50	11.18	8769.66	11.21	12214.34	11.21	10924.56
8	12.14	4782.24	12.08	4353.60	12.34	7229.00	12.41	10322.46	12.42	9007.14
6	7.23	8968.09	7.26	8218.10	7.29	12130.97	7.30	15639.13	7.30	13116.92
6	8.18	7351.30	8.17	6622.20	8.22	9733.55	8.29	13975.30	8.29	12272.70
6	9.06	5906.19	9.02	5349.52	9.14	8277.36	9.17	11689.83	9.22	10420.45
6	10.04	4098.71	10.04	3941.87	10.34	6813.09	10.42	9909.40	10.39	8433.53
4	5.24	8222.50	5.24	7731.08	5.24	11401.39	5.27	15550.41	5.27	13308.07
4	6.17	6702.29	6.14	6112.85	6.21	9524.48	6.30	13552.57	6.25	11594.38
4	7.00	5283.46	6.95	4778.15	7.12	7881.40	7.20	11466.17	7.20	9958.06
4	8.05	3755.11	7.97	3578.67	8.32	6425.47	8.42	9592.50	8.39	8083.97
2	3.23	7547.32	3.20	6788.23	3.24	11096.06	3.23	15514.15	3.26	12707.25
2	4.08	5939.11	4.12	5627.25	4.20	9137.56	4.24	12996.31	4.21	10950.23
2	4.93	4696.86	4.85	4270.77	5.10	7537.60	5.14	10911.33	5.19	9552.39
2	5.96	3540.45	5.90	3227.88	6.30	6047.03	6.42	9220.86	6.39	7680.66

Resilient Modulus Test Results for Soil II and Enzyme A (cont)

	S-3-2-1-A (36)		S-7-2-1-A (40)		S-1-2-1-A-2 (163)		S-2-2-1-A-2 (168)		S-7-2-1-A-2 (154)	
Conf (psi)	Mean stress (psi)	MR (psi)	Mean stress (psi)	MR (psi)	Mean stress (psi)	MR (psi)	Mean stress (psi)	MR (psi)	Mean stress (psi)	MR (psi)
8	9.31	9168.52	9.28	12050.98	9.37	13998.44	9.35	14277.76	9.35	26320.12
8	10.25	7357.15	10.22	10191.88	10.32	12190.65	10.32	14281.68	10.32	25044.51
8	10.99	5637.01	11.10	8392.50	11.29	11222.80	11.34	13929.59	11.32	20661.42
8	12.09	4253.16	12.28	6476.44	12.65	10645.11	12.67	13653.42	12.63	17051.62
6	7.30	8708.03	7.27	11698.24	7.33	12461.20	7.28	13170.31	7.35	26273.28
6	8.18	6706.71	8.20	9880.98	8.32	11902.02	8.33	13534.46	8.33	24592.43
6	9.03	5283.49	9.11	8016.63	9.34	10797.35	9.34	13129.73	9.32	20266.67
6	10.01	3810.12	10.28	5984.68	10.64	10188.65	10.65	12988.63	10.62	16435.97
4	5.24	7445.71	5.25	11396.93	5.36	12292.94	5.32	14575.59	5.35	28191.52
4	6.14	6035.03	6.19	9351.10	6.33	11488.52	6.33	13409.78	6.34	25187.20
4	6.98	4767.53	7.08	7532.80	7.30	10417.21	7.39	13338.58	7.35	20189.00
4	7.91	3388.20	8.22	5459.74	8.66	9925.78	8.68	12714.50	8.67	16393.13
2	3.25	6916.24	3.24	10960.15	3.30	11417.24	3.23	16290.93	3.37	29004.40
2	4.12	5462.51	4.20	9131.72	4.32	11303.67	4.34	14001.73	4.33	24194.18
2	4.89	4176.58	5.06	7072.91	5.35	10282.26	5.40	13438.29	5.36	20217.34
2	5.82	3039.75	6.19	5062.38	6.65	9643.00	6.66	12711.11	6.61	15874.37

Resilient Modulus Test Results for Soil II and Enzyme A (cont)

	S-1-2-05-A (21)		S-1-2-15-A (21)	
Conf (psi)	Mean stress (psi)	MR (psi)	Mean stress (psi)	MR (psi)
8	9.24	15458.51	9.29	11738.19
8	10.19	12917.08	10.21	10000.10
8	11.16	10190.62	11.15	8204.42
8	12.32	7350.89	12.31	6238.96
6	7.21	15532.63	7.27	11089.09
6	8.17	12585.04	8.24	9325.00
6	9.13	9635.30	9.14	7637.08
6	10.34	6764.81	10.31	5690.46
4	5.12	16730.72	5.24	10115.52
4	6.17	12081.66	6.21	8684.49
4	7.08	8972.03	7.09	6948.30
4	8.24	6125.58	8.19	5073.49
2	3.23	17022.48	3.24	9678.04
2	4.13	11865.37	4.17	8143.98
2	5.04	8550.40	5.07	6476.99
2	6.18	5659.38	6.14	4639.69

Resilient Modulus Results for Untreated Soil I

	S-1-1-0 (27)		S-1-1-0-2 (20)		S-2-1-0 (26)		S-3-1-0 (82)		AVERAGE UNTREATED		Std. Dev	
Conf (psi)	Mean stress	MR (psi)	Mean stress	MR (psi)	Mean stress	MR (psi)	Mean stress	MR (psi)	Mean stress	MR (psi)	Mean stress	MR (psi)
8	9.32	15348.99	9.34	17684.93	9.29	10515.16	9.32	8843.69	9.31	13098.19	0.02	4118.13
8	10.28	14328.66	10.31	18307.63	10.28	10370.03	10.32	8849.06	10.29	12963.84	0.02	4245.71
8	11.23	13384.57	11.30	17104.74	11.27	10173.85	11.31	8630.09	11.27	12323.31	0.04	3752.71
8	12.49	11983.19	12.63	15868.95	12.58	9940.77	12.58	8290.94	12.55	11520.96	0.06	3268.47
6	7.28	14407.73	7.36	18521.89	7.25	10056.11	7.29	8387.82	7.27	12843.39	0.04	4557.54
6	8.26	14034.19	8.27	17572.09	8.27	10068.70	8.27	8366.38	8.27	12510.34	0.01	4126.24
6	9.25	13295.93	9.33	16881.80	9.29	10022.80	9.32	8108.97	9.29	12077.37	0.04	3853.00
6	10.50	11809.35	10.66	15890.00	10.54	9596.68	10.61	8093.82	10.55	11347.46	0.07	3391.14
4	5.24	14149.60	5.29	16579.21	5.22	9600.36	5.23	7826.51	5.23	12038.92	0.03	4031.55
4	6.26	14229.87	6.36	17285.55	6.26	9671.60	6.29	7923.82	6.27	12277.71	0.05	4267.61
4	7.27	13202.06	7.32	16797.20	7.28	9595.11	7.31	7813.74	7.29	11852.03	0.02	3986.59
4	8.50	11673.13	8.63	15727.72	8.52	9271.79	8.42	7749.91	8.48	11105.64	0.09	3478.97
2	3.26	14324.45	3.29	16513.55	3.22	9129.92	3.22	7365.93	3.23	11833.46	0.03	4296.36
2	4.23	13776.31	4.36	16945.16	4.24	9272.22	4.26	7654.40	4.25	11912.02	0.06	4238.86
2	5.22	12916.76	5.35	16814.56	5.31	9389.34	5.31	7495.23	5.28	11653.97	0.06	4108.93
2	6.46	11456.69	6.57	15523.13	6.48	8890.53	6.30	7279.65	6.41	10787.50	0.11	3595.25

Resilient Modulus Results for Soil I and Enzyme B

	S-1-1-1-B (21)		S-1-1-1-B-2 (46)		S-2-1-1-B (21)		S-2-1-1-B-2 (46)		AVERAGE		Std. Dev	
Conf (psi)	Mean stress	MR (psi)	Mean stress	MR (psi)	Mean stress	MR (psi)	Mean stress	MR (psi)	Mean stress	MR (psi)	Mean stress	MR (psi)
8	9.31	20672.97	9.32	18480.32	9.30	26476.58	9.32	22368.88	9.31	21999.69	0.01	3382.55
8	10.30	20415.68	10.28	15973.39	10.25	24361.84	10.31	22313.76	10.29	20766.17	0.03	3578.52
8	11.31	18836.77	11.28	14170.15	11.25	21809.70	11.28	22491.49	11.28	19327.03	0.03	3786.43
8	12.34	16682.14	12.59	12815.62	12.54	18170.55	12.60	22035.96	12.52	17426.07	0.12	3812.92
6	7.29	20402.66	7.30	19199.28	7.28	28514.43	7.29	20484.16	7.29	22150.13	0.01	4283.34
6	8.30	20713.80	8.25	15928.34	8.26	27687.46	8.34	18691.22	8.29	20755.21	0.04	5020.51
6	9.31	18819.02	9.28	14085.69	9.29	22802.96	9.30	21665.10	9.29	19343.19	0.01	3884.90
6	10.35	16585.16	10.57	12757.07	10.57	18443.25	10.66	21604.76	10.54	17347.56	0.13	3695.86
4	5.29	21315.60	5.29	19953.07	5.27	33449.98	5.30	18347.70	5.28	23266.59	0.01	6896.44
4	6.30	20520.32	6.25	16736.45	6.27	28614.91	6.31	22176.16	6.28	22011.96	0.03	4955.87
4	7.01	19244.88	7.27	14370.20	7.23	22965.81	7.31	22036.95	7.20	19654.46	0.13	3861.46
4	8.56	16750.59	8.61	12915.88	8.60	18794.47	8.63	21116.03	8.60	17394.24	0.03	3477.66
2	3.30	20985.57	3.24	22895.16	3.30	33652.24	3.31	21081.33	3.29	24653.57	0.03	6063.09
2	4.30	21338.20	4.27	17411.87	4.26	28878.30	4.31	19700.01	4.29	21832.09	0.02	4965.79
2	5.12	19328.60	5.24	14500.47	5.29	23609.72	5.30	21260.39	5.24	19674.79	0.08	3868.30
2	6.46	16737.07	6.60	12781.83	6.57	18770.13	6.66	21622.89	6.57	17477.98	0.09	3717.20

Resilient Modulus Results for Soil I and Enzyme B (cont)

	S-1-1-05-B (21)		S-1-1-15-B (21)	
Conf (psi)	Mean stress	MR (psi)	Mean stress	MR (psi)
8	9.32	16918.25	9.27	16740.70
8	10.31	17065.26	10.26	16307.74
8	11.31	16205.36	11.28	15174.68
8	12.34	14889.28	12.53	13163.23
6	7.29	17738.66	7.32	17247.61
6	8.32	17189.91	8.20	16223.45
6	9.30	16057.90	9.28	15177.60
6	10.29	14754.90	10.59	13315.17
4	5.31	18537.02	5.23	17548.00
4	6.33	17500.62	6.26	16570.93
4	7.28	16102.10	7.26	15231.67
4	8.28	14787.34	8.53	12997.76
2	3.28	18651.84	3.20	17394.41
2	4.32	17045.48	4.26	16846.43
2	5.07	16022.06	5.29	15601.15
2	6.32	14831.33	6.54	13120.57

Resilient Modulus Results for Untreated Soil II

	S-2-2-0 (35)		S-3-2-0 (15)		S-5-2-0 (15)		AVERAGE UNTREATED		Std. Dev	
Conf (psi)	Mean stress (psi)	MR (psi)	Mean stress (psi)	MR (psi)	Mean stress (psi)	MR (psi)	Mean stress (psi)	MR (psi)	Mean stress (psi)	MR (psi)
8	9.27	8122.36	9.31	12310.77	9.19	5042.39	9.29	10216.56	0.06	3648.25
8	10.16	6811.15	10.25	11193.29	9.62	5209.76	10.21	9002.22	0.34	3097.59
8	11.05	5758.04	11.17	9592.29	10.82	3521.83	11.11	7675.16	0.18	3070.09
8	12.10	4500.59	12.47	7674.35	11.89	3265.26	12.29	6087.47	0.29	2274.46
6	7.27	7674.72	7.29	12008.02	7.16	4738.69	7.28	9841.37	0.07	3656.98
6	8.15	6271.05	8.25	10676.69	7.95	3691.67	8.20	8473.87	0.15	3532.07
6	9.03	5260.12	9.03	9066.28	8.77	3213.97	9.03	7163.20	0.15	2969.93
6	10.05	4027.14	10.45	7085.80	9.82	3030.83	10.25	5556.47	0.32	2113.09
4	5.21	6647.52	5.26	11110.18	5.09	3993.13	5.23	8878.85	0.09	3596.61
4	6.12	5783.43	6.22	9824.88	5.92	3290.67	6.17	7804.15	0.15	3297.55
4	6.94	4664.01	7.16	8447.97	6.69	2845.47	7.05	6555.99	0.23	2858.13
4	7.99	3631.79	8.39	6476.42	7.75	2774.80	8.19	5054.11	0.32	1937.71
2	3.20	6046.13	3.23	10568.58	3.03	3349.62	3.21	8307.36	0.11	3647.76
2	4.07	5099.74	4.14	9192.33	3.81	2760.96	4.10	7146.04	0.17	3255.30
2	4.92	4215.49	5.15	7786.43	4.57	2471.78	5.03	6000.96	0.29	2709.17
2	5.89	3250.01	6.35	5963.41	5.62	2510.28	6.12	4606.71	0.37	1818.14

Resilient Modulus Results for Soil II and Enzyme B

	S-1-2-1-B (35)		S-2-2-1-B (35)		S-3-2-1-B (35)		S-4-2-1-B (30)		S-5-2-1-B (30)	
Conf (psi)	Mean stress (psi)	MR (psi)	Mean stress (psi)	MR (psi)	Mean stress (psi)	MR (psi)	Mean stress (psi)	MR (psi)	Mean stress (psi)	MR (psi)
8	9.24	10202.97	9.27	11342.39	9.34	11631.71	9.28	11985.00	9.29	12071.26
8	10.19	8907.03	10.24	10130.75	10.22	9380.61	10.21	10232.66	10.20	10451.26
8	11.06	7511.63	11.12	8496.29	11.12	8072.64	11.10	8869.76	11.11	9025.87
8	12.20	6065.73	12.32	6818.20	12.26	6251.41	12.23	7013.27	12.27	7146.42
6	7.29	10470.00	7.25	11174.90	7.27	10905.77	7.22	11238.89	7.24	11643.73
6	8.17	8342.72	8.20	9499.79	8.22	9137.40	8.18	9958.33	8.19	10144.08
6	9.02	6965.43	9.10	8008.43	9.10	7583.39	9.04	8312.69	9.11	8601.73
6	10.15	5512.57	10.31	6292.45	10.23	5740.78	10.20	6522.36	10.26	6657.69
4	5.21	9497.31	5.23	10508.16	5.25	10232.88	5.22	11053.56	5.24	11390.33
4	6.10	7712.78	6.20	9211.70	6.21	8705.97	6.16	9459.24	6.21	9962.37
4	6.98	6470.67	7.07	7554.71	7.06	7075.93	7.03	7938.51	7.07	8136.79
4	8.10	5063.48	8.23	5741.45	8.20	5296.58	8.19	6154.11	8.23	6260.28
2	3.20	8816.37	3.21	10056.71	3.23	9923.39	3.19	10603.42	3.24	11343.56
2	4.09	7319.98	4.18	8679.72	4.19	8263.28	4.18	9225.31	4.21	9716.66
2	4.97	6071.46	5.05	7101.13	5.05	6720.64	5.05	7728.90	5.07	7860.50
2	6.06	4669.63	6.20	5381.37	6.13	4908.22	6.09	5721.92	6.15	5868.43

Resilient Modulus Results for Soil II and Enzyme B (cont)

	S-6-2-1-B (143)		S-7-2-1-B (55)		S-1-2-1-B-2 (148)		AVERAGE		Std. Dev	
Conf (psi)	Mean stress (psi)	MR (psi)	Mean stress (psi)	MR (psi)	Mean stress (psi)	MR (psi)	Mean stress (psi)	MR (psi)	Mean stress (psi)	MR (psi)
8	9.36	22886.76	9.30	17770.24	9.34	16636.11	9.30	14315.81	0.04	4378.68
8	10.35	21276.37	10.27	16465.89	10.33	16033.73	10.25	12859.79	0.06	4500.73
8	11.31	19646.33	11.26	15225.79	11.29	14369.38	11.17	11402.21	0.10	4441.95
8	12.62	17353.15	12.47	13050.37	12.67	13477.29	12.38	9646.98	0.18	4329.07
6	7.35	21901.07	7.29	17427.70	7.32	16323.86	7.28	13885.74	0.04	4186.10
6	8.31	20592.80	8.27	16428.39	8.31	15549.40	8.23	12456.61	0.06	4469.12
6	9.34	18974.53	9.23	14600.90	9.33	14235.42	9.16	10910.32	0.13	4421.33
6	10.67	17141.92	10.50	12645.75	10.65	13171.64	10.37	9210.65	0.21	4446.04
4	5.36	22010.98	5.32	18368.65	5.35	16250.56	5.27	13664.06	0.06	4622.51
4	6.33	20159.05	6.27	16038.83	6.27	15215.76	6.22	12058.21	0.07	4485.04
4	7.33	18553.84	7.23	14317.58	7.34	13794.09	7.14	10480.26	0.14	4457.21
4	8.64	16401.52	8.48	12291.95	8.66	12614.70	8.34	8728.01	0.22	4367.55
2	3.32	21807.27	3.30	17744.15	3.30	14770.07	3.25	13133.12	0.05	4585.46
2	4.34	19670.81	4.30	15732.11	4.34	14576.57	4.23	11648.05	0.09	4443.62
2	5.34	18264.62	5.23	13985.16	5.36	13386.47	5.14	10139.86	0.15	4469.32
2	6.67	16060.12	6.48	11943.21	6.69	12213.30	6.31	8345.78	0.26	4384.24

Resilient Modulus Results for Soil II and Enzyme B (cont)

	S-1-2-05-B (21)		S-1-2-15-B (21)	
Conf (psi)	Mean stress (psi)	MR (psi)	Mean stress (psi)	MR (psi)
8.00	9.30	12314.55	9.30	12619.70
8.00	10.25	10594.04	10.23	10420.44
8.00	11.17	8873.76	11.19	8808.90
8.00	12.41	7052.83	12.27	7319.50
6.00	7.28	11627.31	7.31	12827.50
6.00	8.24	10039.48	8.27	10086.80
6.00	9.17	8270.94	9.18	8162.29
6.00	10.39	6450.88	10.14	6766.35
4.00	5.27	10897.40	5.24	11174.19
4.00	6.23	9615.52	6.23	9283.51
4.00	7.16	7704.92	7.18	7543.01
4.00	8.37	5910.80	8.09	6096.61
2.00	3.24	10849.40	3.24	11612.15
2.00	4.19	8739.33	4.20	8770.31
2.00	5.14	7098.18	5.10	6751.06
2.00	6.36	5437.98	6.02	5447.90