STABILIZATION OF SOFT CLAY WITH BIO-ENZYME FOR ROAD PAVEMENT APPLICATIONS

BY

GBAYAN, Nguer Theresa
MEng/SEET/2017/ 7068

CIVIL ENGINEERING DEPARTMENT
FEDERAL UNIVERSITY OF TECHNOLOGY, MINNA

AUGUST, 2021

ABSTRACT

This study evaluated the compaction characteristics and strength of selected clay soils using non-toxic, bio-degradable liquid concentrate bio-enzyme fermented from vegetable extracts. The soils were stabilized with 5, 10, 15 and 20% urease bio-enzymes. Index properties tests including, moisture content, specific gravity, sieve analysis, Atterberg limits, as well as compaction, California bearing ratio (CBR) and unconfined compressive strength (UCS) were conducted. The liquid limit values obtained are 41 and 50.5%, plastic limit values were 28.1 and 35.9%, while plasticity index values were in 12.9 and 14.6% for samples A and B, respectively. The moisture content and specific gravity values for samples A and B were 16.9 and 21.6% and 2.79 and 2.75, respectively. The percentage passing through sieve No. 200 for sample A was 61.8 and 84.0% for sample B. Sample A was classified as clay soil (CL) or A-7-6 while sample B classified as CL or A-7-5 soils according to unified soil classification system (USCS) and American association for state and transportation officials (AASHTO) systems, respectively. According to the classification, the soils belong to weak class of clay soil that require stabilization and/or enhancement in order to support structural loads. Analysis were carried out to evaluate the compaction characteristics and strength of the soil. The results revealed that the optimum moisture content (OMC) decreased from 12.6% to 9.6% with an increase in enzyme percentage from 0% to 20% for sample A, while the same decreases were observed for sample B from 17.2 to 14.5%. The decrease was accompanied by a corresponding increase in maximum dry density (MDD) from 1.91 to 1.99g/m^3 for sample A and 1702 to 1880Kg/m^3 for sample B, which indicates that the soil has achieved a greater compaction at minimum compactive effort as the voids decreased. In addition, the work revealed an improvement in the CBR value from 21 to 45% (88% increment) for A-7-6 clay and 10 to 41% (178% increment) for A-7-5 clay when both samples were cured for 28 days with 0 to 20% Urease bio-enzymes. Finally, there is improvement in UCS of enzyme-stabilized clay after 28 days curing, therefore urease bio-enzymes is recommended as suitable for the stabilization of soft clay soils for road pavement applications.

TABLE OF CONTENTS

Content **Page**

Page

LIST OF TABLES

LIST OF FIGURES

LIST OF PLATES

ABBREVIATIONS, GLOSSARIES AND SYMBOLS

AASHTO	American Association for State Highway and Transportation Officials
ASTM	American Society for Testing and Materials
BS	British Standard
BSL	British Standard Light
$CaCO_3$	Calcium carbonate
CBR	California Bearing Ratio
CH	Clay of High Plasticity
CL	Clayey soil
Gs	Specific gravity
LL	Liquid Limit
M	Mass
m	Metre
MDD	Maximum Dry Density
M_s	Mass of compacted soil
$MgSO_4$	Magnesium sulphate
NA	Nutrient agar
Na_2HPO_4	Disodium hydrogen phosphate
OMC	Optimum Moisture Content
OPC	Ordinary Portland Cement
PI	Plasticity Index
PL	Plastic Limit
UCS	Unconfined Compressive Strength

USCS Unified Soil Classification System

V_e Volume of sand in bio-enzyme

V_e Volume of sand in bio-enzyme

Bulk density

XRD Dry density Natural moisture content

X-ray diffraction

V

CHAPTER ONE

1.0 INTRODUCTION

1.1 Background to the Study

Clay is a natural material composed primarily of fine-grained minerals. It consists of tiny particles that have plastic and adhesive properties. Clay also possesses very small voids or pores which is responsible for its ability to retain water. In this condition, it tends to expand/heave with moisture and drastically shrink/recede without moisture which can lead to settlement. They are expansive and collapsible soils (Abolarinwa, 2010).

When exposed to increments of water, clay tends to soften and liquefy. Clay often causes difficulties in construction with its low strength and stiffness. This has caused serious problems in geotechnical engineering because weak soil may cause damage to the foundation of buildings and cracks along the road pavement, hence the need for soil stabilization.

Soil stabilization is crucial for construction jobs such as road. It is altering the soils physical, geotechnical properties like gradation, permeability, swelling, shear strength, to meet the design requirements of the proposed structure. Soil stabilisation generally refers to the procedure in which a special soil, cementing material, or other chemical materials are added to a natural soil to improve one or more of its properties. Stabilization therefore looks at the various methods employed for modifying the properties of a soil to improve its engineering performance. One may achieve soil stabilization through mechanically mixing the natural soil and stabilizing material together so as to achieve a homogeneous mix or by adding stabilizing material to undisturbed soil deposit and obtaining interaction by letting it permeate through soil voids (Njideka & Ben, 2018).

1

When soil is stabilized by use of additives it improves the properties of less-desirable road soils. When used these stabilizing agents can improve and maintain soil moisture content, increase soil particle cohesion and serve as cementing and water proofing agents. Since foundation of any structure including road is a critical aspect of the structure in terms of load transfer to the earth, it is usually a difficult problem in civil engineering works when the sub-grade is found to be clay soil. Soils having high clay content have the tendency to swell when their moisture content is allowed to increase (Njideka & Ben, 2018).

The most common methods of soil stabilization of clay soils in pavement work are cement and lime stabilization. Lime or calcium carbonate is oldest traditional chemical stabilizer used for soil stabilization.

Cost effective roads are very vital for economic growth in any country. There is an urgent need to identify new materials to improve the road structure and to expand the road network. Commonly used materials are fast depleting and this has led to an increase in the cost of construction. Hence, the search for new materials and improved techniques to process the local materials has received an increased impetus. When unsuitable material is available at the construction site, the best option is to modify the properties of the soil to meet the pavement design requirements which is the reason for the development of soil stabilization techniques. Since the nature and properties of natural soil vary widely, a suitable stabilization technique has to be adopted for a particular situation after considering the soil properties. (Njideka & Ben, 2018).

Soil improvement by mechanical or chemical means is widely adopted. In order to stabilize soils for improving strength and durability, a number of chemical additives, both inorganic and organic have also been used.

Recently bio-enzymes have emerged as a new chemical for soil stabilization. Bio-Enzymes are chemical, organic, and liquid concentrated substances which are used to improve the stability of soil sub-base of pavement structures. It is a natural, non-toxic, non-flammable, non-corrosive liquid enzyme formulation fermented from vegetable extracts that improves the engineering properties of soil, facilitates higher soil compaction and increases strength (Joydeep & Jitendra, 2015).

The bio-enzyme stabilization has shown little to very high improvement in physical properties of soil. This little improvement may be due to chemical constituent of the soil, which has low reactivity with enzyme. In the cases of high clay moderate soil, like silty soil to sandy soil, the effect of stabilization has improved the CBR and unconfined compression strength. Bergmann (2000) concluded that the Bio-Enzymes require some clay content in the aggregate material in order to create the reaction that will strengthen the material. The successful stabilization could be achieved with as little as two (2) percent clay in the aggregate material but best result seems to be achieved with 10 to 15 percent clay.

1.2 Statement of the Research Problem

Weak clay is a problematic soil in geotechnical engineering practice. It is amongst wide spread soil type available for construction in Nigeria. Its vulnerability to severe shear strength loss is one of its properties when its natural structure is disturbed due to remoulding (Brand & Brenner, 1981). These clays cannot be safely and economically used for the Construction of Civil Engineering structures without adopting some stabilization measures (Abolarinwa, 2010).

More so, available stabilizing agents such as cement, fly ash, lime and others seem to be very expensive methods of soil stabilization that are not easily affordable. Besides the economic factor, some of the traditional stabilizing agents are harmful to the environment.

Therefore, the urgent need to identify naturally existing, non-harmful and readily available stabilizing agents such as urease bio-enzymes, and the need to explore the suitability or otherwise of bio-enzymes in soil stabilization process are the focus of this study.

1.3 Aim and Objectives of Study

The aim of the study is to investigate effect of bio-enzymes addition on the stabilization of soft clay soil for pavement applications.

To achieve this aim, the objectives are:

1. Determination of the physical and mineralogical composition of clay samples and bio-enzyme
2. Determination of the index properties of clay sample-bio enzyme mix.
3. Determination of the compaction and strength properties of clay sample-bio enzyme mix at 5, 10, 15 and 20%.

1.4 Scope of Study

This study was limited to the stabilization of selected clay soils collected from 0.5-2.0m depth in borrow pit located in LapaiGwari sites, Minna using non-toxic, non-corrosive bio-degradable liquid concentrate bio-enzyme fermented from vegetable extracts. The sample were tested and analysed in the Civil Engineering laboratory of Federal University of Technology Minna. The geotechnical prospect of the clay soil with Bio-Enzymes in pre-determined percentages of 5, 10, 15 and 20% was explored in this study.

1.5 Justification of the Study

It is necessary to understand that Nigerian roads are characterized with potholes, longitudinal cracks and lots of other pavement defects known to road construction. One can hardly travel a mile without coming across potholes on Nigeria roads, which have contributed greatly to the high rate of accidents and a dip on the nation's economic development. All structures designed and built have a stipulated design life but most times fail long before the expected date due to poor or weak granular subgrade materials which may cause shrinkage, expansion and settlement (Njideka & Ben, 2018).

Since primary methods of soil stabilization which ranges from mechanical to chemical stabilization are relatively expensive to be implemented by developing nations, there is need to explore locally available materials which are relatively cheap and affordable to construction practitioners.

This study will therefore provide an effective, non-toxic, easily accessible and low cost method of soil stabilization using bio-enzymes as an alternative material to cement and other materials that are not easily available or high cost effective.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Preamble

The use of enzyme for stabilization in pavement construction 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 increased the wetting and bonding capacity of the soil particles (Tingle *et al*., 2007).

An enzyme is defined as an organic catalyst that facilitates a chemical reaction without becoming a part of the end product. Since the enzymes do not become the part of end product and are not consumed by the reaction, a very small amount of bio enzyme is required for soil stabilization. They are organic molecules that catalyse very specific chemical reactions if conditions are conducive to the reaction they facilitate. For an enzyme to be active in a soil, it must have mobility to reach at the reaction site. The pore fluid available in the soil mass provides means for mobility of the molecules of bio enzyme, the specific soil chemistry provides the reaction site, and time is needed for the enzyme to diffuse to the reaction site. An enzyme would stay active in a soil until there are no more reactions to catalyse. Enzymes are soil specific (Tingle *et al*., 2007).

Each enzyme is specifically tailored to promote a chemical reaction within or between other molecules. The enzymes themselves are unchanged by these reactions. Enzymes serve as a host for the other molecules, greatly accelerating the rate of normal chemical and physical reactions. The enzyme allows soil materials to become more easily wet and more densely compacted. They also improve the chemical bonding between soil particles and creating a more permanent structure that is more resistant to weathering, water penetration as well as wear and tear (Tingle *et al*., 2007).

2.2 Stabilization of Expansive Clay Soil

Clay has the smallest particle size of any soil type, with individual particles being so small that they can only be viewed by an electron microscope. This allows a large quantity of clay particles to exist in a relatively small space, without the gaps that would normally be present between larger soil particles. Because of the small particle size of clay soils, the structure of clay-heavy soil tends to be very dense (Guggenheim *et al*., 2006).

Expansive soils are present throughout the world that have tendency to swell upon increase in moisture content. There is extensive damage caused by soil expansion reported from many countries worldwide. Such soils impose a potential risk to safety of civil engineering structures including highways, bridges, railways, airports, and seaports constructed on expansive soils (Dang *et al.,* 2016). When behaviour of soils changes, it promotes severe land degradation. Thus, it is very important to stabilize expansive soils.

Soil stabilization is the most common ground improvement technique that modifies soils to improve the engineering properties including strength of soils. Additives are commonly used to improve the engineering or geotechnical properties of expansive soils using traditional stabilizers such as cement, gypsum, limestone, and industrial by products including fly ash. However, in order to reduce the cost and environmental impact due to secondary pollutant nature of some of these additives (Cokca, 2001) industrial by products are materials of choice by many researchers.

Soil stabilization process refers to the mixing of two or more different soils or to the mixing of a soil with another geomaterial or with chemicals that can amend its geotechnical properties as per the project requirements. Soil stabilization is a very common process for almost all the road projects. Soil stabilization aims at improving soil strength and increasing resistance to softening by water through bonding the soil particles, water proofing the particles or combination of the two (Sherwood, 1993). Usually, the technology provides an alternative provision structural solution to a practical problem. The simplest stabilization processes are compaction and drainage (if water drains out of wet soil it becomes stronger). The other process is by improving gradation of particle size and further improvement can be achieved by adding binders to the weak soils (Rogers *et al.*, 1993).

The major reasons for soil stabilization are as follows (Behnood, 2018):

i. Enhancement of the strength and hence bearing capacity, as well as some of the other engineering properties of soils.
ii. Dust control for a good working environment.
iii. Waterproofing for conservation of natural or man-made structures.
iv. To promote the use of waste geomaterials in constructions.

2.2.1 Clay mineralogy

Clay refers to naturally occurring material composed mainly of fine- grained minerals, which is generally plastic in nature at appropriate water contents and will harden when fired or dried. Clay minerals are important components of soil and sedimentary rocks. For example, the quantity of clay minerals is up to approximately 35 wt. % of sedimentary rocks (Garrels & Mackenzie, 1971). Clay minerals have been confirmed to affect the properties of soils and rocks because they possess unique structures and surface properties, such as swelling (Moore & Reynolds, 1997, Yuan *et al*., 2008, 2009, Fan *et al*., 2009), cation exchange capacity (Środoń & McCarty, 2008, He *et al*., 2010) and solid acidity (Okada *et al*., 2006, Liu *et al*., 2011, Liu *et al.*, 2013a 1, Liu *et al*., 2013b, Liu *et al*., 2013c, Liu *et al*., 2013d, Liu *et al*., 2013e). They are also "recorders" of geological processes such as (weathering, transportation, sedimentation, and diagenesis) and can provide abundant information on soil or rock transformations (Griffin *et al*., 1968, Rateev *et al*., 1969, Jacobs and Hays, 1972, Singer, 1984, Środoń, 2002, 2013). The identification of essential information on mineralogical properties and geological processes is affected by quantitative analysis of clay mineral compositions (Środoń, 2002). For example, the adsorption of methane in the hydrocarbon source rock of a reservoir is strongly related to the amount of clay minerals (Ji *et al*., 2012, Liu *et al.*, 2013b, Liu *et al.*, 2013a). In this case, it is of great significance to obtain clay mineral compositions of soil and sedimentary rocks for researching the source mineralogy (Środoń *et al*., 2006).

The minerals found in clay are generally silicates less than 2 microns (one millionth of a meter) in size, about the same size as a virus. Clay is very abundant at the earth surface therefore they tend to form rocks known as shales and are a major component in nearly all sedimentary rocks. The small size of the particles of clay are their unique crystal structures giving clay minerals special properties, including cation exchange capabilities, plastic behaviour when wet, catalytic abilities, swelling behaviour and low permeability.

The main minerals that could be found in clays are quartz, plagioclase, feldspar, amphibole, calcite, dolomite, phyllosilicates and amorphous matter (Leroueil, 1999). It was found that tectosilicate minerals, such as quartz, feldspar, and plagioclase, dominate the mineral content of the Champlain Sea clay (Locat, 1996; Torrance, 1988; Berry, 1988). Quartz, feldspar, and plagioclase minerals differ in quantities from one region to another. So, any one or two of them could be present in more dominant quantities than the Clay minerals are hydrous silicates, largely of aluminium, magnesium, and iron that on heating lose adsorbed and constitutional water and yield refractory material at high temperatures. Plasticity is a characteristic of clay minerals and is largely due to an affinity of the clay surface for water, resulting from a net negative charge on the surface of a clay particle that causes it to adsorb water and other fluids. Clay minerals can therefore profoundly affect a soil's engineering behaviour, even when present in small quantities. As the clay content of a soil sample increases, the influence the clay fraction will have on the behaviour of the sample also increases. The strong influence of clay minerals on soil behaviour can be illustrated by the addition of bentonite to a granular soil. Bentonite is a clay mineral composed largely of the clay mineral sodium montmorillonite.

Sabat (2012) conducted series of test and concluded that the addition of brick dust decrease liquid limit, plastic limit, plasticity index, optimum moisture content, maximum dry density and angle of internal friction of clay soil. Further work is required on this especially to explore the effect bio-enzyme addition on strength and other engineering characteristics of this type of soil.

2.3 Bio-Enzymes as Soil Stabilizer

Bio-Enzyme is a natural, non-toxic, non-flammable, non-corrosive liquid enzyme formulation fermented from vegetable extracts that improves the engineering properties of soil, facilitates higher soil compaction and increases strength. Enzymes catalyse the reactions between the clay and the organic cat-ions and accelerate the cat-ionic exchange process to reduce absorbed layer thickness (Joydeep & Jitendra, 2015). For other types of chemical stabilization, chemicals are mixed with soil, which is difficult to mix thoroughly, but bio-enzyme is easy to use as it can be mixed with water at optimum moisture content and then it is sprayed over soil and compacted (Shankar & Mithanthaya, 2009). By altering the physical and chemical characteristics of soil, materials treated with bio-enzyme retain higher performance levels and extended life span. Bio-Enzyme may be used to increase the Maximum Dry Density (MDD) and Unconfined Compressive Strength (UCS) values of a marginal material to achieve specified standards for a base course (Andrew *et al.,* 2003). Bio-Enzyme manufactured in USA and Netherlands also increase the unconfined compressive strength (UCS) and California Bearing Ratio (CBR) of sub-grade soil.

Among the soil materials stabilized by the Bio-Enzymes in the trials are sandy clay, silty clay, sandy silt, plastic and non-plastic clay, sandy loam, fine loam, and loam mixed with clay. The dosage levels of the Bio-Enzymes vary from 1 to 5 litres for $5m^3$of soil depending on the soil type, soil characteristics, and product concentration. The amount of dilution water depends on in-situ moisture content of soil.

Lacuoture and Gonzalez (1995) conducted a comprehensive study of the TerraZyme soil stabilizer product and its effectiveness on sub-base and sub-grade soils. The variation in properties was observed over a short period only and it was found that in cohesive soils there was no major variation in properties during the early days but the soil showed improved performance progressively.

Hitam and Yusof (1998) of Palm Oil Research Institute of Malaysia conducted field studies on improvement on plantation roads. TerraZyme was treated to 27.2 km of the road, which was having serious problems during the monsoon season or after heavy downpour. The sections were then monitored on the surface erosion due to rainwater and wear due to usage. After two monsoon seasons the road was found to be in very good condition in spite of large exposure to heavy rainfall. No surface damage was observed, thus requiring no repair works to the road section.

Brazetti and Murphy (2000), conducted field experiments to study the use of Terrazymeas the bio-enzyme stabilizer for road construction. Different soils like sandy clay, silty clay, sandy silt, plastic and non-plastic clay, sandy loam, loam mixed with clay were selected as pavement materials and field stretches were periodically tested with Dynamic Cone Penetrometer and concluded that the enzyme stabilization is a good technique for the effective and economic solution for pavement construction.

Andrew *et al*. (2003) laboratory scale testing program to evaluate the effectiveness of enzyme treatment on sub grade soil. The effectiveness of enzyme treatment was evaluated on the basis of CBR, strength, soil stiffness and soil modulus.

Isaac *et al.* (2003) conducted a comprehensive study of the TerraZyme and its effectiveness on lateritic soil and clay type soil collected from Kerala. The reactions of the soils treated with the enzyme was observed, recorded and compared to the untreated samples for the period of 8 weeks. It was found that in all soil types considered, the CBR value increased by addition of TerraZyme, which proved its suitability as a stabilizing agent. The increase in CBR was of the range of 136 to 1800 times that of the original value. TerraZyme is useful for clay soil and sand but is less significant to silty soils and clayey and sandy soil had increase in CBR by 700 percent.

Manoj *et al.* (2003) conducted a study to assess the suitability of Bio-Enzyme as soil stabilizer on five types of soils with low clay content to very high clay content. Laboratory tests were conducted to determine the engineering properties of soil and strength characteristics of soil with and without stabilization with bio-enzyme.

Laboratory tests were conducted to determine the engineering properties of soil and strength characteristics of soil with and without stabilization with bio-enzyme. The bio-enzyme stabilization has shown little to very high improvement in physical properties of soil. This little improvement may be due to chemical constituent of the soil, which has low reactivity with Bio-Enzyme. In the cases of highly clay moderate soil, like silty soil to sandy soil, the effect of stabilization has improved the CBR and unconfined compression strength.

Bergmann (2000) concluded that the Bio-Enzymes require some clay content in order to create the reaction that will strengthen the soil. The successful stabilization could be achieved with as little as 2 percent clay but best result with 10 to 15 percent clay.

2.3.1 Mechanism of soil stabilization using bio-Enzyme

In clay water mixture positively charged ions (cat-ions) are present around the clay particles, creating a film of water around the clay particle that remains attached or absorbed on the clay surface. The absorbed water or double layer gives clay particles their plasticity. In some cases the clay can swell and the size of double layer increases, but it can be reduced by drying. Therefore, to truly improve the soil properties, it is necessary to permanently reduce the thickness of double layer. Cat-ion exchange processes can accomplish this. By utilizing fermentation processes specific micro-organisms can produce stabilizing enzyme in large quantity. These soil-stabilizing enzymes catalyze the reactions between the clay and the organic cat-ions and accelerate the cat-ionic exchange without becoming part of the end product (Joydeep & Jitendra, 2015).

Kestler (2009) suggested that enzymes are proprietary of their supplier; unless they provide the composition, it is very difficult to determine the precise composition and stabilization mechanism. He also recommended that some commercial enzymes, for example, Bio Cat 300-1, EMCSQUARED, PermaZyme 11-X, TerraZyme, and UBIX No.0010, should contain protein molecules which react with soil molecules to bind the soil particles together, thus decreasing the affection of soils for moisture. Scholen (1992) proposed that enzymes increase the rate of chemical reaction, which occurs at a much slower speed in the absence of enzymes, without becoming a component of the final product. Enzymes combine with big organic molecules to generate a reactant mediator, which swaps ions with the clay structure and breaks up the clay lattice. As a result, this produces a covering effect, which blocks further absorption of water and loss in density. This reaction regenerates the enzymes again, which sets out and reacts yet again. The enzymes are absorbed by the clay lattice and are afterwards freed upon exchange with metals cations. They have a significant role in the behaviour of the clay lattice, first causing them to get bigger and then to stiffen.

Rauch *et al*. (2003) through different chemical and physical tests, endorsed the hypothesis proposed by Scholen (1992) that states that enzymes unite with the large organic molecules and adhere to clay surfaces, thus jamming potential cation exchange sites and preventing absorption of moisture and subsequent swelling. Resulting ionized water forms linkages among packed particles to provide a binding effect.

Enzyme manufacturers and suppliers claim that enzymes, when used in soil stabilization, can enhance the wetting and bonding properties of the soil particles. The enzymes make the soil more workable, which can be compacted more heavily. Furthermore, the enzymes enhance the chemical bonding of soil particles, which aids in combining them. Thus, a more durable soil structure is built that is more resistant to weathering, traffic, and water infiltration.

Strength tests have shown a considerable increase in strength for soils treated with bio-enzymes, Lacuoture and Gonzalez (1995) studied the effect of the TerraZyme soil stabilizer product on sub base and subgrade soils. Variation in properties and progressive improvement were observed, but no significant improvement was reported during the early days. Hitam *and* Yusof (1998) also studied the effect of TerraZyme on plantation roads through field observations. He noticed that the roads, which had serious problems due to monsoons in the past, remained intact after two monsoon seasons.

2.4 Commonly Available Bio-enzyme Products and their Probable Stabilization Mechanism

2.4.1 Renolith

The Renolith patented product was developed in Germany. Renolith and the cement polymer-forming road stabilization chemical was further developed in Australia in 1995–96. Renolith significantly improves the strength of soil in the cement stabilization process in a variety of roads such as heavy haul roads, highways, rural roads, pathway construction, hard stands and rail earthworks capping. It also improves the flexibility of standard cement stabilized pavements (NRG, 2012).

Renolith's usual application is as a mixture with water in specific proportions. This mixture is then applied to a cement-based aggregates or in-situ soils from fine sands to high plasticity clays. Renolith when thoroughly mixed and stabilized with a soil or road pavement material, cement and water produces an exothermic chemical reaction and forms a polymer which when compacted provides a very dense layer(www.nrgrenolith.com). It is a cost-effective method of subgrade enhancement and pavement rehabilitation.

2.4.2 Permazyme

Permazyme is a compaction enzyme, when it is added to a soil and aggregate mixture, it causes the compaction of clays and silts with a much faster rate than that occurs in nature. According to the manufacturer, this enzyme is a natural organic compound, similar to proteins, which acts as a catalyst (Vijay & Suneet, 2014). Their large molecular structures contain active sites that assist molecular bonding and interaction. The organic formulation is designed to maximize compaction and increase the natural properties of soil to optimal conditions. This enzymatic stabilizer increases the wetting action of water to help achieve a higher density during compaction and the formulation accelerates cohesive bonding of soil particles, creating a tight permanent stratum (Vijay & Suneet, 2014).

2.4.3 Fujibeton

The Fujibet on material, developed in Japan, is climatically stable material and suitable for stabilization of all types of soils. Basically, the product is an inorganic polymer that chemically binds with all compounds, when blended with ordinary Portland cement. The blended mix is called Fujibet on Mix, which is used for soil stabilization to improve the engineering properties of soil. The design concept is based on the unconfined compressive strength results determined on the given soil for different proportions of soil-Fujibet on mix and calculation of the thickness of the stabilization layer based on design CBR, wheel load and volume of traffic. The top layer of the pavement should be covered with 3 to 5 cm asphalt concrete (Chandrasekhar, 2006).

2.4. 4 Terrazyme

TerraZyme is a natural, non-toxic liquid, formulated using vegetable extracts and accepted all over the world as a sound and resourceful road building practice, which completely replaces the conventional granular base and the granular sub base, it emphasizes on strength, performance and higher resistance towards deformation. TerraZyme is specially formulated to modify the engineering properties of soil. They require dilution in water before application. The use of TerraZyme enhances weather resistance and also increases load bearing capacity of soils. These features are particularly evident in fine-grained soils such as clay in which the formulation affects the swelling and shrinking behaviour. This formulation has the ability to change the matrix of the soil so that after compaction the soil loses its ability to reabsorb water and the mechanical benefits of compaction are not lost even after water is reapplied to the compacted soil (Sureka & Gangadhara, 2010). Once the enzyme reacts with the soil, the change is permanent and the product is bio-degradable.

2.4. 4.1 *Mechanism of stabilization using terraZyme*

TerraZyme reacts with the adsorbed water layer of clay particle and reduces the thickness around the soil particle due to which void between the soil particles reduces and the soil particle gets closer orientation with lower compactive effort. This decreases the swelling capacity of the soil particles and also reduces permeability (Velasquez *et al.,* 2005).

2.4.4.2 Advantages of Terrazyme

1. TerraZyme increases the durability of pavement and reduces swelling properties of soil.

2. Reduces construction cost by about 20-40% due to reduction in the transportation of materials and reuse of onsite materials.

3. The use of TerraZyme enhances weather resistance and improves load bearing capacity of soils.

TerraZyme stabilization can convert the road to an all-weather road that has minimum destruction in hot and wet season.

2.4.5 Urease

Urease bio-enzyme catalyses the hydrolysis of urea forming ammonia and carbon dioxide. Found in large quantities in jack beans, soybeans, and other plant seeds, it also occurs in some animal tissues and intestinal microorganisms. Urease is significant in the history of enzymology as the first enzyme to be purified and crystallized (James, 1926). This achievement laid the groundwork for the subsequent demonstration that urease and other enzymes are proteins. The potential use of urease has been studied in several laboratory studies, that is in biotechnology and engineering applications. The direct use of urease compared to the use of urease producing bacteria has the advantage of not having to consider the growth and storage of the bacteria (Krajewska, 2017).

The enzyme are supplied in liquid form and are easily soluble in water, which is used for soil compaction. This saves time and costs normally consumed by the mixing of traditional solid stabilizers with soil. Kestler (2009) suggested that most of the information about enzymes is provided by enzyme suppliers, and, therefore, independent testing information is not readily available.

2.5 Methods of Stabilization

Soil stabilization aims at improving soil strength and increasing resistance to softening by water through bonding the soil particles together, water proofing the particles or combination of the two (Sherwood, 1993). It provides an alternative provision structural solution to a practical problem. The simplest stabilization processes are compaction and drainage (if water drains out of wet soil it becomes stronger). The other process is by improving gradation of particle size and further improvement can be achieved by adding binders to the weak soils (Rogers and Glendinning 1993). Soil stabilization can be accomplished by several methods which fall into two categories; namely mechanical stabilization and chemical stabilization.

2.5.1 Mechanical stabilization

Mechanical stabilization includes all improvements to either soil or soil mass properties without the addition of stabilizing agents. The central idea of mechanical stabilization of expansive clay soils is production of a soil or soil mass that (a) will not or cannot change in volume, (b) has sufficient strength to safely sustain the loads applied to it, or c) causes no damage to transportation facilities as its volume changes. Under this category, soil stabilization can be achieved through physical process by altering the physical nature of native soil particles by either induced vibration or compaction or by incorporating other physical properties such as barriers and nailing (Hall *et al.,* 2012).

2.5.2 Chemical stabilization

Chemical stabilization of expansive clay soil consists of changing the physicochemical environment around and inside clay particles, changing the nature of the water that moves into and out of the voids, and effecting behavioural changes in the soil mass as a whole. These methods include making the clay require less water to satisfy the charge imbalance, making it difficult for water to move into and out of the system, flocculating the clay to cause agglomeration, and, perhaps, cementing particles together to reduce volume change. Hall *et al.,* (2012) under this category, soil stabilization depends mainly on chemical reactions between stabilizer (cementitious material) and soil minerals (pozzolanic materials) to achieve the desired effect.

A chemical stabilization method is the fundamental of this review and, therefore, throughout the rest of this report, the term soil stabilization will mean *chemical stabilization.*

Through soil stabilization, unbound materials can be stabilized with cementitious materials (cement, lime, fly ash, bitumen or combination of these). The stabilized soil materials have a higher strength, lower permeability and lower compressibility than the native soil.

This method of soil stabilization can be achieved in two ways, namely;

(1) In situ stabilization and

(2) Remoulded stabilization. Stabilization is not necessary a magic by which every soil properties can be improved for better (Ingles & Metcalf, 1972). The decision on which method is to be adopted depends on which soil properties have to be modified. The chief properties of soil which are of interest to engineers are volume stability, strength, compressibility, permeability and durability (Ingles & Metcalf, 1972; Sherwood, 1993; EuroSoilStab, 2002).

For a successful stabilization, a laboratory tests followed by field tests may be required in order to determine the engineering and environmental properties. Laboratory tests although may produce higher strength than corresponding material from the field, but will help to assess the effectiveness of stabilized materials in the field. Results from the laboratory tests, will enhance the knowledge on the choice of binders and amounts (EuroSoilStab, 2002).

2.6 Factors affecting the Strength of Stabilized Soil

There are many factors that may affect the strength of stabilized soil. Some of these factors such as the presence of organic matters, sulphates, sulphides and carbon dioxide in the stabilized soils may contribute to undesirable strength of stabilized materials. Others such as compaction, moisture content, Temperature, freeze-Thaw and Dry-wet effect as enumerated below also affects the strength of stabilized soil. (Netterberg & Paige-Green, 1984; Sherwood, 1993).

2.6.1 Organic matter

In many cases, the top layers of most soil constitute large amount of organic matters. However, in well drained soils organic matter may extend to a depth of 1.5 m (Sherwood, 1993). Soil organic matters react with hydration product for example, calcium hydroxide ($Ca(OH)_2$) resulting into low pH value. The resulting low pH value may retard the hydration process and affect the hardening of stabilized soils making it difficult or impossible to compact.

2.6.2 Sulphates

The use of calcium-based stabilizer in sulphate-rich soils causes the stabilized sulphate rich soil in the presence of excess moisture to react and form calcium sulphoaluminate (ettringite) and or thamausite, the product which occupy a greater volume than the combined volume of reactants. However, excess water to one initially present during the time of mixing may be required to dissolve sulphate in order to allow the reaction to proceed (Little & Nair, 2009; Sherwood, 1993).

2.6.3 Sulphides

In many of waste materials and industrial by-product, sulphides in form of iron pyrites (FeS_2) may be present. Oxidation of FeS_2 will produce sulphuric acid, which in the presence of calcium carbonate, may react to form gypsum (hydrated calcium sulphate) according to the reactions in equations (2.2) and (2.2)

$$2 \quad _2+2\ _20+70_2 = 2 \quad 0_4+2\ _2\ 0_4 \qquad (2.1)$$

$$0_3 + \quad _2\ 0_4 + \quad _20 = \quad 0_4.2\ _20 + \quad 0_2 \qquad (2.2)$$

The hydrated sulphate so formed, and in the presence of excess water may attack the stabilized material in a similar way as sulphate (Sherwood, 1993). Even so, gypsum can also be found in natural soil (Little & Nair, 2009).

2.6.4 Compaction

In practice, the effect of addition of binder to the density of soil is of significant importance. Stabilized mixture has lower maximum dry density than that of unstabilized soil for a given degree of compaction. The optimum moisture content increases with increasing binders (Sherwood, 1993). In cement stabilized soils, hydration process takes place immediately after cement comes into contact with water. This process involves hardening of soil mix which means that it is necessary to compact the soil mix as soon as possible. Any delay in compaction may result in hardening of stabilized soil mass and therefore extra compaction effort may be required to bring the same effect. That may lead to serious bond breakage and hence loss of strength. Stabilized clay soils are more likely to be affected than other soils (Figure 2.1) due to alteration of plasticity properties of clays (Sherwood, 1993). In contrary to cement, delay in compaction for lime-stabilized soils may have some advantages. Lime stabilized soil require mellowing period to allow lime to diffuse through the soil thus producing maximum effects on plasticity. After this period, lime stabilized soil may be remixed and given its final compaction resulting into remarkable strength than otherwise (Sherwood, 1993).

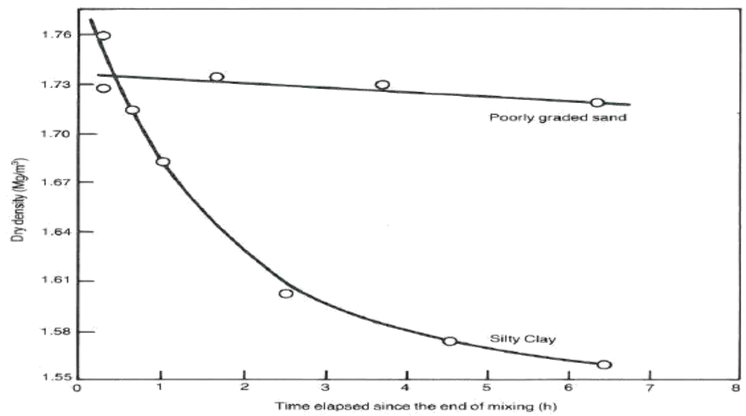


Figure 2.1: Dry density variation with elapse time of stabilized clay (Source: Sherwood, 1993)

2.6.5 Moisture content

In stabilized soils, enough moisture content is essential not only for hydration process to proceed but also for efficient compaction. Fully hydrated cement takes up about 20% of its own weight of water from the surrounding (Sherwood, 1993). On other hand, Quicklime (CaO) takes up about 32% of its own weight of water from the surrounding (Rogers and Glendinning 1993; Sherwood, 1993). Insufficient moisture content will cause binders to compete with soils in order to gain these amounts of moisture. For soils with great soil-water affinity (such as clay, peat and organic soils), the hydration process may be retarded due to insufficient moisture content, which will ultimately affect the final strength.

2.6.6 Temperature

Pozzolanic reaction is sensitive to changes in temperature. In the field, temperature varies continuously throughout the day. Pozzolanic reactions between binders and soil particles will slow down at low temperature and result into lower strength of the stabilized mass. In cold regions, it may be advisable to stabilize the soil during the warm season (Sherwood, 1993; Maher & Ho*,* 1994).

2.6.7 Freeze-thaw and dry-wet effect

Stabilized soils cannot withstand freeze-thaw cycles. Therefore, in the field, it may be necessary to protect the stabilized soils against frost damage (Maher *et al.*, 2003; Al-tabbaa & Evans, 1998). Shrinkage forces in stabilized soil will depend on the chemical reactions of the binder. Cement stabilized soil are susceptible to frequent dry-wet cycles due to diurnal changes in temperature which may give rise to stresses within a stabilized soil and, therefore, should be protected from such effects (Sherwood, 1993; Maher *et al*., 2003).

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Materials

Materials that were used in the course of this study include the following:

3.1.1 Soil

Clay soil samples were used for this study. They were finely-grained natural soil material with 0% of gravel. The sample were obtained from LaipaiGwari borrow pits in Brigi Community, Niger State. The samples were collected at a depth not less than 0.5m from the ground.

3.1.2 Urease

Urease is a natural bio-enzymatic soil stabilizer non-toxic, noncorrosive and non-inflammable liquid, produced by formulating vegetable extracts. The urease bio-enzyme used for the clay stabilization were produced in the Microbiology laboratory of the Federal University of Technology, Minna, Niger State. It was extracted from the bacteria *Lysinibacillus fusiformis Strain* and grown in B4 medium.

3.1.2.1 *Preparation of media*

Nutrient Agar (NA) was prepared in accordance with the manufacturer's instruction (MacFaddin, 2000). Analytical balance was used to weigh 2.8 g of powdered NA into 250 mL conical. Hundred millimetres (100 mL) of distilled water was used to dissolve the powdered NA which was preheated using a heating mantle before sterilizing in the autoclave at 121°C for 15 minutes; it was allowed to cool to 37^{o}C before dispensing into Petri plates to be used.

Urea Agar Base media was prepared according to manufacturer's instruction. A 2.1 g of the media was dissolved into 90 mL of distilled water and preheated using the heating mantle before sterilizing using the autoclave at 121°C for 15 minutes. Four grams (4 g) of urea was weighed and dissolved into 10 mL of sterile distilled water. After sterilizing the urea agar base media, it was allowed to cool to 37^{O}C before adding the dissolved urea solution aseptically using a sterile filter paper and mixed gently before dispensing into the Petri dishes and was allowed to gel before used.

3.1.2.2 ***Collection of isolate***

The isolate identified as *Lysinibacillus fusiformis* strain5B was obtained from Department of Microbiology, Federal University of Technology Minna. The purity of the isolate was confirmed by streaking on nutrient agar plate. This was followed by incubation at 37^{O}C for 24 h.

3.1.2.3 ***Confirmatory test for urease production***

The isolates were aseptically streaked onto urea agar base media and incubated at 37°C for 24 h. After incubation period, isolates were observed for urease production. A change in the agar medium to pink indicates hydrolysis of urea by urease.

3.1.2.4 ***Fermentation medium***

The fermentation medium for urease production was carried out in a chemically defined basal medium of Bakhtiari *et al.* (2006) that was modified slightly with a composition of glucose 5.0 g/L, disodium hydrogen phosphate Na_2HPO_4 2.0 g/L, magnesium sulphate $MgSO_4 \cdot 7H_2O$ 0.5 g/L, calcium carbonate $CaCO_3$ 0.1 g/L, mono-potassium phosphate KH_2PO_4 0.8g/L, Sodium Chloride 2.5 g/L, calcium chloride, cement 5g/L. it was measured in a 1000 ml conical flask, and 500 ml of distilled water was and autoclaved for 15 minutes at 121^{O}C, cooled, and after which 2% (v/v) inoculums from the already prepared inoculum was aseptically added with the aid of sterile pipette. The culture was incubated for six days at 32°C in a rotary shaker at 150 rpm. After the completion of the incubation period, the final liquid culture was centrifuged for 10 minutes at 7000 rpm at 4°C in order to remove the bacterial cells. The resulting supernatant was separated and then filtered using a 0.22 μm pore size filter membrane in order to obtain a cell-free supernatant. The cell-free supernatant was taken as the crude urease enzyme produced, and stored in the refrigerator at freezing temperature for clay stabilization analysis.

3.1.2.5 Effect of temperature on urease enzymes

Bio-enzyme just like water, functions well under the same atmospheric conditions during compaction. Any temperature that keeps the enzyme in a continuously liquid state will not affect or change the properties of the enzyme. Extremely high temperature is not advisable especially as the temperature approaches the boiling point of the enzyme which is 100^{o}c. The best result is achieved when the enzyme is used at room temperature.

3.2 Methods

3.2.1 Sample collection

The soil sample were collected from selected borrow pits in LaipiGwari site in Birgi Community, Niger State at depths of 0.5, 1.0, 1.5 and 2.0m. About 50 kg sample were collected as shown in plate I each using a spade, hand auger, trowel and transported to the Civil Engineering laboratory of the Federal University of Technology Minna for analysis.

The sample were kept safe and packed in an air-tight bag to retain its natural moisture. The natural moisture contents of the samples were determined immediately in the laboratory. The samples were then be pulverized and dried in the soil laboratory of Civil Engineering.

Marks were placed on them to indicate soil descriptions, sampling depths and dates of sampling. The samples were spread on different flat platforms to facilitate air drying. All the clods and lumps in the samples were broken down and reduced to fine particles (pulverization) before conducting various tests on them. The tests werecarried out in accordance to BS1377 (1990) specification.

Plate I: Sample collection at Birgi-LapaiGwari, Minna

3.2.2 Procedure

This study was carried out in two stages; in the first stage, soil characterization was done, while in the second stage, Urease as a bio-enzyme was added to the soil in dosages of 5, 10, 15 and 20%. The soil was oven dried and pulverized and then mixed with the predetermined dosage of 5, 10, 15 and 20% urease. Thereafter, the analysis was conducted with soil with 0% enzyme, as well as soil stabilized with urease containing different percentages.

3.3 Analysis

To assess the suitability of bio-enzyme as soil stabilizer, laboratory test were conducted to determine the engineering properties and strength characteristics of clay soil with and without stabilization with bio-enzyme. The clay soil samples considered for this study were first tested for engineering properties and then for strength parameters such as CBR and unconfined compressive strength of stabilized soil mixes, with 0% and specified percentages of Urease Bio-enzymes. The samples were cured for 28 days to achieve maximum effect.

3.3.1 Tests conducted

The following tests and analyses were carried out in the laboratory according to BS1377(1990) specification to:

(a) Determination of the index properties of the soil sample before stabilization

i. Natural moisture content
ii. Sieve analysis
iii. Atterberg Limits
iv. Specific gravity (by Density Bottle)

(b) Tests conducted on urease treated soil samples

i. Compaction Test
ii. California Bearing Ratio Test
iii. Unconfined Compression

3.3.1.1 ***Index properties***

Natural moisture content, specific gravities, particle size analysis and Atterberg limits tests were conducted in accordance with tests procedures specified in BS 1377: 1990.

3.3.1.2 ***Compaction characteristics***

Compaction improves soils characteristics by increasing soil strength, decreases permeability, increases slope stability of an embankment and reduces foundation settlement. Compaction of stabilized soft clay specimens with and without bio-enzymes was conducted in accordance with the guidelines specified in BS 1377 (1990) to compute the required parameters. The British Standard Heavy (BSH) method compactive effort was used. The BSH compaction is the energy resulting from 4.5 kg rammer falling through a height of 45 cm onto five layers, each receiving 27 blows.

3.3.1.3 ***Unconfined compressive strength (UCS)***

The UCS test was conducted in accordance with the procedure specified in BS, 1377: (1990). The treated specimens were stabilized with 5, 10, 15, and 20 % of Urease bio-enzymes and compacted with BSH compactive energy. The compacted specimens were cured for 24 hours in the moulds before extrusion and trimming as well as further cured for another 28 days in the laboratory at temperature of $24 \pm 2^{o}C$

3.3.1.4 ***California bearing ratio test (CBR)***

California bearing ratio (CBR): The soaked CBR of soft clay stabilized with 5, 10, 15 and 20% of bio-enzymes was conducted in accordance with the guidelines specified in BS 1377 (1990) and cured for 28days to compute the required parameters. 6 kg of pulverized mixed samples divided to five parts were poured into CBR mould and rammed with 4.5 kg rammer into five layers, each receiving 62 blows. The attached upper and lower dial gauges measures the upper and lower penetrations of the plunger.

Percentage (%) Increase =/ × 100 (3.1)

3.3.1.5 ***Free swell index*****:** Frees swell index is the increase in volume of a soil without any external constraints on submergence in water. Free swell index determination of soil helps to identify the potential of a soil to swell which might need further detailed investigation regarding swelling and swelling pressures under different field conditions. The free swell index was conducted in accordance with the procedure specified in BS, 1377: (1990). And the value obtained from the formula.

Free Swell Index = − / × 100 (3.2)

V_d = Volume of sand in distilled water

V_e=Volume of sand in bio-enzyme

3.3.1.6 ***X-ray diffraction***

The X-ray diffraction patterns were obtained using a Philips X-ray diffractometer (model 1040) fitted with a Cu target and a Ni filter. The clays were X-rayed after three different treatments, Mg-saturated glyceral solvated, NH~ -saturated air dried and NH~-saturated heated to 550°C for

3 hours in Chemistry Department Laboratory, Ahmadu Bello University, Zaria. The summary of the analysis carried out including materials used and BS codes are shown in Table 3:1

Table 3.1: Summary of Analysis Carried out

S/N	Test	Materials	Output	Code
1	Specific Gravity	Pycnometer, Balance, Vacuum pump, Funnel, Spoon	Specific gravity of soil	BS 1377, 1990
2	Grain size distribution	Soil sample, Sieve Shaker, BS410 Standard Sieves, 0.1g accuracy balance, oven, porcelain dish and spatula, receiving pan, cleaning brush and clock.	% Passing sieve	
2	Atterberg Limits: Liquid Limit	Casagrande apparatus, sieve No. 40(ASTM), Spatula, Containers, Tray, Electric Oven, Balance, Glass plates, Glass cup.	Liquid limit, Plastic limit, Plasticity Index, Shrinkage limit	BS 1377,1990
3	Swelling Index	425 micron IS sieve, Graduated glass cylinders 100 ml capacity 2 N0s (IS 878-1956), Glass rod for stirring, Balance of capacity 500g and sensitivity 0.01g.	Increase in volume of the soil.	BS 1377,1990
4	Compaction		OMC, MDD	BS 1377,1990,1924
5	California Bearing Ratio			BS 1377, 1990, 1924
6	Unconfined Compress Strength			

CHAPTER FOUR

4.0 DISCUSSION OF RESULTS

4.1 Presentation of Results

The result of Stabilization of Soft Clay with Bio-Enzymes for pavement applications (Index properties Specific gravity, Moisture content, mineralogical composition) as well as the strength and compaction analysis of soft clay soil stabilized with 5, 10, 15 and 20% of Urease Bio-enzymes are presented and discussed.

4.1.1 The physical and mineralogical composition of clay samples and bio-enzyme

Using the Atterberg limits and sieve analysis, the soil samples used were classified as CL or, A – 7 – 6 and A – 7 – 5 according to Unified Soil Classification and AASHTO soil classification systems respectively (AASHTO, 1986; ASTM, 1992). The Physical and mineralogical composition of the test samples used and urease bio-enzymes from the X-ray diffraction spectroscopy are shown in Figures 4.1-4.2. The mineralogical composition of test samples are shown in Appendix A. X-ray diffraction (XRD) analyses for test samples indicate that Kaolinite is the principal clay mineral for the two A – 7 groups of A – 7 – 5 and A – 7 – 6. The percentage of Kaolinite for the A – 7 – 5 samples is 4.24%, while that of A – 7 – 6 is 5.40%.

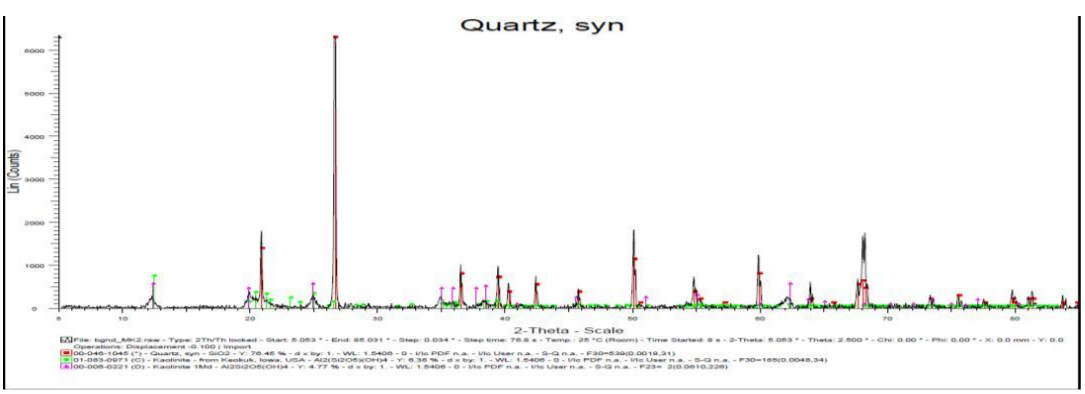


Figure 4.1: X-ray diffraction graph for A – 7 – 5 Soil Sample

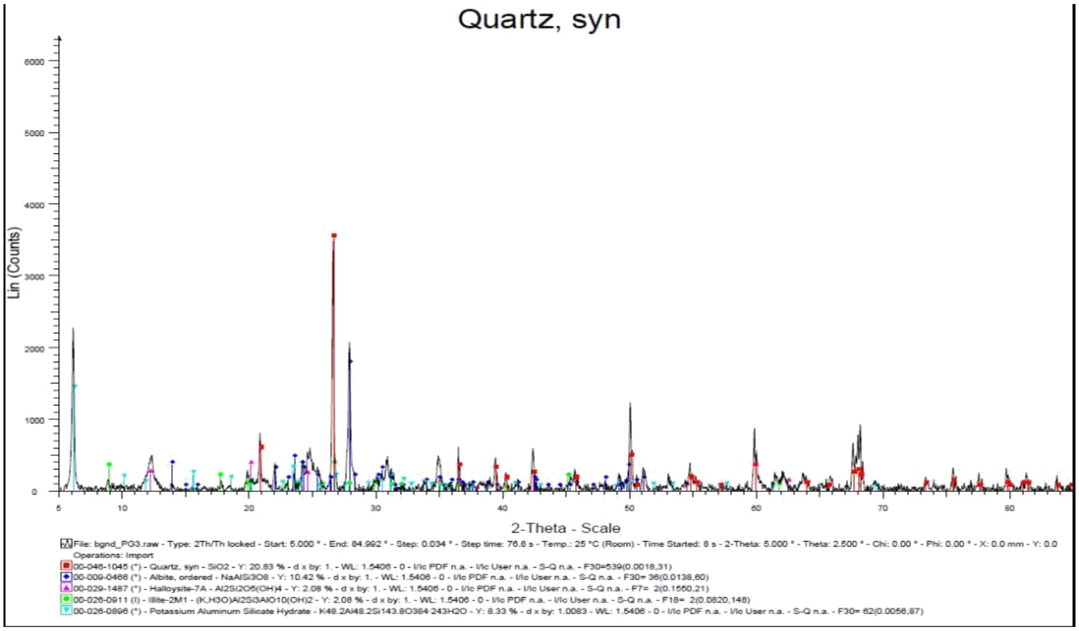


Figure 4.2: X-ray diffraction graph for A – 7 – 6 Soil Sample

The predominant oxides in both A – 7 – 5 and A – 7 – 6 are Iron oxide, Silicon oxide, Aluminium oxide and Titanium oxide. Other oxides present in the test samples as shown in Appendix B. A – 7 – 6 has higher values of Fe_2O_3, Al_2O_3 and CaO than A – 7 – 5. However, the latter has greater value of TiO_2, MnO, MgO and SiO_2 than the former. However both classes A – 7 Clay have the same value of Cr_2O_3.

The minerals identified include; Kaolinite, Montmorillonite, Albite, Halloysite, Illite, Sodium Magnesium Aluminium Oxide, Potassium -Aluminum Silicate Hydrate, Beidellite, Sepiolite and Muscuvite, Silicon and quartz. Quartz was the predominant mineral found in the two samples. Calcium silicide value in A – 7 – 5 and A – 7 – 6 samples are 6.05. % and 6.15% respectively.

Kaolinite is a low swelling clay mineral which has mild affinity for water. Muscovite, also a low swell potential clay mineral was identified in sample A – 7 – 5 at a value of 1.69%.Illite and Halloysite were identified at a value of 2.22% and 2.05% for A – 7 – 5 and A – 7 – 6 samples respectively. A – 7 – 5 and A – 7 – 6 samples contained 6.74% and 6.25% of Montmorillonite respectively. The results obtained from the mineralogical analysis are in conformity with Ola (1975). The work noted that, most Nigerian lateritic soils contained quartz as their most predominant mineral with Kaolinite as the most occurring clay mineral. The oxide contained in the samples are presented in Appendix B. The composition of fermentation medium for the urease bio-enzymes is shown in Table 4.3. Glucose and Calcium chloride are highest at 5.0 g/L. This is closely followed by Sodium chloride (2.5 g/L), Disodium hydrogen phosphate (2.0 g/L), Monopotassium phosphate (0.8 g/L), Magnesium sulphate (0.5 g/L), Calcium carbonate and (0.1 g/L).

4.1.2 Index properties of the natural Soil: The index properties of the natural clay soil and urease enzymes are shown in Appendix E and F. From appendix E, the fraction passing No 200 sieve is 61.83 and 84.01% for A-7-6 and A-7-5 soils respectively. An average value of 16.9% and 21.6% were obtained as natural moisture content for A-7-6 and A-7-5 soils respectively. The mean Specific gravity value for A-7-6 was 2.76g/cm^3 while that for A-7-5 soil was 2.75g/cm^3. According to Ramamurty and Sitharam (2005) the studied soils can be classified as inorganic soils on the basis of the specific gravity.

Appendix E also shows that the plasticity index of the soils are higher than 12% and hence will not be suitable for use as sub-base materials for roads and bridges unless some stabilization method is adopted as specified by (FMWH 1997), Also, there was an observed decrease in the liquid limit of both sample A and B from 41 to 40.4% and 50.50 to 48.5% and an increase in the plastic limit from of both samples from 28.08 to 30.2% and 35.86 to 36.60%, respectively which indicate an improvement in the properties of the soil as the enzymes were used. Ola (1975) indicated that the studied soils will exhibit low swelling potential based on plasticity index in the range of 0-15. (Casagrande chart classification of the soils, 1948) (Appendix D) show soils of medium plasticity and hence compressibility. The soils are not expected to suffer any significant deformation on placement of axle loads.

According to Kestler (2009), enzymes may work suitably for soils containing 12–24% clay fraction with a plasticity index between 8 and 35%. Sample A and B soil fall into this category, and therefore are quite fitting for enzyme functioning and performance in soil stabilization. The summary of geotechnical properties of test samples is shown in Table 4.4. The properties of the urease enzymes according to (Peng *et al*., 2011) is shown in Table 4.5. The grading curves from the sieve analysis is shown in Figures 4.3 and 4.4.

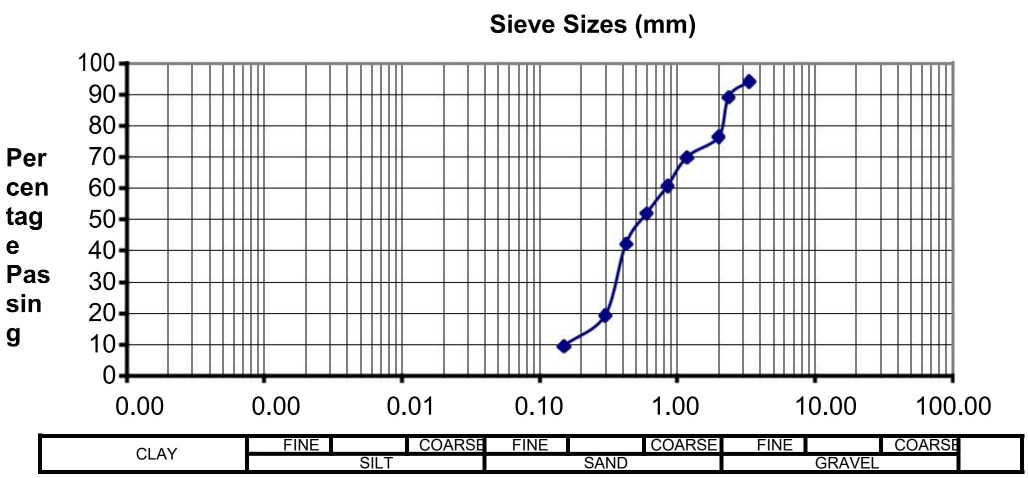


Figure 4.3: Particle size distribution curve for soil sample A

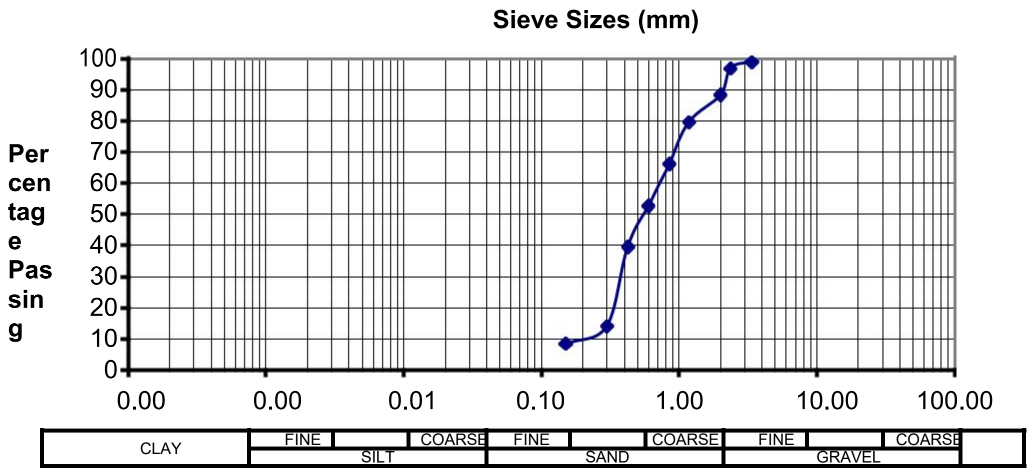


Figure 4.4: Particle size distribution curve for soil sample B

4.1.3 Compaction Characteristics of enzyme-stabilized clay

The densification of soil with the help of mechanical force is known as compaction. For compaction Standard Proctor Test was performed in laboratory. In this test optimal moisture content at maximum dry density for the given soil was determined.

Compaction characteristics of soft clay soil (untreated) were determined using the standard compaction effort (ASTM D698), and the same procedure was used to identify any change in compaction characteristics due to enzymes. The Enzyme -water dilution and application dosage are show in Appendices G and H. The compaction result of the soil stabilized with 5, 10, 15 and 20% of enzymes is shown in Appendix I and represented graphically in Figure 4.6 and 4.7 for A-7-6 and A-7-5 soils respectively. It is worthy to note that three important factors that affect the compaction of soil are moisture content, soil type, and compaction effort.

Appendix I shows that the OMC and MDD for the compacted soil stabilized with 0% enzymes are 60% and 1910kg/m^3 as well as 17.20% and 1720kg/m^3 for A-7-6 soil and A-7-5 clay soils respectively. For A-7-6 soils, there is a decrease in OMC from 12.60 to 10.80 due to the addition of 5% enzymes which further decreased to 10.50% OMC with an increase of 10% enzymes. This is accompanied with a considerable increase of MDD from 1.91 to 1908kg/m^3 for A-7-6 soils. The same decrease in OMC and increase in MDD is observed with A-7-5 soils up to 20% enzymes. It is observed that the OMC for both A-7 sub-class soils decreases with increasing MDD.

The analysis revealed that after the addition of urease enzymes, the OMC decreases and maximum dry density increases for both A-7-6 and A-7-5 soils. It indicates that the voids between the soil particles have decreased and the soil has achieved greater compaction at minimal compactive effort. The variation in compaction parameters of stabilized soil is shown in Appendix I and the compaction curve are shown in Figures 4.5-4.6. From these Figures, Figures 4.6-4.7, urease enzyme addition has little effect on MDD of stabilized soil than the OMC.

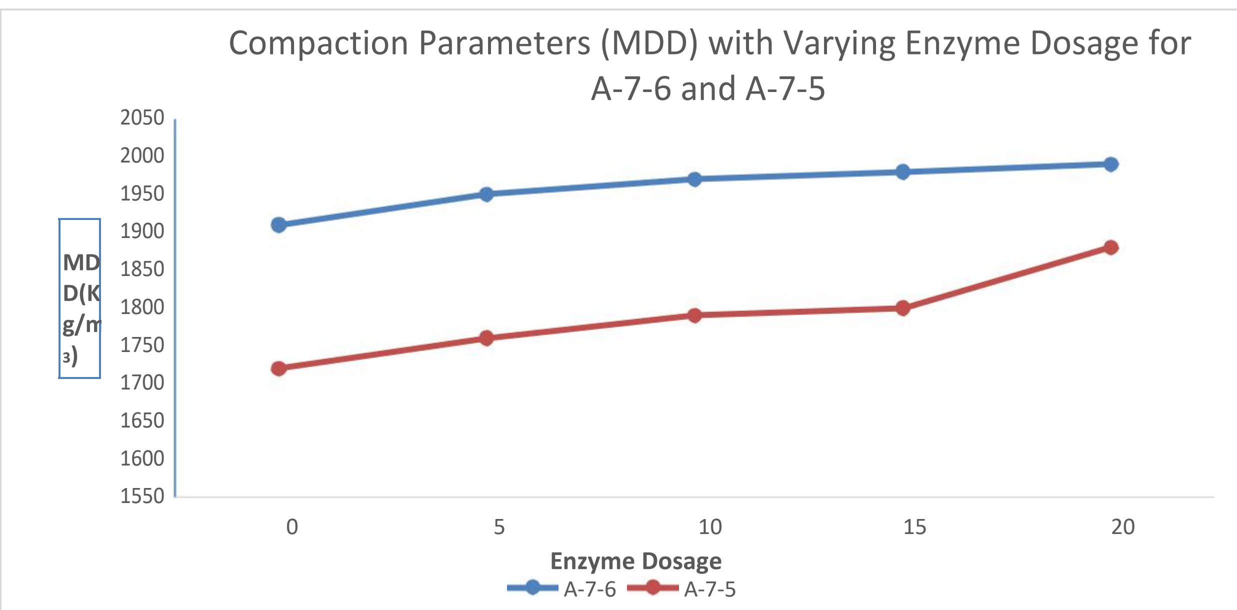


Figure 4.5: Variation of compaction characteristic with enzyme dosage for A and B Sample

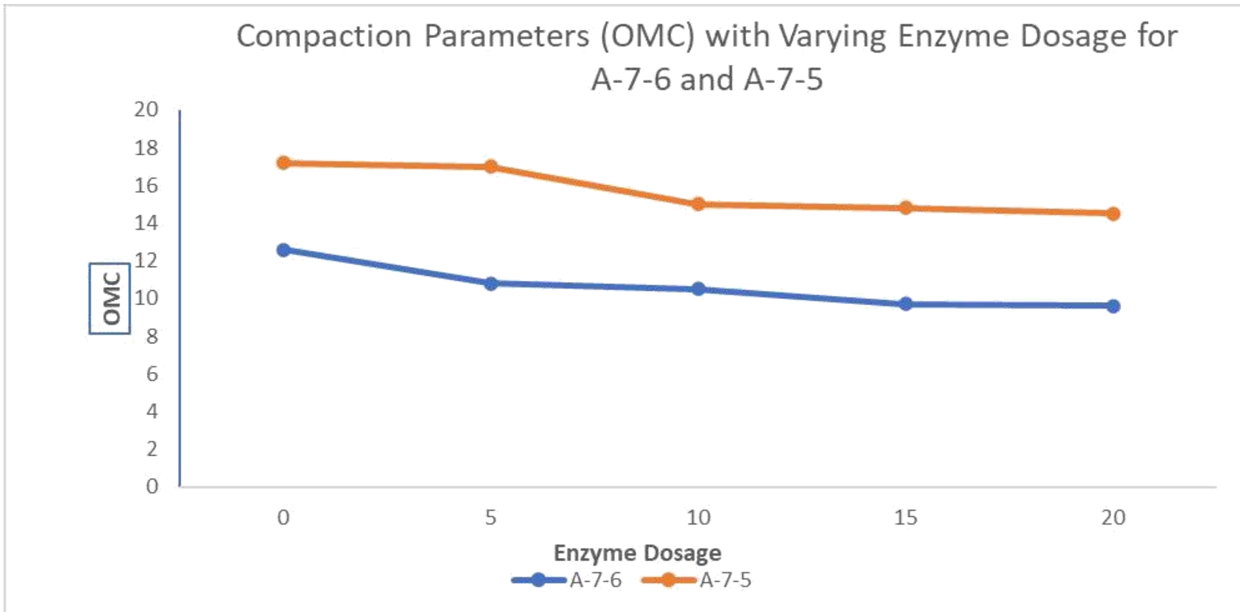


Figure 4.6: Variation of compaction characteristics with enzyme dosage for A and B Sample

4.1.4 California Bearing Ratio (soaked) of enzyme-stabilized clay

California Bearing Ratio is the ratio of force per unit area required to penetrate a soil mass with standard circular piston to that requires for the corresponding penetration of standard material. This test is usually needed to determine the sub-grade strength of the soil in pavements.

The results of the soaked CBR is displayed in Appendix J and graphically in figure 4.8 which influence of soaking is evident in the results obtained. Both the CBR and UCS are often used to estimate the bearing capacity of highway sub-grade and sub-base soils (Gidigasu, 1980). Urease Enzymes was added in varying percentages of 5, 10, 15 and 20% and soaked for 28 days. From the result, it is evident that the CBR values increases with the increase in urease dosage. This is because of the increased compaction which creates a stronger bond between the soil particles, helping them to resist penetration more appreciably (Agarwal *et al*., 2014).

Enzymes primarily attaches to the clay molecules, alters clay molecules and later detaches itself from the modified clay by shifting to its original form after the completion of the reaction. When an enzyme-substrate complex is formed, the enzymes convert the local conditions in the reaction site entirely different to those outside the reaction site. This way, the changes in pH and temperature do not hinder the clay modification. The improvement in soaked CBR Values is due to modification of clay molecules. With the addition of 5% enzymes, the soaked CBR values increased to 29.01 and 17.48 % for A-7-6 and A-7-5 soils respectively. There is a progressive increase to 45.11 and 41.79 % with an increase to 20% enzymes for both A-7-6 and A-7-5 soils respectively. It is observed that the increase effect is more on A-7-6 soils than A-7-5 soils generally. Therefore enzyme stabilization increased the CBR of A-7-6 by 88% (from 20.79 to 45.11%), and A-7-5 by 178% (from 10.06 to 41.79%). With the CBR values of 45.11% and 41.79%, these enzyme-stabilized clay are suitable for road base and sub-base application according to FMWH (1997) and Nigerian General Specification (1997).

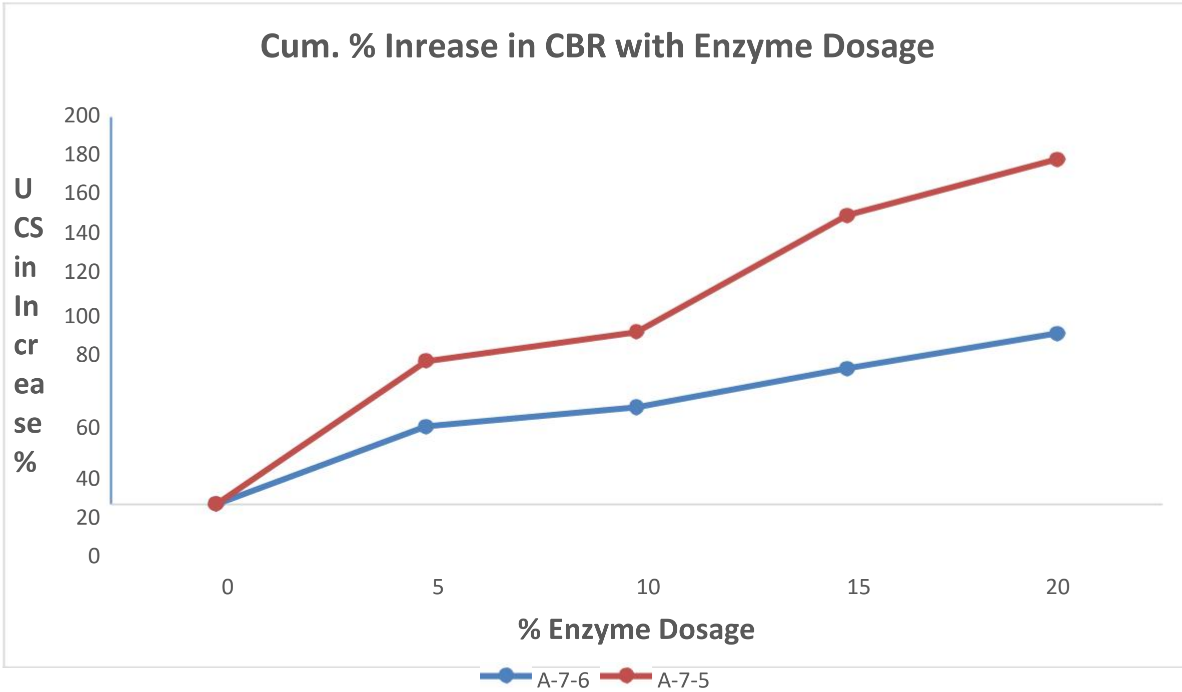


Figure 4.7: Variation of CBR values of clay with enzymes dosage

4.1.5 Effect of urease enzymes addition on unconfined compressive strength (UCS)

The Unconfined Compressive Strength is the maximum axial compressive stress a right cylindrical sample of soil or any other material can withstand under unconfined (confining stress is zero) conditions. UCS test basically gives the strength of the soil so to determine the effect of Urease Enzymes, it is necessary to know the changes on UCS.

The Unconfined Compressive Strength of soft clay soil was evaluated by stabilization with different dosages of urease at 5, 10, 15 and 20% and cured in desiccators for 28 days. The positive effect of curing is evident in the observed values of Unconfined Compressive Strength (UCS) as displayed in Appendix K and graphically in Figure 4.9, The UCS was observed to increase with the increase in the enzyme percentage for samples A-7-6 and A-7-5 both cured for 28 days. This phenomenon according to (Adeyemi & Abolurin, 2000) is as a result of moisture affinity of grains of soil attributable to surface chemical reaction. Figure 4.0 presents the Unconfined Compressive Strength of A-7-6 and A-7-5 soils stabilized with and without urease and the effect of curing on the Unconfined Compressive Strength Value.

The Unconfined Compressive Strength of A-7-6 stabilized with urease has shown tremendous improvement with increase in dosage amount. The Unconfined Compressive Strength of 0% enzymes soil was 232.3 and 370.04 kN/m^2 for A-7-6 and A-7-5 soils respectively, There is a progressive increase from 349.40 to 407.73 kN/m^2 with 5-20% increase of urease enzymes on A-7-6. A similar increase is observed on A-7-5 soil from 463.50 to 576.64 kN/m^2 with 5 to 20% increase in enzymes. The increment was noticed after the both samples were cured after 28 days as shown in Appendix K and Figure 4.9 this increments makes the enzyme an ideal solution for soil stabilization (Venkatasubramanian & Dhinakaran, 2011).

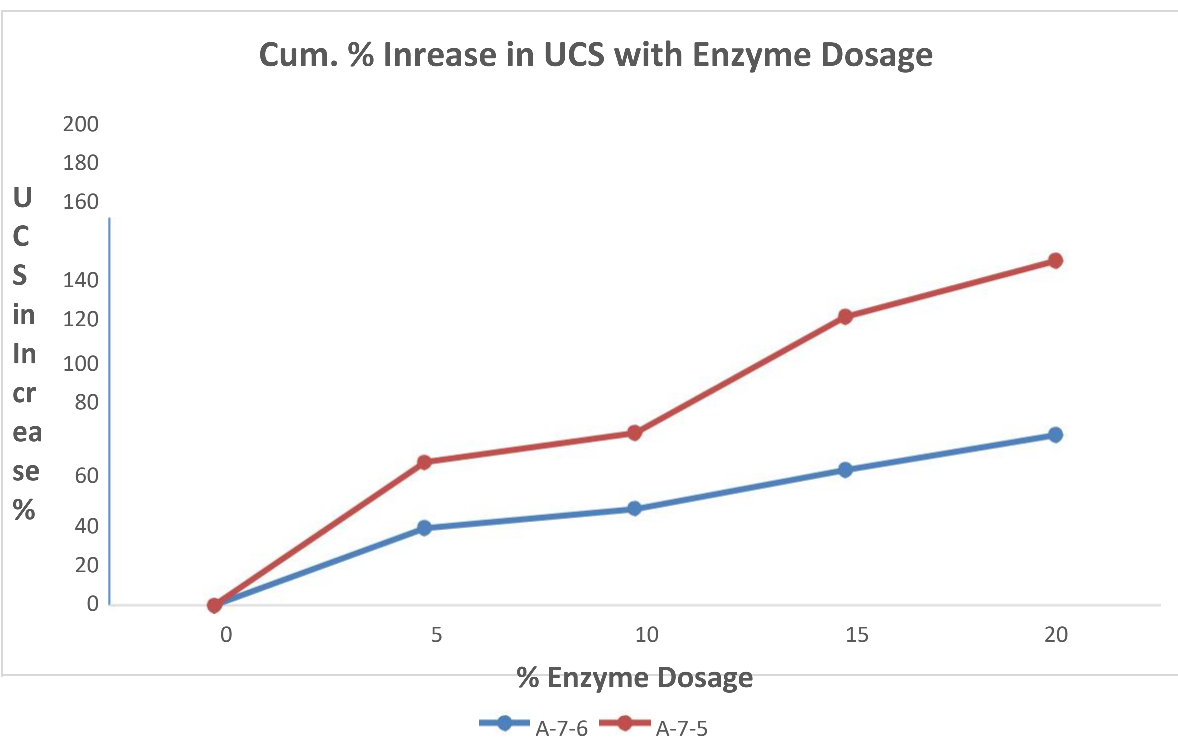


Figure 4.8: Value of UCS of Soil with enzyme dosage

4.1.6 Free swell index (FSI)

The possibility of damage to structures due to swelling of soft clays need to identified at the outset by investigating the soil likely to possess undesirable expansion characteristics. Samples compacted at optimum moisture content were subjected to swell percentage tests using graduated glass cylinders of 100ml capacity.

The Free Swell index (FSI) of the soils after I day curing was obtained as 113.6 and 83.3% for A-7-6 and A-7-5 soils respectively as shown in Appendix L. There was a considerable decrease in the swell from 23.5 to 11 and 16.5 to 9.0 for A-7-6 and A-7-5 soil respectively when cured with enzyme. The FSI of test samples fell within range (>50) %which makes them to be classified as highly expansive clay soil according to (Sridharan & Prakash, 2000a).

CHAPTER FIVE

5.0 CONCLUSION AND RECOMMMENDATIONS

5.1 Conclusion

From the investigation of effect of addition of urease enzymes content on selected geotechnical properties of some soft clay from borrow pits in LapaiGwari, Birgi community, a suburb of Minna, Niger, Nigeria, the following conclusions were drawn;

The dark-brown and reddish samples were classified as CL or A – 7 – 6 and A – 7 – 5 according to Unified Soil Classification and AASHTO soil classification systems respectively. Mineralogically, the soft clay consist of Quartz, Ankerite, Kaolinite, Calcium Silicide, Montmorillonite, Anorthite, Sodium Aluminium Silicate Hydrate, Anorthoclase and Orthoclase. The predominant oxides in both A – 7 – 5 and A – 7 – 6 are Iron oxide, Silicon oxide, Aluminium oxide and Titanium oxide. In summary, the work noted that the clay soils contain quartz as their most predominant mineral with kaolinite as the most occurring clay mineral. The composition of the Bio-Enzyme used for this research(urease) includes, Glucose 59g/l, disodium hydrogen phosphate Na_2HPO_4 2.0 g/L, magnesium sulphate $MgSO_4 \cdot 7H_2O$ 0.5 g/L, calcium carbonate $CaCO_3$ 0.1 g/L, mono-potassium phosphate KH_2PO_4 0.8g/L, Sodium Chloride 2.5 g/L, calcium chloride and cement 5g/L all in 500ml of distilled water in a conical flask.

There was an improvement in the index properties of the clay-sample Bio-Enzyme mix compared to the index properties of the natural soil. The plasticity index of the enzymes mix soils (<12%), and the liquid limit of both sample A and B decreased from 41 to 40.4% and 50.50 to 48.5% while the plastic limit of both samples increased from 28.08 to 30.2% and 35.86 to 36.60%, respectively which indicate an improvement in the properties of the soil as the enzymes were used and that it is suitable for use as sub-grade materials in road construction.

With the use of urease bio-enzyme, the strength of the soil increases which is evident by the increase in UCS and CBR values. Urease bio-enzyme decreases the voids between the soil particles and thus increase the compaction and density of the soil. Stabilization of the soils with urease enzymes increased the MDD, CBR and thus produced denser and stronger samples while there was a reduction in OMC as enzyme was progressively added.

5.2 Recommendations

From the investigation of effect of addition of urease enzymes content on selected geotechnical properties of some soft clay, the following recommendations are given;

1. The utilization of the urease bio-enzymes as an alternative compared to other expensive stabilizing agents especially on clay soil, would reduce construction cost.
2. Urease bio-enzymes is a non-toxic, biodegradable extract, which is less hazardous to construction workers and other participants on site is recommended for stabilizing clay soil.
3. The plasticity index of the enzyme stabilized-soils is <12%, and therefore suitable as sub-grade materials in road construction.
4. It is pertinent to conclude that replacing water with enzyme at OMC for compaction will yield the highest improved properties of the both A-7-6 and A-7-5 soils for civil engineering construction, however, considering the cost implication of replacing water with the enzyme at OMC, 75% and above of enzyme is advised to e used for 3kg of soil for maximum compaction for the required result.

5.3 Contribution to Knowledge

This work established that A-7-5 and A-7-6 soils treated with 20% bio-enzyme recorded California bearing (CBR) ratio values. (that is soaked condition) of 130% and 70% respectively, which are adequate for sub-base road construction works.

REFERENCES

Abolarinwa, A. (2010). Geotechnical Properties of Major Problem Soils of Nigeria. *FUOYE Journal of Engineering and Technology,* doi:10.46792/fuoyejet.v1i1.20

AASHTO (1986). American Association of State Highway and Transport Officials. *Standard Specifications for Transport Materials and Methods of Sampling and Testing*. 14th Edition, Washington, D.C. AASHTO,

ASTM, D 4208 (1992). American Standards Testing Methods. *Annual book of ASTM standards,* Philadephia.

ASTM D 698-00a. (2002). Standard Test Methods for Laboratory Compaction Characteristics of Soil Using Standard Effort (12,400 ft-lbf/ft3(600 kN-m/m3)). *Annual Book of ASTM Standards, American Society for Testing and Materials.* West Conshohocken, PA 1–7.

Al-Tabbaa A, and, Evans W.C. (1998). Pilot in situ auger mixing treatment of a contaminated site-Part 1: treatability study. In: Proceedings of the Institution of Civil Engineers Geotechnical Engineering, 131, 52–59, https://doi.org/10.1680/igeng.1998.30005

Adeyemi, G.O. and Abolurin, S.B. (2000). Strength characteristics of a chemically stabilized lateritic soil from kilometre 4, Ile-Ife/Sekona road, South-western Nigeria. *Journal of Mining and Geology*, 36, 113-119. Retrieved from http://bowenstaff.bowen.edu.ng

Andrew, R. Tolleson, Fadi, M. Shatnawi, Nicholos, E. Harman and Elahe M. (2003). An Evaluation of Strength Change on Sub-grade Soils Stabilized with an Enzyme Catalyst Solution Using CBR and SSG Comparisons, USA, *Geomatrics, Inc. Columbia.*

Agarwal, P, and Suneet, K (2014). Effect of Bio-Enzyme Stabilization on Unconfined Compressive Strength of Expansive Soil. *International Journal of Research in Engineering and Technology*, 3.5, 30-33, Retrieved from https://ijret.org/volumes/2014v03/i05/IJRET20140305007.pdf

Bakhtiari, M. R., Faezi, M.G., Fallahpour, M., Noohi, A., Moazami, N., Amidi, Z. (2006). Medium optimization by orthogonal array designs for urease production by Aspergillus niger PTCC5011. *Process Biochemistry*, 41(3), 547-551

Brand, E. W., and Brenner, R. P. (1981). *Soft clay engineering.* USA: Elsevier Science Ltd.

Brazetti, R. and Murphy, S.R. (2000); General Usage of Bio-Enzyme Stabilizers in Road Construction in Brazil, *32nd Annual Meeting on Paving Brazil*.

Bergmann, R (2000). *Soil stabilizers on universally accessible trails.* USDA Forest Service, San Dimas Technology and Development Center.

Berry A.J. (1988). The Dynamic Penetration of Clays. Proc. *1st International Symposium on Penetration Testing.* SOPT 1(2), *Canadian Geotechnical Journal* https://doi.org/10.1139/cgj-2017-0451

Behnood, A. (2018). Soil and clay stabilization with calcium and non-calcium-based additives: A state of the art review with challenges, approaches and techniques. *Transportation Geotechnics,* 17, 14-32.

BS 1377 (1990). *Method of Testing Soils for Civil Engineering Purposes*. London British: Standard Institute, BSI.

BS 1924 (1990). *Stabilized Material for Civil Engineering Purposes:* Method of Test for Cement Stabilized and lime Stabilized Materials. London, United Kingdom: British Standard Institute.

Casagrande, A. (1948). Classification and identification of soils. *Transactions, ASCE*, 113, 901-930.

Chandrasekhar, B.P. (2006). *A Critical reviews of innovative rural road construction techniques and their impact*, New Delhi: NRRDA.

Cokca, E. (2001). Use of class C fly ashes for the stabilization of an expansive soil. *J. Geotech technical Geoenvironmental Engineering.* 127, 568–573.

Dang, L. C., Fatahi, B. and Khabbaz, H. (2016). Behaviour of expansive soils stabilized with hydrated lime and bagasse fibres. *Procedia Engineering.*143, 658–665.

EuroSoilStab. (2002). Development of Design and Construction Methods to Stabilize Soft Organic Soils: Design Guide for soft soil stabilization. CT97-0351, *European Commission, Industrial and Materials Technologies Programme (*Rite-EuRam III) Bryssel.

Fan, P., Yuan, J., Zhu, T., Chen, A., Yuan, H., He, K., Chen, D., and Liu C. (2009). shell structured iron nanoparticles well dispersed on montmorillonite *J. Magnetic Materials*, 3515-3519

Federal Ministry of Works and Housing (1997). *General Specification for Roads and Bridges*, Lagos, Nigeria: Federal Highway Department, FMWH.

Garrels, R.M. and Mackenzie, F.T, (1971). *Evolution of Sedimentary Rocks.* New York: Norton.

Guggenheim, S., Adams, J.M., and Bain, D.C. (2006). *Summary of recommendations of nomenclature committees relevant to clay mineralogy*: report of the Association Internationale pour l'Etude des Argiles (AIPEA) Nomenclature Committee for 2006. Clay Minerals 41, 863–877

Gidigasu, M.D. (1980). Geotechnical evaluation of residual gravels in pavement construction. *Engineering Geology,* 15, 173-194.

Griffin, J.J., Windom, H., and E.D. Goldberg, E.D.(1968). The distribution of clay minerals in the world ocean Deep Sea Reserve. *Oceanography. Abstract*, 433-459

Hall, M.R., Najim, K.B., and Dehdezi, P.K. (2012). Soil Stabilization and earth Construction: Materials, properties and techniques. United Kingdom: *Woodhead Publishing Limited*

He, H., Ma, Y., Zhu, J., Yuan, P.,Y. (2010). QingOrgano clays prepared from montmorillonites with different cation exchange capacity and surfactant configuration *Applied Clay Science*., 67-72.

Hitam, A. and Yusof, A. (1998). Soil Stabilizers for Plantation Road, National Seminar on Mechanisation in Oil Palm Plantation, 30 June 1998, Selangor, Malaysia.

Isaac, K.P. Biju and Veeraragavan (2003). Soil Stabilization Using Bio-Enzyme for Rural Roads presented at the *IRC Seminar "Integrated Development of Rural An Arterial Road Networks,* Retrieved from https://doi.org/10.1155/2015/798965

Ingles, O.G. and Metcalf, J.B. (1972). *Soil Stabilization: Principles and Practice*. Butterworths, Sydney

James, B. Sumner (1926. Isolation of Urease Crystals. New York, USA: Cornell University, Ithaca.

Jacobs, M.B., and Hays, J.D. (1972). Paleo-climatic events indicated by mineralogical changes in deep-sea sediments J. *Sedimental Petrol*, 889-898.

Ji, L., Zhang, T., Milliken, K.L, Qu, J., Zhang, X. (2012). Experimental investigation of main controls to methane adsorption in clay-rich rocks. *Applied Geochemistry,* 2533-2545

Joydeep, S. and Jitendra, P. S. (2015). Stabilization of Black Cotton Soil using Bio-Enzyme for a Highway Material. *International Journal of Innovative Research in Science, Engineering and Technology, 4,* Issues, 12, Retrieved from https://www.irjet.net ›

Krajewska, B. (2017). Urease-aided calcium carbonate mineralization for engineering applications: A review. *Journal of Advanced Research,* 13, 59-67, Retrieved from https://doi.org/10.1016/j.jare.2017.10.009

Kestler, M.A. (2009). Stabilization Selection Guide for Aggregate and Native-Surfaced *Low Volume Roads, Forest Servic*e, San Dimas Technology & Development Center USA, San Dimas, Calif, USA.

Lacuoture, A. and Gonzalez, H. (1995). Usage of Organic Enzymes for the Stabilization of Natural Base Soils and Sub-bases in Bagota, Pontificia Universidad Jevariana, Faculty of Engineering.

Leroueil, S. (1999). Geotechnical characteristics of eastern Canada clays. *In Proceedings of the International Symposium on the characterization of soft marine clays* 3, 32. A.A Balkema, Yokosuka, Japan.

Little, D. N., and Nair, S. (2009). Recommended Practice for Stabilization of Subgrade Soils and Base Materials. *National Cooperative Highway Research Program*, Transportation Research Board of the National Academies

Liu, D., Yuan, P., Liu, H., Cai, J., Qin, Z., Tan, D., Zhou, Q., He, H., (2011). Influence of heating on the solid acidity of montmorillonite: a combined study by DRIFT and Hammett indicators. *Applied Clay Science,* 358-363

Liu, D., Yuan, P., Liu, H., Cai, J., Tan, D., He, H., Zhu, J., Chen, T., (2013a). Quantitative characterization of the solid acidity of montmorillonite using combined FTIR and TPD based on the NH3 adsorption system. *Applied Clay Science,* 407-412

Liu, D., Yuan, P., Liu, H., Li, T., Tan, D., Yuan, W., He, H., (2013b). High-pressure adsorption of methane on montmorillonite, kaolinite and illite. *Applied Clay Science,* 25-30

Liu, D., Liu, P., Yuan, D., Tan, J., Cai, H., He, J., Zhu, Z., (2013c). Song Studies on the solid acidity of heated and cation-exchanged montmorillonite using n-butylamine titration in non-aqueous system and diffuse reflectance Fourier transform infrared (DRIFT) spectroscopy Physics and *Chemistry of Minerals,* 479-489

Liu, H., Yuan, P., Liu, D., Tan, D., He, H., Zhu, J., (2013d). Effects of solid acidity of clay minerals on the thermal decomposition of 12-aminolauric acid J. Thermal *Analysis Calorim*., 125-130

Liu, H., Yuan, P., Qin, Z., Liu, D., Tan, J., Zhu, He, H., (2013e). Thermal degradation of organic matter in the interlayer clay–organic complex: a TG-FTIR study on a montmorillonite/12-aminolauric acid system. *Applied Clay Science,* 398-406

Locat, J., (1996). On the development of microstructure in collapsible soils, lessons from the study of recent sediments and artificial cementations. In. *Genesis and properties of collapsible soils, Dordrecht*, 93-128

Maher, M.H and Ho, Y.C (1994). Mechanical properties of kaolinite/Fibre Soil composition. *Journal of Geotechnical Engineering,* 120 (8), 1382-1393. Retrieved from doi:10.1061/(ASCE)0733-9410(1994)120:8(1381

Maher, A., Bennert, T., Jafari, F., Doglas, W.S and Gucunski, N. (2003). Geotechnical Properties of Stabilized Dredged Material from New York-New Jersey Harbor. *Journal of the Transportation Research Board*, 86-96. Retrieved from https://doi.org/10.3141/1874-10

Manoj, S., Sunil, B and Sikdar, P.K. (2003). Bio-Enzyme for Stabilization of Soil in Road Construction- A Cost Effective Approach

MacFaddin J. F., (2000). Biochemical Tests for Identification of Medical Bacteria, 3rd Ed., Lippincott, Williams and Wilkins, Baltimore

Moore, D.M., R.C. Reynolds, R.C. (1997). X-ray Diffraction and the Identification and Analysis of Clay Minerals (2nd ed.), Oxford University Press, Oxford.

Netterberg F and Paige-Green P (1984). *Carbonation of Lime and Cement Stabilized Materials in Road Construction.* South Africa: NITRR,CSIR, Pretoria,

Njideka, O and Ben, N. (2018). Soil Stabilization for Road Construction: Comparative Analysis of a Three-Prong Approach, IOP Conference Series: *Materials Science and Engineering*. 413

NRG Technology International Ltd (2012). NRG Renolith- Presentation, *International Journal of Research in Engineering and Technology*, http://www.ijret.org

Okada, K., Arimitsu, N., Kameshima, Y., Nakajima, A., MacKenzie K.J.D(2006). Solid acidity of 2: 1 type clay minerals activated by selective leaching. *Applied Clay Science,* 185-193

Ola, A.S. (1975). Stabilization of Nigerian Lateritic soil with cement, bitumen and lime. *Proceedings of the 6th Regional Conference for Africa on Soil Mechanics and Foundation Engineering*, I, 145-152. Retrieved from https://doi.org/10.1016/0013-7952(77)90036-9

Peng, H. T., Su, H.T., Zhang, X.P. and Wang, J. (2011). An experimental comparison of compressive strengths of soils stabilized with enzyme and ground quicklime, *Advanced Materials Research*, 280, 9–12. Retrieved from https://iopscience.iop.org

Rauch, A. F., Katz, L.E. and Liljestrand, H.M. (2003). An Analysis of the Mechanisms and Efficacy of Three Liquid Chemical Soil Stabilizers, *Center for Transportation Research.* The University of Texas at Austin.

Ramamurthy, T.N., and Sitharam, T. (2005). *Geotechnical Engineering:* Basics of Soil Mechanics. DELHI, India: S Chand & Company Limited

Rateev, M.A., Gorbunova, Z.N., Lisitzyn, A.P., Nosov., G.L (1969). The distribution of clay minerals in the ocean. *Sedimentology*, 13(1).21-43. Retrieved from doi:10.1111/j.1365-3091.1969.tb01119.x

Rogers, C.D.F. and Glendinning, S. (1993). Modification of clay soils using lime. In C. a. Rogers (Ed.), *Proceeding of the Seminar held at Loughborough University on Lime Stabilization* (99-114). London: Thomas Telford.

Sabat, A. K. (2012). Stabilization of Expansive Soil Using Waste Ceramic Dust. *Electronic Journal of Geotechnical Engineering*. 17,3915-3926. Retrieved from http://www.ejge.com/2012/Ppr12.369alr.pdf

Scholen, D. (1992). *Non-standard stabilizers:* Report FHWA-FLP-92- 011, Washington, DC Department of Transportation.

Sherwood, P. (1993). Soil stabilization with cement and lime. State of the Art Review. London: *Transport Research Laboratory*, HMSO.

Shankar, A., Rai, H.K, and Mithanthaya. H. (2009). Bio-enzyme stabilized lateritic soil as a highway material, *Indian Roads Congress Journal,* 70(2):124-130. Retrieved from http://worldcat.org/issn/0046905X

Singer, A. (1984). The paleoclimatic interpretation of clay minerals in sediments- a review. *Earth Science Revision*., 251-293

Sridharan, A., and Prakash, K. (2000a). Classification procedures for expansive soils. *Geotechnical Engineering*. 143, 235-240

Środoń, J. (2002). Quantitative mineralogy of sedimentary rocks with emphasis on clays and with applications to K-Ar dating. *Mineralogical Magazine*, 66 (5), 677-687. Retrieved from doi: https://doi.org/10.1180/0026461026650055

Środoń, J., Kotarba, M., Biroň, A., Such, P., Clauer, N. (2006). Diagenetic history of the Podhale-Orava Basin and the underlying Tatra sedimentary structural units (Western Carpathians): *Evidence from XRD and K-Ar of illite-smectite Clay Miner*., 751-774

Środoń, J., McCarty, D.K (2008). Surface area and layer charge of smectite from CEC and EGME/H2O retention measurements. *Clay Minerals*, 155-174

Środoń, J. (2013). Identification and Quantitative Analysis of Clay Minerals. *Handbook of Clay Science,* Institute of Geological Sciences, Poland, (25–49).

Sureka, N. and Gangadhara, S. (2010). Swelling Properties of Bio-enzyme Treated Expansive soil, *International Journal of Engineering* Studies, 2(2), 155–159. Retrieved from https://www.irjet.net/archives/V5/i3/IRJET-V5I3358.pdf

Tingle, J.S., Larson, S.L., Weiss, C.A., Newman, J.K, Peters, J.F., Tardy, B and Berney, E.S. (2007). Stabilization Mechanisms of Non-traditional Stabilizers. In Transportation Research Record: *Journal of the Transportation Research Board*, 1989(2),59–67. Retrieved from https://doi.org/10.3141/1989-49

Torrance, J. K. (1988). Mineralogy pore-water chemistry and geotechnical behaviour of Champlain sea and related sediments. In. Gadd, N.R. (ed) The late Quaternary Development of the Champlain Sea Basin. *Geological Association of Canada*, 35, 259-275.

Velasquez, R., Marasteanu, M.O., Hozalski, R andClyne, T. (2005). *Preliminary Laboratory investigation of Enzyme solutions as a soil stabilizer*. Research Report MN/RC-2005-25. USA: University of Minnesota

Venkatasubramanian, C. and Dhinakaran, G. (2011). Effect of bio-enzymatic soil stabilisation on unconfined compressive strength and California Bearing Ratio. *Journal of Engineering and Applied Sciences*, 6(5), 295–298, Retrieved from doi: 10.3923/jeasci.2011.295.298

Vijay, R. and Suneet, K. (2014). A Review on Stabilization of Soil Using Bio-Enzymes. *International Journal of Research in Engineering and Technology*. 2319-1163, doi:10.15623/IJRET.2014.0301011

Yuan, P., Annabi-Bergaya, F., Tao, Q., Fan, M., Liu, Z., Zhu, J., He, H., Chen, T., (2008). A combined study by XRD, FTIR, TG and HRTEM on the structure of delaminated Fe-intercalated/pillared clay. *James Colloid Interface Science.,* 142-149.

Yuan, P., Fan, M., Yang, D., He, H., Liu, D., Yuan, A., Zhu, J., Chen T. (2009). Montmorillonite-supported magnetite nanoparticles for the removal of hexavalent chromium [Cr(VI)] from aqueous solutions. 821-829. Retrieved from doi: 10.1016/j.jhazmat.2008.11.083.

APPENDICES

APPENDIX A: Mineralogical composition of test soft clays samples

Description	Quantity (%)	
	A-7-5	**A-7-6**
Quartz	50.15	50.05
Ankerite (%)	5.22	5.25
Calcium Silicide (%)	6.05	6.15
Montmorillonite (%)	6.74	6.25
Anorthite (%)	7.55	7.02
Kaolinite (%)	4.24	5.40
Sodium Aluminium Silicate Hydrate (%)	6.24	6.20
Anothoclase (%)	6.33	6.23
Orthoclase (%)	7.48	7.45
Total	100.00	100.00

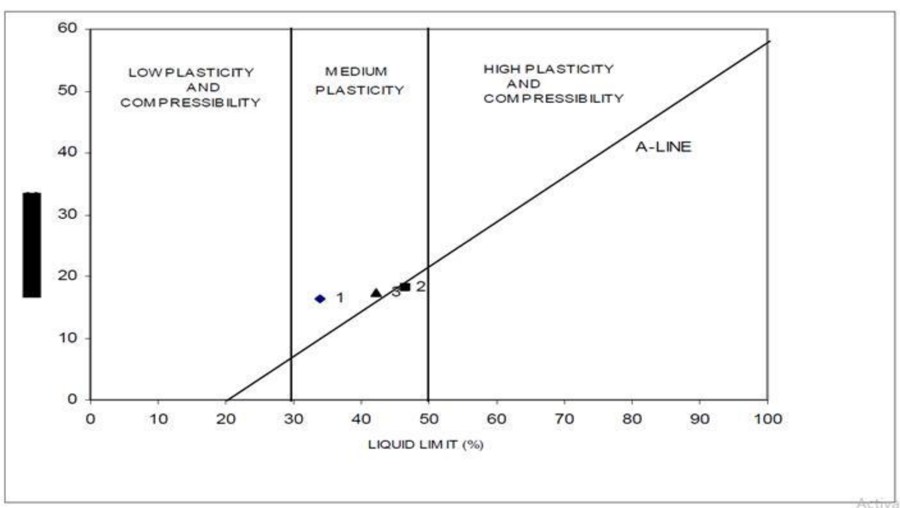
APPENDIX B: Oxide composition of clay sample using XRF (Cu-Zn method)

.	**A–7–5**	**A–7–6**
CuO	0.007	0.004
NiO	0	0
Fe_2O_3	5.483	7.475
MnO	0.059	0.046
Cr_2O_3	0.019	0.019
TiO_2	0.502	0.014
CaO	0.305	0.506
Al_2O_3	0.450	0.679
MgO	0.985	0
ZnO	0.101	0.005
SiO_2	4.085	3.619
Total	11.996	12.367
Balance	88.004	87.633

APPENDIX C: Composition of fermentation medium of urease bio-enzymes

Description	Quantity (g/L)
Glucose	5.0
Disodium hydrogen phosphate	2.0
Magnesium sulphate	0.5
Calcium carbonate	0.1
Monopotassium phosphate	0.8
Sodium Chloride	2.5
Calcium chloride	5.0

APPENDIX D: Casagrande chart classification of soils (Unified/ASTM soil classification)



APPENDIX E: Geotechnical Properties of Natural Soils

Properties (Average)	**A** **A-7-6**	**A'** **Enzyme**	**B** **A-7-5**	**B'** **Enzyme**
Specific gravity of soil	2.79		2.75	
Natural moisture content (%)	16.9		21.6	
Atterberg Limits				
Liquid limit (%)	41	40.4	50.50	48.5
Plastic limit (%)	28.08	30.2	35.86	36.60
Plasticity index	12.92	10.2	14.64	11.90
% Passing No. 200 sieve	61.83		84.01	
Classification				
USCS	CL		CL	
AASHTO	A-7-6		A-7-5	

APPENDIX F: Properties of Urease Enzymes

Identification as it appears	**Urease Enzymes**
Hazardous Compounds	None
Boiling point	100^{o}C
Specific Gravity	2.70
Evaporation Rate	Same as water
Melting Point	Liquid
Solubility in water	Complete
Colour	Amber
Odour	Non-Obnoxious

APPENDIX G: Enzyme-diluted water dosage for A-7-6 soil

%	Compaction		CBR		UCS	
Enzyme	**Enzyme(g)**	**Water(g**	**Enzyme(g)**	**Water**	**Enzyme**	**Water**
0	0	678.9	0	756	0	10.1
5	33.9	644.9	37.8	718.2	0.51	9.59
10	67.9	611.0	75.6	680.4	1.01	9.09
15	101.8	577.1	113.4	642.6	1.50	8.60
20	135.8	543.1	151.2	604.8	2.02	8.08

APPENDIX H: Enzyme-diluted water dosage for A-7-5 soil

%	Compaction	Water(g)	CBR		UCS	
Enzyme	**Enzyme(g)**		**Enzyme(g)**	**Water**	**Enzyme**	**Water**
0	0	678.9	0	756	0	13.76
5	33.9	644.9	51.6	980.4	0.67	13.09
10	67.9	611.0	103.2	928.8	138	12.38
15	101.8	577.1	154.8	877.2	2.06	11.70
20	135.8	543.1	206.4	825.6	2.75	11.01

APPENDIX I: Compaction parameters of enzyme-stabilized soil

Sample	A-7-6		A-7-5	
Enzyme %	OMC%	MDD(Kg/m^3)	OMC (%)	MDD(Kg/m^3)
0	12.60	1910	17.20	1720
5	10.80	1950	17.00	1760
10	10.50	1907	15.00	1790
15	9.70	1908	14.80	1800
20	9.60	1990	14.50	1880

APPENDIX J: Variation California Bearing Ratio (Soaked) with Urease Enzymes

Sample Urease %	A-7-6 CBR	Cum. Incr. CBR %	A-7-5 CBR	Cum. Incr. CBR%
0	20.79	0	10.06	0
5	29.01	40	17.48	74
10	31.81	50	20.17	89
15	38.07	70	32.32	149
20	45.11	88	41.79	178

APPENDIX K: UCS of enzymes stabilized clay soil

Sample Urease %	A-7-6 UCS (kN/m^2)	Cum.% Incr. in UCS	A-7-5 UCS (kN/m^2	Cum% Incr. in UCS
0	232.30	0	370.04	0
5	349.40	50	463.50	25
10	357.58	52	529.60	39
15	372.27	56	553.97	44
20	407.73	66	576.64	48

APPENDIX L: Effect of liquid enzyme solutions on the swell percentage of the soils.

Sample	**V_d(ml)**	**V_e(ml)**	**FSI(%)**
A-7-6	23.5	11	113.6
A-7-5	16.5	9	83.3