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History and Science of Enzymes as Soil Stabilizers

Enzymes as soil stabilizers have been used to improve the strength of sub-grades due to low cost and relatively wide applicability compared to standard stabilizers. The use of enzymes as stabilizer has not been subjected to any technical development and is presently carried out using empirical guidelines based on previous experience. Therefore, it becomes an important priority to study and determine the effects of the enzymes on the strength of different soils prior to being used.

1.0 INTRODUCTION

In recent years, more attention has been given to the use of enzymes as soil stabilizers due to experimentation, development, low cost, and relatively wide applicability compared to standard stabilizers (hydrated lime, Portland cement, and bitumen) which require large amounts of stabilizers to stabilize soils (high costs). Although enzyme-based soil stabilizers appear to have many advantages compared to conventional chemical stabilizers, it is unclear to many in the Industry as to how these products work and under what conditions. It is very difficult to conduct Laboratory Tests that replicate real life conditions; therefore the process has been subjected to a rigorous in field testing instead. Technical investigation continues to be tested using empirical guidelines based on experience. It becomes important to perform a, Site Specific Soil Test, which can give objective support to the use of enzymes as a soil stabilizer. The main objective is to investigate the stabilization mechanism of some of the commercially available enzyme-based products to better understand their potential value for road construction. Limited laboratory experiments are performed to determine if these products improve the material properties of sub-grade soils and if they offer superior mechanical properties compared to other types of stabilization for which comprehensive laboratory and field performance already exists.

2.0 SOIL STABILIZATION

Solidification or Stabilization (S/S) is a treatment technology for contaminated soils, either for clean up or remediation alone or as part of a brown field redevelopment. Portland cement, often augmented with other materials, such as fly ash, lime kiln dust, cement kiln dust, and lime, is

used as a binding reagent in stabilization because of its ability to solidify, change the physical properties and stabilize the changes in the chemical properties of a wide range of hazardous materials. These types of stabilization materials are termed as soil stabilizers. Solidification increases the compressive strength, decreases the permeability, and encapsulates toxic elements. Stabilization converts hazardous elements into less soluble, mobile or toxic forms. Mixing the right combination of binding reagents into contaminated soils allows them to be either excavated and disposed of in a landfill, or re-used on site to support redevelopment. The solidification treatment has the further benefit of improving the structural properties of the site as whole.

2.1 USES

Stabilization is used to remediate or reclaim a contaminated site. Specific treatment effects include:

- λ Chemically binding free liquids in waste mater
- λ Reducing the permeability of waste matter
- λ Encapsulating waste particles
- λ Chemically fixing hazardous elements
- λ Helping reduce the toxicity of contaminants.

2.2 TYPES OF SOIL STABILIZERS

There are mainly two types of soil stabilizers, standard stabilizers and non-standard stabilizers. The non-standard stabilizers, when applied to the appropriate soil and aggregates using the right construction techniques, can produce dramatic improvement on these materials. These non-standard stabilizers are by-products of unrelated processes, modified specifically for use as stabilizers. Unlike the standard stabilizers such as Portland cement, lime and bitumen, these stabilizers have no laboratory tests that can be used to predict their field performance.

2.3 TERMS IN SOIL STABILIZATION WITH NON-STANDARD STABILIZERS

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

2.3.1 Soil Electrolyte Systems

Many sub-grades, aggregates and mixtures of crushed rock and soils are known to behave as electrolyte systems where ion exchanges occur within the material. Knowledge of the layered lattice structure of clay materials, and of colloid transport and osmotic pressure gradients is critical in understanding the behavior of these electrolytes soils. Most clay has a molecular

structure with a net negative charge. To maintain the electrical neutrality, cations (positively charged) are attracted to and held on the edges and surfaces of clay particles. These cations are called “exchangeable cations” because in most cases cations of one type may be exchanged with cations of another type. When the cation charge in the clay structure is weak, the remaining negative charge attracts polarized water molecules, filling the spaces of the clays structure with ionized water.

2.3.2 Osmotic Pressure Gradients

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

2.3.3 Colloid Activity

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

The physical phenomena are related to Brownian motion, laminar shear velocity and pore-size distribution. Brownian motion overcomes the effects of gravitational force and prevents deposition; the laminar shear velocity affects the rate of cation exchange with the clay structure; and the pore-size distribution determines the shear velocity and how close the clay lattice is to the passing colloids and cations. The electrochemical effects are related to the forces between positive and negative particles (Van der Waals forces), and to the repulsion forces between ions of the same charge. If a solution with cations is introduced into the clay structure, a micro environment is created in which the cations are prevented from dispersing by their adjacent clay lattice. If the soil is not completely saturated, the liquid phase will move in laminar flow through the soil pores by capillary forces, leaving the higher concentration of cations close to the surface.

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

2.4 MECHANISM OF NON-STANDARD STABILIZERS

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

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

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

Organic cations created by the growth of vegetation also have the capacity to exchange charges with other ions in the clay lattice. Some of the organic cations are huge in size equaling the size of the smaller clay particles. These larger organic cations can blanket an entire clay molecule, neutralizing its negative charges, and thus reducing its sensitivity to moisture. Soil bacteria make use of this process to stabilize their environment, producing enzymes that catalyze the reactions between clays and organic cations to produce stable soil.

The Non-Standard stabilizers can be classified in two groups: Chemical stabilizers and Pozzolan stabilizers. The chemical stabilizers are also subdivided into five groups: Sulfonated Oils, Ammonium Chloride, Enzymes, Mineral Pitches and Acrylic Polymers.

2.5 CHEMICAL STABILIZERS

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

The sulfonated naphthalene and D-limonene produce powerful hydrogen ions, which penetrate into the clay lattice, producing the breakdown of the structure and the further release of moisture resulting in a dense soil structure. The ammonium chloride produces NH_4^+ ions that adhere strongly to the edge of the clay, releasing the surface water and altering the surface structure to reduce capillarity.

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

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

2.6 POZZOLAN STABILIZERS

The pozzolans come from coal-burning power plants. These non-standard stabilizers differ from other chemical stabilizers because they are a waste or byproduct from other industrial processes and lack the quality control of chemical commercially produced stabilizers. One of the main products is lime. When lime is introduced into a soil with trapped moisture, it ionizes and produces calcium cations that can exchange with the clay lattice. The calcium cation exchanges with the sodium and potassium in the clay structure in the same way that the chemical stabilizers exchange ions. Because the calcium cation is large it cannot move far into the clay structure; adequate mixing is therefore required to obtain the benefits of this type of stabilization. The stronger ionization energy of calcium pulls together the structure of the clay, releasing the water in excess and breaking down the clay lattice.

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

3.0 ENZYMES AS A SOIL STABILIZER

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

An enzyme is by definition an organic catalyst that speeds up a chemical reaction, that otherwise would happen at a slower rate, without becoming a part of the end product. The enzyme combines with the large organic molecules to form a reactant intermediary, which exchanges ions with the clay structure, breaking down the lattice and causing the cover-up effect, which prevents further absorption of water and the loss of density. The enzyme is regenerated by the reaction and goes to react again. Because the ions are large, little osmotic migration takes place and a good mixing process is required. Compaction of aggregates near the optimum moisture content by construction equipment produces the desired high densities characteristic of shale. The resulting surface has the properties of durable “shale” produced in a fraction of the time (millions of years) required by nature.

The idea of using enzyme stabilization for roads was developed from the application of enzyme products used to treat soil in order to improve horticultural applications. A modification to the

process produced a material, which was suitable for stabilization of poor ground for road traffic. When added to a soil, the enzymes increase the wetting and bonding capacity of the soil particles. The enzyme allows soil materials to become more easily wet and more densely compacted. Also, it improves the chemical bonding that helps to fuse the soil particles together, creating a more permanent structure that is more resistant to weathering, wear and water penetration.

3.1 THE CONCEPT OF ENZYME STABILIZATION

Enzyme stabilization is commonly demonstrated by termites and ants in Latin America, Africa and Asia. Ant saliva, full of enzymes, is used to build soil structures, which are rock hard and meters high. These structures are known to stand firm despite heavy tropical rain seasons. For many years, road engineers have used additives such as lime, cement and cement kiln dust to improve the qualities of readily available local soils. Laboratory and field performance tests have confirmed that the addition of 6% to 10% of such additives can 'increase the strength and stability of such soils. However, the cost of introducing these additives has also increased in recent years. This has opened the door widely for the development and introduction of other kinds of soil additives including highly cost-effective liquid enzyme formulations.

Liquid enzyme soil stabilizers can significantly enhance the properties of the soil used in the construction of road infrastructure. Results include a better and longer lasting road with increased loading capacity (CBR) and reduced soil permeability. Stabilization with the right brand of liquid enzymes can lower a road's construction and maintenance costs while increasing the overall quality of its structure and surface.

The promise that soil stabilization technology can actually improve the mechanical qualities of local road soil so that stronger, more durable roads can be built has prompted multi-lateral banks and national road ministries around the world to conduct extensive testing to verify that this new technology is truly cost-effective. For example, a comprehensive two year World Bank study in Paraguay confirmed the benefits of soil stabilizers.

The result is that this new advance in "soil stabilization" technology is increasingly being used in both constructing and improving/rehabilitating subsurface and paved roads worldwide. It has been used with excellent and consistent results in more than 30 countries including the US and Canada, and countries in Latin America, Africa, and Asia. Scores of successful projects have been completed, including rehabilitation of nearly 300 kilometers in Honduras and almost 1,000 kilometers in Malaysia.

3.2 REVIEW OF PREVIOUS STUDIES ON ENZYME BASED SOIL STABILIZER

Wright-Fox (1993) carried out a study to assess the stabilization potential of enzymes. Standard soil tests were used for the study as no specific standards are available for enzyme-stabilized materials. Results from strength and index tests (e.g. liquid and plastic limit) conducted by Wright-Fox showed an increase in the unconfined compressive strength of the stabilized material as compared to control specimens. There was a 15% increase in the un-drained shear strength of the stabilized material. The soil used was silty clay with a liquid limit of 66% and

plasticity index of 42%. The index tests performed did not show any variation from the control specimen. Thus the enzymes might not offer waterproofing qualities using the recommended rate of application. Wright-Fox (1993) concluded that enzymes may provide some additional shear strength for some soils and that the soil stabilization with enzymes should be considered for various applications but only on a case-by-case basis.

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

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

3.3 DESCRIPTION OF INVESTIGATION

Based on the investigations done before, here is a brief description about the experiment methods, their results and improving properties of the soil using enzymes. Enzymes are natural organic compounds which act as catalysts. Their large molecular structures have active sites, which assist bonding and interactions. Enzymes are blended with a biodegradable surfactant to reduce the surface tension and promote enzymatic reactions, which has a wetting action that improves compaction, allowing higher dry densities to be achieved. The treatment with this product is permanent and the treated layer becomes impermeable.

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

Enzymes can be used to stabilize a wide variety of soils. The field testing shows the following advantages of using Enzymes for soil stabilization: low cost, easy application, wide applicability, and environmentally friendly. In addition, it results in a soil with a high resistance to frost heaving.

The Civil Engineering Research Foundation (CERF) funded by the Federal Highway Administration made an evaluation of the environmental impact of the use of Enzymes as a soil stabilizer. The study found that there are seven chemicals in the enzyme soil solution.

The chemical concentrations in soil were compared with Risk-Based Concentrations (RBC) in residential soil, which was developed by the Environmental Protection Agency (EPA) as a screen level for contaminants on a concerned site. It was found that the Enzymes did not increase risk-based concentrations (RBC) levels of soils and it was practically nontoxic in all the toxicological analyses.

Enzymes are a natural organic compound derived from crop-plant biomass. It is similar to

proteins and acts as a catalyst. The large molecular structures contain active sites that assist molecular bonding and interactions. Enzymes accelerate the cohesive bonding of soil particles and creates a tight permanent layer. Unlike inorganic or petroleum-based products that have a temporary action Enzymes create a dense and permanent base and sub-grade that resists water penetration, weathering and wear.

In normal road construction methods, compaction levels in the range of 90-95 percent are usually obtained, while with Enzymes compaction densities of up to 100-105 percent may be reached. Enzymes can be applied to most soils, which contain a minimum of eight to eleven percent of cohesive fines. The basic effects of the action of an Enzyme added into the structure of the soil can be summarized as follows. Initially, the film of absorbed water is greatly reduced and in fact entirely broken.

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

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

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

The hydrogen ions, which are liberated in the dissociation of the water molecules, can once again react with free hydroxyl ions and form water along the gaseous hydrogen. It is important to note that the moisture content of the soil affects the surface tension and is thus a factor affecting compaction. The enzyme reduces surface tension making the soil compaction easier to perform.

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

Some of the properties modified by the Enzymes stabilization process are listed below:

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

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

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

3.4 CASE STUDY

The enzyme products have been used in more than 40 countries in the construction of structures from rural roads to highways for the past 30 years. Based on our research in the overwhelming majority of the cases enzyme stabilization provided a tool that enhanced the life-cycle and quality of the resulting product. A short review of some of the projects where enzymes were used as a road stabilizer is presented below.

A World Bank study on soil stabilization using enzymes in Paraguay reported consistent road improvements and better performance from soil stabilizer treated roads compared to untreated roads. The conclusions were drawn based on data gathered on a large-scale study from multiple sites using commercial enzymes and documentation of road performance for up to 33 months. Stabilization with enzymes has been used in India. Good performance of these roads despite the heavy traffic and the high rainfall has been found. Besides an increase in the strength and durability of the roads, a reduction in project cost has also been achieved.

Enzymes have been used successfully to stabilize roads in Malaysia, China and the Western USA at low cost. In Mendocino County, California Department of Transportation has conducted

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

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

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

A number of projects have been completed in Panaji (India) with the use of enzymes. A rural road and a city road in Maharashtra have lasted for more than two years without any damage. Road sections placed in western Pennsylvania in the fall of 1992 passed subfreezing winters and over forty freeze-thaw cycles and required no maintenance for ruts, potholes or wash boarding during three years. The road sections then received chip-seal coats and asphalt surfaces with no requirement for repairs to the stabilized base. Enzymes have been used to stabilize more than 160 miles of sub-grades and road surfacing in sites located across the National Forest land of the United States Department of Agriculture, where intense rainfall, highly erosive aggregate surfacing and expansive clay are found. The performance of the test sections shows improvement over non stabilized control sections and historical performances of these sections before stabilization. Failures in the test sections have been related with the misuse of the enzymes, such as application over the wrong type of soil and gradation.

4.0 EXPERIMENTAL APPROACH

The seminar is not detailing the experimental approach of enzyme soil stabilizers, only discussing some examples from previous experiments. The use of enzymes as stabilizer has been subjected to many test in Laboratories without success as it is very difficult if not impossible to replicate field conditions. Technical development and Testing is presently carried out using empirical guidelines based on previous experience. Testing does not make it clear how and under what conditions these products work all we know it that it does.

4.1 CHEMICAL ANALYSIS

It is almost impossible with any level of confidence to determine the precise formula of the various Enzyme Stabilizers

4.1.1 Introduction

A chemical analysis of the stabilizing solutions is performed to obtain information relevant to understanding the stabilization process. The analysis includes determining the solution pH, the protein content (enzyme content), metals concentration, total organic carbon concentration and inorganic anion concentration.

The composition and activity of soil stabilizers were evaluated using both standard and innovative analytical techniques. The goal of these analyses was to determine how the soil stabilizers work (what is the mechanism of stabilization).

4.1.2 Experimental Methods

Full-strength sub-samples or diluted solutions of the soil stabilizers were used in the analyses. Dilutions were prepared using high-purity deionized (DI) water or tap water (for surface tension tests only) and the resulting solutions were analyzed for pH, metals concentrations (e.g., Ca, Fe, and Al), total organic carbon concentration, and inorganic anion concentrations (e.g., Cl⁻, NO₃⁻, SO₄²⁻).

4.1.3 Protein Content and Enzymatic Activity

The protein content (a measure of enzyme content) and enzymatic activity of the product were evaluated. Probe compounds were used to analyze for the presence of active aminopeptidase (protein degrading), lipase (lipid degrading), or glucosidase (sugar degrading) enzymes. The objectives of these analyses were to:

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

Standard methods used in chemical analysis

Analysis Method

Ph pH meter

Dissolved metals ICP-MS¹

Protein content Lowry method²

Inorganic anions

Ion chromatography³

¹ ICP-MS = inductively coupled plasma – mass spectrometry

² Lowry et al. (1951)

³ 761 Compact IC with 766 IC Autosampler, Metrohm-Peak, Houston, TX

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

4.1.4 Surface Tension

The surfactant-like behavior of product was assessed by measuring the surface tension of product solutions over a range of concentrations. Proteins are large macromolecules that resemble surfactants in chemical structure and behavior (e.g., protein solutions exhibit foaming when shaken). The surface tensions of the test solutions were measured with a tensiometer (Fisher Surface Tensiomat, Model 21, Fisher Scientific, and Pittsburgh, PA). The results from the analyses of the products solutions were compared with those obtained from the analysis of solutions of a common surfactant (sodium dodecyl sulfate or SDS).

4.1.5 Result

The pH was 4.77 while the pH of Base-1 was 11.34. Thus, the product is acidic and the Base 1 is basic. The concentrations of metals and common inorganic anions (Cl⁻ and SO₄²⁻) in the two soil stabilizers are provided in Table 3. and Table 4, respectively. The main conclusions from these data are that the product A has a very high concentration of potassium (K), and moderate-to-high concentrations of calcium (Ca), magnesium (Mg), and sodium (Na). These results seem to indicate that these metals do not play a significant role in the soil-stabilizing activity. On the other hand, the extremely high concentrations of Na and silicon (Si) in the Base-1 solution suggest that this product primarily contains sodium silicates. In the presence of sufficient calcium (Ca) and water, the silicates should form a calcium silicate hydrate or cement-like material similar to that formed in concrete.

Protein Concentration and Enzyme Activity

The protein concentration in the undiluted product A was 9230 mg/L. Proteins are biomolecules comprising of amino acids that may or may not exhibit enzymatic activity. Enzymatic activity would be indicated by the ability to catalyze a reaction, such as the breakdown of glucose. Thus, the presence of protein alone does not indicate that the solution will exhibit enzymatic activity. In the enzyme activity tests, the fluorescence readings of the product A test solutions were typically less than those in the negative controls, which suggests quenching of the fluorescence by substances in product A (data not shown). In any event, the presence of product A did not result in an increase in the slope of the fluorescence versus time curve for any of the substrates. Thus, it was concluded that the product A exhibited no detectable enzymatic activity for the aforementioned substrates.

The three substrates used in these experiments test for the activity of three major classes of enzymes. The inability of product A to catalyze the degradation of these compounds does not definitively preclude the presence of active enzymes in the samples as there are thousands of enzymes that catalyze the breakdown of virtually all organic compounds.

Nevertheless, the absence of enzymatic activity in these experiments is curious, and suggests that either:

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

Surface Tension

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

It is therefore hypothesized that the surfactant-like character of the product A may be responsible for its soil stabilizing performance, by enhancing the ability to compact the soil and remove water. More work is needed, including soil testing, to confirm this hypothesis. On the other hand, product B did not reduce the surface tension of water and no foam production during mixing was observed, therefore, product B does not behave like a surfactant.

4.1.6 Summary

Two soil stabilization products, product A and Base-1, were first tested to determine their chemical composition and mode of action. The product A contains a high concentration of protein, but did not appear to contain active enzymes based on standard enzymatic activity assays. The results from quantitative surface tension testing and qualitative observations on product A and on an additional product B made available later in the study suggest that product A behaves like a surfactant and product B does not behave like a surfactant; this behavior may play a role in its soil stabilization performance. Base-1, on the other hand, contains high concentrations of sodium and silicon, which suggests that it acts like cement by forming hydrated calcium silicate when added to soil.

4.2 MECHANICAL TESTING

4.2.1 Specimen Preparation

Laboratory compaction methods that reproduce the same effects as those produced by compaction equipment in the field are required for specimen preparation. Static compaction for clayey soils seems to poorly represent field compaction. Kneading compaction procedure instead represents a better way to reproduce the effects of field compaction (tamping feet). The “three kneading feet tool” was used as a laboratory compaction device for the specimen preparation. Dry densities of the samples are close to the in situ densities if the kneading compaction technique is used with five layers and a pressure of 1.25 MPa.

The three kneading feet tool was made of a wood disk (100 mm diameter) under which three

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

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

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

- First the soil was dried for 24 hrs at a temperature of 140°F.
- Then the soil was chopped into small pieces and pushed through the sieve No 4.
- The soil and the additive were mixed using the target density and optimum moisture content (enzyme is part of the water added to obtain 95% of the maximum dry).
- The mixture (or blend) was placed in five layers in the 4" mold for compaction using a static load frame.
- Each layer was compacted using the kneading compactor platen eight times to cover the surface of the sample. After compaction, the dry density of the specimens was calculated.

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

5.0 CONCLUSIONS AND RECOMENDATIONS

Based on many researches and experiments, some conclusions and recommendations were made by investigators. They are listed below;

1. The specimen preparation process showed that product reduced the compaction effort and improved soil workability. Thus, less pressure was used to obtain the target density of the treated specimens compare to the untreated specimens.
2. By the addition of enzymes in soils, it will increase the stiffness of soil to a average value of 69-77%.
3. The type of soil affected significantly the effectiveness of the treatments. The percent of fines and the chemical composition are properties that affect the stabilization mechanism. Therefore, special attention should be paid to select the proper treatment to be used for different soils.
4. Limited number of tests showed that at least four to six months of curing time are needed to observe an improvement on the shear strength of both soils.
5. Addition of enzyme in soil increases the shear strength of soil by a average value of 9% - 39%.

6. A better and longer lasting road with increased loading capacity (CBR) and reduced soil permeability.

The conclusions presented above refer to a limited number of soil and enzyme stabilizers combinations tested in laboratory conditions and should not be extrapolated to other combinations of materials. These results should be validated with field experiments that involve the same combination of materials used in this study.

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