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ChemTech

International Journal of ChemTech Research
CODEN (USA): IJCRGG ISSN : 0974-4290
Vol.6, No.14, pp 5733-5739, Nov-Dec 2014

Extraction and Biochemical Studies of Soil Binding Proteins from Termite Mound

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Abstract: In this report we look at issues related with existing technologies and techniques which are used to improve the soil stabilization. It is a known fact that all structures, roads deteriorate over time. Deterioration is primarily due to accumulated damage from vehicles or environmental effects such as frost heaves, thermal cracking and oxidation. These deterioration problems can only be overcome by strong stabilization of the soil.
In this Report we study about the stabilizing properties of the proteins obtained from the termite saliva. The stabilizing agents are extracted with a series of procedures and are mixed with the soil and studies are done on its soil binding properties. After a series of studies of the soil stabilizing agents on the soil it was found that the soil stabilizing agent itself is a Glycoprotein. The appropriate amount of the saturated stabilizing agent to be mixed with soil is estimated to be 75%. The comparative compression test of the soil mixed with water and soil binding agent was studied and it was found that the diluted sample of our soil binding agent in the ratio 1:4 gave 0.146 N/mm^2 suggesting that the sample when mixed with laterite soil and compacted can withstand far more stress when compared to laterite soil mixed with water.
Keywords: Terrazymes, Soil Binding Proteins, Confined compression test.

1. Introduction

A road is an identifiable thoroughfare, route, way or path between two places which may or may not be available for use by the public; public roads, especially major roads connecting significant destinations are termed highways. Erosion and sediment controls are constructed to prevent detrimental effects. [18].

Processes during road construction include excavation, removal of material to spoil, filling, compacting, construction and trimming. If rock or other unsuitable material is discovered it is removed, moisture content is managed and replaced with standard fill compacted to 90% relative compaction. When a depression must be filled to come up to the road grade the native bed is compacted after the topsoil has been removed. The fill is made by the "compacted layer method" where a layer of fill is spread then compacted to specifications; the process is repeated until the desired grade is reached [18].

Like all structures, roads deteriorate over time. According to a series of experiments carried out in the late 1950s, called the AASHO Road Test, it was empirically determined that the effective damage done to the road is roughly proportional to the 4th power of axle weight. Potholes on roads are caused by rain damage and vehicle braking or related construction works. Virtually all roads require some form of maintenance before they come to the end of their service life [11].

Using the environmental friendly enzymes indicates minimization, elimination of the use of aggregates and is referred to as Aggregate-Free Pavement Technology. Such materials can also be tried in the rural roads construction after proving their efficacy in the Indian conditions, through series of trial projects [13].

The termites are a group of eusocial insects usually classified at the taxonomic rank of order Isoptera. As eusocial insects, termites live in colonies that, at maturity, number from several hundred to several million individuals. Mounds (also known as "termitaria") occur when an above-ground nest grows beyond its initially concealing surface.

These mounds despite severe climatic condition can withstand and survive the test of time. It is this characteristic feature that has caught the eyes of many. No matter what the climatic conditions are, these structure stands erect and provides shelter to all its inmates.

If this feature of the mounds can be brought to the roads they will be able to survive for a longer period of time despite all the harsh climatic condition.This project has been undertaken in order to study the interactions of the binding agent in the soil and its properties.

2. Experimental

Extraction of soil binding samples from the termite mounds is a complicated process and it is a requirement to undergo a series of steps before the proteins are extracted. After the extraction of the soil binding samples from the termite mounds is done, characterization of the extracted sample is done. Various experiments are conducted to study the effect of the samples on soil and its impact on the compression strength of the soil. The steps involved in the extraction and characterization of the soil binding samples are mentioned below.

2.1 Extraction of Soil Binding Samples from Termite Mound

The following steps were followed to extract the soil binding samples from the termite mound: Initially the top end of the termite mound is to be broken and wait for a period of a 24 to 48 hours so that the termites could reconstruct the broken area. This step allows us to extract fresh samples from the termite mounds.

After a standby period of 24 to 48 hours the freshly formed mound is collected and the appropriate amounts of termite mound is weighed and dilute it with distilled water (pH 7.0) in the ratio 1:4 making it to 2 liters. Allow the mixture to sediment for a few days within a controlled environment.

Collect the supernatant without disturbing the sediment and Filter the supernatant using Whatman filter paper to remove the suspended residues.

After collecting the supernatants a series of experiments were conducted on the collected solution which contains a mixture of soil binding proteins.

2.2 Biochemical Characterization

Biochemical characterization studies were conducted with a series of experiments like Molisch's Test, Anthrone Test, Iodine Test, Barfoed's Test, Seliwanoff's Test, Fehling's Test, Benedict's Test, Bial's Test, Ninhydrin Test, Xanthoproteic Test and Biuret test to Identify if the extracted solution is a carbohydrate or Protein.

After the process of biochemical characterization is done. The next step involves precipitation and characterization of the sample solution.

2.3 Precipitation and Concentration of the Sample.

3.3.1 Ammonium Sulphate Precipitation: [12]

The first step of precipitation and concentration of the sample is ammonium sulphate precipitation followed by dialysis.

Ammonium sulphate precipitation is a method used to purify proteins by altering their solubility. It is a specific case of a more general technique known as salting out. Ammonium sulphate is commonly used as its solubility is so high that salt solutions with high ionic strength are allowed. The commonly used salt is ammonium sulphate, as it is very water soluble and has no adverse effects upon enzyme activity.

3.3.2 Dialysis: [7]

In working with proteins and nucleic acids, it is often necessary to eliminate small molecular weight substances such as reducing, non-reacted crosslinking or labeling reagents or preservatives that might interfere with a subsequent step in the experimental procedure. Similarly, it is often desirable to exchange the protein sample into a different buffer system for downstream application such as electrophoresis, ion exchange or affinity chromatography. Dialysis is one method for accomplishing both contaminant removal and buffer exchange for macromolecular samples such as proteins.

3.4 Quantitative Estimation of Proteins and Carbohydrates

3.4.1 Phenol Sulphuric Acid Assay for Total Sugars: [2]

It is sometimes necessary to quantify the amount of sugar in a certain medium. Whether the sugar is in the presence of various salts or protein residues, or attached to a polymer, the phenol-sulphuric acid assay can be performed. The amount of sugar present is determined by comparison with a calibration curve using a spectrophotometer.

The concentration carbohydrates in the given sample can be estimated by using the given formula:

Conc. of Unknown = [OD of Unknown X Conc. of Standard] / OD of Standard

3.4.2 Bradford's Colorimetric Assay For Protein [3]

The Bradford assay, a colorimetric protein assay, is based on an absorbance shift in the dye Coomassie when the previously red form Coomassie reagent changes and stabilizes into Coomassie blue by the binding of protein. Binding of the protein stabilizes the blue form of Coomassie dye, thus the amount of complex present in solution is a measure for the protein concentration by use of an absorbance reading.

The concentration proteins in the given sample can be estimated by using the given formula:

Conc. of Unknown = [OD of Unknown X Conc. of Standard] / OD of Standard

3.4.3 Dnsa Assay For Reducing Sugars [7]

On boiling with reducing sugars 3, 5 dinitrosalycylic acid (DNSA) reagent changes from yellow to red.

The concentration of reducing sugars in the given sample can be estimated by using the given formula:

Conc. of Unknown = [OD of Unknown X Conc. of Standard] / OD of Standard

3.5 Soil Binding Studies

3.5.1 Standard Proctor Compaction Test [1].

The Proctor compaction test determines the maximum density of a soil needed for a specific job site. The test first determines the maximum density achievable for the materials and uses this figure as a reference. Secondly, it tests the effects of moisture on soil density. The soil reference value is expressed as a percentage of density. These values are determined before any compaction takes place to develop the compaction specifications.

The bulk density 'γb' and the corresponding dry density 'γd' for the compacted soil are given by

$\gamma b = w/v$

$\gamma d = \gamma b / (1 + w)$

where, w = weight of compacted wet soil (gm), v = volume of mould (cm^3), w = water content (%)

3.5.2 Unconfined Compression Test [4].

The objective of the unconfined compression test is to determine the UU (unconsolidated, undrained) strength of a cohesive soil in an inexpensive manner. Fine-grained soil is tested in compression. Undisturbed

specimens cut from tube samples and disturbed specimens are loaded in compression, recording load and deflection measurements. The unconfined test uses axial loading without lateral confining pressures, making it the simplest and easiest laboratory method of estimating strength.

Equations for Unconfined strength:

$$q_u = \frac{P}{A}$$

q_u = unconfined compressive strength (psi)

P = compressive force (lbs)

A = cross sectional area (in^2)

Since soils tend to deform much more than concrete, the area of the specimen changes to maintain constant volume through the test (bulging). Thus, the average cross sectional area at a particular deformation during the test is calculated using:

$$A = \frac{A_o}{1-\varepsilon}$$

A = average cross sectional area (in^2)

A_o = initial cross sectional area (in^2)

ε = axial strain (in / in)

$$= \frac{\Delta L}{L_o}$$

ΔL = change in length (in)

L_o = initial length (in)

4 Results

A couple of biochemical characterization tests like Molisch's test, Anthrone Test, Fehlings test on carbohydrates showed positive result along with tests on proteins like Ninhydrin test, Biuret test also showed positive confirmation.

When tests on quantitative estimation of proteins and carbohydrates were done it showed that the sample at 75% saturation has the highest carbohydrate concentration. The sample also showed it has highest protein concentration at 75% saturation. It was also observed that the sample at 75% saturation has highest concentration of reducing sugars. [TABLE: 1,2,3]

Table 1 – Total Sugar Estimation Using Phenol Sulpuric Assay

Sl. No.	Sample (µl)	Distilled Water (µl)	Phenol Soln. (ml)	Conc. H_2SO_4 (ml)		OD 490 nm	Conc. Of Sugar (mg/ml)
T1 (25)	500	500	1.0	5.0	**INCUBATE AT ROOM TEMP. FOR 20 MINS**	0.24	0.80
T2 (50)	500	500	1.0	5.0		0.19	0.63
T3 (75)	500	500	1.0	5.0		0.28	0.93
T4(100)	500	500	1.0	5.0		0.26	0.87

Table 2 – Total Protein Estimation Using Bradford's Colorimetric Assay

Sl. No.	Sample (µl)	Distilled Water (µl)	Bradford's Reagent (Ml)		Distilled Water (Ml)	Od 620 Nm	Conc. Of Protein (Mg/Ml)
T1(25)	500	500	1.0	Incubate At Room Temp. For 10 Mins	5.0	0.24	1.60
T2(50)	500	500	1.0		5.0	0.30	2.00
T3(75)	500	500	1.0		5.0	0.53	3.53
T4(100)	500	500	1.0		5.0	0.36	2.40

Table 3 – Total Reducing Sugar Estimation by Dnsa Assay

Sl. No.	Sample (µl)	D / W (µl)	Incubate For 3 Mins At Room Temp.	Dnsa (Ml)	Keep In Boiling Water bath For 5 Mins	D / W (Ml)	Od 540 Nm	Conc. Of Reducing Sugar (Mg/Ml)
T1(25)	500	500		1.0		8.0	0.21	0.44
T2(50)	500	500		1.0		8.0	0.14	0.29
T3(75)	500	500		1.0		8.0	0.36	0.75
T4(100)	500	500		1.0		8.0	0.34	0.70

Table 4 – Standard Proctor Compaction Test for Soil and Water

Determination No.	1	2	3	4	5	6
Wt. Of Mould + Soil (Gm)	6440	6630	6690	6690	6650	6600
Wt. Of Mould (Gm)	4800	4800	4800	4800	4800	4800
Wt. Of Compacted Soil (Gm)	1640	1830	1890	1890	1850	1800
Bulk Density (Gm/Cm^3)	1.631	1.820	1.880	1.880	1.840	1.799
Dry Density (Gm/Cm^3)	1.431	1.596	1.649	1.649	1.614	1.578
Water Content (%)	8	11	14	17	20	23

Table 5 – Standard Proctor Compaction Test for Soil and Soil Binding Agent

Determination No.	1	2	3	4	5	6
Wt. Of Mould + Soil (Gm)	6380	6540	6620	6700	6700	6670
Wt. Of Mould (Gm)	4800	4800	4800	4800	4800	4800
Wt. Of Compacted Soil (Gm)	1580	1740	1820	1900	1900	1870
BULK DENSITY (Gm/Cm^3)	1.579	1.739	1.819	1.899	1.899	1.869
DRY DENSITY (Gm/Cm^3)	1.385	1.525	1.595	1.657	1.657	1.639
Sample Content (%)	8	11	14	17	20	23

On the completion of a standard proctor compaction test. It was observed that dry density is best at 14% moisture content in the case of water sample and similarly dry density is best at 17% moisture content with termite mound sample [TABLE 4,5]. The corresponding reading from the water content is further used in unconfined compression test (U.C.C) for finding out the stress and strength of the sample and control. The compression strength for soil with water was observed as 0.092 N/mm^2 and the compression test for soil with the extracted sample was observed as 0.146 N/mm^2. It is evident that the compressive strength is far more superior in the soil sample which is treated with the soil binding agent than that of the normally compacted soil with water.

5 Discussion

On comparison with results of U.C.C from literature of terrazyme it was found that terrazyme being highly purified gave results in the ranges of 0.237N/mm^2 to 0.246N/mm^2 on the contrary a diluted sample of our soil binding agent in the ratio 1:4 gave 0.146 N/mm^2 suggesting that; had our sample been as purified as terrazyme was, we would have got compressive strength results either as good as or better than that of terrazyme.

It was also found from literature that terrazyme increases the C.B.R of soil sub grade by more than 100%, impedes dust from loose materials from surface of soil, reduces the cost of construction by 15% to 20% and minimizes the material loss of gravel by erosion or abrasion of traffic.

As the U.C.C characteristics of our soil binding agent and terrazyme are almost similar we expect that our soil binding agent too should share these advantages.

6 Conclusion

It can be concluded from the research conducted that the sample taken from the termite mound is a glycoprotein. It is also clearly evident that the sample when mixed with laterite soil and compacted can withstand far more stress when compared to laterite soil mixed with water and compacted.

From Bradford's colorimetric assay for proteins and DNSA assay for reducing sugar, it was found that at 75% saturation of ammonium sulfate, protein concentration was 3.53mg/ml and for that of reducing sugar was 0.75mg/ml.

For soil binding studies using compaction test the maximum water holding capacity/dry density was found to be at 14% for water content and 17% for sample content for laterite soil.

At the end of U.C.C test there was an increase in compressive strength from 0.092N/mm^2 of soil with water to 0.146 N/mm^2 of soil with sample.

On examining the various literatures available on terrazyme, the glycoprotein isolated from the termite mound gave comparable results with that of terrazyme.

This justifies our research as to how well soil binding glycoprotein from termite mound can bind to soil and form a rigid surface. This sample if made in bulk can be used for making rural roads and road base that cannot be leached out easily over which a tarred surface can be made. A road made in such a manner lasts longer than roads that are made in the conventional manner. This is because the soil under the tar can last longer and cannot be easily eroded due to climatic factors.

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