INFLUENCE OF BACTERIA ON STRENGTH CHARACTERISTICS OF A SWELLING SOIL WITH CALCITE

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ABSTRACT

Studies on microorganisms in soils is necessary for understanding the behaviour of soils and mechanism developing for deciding the nature of the soil. The microorganisms may exist in soil or induced into the soil. These studies are referred to with different names based on the intended purpose. Soil Microbiology encompasses the study of microorganisms and their processes in soil. The interaction of organisms with each other and their environments involve Soil Ecology. Soil Biochemistry includes microbial processes, soil enzymes, and the formation and turnover of soil organic matter. Microorganisms/ Microbes are classified as Bacteria and Virus. Most of the Bacteria are useful to humankind and virus have provoking effects on the environment as well as on human beings. At present bacteria is used in several civil engineering applications as listed in recent research work in literature. From the Civil Engineering perspective, When the water content of some soils changes, problems arise in structures resting on swelling soils, mainly the strength of swelling soils decrease drastically. In the present paper chemical stabilization using bacteria is considered to improve the strength and reduce swelling behaviour by mixing different dosages of Bacteria to selected soil and waiting for different curing periods. The methodology is used for induction of microbes into selected soil and inspect the strength, based on the results of Unconfined Compressive Strength(UCS). The mechanism that occurred is analyzed using Scanning Electron Microscope (SEM) studies. Bacteria is isolated from vegetable peel waste and induced to the selected soil for finding variation in the strength of soil so as to ascertain the optimum dosage for bacteria induction and curing time for better strength improvement. The test results show an intensification of the strength of the soil as a result of the process of enzymatic Calcium Carbonates (CaCO_3) precipitation.

Keywords: Swelling soil, Microbes, Unconfined Compressive Strength (UCS), Scanning Electron Microscope (SEM), Dosages, Curing period, Isolation and Bacteria.

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1. INTRODUCTION

Construction industry necessitates, maintaining a balance between recital and expenditure, while at the same time satisfying environmental regulations, has become a challenge for building materials and contractors. Soil properties enhancement by mixing chemical and mechanical resources has been widely adopted, for improving the strength of the soil and its durability using a variety of chemical additives, both inorganic and organic types. The major benefit of chemical stabilization is that setting time and curing time can be controlled. Chemical stabilization is however generally more expensive than other types of stabilization. An enzyme is a substance produced by a living organism that acts as a catalyst to bring about a specific biochemical reaction. Soil enzymes are a group of enzymes whose usual inhabitants are the soil and are continuously playing an important role in maintaining soil ecology, physical and chemical properties, of soil. Soil structure depends on stable aggregates of soil particles. Soil Organisms play an important role in soil aggregation. Constituents of soil are Organic matter, Polysaccharides, Lignins. Gums/Enzymes synthesized by soil microbes play an important role in cementing of soil particles known as Microbial cementation (or Structural Microbial Grouting). The cementation is due to the formation of white precipitate rich in Calcium Carbonate(Calcite) known as Microbially Induced Calcite Precipitation(MICP). Soil particle-binding material is to form after the introduction of microbes and specific additives into the soil. It is different from bio-binding, which is the formation of the particle-binding cellular chains. The effectiveness of the Microbial Cementation soil improvement technique is determined by comparing the shear strength of the control and the treated soil specimens. The Calcite formed in the treated soil is further examined using a Scanning Electron Microscope (SEM).

2. MICROBIAL INDUCED CALCITE PRECIPITATION

In recent times, a reasonably clean and sustainable technique, called Microbial Induced Calcite Precipitation (MICP) has been introduced in the field of ground improvement. There are a number of references presenting further tests, for example, shear strength and triaxial drainage tests (Feng et al.,2016, Dejong et al., 2013). The applications of this method show the possibility in various fields like improvement of strength and durability of concrete, bricks, sand impermeability, soil strength etc., and various works are being conducted on these fields in the current circumstances. Chou et al. (2011, Dejong et al. (2006), Whiffin et al. (2007), and Van Paassen (2009).

3. MATERIALS AND METHODOLOGY

Since soil is complex in nature, testing of soils and mixing additives is necessary to enhance the soil properties, mostly strength. In this investigation, an attempt has been made to study the effect of microbial induction into the soil which comes under chemical stabilization. Swelling soil is selected and Bacteria is isolated from vegetable peel waste and sterilized. The sterilized solution is inoculated and incubated for 24 hours for developing the morphology colonies. This process is known as pure culturing. Pure Culturing stage is followed by subculturing stage based on the more extent spread bacteria colony which is selected and mixed with sterilized Nutribroth solution.

3.1. Soil

The soil used in this study was obtained from chennur, near Kadapa (K). The basic properties of the collected sample and classification are tabulated in table 1.

Table 1 Tested Soil Properties

S.No	TESTS	Value
		Soil (K)
1	Sieve Analysis	
	a.Gravel(%)	3.6
	b.Sand(%)	10.4
	c.Silt(%)	40.0
	d.Clay(%)	46.0
2	Liquid Limit(%)	58.0
3	Plastic Limit(%)	20.0
4	Plastic Index(%)	38.0
5	IS Classification of Soil	CH (Highly Compressible Clay)
6	Free Swell Index(%)	110.0
7	Differential Free Swell Index (%)	10
8	Swelling Pressure(kPa)	190
9	Specific Gravity	2.65
10	Compaction Characteristics	
	a.Optimum Moisture Content (OMC)(%)	17.0
	b.Maximum Dry Density (MDD) (kN/m ³)	15.5
9	Unconfined Compressive Strength (kPa)	233.0
	a.Cohesion (kPa)	116.50
10	CBR(%)	
	a. Unsoaked Condition	11.86
	b. Soaked Condition	6.27

3.2. Bacteria

Bacteria are extracted from vegetable peel waste. The complete support for the whole process of developing bacteria is taken from the Microbiology Department in S.V. UNIVERSITY, Tirupati.

3.1.1. Preparation of Bacteria Sample

A sample is taken from vegetable peel waste in the form of liquid. Aseptic technique is applied to prevent contamination during taking the sample.

3.2.2. Isolation of Microbes

The sample was mixed with 1L(one litre) pure water to make a solution sample. This solution sample was diluted up to 10^{-5} serial dilutions. Serial dilution is a process of diluting a sample

several times. The sterile Petri dish was labelled. Then 90ml of Phosphate Buffer was transferred into each of 5 test tubes using sterile pipettes (10ml) with aseptic technique. By using a sterile pipette (1ml), 1ml of water sample was transferred into a 10^{-1} tube and mixed the test tube properly. Dilution with the aseptic technique was continued for test 10^{-2} until 10^{-5} . After that, each of serial dilution was transferred into nutrient agar plate by using spread plate method. 0.1ml of an appropriately diluted culture was spread over the surface of agar using a sterile glass spreader. The plate was then incubated until the colonies appear. It is important that the surface of the plate be fairly dry so that the spread liquid soaks in.

3.2.3. Isolation Results

The isolation process is a procedure of isolation of the mixture of colonies to a single colony. This process was done by using the streaking method to obtain pure cultures. The soil sample was added with one Litre pure water to obtain a solution sample before transferred into a nutrient agar plate. It is important that the numbers of colonies developing on the plates are not being too large. On crowded plates, some cells may not form colonies, and some colonies may fuse, leading to erroneous measurements. So, to obtain the appropriate colony number, the samples need to be diluted. This solution samples were diluted up to 10^{-5} . By using the spread plate method, the diluted samples were transferred into a nutrient agar plate and the bacteria were grown on it. This bacteria was grown used for Nutri-both media. From the observation, these samples take about three to four days for growth on the plate.

3.2.4. Preparation of Sample

Initially, the specimens were prepared by taking dry soil with varying percentages of dosages with respect to the weight of soil taken. Bacteria and cementation reagents (3g of Nutri-both and 15g of Calcium Lactate) were added to the water content and mixed to the soil and mixed it thoroughly. The soil was compacted as layer by layer in a split mould having a diameter of 38mm and height of 76mm. Soil specimens were removed from the split mould and then placed on Unconfined Compressive testing machine platform and the test was conducted as per IS:2720 - (part-10) -1995. The dosage amount of bacteria and cementation reagent were added to soil and were ensured for the proper distribution of bacteria in soil. In order to develop Calcite Precipitation in bacteria added to soil to the treated samples, the curing period was allowed for 0, 3, and 7 days to provide sufficient waiting period for chemicals to react and further allow Calcite precipitates to develop. The treated samples were kept under room temperature covered with moist gunny bags.

4. RESULTS AND DISCUSSIONS

Bio-cementation enhances of improving the soil particle binding, as the results of induction bacteria and cementation reagents into the soil. The soil cementation materials are mostly carbonates, silicates, phosphates, sulphates and hydroxides. Calcium Lactate is an attractive element to be in bio-cementation because Calcium Lactate formation is commonly found in nature. Cementation reagents are a very important component for MICP Process. Generally, the growth of isolated bacteria is observed with the addition of growth-promoting or favouring agents called Nutri-broth. This is mixed with distilled water in definite proportions i.e., 13gm to prepare 1000ml solution. But, the additional cementation reagents consist of 3g of Nutria-broth and 15g of Calcium Lactate in water. Cementation reagents concentration of 0.5M was used throughout the MICP process, it was found to be the most suitable concentration for efficient bio-cementations of soils. (Soon et al.,2014, Sharama& Ramakrishna ., 2016, Umar et al., 2017).The treatment with cementation fluids only was used as an indirect indicator for the existence of naturally inhibited Calcium Lactate forming bacteria in the soil specimens. Different control specimens are tested for finding strength improvement. The maximum

strength developed at particular dosage for different curing periods is analyzed based on UCC tests and that control conditions are used for further studies at different dosages or concentrations.

4.1. Effect of Bacteria Induction on Strength of Tested Soils

Bio-cementation improves the shear strength of soil during the fabrication of soil particle-binding materials, as the result of introducing bacteria and cementation reagents into the soil. Calcium Lactate (Calcite) is an attractive element to be studied in bio-cementation because Calcite formation is commonly found in nature. Here isolated bacteria is developed using Nutribroth and Calcium Lactate, then the inoculation is done and incubated for 24 hrs, finally bacteria is formed. Besides the soil samples were induced with bacteria that is develop from above process and cementation fluids, another two sets of control specimens were also prepared, i.e. untreated specimens, and specimens treated with cementation fluids only. The maximum strength developed at particular dosage for different curing periods is analyzed based on Unconfined Compressive Strength (UCS) tests and that control conditions are used for further studies at different concentrations of bacteria (T_1 , T_2 , T_3 , and T_4).

4.2.1. Effect of bacterial growth on UCS

The soil specimens that underwent different bacteria treatment conditions were tested for their shear strength. The shear strength was obtained by performing the Unconfined Compression Test on 38 mm diameter and 76 mm height saturated specimen. It is recommended to select a Calcite forming bacteria that exist most favorable in soil temperature. The supply of nutrient into soil specimen during soil treatment process is essential. The tests are conducted in different dosages and different curing periods.

Figure 1 Variation of UCS with curing period (days) at different dosage induction of isolated for different bacteria

Figure 2. Variation of UCS with curing period (days) at different dosage induction of isolated for different bacteria mixed with Nutri-both.

Figure 3 Variation of UCS with curing period (days) at different dosage induction of isolated for different bacteria mixed with Nutri-both and Calcite.

The variation of Unconfined Compressive strength (UCS) with D₁, D₂, D₃ dosages for different curing periods 0, 3, 7 days was observed from the graphs that the strength of soil increases with the addition of dosages. Maximum strength is observed for T₃ bacteria with D₂ dosage when compared to remaining treatments. An increase in soil UCS treated with MICP to a cumulative value of 470 kPa (Putra et al., 2016). An increase in UCS from 150 to 198 kPa was also reported compared with values in the range of 119–137 kPa for the control specimen (Dove et al., 2011).

5. EFFECT OF BACTERIA ON SOIL COMPOSITION

The maximum Unconfined Compressive Strength (UCS) for Bacteria and Cementation reagents mixed with highly compressible soil with respect to curing period (days) is considered for soil composition studies.

5.1. Effect on Carbonates

Maximum Unconfined Compressive Strength (UCS) value was obtained for soil chemical with 0.2 ml of T₃ bacteria for 7 days curing. This may be due to an increase in carbonate's percentages reading to cementation and bonding nature of the admixed soil when compared to original soil. The Soil Composition of selected soil treated with bacteria T₃ presented. Carbonate ions are produced during MICP processes, and Calcite in the presence of excess dissolved calcium ions,

The crystals have been precipitated. These crystals form a link between them. The grains of the soil, which ultimately increases the strength and soil stiffness. This process is called bio-cementation (Weaver et al., 2011).

5.2. Effect on Sulphates

The maximum UCS of bacteria admixed highly compressible soil with respect to the curing period is considered for soil composition studies. It can be observed that the sulphates (%/ mass) increased with the curing period when compared to the original soil. The increment in Sulphates may be due to the increase in the pH value of the alkaline environment.

Effect of Chlorides on treated soil

The chlorides have increased for 7 days of curing. The utilized chlorides can increase the flocculation structure of soil and also increase the carbonates which give the cementation and strength to the soil, which exhibit a slow rate of mineral precipitation, microbial metabolic activities often contribute to selective cementation making the deposition process much faster. Cement soil materials are usually carbonates, silicates, Phosphates, sulfides, and hydroxides (Ivanov et al., 2008).

Effect on pH

The pH of the soil can influence its behaviour inducing acidic or alkaline nature. The soil generally becomes more flocculated with an increase in pH. Further Increase in pH can increase the Cation Exchange Capacity of soil which in turn can alter the properties. The pH values of maximum strength (UCS) attained soil i.e dosage (D₃) of T₃ Bacteria admixed highly compressible soil is tested at different curing periods (days). The pH Bacteria admixed highly compressible soil with 7 days curing period. The pH value shows the alkaline nature of the soil increases as a result the strength of soil, increases due to the increase in the cementation environment. Calcite precipitation is affected by pH because urease is only activated at a pH-specific point where urea hydrolysis occurs. MICP is performed at a pH of between 7.0 and 9.5 (Dupraz et al., 2009).

Table 2 Soil Composition of highly compressible clay treated with T₃bacteria

Soil Composition	Dosages(ml)		
	D ₁	D ₂	D ₃
CaCO ₃ (%)	29.23	28.75	27.15
Sulphates (%/mass)	1.39	1.45	1.73
Chlorides (mg/L)	22.48	17.35	18.76
pH	9.01	9.1	9.14

6. SCANNING ELECTRON MICROSCOPE ANALYSIS

The fabric changes are inferred by Scanning Electron Microscope (SEM) studies. Examining the morphology of the expansive soil and soils with stabilizer, SEM demonstrated the MICP-based mechanism that leads to the stabilization of the soil (Li.M., et al., 2018). Often one of the problems encountered in microbial stabilization of soils is that the cementation and carbonate precipitation concern. It is found that from the Unconfined Compressive Strength point of view, the induced Bacteria in soil with T₃ concentration for 7 days curing period gave a maximum improvement in strength. In order to understand the SEM studies on untreated and treated soil at the particular dosage and curing periods, are a mechanism in strength improvement performed. SEM-EDX analysis provided direct and comprehensive information about the MICP process and change of surface compositions in various expansive soil specimens(Li.M., et al., 2018).

Figure 4 Scanning Electron micrograph of Highly Compressible Soil without bacteria induction.

Figure 5 Scanning Electron Micrograph with D₁ dosage of bacteria induction for 7 days curing .

Figure 6 Scanning Electron Micrograph with D₂ dosage of bacteria induction for 7 days curing

Figure 7 Scanning Electron Micrograph with D₃ dosage of bacteria induction for 7 days curing.

The formations of Calcite crystal were found in abundance for the specimen with bacteria and cementation. On closer observations, rod-shaped bacteria were found in near contact with calcite crystals are shown Figs from 5.2 to 5.4. The micrographs illustrate the soil particles with an intertwining bunch with regular aggregation due to the flocculation of clay particles.

7. CONCLUSIONS

From the investigation results, it can be inferred that the induction of bacteria growth at D₂ dosage for T₃ bacteria concentration with 7 days curing period is better for Bio-Cementation which indirectly improves the strength of soil when compared T₁ and T₃ bacteria treatments. Bacterial growth in the soil for strength improvement is most economical and time saving compared to other admixtures. Bacterial growth is non-pathogenic, aerobic, alkaline in nature can be grown at room temperatures, and also eco-friendly nature and it is not harmful to human health. The micrograph illustrates the cementitious particles are rod-shaped flaked structure with Calcite precipitation, thereby contributing to improvement in strength due to cementation or aggregation.

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