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AN EXPERIMENTAL STUDY ON STABILIZING COHESIVE SOIL USING MICROBIALLY INDUCED CALCIUM CARBONATE PRECIPITATION

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Abstract - Bio-cementation is an environmentally friendly procedure grounded on microbial induced carbonate precipitation mechanism, occurring in environments abundant in calcium. In the effort to bolster soil stability, traditional urea is combined with bacteria to enhance the mechanical properties of soil. The research delves into the microbial mechanisms involved in microbially induced calcite precipitation, highlighting the role of urease-producing bacteria in facilitating calcium carbonate precipitation within the soil structure. This also entails pivotal laboratory and field experiments, evaluating the efficacy of microbially induced calcium carbonate in improving soil engineering characteristics, such as strength, permeability, and durability. Furthermore, this process can be employed across various soil types, offering adaptability in geotechnical applications. Additionally, we investigate the environmental consequences and sustainability aspects by underscoring its potential as an eco-friendly substitute for soil enhancement. Nonetheless, challenges such as optimizing bacterial activity, regulating the precipitation process, and ensuring even distribution of calcium carbonate within the soil necessitate further exploration. Based on the envisioned review, it contributes to the comprehension of MICP as a promising method for soil stabilization, providing insights for researchers, practitioners, and policymakers keen on sustainable and inventive approaches to geotechnical engineering.

Key Words: Bio-geotechnical engineering, soil stabilization, Microbially induced calcium carbonate Precipitation, Soil improvement.

1.INTRODUCTION

Microbially Induced Calcium Carbonate Precipitation (MICP) has emerged as a promising technique for soil stabilization, offering a sustainable and environmentally friendly approach to address soil instability issues. MICP involves the precipitation of calcium carbonate within the soil matrix through the metabolic activities of urease-producing bacteria. Among the various urease enzymes, Jack Bean Urease stands out as a prominent catalyst for MICP due to its effectiveness in catalyzing the hydrolysis of urea to carbonate ions and ammonia. When combined with suitable bacterial strains such as E. coli, Jack Bean Urease can facilitate the formation of calcium carbonate, thereby enhancing the mechanical properties of cohesive soil.

This comprehensive review aims to provide a thorough examination of the utilization of MICP catalyzed by Jack Bean Urease for stabilizing cohesive soil, particularly focusing on the integration of E. coli as the bacterial agent. The review will encompass a wide range of aspects related to this innovative technique, including the underlying principles of MICP, the role of urease enzymes in calcium carbonate precipitation, the selection and optimization of bacterial strains, laboratory and field experiments evaluating soil stabilization effectiveness, environmental implications, challenges, and future directions.

MICP improves all engineering properties in soil which is helpful in geotechnical, concrete remediation, heavy metal solidification and etc, which also provides a potentiality to induce soil stabilization in cohesive soil that reduces the voids by precipitation, which enhance the bearing capacity and resisting liquification. By which, the MICP utilizes bacteria to hydroyze urea to give CO32- to produce calcite which binds and increase the mechanical properties of cohesive soil and also to avoid ground settlement, CBR test is conducted to analyze strength and also direct shear test is conducted to resist the shearing of soil. To analyze the maximum dry density and optimum moisture content, standard proctor compaction test has been conducted and also SEM and XRD are used for quantities analysis of crystalline calcium carbonate precipitation.

The discussion will begin by elucidating the fundamental mechanisms of MICP, emphasizing the biochemical reactions involved in the microbial-induced precipitation of calcium carbonate. Special attention will be given to the catalytic activity of Jack Bean Urease, exploring its kinetic properties and substrate specificity in the hydrolysis of urea. The review will also delve into the genetic engineering approaches employed to enhance the urease production in bacterial strains like E. coli, thereby maximizing the efficiency of calcium carbonate precipitation.

Furthermore, the review will present a comprehensive analysis of laboratory and field experiments conducted to assess the efficacy of MICP in stabilizing cohesive soil using E. coli as the microbial agent. These experiments

will encompass various soil types, ranging from clayey to silty soils, to provide insights into the versatility and applicability of MICP across different soil conditions. Key parameters such as soil strength, permeability, and durability will be evaluated to quantify the improvements achieved through MICP-mediated soil stabilization.

In addition to engineering properties, the review will address the environmental implications of MICP, emphasizing its potential as an eco-friendly alternative to conventional soil stabilization methods. By utilizing naturally occurring urease-producing bacteria and biocompatible substrates such as urea and calcium chloride, MICP minimizes the environmental footprint associated with soil remediation activities. Moreover, the review will discuss the potential for carbon sequestration through the incorporation of calcium carbonate into the soil matrix, contributing to climate change mitigation efforts.

The stabilization is performed by mechanically mixing the natural soil and stabilizing material together so as to achieve a homogeneous mixture or by adding stabilizing material to an undisturbed soil deposit and obtaining interaction by letting it permeate through soil voids. By using these stabilizing agents can improve and maintain soil moisture content, increase soil particle cohesion and serve as cementing and water proofing agents (Perloff, 1976; Chen, 1981; Afrin, 2017).

Despite its promising potential, MICP faces several challenges that warrant further investigation and optimization. These challenges include the need to optimize bacterial activity, control the precipitation process, ensure uniform distribution of calcium carbonate within the soil, and address cost considerations associated with microbial cultivation and substrate supply. The review will critically evaluate current research endeavors aimed at overcoming these challenges, highlighting ongoing efforts to streamline the MICP process and make it economically viable for widespread adoption in geotechnical engineering applications.

The methods are needed that could offer enhanced soil stability, without the problems current approaches face. The MICP offers an alternative soil stabilization approach and, if applied properly, can be a cost efficient, long term, and a relatively environmental friendly approach (Ivanov and Chu, 2008; Anbu et al., 2016). The MICP typically involves the use of common soil borne microbes to promote calcite precipitation; calcite in return acts as the cementing agent for loose soils (Dhami et al., 2013; Cheng and Shahin, 2019).

In conclusion, MICP catalyzed by Jack Bean Urease represents a novel and effective approach for stabilizing cohesive soil, with E. coli serving as a promising microbial agent. This review aims to consolidate existing knowledge and insights into the utilization of MICP for soil stabilization, providing researchers, practitioners, and policymakers with a comprehensive understanding of this innovative technique's potential, challenges, and future directions. Through interdisciplinary collaboration and continued research efforts, MICP holds the promise of revolutionizing soil stabilization practices, paving the way for sustainable and resilient infrastructure development.

2. MATERIALS

2.1 SOIL ATTRIBUTES

A mixture of minerals, organic matter, gasses, liquids, and innumerable species that collectively support life on Earth is referred to as soil. It is constantly developing due to a variety of physical, chemical, and biological activities, such as evaporation, which raises the soil's plastic limit. The ideal characteristics aren't always present in the soil. Because of this, in order to acquire the required qualities, soil intervention is required (Afrin, 2017).

Numerous techniques are used to improve the geotechnical qualities of soils. One of the most crucial problems in geotechnical engineering procedures is soil stability. Because of its advantages, including affordability, workability, and safety, cement-based materials have been extensively employed for a variety of soil restoration techniques (Achal et al., 2010; Choi et al., 2016). Soil stabilization is the process of enhancing a soil's engineering qualities. According to Andavan and Kumar (2020), soil stabilization results in a decrease in permeability and compressibility as well as an increase in shear strength.

It is a well-established fact that stabilization is not a magic bullet that can enhance every aspect of soil properties. The choice of which soil qualities need to be changed determines how technology is used. The stabilization process combines a number of techniques used to alter a soil's characteristics in order to enhance its engineering performance.

In order to establish a homogenous combination, the stabilizing material and natural soil are mechanically mixed. Alternatively, the stabilizing material is added to an undisturbed soil deposit and interaction is obtained by allowing it to infiltrate through soil voids. These stabilizing compounds can be used as cementing and water-proofing agents, to maintain and raise soil moisture content, and to increase soil particle cohesion (Perloff, 1976; Chen, 1981; Afrin, 2017).

Several techniques are used in the application of soil stabilization, including grouting, in-situ densification, precompression, vertical drains, reinforcement, and admixture stabilization. Every one of these techniques works well with a particular kind of soil when applied to the right kind of soil. To successfully stabilize soil in geotechnical applications, it is crucial to use the appropriate stabilization technique for the kind of soil.

It is necessary to find techniques that could improve soil stability without having the drawbacks of the current strategies. When implemented appropriately, the MICP can be a long-term, reasonably priced, and ecologically benign alternative to traditional soil stabilization techniques (Ivanov and Chu, 2008; Anbu et al., 2016). In order to facilitate calcite precipitation,

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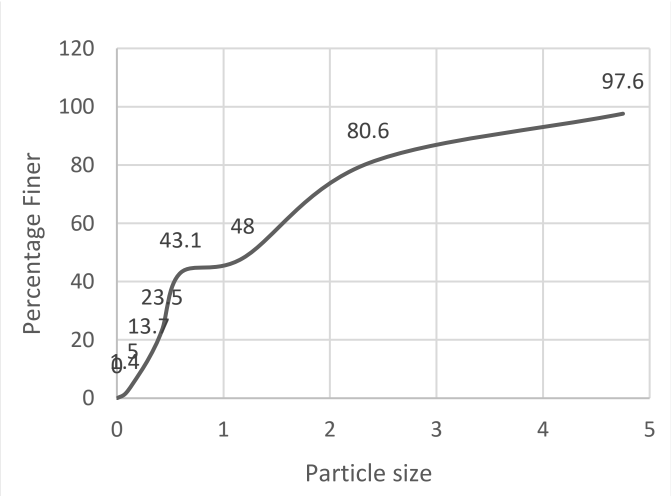
common soil-borne microorganisms are usually used in the MICP; calcite then serves as a cementing agent for loose soils (Dhami et al., 2013; Cheng and Shahin, 2019).

The ultimate product of the MICP is comparable to that of geochemical calcite precipitation, as both processes use calcite as the cementation agent. But because it uses less mechanical energy and artificial materials in its application, the MICP is more cost-effective and energy-efficient (DeJong et al., 2010; Mujah et al., 2016; Saneiyan et al., 2018).

Table -1: Particle size distribution of cohesive soil

No. of Particles	Particle Sieve Size	Percentage Finer
1	0.075	1.4
2	0.15	5
3	0.3	13.7
4	0.425	23.5
5	0.6	43.1
6	1.18	48
7	2.36	80.6
8	4.75	97.6

Chart 1: Graphical representation of Particle size



2.2 ESCHERICHIA COLI

Escherichia coli is a key player in the process of Microbially Induced Calcium Carbonate Precipitation (MICP). This nonpathogenic bacterium has a special characteristic that allows it to live in subterranean environments: it can make urease enzymes. E. coli breaks down urea during MICP, releasing carbonate and ammonium ions in the process. Solid calcium carbonate (CaCO3) crystals are created when these carbonate ions combine with calcium ions in the soil solution. These crystals improve the qualities of soil by acting as a natural "bio cement." Temperature has a major impact on MICP efficiency, according to research. Although bacterial ureolytic activity is initially increased by higher temperatures, it decreases more quickly than it does at lower temperatures. Better treatment outcomes are produced by the longer retention period of bacterial activity at lower temperatures.

2.3 PREPARATION OF BACTERIAL SOLUTION

Commercial nutrition powder is dissolved in distilled water to create the nutrient broth. After that, an autoclave was used to sterilize the solution in order to remove any impurities. The soup was sterilized and then allowed to cool to room temperature in order to use it as a growth medium for Escherichia coli cultures. The autoclave method was used to cultivate Escherichia coli (E. coli) in nutritional broth, guaranteeing sterility and ideal growth conditions. After being incubated for 48 hours, the bacterial culture attained the desired density. Four distinct variations of the cultured E. coli were now added to soil samples for comparative study. The purpose of this experiment is to look at how E. coli affects soil dynamics and possible uses in environmental or agricultural contexts.

2.4 CRUDE UREASE EXTRACTION FROM JACK BEANS

We bought dry jack beans (Canavalia gladiata) from the neighbourhood store on a business basis. To create a powder with particles smaller than 0.15 mm, the jack beans were ground in a kitchen blender and sieved. Before being used, the jack bean powder was kept in a refrigerator at 4°C. A magnetic stirrer was used to combine the jack bean powder with the deionized water at a specific dosage, creating a homogenous suspension of jack bean urease solutions. For a whole day, the suspension was kept in a refrigerator set at 4°C. After that, the suspension was centrifuged for 15 minutes at 4°C and 3500 rpm.

2.5 UREASE ACTIVITY TEST

It was looked into how the concentration of jack beans affected the urease activity. The electrical conductivity (EC) method, as used by Whiff net al. (2007), was used to measure the activity. The increase in electrical conductivity was brought about by the hydrolysis of urea with ammonium and carbonate. 3 mL of crude urease were added to 27 mL of testing solution containing 1.11 mol/L of urea in order to prepare the test sample. When testing at a standard concentration of 1 mol/L at 20°C and pH 7 is maintained, the activity, or the rate at which urea hydrolyses, is equal to the increasing rate of 1 mS/cm/min of EC. For five minutes, the relative electrical conductivity change at mS/cm/min was recorded. The real activity values should account for the 10-fold dilution factor, since the urea testing solution had to be added in 27 mL to 3 mL of urease liquid in order to measure the activity. For the experiment, crude urease liquid samples were generated for the activity testing with additions of 10g/L, 20g/L, 25g/L, 35g/L, 40g/L, 50g/L, 70g/L, 90g/L, and 100g/L of jack bean powder.

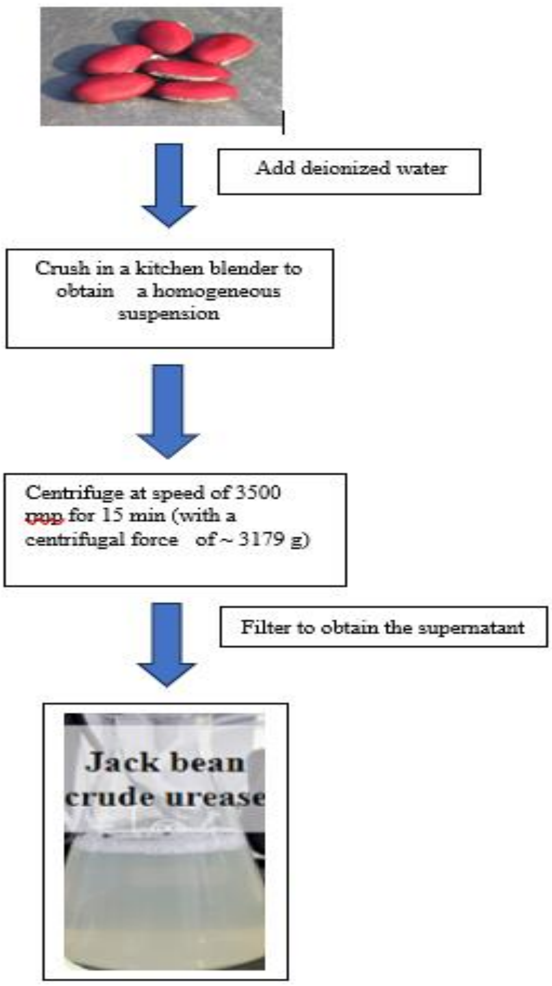
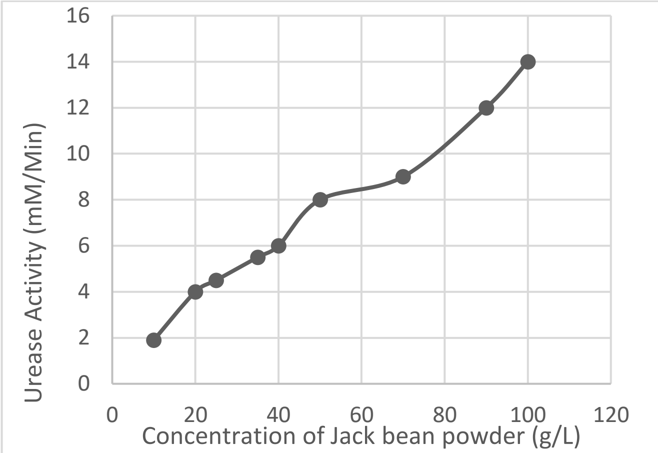


Fig -1: Schematical for preparing jack bean crude ureases

Table -2: Urease Activity

Case No.	Dosage(g/L)	Urease Activity(mM/Min)
1	10	1.9
2	20	4
3	25	4.5
4	35	5.5
5	40	6
6	50	8
7	70	9
8	90	12
9	100	14

Chart 2: Graphical representation of Urease Activity



2.6 TEST FOR UREASE REACTION

Under batch settings, the reaction of the urease-catalysed calcium carbonate precipitation by microbial induction was studied. For comparison, ureolytic bacteria (Cyanobacteria) were also employed in certain experiments. A test sample was prepared by mixing 60 mL of urea-calcium chloride solution with 20 mL of crude urease/bacterial liquid. The use of intact or crude urease, the activity of the urease/bacteria, and the urea and calcium chloride concentrations were among the testing variables. The tasks had to do with how much bacteria or jack beans were used. The concentrations of calcium and ammonium were monitored during the testing. Samples of liquid were collected at specific intervals. Nessler's reagent was used to quantify the ammonium concentration, and the EDTA titrimetric method (IOS, 1984) was used to determine the calcium concentration.

2.7 MICP MECHANISM

A bio-geochemical process called the MICP technique causes calcium carbonate to precipitate out of the soil matrix. The induced mineral precipitation in the soil stabilization process via the MICP mechanism binds the sand grains together at the particle-particle contacts, increasing the soil's strength and stiffness (Mortensen et al., 2011). By using this technique, urea is broken down by bacteria into carbonate and ammonium ions. Sand grains can be cemented by precipitated calcium carbonate, which is created when carbonate ions (CO32-) mix with calcium ions (Ca2+). According to several studies (Choi et al., 2017; Harkes et al., 2010; Inagaki et al., 2011; Montoya et al., 2013; Choi et al., 2020), the mineralogy of precipitated calcium carbonate primarily reveals calcite.

It is a well-known fact that the MICP develops spontaneously as a result of the bio-mineralization process, in which soil microorganisms cause calcium carbonate to precipitate. The MICP mechanism results from bacterial production of urease enzyme and the hydrolysis of urea (Boquet et al., 1973; Stocks-Fischer et al., 1999; DeJong et al., 2010; Siddique and Chahal, 2011; Al-Salloum et al., 2017; Wang et al., 2017; Choi et al., 2020). Therefore, bacteria that are capable of producing urease enzyme can be employed for MICP.

The urease enzyme is created by the bacteria, which then causes urea to hydrolyze and break down into carbon dioxide (CO2) and ammonia (NH3) (Güllüce, 2019). Local pH rises as a result of the hydroxide ions (OH-) and ammonium ions

(NH4+) produced when ammonia (NH3) dissolves in water (Eq. 2). Bicarbonate ions (HCO3) and hydrogen ions (H+) are produced when carbon dioxide (CO2) dissolves in water (Eq. 3). In the high pH environment, this bicarbonate (HCO3) combines with the hydroxyl ions (OH) to form the carbonate ions (CO^2) (Eq. 4). Because of its poor solubility in water, calcium carbonate (CaCO3) is consequently generated in the presence of calcium ions (Ca2+) and quickly precipitates out (Eq. 5) (Choi et al., 2020).

CO(NH2)2 + H2O → 2NH3 + CO2 (1)

2NH3 + 2H2O → 2NH4+ 2OH- (2)

CO2 + H2O → HCO3- + H+ (3)

HCO3- + H+ + 2OH- ↔ CO32- + 2H2O (4)

Ca2+ + H+ CaCO3 (S) ↓ (5)

There are three different anhydrous polymorphs of calcium carbonate minerals in nature, and calcite is the most stable of them all. According to several studies (de Leeuw and Parker, 1998; DeJong et al., 2010; Rodriguez Navarro et al., 2012; Shahrokhi-Shahraki et al., 2013; Choi et al., 2020), calcite makes up the majority of the calcium carbonate minerals formed by the MICP process. The procedure by which bacteria consume urea and the MICP occurs is depicted in the previous equations. An illustration showing how urease-positive microorganisms cause calcite formation and precipitation when they are in the presence of water, urea, and calcium chloride.

3. RESULT AND DISCUSSION

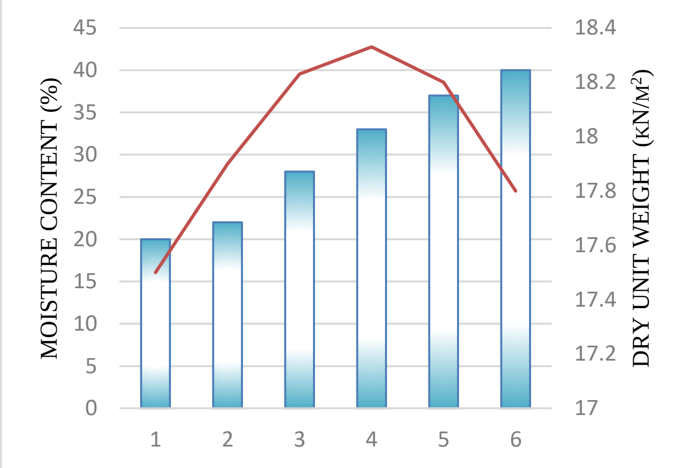
3.1 STANDARD PROCTOR COMPACTION TEST:

The result of optimum moisture content of microbially treated soil sample is increased by 20% to 31% and the dry unit weight is shown in (Chart.3). By this result the variant JCUE880 and JCUE800 had high efficiency in maximum dry density and OMC. From the results obtained, the bacterial solution was above 6% and JCU were minimum of of 80g/L.

Table- 3: Optimum moisture content and Dry unit weight

Variant Name	OMC	Dry Unit Weight (kN/m^2)
JCUE680	20	18
JCUE600	27	18.04
JCUE880	32	18.24
JCUE800	33	18.33

Chart 3: Optimum moisture content and Dry unit weight



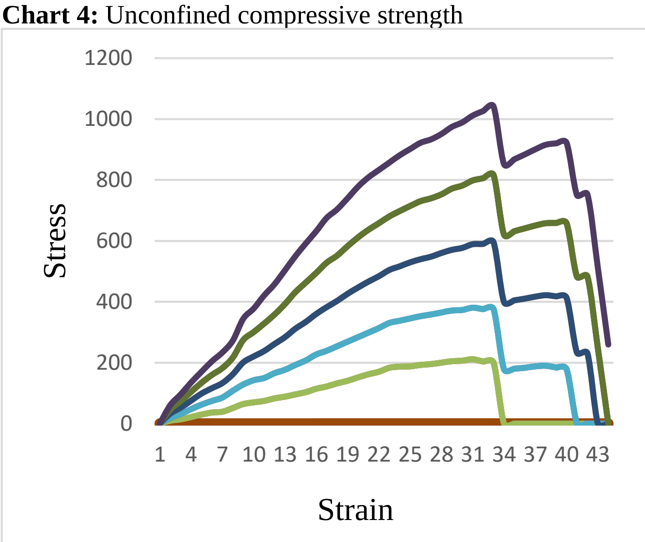
3.2 UNCONFINED COMPRESSION TEST:

The firm clay soil sample is treated and increased its UCS strength of 269.7175353 kN/m2 at maximum. The test is conducted at optimum moisture content, the results were possibly influenced by 8% microbes which has high density of growth. The high compressive strength and the shear strength shows that the voids between soil particles were filled by calcium carbonated precipitation.

Table- 4: Unconfined compressive strength

Variant Name	UCS (N/mm^2)
JCUE680	0.1897
JCUE600	0.2343
JCUE880	0.2520
JCUE800	0.2697

Chart 4: Unconfined compressive strength



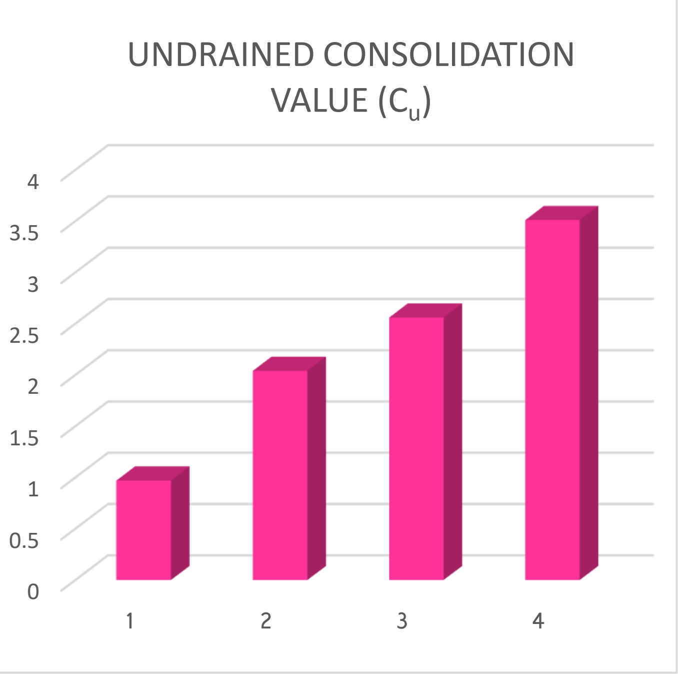
3.3 TRIAXIAL TEST:

The undrained consolidation value is increased by 0.97 cm^2/sec to 4.48 cm^2/sec by treating with four variant of soil sample. It results in increasing the soil stiffness of cohesive soil. The variant JCUE800 had high undrained consolidation value that shown in the Table.5.

Table- 5: Undrained Consolidation strength

Variant Name	Undrained Consolidation (cm^2/sec)
JCUE680	2.04
JCUE600	2.56
JCUE880	3.51
JCUE800	4.84

Chart 5: Undrained consolidation



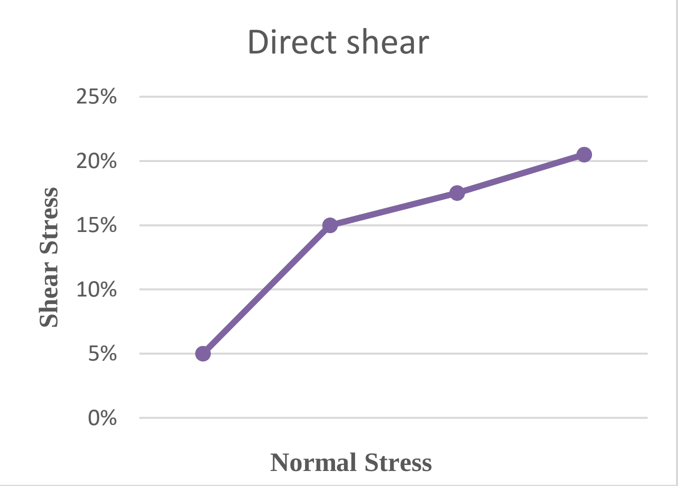
3.4 DIRECT SHEAR TEST:

The shearing strength of the treated soil is increased by 20% than the untreated soil sample. The effectiveness of shearing capacity is shown in the Chart 6. Internal friction between the particle were increase the shearing resistance. The JCUE800 having the high resistance to the shearing capacity.

Table- 6: Shear strength

Variant Name	Shear Strength (kPa)
JCUE680	0.054
JCUE600	0.065
JCUE880	0.074
JCUE800	0.086

Chart 6: Shear strength



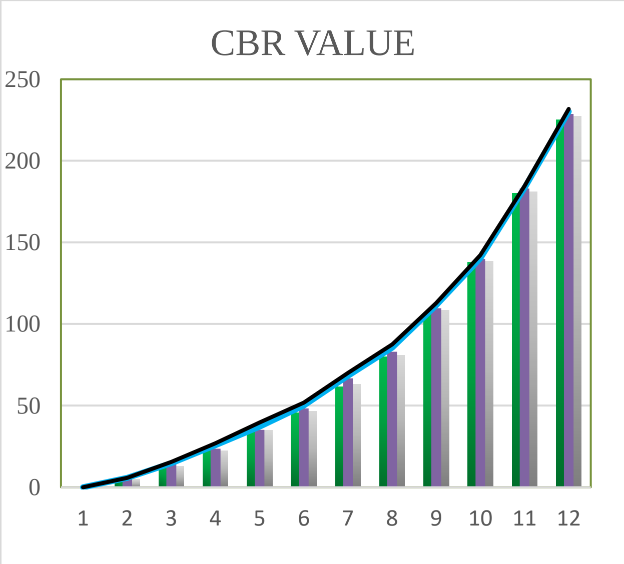
3.5 CALIFORNIA BEARING RATIO:

The CBR test is carried out to determine the strength of subgrade soil. From this test the gradual increase in the strength is shown in Chart 7. The MICP were increase the strength of the soil to avoid settlement. The EICP and MICP were employed to attain the appropriate range of strength.

Table- 7: CBR value

Variant Name	Penetration at 2.5 mm	Penetration at 5 mm
JCUE680	46.58	108.32
JCUE600	48.37	109.68
JCUE880	50.12	111.5
JCUE800	51.95	112.92

Chart 7: Shear strength



4. CONCLUSION:

The incorporation of microbial solution into the 4 variants of soil sample with combination of jack bean crude urease. The calcium carbonate precipitation will decrease voids and increase the mechanical strength.

The measured shear strength, unconfined compressive strength, strength of subgrade and other mechanical properties of soil samples are summarized. From the test results, it can be observed that the engineering properties and mechanical properties of each variant is enhanced by the E.coli and JCU of different ratios that mentioned.

The mix ratio of JCUE880 and JCUE800 shows that the higher efficiency of stabilized soil. As comparing with untreated soil sample, the MICP treated sample shows better results. Combination of MICP and EICP plays major role in stabilization of cohesive soil.

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