A Review and Study on stabilization of Sand using Sugarcane Bagasse, Yeast, Human Hair Fibers and Lime

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Abstract - *During earthquakes medium to fine sands become unstable due to liquefaction as a result of application of cyclic loads during earthquakes (the resulting high porewater pressures reduce the effective stresses to zero). Several microbiology-based techniques have been manifested with time to counteract this and one amongst them is Microbially Induced Calcite Precipitation (MICP). Conventionally, MICP includes the bacteria with ureasic activity. The carbonate ions produced, by the metabolism of bacteria in presence of urea, react with externally added calcium ions to give calcium carbonate, a cementitious material called bio cement, thereby improving the shear strength of sand. In this work, an effort has been made to check the adequacy of certain unprecedented materials in MICP after a thorough study. For this, Sugarcane Bagasse (SCB) has been used in place of Urea and edible Yeast cells instead of urea bacteria. Sugarcane bagasse, being a potential solid waste, has been found to have some sucrose content and moisture. Thus, by alcoholic fermentation of sucrose and/or plant glucose present in bagasse, through yeast, in an anaerobic saturated environment within sand pores, CO_2 can be produced, which get converted to carbonate ions to react with externally added calcium ions (from added lime) to produce calcium carbonate (bio cement). Additionally, Human Hair Fibers of requisite aspect ratio have been used as reinforcement, which would further increase the shear strength of sand. The aim has been to develop only the stabilized samples of sand for Unconfined Compressive Strength test in compliance with the IS code.*

Key Words: Microbially Induced Calcite Precipitation, Sugarcane Bagasse, Yeast, Human Hair Fibers, Lime

1.INTRODUCTION

Sands too, are susceptible to damages under earthquakes due to liquefaction. Stabilization of sand is an effective technique for reducing the damages during liquefaction. Liquefaction is a geotechnical problem which arises due to the application of dynamic loads during earthquakes (seismic activities), in which soils get loaded under undrained conditions as dissipation of pore water is not possible in such a small time. This results in high porewater pressures which reduce the effective stresses to absolute zero. Sands under such conditions become unsuitable to support any overlying structure as the shear strength of sand (cohesionless) is totally governed by the overburden effective stresses. Increasing the shear strength and stiffness of sand with the help of some stabilization methods to tackle such situations, can avert great damages like building collapse, tilting and toppling. Any soil stabilization technique aims at increasing the shear strength of soil and subsequently its bearing capacity and decreasing its permeability and compressibility. Several works have been done for improvement of soil taking cost also as a governing factor. Soil improvement techniques can be broadly divided into four main categories [11]:

(1) Soil improvement without admixtures (for non-cohesive soils: vibro-compaction, dynamic-compaction, explosive compaction, electric pulse compaction and surface compaction, and for cohesive soils: soil replacement/displacement method, preloading using fill and prefabricated using vertical drains, preloading using vacuum, dynamic consolidation with enhanced drainage and vacuum, electro-osmosis or electro-kinetic consolidation, thermal stabilization using heating or freezing etc.). (2) Soil improvement with admixtures or inclusions (vibro-replacement or stone columns, dynamic replacement, sand compaction piles, geotextile confined columns, controlled modulus columns, multiple stepped piles etc.). (3) Soil improvement using stabilization with grouting type admixtures (particulate grouting, jet grouting, chemical grouting, mixing methods etc.). (4) Earth Reinforcements (using Geosynthetics, anchors and biological reinforcing with vegetation roots).

Now a days, interests towards 'Microbiological Geotechnology' are widening which includes the processes of bio clogging and bio cementation seeking to reduce the permeability and to increase the shear strength of the soil. These biological techniques have been successfully implemented in sands for controlling their properties. Soil bio-cementation can be achieved through a very commonly adopted process that is; 'Microbially Induced Calcite Precipitation (MICP)'. In this technique, the bacteria secrete certain metabolic products under favourable conditions, which react with the ions present in the external environment to develop a cementing compound, Calcium Carbonate ($CaCO_3$) also called bio cement. This has also been categorized as Biologically Induced Mineralization (BIM) and is a most important sustainable technique [1][17]. This work uses the same concepts of MICP (or bio cementation) but with certain new categories of bacteria and ions, clubbed with mechanical stabilization for medium to fine sands.

2. BACKGROUND STUDY

Bio clogging is the process of clogging of the pores within the soil mass by the metabolic secretions of the microorganisms or by their body clusters. This reduces the permeability and compressibility of the soil. Similarly, bio cementation is the process of binding of the soil grains together by cementing material called bio cement formed by the combination of microbial secretions and externally supplied ions or compounds [30]. This increases the shearing strength of the soil and its bearing capacity. Calcium Carbonate is the most common cementitious material which precipitates naturally in marine water, fresh water as well as in soils and accounts for 4% of the earth's crust by weight [9]. Microbially Induced Calcite Precipitation (MICP), also called Microbially Induced Calcium Carbonate Precipitation (MICCP), as stated, is a new soil improvement technique that has been developed in recent years to solve the liquefaction related problems and other geotechnical engineering problems in sands by the deposition of calcium carbonate in the pores [21]. Precipitation of calcium carbonate by microbial action refers to the formation of calcium carbonate within an oversaturated solution (calcium rich environment) in the presence of microorganisms through their biochemical metabolic activities [10]. During this process, microorganisms secrete one or more metabolic products (possessing CO_3^{2-} ions) that react with ions (Ca^{2+}) found in the local environment leading to subsequent bio cement or microbe cement ($CaCO_3$) precipitation [35]. The effective role of microbes in inducing calcite precipitation makes bio cement a most important metabolic product of Biomineralization, which has been found to remediate and restore the disturbances in structures as well, like minor cracks [30].

2.1 Conventional method of MICP using Urea, Ammonifying bacteria and Calcium source.

Various studies conducted by many research professionals in this field over time show that MICP can be suitably used for the repair of the buildings, soil improvements, preparation of self-healing concrete and bio grouting. There are many biological processes that can lead to MICP like cementation by urease producing bacteria (UPB), cementation by iron hydroxides using iron reducing bacteria (IRB), hypothetical cementation by ferrous sulfide using sulphate reducing bacteria (SRB) and cementation by microorganisms involved in Nitrogen cycle [30]. But the urease producing bacteria also called ammonifying bacteria are getting more attention because of their widespread availability. Ureases are a group of enzymes that hydrolyze urea to promote bio mineralization in nature. Although, there is a wide availability of microorganisms with the ureasic activity, but the Bacillus group is well renowned for its high levels of urease production [1]. Especially, *Sporosarcina pasteurii* (with 21mM hydrolyzed urea per minute) is found to be the most apt bacteria for MICP ([1][10]) as it can grow under harsh conditions of pH 9 and is nonpathogenic [10][20]. Thus, it can be well said that the conventional MICP process involves the urea bacteria or ammonifying bacteria for calcium carbonate precipitation. The set of reactions involved in this MICP process are as follows [10]:

a. Urease catalyzes urea hydrolysis to produce ammonium and carbonate. In this reaction, one mole of urea is hydrolyzed which forms one mole of ammonia and one mole of carbamic acid.

$$CO(NH_2)_2 + H_2O + \text{Microbial urease} \longrightarrow NH_2COOH + NH_3$$

b. Carbamic acid is hydrolyzed spontaneously to another ammonia and carbonic acid molecule which get balanced in an aqueous medium.

$$NH_2COOH + H_2O \longrightarrow NH_3 + H_2CO_3$$

c. The carbonic acid molecule forms bicarbonate and hydrogen ion. Added to this, two moles of ammonia get converted into two moles of ammonium and two moles of hydroxyl ions. The latter increase the pH of the medium to 7.5 – 8 affecting the equilibrium of bicarbonate ions which leads to the subsequent formation of carbonate ions.

$$H_2CO_3 \longrightarrow HCO_3^- + H^+$$

$$2NH_3 + 2H_2O \longrightarrow 2NH_4^+ + 2OH^-$$

$$HCO_3^- + H^+ + 2OH^- \longrightarrow CO_3^{2-} + 2H_2O$$

d. This causes the metal ions to precipitate in conjunction with carbonate anions as calcite. The NH^{4+} ions, so produced, increase the pH of the medium and the reaction continues spontaneously towards calcium carbonate (bio cement) formation [16], provided there is sufficient calcium and carbonate ion concentration in the solution. Here, the bacterial cell acts as an interface for this reaction to complete [44].

$$Ca^{2+} + \text{Bacterial Cell} \longrightarrow \text{Cell} - Ca^{2+}$$

$$\text{cell} - Ca^{2+} + CO_3^{2-} \longrightarrow CaCO_3 + \text{Cell}$$

e. These compounds are generated in the nearby aqueous environment, totally dominated by pH and suitable chemical conditions of the surrounding solution. The production of hydroxyl ions (associated with the conversion of ammonia to ammonium) and the production of bicarbonate ions (by the conversion of carbonic acid) bring in the availability of carbonate ions, which in the presence of external calcium cause the calcium carbonate precipitation under supersaturated conditions in aqueous medium. Under such conditions, calcium carbonate precipitates (MICP) on the microbial cell walls near soil particle-particle contacts [13]. Fig-1 ([10][44]) illustrates these reactions. Thus, the most popular and conventional method of MICP is this aforementioned way which involves 'urea hydrolysis' [13][20].

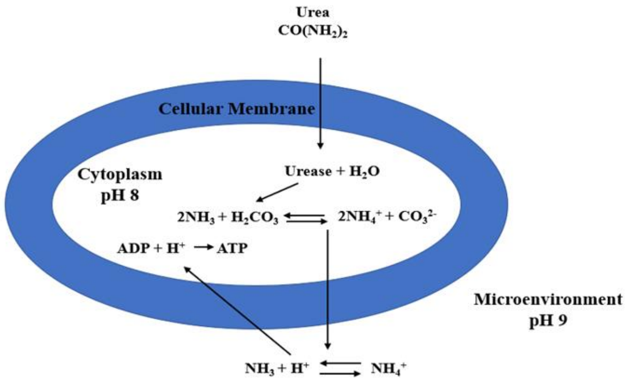

Fig-1: Ureasic activity by bacteria involved in calcite precipitation [10][44].

Interestingly, as per [41], with MICP treatment, the improvement ratios in the shear strength of the residual soil specimens were found to be higher (1.41 to 2.64) when compared with those of sand specimens (1.14 to 1.25), whereas the reduction in the hydraulic conductivity values are more in sand specimens (final to initial ratio as 0.09 to 0.15) contrary to residual soil specimens (0.26 to 0.45). Apart from this, bio cementation (MICP) using urea hydrolysis suffers from serious limitations. The production of ammonia within this process can be fatal to humans ([21]) and also this ammonia production step is quite slow. This process is very difficult to control and depends upon several factors including pH, temperature, concentrations of species, nutrients etc. [27]. Also, there are serious economic limitations in the acquisition of nutrients during field works [10]. The extraction of pure strains of bacteria with effective ureasic activity could also be difficult. Thus, it is important to look out for some other alternatives in the field of soil improvement using bio cementation (MICP).

3. A REVIEW ON PROPOSED MATERIALS

3.1 Possibility for a new MICP process using Sugarcane Bagasse and Yeast

In the previous section, the conventional method of MICP has been mentioned and studied. But, in this work an effort has been made to check the use Sugarcane bagasse (SCB) and Yeast cells instead of Urea and Urea bacteria for MICP. The methodology of Urea Hydrolysis has been replaced by Alcoholic Fermentation for MICP with further addition of Human Hair Fibers as reinforcement and Lime for mechanical stabilization for sand.

Sugarcane Bagasse is a waste available from sugar cane after extracting available sucrose (sugar juice) from it. It is a potential solid waste and takes lots of time for bio degradation because of high cellulose and lignin content in it [18]. Bagasse has 1-2% sucrose in it and has a moisture content of 12.6 % on an average [3]. Although being a waste product of the sugar industry, it is also burned as fuel in sugar mill boilers [19][42]. The use of SCB ash is very renowned now in stabilizing clayey soils and in formation of compacted soil blocks [2]. SCB ash along with lime can effectively work as chemical stabilizers in compacted soil blocks such that after proper curing of these blocks for 7, 14 and 28 days, an improved compressive strength and durability could be achieved when compared with only lime addition [2]. As per [8], approximately 270 to 280 kg of bagasse is generated from processing each ton of sugarcane which is 25% by weight along with 140 kg of straw. Around 200 million tons of Lignin (for polymer composites) are produced annually from SCB [40]. Also, the steam explosion pretreatment of raw SCB at 215˚C for 5 minutes results in an overall glucose yield of 86.8% of the total content [3]. The amount of glucose in bagasse is 41.9 % by dry weight of unaltered bagasse [3]. As per [19], the low cost, low density, and acceptable mechanical properties of SCB fibers also make them eligible to be used as reinforcements in plastic composites. SCB with the aspect ratio of 80 ([12]) can act as reinforcements in composites as it is composed of approximately 50% cellulose, 25% hemicellulose and 25% lignin ([19]) giving it a tensile strength in range of 170 to 290 MPa and modulus of elasticity of 15 to 19 GPa [45].

We also cannot neglect an important attribute associated with SCB, that is, alcoholic fermentation. Alcoholic fermentation is the process of converting sugar (glucose, $C_6H_{12}O_6$) into alcohol (ethanol, C_2H_5OH) and carbon dioxide (CO_2) following anaerobic conditions [15]. As per [15], stoichiometry shows that 180 g of glucose/simple sugar can produce approximately 92 g of alcohol with 88 g of carbon dioxide gas (48.9% by weight of glucose) which can remain dissolved in the solution but a part of it can even escape. Such a process takes place in the absence of oxygen when certain species like yeast act on simple sugars/glucose for deriving energy [46]. The alcohol produced in this process of fermentation is ethanol (popularly called bio ethanol) which has maximum concentration at a pH of 4.5 and temperature of 35˚C. These pH and temperature conditions are also optimal for yeast cells to grow [46]. The fermentation of sucrose (or glucose) present in SCB with the help of yeast can take place in the same manner as the fermentation of sugars within an anaerobic atmosphere. A kinetic study for the production of bioethanol from SCB using yeast strains has also been conducted to establish the success of the process [23]. From each mole of sucrose, four moles of ethanol (alcohol) and four moles of carbon dioxide are produced by this fermentation process compared to only two moles of each from one mole of glucose or fructose [31]. Sucrose (being a disaccharide) undergoes hydrolysis to produce glucose and fructose as shown [31]:

$$\text{Sucrose} + H_2O \longrightarrow \text{Glucose} + \text{Fructose}$$

The fermentation reactions of sucrose, glucose and fructose are given separately [31]:

$$\text{(Sucrose) } C_{12}H_{22}O_{11} + H_2O + \text{yeast} \longrightarrow 4C_2H_5OH + 4CO_2$$

$$\text{(Glucose) } C_6H_{12}O_6 + H_2O + \text{yeast} \longrightarrow 2C_2H_5OH + 2CO_2$$

$$\text{(Fructose) } C_6H_{12}O_6 + H_2O + \text{yeast} \longrightarrow 2C_2H_5OH + 2CO_2$$

Through the above processes enough CO_2 is produced in the aqueous anaerobic environment. The CO_2 so released at acidic pH can be used in carbonate ion formation at basic pH. These ions can react with externally added calcium ions to produce calcium carbonate (Bio cement). It has also been found that the fermentation rates of sucrose, glucose and fructose are more or less the same (sucrose having higher rate than glucose and fructose [31]).

3.2 The use of Human Hair fibers and Lime

Furthermore, a very wide application possibility of Human Hair fibers to be used as reinforcements in sands or sandy soils are available in literature. Human Hair, being non-biodegradable [7] and a potential solid waste [14], is directly dumped into the soil where it creates several health and environmental issues [29]. Sand has shown great improvement in its mechanical properties with several polymeric reinforcements. As per [38], poorly graded sand reinforced with 51 mm long monofilament polypropylene showed high values of Unconfined Compressive Strength at an optimum fiber content of 1% (1.5% when large deformations were allowed) and such fiber-stabilized sand subgrades can be beneficially used in airfields. Also, as per [28], when such polypropylene fibers of lengths 6 mm and 12 mm were added to sand, it showed a resistance against liquefaction, the maximum resistance at a fiber content of 1% and relative density of 50% for 12 mm long fibers. The Cyclic Stress Ratios increased with the increase in the fiber length. Apart from these polymeric fibers, Human Hair fibers are also now in use for reinforcing sands however, they were formerly used extensively for stabilization of cohesive soils. As per [7], Human Hair fibers can be used within clayey soil to increase its strength and CBR value. Presence of Human Hair fibers within soil can also reduce its cracking. Such reinforcements are incombustible and durable thereby, increasing the service life of the construction soil, reducing the lateral spreading and providing higher confinement. According to [6], the inclusion of randomly oriented fibers (Synthetic, Coir and Human Hair) in Kaolinite Clay showed an increase in the Liquid limit, Hydraulic conductivity, Unconfined Compressive Strength, Stiffness and Optimum moisture content and a subsequent reduction in Compressibility and in Maximum Dry Density due to their light weight. Human Hair fiber reinforced Kaolinite clay showed the highest value of Maximum Dry Density amongst all but, the mixing of fibers above a content of 2% could be difficult with no further improvement in engineering properties. Parallelly, the work of [33], shows that an addition of 2% of Human Hair fibers to Kaolinite Clay causes a 200% increase in the Unconfined Compressive Strength value of the clay. The Maximum Dry Density reduces slightly (Optimum moisture content increases marginally) with this addition and then remains same. Similarly, [29] showed a maximum increase of 163.2% in California Bearing Ratio value and 167.4% increase in Unconfined Compressive Strength value of Kancheepuram clay at 1.2% of Human Hair fiber inclusion. As per [39], a maximum increment of 67.2% was obtained in the Unconfined Compressive Strength of the cohesive soil with an inclusion of 1.2% of Human Hair fibers. The same soil sample when treated with chloride compounds (Sodium Chloride and Calcium Chloride) along with Human Hair fibers showed an improvement of nearly 180% in both Unconfined Compressive Strength and California Bearing Ratio. [4] showed that an addition of Human Hair fibers to the marine clay along with jute fibers of length 2 cm, the Maximum Dry Density, Unconfined Compressive Strength and California Bearing Ratio values increased, and Optimum moisture content and Liquid limit values decreased. The optimum amount of Human Hair fibers was 1.5% in context with compressive strength. According to [14], an addition of 9% lime and 1.5% Human Hair fibers to Kuttanad clay led to an increase of 90.4% in the compressive strength with the rate of strength achievement higher for 2-14 days of lime curing than 14-28 days. However, lime and Human Hair fibers alone showed lesser strength improvement.

Also, Human Hair fiber reinforcement has shown good results in sandy soils or sands too. As per [36], the Yamuna sand showed a higher dilative behavior when reinforced with Human Hair fibers. The peak shear strength of sand specimens increased after the addition of Human Hair fibers as per Direct Shear Test results along with an increase in angle of shearing resistance (maximum value of 45.3˚ at 2% fiber addition), but the cohesion intercept decreased as fiber content increased. Similarly, [37] showed, through a series of Cyclic Triaxial Tests conducted on Human Hair fiber reinforced sand at two different relative densities of 50% and 80%, that the Shear modulus had a maximum increase under medium shear strains and at 0.5% fiber inclusion for 80% relative density.

For stabilization of local soils, Lime has been used since quite a long time now. It is the great success of lime in improving the mechanical properties of soil that Lime stabilization is so popular especially in pavement construction [5][22]. As per [32], local soil mixed with lime showed an increase in strength and in load bearing capacity required for the road construction. After a curing period of 28 days such a mixture can work as a subbase material for flexible pavements. According to [43], dune sand can be stabilized with Bentonite and Lime mixture. The Unconfined Compressive Strength in dune sand samples was found to be maximum when the Bentonite content was 15% and Lime content was 3% where the curing environment had a significant influence on the strength development. Thus, lime could be used beneficially in improving the properties of sands as well.

4. METHODOLOGY ADOPTED

Having seen that sand could undergo bio cementation using this new MICP process involving SCB and yeast cells with further improvement using Human Hair fiber reinforcement in the presence of lime, some experimentation was conducted to get the sand samples. Table-1 shows the properties of sand used in this experiment. Sand was first washed over 75-micron sieve and then dried before its use. Fig-2 shows the Grain Size Analysis chart developed using the sieve analysis (as per IS 2720 Part-4) for the sand. The sand chosen was poorly graded (SP) with specific gravity of 2.526 (as per IS 2720 Part-3) and with hydraulic conductivity of 0.009095 cm/s from constant head permeability test. The sand so chosen had a maximum void's ratio of 0.81 and minimum void's ratio of 0.646. Sugarcane Bagasse (SCB) was collected from local juice centres. For the ease of work, the outer green portions were removed, and inner white portions were used (which were soft with some moisture/sucrose in them). Such portions were cut out in lengths of 10 to 20 mm so that they can be properly incorporated within the sand samples. Yeast (baker's yeast) was collected from a grocery shop. To do a comparative analysis on its performance in relation to pure laboratory strains, Saccharomyces Cerevisiae yeast was collected from the Biotechnological Department of Birla Institute of Technology, Mesra. Human Hair was collected from local barber shops and parlours. These Human Hair fibers were first washed and then air dried before their use. Quick Lime (CaO) was used in powdered form for strength improvement and in the form of lime water for calcium ion source in the MICP process. For lime water preparation, powdered lime was mixed with water and filtered over a filter paper. The filtrate so obtained was used.

Table-1 Properties of the Sand used in the experiment

Properties of Sand	Values
D_{60}	1.225 mm
D_{30}	0.552 mm
D_{10}	0.331 mm
Coefficient of Uniformity (C_u)	3.70
Coefficient of Curvature (C_c)	0.751
Classification as per ISSCS	Poorly Graded Sand (SP) (Uniformly Graded)
Specific Gravity of solids (G_s)	2.526
Coefficient of Permeability (k)	0.009095 cm/s
Maximum Void's Ratio (e_{max})	0.810
Minimum Void's Ratio (e_{min})	0.646

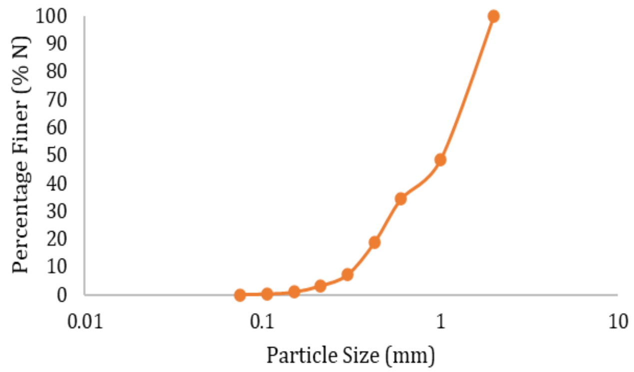

Fig-2: Grain Size Analysis Chart of the Sand used.

Fig-3 shows a collage of the various materials used in the sand sample preparation. The aim was to obtain only the stabilized sand samples with dimensions conforming with standard Unconfined Compressive Strength test (as per IS 2720 Part-10). This could ensure a success of this new stabilization method for sand.

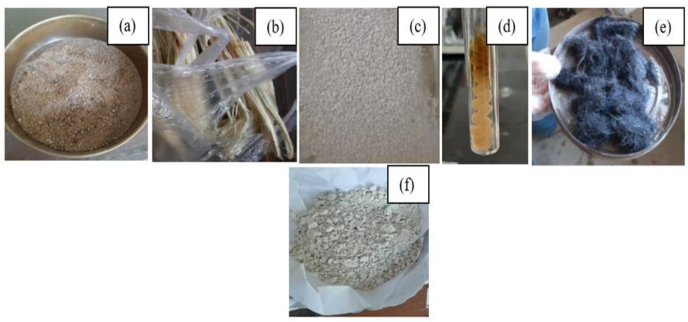

Fig-3: The materials used in the experiment: (a) Sand to be used in sample preparation, (b) Sugarcane Bagasse, (c) Yeast commercially available, (d) Yeast (*Saccharomyces Cerevisiae*) obtained from Biotechnological Laboratory, (e) Human Hair fibers and (f) Lime to be used in powdered form and in lime water.

4.1 A Feasibility Experiment to check CO_2 production

To check the feasibility of production of carbon dioxide (CO_2) from the Alcoholic fermentation of SCB using yeast, an experiment was conducted. The carbon dioxide so produced can be used in carbonate ion formation for calcium carbonate precipitation between sand particle-particle contacts. For such an experiment (where only escaping carbon dioxide can be used), (1) 50 g of Sugarcane Bagasse (white portions) was taken in a beaker and 200 mL of distilled water was added to it. To this, a distilled water solution of yeast (obtained from Biotechnological Laboratory) was added, and the setup was covered with a paper to block the release of CO_2 gas which would be produced by anaerobic fermentation. (2) Through a hole made in this paper cover, a pipe was taken into a cylindrical measure containing lime water which would turn milky if sufficient amount of CO_2 gas produced moves to the lime water from the beaker. This could prove that fermentation for SCB is taking place due to the presence of yeast. The joints were sealed with clay. (3) The apparatus was left undisturbed for fermentation for 15 days and the reaction was monitored every alternate day. (4) After conducting this test, a similar setup was prepared using 5 g of commercially available Baker's yeast and the observations were recorded for 15 days. Fig-4 gives a representation of this feasibility experiment.

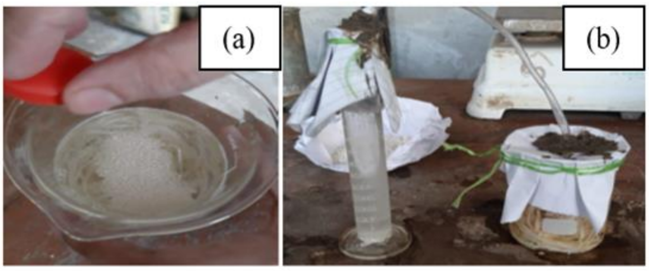

Fig-4: The setup preparation for feasibility experiment (a) addition of commercial yeast to SCB and water (b) final setup kept for observation with fermentation taking place in beaker and lime water in the cylindrical measure connected with a pipe.

4.2 Preparation of the Sand Samples

The sand samples were prepared using the admixtures and molded in the size suitable for Unconfined Compressive Strength test (as per IS 2720 Part-10). To do that (1) 15 g of Human Hair fibers (1.5%) were added to 1 kg of dry sand and the mixture was mixed with sand till uniform consistency (Fig-5(b)). (2) 50 g of finely powdered lime (5%) was added at this stage and then mixed thoroughly for initial stiffness. (3) Now, the next was the addition of Yeast and SCB to this mixture of sand, lime and Human hair. Separately a mixture of Yeast and SCB was prepared. As an initial step, 15 g of SCB cut out in lengths of 10 to 20 mm (white inside portions) was taken and yeast was added to it in two ratios 1:6 and 1:10 by weight (Yeast to SCB). The smallest dimension of the SCB strips taken, was kept lesser than one-eighth of the sand sample size (as per IS 2720 Part-10). These were properly kneaded with wet hands to provide good contact of yeast with bagasse (Fig-5(c)). (4) However, for comparison, these two Yeast-SCB mixtures were added to the sand-lime-Human hair mixture in layers and by a thorough mixing. Thus, a total of four stabilized sand samples (two mixed samples and two layered samples) were prepared (Fig-6). (5) The samples are required to be of cylindrical shape with 38 mm diameter and 76 mm height (height to diameter ratio of 2) as per standards. So, for this, 38 mm diameter plastic pipe was taken which was cut in pieces of suitable lengths and the stabilized sand samples were prepared within these pipes. Also, it was tried that all the samples remain fully saturated. (6) To allow saturation water and lime water to pass through them and for full drainage, a filter arrangement was developed within these pipes using scrubbers and gravels (8 to 10 mm size) placed suitably (Fig-6). (7) After, the suitable preparation of sand samples (layered and mixed) within the mold pipes, these were made fully saturated by adding water from one side and collecting the effluent from the other. This was repeated for several times from both the sides of the sample pipes. Furthermore, the effluent was again passed through the samples to reduce the chances of loss of yeast cells. (8) The samples within the mold pipes were allowed to undergo decomposition and fermentation for 5 days so that enough carbon dioxide (as per feasibility experiment) could be produced. From day 6 onwards, lime water was added on each alternate day so that increasing pH can facilitate carbonate ion formation and subsequently calcium carbonate (bio cement) precipitation between particle-particle contacts using calcium ions from the lime water. In this process also, the effluent so obtained was re-entered from the top (maintaining the same flow direction). This was repeated several times to make the best use of carbon dioxide dissolved in the effluent. The samples were removed from the mold pipes on day 15. An average temperature of nearly 30 ˚C was maintained during the complete process.

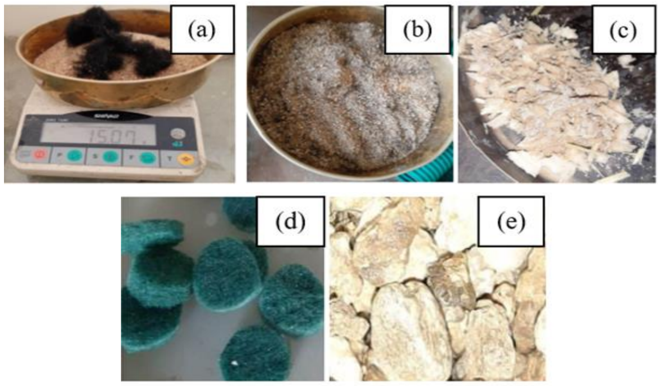

Fig-5: (a) 15 g of Human Hair fibers taken for 1 kg of dry sand, (b) the sand is mixed properly with Human Hair, (c) the Yeast-SCB mixture prepared, (d) Scrubbers used in the mold pipes in filter arrangement and (e) Gravels used in filter arrangement within the mold pipes.

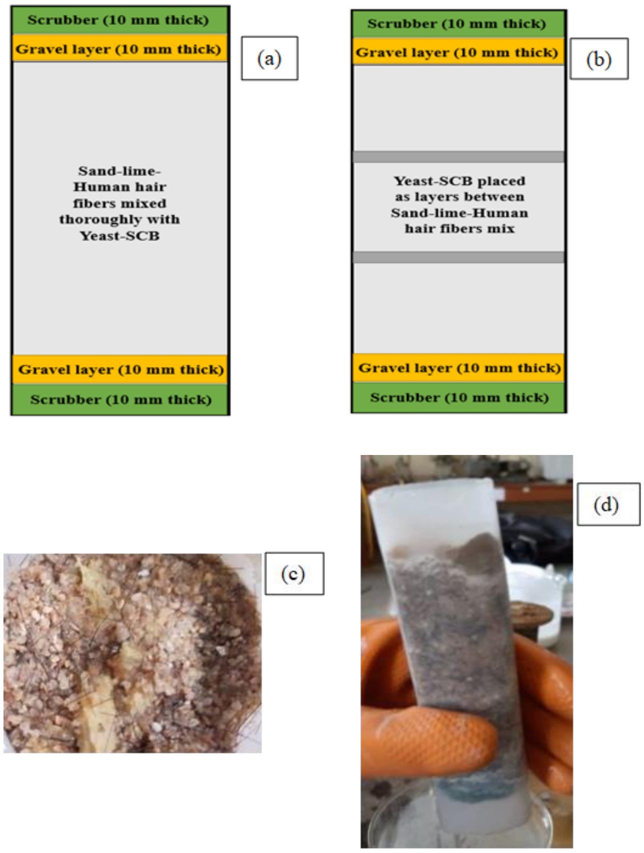

Fig-6: Proper arrangement of the sand-lime-Human Hair mixture and Yeast-SCB mixture for (a) mixed sample, (b) layered sample, (c) mix within a mixed sample and (d) lime water addition in a mixed sample.

5. RESULTS AND ANALYSIS

The feasibility experiment showed that there is a possibility of the fermentation of sucrose/glucose present in SCB using Yeast under anaerobic conditions (Fig-7). It was observed, after 15 days, that the complete lime solution did not fully turn milky but, a stretch of the pipe connecting the fermentation area with lime water turned white and also a part of cylindrical vessel containing lime water turned white as calcium carbonate precipitated therein. Table-2 gives the day wise observations of the setup. The results obtained by using Yeast (*Saccharomyces Cerevisiae*) obtained from Biotechnological Laboratory and by using commercially available Yeast (Baker's Yeast) were same. It was also observed that the bubbling of carbon dioxide as a result of fermentation was more concentrated at the bottom of the beaker (due to anaerobic conditions) while the top of the beaker showed aerobic decomposition (in the form of dark patches) whose rate increased after day 7. Although, the carbon dioxide bubbling could be seen but the intensity of production was low probably because the ideal temperature of 35 ˚C could not be maintained. Also, the ethanol so produced during fermentation, being acidic, could reduce the pH to suit further carbon dioxide production. This explains the increase in the bubbling intensity with the passing of days.

Table-2: Day wise observations in the setup of the feasibility experiment

Days	Observations
Day 1	No Change
Day 3	Some bubbling at the bottom of the vessel.
Day 5	Vigorous bubbling at the bottom of the vessel.
Day 7	More bubbling and aerobic degradation starts at the top of the beaker.
Day 9	The beaker starts stinking due to aerobic action but still CO_2 bubbling at the bottom takes place due to anaerobic yeast action.
Day 11	The connecting pipe starts turning white but the bubbling starts reducing.

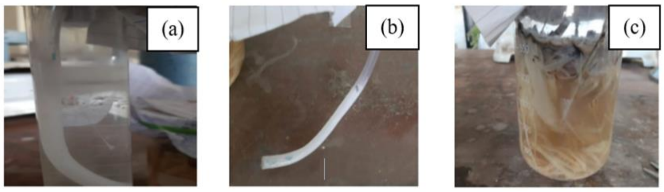

Fig-7: The results of the Feasibility Experiment (a) slight haziness in lime water, (b) the deposition of calcium carbonate in the connecting pipe and (c) the beaker containing SCB, Yeast and water after 15 days.

The sand-lime-Human Hair mix along with Yeast-SCB mixture in ratios of 1:6 and 1:10 (by weight) were used in Layered and Mixed sample preparation within the mold pipes. When these sand samples were removed from the mold pipes, the samples could retain their shapes of 38 mm diameter and height 76 mm successfully (Fig-8 and 9). Hence, it could be said that this MICP process based on 'Fermentation Geotechnology' along with Human Hair fibers reinforcement and lime can produce well stabilized sand samples. This could be attributed to the fact that within the fully saturated sand pores anaerobic conditions exist supporting fermentation by yeast. The day wise observations developed on the general behavior of the layered and mixed samples during lime water addition are given in Table-3. The permeability of the layered sample(s) was found less than the permeability of the mixed sample(s). This might be so because in layered samples the fermenting Yeast-SCB zones were placed in between the sand layers which broke down to simpler small pieces hindering the easy water passage. For both the layered and mixed samples, the ones having Yeast-SCB ratio of 1:6 (by weight) showed lesser permeability than those with 1:10 Yeast to SCB ratio because of more fermentation and stabilization of SCB due to high microbe (Yeast over SCB) content. Certain dark patches of decomposition could be seen along with a consistent pungent smell. When sand was mixed with Human Hair fibers then it showed a quick response when shaken and squeezed between the palms under saturated conditions. The SCB particles left within the samples could also work as reinforcements and improve the mechanical properties of sand furthermore.

Table-3: Day wise observations in the Layered and Mixed sand samples

Days	Layered Samples	Mixed Samples
Day 1	The samples were set in both 1:6 and 1:10 Yeast-SCB ratio. They were made fully saturated with water.	The samples were set in both 1:6 and 1:10 Yeast-SCB ratio. They were also made fully saturated. The permeability was higher than the layered sample(s).
Day 6	Lime water was added for the first time. Pungent smell was noticed.	Lime water was added. Harder pungent smell was observed than layered sample(s).
Day 8	Lime water was again added. The flowing speed indicated lesser permeability.	Lime water was again added. The flowing speed indicated lesser permeability.
Day 10	Lime water was again added. Pungent smell got strengthened.	Lime water was added. Pungent smell was much more than the layered ones.
Day 12	Lime water was added. No new observation.	Lime water was added.
Day 14	Last dose of lime water was added. The sample(s) had much reduced permeability compared to the first day. Stretches of black decomposition patches could be observed near the SCB layers.	Last dose of lime water was given. More permeability was observed compared to the layered sample(s). The sample(s) had no dark patches of decomposition of SCB.
Day 15	The sample(s) was gently removed from the mold pipe(s).	The sample(s) was gently removed from the mold pipe(s). It had very high pungent smell owing to more decomposition compared to layered sample(s).

Fig-8: Removing the stabilized sand samples from the mold pipes

It was also found that the bio-cementation using this approach is not that widespread as that happens in the conventional process. This was so because the extend of CO_2 production was lesser using SCB in presence of yeast (when compared to any sugar solution). Also, there could have been fluctuations in pH and temperature during the fermentation phase. However, it was seen that, visually, there were not many differences between layered and mixed samples (Fig-9) and samples were stiffer in behavior.

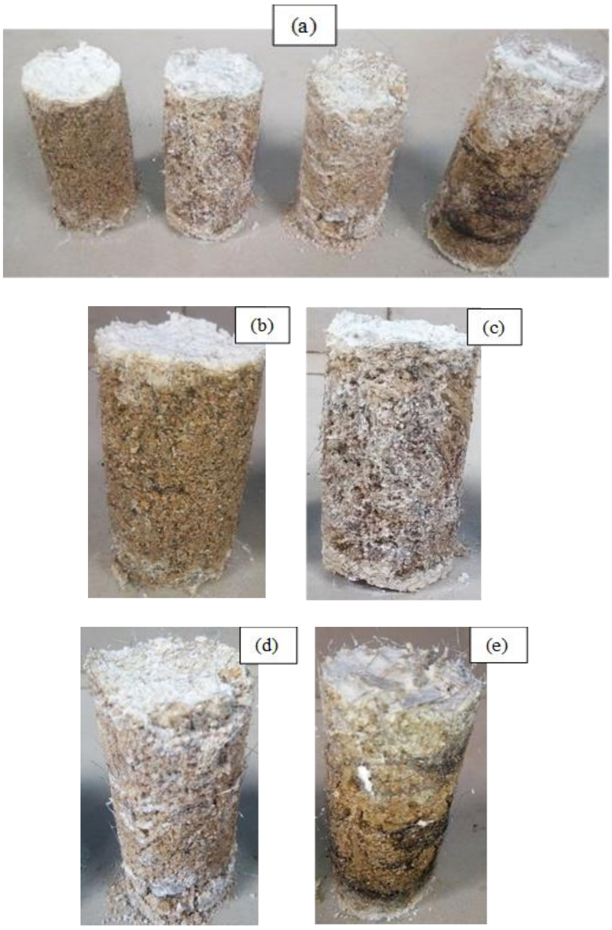

Fig-9: (a) All the samples after removing from the mold pipes (b) Mixed Sample with Yeast to SCB ratio of 1:6 (c) Mixed Sample with Yeast to SCB ratio of 1:10 (d) Layered Sample with Yeast to SCB ratio of 1:6 and (e) Layered Sample with Yeast to SCB ratio of 1:10.

6. CONCLUSION

The aim of this work was to develop the stabilized sand samples of standard sizes of 38 mm diameter and 76 mm height as per Indian Standards, based on 'Fermentation Geotechnology' and reinforcing with Human Hair fibers in presence of lime. Several works have been done to determine the adequacy of each one of these materials in the stabilization of sand however, an effort for a collective study has been made here with a special emphasis on the use of the fermentation principle in bio cementation (MICP) using

Yeast under anaerobic conditions prevalent within fully saturated sand pores. Instead of pure sugar or glucose source, Sugarcane Bagasse (SCB), a waste material was chosen to serve as a source of fermentation and also to act as reinforcement within sand. The general conclusions are as follows:

a. Medium to fine sand is prone to serious Liquefaction effects during earthquakes and this can be reduced only by improving the engineering properties of sands. Therefore, sand is mixed with certain admixtures for its stabilization in order to tackle such conditions. The aim has been to impart Unconfined Compressive Strength to sand so that the stabilized sand samples could retain their shapes. As natural sand lacks Unconfined Compressive Strength (and so cohesion), therefore, the objective of getting firm samples with standard dimensions can only be fulfilled using bio cementation (MICP) process. Conventionally, urea bacteria are made to undergo urea hydrolysis for carbonate ion formation within the pores which react with calcium ions (available from any external source) to form calcium carbonate (bio cement) at sand particle-particle contacts. However, this suffers with certain limitations. Therefore, a new method of bio cementation (MICP) was targeted here with the same carbonate ion production mechanism but by fermenting SCB with the help of yeast cells within the anaerobic environment present within the pores. The CO_2 thus produced, have got converted to carbonate ions which in the presence of lime water, precipitated as Calcium Carbonate (bio cement) at the contact points.

b. With the inclusion of 1.5% Human Hair fibers in sand, it showed a quick response in shaking and squeezing behaviors under fully saturated conditions. 5% of finely powdered lime was also added to this for initial stiffness. An observative study was also carried out on different 'Layered' and 'Mixed' samples with different Yeast-SCB mixtures (i.e., with Yeast-SCB ratios of 1:6 and 1:10 by weight). After 15 days of curing and fermentation, the stabilized sand samples were removed from the mold pipes and they could retain their shapes indicating that the method of MICP developed in this work can effectively produce stabilized sand samples for Unconfined Compressive Strength test with standard dimensions.

Although the work demonstrates the conversion of sand with zero Unconfined Compressive Strength value to stabilized sand with some Unconfined Compressive Strength value, but the work is not complete yet. A better study and experimentation on this newly adopted MICP process (Fermentation Geotechnology) using Scanning Electron Microscope images along with measured improvements in Unconfined Compressive Strength value and Liquefaction resistance using Cyclic Triaxial tests for this stabilized sand would be presented in future.

REFERENCES

[1] Achal V, Mukherjee A, Kumari D, Zhang Q (2015) "*Biomineralization for sustainable construction – A review of processes and applications*" Earth-science reviews 148: 1-17. https://doi.org/10.1016/j.earscirev.2015.05.008

[2] Alavez-Ramirez R, Montes-Garcia P, Martinez-Reyes J, Altamirano-Juarez DC, Gochi-Ponce Y (2012) "*The use of sugarcane bagasse ash and lime to improve the durability and mechanical properties of compacted soil blocks*" Construction and Building Materials 34: 296-305. https://doi.org/10.1016/j.conbuildmat.2012.02.072

[3] Amores I, Ballesteros I, Manzanares P, Saez F, Michelena G, Ballesteros M (2013) "*Ethanol Production from sugarcane bagasse pretreated by Steam Explosion*" Electronic Journal of Energy and Environment 1(1): 25-36. https://doi.org/10.7770/ejee-V1N1-art486

[4] Anjanadevi KA, Rehman A, Merine G, Saumya M, Sruthi MG (2019) "*Soil Stabilization using Jute and Human Hair Fiber*." International Research Journal of Engineering and Technology (IRJET) 6(5): 5117-5121.

[5] Arora MG, Khanna SK, Vasan RM (1970) "*Durability and strength characteristics of lime-flyash-soil mixture for road construction*". Indian Roads Congress Road Research Bulletin No. 14.

[6] Ayothiraman R, Bhuyan P, Jain R (2014) "*Comparative studies on performance of human hair and coir fibers against synthetic fibers in soil reinforcement*." In: Proceedings of 2nd Annual International Conference on Architecture and Civil Engineering (ACE 2014) Singapore, pp. 222-227.

[7] Butt WA, Gupta K, Naik H, Bhat SM (2014) "*Soil sub-grade improvement using human hair fiber*". International Journal of Scientific and Engineering Research 5(12): 977-981.

[8] Canilha L, Chandel AK, Milessi TSDS, Antunes FAF, Freitas WLDC, Felipe MDGA, da Silva SS (2012) "*Bioconversion of sugarcane biomass into ethanol: An overview about composition, pretreatment methods, detoxification of hydrolysates, enzymatic saccharification, and ethanol fermentation*". Journal of Biomedicine and Biotechnology 2012 hindawi.com Article ID 989572. https://doi.org/10.1155/2012/989572

[9] Castanier S, Le Metayer-Levrel G, Perthuisot JP (1999) "*Ca-carbonates precipitation and limestone genesis-the microbiologist point of view.*" Sedimentary Geology 126(1-4): 9-23. https://doi.org/10.1016/S0037-0738(99)00028-7

[10] Chaparro-Acuna SP, Becerra-Jimenez ML, Martinez-Zambrano JJ, Rojas-Sarmiento HA (2017) "*Soil bacteria that precipitate calcium carbonate: mechanism and applications of the process*." Acta Agronomica 67(2): 277-288. https://doi.org/10.15446/acag.v67n2.66109

[11] Chu J, Varaskin S, Klotz U, Mengé P (2009) "*Construction processes*." In: Proceedings of the 17th International Conference on Soil mechanics and Geotechnical Engineering Amsterdam (Volumes 1,2,3 and 4), pp. 3006-3135.

[12] da Luz SM, Gonclaves AR, Del'Arco Jr AP (2007) "*Mechanical behavior and microstructural analysis of sugarcane bagasse fibers reinforced polypropylene composites*." Composites Part A: Applied Science and Manufacturing 38(6): 1455-1461. https://doi.org/10.1016/j.compositesa.2007.01.014

[13] DeJong JT, Mortensen BM, Martinez BC, Nelson DC (2010) "*Bio-mediated soil improvement*." Ecological Engineering 36(2): 197–210. https://doi.org/10.1016/j.ecoleng.2008.12.029

[14] Elias T, George S, Zachariah A, Sulfi S, Sreedevi P S (2016) "*Comparative study of soil stabilization using human hair and lime*." International Journal of Scientific & Engineering Research (IJSER) 7(2): 1323-1326 ISSN 2229-5518.

[15] Elshani A, Pehlivani K, Kelmendi B, Cacaj I (2018) "*Possibility and Determination of the use of CO2 produced by the production of beers*." Journal of Pharmaceutical Sciences and Research 10: 1229-1230.

[16] Ferris FG, Stehmeier LG, Kantzas A, Mourits FM (1997) "*Bacteriogenic mineral plugging*." Journal of Canadian Petroleum Technology 36(9). https://doi.org/10.2118/97-09-07

[17] Frankel RB, Bazylinski DA (2003) "*Biologically induced mineralization by bacteria*." Reviews in Mineralogy and Geochemistry 54(1): 95-114. https://doi.org/10.2113/0540095

[18] Haghdan S, Renneckar S, Smith GD (2016) "*Sources of Lignin (Lignin in Polymer composites).*" In: Frank O, Sain M (ed) Lignin in polymer composites, William Andrew Publications, Elsevier, pp. 1-11.

[19] Hajiha H, Sain M (2015) "*The use of sugarcane bagasse fibers as reinforcements in composites*." Biofiber Reinforcements in Composite Materials 525-549. https://doi.org/10.1533/9781782421276.4.525

[20] Hammes F, Boon N, de Villiers J, Verstraete W, Siciliano SD (2003) "*Strain-Specific ureolytic microbial calcium carbonate precipitation*." Applied and Environmental Microbiology 69(8): 4901–4909. https://doi.org/10.1128/AEM.69.8.4901-4909.2003

[21] Harkes MP, van Paassen LA, Booster JL, Whiffin VS, van Loosdrecht MCM (2010) "*Fixation and distribution of bacterial activity in sand to induce carbonate precipitation for ground reinforcement.*" Ecological Engineering 36(2):112–117. https://doi.org/10.1016/j.ecoleng.2009.01.004

[22] Herrin M, Mitchell H (1961) "*Lime-soil mixtures*." In: Proceedings of 40th Annual Meeting of the Highway Research Board, Highway Research Board Bulletin 304. http://onlinepubs.trb.org/Onlinepubs/hrbbulletin/304/304-008.pdf

[23] Iram M, Asghar U, Irfan M, Huma Z, Jamil S, Nadeem M (2018) "*Production of bioethanol from sugarcane bagasse using yeast strains: A kinetic study*." Energy Sources, Part A: Recovery, Utilization and Environmental Effects 40(3): 364-372. https://doi.org/10.1080/15567036.2017.1422056

[24] IS 2720 Part 10 (1991) "*Indian Standard, method of tests for soils: determination of unconfined compressive strength*." Second Revision. Bureau of Indian Standards, New Delhi

[25] IS 2720 Part 3 (1980) "*Indian Standard, method of tests for soils: determination of specific gravity*." First Revision. Bureau of Indian Standards, New Delhi

[26] IS 2720 Part 4 (1985) "*Indian Standard, method of tests for soils: grain size analysis. Second Revision*." Bureau of Indian Standards, New Delhi

[27] Ivanov V, Chu J (2008) "*Applications of microorganisms to geotechnical engineering for bioclogging and biocementation of soil in situ.*" Reviews in Environmental Science and Biotechnology 7(2): 139–153. https://doi.org/10.1007/s11157-007-9126-3

[28] Karakan E, Eskisar T, Altun S (2018) "*The liquefaction behavior of poorly graded sands reinforced with fibers*." Advances in Civil Engineering 20 hindawi.com Article ID 4738628. https://doi.org/10.1155/2018/4738628

[29] Narayanan KS, Sharmila SMR (2017) "*Stabilization of clay with human hair fiber.*" International Journal of Civil Engineering and Technology (IJCIET) 8(4): 662:667.

[30] Parmar S, Marjadi D (2017) "*Biocementation: a novel technique and approach towards sustainable material*."

World Journal of Research and Review (WJRR) 4(3): 34-41 262839.

[31] Pepin C, Marzzacco C (2015) "*The fermentation of sugars using yeast: A discovery experiment*." Melbourne FL, Chem 13 News Magazine.

[32] Pereira RS, Emmert F, Miguel EP, Gatto A (2018) "*Soil stabilization with lime for the construction of forest roads.*" Floresta e Ambiente 25(2). https://doi.org/10.1590/2179-8087.007715

[33] Pillai RR, Ramanathan A (2012) "*An innovative technique of improving the soil using human hair fibers*." In: Proceedings of the 3rd International Conference on Construction in developing Countries (ICCIDC-3) pp. 4-6.

[34] Qabany, AA, Mortensen B, Martinez B, Soga K, DeJong, J (2011) "*Microbial carbonate precipitation: correlation of S-wave velocity with calcite precipitation*." Geo-Frontiers 2011: Advances in Geotechnical Engineering 3993-4001. https://doi.org/10.1061/41165(397)408

[35] Rong H, Qian CX, Li L (2012) "*Study on microstructure and properties of sandstone cemented by microbe cement*." Construction Building Materials 36: 687–694. https://doi.org/10.1016/j.conbuildmat.2012.06.063

[36] Sahu R, Ayothiraman R, Ramana GV (2018) "*Shear behavior of Sand Reinforced with Human Hair Fibers*." In: Proceedings of 19th Southeast Asian Geotechnical Conference and 2nd AGSSEA Conference (19 SEAGC and 2 AGSSEA), pp. 3-5.

[37] Sahu R, Ramaiah BJ, Ayothiraman R, Ramana GV (2020) "*Dynamic properties of human hair fiber- reinforced yamuna sand*." Journal of Testing and Evaluation 48(6). https://doi.org/10.1520/JTE20180133

[38] Santoni RL, Webster SL (2001) "*Airfields and road construction using fiber stabilization of sands*." Journal of Transportation Engineering 127(2): 96-104. https://doi.org/10.1061/(ASCE)0733-947X(2001)127:2(96)

[39] Sharmila SMR, Narayanan KS, Arun S (2019) "*Experimental investigation of soil reinforced with human hair fibre and chloride compounds*." Engineering Research Express 1(1) 015017.

[40] Singh R, Singh S, Trimukhe KD, Pandare KV, Bastawade KB, Gokhale DV, Varma AJ (2005) "*Lignin-carbohydrates complexes from sugarcane bagasse: Preparation, purification, and characterization*." Carbohydrate polymers 62(1): 57-66. https://doi.org/10.1016/j.carbpol.2005.07.011

[41] Soon NW, Lee LM, Khun TC, Ling HS (2013) "*Improvements in engineering properties of soils through microbial-induced calcite precipitation*." KSCE Journal of Civil Engineering 17(4): 718–728. https://doi.org/10.1007/s12205-013-0149-8

[42] Tyagi S, Lee KJ, Mulla SI, Garg N, Chae JC (2019) "*Production of bioethanol from sugarcane bagasse: current approaches and perspectives*." Applied Microbiology and Bioengineering pp. 21-42. https://doi.org/10.1016/B978-0-12-815407-6.00002-2

[43] Wayal AS, Ameta NK, Purohit DG (2012) "*Dune Sand Stabilization using Bentonite and Lime*." Journal of Engineering Research and Studies 3(1): 58-60.

[44] Whiffin, VS (2004) "*Microbial $CaCO_3$ precipitation for the production of biocement*." Dissertation, Murdoch University, Perth, Australia.

[45] Wirawan R, Sapuan SM, Yunus R, Abdan K (2011) "*Properties of sugarcane bagasse/poly (vinyl chloride) composites after various treatments*." Journal of Composite Materials 45(16): 1667-1674. https://doi.org/10.1177/0021998310385030

[46] Wong YC, Sanggari V (2014) "*Bioethanol production from sugarcane bagasse using fermentation process.*" Oriental Journal of Chemistry 30(2): 507-513. http://dx.doi.org/10.13005/ojc/300214