

“GANGA RIVER SAND IS BIOCALCIFIED TO INCREASE SHEAR STRENGTH BEHAVIOUR FOR EROSION CONTROL.”

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Abstract - For various geotechnical applications, the properties of subsoil, such as their bearing capacity, shear strength, settlement characteristics, drainage, etc., are frequently improved using ground improvement techniques. Microbially induced calcite precipitation has been used to enhance the soil's physical characteristics. These methods can be applied to a fine-grained soil. The findings in this paper represent the first attempt to improve the Microbially induced calcite precipitation process. It has been discovered that MICP can decrease soil permeability while also increasing soil strength, stiffness, liquefaction resistance, and soil erosion resistance. A number of biochemical processes, including Microbially induced calcite precipitation processes, are influenced by both internal and external factors. The composition and concentration of the cementation solution, inoculation techniques, bacterial species, bacterial concentration, temperature, pH, soil properties, and injection strategies are just a few of the influential factors discussed in this paper. The bacteria culture – Bacillus SP 77, Sphaericus 78, Subtilis 79 filling in sand column tests indicated that a urea- calcium promoted the precipitation of calcite by bacteria. After determining the ideal urea- Ca² (Calcium) input rate, bacterial cultures were added to samples of Ganga River Sand, fed with the required chemical mixture, and kept at room temperature (250 –300 C) for 18 days. The samples' stiffness and shear strength increased along with the precipitated calcite content. Microbially induced calcite precipitation (MICP) activity to improve the soil's characteristics. The paper concludes by outlining some potential areas that ought to receive attention if biogeotechnology is to be applied in a way that is efficient, affordable, long-lasting, and durable under various construction circumstances

Key Words: Calcium Carbonate, MICP, temperature, Soil erosion, Soil improvement

1.INTRODUCTION In terms of geotechnical work, soft soil presents a number of challenges, including high water content, low shear strength, and low stiffness. These

characteristics have the potential to cause infrastructure damage or ground collapse during or after the construction phase. Soft soil can be strengthened using a variety of methods, including compaction, the use of prefabricated vertical drains, stabilization with cement and lime, and chemical grouting. (Arpajirakul, Pungrasmi, & Likitlersuang, 2021). For ground improvement there are various types of ground technique like physical, chemical and biological treatment. Of the options listed, physical methods are typically expensive and energy intensive, while chemical based methods can increase the material's embodied energy, produce a lot of CO₂ from hydration reactions, and produce more chemical residues during the production and execution processes that are launched into the environment. Fortunately, sustainable, eco-friendly, and energy-efficient biological methods have been suggested as alternatives to these physical and chemical methods (Wang, 2008). Microbial-Induced Calcium Carbonate Precipitation (MICP) has emerged as a promising biotechnological process that utilizes microorganisms to induce the formation of calcium carbonate minerals in soil and concrete structures. This innovative approach offers numerous advantages over traditional stabilization and repair methods, including its environmentally friendly nature, compatibility with existing materials, and potential for wide-ranging applications. Bacteria basically of two types pathogenic and non-pathogenic. We are using non-pathogenic bacteria for Microbially induced calcite precipitation (MICP). Non – pathogenic bacteria is easily survive in soil, easily adjust in the environment without any harm to human beings. The bacteria that cannot cause disease are known as non-pathogenic bacteria. Additionally known as non-infectious agents. They may even be advantageous to some hosts because they do not harm them. The most common microorganism for MICP are Lactobacillus, B. cereus, Bacillus lichenformis, B. megaterium, Bacillus pseudofirmus, B. Sphaericus (Jha, February 2022). The chemical breakdown

of urea into ammonia by ureolytic microorganisms raises pH (8–9.2) and produces two moles each of the ions ammonium and hydroxide from one mole of urea, while the one mole about carbonate ion that is produced raises the environment's alkalinity. The calcite that has built up in the soil's pore spaces has improved the soil's stiffness, strength, and behaviour (Sharma, Satyama, & Reddy, 2021). A good potential technology, MICP has the potential to be used in many different engineering applications. To fully comprehend the mechanisms of the process and to optimize the use of microorganisms for particular applications, more research is however required. (Arpajirakul, Pungrasmi, & Likitlersuang, 2021).

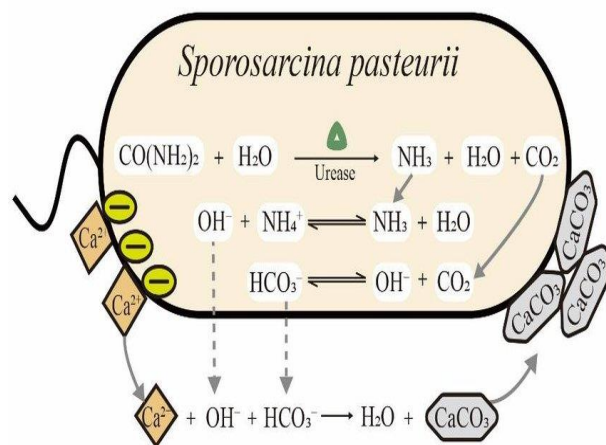


Fig.1.1 - Schematic-of-microbially-induced-calcite-precipitation

1.1 Objectives of Study

- Increase the strength and weight-bearing capacity of weak or loose soils by encouraging the precipitation of calcium carbonate within soil particles.
- Erosion Control: Using MICP treatments to reduce soil erosion and increase the stability of slopes, embankments, and riverbanks.
- Green building aims to promote environmentally responsible and sustainable building methods by lowering the demand for materials with a high energy content and lowering environmental impact.
- Cost Savings: To investigate the potential cost savings in construction projects by utilizing MICP for concrete repair and soil Stabilisation.
- By using MICP to increase the soil's resistance to erosion, to stop soil erosion and stabilize weak slopes and embankments in environmentally sensitive areas.

1.2 Materials & Methods

A proposed work on Microbial-Induced Calcium Carbonate Precipitation (MICP) will typically include

several important elements in its theoretical aspects. Here are some crucial factors to remember:

- Microorganism Selection:** A thorough assessment and selection of microorganisms appropriate for MICP should be a part of the proposed work. Finding microorganisms that can metabolize particular nutrients and produce calcium carbonate as a metabolic byproduct is a part of this process. (Almajed, Tirkolaei, & Kavazanjian, 2018) (Sharma, Satyama, & Reddy, 2021) (Jha, Feburary 2022)
- Nutrient Availability and Optimization:** In order to encourage calcium carbonate precipitation, nutrients must be both available and optimized. The proposed work should evaluate the nutrient needs of the chosen microorganisms and determine the best sources and concentrations of nutrients for effective precipitation. Consideration should be given to nutrient supplementation techniques such as pH leveladjustment and provision of essential elements. (Mujah, Cheng, & Shahin, 2019) (Jha, Feburary 2022).
- Environmental Aspects:** The theoretical aspects should take into account how the environment may affect MICP. Microbial activity and the rate at which calcium carbonate precipitates can be influenced by variables like temperature, moisture content, pH, and oxygen availability. For the proposed work, it is crucial to comprehend the ideal ranges for these environmental parameters and how they affect the MICP process. (Sharma, Satyama, & Reddy, 2021) (Yang , et al., 2020).

2. LITERATURE REVIEW

- Arvind Kumar Jha (2022)** The implications of biogeotechnology for enhancing various soil types through illustrating a summary of the underlying mechanisms (ex. bio stimulation and bioaugmentation-induced calcite precipitation) involved. The suitability, restrictions, and related bacteria and strains that are employed in the biotreatment of soils of the three most frequently used biological processes-ureolysis or urea hydrolysis, denitrification, and sulphate (SO42-) or iron (Fe) reduction-will be contrasted. Compared to sandy or silty soils, clayey soils need more attention from biogeotechnology to improve their behaviour.
- Do Jinung (2021)** As compared to the untreated soil surface, the treated soil surface displayed a different pattern of erosion and fewer changes in landform. Instead of having smooth particle transport, the cemented surface eroded and became fragile.. Due to the rapid injection seepage, cementation was less severe in the area close to the injection source. Due to variables

like the equilibrium between reaction speed and seepage speed, the impact the effects of gravity on the flow, and the boundary at the sample's edge, the area higher cementation was visible the farther away from the injection source.

[3] **Dubey Anant Aishwarya (2021)** The enrichment media (NB5U) successfully supported the growth of the soils' indigenous communities. The two subcultures of the cultivated communities were applied with a sterile loop to plates of nutrient agar. that had been additionally with 2% urea after being serially diluted (from 10⁻² to 10⁻⁶). It had 36 morphologically distinct single colonies on the urea agar base plates. subsequently got based on visual observation.

[4] **Zhao Jitong (2021)** To find three various types of fibres perform on MICP-treated calcareous sands, a series of water absorption, UCS, and SEM tests were performed on three different fiber-reinforced biocemented sands. The ideal glass fibre and polyester fibre contents were, respectively, 0.20% and 0.25%. The ideal concentration of hemp fibre fell between 0.20 and 0.25%. The results of the tests for unconfined compressive strength and water absorption showed that polyester fiber being the most efficient, subsequently glass fiber, and that hemp fiber was the least efficient.

[5] **Yang Yang (2020)** In order to bridge sand particles together, this research demonstrated a novel method for improving soil by carbonating RMC-based sand samples with the microbially induced carbonate precipitation (MICP) technique. In a calcium-rich environment, RMC-based samples performed on par with corresponding bio cemented samples using the conventional MICP method., demonstrating the viability of the suggested bio carbonation strategy for use in more widespread soil improvement.

[6] **Donovan Mujah et al. (2019)** The concentrations of bacterial culture (BC) and cementation solution (CS) on the various CaCO₃ precipitates significantly improved the soil strength and stiffness yet preserved a sufficient permeability characteristic of the bio cemented sand in civil engineering projects like transportation subgrades and embankments.

[7] **Shihui Liu et al. (2019)** The results suggested the soil had been treated with MICP had poor resistance to acid rain, wet-dry cycles, as well as freeze-thaw cycles. To improve the strength of MICP-treated sandy soil under

environmental deteriorations, fibre reinforcement and numerous MICP treatments were carried out.

[8] **Chaolin Fang. (2019)** Building materials' cracks can be repaired or improved using the well-established MICP process in cementitious materials, which involves plugging pore their technical characteristics. Consequently, it is expected, and then confirmed that bio stimulation caused calcium carbonate to precipitate on and inside the surface of numerous pores in CSRE samples, which ultimately produced an increase in compressive strength and a decrease in water absorption.

[9] **Jinung Do(2019)**This study showed that by bonding the particles with calcium carbonate, the MICP process increases the strength and resistance to erosion of sand. Depending on the degree of the improvement, the calcium carbonate precipitation was distributed differently. Since cementation is achieved by forming mineral bridges between particle contact points rather than by completely filling the voids, the change in hydraulic conductivity with the introduction of the MICP process under low confining stress is found to be minimal.

3.METHODLOGY

In the current study, Ganga River sand from Patna, Bihar, India. The lists of fundamental size of sand and its physical characteristics of the sand utilised Table 1. The sand used is categorised by the Indian standard (1498 - 1970). We will take Fine gained sand for this process.



Fig.3.1. Map of Bihar

It is most basic form of soil. It is one of the most unsuitable forms of soil for construction since it is also very low in permeability and has poor water retention. The basic physical characteristics, including grain density, shape, and size distribution, were measured. Several index

characteristics, such as the maximum and lowest void ratios, that describe physical attributes of this soil.

Chart 3.1.1: Sand Classification

Sand Classification	Size of Sand As per IS Sieve
Coarse	4.75 mm to 2.0 mm
Medium	2.0 mm to 425 micron
Fine	425 micron to 75 micron

Properties	Site (Ganga riverbank)
Coordinates	25°34'15"N 84°47'54"E
pH	9.5
Electrical conductivity (µS/cm)	9
Cation exchange capacity (meq/100g)	0
Predominant minerals	Quartz, mica
Environmental temperature	32 +- 3 °C
Specific gravity 2.7 2.65	2.6 to 2.8
Coarse sand content	
Silt content % (0.002–0.075 mm)	4 %
Clay content % (≤ 0.002 mm)	0
USCS classification	SP
USDA classification	SAND
Maximum dry density (kN/m3)	15.36

Chart 3.1.2 ; Properties of Sand

3.1 Characteristics of the Ganga River

The geological characteristics of the area and the river's dynamics have an impact on the Ganga River's sediment characteristics in Patna, Bihar. The Ganga River in the Patna region exhibits the following significant sediment characteristics:

1. Grain Size: Sand, silt, and clay are among the different types of grains found in the sediments of the Ganga River in Patna. Along the river channel and the floodplain, the distribution of grain sizes can change. Typically, sediments that have been deposited on the floodplain are finer grained than those that have been deposited closer to the river channel.

2. Alluvial Deposits: Alluvial sediments are transported and deposited by the Ganga River in Patna. Particles from upstream sources and the erosion of rocks in the Himalayas make up the majority of these sediments. The Ganga River's floodplain and riverbanks in the area frequently contain alluvial deposits.

3. Organic matter: The Ganga River sediments in Patna may contain a small amount of organic matter, which comes from plant matter and organic debris that the river has carried. The organic content of the sediments may have an impact on the stability of the sediment, the cycling of nutrients, and the overall health of the ecosystem.

It is important to note that depending on regional factors, river dynamics, and anthropogenic influences, sediment characteristics can differ at various locations along the Ganga River in Patna

4. Experimental Study

- Indian Standard Sieve: - To categorize different types of soil. Grain size distribution is the procedure in consideration.
- Tray frame 300 mm long, 225 mm wide, 50 mm height, Conical flask, measuring cylinder, Pipette.
- Bio safety cabinet: - It is also known as Laminar Flow. In this apparatus, air is travelling in the same direction and at the same speed. The system functions by allowing air to enter after passing through a pre-filter. The air is then directed towards the HEPA filters by the blower or fan (High Efficiency Particulate Air). Microbial culture is transferred aseptically using laminar air flow. For procedures that are sensitive to contamination, it serves as a superb aseptic work table.

- Autoclave: - An autoclave is a pressurized chamber used for the of sterilization and disinfection by combing four factors: time, pressure, temperature and steam
 - Hot Air Oven: - Hot air ovens are devices which use dry heat to sterilize the product for a given time period under particular circumstances, such as humidity, pressure, and other environmental variables.
 - Shaking Incubator: - The shaking incubator can be used for growth of cell including microbes or bacterial cultures, tissue cultures, and yeast..
 - Ph Meter: - The pH scale establishes the acidity or basicity of water. The range is 0 to 14, with neutrality denoted by 7 on the scale. A pH of higher than 7 denotes a base, whereas one less than 7 suggests acidity.
 - UV spectrophotometer: - It is a numerical method to figure out how much light an element of chemistry will absorb. By contrasting the total amount of light that travels through a sample with that which passes through an identical sample or a blank, this is achieved.
 - XRD - X-ray diffraction: - A common method for determining the crystallinity and structure of solid materials is X-ray diffraction (XRD). In conclusion, X-rays are scattered by the electrons of atoms present in the sample without changing their wavelength, which causes the crystal X-ray diffraction.
 - Titration: - Titration is a method of chemical analysis where the amount of a sample's ingredient is determined by adding a precise amount of a different substance whose reaction the desired constituent will have in a specific, known proportion to the sample being tested.
 - Uniaxial compressive strength (UCS): - The primary objective of the unconfined compression test is to quickly determine a measurement of the UCS of rocks or fine-grained soils that have sufficient cohesion to permit testing in the unconfined condition.
- #### 4.1 The sample's preparation and the MICP treatment procedure
- The untreated sand from the Ganga River sand was subjected to MICP treatment employing bacteria that produce urease under the following circumstances and setups:
1. Step 1st - First, we collect the soil sample from Ganga River, Patna, Bihar for microbial induced calcite preparation (MICP) for treatment of soil. Fig.1.
 2. Step 2nd - We make Solution (Nutrition broth) in flask of Peptone A + Peptone B + NaCl (Sodium Chloride) and its ratio of mixing solution ratio is 10: 10: 5 per gm for 1 litre then flask is sealed with aluminum foil. After making solution then kept in autoclave machine for around 2 to 3 hrs.
 3. Step 3rd - After completing process of autoclave then kept in Bio safety cabinet while doing this process first and equipment used in this machine with IPA (Isopropyl alcohol) then kept it bio safety cabinet for 15 minutes under UV rays to remove all the microbes or bacteria contamination from it after completing the process then we use sprit lamp box before starting any experiments in it then we add microbes in solution we made.
 4. Step 4th - The next step is to isolate the bacteria, such as Bacillus, Sphaericus, and, Subtilis from the sample. This is usually done through selective culture techniques. After adding bacteria culture (SP 77, SPH 78, SUB 79) in Nutrient Broth (NB) then kept in Shaking incubator for 3 days at 35^o C at 120 rpm to growth the culture.
 5. Step 5th: - We used local sand (from the Ganga River) in this method. To get 75-micron above sand, we first sieve it. Next, we wash it in normal water 10 or more times until the silt particles are removed, and then we add a 12-hour soak in HCL (to remove calcium carbonate from soil) to further remove the silt.
 6. Step 6th - After the 12 hours are up, wash it with regular water and then soak it in a solution of NaOH and water for 12 hours to remove the HCL. After the 12 hours are up, wash it with plain water and distilled water to remove the NaOH. To unsaturated dried sand, keep it in a hot air oven for 24 hours at 105^o C. Wet sieve will be used to measure the amount of silt in it after 24 hours have passed.
 7. Step 7th - Preparation of sample PVC tube with cap, 8 mm silicon pipe, a Scotch Brite pad used as a filter. For the PVC tube to remain water-tight, use m-seal(pidilite)and grout (Asian paint). Fig 1.1
 8. Step 8th - We'll test the sand pH, after thoroughly mixing 150 ml of distilled water and 60 gram of sand for 15

minutes, we measured the water pH level. We get pH value of sand 9.79 with 4% silt in it.

- Step 9th - We made SP 77, SPH 78, and SUB 79 cultures. Now we measure the OD of the culture using a UV spectrometer to determine turbidity, which should be between range 0.8 and 1.2. Optimum Density (OD) of Culture (bacteria).

SP 77 - 1.09
 SPH 78 - 1.1
 SUB 79 - 0.998

- Step 10th - After taking OD of culture, we add every culture in the sample tube for 24 hours after that nutrient growth will be provided to them.



Fig.4.1.1 Ganga River Soil collection.



Fig.4.1.2 Oven (to make sand dry)

- Making of cementation solution preparation: Nutrition broth (NB) ratio for per litre (10:10:5) Peptone A (type 1 bacteriological) + Peptone B + NaCl (sodium chloride). Then mix it properly so it is totally dissolved in DI water and then kept in Autoclave for 24 hours (DeJong, Fritzges, & Nüsslein, 2006) . After completing 24 hours then add Urea Solution – For 0.5 Mol in it.
 NH₄CL - Ammonium Chloride
 NaHCO₃ - Sodium bicarbonate
 CaCl₂ 2H₂O - Calcium chloride dihydrate.
- Collecting every sample drain water to check their pH and calcium content in it.
- Every treatment cycle began with the draining of the earlier provided cementation solution and the injection of freshly produced cementation solution that corresponded with the pore volumes.
- Testing the pH and titrating each sample tube daily to determine how hardness in the water is and how much calcium is present in the tube
- After competing 18 days of treatment then we will do strength test on the Uniaxial compressive strength (UCS), splitting tensile strength (STS) to determine the strength.



Fig 4.2.1. Bacillus, SP 77, SPH 78, SUB 79.

4.2 MICP treatment process:

The following is a description of the MICP treatment process:

- The cylindrical samples were prepared using PVC tube with diameter 40 mm length 200 mm an open from the top and a discharge nozzle at the bottom and hang it on a board.
- Adding 170gm of soil in it, after adding we will add 30 ml of distilled water in it to saturate the soil after saturation, we will add culture species of SP 77, SPH 78, SUB 79 in each 5 sample for 24 hours to survive in this environment.
- The Solution was drained out through silicon pipe from the nozzle at the bottom of PVC tube then we add cementation solution (urea and calcite precipitation). Therefore, in this investigation, the biotreatment or microbial induced carbonate precipitation was carried out for up to 18 days.



Fig.4.2.2 UV Spectrometer



Fig.4.2.3 PVC tube sample & Autoclave

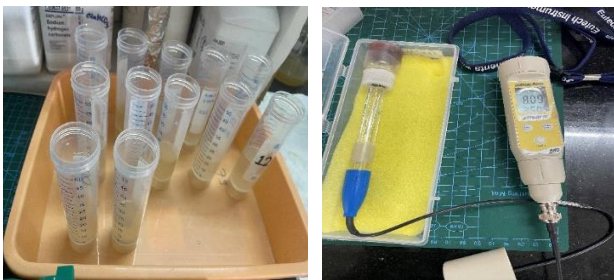


Fig. 4 The MICP treatment procedure is used to prepare and test UCS and STS samples.

5. RESULT AND ANALYSIS

The mechanical properties of soil are improved through a procedure called microbially induced calcite precipitation (MICP), which involves adding a solution containing bacteria, calcium carbonate, and urea into the soil. Calcium carbonate, and this can fill soil pores and bind soil particles together to increase soil strength and decrease soil permeability, is a byproduct of the bacteria's consumption of a substrate (like urea). The bacteria – Bacillus SP 77, Sphaericus 78, Subtilis 79 are used in the process and, the adding technique, and the type and conditions of the soil all affect how well MICP treatment works on soil.

However, in some soils, such as those with high organic content or those containing specific minerals that can inhibit bacterial growth, the efficacy of MICP treatment may be constrained. It was discovered that by using a highly concentrated calcium and urea solution and retaining up to half of the permeability, bio-cemented sand samples' UCS achieved two times higher strength (with the same number of crystals produced) than that of MICP treatment, indicating a promising future. Depending on the

needs of the project and the conditions of the site, the cost and viability of MICP treatment may also change. MICP treatment has the potential to be a useful technique for geotechnical engineering and environmental applications and has shown promising results for soil improvement.

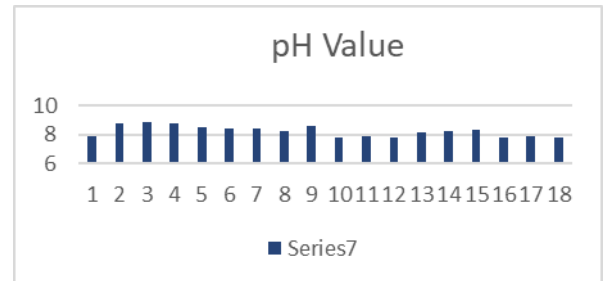


Chart.5.1: pH table

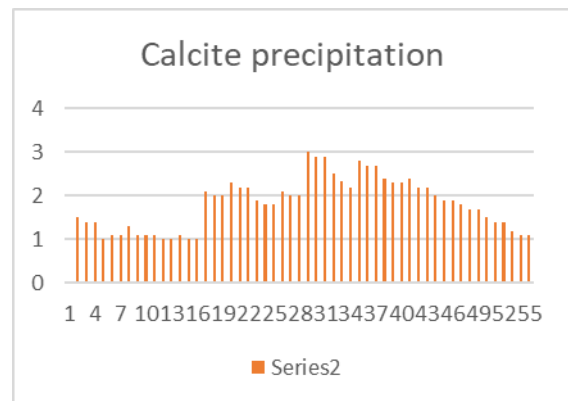


Chart:5.2- Hardness Test (Calcite precipitation) by using Titration

CONCULOSION

The present study looks at the use of MICP, or microbially induced calcium carbonate precipitations, to enhance soil, is a procedure that uses bacteria to induce the precipitation of calcium carbonate to produce materials that resemble cement. Numerous studies have been conducted on this technology, and it has been used in a variety of industries, such as construction, environmental remediation, a geotechnical engineering. According to MICP, soil stabilization, bioremediation, and construction could all be revolutionized by this promising new technology. It is simple to control and has a quick higher production efficiency of CaCO₃, urea hydrolysis is the most popular CaCO₃ precipitation mechanism. Highly urease active bacteria are among the best bacteria for soil bio-cementation, but they are not the only one. This innovative and fascinating area of biogeotechnology has a wide range of potential applications because it provides a long-term solution for a better future. Despite the difficulties, MICP has potential for addressing some of the current issues with soil improvement. Before the MICP process is directly applied in the field, more study should

be focused on optimizing it at both the micro and macro levels. The following is an overview of the key findings of the study.

- a) The particle size distribution affects how well MICP improves the shear strength parameters of sand mixtures.
- b) The effectiveness of MICP treatment may be decreased by the presence of fine particles.
- c) As the fine content rises, the impact of produced CNPs on shear strength decreases.
- d) Plastic fines have a greater negative impact on sandy soil MICP treatment efficiency than non-plastic fines. When shear failure occurs, sands' contractionary behavior changes to dilatatory behavior due to MICP treatment and a high rate of biocementation.
- e) When compared to pure sand, sands mixed with fines reach their shear strength peaks after undergoing significant shear strain.
- f) Under all normal stresses, the strain in treated SC samples is greater than that in treated SM samples at the time of shear failure.
- g) The first 7 days of MICP treatment are when shear strength parameters improve the most. As a result of the biological and physical problems, the rate of improvement declines to a negligible amount after the 18th day.

It's important to remember that the specific outcomes and results of MICP can change depending on the study's goals, available resources, and potential application scenarios. To fully grasp and utilize the potential of MICP in various fields, additional study, experimentation, and optimization are required

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