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Research Article

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Thermal Effects on the Strength Behavior of Reinforced Organic Soil Treated with Microbially Induced Calcite Precipitation

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Abstract

Organic soils have been noted for their weak structural strength, high compressibility, and poor bearing capacity. In this study, the use of MICP and Basalt fibre is investigated to improve the properties of organic soil obtained from Ziro Valley. The UCS of untreated organic soil was recorded to be 96 kPa, but it was observed to improve drastically with the treatments used. For the case of the use of MICP, treatment at 1.5 OD and 1.5 M resulted in a UCS of 487 kPa, while basalt fibre treatment at 1% resulted in a UCS of 215 kPa. With the application of both treatments, there was an observed synergy resulting in a maximum UCS of 585 kPa at 1.5 OD, 1.5 M, and 2% fibre. The optimum maximum dry density improved from 13.12 to 15.6 kN/m³ with reduced plasticity. The freeze-thaw results revealed that the treated samples had improved strength compared to the untreated organic soil samples**.**

Keywords: Organic soil, MICP, Basalt fibre reinforcement, UCS, Bio-cementation, Freeze–thaw resistance

1. Introduction

The increase in urban development coupled with an increased population and lack of adequate land necessitates consideration of other types of soils that were initially disregarded. One of these challenging soils is organic soil. Organic soils can be described as soils found in wetlands, marshes, and bogs containing a significant proportion of plant material in a state of decomposition referred to as peat and muck. As a result of the high organic matter in these soils and the moisture content, their engineering properties are not favorable and unreliable.

One of the issues related to organic soils is their very poor bearing capacity. This arises due to the high water content in addition to the weak structure formed by the decomposing organic matter [1]. In these soils, there is primary and secondary compression when the soils are subjected to any load. Primary compression occurs early in consolidation and results in considerable volume reduction. However, even after consolidation, secondary compression takes place owing to repositioning and decomposition of the organic matter [2]. Therefore, the organic soils have low shear strength and high compressibility, thus, cannot support loads unless treated. One problem that arises is that of over-consolidation, which can cause instability in structures constructed on such soils [3]. The presence of a high degree of moisture can also cause shrinking and swelling, as well as permanent deformation [4]. Furthermore, because of the low shear strength and high compressibility nature of the soils, it becomes very hard to compact them in the field [3][4]. This is why soil stabilization techniques have been developed to enhance their engineering characteristics [5].

The study involves the collection of soil samples from Ziro Valley in Arunachal Pradesh, India. This place is located at an elevation of approximately 1,500 m above sea level, where there is heavy vegetative cover and distinctive climatic features [6]. All these parameters help form organic soils with unique engineering characteristics [6]. Hence, it is essential to investigate various stabilization techniques for enhancing their engineering characteristics.

One approach that has been recently introduced is Microbially Induced Calcite Precipitation (MICP). It is a biological method where ureolytic bacteria, like Sporosarcina pasteurii, hydrolyze urea and release carbonate ions. In turn, the carbonate ions react with calcium ions to form calcite ($CaCO_3$) [7]. The resulting calcium carbonate bonds soil grains together, which increases their strength and stiffness but lowers their permeability

[8]. Additionally, when compared to conventional stabilizing materials, such as cement and lime, MICP is more eco-friendly [7]. Moreover, previous research indicates that the technique positively impacts the unconfined compressive strength and longevity of soils via biocementation [7] [8]. Fiber reinforcement is another technology utilized for the improvement of soil. One type of fibers, which is gaining popularity among others, is basalt fibers due to their tensile strength, chemical stability, and environmentally friendly production process [9]. Basalt fibers are obtained from volcanic rocks and show improved mechanical properties compared to synthetic fibers [9]. When applied to soil, such fibers form a reinforcing skeleton in soil and improve its ability to resist deformation and increase shear strength [10]. Fiber reinforcement of soils also reduces brittleness and increases their bearing capacity [9] [11].

Recently, research studies began to combine two mentioned methods in order to benefit from both bonding and reinforcement at once. Thus, basalt fibers are combined with calcite precipitates obtained with MICP technology [12]. Due to the interaction of calcite precipitation (providing bonding of soil particles) and fibers (reinforcement and increase of ductility), an increased stability and bearing capacity can be achieved with the combination of technologies [13] [14].

In addition to the strength, resistance to environmental variations also plays a significant role [15]. In cases where the soil is subjected to freeze-thaw cycles due to low temperatures or altitudes, this phenomenon becomes a crucial aspect that impacts the soil behavior to a great extent [16]. The formation of ice within the pores creates disturbances in the structure, and during thawing, the soil loses strength. Therefore, it becomes vital to test the performance of the stabilized soils against temperature variations [17].

This paper aims at evaluating the improvement of organic soil samples retrieved from the Ziro valley through the use of microbial-induced calcite precipitation (MICP) technique along with basalt fiber reinforcement. First, the soil samples were subjected to compaction tests to determine the maximum dry density (MDD) and optimum moisture content (OMC). Then, the soil samples were stabilized using MICP technique using Sporosarcina pasteurii bacteria, followed by basalt fiber reinforcement. Afterward, the combined effect of both techniques on the soil was assessed. In the current research, freeze-thaw analysis was carried out on the stabilized soil samples to expose them to environmental changes by applying the freeze-thaw cycles to check their performance under such environmental circumstances. In other words, the purpose of this experiment is to see whether the stabilized organic soil can withstand temperature variations while keeping its sufficient stability and strength.

2. Materials

2.1 Soil (S): Soil samples have been collected from a depth of 1 meter of the soil profile in Ziro Valley, which is positioned at an altitude of around 1,500 meters above sea level. Ziro Valley is positioned at the latitude of around 27.5°N and the longitude of 93.8°E.



Fig 1: Organic soil sample at the site.

2.1.1 Physio Chemical Properties of Soil

A series of soil property tests were conducted in the lab, complemented with visual inspection of the soil. The natural water content of the soil turned out to be very high – 71.2%, which indicates its good moisture retention capability. This trait is rather characteristic of organic soils [18]. Specific gravities varied between 1.33 and 1.35, which is significantly lower than for mineral soils, indicating a lot of organic

matter present [19]. The soil does not include a gravel component; instead, its texture is 15.46% sand, 6.12% silt, and 78.42% clay. Therefore, one can conclude that the soil is mainly fine-grained [19]. Based on the Indian Standard soil classification method, the soil belongs to OH class, meaning that it is an organic soil that features a high level of plasticity [20]. The liquid limit and plastic limit of the soil were estimated to be equal to 53.04% and 20.13%, respectively [20]. The optimum moisture content was determined to be 23.44%, and the maximum dry density was calculated to be 13.12 kN/m³. These properties are relatively low, considering that they are compared to mineral soils, but this is because of the very high level of organic matter found in the sample. The organic matter present in the sample is also estimated to be 60.62% and, as such, indicates that it is highly organic soil [18]. The appearance of the soil sample, depicted in Figure 1, shows that this is so. It can be seen to be dark brown or black in color and has visible fibrous plant materials and decaying organic matter present [18]. It is also spongy and fibrous in nature.

In summary, the above features show that the soil is weak, highly compressible, and has a low load bearing capacity. Thus, it is unsuitable for direct construction purposes.

Table 1: General properties of the natural soil.

Property	**Result**
Water Content	71.2%
Specific Gravity	1.33-1.35
Sieve analysis	
Gravel size (> 4.75mm)	0%
Sand size (0.075mm - 4.75mm)	15.46%
Silt (0.075 mm - 0.002 mm)	6.12%
Clay (<0.002mm)	78.42%
IS Classification of soil	OH (peat)
Liquid limit	53.04%
Plastic limit	20.13%
Plasticity Index	Medium plastic
OMC	23.44%
MDD	13.12 KN/m²
Organic content	60.62%

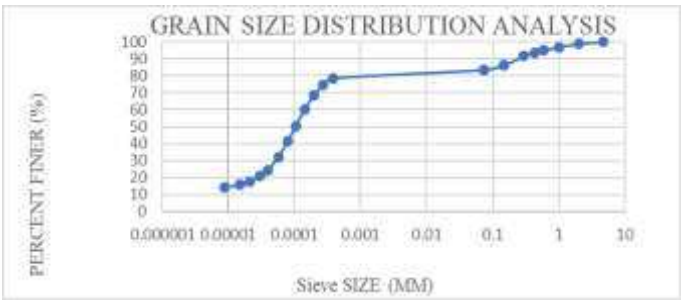


Fig. 2: Grain size distribution curve

Table 2: Basic characteristics of the soil sample.

Soil possesses the features usually found in weak and difficult geomaterials. The acidity of its pH level (4.8–5.0) indicates weak aggregation and less bonding of particles, resulting in poor mechanical strength. Due to the low electrical conductivity and low TDS level, one can conclude that there is a lack of minerals in solution. As a result, the number of ions for cementing particles together is insufficient. Even though

soil is highly resistant to electrical current and moderately hard, this indicates that there is not much ion mobility, which prevents any interaction of charges and nutrients in the soil [22].

Ph	4.8-5
ORP (mV)	138-140
Resistance	2340.2-2348.4
Conductivity (mS)	0.427-0.464
TDS (ppm)	284-289
Salt (%)	0.01
Hardness (PPM)	213-215
Dissolved Oxygen (mg/l)	6.3-6.5



Fig. 3: Determination of the organic content of a sample using a muffle furnace.



Fig.4: Burned Organic soil



Fig. 5: Determination of the physical and chemical parameters of a soil sample using a multiparameter device.

2.2 *Sporosarcina pasteurii:*

Bacillus Sp (MTCC No. 4445) bacteria will be used in the study and has been obtained in a powder form from the Microbial Type Culture Collection and Gene Bank in Chandigarh, India. The bacteria collected is kept in airtight containers to prevent contamination and moisture ingress.



Fig.6 : Bacillus Sp. Bacteria in powder form

2.3 Bacterial Solution (BS):

A standard nutrient broth was prepared using the following composition (per liter): peptone 5.0 g, beef extract 3.0 g, and sodium chloride 3.0 g, given in table 1. the final pH was adjusted to 6.8 ± 0.2 at 25°C. The powdered medium was dissolved in distilled water, and its pH was accurately adjusted to 7.0. The solution was then sterilized in an autoclave at 15 psi (121°C) for 15 minutes. After autoclaving, the medium was exposed to ultraviolet light for 15 minutes under sterile conditions to reduce contamination risk. The powdered bacteria was aseptically introduced into the sterile medium within a laminar-flow cabinet. The culture was incubated at 30°C. Bacterial suspensions (BS) were harvested and diluted to optical densities (OD600) of 0.5, 1.0, and 1.5, measured using a spectrophotometer. Aliquots were stored at 4°C for subsequent use, with subculturing performed as needed.



Fig 7: Preparation of Nutrient Broth



Fig 8:Nutrient Broth



Fig.9: *Autoclaving Nutrient Broth.*



fig 10: Control Media



Fig.11: Inoculation of bacteria



Fig. 12: Sample kept in incubator



Fig. 13: After 24 hr Incubation.



Fig. 14: Spectrophotometer

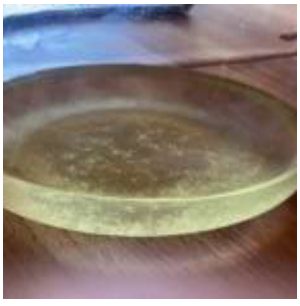


Fig.15: 48-hour incubated bacterial culture

Bacterial stock suspension was made using 500 ml of nutrient broth. This volume was poured into four sterile culture tubes, which were later sterilized at 121°C for 15 minutes (15 psi) in an autoclave. Following sterilization, these culture tubes were exposed to ultraviolet light in a laminar flow cabinet for 15 minutes. Bacteria inoculum contained in a sealed glass ampoule was added to three tubes aseptically; while remaining one was kept aside without inoculation for serving as the control for OD measurement. These culture tubes were then incubated at 30°C aerobically. Growth of bacteria in these culture tubes was estimated by taking OD values using a spectrophotometer at 600 nm. In general, OD value became 0.5 at 12 h, OD value became 1.0 at 24 h, and OD value became 1.5 at 48 h of incubation time. Such suspensions having growth were stored in the refrigerator at 4°C, and fresh cultures were obtained when needed.

Table 3: Nutrient broth composition

Sl. No.	Composition	Quantity
1.	Peptone	5g
2.	NaCl	3g
3.	Beef extract	3g
4.	Distilled water	1000 g

2.4 Cementation Solution:

The MICP treatment employed in this research was done by mixing ESP with urea as the main ingredients for microbial precipitation of $CaCO_3$ using the urease enzyme secreted by Sporosarcina pasteurii. Eggshells were initially washed with distilled water before being dried in an oven at a temperature of 50–60 °C for 2–3 hours. The eggshells were later grounded into fine powder after the drying process. The eggshell powder was then incorporated in the soil mixture at 0.5 % by mass of soil. Even though eggshells are majorly composed of $CaCO_3$, the amount of calcium extracted from eggshell powder alone is unlikely to supply the necessary concentration of Ca^{2+} ions needed for calcite precipitation. Therefore, calcium chloride ($CaCl_2$) was also included in the cementation solution to ensure an adequate supply of calcium ions for microbial calcite formation. The cementation solution used for the MICP treatment was prepared at a concentration of **0.5 M**, as lower molar concentrations are known to produce more uniform calcium carbonate precipitation in fine-grained and organic soils. Organic soils contain high water content and organic matter, which can reduce the efficiency of MICP at higher concentrations due to rapid and uneven precipitation of calcium carbonate. Therefore, a moderate concentration was selected to obtain better bonding between soil particles and more stable improvement [24][25]. Since **eggshell powder (ESP)** was also added in this study as an additional calcium source, using a lower molar concentration helps to control excessive calcite formation and provides more uniform cementation throughout the soil matrix. Similar behaviour has been reported in studies on bio-cemented and calcium-treated soils, where moderate cementation concentration produced better consistency and strength improvement [44]For preparing 0.5 M cementation solution, urea and calcium chloride were dissolved in distilled water in equal molar concentration. For one litre of solution, 30 g of urea ($CO(NH_2)_2$) and 73.5 g of calcium chloride dihydrate ($CaCl_2 \cdot 2H_2O$) were used. The chemicals were mixed thoroughly until complete dissolution, and the prepared solution was used along with bacterial solution and 0.5% eggshell powder (ESP) for the treatment of organic soil specimens. Although calcium chloride was used in the cementation solution as the main calcium source for the MICP process, eggshell powder (ESP) was also added to improve the efficiency of soil stabilization. Calcium chloride provides soluble calcium ions for calcite formation, but the reaction can occur very quickly and may lead to non-uniform precipitation, especially in fine-grained and organic soils. The inclusion of ESP that has a large quantity of calcium carbonate functions as another slow calcium source to ensure uniform bonding of the soil particles [24].

The use of extra calcium in organic soils due to ESP contributes to ensuring continuous formation of calcite and makes the soil more stable. Similar improvement was observed during a study involving eggshell powder in soil stabilization that led to enhanced particle bonding and engineering behavior [45].



Fig. 16: Preparation of Cementation Solution



Fig.17: Cementation Solution Mixing Process

2.5 Basalt fiber:

The basalt fiber used in this research was sourced from IndiaMART, an online business platform that deals with different kinds of industrial products. The basalt fibers that will be used in the study will be between 9 millimeters and 12 millimeters. This choice of the lengths of basalt fiber was made after a careful evaluation of literature on the topic. According to the information in the literature, the chosen lengths of basalt fibers should provide better results for the purposes of the experiment..



Fig. 18: 9 mm to 12 mm length basalt fibre

3. Methodology

3.1 Details of Laboratory Tests:

All laboratory investigations have been done using procedures specified in the relevant Indian Standard (IS) code. As majority of the soil particles had passed the 75 μm sieve test, the hydrometer analysis method has been used to find out the size distribution of the finer particles in the soil. The soil size distribution curve has been prepared on the basis of dry sieve analysis test results and hydrometer test results. The specific gravity of the soil samples was found out by using a pycnometer as per IS Code requirements. Both liquid limit and plastic limit tests for natural soil and soil with additives have been done using the cone penetrometer.

The Standard Proctor Compaction Test has been performed to determine MDD and OMC of the soil. Unconfined Compressive Strength (UCS) tests were carried out on both untreated and stabilized soil samples after curing periods of 0, 7, 14, and 28 days. For each mix and curing duration, three specimens were prepared and tested, and the average value was taken as the representative UCS strength obtained from the experiment.

To evaluate the durability of the stabilized soil, freeze–thaw tests were also performed on the UCS specimens. The samples were subjected to 0, 3, 5, 7, and 10 freeze–thaw cycles**.** One freeze–thaw cycle consisted of freezing the sample at −20°C for 12 hours followed by thawing at 0°C for 12 hours. Thus, 3 cycles indicate that this freezing and thawing process was repeated three consecutive times before conducting the UCS test.

For the Microbially Induced Calcite Precipitation (MICP) treatment, the prepared soil specimens were wrapped in plastic to maintain moisture and prevent contamination during the curing period. The MICP-treated samples were cured for 3, 7, and 14 days to study the influence of curing duration on microbial activity and calcite precipitation. Previous studies have shown that microbial activity and calcium carbonate formation increase during the initial curing period, with 7 days often showing the most effective calcite precipitation and strength improvement. Therefore, the curing period that showed the maximum microbial activity and strength gain was considered as the optimum curing duration and was adopted for further UCS testing of the MICP-treated soil samples.

To better understand the mechanisms responsible for strength improvement, Scanning Electron Microscopy (SEM)**,** Energy Dispersive X-ray Analysis (EDX)**,** and X-ray Diffraction (XRD) tests were carried out on the optimum stabilized mixes. These tests allowed for an analysis of the microstructural modifications, mineralization, and elemental makeup of the soil samples treated, specifically the formation of calcium carbonate (calcite), which occurs by the MICP treatment method and results in the better bonding of soil particles and increases soil strength.

Sample Preparation:

The first test done was the compaction test, which gave the optimum moisture content needed to make the soil specimens. This helped determine the amount of water that should be put into each specimen.

In preparation of the specimens with basalt fiber reinforcement, basalt fibers were added to dry soil in the quantities of 0.5%, 1.0%, and 1.5%. These materials were mixed well to achieve a homogeneous mixture after which they were combined with water corresponding to the optimum moisture content. The addition of fibers is meant to increase the soil strength while reducing cracks.

In case of the MICP treated samples, a bacterial suspension (BS) of 0.5 OD, 1.0 OD, and 2.0 OD as well as a cementation solution (CS) of 0.5 M, 1.0 M, and 1.5 M was used. The bacterial and cementation solution were mixed in equal proportions and then added to the soil to achieve a mixture. The specimens made were later extruded then wrapped in plastic. As far as the MICP and basalt fiber composite specimens are concerned, the initial procedure involved mixing the basalt fibers, at percentages of 0.5%, 1.0%, and 1.5% with dry soil. Afterwards, the bacterial suspension and cementation solution were added in the specified proportions. Once the blend was well-mixed, the specimens were compacted into the mold to prepare the UCS specimens.



Fig 19: Preparation and curing of UCS soil specimens sealed with plastic for MICP treatment



Fig 20: Materials used for sample preparation

3.2 Mix Designation of the Soil Samples

The naming convention adopted for various materials has been stated as follows: S = untreated parent soil, BS = bacterial suspension, CS = cementation solution, and BF = basalt fiber. The systematic mix nomenclature system was adopted to make it easier to recognize which material and how much of each was being introduced into the soil sample. All the combinations used in the experiment have been summarized in Table 4.

A systematic way of designating the mixes was achieved through numerals preceding the various symbols representing their respective concentrations or percentages. For example, the notation S + 0.5OD + 0.5M would represent a specimen made up of S (soil), bacterial suspension of concentration 0.5OD, and cementation solution of concentration 0.5M. Likewise, notation such as S + 0.5OD + 0.5M + 0.5% BF would mean that the specimen included S (soil) along with a bacterial suspension of concentration 0.5OD, cementation solution of concentration 0.5M, and basalt fiber of 0.5%.

Table 4. Mix Designation of the Soil Samples

Designations	Mix	Bacterial solution (OD)	Cementation solution (M)	Basalt Fiber (%)	Curing (days)
S	Soil				0,3,7,14,28
BS	Bacterial solution	0.5, 1.0, 1.5			
CS	Cementation solution		0.5		
BF	Basalt Fiber			0.5, 1.0, 2.0	

S+BF	Soil-Basalt fiber			0.5, 1.0, 1.5	
S+BS+CS	Soil-Bacterial solution-Cementation solution	0.5, 1.0, 1.5	0.5		
S+BS+CS+BF	Soil-Bacterial solution-Cementation solution-Basalt Fiber	0.5, 1.0, 1.5	0.5, 1.0, 1.5	0.5, 1.0, 2	

4. Results and Discussion:

4.1 Liquid Limit and Plastic Limit

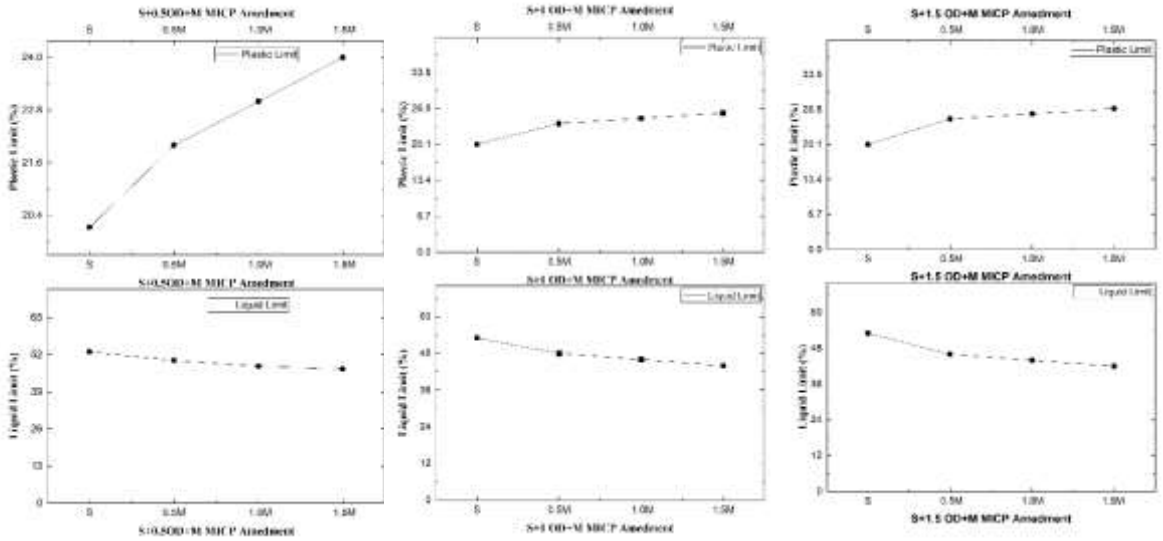


Fig.21 Variation of Liquid Limit and Plastic Limit at Different Optical Densities

As shown in Fig.21, the untreated organic soil had a liquid limit of 53.04% and a plastic limit of 20.13%. The result indicated that the untreated organic soil is characterized by plasticity properties that are prevalent among organic and clayey soil [7]. On the other hand, upon treatment with the MICP and eggshell powder, the liquid limit showed a progressive reduction, while the plastic limit had a slight increase. The reason for this could be explained by the bio-cementation process, wherein precipitation of calcium carbonate creates bonds between soil particles, decreasing the thickness of the diffuse double layer and thus the soil's ability to absorb water [23][24].

With a constant optical density of 0.5 OD, the increasing concentration of MICP reduced the liquid limit from 50% to 47%. This means that the increased concentration of MICP results in the increased precipitation of calcite which fills up the voids and binds soil particles effectively [25][26]. The plastic limit rose from 22% to 24%, implying that the treated soil required slightly higher moisture to achieve its plastic state because of its increased stiffness and strong particle bonds resulting from calcite formation [8].

The addition of eggshell powder also played a key role in reducing plasticity because eggshell powder is rich in calcium carbonate that helps to act as a stabilising material to enhance cementation processes for fine-grained soils [27][28]. These reductions in the liquid limit and increase in plastic limit are similar to the findings of previous research done in clay and organic soils using calcium-based additives and bio-cementation techniques [25][29]. It can be concluded from the above results that the combination of both MICP and additives has improved the consistency of organic soils. At 1.0 OD, organic soils treated with different concentrations of MICP revealed a reduction in the liquid limit from 48% to 44%, while the plastic limit was increased from 24% to 26%.This implies the improved consistency behavior of the soil, which is brought about by the bio-cementation process, where the precipitation of calcium carbonate cements soil particles together and lowers their water retention capability [30].. An increase in the concentration of MICP will lead to more calcite production, thus filling the pores and reducing the plasticity of the soil while increasing its rigidity. The increment in the plastic limit value signifies more

binding among particles owing to the cementation process, which requires more water to attain a plastic state of the soil [32]. The incorporation of eggshell powder also increases the efficiency of this process since it is a rich source of calcium carbonate, which promotes particle cementation and lowers the affinity for water in the soil [33]. At 1.5 OD, the liquid limit was found to reduce from 46% to 42%, whereas the plastic limit was increased from 25% to 27% due to the rise in MICP concentration. This is attributed to the greater decrease in liquid limit due to high bacterial density resulting in more calcium carbonate production via MICP reactions. Calcium carbonate deposition enhances the density of soil structure and lowers its capacity for holding water between soil particles [26]. An increase in plastic limit indicates that the treated soil gains stability and resistance to water content changes, owing to enhanced particle binding. High bacterial activity promotes better soil cementation, increasing soil stiffness and plastic limit at high water content [23].

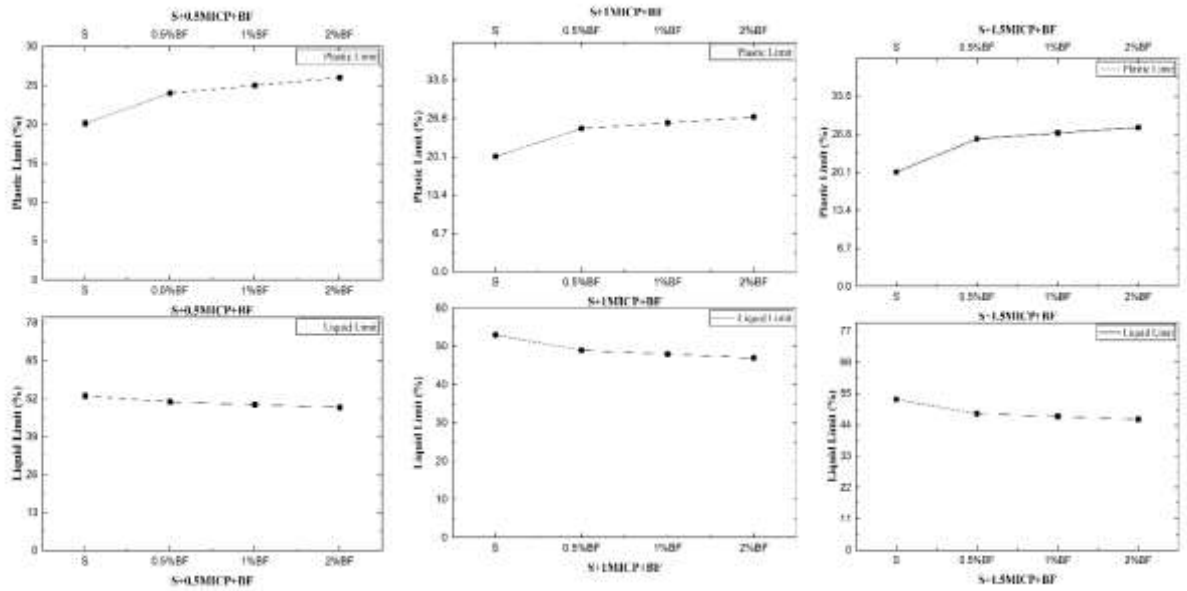


Fig.22: LL and PL at different BF (%)

With the addition of basalt fiber, the liquid limit increased from 54% to 56% and the plastic limit increased from 22% to 24% as the fiber content increased from 0.5% to 2%. This increase may be due to the higher surface area of fibers, which requires more water for proper mixing and uniform distribution in the soil [15]. The rise in plastic limit indicates better interlocking between soil particles due to the presence of fibers, which makes the soil more resistant to deformation and requires more water to reach the plastic state [34]. Similar behaviour has been reported in fiber-reinforced soils, where the addition of fibers slightly increases the Atterberg limits because of changes in soil structure and moisture distribution [11].

4.2 Compaction

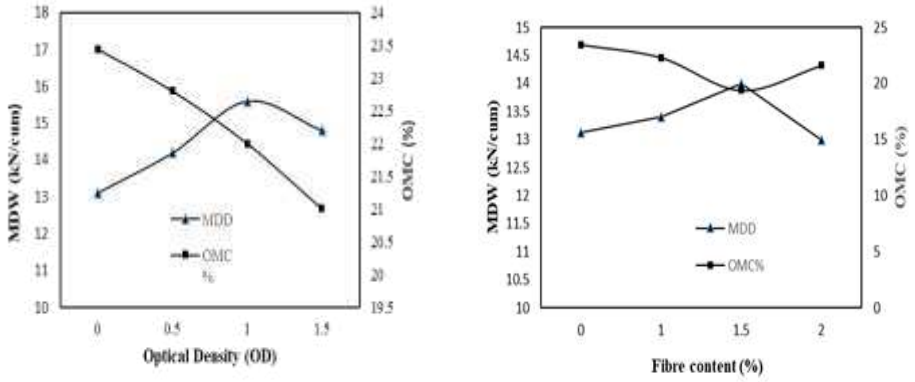


Fig.23: Variation of MDW and OMC with optical density and fibre content.

Compaction tests were carried out on the parent soil and soil treated with different optical densities (OD) of bacterial solution to determine the maximum dry unit weight (MDW) and optimum moisture content (OMC). The untreated soil (OD = 0) showed a maximum dry unit weight of 13.12 kN/m³ at an optimum moisture content of 23.44%. After adding the bacterial solution, clear changes in the compaction behaviour of the soil were observed.

Fig 23 shows that as the optical density increased, the MDW value increased up to 1.0 OD and then slightly decreased at higher concentrations. At 0.5 OD, the MDW increased to 14.2 kN/m³ with OMC of 22.8%, while the highest MDW of 15.6 kN/m³ was obtained at 1.0 OD with OMC of 22%. When the optical density was further increased to 1.5 OD, the MDW slightly decreased to 14.8 kN/m³, and the OMC reduced to 21%. The increase in dry unit weight with bacterial treatment may be due to the precipitation of calcium carbonate during the MICP process, which fills the voids between soil particles and makes the soil structure denser [7] [24]. Because of this improved particle bonding, less water is required for compaction, resulting in a decrease in OMC [8]. This indicates that bacterial treatment improves the compaction characteristics of the soil up to an optimum concentration, after which the improvement becomes less significant [26]. Compaction tests were also conducted on the soil mixed with different percentages of basalt fibre to study the variation in maximum dry unit weight (MDW) and optimum moisture content (OMC). The untreated soil (0% fibre) showed an MDW of 13.12 kN/m³ at an OMC of 23.44%. After the addition of basalt fibre, changes in the compaction characteristics of the soil were observed.

With the inclusion of fibre, the MDW initially increased and then decreased at higher fibre content. At 1% fibre, the MDW increased slightly to 13.4 kN/m³ with OMC of 22.3%. The maximum MDW of 14 kN/m³ was obtained at 1.5% fibre, along with a reduced OMC of 19.4%. However, when the fibre content was increased to 2%, the MDW decreased to 13 kN/m³, while the OMC increased to 21.6%.

The initial increase in MDW may be due to the reinforcing effect of basalt fibres, which helps in better interlocking of soil particles and reduces the void spaces, resulting in higher density[34]. At higher fibre content, the MDW decreases because excess fibres create obstruction in proper compaction and increase the void ratio [15]. The variation in OMC indicates that the presence of fibre changes the water requirement of the soil, as fibres absorb some moisture and also affect the arrangement of soil particles during compaction.

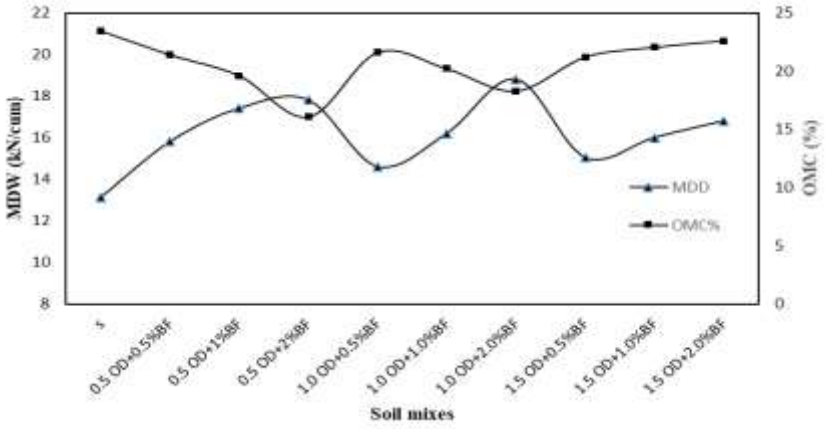


Fig.24: Variation of MDW and OMC of various soil mixes

The figure 24 shows the variation of maximum dry unit weight (MDW) and optimum moisture content (OMC) for soil treated with different combinations of bacterial solution (optical density) and basalt fibre content. The untreated soil shows a MDW of about 13.12 kN/m³ with OMC around 23%. After the addition of bacterial solution and fibre, noticeable changes in compaction behaviour can be observed.With the addition of bacterial solution along with basalt fibre, the MDW generally increases up to an optimum combination and then decreases at higher contents. The increase in MDW can be attributed to the precipitation of calcium carbonate during the microbial-induced calcite precipitation (MICP) process, which fills the voids between soil particles and increases the density of the soil mass [8] [12]. The presence of basalt fibre further improves the interlocking and bonding between soil particles, resulting in better compaction characteristics [9]. Because of this combined effect, higher density can be achieved at lower moisture content.At higher fibre percentage or bacterial concentration, the MDW starts decreasing. This may be due to excess fibre creating resistance to particle rearrangement during compaction, leading to an increase in void ratio [35]. Similarly, higher bacterial content may cause non-uniform calcite precipitation, which reduces compaction efficiency [24]. The OMC values show an opposite trend to MDW in most of the mixes, indicating that improved bonding between particles reduces the amount of water required to reach maximum compaction.Overall, the results indicate that an optimum combination of bacterial solution and basalt fibre exists at which the soil attains higher dry unit weight and lower moisture requirement compared to untreated soil, showing improvement in compaction behaviour. Similar trends have been reported in previous studies on bio-cemented and fibre-reinforced soils.

4.3 Unconfined Compression Strength Tests

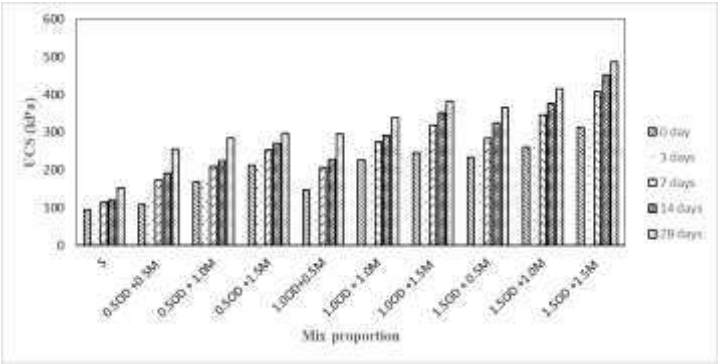


Fig.24: UCS of soil for different OD and cementation solution molarity at various curing periods

The unconfined compressive strength (UCS) values obtained for soil treated with different optical densities (OD) of bacterial solution and different molarities (M) of cementation solution at various curing periods are presented in Fig. 24. The untreated soil showed a UCS value of 96 kPa at 0 day curing, which gradually increased to 153 kPa at 28 days. This small increase may be due to slight densification and moisture redistribution with time. However, when the soil was treated with bacterial solution and cementation solution, a considerable improvement in strength was observed at all curing periods.

For all the mixes, the UCS increased with increase in curing time from 0 to 28 days. This behaviour indicates the gradual development of bonding within the soil matrix. The enhancement in strength can be attributed to microbially induced calcite precipitation (MICP). As per this mechanism, bacteria existing in the solution produce an enzyme called urease, which breaks down urea available in the cementation solution into carbonate ions. These carbonate ions react with calcium ions to produce calcium carbonate, which precipitates in the pores existing in between the soil particles. Calcium carbonate serves as the cementing agent and increases the strength of soil [7][24].

From the above data, it is also evident that UCS values increased when there was an increase in the optical density of the bacterial solution and the molarity of the cementation solution. The increase in optical density indicates a higher number of bacterial cells leading to enhanced urease activity and production of calcite. Likewise, an increase in the molarity of the cementation solution will result in increased calcium ions for precipitation [8].

Among all the mixes, the combination of 1.5OD + 1.5M gave the highest UCS value at all curing periods, reaching 487 kPa at 28 days. This shows that sufficient bacterial concentration, along with an adequate cementation solution, is necessary for effective bio-cementation.

The continuous increase in strength with curing time indicates that the precipitation of calcium carbonate is a time-dependent process and requires proper curing for the bonding to develop fully. Similar trends of strength improvement with an increase in bacterial concentration, cementation solution content, and curing period have been reported by earlier researchers working on MICP-treated soils [7] [23]. The results clearly show that the use of bacterial solution together with cementation solution is effective in improving the strength characteristics of organic soil.

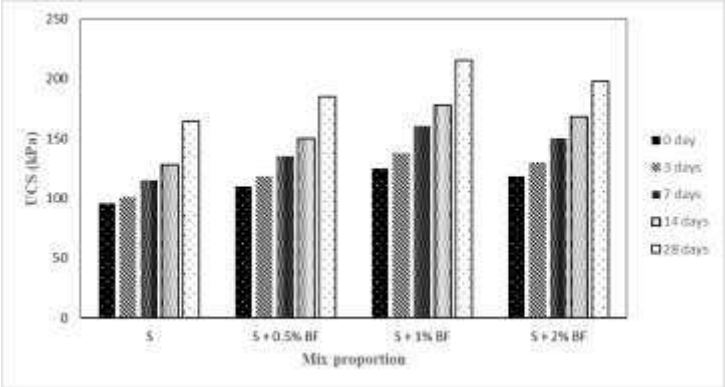


Fig.25: Unconfined compressive strength of soil reinforced with basalt fiber at different curing periods

The UCS values of soil mixed with different percentages of basalt fiber at various curing periods are presented in fig.25 The untreated soil showed a UCS of 96 kPa at 0 day curing, which increased to 164 kPa at 28 days. With the addition of basalt fiber, the strength of the soil increased for all curing periods. The maximum UCS was obtained for the mix containing 1% basalt fiber, reaching 215 kPa at 28 days. The improvement in strength may be due to the reinforcing effect of fibers, which enhances the interlocking between soil particles and helps in resisting crack development during loading. However, when the fiber content increased to 2%, a slight reduction in strength was observed, possibly due to poor fiber distribution and increased voids within the soil matrix. Similar behaviour of optimum fiber content improving soil strength has been reported in earlier studies [9] [35].

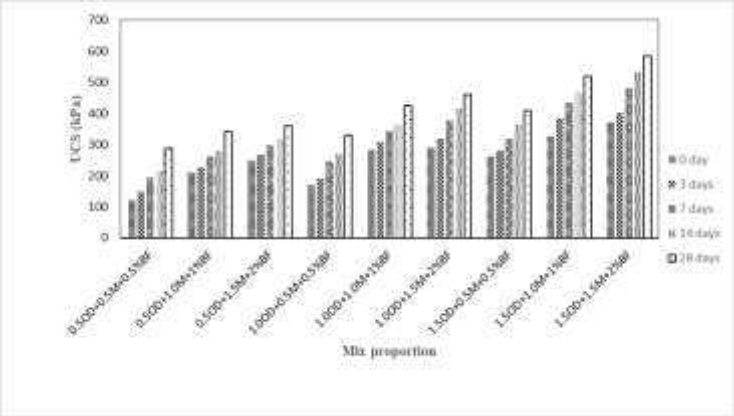


Fig.26: Variation of UCS of bio-cemented soil reinforced with basalt fiber at different optical densities and cementation solution molarities

The unconfined compressive strength (UCS) results for soil samples reinforced with various optical density (OD), molarities (M), and percentages of basalt fiber at different ages of curing are illustrated in Fig. 26. It is observed that there was an increase in the strength of soil with a combination of microbial and fibre treatment as compared to untreated soil. In all cases, the UCS values were found to increase with the curing age of 0-28 days due to the progressive bond formation between soil particles.

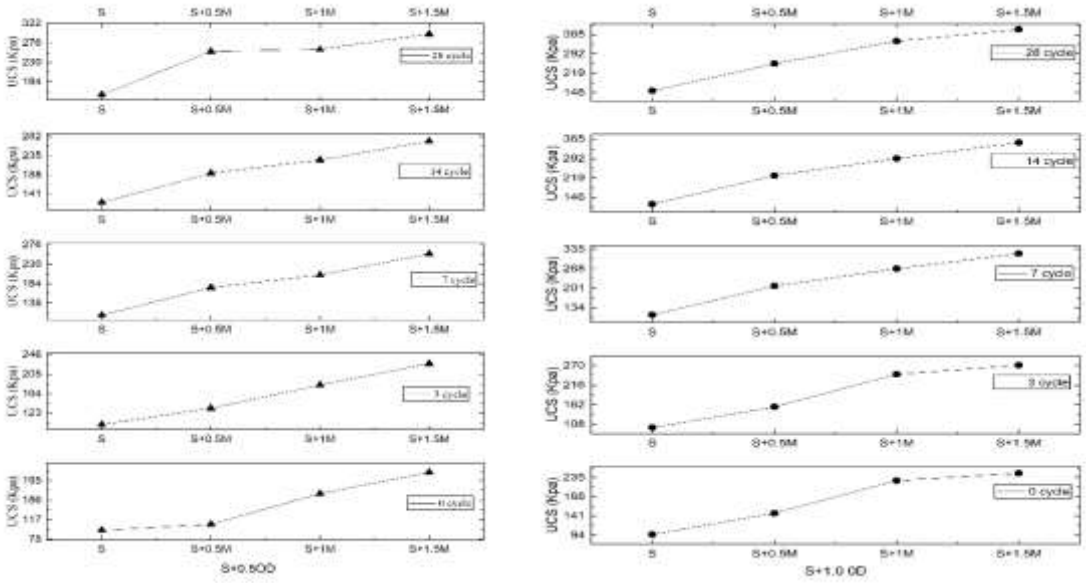
The improvement in soil strength can be explained by the phenomenon of microbial induced calcite precipitation (MICP). In MICP process, bacteria available in soil act as urease enzyme that hydrolyses urea content in cementing solution. This reaction results in the production of carbonate ions which combine with calcium ions to precipitate calcium carbonate in pores of soil, thus increasing the soil strength and stiffness [36][37]. A higher optical density of the bacterial suspension offers a higher number of active bacteria, whereas high molarity of the cementing liquid ensures the availability of high calcium ions leading to increased calcite production and increased bonding.

The incorporation of basalt fibers resulted in greater strength improvement in the treated soils. The presence of fibers in the soil body acts as a reinforcement mechanism and plays an important role in the distribution of load, thus avoiding cracking when subjected to compressive forces. As a result, ductility is improved with reinforced fibers, which prevents failure by delaying it and thus gives higher UCS than that of soil without any fibers [38]. It can be observed that there was a direct relationship between strength and increasing OD, molarity, and fibers content up to an optimum level. In this context, the 1.5OD + 1.5M + 2% BF mix achieved the highest UCS value, i.e., 585 kPa at 28 days.

The consistent rise in the value of strength with respect to the curing time implies that the process of crystallization of calcium carbonate and inter-particle bonding is time-dependent. Such findings have also been observed in other experiments conducted in the past for bio-cemented and fiber-reinforced soils, wherein a high amount of bacteria, sufficient cementing agent, and optimum fiber content had contributed to improved strength parameters [36] [37]. The findings indicate that the combination of bacteria, cementing agent, and basalt fibers is beneficial for organic soil.

5. Effect of Freeze–Thaw Cycles on Unconfined Compressive Strength (UCS)

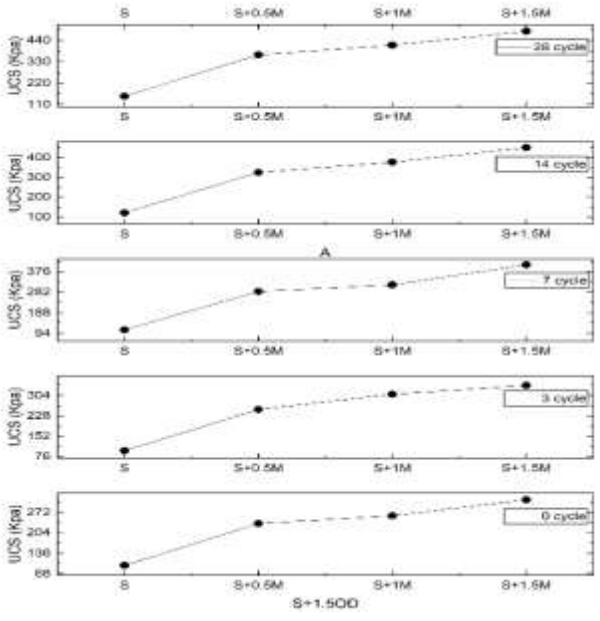
5.1 Freeze-Thaw cycles for different optical densities.



F*ig.27: variation of Unconfined Compressive Strength (UCS) with Freeze–Thaw Cycles for Optical Density (OD) Levels of 0.5 and 1.0.*

The freeze–thaw test results show that the unconfined compressive strength (UCS) of both untreated and treated soil increased gradually with the number of freeze–thaw cycles (0, 3, 7, 14, and 28 cycles). The untreated soil (S) exhibited very low strength, which increased only slightly from 96 kPa at 0 cycles to 153.11 kPa at 28 cycles. This indicates that the natural soil has poor resistance to freeze–thaw action. Such behaviour is expected for organic soil because of its weak particle bonding and high compressibility, which makes the soil structure easily disturbed during freezing and thawing [39].

In the treated samples, the addition of bacterial solution with different optical density (OD) and different cementation solution molarity (M) resulted in a significant improvement in strength. In general, UCS increased with an increase in freeze–thaw cycles, OD, and molarity. This suggests that bio-cementation



continued during the curing and freeze–thaw process, leading to stronger bonding between soil particles due to calcium carbonate formation [12].

Fig.28: variation of Unconfined Compressive Strength (UCS) with Freeze–Thaw Cycles for 1.5 Optical Density (OD)

For the mixes with 0.5 OD, the strength increased as molarity increased from 0.5M to 1.5M, where the UCS at 28 cycles increased from 256.34 kPa to 297.18 kPa. A similar trend was observed for 1.0 OD, where the UCS reached 383.87 kPa at 1.5M after 28 cycles. The highest strength was obtained for the mix with 1.5 OD and 1.5M, where the UCS increased from 314 kPa at 0 cycle to 487 kPa at 28 cycles, showing the best resistance to freeze–thaw cycles. Higher OD means more bacterial concentration, and higher molarity provides more cementation chemicals, which results in greater calcium carbonate precipitation and stronger soil structure [36].

The strain values also increased with OD and molarity, indicating that the treated soil became stronger while maintaining better deformation capacity compared to untreated soil. This shows that bio-cementation not only increases strength but also improves the overall stability of the soil under repeated freezing and thawing. Similar observations have been reported in previous studies on bio-treated soils [24].

Overall, the results show that increasing optical density (OD) **and** cementation molarity (M) improves the freeze–thaw durability of organic soil, and the mix S + 1.5OD + 1.5M gave the best performance among all the combinations.

5.2 *Three-Dimensional Analysis Comparing Unconfined Compressive Strength (UCS) under Freeze–Thaw Cycles and Normal Curing Conditions of Optimum Mixes.*

A three-dimensional analysis was performed to compare the variation of Unconfined Compressive Strength (UCS) of the optimum mixes under freeze–thaw cycles and normal curing conditions.

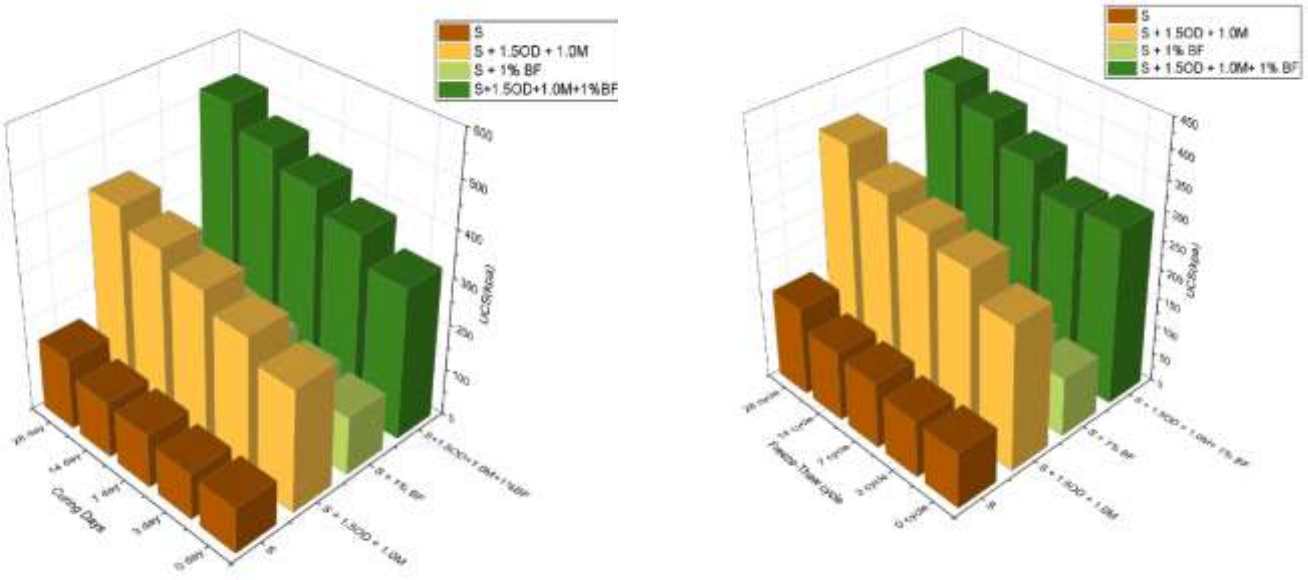


Fig. 29: Three-Dimensional Analysis Comparing Unconfined Compressive Strength (UCS) under Freeze–Thaw Cycles and Normal Curing Conditions

This section compares the optimum mixes under normal curing and freeze–thaw conditions based on UCS values. The results show that all treated soils have much higher strength than the control soil, but a reduction in UCS is observed after freeze–thaw cycles. Under normal curing, the UCS of the control soil

increases from 96 kPa to 153 kPa, whereas the optimum mix *S + 1.5OD + (1.0M + 0.5ESP) + 1% BF* increases significantly from 326 kPa to 520 kPa, indicating strong improvement due to combined stabilization and fiber reinforcement. Nevertheless, in the case of freeze-thawing, the same mix exhibits lower values, rising from 302 kPa to 410 kPa, obviously below the strengths recorded during normal curing. The trend is almost identical for S + 1.5OD + (1.0M + 0.5ESP); the UCS decreases from 415 kPa to 384 kPa when subjected to freeze-thawing. This weakening of the soils is primarily caused by the expansion of the pore water as a result of the frozen conditions, leading to the generation of internal stresses within the soils. After thawing, large pores are left, as well as tiny cracks in the soil; this leads to deterioration of the soil structure as a whole. Under continuous repetitions of this cycle, the process causes an accumulation of weaknesses resulting in reduced bond between the soil particles [40][41]. Nonetheless, even at this stage, the treated soils still exhibit improved characteristics than the control soil sample; this is because of the improvement in bond and reduction of voids brought about by stabilization, as well as binding of soil through fiber reinforcement that limits the development of cracks [9].

6. Regression Analysis:

The regression analysis method was employed to investigate the interaction between the unconfined compressive strength (UCS) and curing time for the soil without any treatment, the soil mixed with bacterial solution and cementation solution, the soil reinforced with basalt fibre, and the composite soil mixed with bacterial solution, cementation solution, and basalt fibre reinforcement.

As the curing times taken into account in this study include 0, 3, 7, 14 and 28 days, the equation of linear regression was applied to describe the variation of UCS against time. The regression equation is expressed as

$$UCS = a\,t + b6.$$

where UCS is the unconfined compressive strength (kPa), t is curing time (days), a is the strength gain rate (kPa/day), b is the intercept representing initial strength, and R^2 is the coefficient of determination.

The regression results show that R^2 values for all mixes are close to 1, indicating a good linear relationship between UCS and curing time. Untreated soil showed the lowest strength gain rate, while treated soils showed higher slope values. Mixes containing bacterial solution and cementation solution showed higher strength gain due to calcite precipitation, and the addition of basalt fiber further improved the strength by providing reinforcement. The highest strength gain rate was observed for the combined mixes with higher optical density, molarity, and fiber content, showing the effectiveness of bio-cementation and fiber reinforcement in improving soil strength.

Table 5: Regression Analysis

Mix	**Regression Equation (UCS = a·t + b)**	**R^2**
S	UCS = 2.02t + 95.35	0.98
S + 0.5OD + 0.5M	UCS = 5.02t + 120.35	0.96
S + 0.5OD + 1.0M	UCS = 4.04t + 172.21	0.99
S + 0.5OD + 1.5M	UCS = 2.92t + 221.65	0.92
S + 1.0OD + 0.5M	UCS = 5.29t + 152.20	0.96
S + 1.0OD + 1.0M	UCS = 3.82t + 236.03	0.97
S + 1.0OD + 1.5M	UCS = 4.76t + 264.70	0.88
S + 1.5OD + 0.5M	UCS = 4.71t + 243.43	0.96
S + 1.5OD + 1.0M	UCS = 5.01t + 289.29	0.87
S + 1.5OD + 1.5M	UCS = 6.08t + 337.18	0.87
S + 0.5% BF	UCS = 2.65t + 111.99	0.99
S + 1% BF	UCS = 3.14t + 130.58	0.98
S + 2% BF	UCS = 2.80t + 123.72	0.97
S + 0.5OD + 0.5M + 0.5% BF	UCS = 5.63t + 134.81	0.96
S + 0.5OD + 1.0M + 1% BF	UCS = 4.54t + 217.83	0.98

S + 0.5OD + 1.5M + 2% BF	UCS = 3.78t + 260.05	0.95
S + 1.0OD + 0.5M + 0.5% BF	UCS = 5.58t + 183.00	0.94
S + 1.0OD + 1.0M + 1% BF	UCS = 4.77t + 295.34	0.97
S + 1.0OD + 1.5M + 2% BF	UCS = 5.90t + 311.09	0.90
S + 1.5OD + 0.5M + 0.5% BF	UCS = 5.28t + 272.45	0.96
S + 1.5OD + 1.0M + 1% BF	UCS = 6.31t + 361.40	0.88
S + 1.5OD + 1.5M + 2% BF	UCS = 7.53t + 396.04	0.89

7. Microstructure Analysis:

Microstructural characterization was carried out to better understand the mechanisms responsible for the improvement in strength of organic soil, both in its untreated state and when treated with basalt fibre, MICP, and their combined application. The analysis focused on identifying changes in surface morphology, elemental composition, and mineralogical structure. For this purpose, Scanning Electron Microscopy (SEM), Energy Dispersive X-ray (EDX), and X-ray Diffraction (XRD) tests were conducted. The photographs of the equipment used for SEM and XRD analysis are presented in Figure 30(a) and Figure 30(b), respectively.

7.1 Scanning Electron Microscopy (SEM):

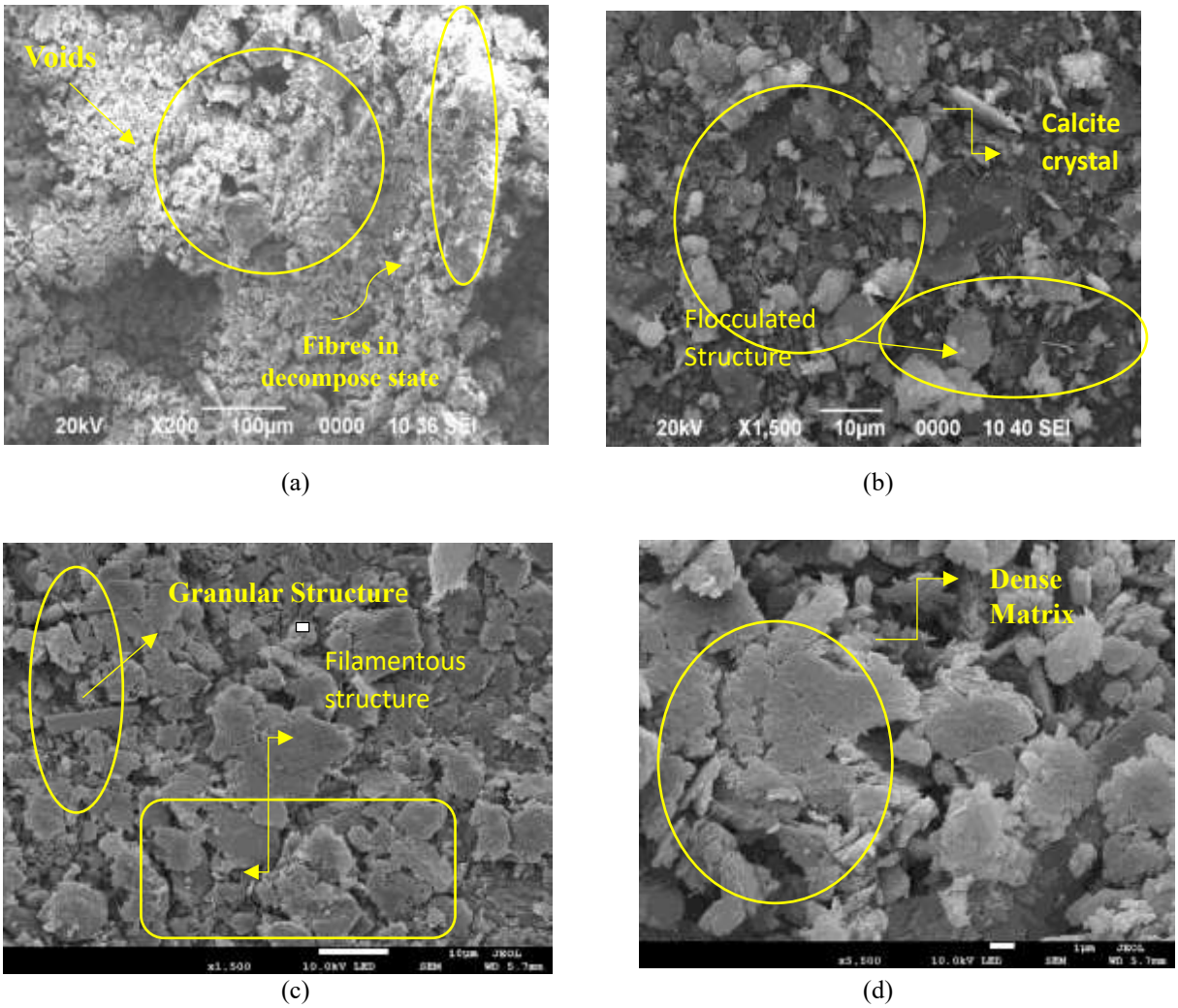


Fig.30: SEM images of a) organic soil, b) S+MICP , c) S+MICP+BF, d) S+MICP+BF

The SEM images clearly illustrate the changes in soil microstructure due to different treatments. As seen in Figure 30(a), the microstructure of the untreated organic soil exhibits a loose and fibrous nature, containing many voids, reflecting low cohesion and highly porous nature. As seen in Figure 30(b), the presence of calcite crystals, created through MICP treatment in the soil, binds the soil particles together, improving the microstructure of the material. As seen in Figure 30(c) under magnification (1500×) of the soil, MICP, and basalt fibre (combined mixture), the microstructure appears to be both fibrous and granular in nature, suggesting that fibres are involved in bridging of particles, while calcite precipitation results in improved cohesion in the microstructure. Figure 30(d) displays a dense and compact microstructure, where most of the void spaces are filled, forming a well-packed soil structure. Such microstructure accounts for the increased strength of the modified soil. Such behaviour of the microstructure has also been observed in earlier research where calcite precipitation causes reduced pore spaces and increased cohesion between the particles, whereas fibre reinforcement causes improved microstructure [25][36].

7.2 The Energy Dispersive X-ray (EDX) analysis

Energy Dispersive X-ray (EDX) analysis was used to identify the elemental composition of the soil before and after treatment. The untreated soil mainly showed silicon (Si), aluminium (Al), and oxygen (O), with very low calcium content. After treatment with MICP and calcium-based additives, an increase in calcium (Ca) and carbon (C) was observed, confirming the formation of calcium carbonate within the soil matrix.

Table 6: Elemental composition of treated and untreated soil obtained in EDX

Element	Natural Soil (wt.%)	Soil + MICP (wt.%)	Soil + BF (wt.%)	Soil + MICP +BF (wt.%)
C	10.2	13.5	11.4	17.1
O	48.1	49.8	47.6	51.2
N	3.1	10.5	3.0	14.6
Si	20.4	12.0	19.8	9.6
Al	7.2	4.2	6.5	3.8
Ca	1.6	10.8	2.1	13.9
Fe	3.2	2.0	4.6	3.8
Mg	1.1	0.7	2.8	1.9
K	0.9	0.5	0.8	0.6
Na	—	—	0.7	0.5
Ti	—	—	0.6	0.4
Others	4.2	3.0	3.1	2.6

The EDX results show that the types of elements present in the soil remain mostly the same, but their amounts change after treatment. The natural soil mainly contains oxygen, silicon, and aluminium, which is typical for this type of soil [22]. After MICP treatment, there is a clear increase in calcium and carbon, which confirms the formation of calcium carbonate due to microbial activity [12][24].The increase in nitrogen also indicates the presence of bacteria. When basalt fibre is added, slight increases in elements like iron, magnesium, and titanium are seen, which come from the fibre itself. In the combined treatment (MICP + basalt fibre), the highest calcium and carbon contents are observed, showing stronger bonding due to more calcite formation. At the same time, the decrease in silicon and aluminium suggests that the soil particles are getting coated and bonded together. Overall, MICP mainly improves the soil by forming cementation products, while basalt fibre helps in holding the structure together, and together they give better strength and stability [36].

7.3 X-ray Diffraction (XRD) Analysis:

The obtained results from the X-ray diffraction analysis clearly illustrate that there is a shift in mineralogical composition of soils depending on their treatment. Untreated soil demonstrates broad, low-intensity peaks, indicating the presence of naturally occurring minerals like quartz and alumino-silicates and hence weak mineral bonding. Treatment of soil using the MICP technique produces peaks of higher intensity compared to those produced by untreated soil. For example, an increased intensity peak occurs at $2\theta \approx 29°$-$30°$, suggesting the presence of calcite. This observation illustrates the role of microorganisms in precipitating

calcium carbonate and forming a good soil structure by binding soil particles. This has been observed in similar studies [23][26] in the presence of peaks for calcite formation.

For the soil with basalt fibres (S + BF), there is no significant peak shift as a result of adding basalt fibres. This could be explained by the absence of new formed mineral phases in S + BF soils due to their mechanical effect rather than chemical properties on soil structure. Variations in the peaks' intensities can be observed, which can be explained by the mineral phase of basalt fibres that contain silica, iron, and magnesium [42].For the combined treatment (S + MICP + BF), the XRD pattern shows sharper and more intense peaks compared to the individual treatments. The calcite peak around 29° becomes more prominent, indicating enhanced

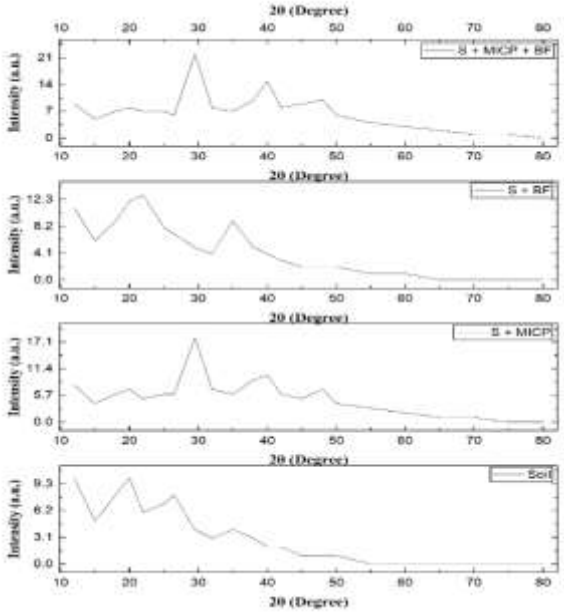


Fig.31: XRD plot of untreated and treated soil

calcium carbonate precipitation. This implies that the availability of fibres contributes towards the increase in the number of nuclei for the precipitation of calcite, which leads to a higher density and uniformity of cementation products. Improved intensity and well-structured nature reveal better crystallinity and lower porosity. Analogous studies have revealed significant synergy between bio-cementation and fibres in increasing soil stability and longevity [43][44].

Overall, the XRD results confirm that MICP plays a dominant role in altering the mineral composition through calcite formation, while basalt fibres contribute to structural reinforcement. Their combined effect leads to a denser and more stable soil matrix, which supports the improved mechanical behaviour observed in UCS results.

8. Conclusions:

Based on the experimental investigation carried out on organic soil treated with MICP, basalt fiber, and their combined application, the following conclusions are drawn:

1. The unconfined compressive strength (UCS) values of all treated soils were found to increase with increasing curing time, showing that there is time dependence of strength development owing to gradual deposition of calcite. The maximum UCS value occurred after 28 days of curing.

2. MICP technique helped to considerably improve the strength of the organic soil through the precipitation of $CaCO_3$, which served as cementation material for soil particles. High optical density (OD) and cementation solution molarity contributed towards higher strength development. The optimum OD and cementation solution molarity was found at 1.5 OD and 1.5 M.
3. Addition of basalt fibers improved the mechanical properties of the soil by increasing interlocking and preventing cracking. However, the optimum amount of basalt fibers was found at 1% because beyond that strength decreased due to void formation.
4. The combined application of MICP and basalt fiber showed a significant synergistic effect, resulting in higher UCS values compared to individual treatments. The maximum strength of about 585 kPa was achieved for the mix 1.5 OD + 1.5 M + 2% BF**,** while a balanced and practical optimum mix was obtained at 1.5 OD + 1.0 M + 1% BF**.**
5. The compaction properties were enhanced by the treatment. The highest dry density value was achieved and optimum moisture content reduced due to MICP to an optimal value (1.0 OD). Compaction was initially enhanced by fibers but deteriorated at high concentrations.
6. The soil was characterized with Atterberg limits after treatment, with liquid limits reduced, whereas plastic limits were raised, implying less plasticity and improved soil stability due to calcite binding.
7. The soil subjected to freeze-thaw cycles was found to exhibit higher values of strength than the un-stabilized soil. Despite the slight reduction in strength after a few cycles, the soil stabilized showed adequate behavior particularly at higher OD and molarity values.
8. Some microstructures were investigated through SEM, EDX, and XRD. Calcite crystals were detected; void space was reduced, while denser soil matrix was observed. Calcium and carbon contents were found to be raised due to treatment.
9. The regression analysis revealed that there was a high linear correlation between UCS and curing time due to high values of R^2, which suggested that the strength improvement in the treated soil could be predicted.
10. Generally, the research reveals that MICP together with basalt fiber constitutes a promising and sustainable way of strengthening organic soil.

Author Contributions: The Author Miss Nabam Jumsi (523013@nerist.ac.in) played a significant role in writings the paper and performing all the experimental tests.
The Author, Dr. Ajanta Kalita, an associate professor, played a significant role in completing the investigation in every manner.
The Author, Dr. Ajay Bharti helped in experimental tests that has been carried out.
Corresponding author: Miss Nabam Jumsi
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Data Availability Statement: The datasets generated during and/or analysed during the current study are available from the corresponding author on reasonable request.

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