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Review Article

An Art of Review of Improving Soil Strength Using Microbes

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Abstract *- Most of the soil found has very little strength. There are different methods of stabilising and increasing the strength of the parent soil, such as the addition of chemicals or additives. One of the emerging methods is Microbial Induced Calcite Precipitation (MICP). This MICP technique is an eco-friendly advanced technology used for soil stabilising by using microorganisms, i.e. bacteria. The microbial activities are responsible for the change in the properties of the soil. Different studies have been conducted, and different laboratory tests have been performed to analyse the effect of MICP technology on the soil. It is seen that there is the formation of calcium carbonate precipitate which is responsible for the change in the soil parameter. The formation of Calcium carbonate intact the soil particles decreases the voids and increases the friction, stability and strength of the soil. However, the application of MICP is still very rare as very few studies have been done on this topic. This paper reviews the MICP techniques in detail, the strength mechanism, factors that need to be considered while adopting these techniques, the different procedures and approaches followed by the researchers and the change in the soil properties that stabilise the soil.*

Keywords *- Bio cementation, Biotechniques, MICP, Microstructural, Soil stabilisation.*

1. Introduction

The soil in this environment is available in abundance, but this is also the fact that all the soils are not fit for construction purposes. The drastic growth in infrastructural development bounds to extend the constructional works towards the weak or problematic soil. Soils like peat soil, silty sand, or soil of high compressibility, such as expansive soil or highly cohesive or cohesionless soil, are unfit for construction. Due to this behaviour, it becomes challenging to construct any structure on this type of soil. The structures erected on these soils are prone to cracks, sliding of slopes, upliftment and differential settlements, which results in damage to the structure like settlement of the roads causing rutting and fatigue failure, cracks in the buildings, landslides, instability of the slopes causing damages to the retaining walls and sometimes collapse of the structure. So, soil strengthening is considered a wealth in research because of different techniques and implementations [92, 98]. Different studies show soil stabilisation with the help of some additives or chemical stabilizers like lime, cement, fly ash, agricultural wastes, and geosynthetics. [16, 28, 29, 88, 92, 98, 111, 133]. However, these methods of soil stabilisation are laborious and time-consuming. The toxic chemicals released also impact the environment and are highly costly [51, 84, 117]. So, there is a need for some methods that can replace the problem faced in its use and application. At the same time, it is energy-efficient and cost-effective. Various researches have been carried out to solve the engineering problem using some bio-mineralization. Bio mineralization is such a method in which the soil is strengthened with the help of some microorganisms that interact biologically with the soil [42, 60]. The study involves the use of microbes, and there is the formation of calcite precipitate, which the study is also known as Microbial induced Calcite Precipitate (MICP). MICP is one of the biomineralisation processes. Eco-friendly techniques recently came into the picture for improving the properties of the weak soil in which minerals are formed biologically, and it is widely being emerging as they are cost-effective and give better results in stabilising the soil [11, 69, 132]. The effectiveness of MICP is concluded through the UCS strength, formation of calcium carbonate, concentration of ion exchange, SEM test and the reactions undergone through the process. Other tests, such as the one-dimensional consolidation test, swelling test, and other strength tests, were also done on these soils using the MICP technique. The strength and the moisture content-dependent parameters were found to be improved [11, 15, 56, 65, 69]. These techniques were also used for peat soil, and the soil's properties were improved due to the Bacillus licheniformis, in which the activity of this microorganism was increased by scallop powder [41, 102].

MICP is basically a method of strengthening the soil with the help of microbes and bacteria with some chemical reagent. These microbes secrete enzymes that react with the reagent to form calcium carbonate precipitates [73, 90]. This is mainly categorised in Bio mineralization, ferric reduction, urea hydrolysis, denitrification and sulphate reduction [4, 68, 91, 120, 121]. The most efficient is urea hydrolysis, where the formation of calcium carbonate is very high without adding any nutrients to the microorganism; the microbial survival rate is also more comparatively and is easily controllable [12, 18]. The grout penetrated the soil, which improved the strength and stability of the expansive soil by forming calcium carbonate precipitates, filling in the micro pores of the soil and binding the particles together [81]. The MICP techniques much study properties like strength and stability, but the main parameter responsible for the change, the moisture content dependent parameters, are still more to be focused on.

This paper is based on the overview of the work done with the help of MICP, its engineering aspects in the field of soil, the effects of the microbes in increasing the strength, and the factors to be considered in MICP by different procedures. However, the study on these techniques is still very limited and concise, which makes it a recent emerging technique in improving soil strength. So, this study is a potential way to gain in-depth knowledge of improving soil strength using microbes, factors to be considered while studying, methods to be followed, and suggest some future study scope.

2. MICP Interaction with Soil

As mentioned, MICP is used to strengthen the soil using bacteria, and the formation of CaCO3 achieves strength. So, the concern of the study is how the bacteria or the microbes are reacting with the reagents and how the formation of $CaCO_3$ helps strengthen the expansive soil.

2.1. Transportation and Adsorption of Bacteria

The bacteria and the reagents are introduced into the soil in the form of a solution. This solution travels through the pores present in the soil. The bacterial solution's adsorption occurs either by convection or by radiation [3]. The rate of transportation and adsorption depends upon many factors, such as the shape and size of the bacteria, features, mode of mixing, surface charge, and temperature. [3, 54]. The transportation of bacteria is through the soil pores throat which depend upon the bacterial cell compatibility [52]. The bacterial cell has a diameter range of 0.5 µm to 3 µm, because of which they are easily penetrated deep into the soil [50, 52]. The feasibility of bacterial movement within the pores is governed by the effective size of the soil particle. The pore throat size is 20% of the effective size of the soil (D_{10}) via the injection method, while the upper bound of the pore throat size is 5% of the effective size of the soil for bacterial transportation [51, 116]. The interaction between soi and bacteria exists for a shorter distance of less than 1nm and a maximum of 10nm [58, 110]. Bacteria adsorption occurs by attractive and repulsive forces named irreversible and reversible adsorption, respectively. Reversible adsorption has weak electrostatic forces, while irreversible adsorption has attractive Van der Waals forces when the bridge is connected between soil and bacterial cells involving large energy [57]. In irreversible, adsorption is permanent, shear strength is very low and provides large sites for adsorption [10, 38, 58, 70, 75].

2.2. Soil-Bacteria Reaction

The rate of calcium carbonate generation in the environment may not be sufficient to fulfil the requirement of the $CaCO_3$ precipitation [49]. The strength in MICP is mostly due to the deposition of $CaCO_3$ precipitate [4, 16, 56, 65, 69, 81, 91, 132]. The nutrition of bacteria in the reaction involves both autotrophic and heterotrophic. The photosynthesis process and reaction of soil bacteria involves both aerobic anaerobic, methanogenesis and non-methylotrophic, in which amino acids ammonify and sulphate reduce. The most controlled energy efficiency is referred to as the hydrolysis of urea, where denitrification occurs. This energy efficiency in a controlled system makes MICP an emerging topic in the research [51, 77, 133]. During the hydrolysis of urea, the metalloenzyme involved is urea amidohydrolases; the non-pathogenic microorganisms, Sporosarcina Pasteurii and Bacillus Pasteurii, are the most commonly used for the hydrolysis as they can tolerate the harsh conditions [2, 45, 67]. In the process of urea hydrolysis in MICP, the formation of calcium precipitates takes place in steps. The very first steps involve the ureolysis in which there is the formation of ammonia and carbonic acid. The carbonic acid further produces carbon dioxide and ammonia in the second step. In the presence of water, this carbon dioxide reacts to form carbonic acid and bicarbonates in the third step. In the fourth step, ammonia reacts to produce ammonium and hydroxide ions, where there is an increase in the PH. During the fifth step, there is the formation of more bi carbonates. Finally, the calcium soluble forms the calcium carbonate precipitates ($CaCO_3$) [45, 51, 99]. Apart from this, the biological reaction is involved, where functional groups like carboxyl amines negatively charge the biological cell, attracting the calcium ions and hence crystallising [35, 45, 97, 109].

3. Strength Mechanism

The strength mechanism of bio-cementation is basically due to the formation of calcium carbonate crystals on the soil's surface. The crystal forms bind the soil together and impart strength to the soil. MICP is also the method of bio-cementation that follows the same [117]. Although this technique is a bit different from the other in which the pores are filled with some cementitious material in this technique, there is adsorption of bacteria to the surface of the soil and deposition of crystals of $CaCO_3$ on the surface. When adsorbed with the soil, the bacteria locate themselves in the pores of the soil, where they can get the nutrients easily and acquire less shear strength. The calcium precipitates forms accumulate on the soil when moved to the interparticle pores by the fluid movements [51, 52, 133]. The calcium carbonates are yellow-blue threads of crystal-like precipitates that lock the soil particles together and act as friction between the particles. These increase roughness in the soil, hence imparting strength to the soil [16, 51, 62]. Therefore, in bio-cementation, the strength depends upon forming calcium carbonates, the final product after ureolysis. The bacteria present in the soil are responsible for the urease activity, where the formation of ammonia and carbonic acid forms; the reaction increases the pH, making the soil alkaline and finally hydrates to form the final product as calcium carbonates precipitate. Hence, MICP techniques increase the strength and stiffness.

4. Bacteria Growth Culture

The bacteria used for the study are generally S. Pasteuri and B Pasteurii. The microorganisms are cultivated at a controlled temperature and for a specific time when the bacteria grows. Generally, the temperature for the culture is kept at 28ºC for 24 hours and is stored at 4ºC in a refrigerator for not more than 2 days [55, 59]. In MICP, it was seen that different strains of bacteria give different rates of calcium carbonate precipitation and crystallization.

The resulting phenomenon depends on the crystal forms' size, morphology, and polygraph. In the same condition, the growth rate of bacteria may depend upon the organic and inorganic matter, which may form different forms of $CaCO_3$ crystal along different planes [16]. During the urea hydrolysis, the culture medium is highly alkaline, leading to high PH. The phase of different rates of crystallization is governed by polymorph stabilization. The enzymes extracted from the S. Pasteuri bacteria result in the formation of bacterial strain [105, 122]. During ureolysis, different bacterial strains are produced in analogous conditions, which results in the formation of $CaCO_3$ at much higher rates [2, 45]. So, it is seen that there are different levels in MICP that produce the $CaCO_3$ depending upon the bacterial concentration and polymorphs for the strengthening. So, it is difficult to predict the variations in the culture medium of the bacteria to which it can optimum strength in the mechanical or engineering properties.

5. Factors Affecting MICP

The MICP techniques are affected by many factors. The criteria discussed above depend upon the size of the particle, the concentration of the solution used, the temperature at which the bacteria culture has been grown, and other environmental factors [16, 34]. Some of the influencing factors are discussed below.

5.1. Effects on PH and Temperature

Temperature is the most affecting factor in MICP, as is the bacteria growth, degradation, activity of the bacteria, the enzymes' reactivity, the reaction involved, and the precipitate formation; everything depends upon the temperature. Mostly, the experiment is conducted at 20ºC. The urease activity and the bacterial growth are mostly seen at 0ºC to 30ºC. The growth of bacteria and urease activity increases as the temperature increases. According to many researchers, the optimum was seen at 15ºC to 40ºC for bacterial growth, while for urea hydrolysis, it is between 20ºC to 30ºC [1, 81, 130, 131]. Figure 1 shows the growth of bacteria and urease activity with the temperature change. Apart from this, for the formation of CaCO3 crystals, a specific temperature is required to react. The decrease in the temperature shows a decrease in the activity, hence, insufficient formation of precipitates. The enzyme activity was null below the 10ºC [81].

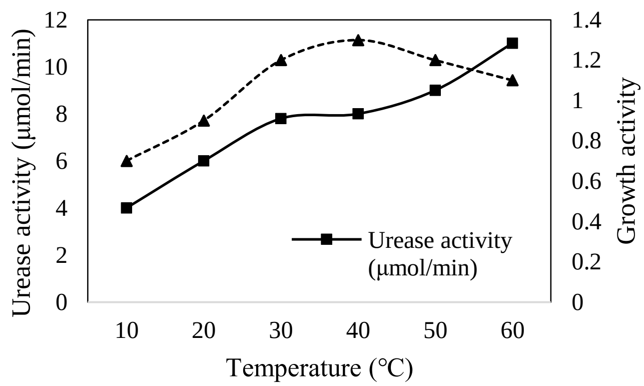

Fig. 1 Bacterial growth and urease activity at different temperatures [Kim, Y.; Roh, Y, 2019]

PH also plays an important role in the MICP. It can influence the ion's concentration and macro-structural properties by changing their charge. Thus, the nutrients of the bacteria are affected by decreasing their absorption and increasing the non-requirable products that pollute the environment [1, 14]. The maximum increase in the PH was seen to be around 5 to 9, whereas, for B. pasteurii and S. pasteurii, it was around 8 and 9, respectively [1, 37, 99]. The variation of urease activity with different PH for B. pasteurii and S. pasteurii is shown in Figure 2.

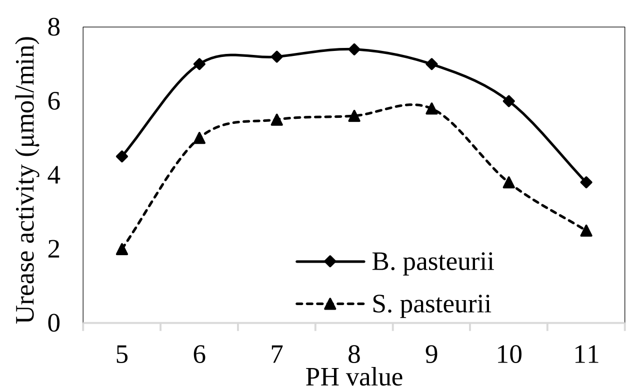

Fig. 2 Urease activity with B. Pasteurii and S. Pasteurii [Yang, Y et al., 2019]

5.2. Impacts of Bacterial Concentration

The concentration of bacteria is also a very important factor to consider for the MICP, as it is the main constituent used. The hydrolysis rate depends upon the bacteria concentration required for the optimum formation of calcium precipitates. Keeping all the other factors the same, the change in the bacterial strain changes the rate of growth of the bacteria and also the crystal formation [91, 117, 45]. Different crystal formation and nucleation rates were seen by varying the S. Pasteurii bacteria, which increases the alkalinity and causes supersaturation. Due to the change in the PH and the precursor solution timing, the polymorph and phase transformation changes. Nucleation rates become faster with high PH while it is slowed in near-neutral [122, 125]. The specific bacterial concentration affects the growth rate of bacteria and their surface properties, the rate of hydrolysis, the ions concentration, nucleation rate, dissolution of the compounds and the organic matter and supersaturation condition [45, 54, 66, 91, 108, 132].

In MICP, calcium carbonate precipitates form in the bacterial cell, which acts as a nucleation site for the bacteria. The growth of the urease activity depends upon the availability of the nucleation sites where the catalytic reaction occurs between the calcium ions. Since the bacteria are adsorbed with the nucleation sites, they are directly linked with the amount of formation of urease activity responsible for the strength improvement [33]. When there is the formation of new sites, they compete with the crystal that already exists. This leads to a more catalytic reaction and the formation of more calcium carbonates and bicarbonates. This shows that the bacterial concentration directly affects the MICP techniques [40].

5.3. Impact of Reagent Concentration

In MICP, the main compounds responsible for the strengthening of the soil are the concentration and amount of $CaCO_3$. The formation of these compounds does not depend upon the bacteria strain used but depends on the polymorphs and morphology of the calcium salts [15]. The important constituent in MICP is calcium, the source of calcium chloride. Calcium is produced by various means by many researchers, either in soluble means by adding some calcium contents products like limestones, eggs, oysters, saline water, or some other biodegradable for improving the strength of the soil [9, 14, 63]. The concentration of the reagent in bio cementation was generally used from 0.1M to 1M. Different researchers took different moles of solution for the study.

It was seen that when the concentration was increased from 0.25 mol/L to 0.5 mol/L and further to 1 mol/L, the strength of the UCS was found to be increasing [85]. This shows that by increasing the concentration of the reagent, the urease activity increases as $CaCO_3$ precipitates, which leads to an increase in strength. Other researchers also concluded that the concentration of 1 mol/L gives a higher strength than the concentration of 0.7 and 0.5 mol/L [80]. Many researchers also concluded that concentration does not increase the strength of the soil; the size and particle properties also hampered the strength criteria [47].

5.4. Effects of Soil Characteristics

5.4.1. Influence of Soil Particle Size

In most of the studies, it is said that the particle size of the soil effects the MICP techniques. Amarakoon et al. [8] found that the sand particle having the range of 0.6 mm to 1.2 mm gives the least strength as the bacteria cannot complete the urease activity, and the formation of calcium carbonate precipitate is also less. Apart from this, the soil's strength, cohesion and internal friction angle also change with the change in the particle size. The cohesion and internal friction of the reinforced soil were found to be increasing with the increase in the particle size [24]. Some researchers concluded that the small-sized sand particles have small gaps and inter-particle pores, where the bacteria occupy and create a nucleation site for hydrolysis. The pores present are then filled by calcium carbonate precipitates. This compacts the soil and binds the particles together, which increases their strength. The sand particles having large sizes are not that effective as the cementation is not properly seen. The calcium crystals were found in large amounts but could not adhere to the soil particle, resulting in less strength [29].

5.4.2. Influence of Particle Shape and Mineral Composition

The behaviour of the soil strength also depends upon the particle shape and the mineral composition. The rounded soil particle, angular, flaky or different shape of soil particle will show a different characteristic [86, 106, 107]. It was seen that the angular-shaped particle gives comparably greater strength than rounded particles. The strength of the soil was checked by blending it with different percentages of the angular soil particles, and the UCS test concluded that increasing the percentage of angular soil particles increases the strength and stiffness of the soil in MICP. In the angular shape particles, the contact bond between the soil particles is planar or is like a cone-to-plane structure, which makes it more stable to form good bonds between them [106].

Mortensen et al. [9] state that the sand, rich in silica, iron oxide, and calcite, has feldspar with several varieties and conducted the test to check their properties. It was concluded that the sand having calcite in maximum percentage shows the fastest change in their properties. In MICP, the strength is gained with the calcium ions and calcite containing sand contains calcium as their main constituent, so forms $CaCO_3$ precipitates to a much larger extent. The sand particles containing calcareous and silicious compounds were also investigated by some researchers [62, 76]. The mechanical properties were tested using MICP, where the results were with the belief that MICP leads to surface hardness and particles undergo scale failure, but it was a bit hypothetical as the organic compound in the soil affects the properties [62].

Canakci et al. [18] performed a similar test using MICP where the soil was treated with the organic contents, which contain compounds to form $CaCO_3$ precipitates; with 60% organic matter, the formation of precipitates was found to be optimum. This shows that the calcium present in the soil contributes to the formation of calcium carbonate precipitates. It was concluded that the presence of organic content only increases the formation of calcium carbonate crystals by forming a soil matrix. However, very little research is done on this characteristic to understand the effect clearly.

5.4.3. Influence of Degree of Saturation

In MICP, the calcium carbonate precipitates form over the soil, which also depends on the interparticle pores between the soil. These crystals can be controlled by the degree of saturation. Cheng et al. [21] state that sand having a lower degree of saturation gives more strength, keeping all the other conditions the same. The effectiveness was checked for varying percentages of the degree of saturation, and it concluded that the soil gives the best result at the degree of saturation when it is less than 20%. The $CaCO_3$ crystal forming at a lower degree of saturation is more effective than the higher one. Some researchers also concluded that at a higher degree of saturation, the crystals were formed in an undesirable manner, which does not play any role in strength improvement [125]. However, the degree of saturation, microstructure and other properties change after the CaCO3 crystal forms. There is a change in mass transfer and phase transformation due to changes in the volume and water content of the soil.

6. Treatment Method in MICP

In order to increase the strength of the soil using MICP, the main part is to adsorb the bacteria to the soil so that the urease activity can take place. There are many ways researchers have followed to mix the soil with the bacteria. The method of bacteria mixing with the soil will directly affect the strength phenomenon. The different methods adopted by the different researchers are as follows:

6.1. Injection Method

In this method, the soil sample is injected with the bacterial solution before the testing. The solution is prepared by mixing the bacterial solution with the reagent solution, and then it is pumped into the soil sample under a specific hydraulic pressure; it flocculates at a point and forms calcium precipitates. [61, 103, 125]. This method is generally done in the soil where the solution can percolate easily through the pores, i.e., the coarse grain soil with high permeability. This type of procedure comes under one phase where the injection is done only from one side of the sample [79]. Injection techniques are also done using the two-phase method by some researchers, where the bacterial solution is first injected, and then the reagent is injected into the sample. This increases the salinity and ionic strength, not allowing the bacterial cells to stick together. This results in the bacteria adsorbed to the soil [79, 125]. Shahrokhi et al. [103] considered many tests for finding the effective phase method. He suggested that comparably, the stage method is more effective when the reagent solution is injected after a retention time after the injection of the bacterial solution. Although this technique is time-consuming and also costly, it is still found to be the best choice. The nutrient solution prepared for this method is shown in Figure 3.

Fig. 3 Nutrient solution mixed

6.2. Premixing Method

This is the most common technique and is simple to use. In this technique, the bacteria and the reagent solution are mixed before the test. It is then kept in an autoclave for bacteria to grow. The first step is to prepare a reagent solution with different moles of broth solution, and then the bacteria is injected into the broth solution [13, 83, 85]. Figure 4 shows the mixing of the bacterial solution with the reagent solution.

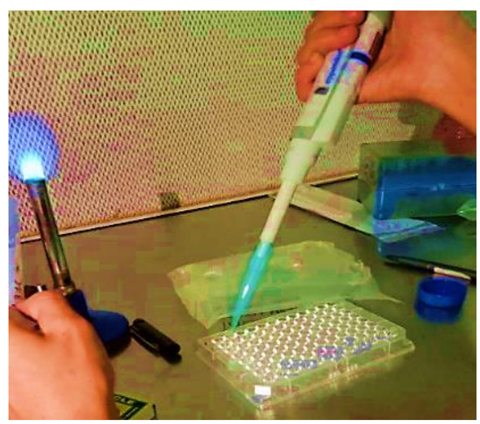

Fig. 4 Mixing of bacterial solution with the reagent solution

This method is adopted for fine-grain soil with low permeability where the injection method could not properly work. This method allows the uniform distribution of the solution throughout the soil [13]. Although this method has quite a few drawbacks, it cannot be used in the actual field where the volume of soil is much higher. Mixing the soil with the bacteria-based solution will be difficult mechanically [83]. Apart from this, mixing with the soil will require much care and precaution.

6.3. Surface Penetration Treatment

In this method, the soil is spread uniformly, and then the solution of bacteria and reagent is spread over the soil surface. The bacterial solution is said to be penetrated up to a depth of 2m. This method is easy and quick as it does not require heavy machinery. However, this method will be difficult for fine-grained soil like silt and clay, where the pores present in the soil are much less, and less permeability exists. The penetration in such soil is less than 1m; hence, it is unsuitable [22].

From the above methods, it is being seen that the injection method is being used mostly in the MICP treatment. The methodology used is that the nutrient solution for bacteria, along with the bacterial solution, is prepared and mixed with the soil using different techniques depending on the types and characteristics of the soil. Different tests are then performed using various mixes of the solutions with the soil samples.

7. Test Results Performed in MICP Soil Sample

7.1. Shear Strength Test

The shear strength of the soil is used to calculate the shear parameters, i.e. cohesion (c) and internal friction (Ø). The test was done on the sand sample with S. Pasteurii bacteria, and a cementitious compound was used to treat it. It was concluded that there was a change in the shear parameter of the soil. Internal friction did not increase, but there was an increase in the cohesion of the bio-cemented soil [36]. Cohesion of the soil was also affected by the use of S. Pasterii bacteria tested on the bio-cemented sample prepared under growing, resting, and dead conditions. It was seen that there was a huge increase in the shear strength. The internal angle was increased to a large extent [26]. The improvement in the soil strength was also seen with the other bacteria. With the bacillus megaterium, the ratio of the shear strength of the treated soil sample to that of the untreated soil sample was seen to be doubled [87]. The shear strength was also related to the degree of saturation. It was said that the cohesion and internal friction of the soil increase drastically at a lower degree of saturation [20].

All researchers show that the soil treated with MICP affects the soil's shear strength. The cohesion of the soil increases as the calcium, being a pozzolanic material, increases the cohesion of the soil, and when the pores are filled with the crystals of calcium precipitates, it increases the friction between the particles; hence, the internal friction of the treated soil with MICP increases.

7.2. Unconfined Compression Test

This test is most common among all researchers. This test is done to find the compressive strength of the soil treated with MICP [20, 21, 44, 46, 89]. Many researchers have concluded that the UCS strength increases in the MICP-treated soil sample. The increase in the strength is found due to the formation of calcium carbonate crystals during the urease activity. It is concluded that the MICP-treated sample shows much larger strength than the untreated sample. The value recorded for untreated was 34 kPa, while the MICP treated shows a value of 34 MPa [125]. The increase in the stress-strain behaviour shows an exponential increase. A sample with the same amount of crystal shows different strengths and depends on the crystal formation mechanism [28, 100, 119]. The increase in strength by some researchers is also said to be linear.

The formation of $CaCO_3$ at low amounts is weak and breaks easily. With an increase in the amount of these crystals in MICP-treated soil, the strength increases to more than three times the original strength [85]. When the interparticle distance is less, there is less contact of the cementitious compound to them; this affects the bonds between them and causes easy failure. The densification of calcium precipitate crystals strengthens the bond and imparts effective strength to the soil.

There are many researchers who mathematically find the co relation between UCS strength and the formation of calcium carbonate crystals. But, there are variations in the conclusion. Some suggested that the relationship between the UCS strength and the calcium carbonate precipitation is linear [85], while others suggested that it follows exponential [62, 119]. However, the strength depends upon many factors like bacterial concentration and reagent concentration, PH, temperature, culture medium, etc. [13, 16].

7.3. Permeability Test

This test is done to check the passage of fluid flowing through the pores [21, 25, 26]. In MICP, mostly sandy or coarse soil is used so the bacterial solution can easily flow through it. The permeability of these soils is very high, as there are more pores present in these soils. The strength increases when the soil densifies, which means that the pores present in it are reduced, which reduces permeability.

MICP must have sufficient pores so that the solution can penetrate or, simultaneously, strength should be improved [25]. In MICP, for the sufficient formation of calcium carbonate precipitates, the hydraulic conductivity suggested is 1.0×10^{-4}m/s for better penetration of bacterial solution in the soil [27]. It is said that at this hydraulic conductivity, there is a uniform formation of calcium carbonate crystal formation.

MICP is a comparably best method to hold the cementitious compound in the soil's pores as it allows the retention of the solution in the pores. Cheng et al. [20] used MICP for the soil treatment, and Ordinary Portland Cement was used. It was suggested that the Ordinary Portland Cement occupies the pores present in the soil, and due to the clogging of cement, the permeability of the soil is reduced. The clogging of the pores is due to the formation of insoluble salts, while in MICP, they are filled with CaCO3 crystal, which imparts strength and reduces permeability.

7.4. Microstructure Examination

The microstructure analysis is done to observe the behaviour of soil in MICP at the micro level. This analysis is done by various means, such as scanning electron microscope, X-ray diffraction, backscattered electrons, etc. In these tests, the micro image is captured and analysed. Various studies have been done to see the microstructural characteristics of the soil treated with MICP, which shows the formation of $CaCO_3$ crystal on the soil, which is responsible for the strength enhancement [16, 20, 50, 52, 62, 104]

7.4.1. Scanning Electron Microscope (SEM)

In this analysis, the micro image of the soil sample is seen through the microscope and the image is then analysed. The SEM image of soil with MICP shows the formation of calcium carbonate crystals on the soil surface. The crystals form between the interparticle gaps between the soil, imparting strength to the soil [16]. The image shows the inter-particle link with the crystals forming a bridge-like bond between the particles of sand soil. The distribution of $CaCO_3$ crystal around the sand particles was of uniform thickness, which means that all the sand particles possess equal amounts of calcium carbonate crystal around them. The bond was so strong that they were equally building the particles together, resulting in an increase in cementation, friction and densification. [20, 32]. The SEM image of formation of $CaCO_3$ crystals treated with MICP is shown in Figure 5.

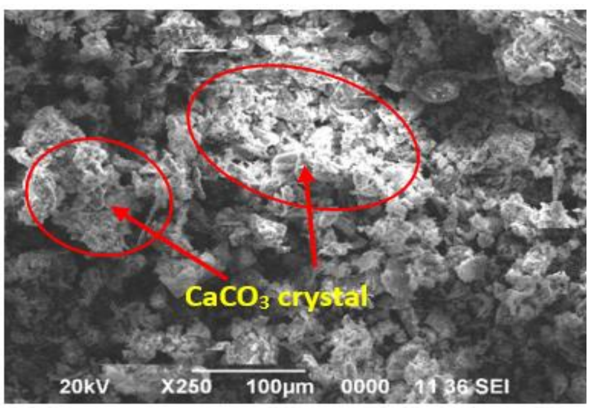

Fig. 5 SEM image of soil sample treated with MICP

7.4.2. Micro-Computed Tomography (Micro-CT)

This technique has recently come into the picture. It is used to analyse the micro behaviour of the bio-cemented soil with a qualitative approach. The X-ray micro-CT image of the soil treated with the MICP is obtained in 3D. The bacterial behaviour and formation of calcium carbonate precipitate are easily seen in the 3D image, and analysis became easy [113, 114]. It was seen that a very less percentage of the soil is uncemented, around 3%, which does not contribute to the formation of $CaCO_3$ precipitation [31]. The behaviour is clearly seen when it is done on a microfluidic chip where the growth of bacteria and distribution of $CaCO_3$ precipitates around the soil is clearly seen [128]. The continuous injection of reagent into the soil sample washed away the bacterial cell, which accumulates in one place and leads to the formation of large crystals at some sites. The formation of precipitates takes up to a stage where, at the very first stage, the crystal forms in an irregular shape and of small size, while in the next, it dissolves to form a large crystal of uniform shape and size [127, 128, 129].

7.4.3. X-Ray Diffraction (XRD)

Using this test, the different polymorphs of the CaCO3 MICP are obtained. In the soil treated with MICP, the polymorphs are mainly found in the calcite, aragonite and vaterite [63]. Some hydrated phases are also seen, such as amorphous calcium carbonate, mono-hydro-calcite, and ikaite. The calcite is said to be the most stable and soluble polymorph, whereas all the polymorphs differ in their characteristics, solubility, and stability [71, 128]. The spectrum found in MICP-treated soil is shown in Figure 6.

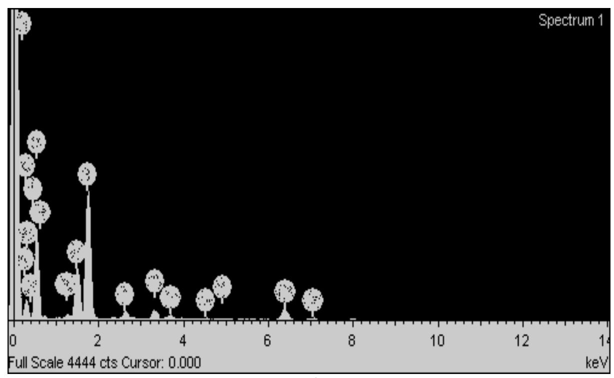

Fig. 6 XRD image of MICP-treated soil

7.4.4. Back Scattered Electron (BSE)

This method is based on the scattering of the light where the light is passed through the sample to get the microscopic view. From the BSE, the microstructure of the soil treated with MICP is obtained. It is seen that the $CaCO_3$ crystals are formed in layers when the bacterial solution is introduced to it [114]. It was also confirmed by the same method that one layer of calcium carbonate forms on the other layers of the existing precipitates. The nucleation sites were also seen in the image, where there was an accumulation of bacteria [53, 83]. This crystal imparts strength to the soil by binding the soil together and densifying it, which enhances its strength. However, the other parameters are also to be considered for strength like PH, temperature, solution bacterial concentration etc [16, 113, 114].

8. Critical Summary

On reviewing all the studies of the researcher, the following points are being emerged:

- MICP is an emerging technique nowadays that is being used for soil stabilization. The bacteria mostly used are the Sporosarcina Pasteurii and Bacillus Pasteurii. The bacteria are grown in a culture medium maintaining a constant temperature of 30ºC to 40ºC and PH of 5 to 7 for their optimum growth by mixing it with a nutrient solution.

- The methods used in MICP are injection, premixing and surface penetration. Out of which injection method is suggested to be the most opted one where the bacterial solution is injected either in one phase, two phases or stages. The stages method takes much time but is more effective than others.
- The bacterial solution is retained in the pores of the soil where the hydrolysis of urea takes place, and the final product comes out to be the calcium carbonate precipitates. These calcium carbonates precipitate crystal forms in layers and impart strength to the soil. The strength of the soil also depends on the size, shape, and other polymorphic characteristics of the crystal forms.
- The strength characteristics also depend on other factors like PH, temperature, degree of saturation, bacterial concentration, nutrient concentration, shape, size and mineral composition of the soil particles. It is said that an increase in the PH and temperature increases the urease activity, and the optimum was seen at PH 9 and a temperature range of 30ºC to 40ºC. Out of other shapes and sizes of the soil, the angular and coarse sandy soil gives more strength when the degree of saturation is kept lower. The maximum minerals found are calcite and felspar, where calcite contributes to the maximum strength of the soil.
- Experimental test result concludes that shear strength test, UCS test, permeability test and macrostructure examination. The shear strength was increasing more than double that of the parent soil. The test is mostly suitable for sandy soil whose permeability is higher, while after the treatment, the permeability of the soil reduces as the pores are filled with $CaCO_3$.
- The microstructure analysis is done by various means like X-ray diffraction, scanning electron microscope, and backscattered electron to see the microstructural characteristics of the soil treated with MICP, which shows the formation of CaCO3 crystal on the soil, which is responsible for the strength enhancement.
- The SEM image formation of calcium carbonate crystal on the soil surface. The X-ray micro-CT image of the soil treated with the MICP is obtained in 3D. The behaviour is seen at the very first stage; the crystal forms in an irregular shape and of small size, while in the next, it dissolves to form a large crystal of uniform shape and size. By XRD, it was concluded that in the soil treated with MICP, the main found polymorphs are calcite, aragonite and vaterite. Some hydrated phases are also seen, such as amorphous calcium carbonate, mono-hydro-calcite, and ikaite. The calcite is said to be the most stable and soluble polymorph, whereas it is solubility and stability.

This study proves that microbial-induced calcium precipitates are an eco-friendly and sustainable technique to be used for increasing the engineering properties of the soil.

Author Contributions

Nisha K. Singh, the corresponding author, reviewed the study and wrote the manuscript. Dr Ajanta Kalita, coauthor, played a significant role in the thorough review of the manuscript.

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