

BIO-MEDIATED SOIL STABILIZATION TECHNIQUES (BIO-POLYMERS AND MICP)

Abstract

Soil stabilization through biologically and eco-friendly ways are not so long-standing attempts because of the extensive research done in the geotechnical engineering field. Among them, are the use of environmentally friendly materials like biopolymers, and other methods namely, microbial-induced calcite precipitation (MICP). Biomineralization mediated by MICP is a phenomenon whereby certain microorganisms produce enzymes that can precipitate calcium carbonate crystals. Most importantly, carbonic anhydrase and urease producers are widely used for MICP in various fields. The mineralization properties of these enzymes are drastically exploited for various purposes including the formulation of self-healing bio-concretes, retention of monuments, sealing of cracks in roads and buildings, etc. Biomineralization through MICP processes can in a way contribute to the carbon sequestration through the active formation of calcites when these enzymes react with atmospheric carbon dioxide. The increased rise of environmental issues upon the use of chemical stabilizing agents made people think of a more sustainable solution through the application of biopolymers, such as guar gum, xanthan gum, alginate, beta 1,3/1,6 glucan and chitosan. In conclusion, both the mentioned alternatives are considered eco-friendly and are helpful in reducing carbon dioxide emissions.

Keywords: Biomineralization, Urease, Carbonic anhydrase, Urea hydrolysis, Soil stabilization, Durability, Shear strength.

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I. INTRODUCTION

Based upon the outcomes of the microbial treatment of soil, there are at least eight different categories of biotechnological techniques used in the construction industry (Fig 1.) [1]. In terms of the fundamental characteristics of research methods, two biological processes projects:

Biocementation: It involves *in situ* microbial production of particle-binding compounds to improve soil shear strength

Bioclogging: It entails using biological processes to produce pore-filling materials in order to significantly reduce the hydraulic conductivity of soil or porous matrix. These techniques enable a comprehensive perspective and significant qualities in the field of soil stabilization and thus serves as additional support for this [2]. Although anaerobic fermenting bacteria have been proposed, microaerophilic bacteria and facultative anaerobic are the best candidates for these approaches (soil bioclogging, biocementation, and bio aggregation) [1].

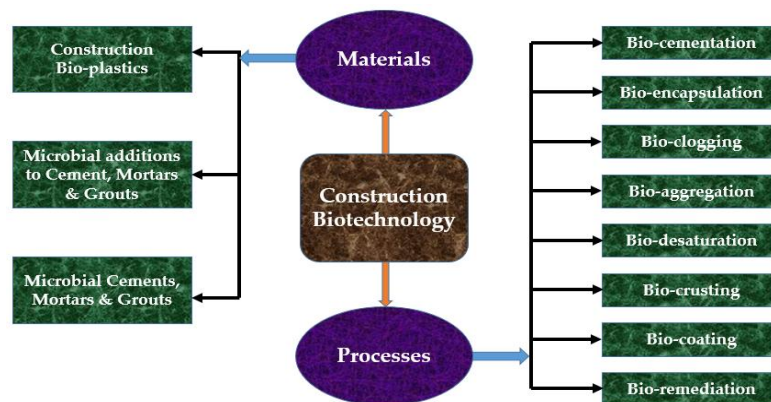
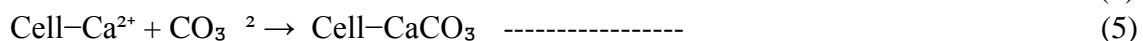
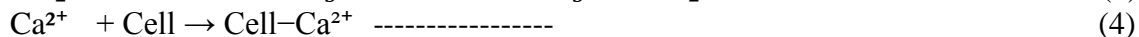
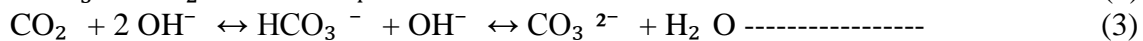
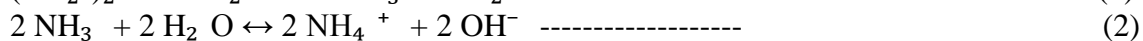
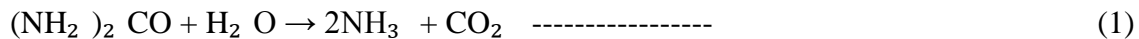


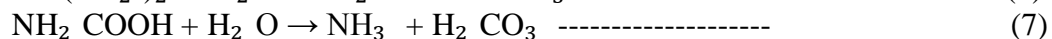
Figure 1: Construction related microbial biotechnology.

1. MICP in soil stabilization : Microbial induced calcite precipitation (MICP), also called microbial urease catalyzed biomineralization, is a biocementation method and has been employed extensively in environmental and geotechnical engineering, and building and materials technologies, mostly focussed at upgrading soil properties and minimizing hazards, like mitigation of internal and surface erosion, liquefaction control, slope stabilization, structural stability, restoration of heavy metal contamination in water and soil, cations (Ca^{2+}) and metalloids, and even in self-healing of bioconcrete, soils or cracks [3, 4, 5]. The term "microbially induced calcite precipitation" (MICP) describes how microbial cells and metabolic processes cause calcium carbonate to precipitate out of a supersaturated fluid. In the course of MICP, organisms are capable of releasing more than one metabolic products (CO_3^{2-}), which when they interact with the ions (Ca^{2+}) in the environment, precipitate minerals. In the past, many mechanisms including photosynthesis, urea hydrolysis, sulphate reduction, anaerobic sulphide oxidation, biofilm, and extracellular polymeric molecules were proposed as being in charge of producing calcium carbonate precipitation. [6]. However, the most popular technique is for bacteria to precipitate calcium carbonate through urea hydrolysis [2].

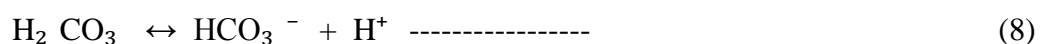
2. Mechanism of microbial-mediated calcium carbonate precipitation: So far scientists have identified the role of urease (EC 3.5.1.5) and carbonic anhydrase (EC 4.2.1.1) in MICP process and substantial research is still going on in the area to talk about the application of MICP for geotechnical and environmental applications. The approach of soil stabilisation that is most frequently investigated is bio-cementation, which precipitates carbonates from urea through a process called carbonate precipitation. The interaction between (HCO_3^-) and calcium ions present in the microenvironment, as well as other metabolic wastes, causes calcium carbonate to develop in MICP. [7]. The most frequent mechanism for urease-producing bacteria, such as *Sporosarcina pasteurii*, *Bacillus megaterium*, and *Sporosarcina aquimarina*, to precipitate carbonate is by the hydrolysis of urea. [3, 8, 9]. Some other representative species include *Bacillus sphaericus* and *Pararhodobacter* sp. The urease enzyme production through bacterial metabolic activities marks the initial step of the process of carbonate precipitation by ureolysis, followed by the development of ammonia (NH_3) and dissolved inorganic carbon (DIC) from the urea catalyzed by the urease enzyme (Equation (1)). This causes a rise in pH (alkalinity) near the bacteria cells (Equations. (2), (3)), and ultimately resulting in the carbonate precipitation (Equations (4), (5)) [3].



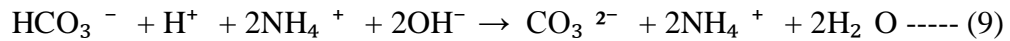
Carbonic anhydrase is a further significant enzyme that performs a key function in addition to urease. The primary goal of carbonic anhydrase-induced calcium carbonate precipitation is to catalyse the interaction between Ca^{2+} and CO_2 in water [10]. The synergistic role of carbonic anhydrase and urease was already reported by Park and Hausinger and Dhami et al. [11, 12]. This is caused by the inclusion of nickel in the urease active centre, which depends on the control of the $\text{CO}_2/\text{HCO}_3^-$ reaction, which is facilitated by carbonic anhydrase. The synergistic process can be discussed in the following way: the initial ingredient, urea, is degraded by the urease enzyme into a mole of ammonia and carbamate (Equation 6), which is followed by the spontaneous hydrolysis of carbamate into a mole of ammonia and carbonic acid (Equation 7).



Then carbonic acid will get converted to bicarbonate (Equation 8) by carbonic anhydrase and ammonia hydrolysis will take place forming two moles of hydroxide and ammonium, similar to the step mediated by urease (Equation 2).



Here too, the pH is elevated around the cell, which will cause calcium carbonate to precipitate when soluble Ca^{2+} is present (Equations 9, 4-5).



In terms of the rate of ureolysis (or urease activity), as well as nucleation and crystallisation, bacterial strains play a crucial part in an efficient MICP. Like other biomineralization processes, calcium carbonate (CaCO_3) precipitation can happen via two major mechanisms: either biologically induced or controlled [13]. The organism significantly influences the process, including the nucleation and growth of the mineral particles, in physiologically regulated mineralization. The creature produces minerals in a way unique to its species, independent of its environment. However, as the sort of mineral generated depends largely on the environmental conditions, and since no additional specialised structures or other particular molecular pathways are considered to be involved, the synthesis of calcium carbonate by bacteria is often referred to as being "induced." Different bacterial species and abiotic factors (including salinity and medium composition) appear to contribute in different ways to calcium carbonate precipitation in a range of circumstances [14].

- 3. Factors affecting MICP:** In MICP, urea and a calcium source are used as the cementation solution, which is subsequently injected into the medium after the introduction of bacteria [15]. A simple and uncomplicated method of MICP that can produce high amounts of CaCO_3 in a short amount of time is calcite precipitation. The seemingly straightforward chemical process of calcium carbonate precipitation depends significantly on the pH, calcium concentration, DIC quantity, and accessibility of nucleation sites [16]. Availability of nucleation sites being the final parameter is crucial for continuous and stable calcium carbonate production, whereas the first three mentioned parameters affect the carbonate ions concentration (CO_3^{2-}) or the saturation state [17]. Most of the ureolytic bacteria that have been discovered and are now in use are aerobes, hence they are employed for MICP in oxic settings. This facilitates their continuous enzymatic activity expression at the surface of soil where the oxygen is adequate. But the activity in anoxic conditions by the same organisms has been reported in many literatures [3, 18]. Each of these variables has a significant impact on the production of CaCO_3 crystals or ureolytic activity. Additionally, a number of environmental factors like temperature and carbon dioxide partial pressure affect the concentration of DIC (for systems exposed to the atmosphere). Additionally, the primary features of the treated (final) products, such as stiffness and strength, depend on the base material's grain characteristics, namely shape, particle roughness, and size, as well as the distribution and morphology of the cement within the medium (that is the crystal shape and size, amount, and location of calcium carbonate) [2, 19]. For example, coarse sand was infrequently chosen for bio-cementation since the microorganisms and particle sizes were incompatible [20]. The creation of a thin, uneven layer of calcium carbonate, which was insufficient to increase the specimens' strength, was blamed for low cementation in coarse soils. In the biomineralization process, when calcium carbonate precipitates along with the bacteria, bacteria act as nucleation sites. The many negatively charged groups found on the cell wall of bacteria allow positively charged metal ions to attach to their surfaces at neutral pH [21]. Later, these linked metal ions, such as calcium, could interact with anions, such as carbonate, to form an insoluble salt (e.g. calcium carbonate). When there is a sufficient excess of the required cations and anions, the metal salt on the cell surface acts as a nucleation site to initiate mineral formation. In addition to calcium carbonate precipitation, the shape of the crystals that are created affects how durable they are.

The method's advantage over traditional procedures is its ability to mitigate geotechnical engineering issues without causing any disruptions, along with the easy application over a large area at ambient temperatures, even under buildings, without disturbing them. While maintaining some of the soil's permeability, MICP gives significant gains in strength, stiffness, and dilative behavior. It has been known that MICP can modify soils' engineering characteristics, including their strength, stiffness, and hydraulic conductivity. The same method can also be used to enhance the quality of sand and soil, as well as to seal concrete with cement. Environmental remediation using MICP has proven to be efficient and ecologically sustainable. MICP is used to sequester atmospheric CO₂ and remove radionuclides and heavy metals from damaged areas. MICP applications are not restricted and are helpful for other applications to create goods that are secure and stable for the environment. The technique of application, which necessitates numerous two-phase cycles of treatment to produce sufficient strength and carbonate precipitation, is one of the major MICP drawbacks, followed by the high cost of implementation [22, 23].

**Table 1: Microorganisms and enzymes used for MICP
(Referred below)**

II. BIOPOLYMERS IN SOIL STABILIZATION

The use of biopolymers in place of traditional admixtures for soil stabilization and enhancement is becoming more and more common [42]. The purpose of using *Xanthan gum* in geotechnical engineering is to improve soil erosion resistance by filling soil pores and reducing the permeability of sandy soils [43, 44]. Through resonant column (RC) testing, Im et al. [45] studied the dynamic characteristics of xanthan gum with typical Korean sand. Although xanthan gum could hold the sand together, moisture exposure significantly reduced its strength. Clay was added, which considerably enhanced the performance by strengthening the polymer. According to the mechanical properties derived from UCS testing, adding clay increases the compressive strength of biopolymer stabilized sands by a significant amount [46]. This study assessed how well the biopolymers guar gum (GG) and xanthan gum (XG) improved the consolidation properties and unconfined compressive strength (UCS) of an expansive soil under various testing conditions. The crosslinking of soil particles by gum strands, which contributes to the increase in strength, was validated by the microlevel experiments [47].

Similarly, gellan gum has been studied for its impact on pore filling to reduce permeability and increase the strength of shallow soils [48]. Through direct shear experiments, Chang and Cho [49] examined how the shear strength and cohesiveness of gellan gum-treated sand-clay mixes increased with increasing overburden stress levels. Agar offers hard textures and has been utilized as a stabilizer when it forms gels [50]. Because polyacrylamide (PAM) is more efficient and reasonably priced, it is frequently utilized for EOR, water treatment, and soil amendment effects [51, 52]. Guar gum was added in concentrations of 0.22 to 5%, which decreased silt and sand permeability and raised sand cohesion stress. Additionally, Chudzikowski [53] used scanning electron micrographs to demonstrate how guar gum (2 % concentration after 5 weeks of curing time) filled pores between soil particles (SEM).

The biopolymer dosage should be chosen keeping in mind the intended strength, curing temperature, and time. To attain a higher UCS, the curing time or temperature (or both) should be raised as the biopolymer dosage increases [46]. The biopolymer concentration in the biopolymer-treated soil mixture, moisture condition, temperature, and dehydration time are the significant elements and soil and environmental conditions affecting various geotechnical aspects of biopolymer-treated soils [54]. Additionally, it would be simple to create and implement artificial intelligence methodologies and algorithms to enhance the performance of biopolymers as a workable soil development technique. For instance, Bayesian networks or genetic programming neural networks have been successfully employed in the investigation of geopolymers concrete and brick, and they may be imitated for biopolymers [55].

In a similar study, a cross-linked polysaccharide biopolymer called -glucan (-G) and a cross-linked protein biopolymer called poly—glutamic acid (-PGA) was used to stabilize the soil. Genetic programming was used to analyze how various variables related to the stabilized pavement design. The study's findings demonstrate that subgrade stabilization can be accomplished using -PGA - -G biopolymer. A tiny (1%) amount of biopolymer results in specimens with a 56-day UCS cure that is 247% stronger [56]. However, given that current stabilization/solidification techniques are susceptible to the effects of Mine Tailing toxicity and salinity, it is crucial to look into the possibility of more environmentally sound, long-term solutions under these circumstances. Armistead et al. (2022) [57] examined the effects of salinity (NaCl, 0-2.5 M) and arid climate temperatures (25 °C, 40 °C) on the stability of MT exemplar and sand (control) soil systems by locust bean gum (LB) biopolymer.

Some of the shortcomings of conventional systems, such as the requirement for microbial and nutrient input, time for cultivation and excrement precipitation, and inappropriateness with clayey soils, are solved by the direct use of exo-cultivated biopolymers for soil remediation. There are some advantages of direct use of biopolymers in soil over traditional biological soil treatment methods. Furthermore, because biopolymers are widely available and innocuous in nature, they can be utilized as a replacement for cement that releases greenhouse gases. Moreover, biopolymers can also be produced in large quantities and react with soil particles rather quickly, making them useful for short-term and urgent applications [58]. Each biopolymer is made of the same biodegradable polymers, but because the physiochemical properties vary, we need to utilize engineering judgment to harness these features effectively [42].

III. PROPERTIES OF MICROBIALLY TREATED SOIL

The section demonstrates the various properties of soil after treating it with various microbial treatment techniques. This section has been categorized as strength and durability properties of soil strengthened with various techniques.

- 1. Strength properties:** Ng et al. [59] applied microbial induced calcium carbonate precipitation (MICP) procedure using *B. megaterium* to evaluate the shear strength, and they observed that the ratio of treated shear strength to that of untreated sample augmented to 2.64 from 1.40. Unconfined compressive strength (UCS) was evaluated to be 800 kPa (prior to freeze-thaw cycling) for the MICP treated samples and that of samples treated with OPC resulted in > 600 kPa (prior to freeze-thaw cycling) and

typically results in severe damage, as anticipated with 40% reduction in strength, was observed by Cheng et al. [60] to validate bio-enzymatic facilitated enhancement of soil. Nafisi reported a tensile strength range of 210 kPa to 710 kPa with the type of sand and mass of carbonate, whereas a minimum compressive strength of 300 kPa was stated by van Paassen et al. [61]. High compressible with reduced shear strength fine-grained soils are pervasive in various regions of the globe [62]. Therefore, enhancing the properties of soil are frequently advised for these soils due to the concerns related to low bearing capacity, differential settlement, and inappropriate sideways motions on loading, when it is provided underneath foundations [63]. In the course of erection of roads, runways in airport, and railway tracks, chemical stabilisation practices have been often employed to improve the soil bearing capacity, with a reduction in permeability and settlements, and regulate the issues related to swelling/shrinking [64-67]. According to extensive research associated with the chemical processes and stabilisation mechanisms for conventional soils treated with additives [68], the engineering properties depend on the nature of chemical employed and the essential characteristics and properties of the stabilised soil.

In triaxial testing, Cheng et al. [60] reported that the mechanical characteristics of sand treated with the bio-cements increased the effective shear strength (i.e., cohesion, frictional angle) with a rise in the concentration of CaCO_3 at all level of saturation. The crystals precipitated have improved the cohesiveness with the aid of coarse sand more effectively at a lower saturation level than by increasing the frictional angle. With the similar level of saturation and CaCO_3 content as fine sand, coarse sand showed a greater friction angle compared to that of fine sand. According to the various researches, the major impacts of tiny soil particles are that they (a) increase the number of inter-particle interaction spots for the MICP practice and (b) lessen the tension occurring at each particle contact. The MICP method works best at a particle interaction right when cementation starts, and it becomes less effective as cementation spreads outward around a particle contact. Due to an increase in inter-particle interactions, moving the CaCO_3 crystals to two interaction places as opposed to one would be additional beneficial. The contact stress likewise reduces in direct proportion to the particle radius squared. As a result, smaller particles have two complementary advantages: improved MICP and reduced particle contact stresses.

Despite the fact that various investigations describe the strength parameters on a broad scale, triaxial tests are indicated to evaluate how biocemented soils react under monotonic and cyclic loadings since they replicate the performance of soil in the site for road applications.

- 2. Durability properties:** Increased soil strength (bearing capacity and shear strength), improved surface erosion resistance, and regulation of hydraulic conductivity and seepage are the primary goals of ground improvement [69].

With soil treated with a precipitation of $100 \text{ kg/m}^3 \text{ CaCO}_3$ resulted in a reduction in the permeability of about 60% [61]; whereas a reduced permeability results was observed by Ivanov et al. as per [70] in the range of 50 to 99% with the help of 1M cementation solution as a result of pore space gets clogged with calcite crystals. This might be due to the formation of crystals at interaction spots, which can sustain the assembly of pores without restraining the pore water agility.

According to Chang et al. [71], a tiny amount of Korean red-yellow soil that had been treated with xanthan gum increased soil erosion resistance and improved plant cultivation. Strong water adsorption during the rainy season and high soil moisture retention during the dry season are both characteristics of xanthan gum-treated soil [45].

Reduced permeability inhibits water from entering the samples, which reduces the amount of biopolymer particles that dissolve into the stabilized specimens [72-73]. Various studies observed that numerous biopolymers such as xanthan gum, chitosan, guar gum, poly glutamic acid, poly-hydroxybutyrate and sodium alginate that are available in the market were proficient in decreasing the soil permeability up to five times [74-77]. Biopolymers in soil have the positive potential to dramatically lower the erodibility of soil by boosting inter-particle cohesion, even if they make up just a tiny portion (0.5–1.0%) of the soil mass [71].

Chang et al. [78] investigated the strength and resilience of Jumunjin sand (standard sand of the Republic of Korea) treated with gellan gum biopolymer were assessed during cyclic wetting and drying. The acquired results show that the repeated wetting and drying of sands treated with gellan gum causes a progressive loss of strength due to the dissociation of the gellan gum monomers during wetting and incomplete recomposition during re-drying, with a loss of strength of around 30% over 10 cycles. Even after several cycles, a certain amount of strength recovery and resistance was seen, suggesting that gellan gum-treated soils may be used in practical construction for short- or medium-term goals.

Table 2 outlines the merits of five frequently utilized biopolymers in the geotechnical engineering field for stabilizing various types of soil.

Table 2: General merits of five frequently used soil stabilizing biopolymers

Biopolymer	Chemical composition	Merits	References
Xanthan gum	$C_{35}H_{49}O_{29}$	Reduced permeability	[58,72,79]
		Strong hydrogen bonds allow for the retention of the water.	
Gellan gum		Strengthening and extending the resilience of soils using thermos-gelation treatment	[71]
Agar	$(C_{12}H_{18}O_9)_n$	Due to the influence of curing time, increase the soil's shear strength.	[79-81]
		Quick gelation without chemical reaction during soil enhancement method	
Polyacrylamide	$(C_3H_5NO)_n$	Upsurge water permeation	[58,82-84]
		Reduction in soil erosion as a result of Polyacrylamide hydrogels	
Guar gum		Decreased bleeding level of the ground granulated blast furnace slag cement grouts	[72,85]
		Condense the penetrability	
		Improved shear strength constraints	

IV. CONCLUSION

Geotechnical engineering has long attempted to and developed methods of enhancing soil characteristics. Out of the many ancient techniques, people are now opting for more eco-friendly and sustainable techniques such as the use of biopolymers and non-pathogenic microorganisms in soil stabilization techniques. The techniques for bio-mediated soil improvement have been developed to lower carbon dioxide (CO₂) emissions during cement manufacture. Biopolymers being the cost-effective technique, have many more advantages over MICP, such as, their use can avoid some difficulties occurring while cultivating microorganisms in soil. The recent researches carrying out on both the areas are hoping to provide better user friendly and sustainable qualities, whereby a complete eradication of cement usage and the subsequent greenhouse gas emissions can be expected.

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Organism	Enzyme	Application	Reference
B. pasteurii NCIM	Urease	To improve the properties of bricks by MICP	[24]
Kocuria. flava CR1	Urease	Investigated the copper bioremediation capacity of K. flava CR1 based on MICP	[25]
Halomonas sp. SR4	Urease	examined how MICP can be used to remove strontium from aquifer quartz sand	[26]
Sporosarcina ginsengisoli CR5	Urease	Investigated the role of MICP in remediating As (III) contaminated soil	[27]
Bacillus megaterium	Urease	Investigated MICP for reducing the hydraulic conductivity (bioclogging) and improving the shear strength (biocementation) of two soil types (tropical residual soil and sand)	[8]
Bacillus megaterium SS3	Urease	Investigated the synergistic role of carbonic anhydrase and urease in calcium carbonate biomineralization	[28]
Methylocystis parvum	Urease	M. parvus OBBP offered a substitute MICP made of calcium formate to address some drawbacks like the emission of ammonia and the creation of nitric acid.	[29]
Lysinibacillus sphaericus CH5	Urease	Investigated the remediation of heavy metal-contaminated (Cd) soil by L. sphaericus CH-5	[30]
Bacillus megaterium	Urease	It has been demonstrated that improving the characteristics of structural concrete through the use of the bio-mineralization mechanism in cementitious materials is a good idea.	[31]
Bacillus aerius	Urease	Studied the strength and permeation properties of concrete employing calcite producing bacteria and rice husk ash	[32]
Bacillus sphaericus	Urease	Used B. sphaericus to examine how high-strength concrete's compressive strength could be improved	[33]
Sporosarcina pasteurii (ATCC 6452)	Urease	Evaluated the efficiency of MICP for enhancing the resistance to internal erosion of gravel-sand mixtures	[34]
Bacillus megaterium	Urease	Examined the effectiveness of sand biocementation to reduce an aeolian sand's susceptibility to wind erosion	[35]
Sporosarcina pasteurii	Urease	Recycled fine aggregates (RFAs) of mortars were shown to have better characteristics as a result of appropriate calcium carbonate precipitation on the mortar surfaces	[36]
Sporosarcina pasteurii	Urease	Investigated the reduction in liquid limit and increase strength via biocementation; increase strength via bioencapsulation of marine clay	[37]
Bacillus subtilis	Urease	It was examined how adding B. subtilis as a unique method affected the concrete's water absorption, electrical resistance, compressive strength, chloride ion penetration, carbonation depth, water penetration depth, and compressive strength in a sulphate	[38]

		environment.	
Sporosarcina pasteurii and Bacillus sphaericus	Urease	The effects of various treatment durations and pore volumes (PVs) of cementation media on MICP were investigated through laboratory procedures carried out outside of sterile and temperature-controlled conditions.	[39]
Sporosarcina Pasteurii (BCRC11596)	Urease	Investigated the effectiveness of MICP in mitigating beach erosion for coastal stabilization	[40]
Streptomyces microflavus	Carbonic anhydrase	Presented a method where CO ₂ is used for the treatment of calcareous sand	[41]

Table 1: Microorganisms and enzymes used for MICP