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Biocementation promoted by bacteria in concrete recovery and soil stabilization.

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ABSTRACT
The use of intelligent and adaptable materials has been gaining prominence in the construction industry, due to the ecological market demand and the awareness of investing in sustainable techniques, with low energy consumption and anthropogenic carbon emission. The development of materials and construction methods, with energy efficiency, adaptability to environmental conditions, and non-polluting has become a requirement for the progress of the sector and the future of cities. Thus, the objective of this bibliographic review is to provide an overview of the application of biocementation promoted by bacteria in the recovery of structures and soil stabilization. This technique uses minerals resulting from bacterial metabolic activities at room temperature. The microbiologically induced precipitation of calcium carbonate (MICP) produces cementitious materials with structural functionalities and durability. Also, it is harmless to the environment. Different applications of this process have been proposed in civil engineering, such as the bioconcrete in the remediation of fissures, the restoration of stone cultural heritage, and the improvement of the physical-mechanical properties of the soil, therefore, changing its resistance and permeability. Understanding how this biotechnology can change current construction strategies and solutions, thus, integrating nature-based systems into construction projects, will be discussed in this study.

KEYWORDS: bioconcrete, biostabilization, calcite

1 INTRODUCTION

Civil construction is in constant transformation, and changing the culture towards sustainability, since the sector is a great consumer of natural resources, has become a challenge, especially in the replacement of materials that cause environmental damage.

Also, the use of ecologically correct physical resources and the efficient use of energy, through the development of alternative materials and construction methods that are adaptable to environmental conditions, represents the future for sustainable cities and communities (DADE-ROBERTSON *et al*., 2017), particularly in the substitution of cementitious materials.

Cement is widely used as a construction material. However, its production generates environmental impacts during all stages of manufacturing. World cement production is responsible for around 10 to 15% of total industrial energy and 5 to 8% of anthropogenic $C0_2$ emissions (USON *et al*., 2013; GONZÁLEZ-KUNZ *et al*., 2017).

Thus, the use of non-polluting materials has been gaining importance in the construction industry, due to the demands of the ecological market and the awareness of investing in sustainable techniques. Providing, therefore, a space for the integration of biotechnology in construction projects.

A biologic alternative to reduce the environmental impacts caused by civil construction is the use of microbiologically induced calcite precipitation (MICP) (ALAZHARI *et al*., 2018). This approach uses the metabolic activity of bacteria and a mineral precursor in the medium to form an inorganic compound as cementing material, calcium carbonate, in the form of calcite, vaterite, and aragonite (AL-THAWADI, 2011; DE MUYNCK *et al*., 2010).

The MICP formed by bacterial cells represents an emerging interdisciplinary field, promising for several areas, as Geotechnology, Paleobiology, and Civil Engineering (DHAMI *et al*., 2013). Also, it combines microbiology with scientific research in the production and maintenance of building materials to design new intelligent, adaptable materials, integrating living cells into construction and architecture (DADE-ROBERTSON *et al*., 2017).

This technique appeared as an effective option to improve, among others, the mechanical properties of soils, concrete, and mortar structures, as well as in the repair of cracks and the protection of the surfaces of these elements against the attack of harmful agents. (DE MUYNCK *et al*., 2010; DEJONG *et al*., 2013; DHAMI *et al*., 2017).

In addition, this biotechnology can provide a solution in places where access is limited or as a safety factor, such as protection of slopes, old buildings susceptible to collapse, tunnel linings, and marine structures. It also reduces the costs of the work of inspection and maintenance.

Hence, the objective of this review is to provide an overview of the application of biocementation promoted by bacteria in the recovery of structures and soil stabilization.

2 ITEMS ABOUT THE TOPICS OF THE REVIEW

2.1 Mechanisms of calcium carbonate production by microorganisms

Different bacteria species, such as cyanobacteria, ureolytic bacteria, nitrate-reducing bacteria, myxobacteria, sulfate-reducing bacteria, and methanogenic archaea, induce the precipitation of calcium carbonate in the form of minerals. It occurs by increasing the pH, the concentration of dissolved inorganic carbon, the concentration of calcium, and the availability of nucleation sites (DHAMI *et al*., 2013, HAMMES *et al*., 2003; HAMMES & VESTRAETE, 2002).

In microbiologically induced calcite precipitation (MICP), pH and the concentration of dissolved inorganic carbon and calcium are essential to the process. However, the availability of nucleation sites is not necessary, as bacteria can behave as active nucleation sites (AL-THAWADI, 2008).

The increase in pH, due to the formation of hydroxyls (OH^{-}) generated from the production of ammonium ions (NH_4^+), is necessary to dissolve and dissociate inorganic carbon. MICP occurs over a pH range of 8.3 and 9.0, in which urease activity remains high (STOCKS-FISCHER *et al*., 1999).

Calcium and urea concentrations between 0.05 and 0.25 M are more effective in precipitating $CaCO_3$ (AL QABANY & SOGA, 2013). Among calcium sources (calcium chloride, calcium oxide, and calcium acetate), calcium chloride is the best for MICP as it provides higher urease activity and higher calcite production (ACHAL & PAN, 2014). In the MICP process, carbonate is produced extracellularly by microorganisms through two metabolic pathways: autotrophic and heterotrophic (SEIFAN *et al*., 2016).

In the autotrophic pathway, microorganisms convert carbon dioxide into carbonate in three forms: non-methylotrophic methanogenesis, by methanogenic archaea (this pathway is more common in marine sediments); oxygenated photosynthesis by cyanobacteria, and anoxygenic photosynthesis by purple bacteria (CASTANIER *et al*., 1999).

Regarding the heterotrophic pathway, there are three main groups of microorganisms involved in the process: organisms that use organic acids, sulfate-reducing bacteria, and organisms involved in the nitrogen cycle (DHAMI *et al*., 2013).

In the first case, different genera of bacteria such as *Bacillus, Arthrobacter*, and *Rhodococcus* use organic compounds (acetate, lactate, citrate, succinate, oxalate, malate, and glyoxylate) as a source of energy. Therefore, converting them into carbonic minerals, like calcium and magnesium carbonate (SEIFAN *et al*., 2016).

According to Knorre *et al*. (2000), aerobic oxidation to form calcium carbonate ($CaCO_3$) from acetate (CH_3COO^-) as an acid source and with the presence of calcium ion (Ca^{2+}) results in Eqs. (1-2-3).

$$CH_3COO^- + 2O_2 \xrightarrow{heterotrophic\ bacteria} 2CO_2 + H_2O + OH\text{-} \tag{1}$$

$$2CO_2 + OH\text{-} \rightarrow CO_2 + HCO_3{}^- \tag{2}$$

$$2HCO_3{}^- + Ca^{2+} \rightarrow CaCO_3 + CO_2 + H_2O \tag{3}$$

In the sulfur cycle, sulfate-reducing bacteria are responsible for dissimilatory sulfate reduction, and hydrogen sulfide (H_2S) is produced in an anaerobic respiration process. Hence, to form calcium carbonate ($CaCO_3$), the source of calcium, organic matter, and sulfate must be present in the medium (DHAMI *et al*., 2013).

Nevertheless, the production of carbonate or bicarbonate through the nitrogen cycle can occur by three main routes: the degradation of urea (ureolysis); amino acid ammonification, and dissimilatory nitrate reduction (ALAZHARI *et al*., 2018; SEIFAN *et al*., 2016). To produce $CaCO_3$, urea hydrolysis is the simplest and most widely used method for precipitating carbonates for different technical applications (DHAMI *et al*., 2013).

In ureolysis, the urease enzyme hydrolyzes urea to produce carbamate and ammonia (Eq. 4), which spontaneously hydrolyzes to another molecule of ammonia and carbonic acid (Eq. 5). These products equilibrate to form bicarbonate in the presence of water. Therefore, creating ammonium and hydroxide ions, leading to an increase in pH (Eqs. 6 –7). In the presence of calcium and an alkaline environment, these reactions pave the way for the precipitation of calcium carbonate (Eq. 8) (HAMMES *et al*., 2003; DHAMI *et al*., 2013).

$$CO(NH_2)_2 + H_2O \xrightarrow{urease\ enzyme} NH_2COOH + NH_3 \tag{4}$$

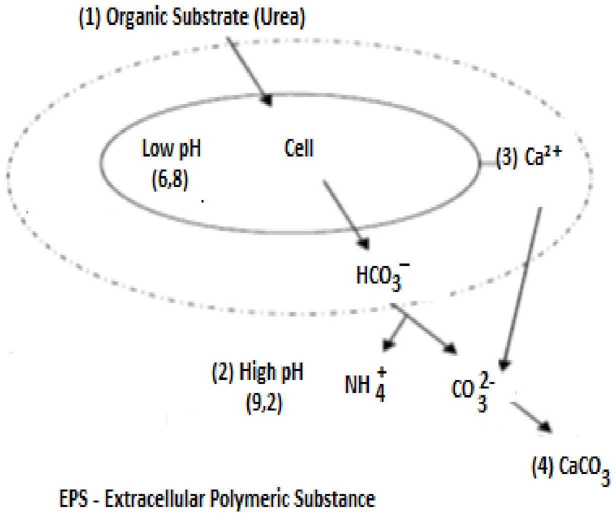
$$NH_2COOH + H_2O \rightarrow NH_3 + H_2CO_3 \tag{5}$$

$$H_2CO_3 \rightarrow 2H^+ + CO_3^{2-} \tag{6}$$

$$NH_3 + H_2O \rightarrow NH_4^+ + OH^- \tag{7}$$

$$Ca^{2+} + CO_3^{2-} \rightarrow CaCO3 \tag{8}$$

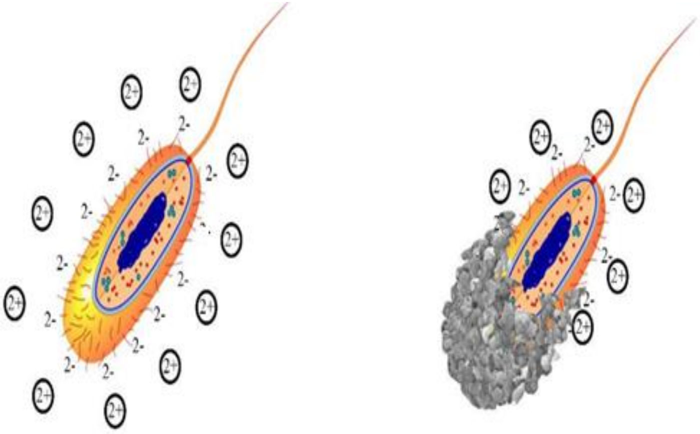
Figure 1 – Mineralization scheme by the ureolitic route.



Source: Al-Thawadi (2008)

Figure 2 shows that calcium carbonate production occurs by the attachment of positively charged calcium ions to negatively charged microbial cell walls by carbonates.

Figure 2. **Production of calcium carbonate through the binding of ions**



Cell wall with a negative charge (anions) and ions with a positive charge (cations)

Biomineral production through cell wall binding ions

Source: Modified by Seifan *et al.* (2016)

According to Dhami *et al*. (2017), in addition to the metabolic routes, the enzymatic pathway of carbonic anhydrase plays a significant role in capturing CO_2 in the form of carbonates in nature. This enzyme acts as a biological catalyst for the hydration of CO_2 into carbonic acid (H_2CO_3), which spontaneously dissociates into bicarbonate ions (HCO_3^-) and hydrogen cation (H^+). In the presence of a source of calcium, it produces calcium carbonate (Eqs. 9 - 10).

$$CO_2 + H_2O \xrightarrow{carbonic\ anhydrase\ enzyme} H_2CO_3 \leftrightarrow HCO_3^- + H^+ \quad (9)$$

$$Ca^{2+} + 2\,HCO_3^- \rightarrow CaCO_3 + HCO_3^- + H^+ \rightarrow CaCO_3 + CO_2 + H_2O \quad (10)$$

2.2 Species and concentration of bacterial cells

Different bacterial species precipitate carbonates in alkaline environments, rich in Ca^{2+} ions. However, the most suitable bacteria for MICP, capable of catalyzing the hydrolysis of urea, are urease positive, especially the genus *Bacillus* and *Sporosarcina* (KUCHARSKI *et al.*, 2012).

Aerobic bacterial species are indicated to produce urease enzymes since they release CO_2 from cellular respiration. The CO_2 production is related to an increase in pH due to the production of ammonium (KUCHARSKI *et al.*, 2012).

Many researchers have evaluated the potential for MICP application by bacteria in soil and sand stabilization, in concrete, and restoration of historic buildings (DHAMI et *al.*, 2013; ABO-EL-ENEIN *et al.*, 2013; JONKERS *et al.*, 2010; DE BELIE & DE MUYNCK, 2008; LEE, 2003; DICK *et al.*, 2006).

The species Bacillus megaterium has been used to improve concrete hardness and to reduce the compressibility of residual soils (LEE *et al.*, 2013).

Achal *et al.* (2011) evaluated mortar cubes treated with Bacillus sp. The result was almost six times less water absorption when compared to untreated samples. Dick *et al.* (2006) also reported a 50% reduction in water absorption by applying *Bacillus sphaericus* to limestone cubes.

Studying *Sporosarcina pasteurii* on the feasibility of calcite precipitation and its effect on the strength and compressibility of organic soil, Canakci *et al.* (2015) concluded that bacterial treatment influenced soil compressibility and shear strength.

Dhami *et al.* (2017) investigated the urease and carbonic anhydrase pathways, through *Sporosarcina pasteurii* and *Bacillus cereus*, respectively. The experiment was under biostimulation and bioaugmentation in sand column consolidation and high nutritional conditions. Both routes had success.

In a study on the recovery of historic buildings, Tiano *et al.* (1999) investigated the consolidating effect of the bacterial biomineral calcite on Pietra di Lecce limestone using *Micrococcus* sp. and *Bacillus subtilis*. Webster & May (2006) used anaerobic sulfate-reducing bacteria, Desulfovibrio desulfuricans and D. vulgaris, to remove black sulfate crusts that often stain buildings.

The main microorganisms of the genus Bacillus and Sporosarcina, used in MICP cited in the literature, are in Table 1.

Table 1: Main microorganisms, used in researches with MICP

Microorganisms	Phylo	Use	References
Bacillus amyloliquefaciens	Firmicutes	Restoration of ornamental stones	Lee (2003)
Bacillus cereus	Firmicutes	Biocement formation, limestone, and sand column consolidation.	Casteneir *et al*. (1999) Dhami *et al*. (2017)
Bacillus cohnii	Firmicutes	Calcite precipitation and concrete remediation	Jonkers & Schlangen (2008)
Bacillus lentus	Firmicutes	Limestone consolidation	Dick *et al*. (2006)
Bacillus megaterium	Firmicutes	Increased compressive strength in concrete, improves durability of building materials, structures, and soil compressibility	Dhami *et al*. (2013) Lee *et al*. (2013). Soon *et al*. (2013)
Bacillus subtilis	Firmicutes	Reduction in water absorption of limestone.	Tiano *et al*. (1999)
Bacillus pseudofirmus	Firmicutes	Repairing concrete cracks, increasing compressive strength, and reducing permeability.	Alazhari *et al*. (2018) Jonkers *et al.* (2010)
Sporosarcina pasteurii (Bacillus pasteurii)	Firmicutes	Biocement formation, sand column consolidation, concrete crack repair, increased compressive strength and reduced permeability, soil stabilization	Dhami *et al*. (2017) Canakci *et al.* (2015) Abo-El-Enein *et al.* (2013)
Lysinibacillus sphaericus (Bacillus sphaericus)	Firmicutes	Concrete consolidation: formation of biocement, repair of concrete cracks, and the reduction of permeability.	Dick *et al*. (2006) De Belie & De Muynck (2008)

Source: Author (2022)

In addition to the species, the concentration of bacterial cells influences the amount of precipitated calcium carbonate and urease activity (HAMMES & VERSTRAETE, 2002). High concentrations produce more urease per unit volume to initiate urea hydrolysis (KADHIM & ZHENG, 2016). Concentrations on the order of 10^6 to 10^8 cells increase the amount of urease (OKWADHA & LI, 2010).

2.3 MICP applications in engineering

MICP technology has potential in cement mortar restoration, limestone monument repair, reduction of permeability and cracks in concrete, pore filling, and consolidation of sand and soils (CHENG *et al*., 2013; DHAMI *et al*., 2013).

A – Self-healing concrete: the bioconcrete

Some microorganisms, in their natural state, can inhabit inhospitable places, and when they come under environmental stress, they form resistant endospores (survival cells), which are tolerant to high temperatures, chemicals, ultraviolet light and can survive for centuries (JONKERS, 2011).

Thus, when using bacteria to produce bioconcrete, most systems require the application of endospores, which have to be immobilized, usually via encapsulation, before being added to the self-healing concrete. To enable the bacteria to remain alive under adverse conditions for a prolonged period. In addition, it overcomes concerns about its viability under the aggressive conditions that occur in concrete hydration (JONKERS *et al*., 2010).

Furthermore, the endospore concentration required to provide calcium carbonate precipitation needs to be higher than 4×10^7 (endospores/ml) (ZHANG *et al*., 2017), as well as Ca^{2+} must be sufficiently available in the concrete. Therefore, allowing a satisfactory formation of calcium carbonate to fill the cracks (ALAZHARI *et al*., 2018).

The process begins when a bioconcrete structure is damaged, then the moisture starts to penetrate the cracks, and bacterial endospores germinate upon contact with water and nutrients and begin to consume sources of calcium, resulting in the production of carbonate. The main mechanism of bacterial crack healing is that the bacteria act widely as a catalyst and transform a precursor compound into a suitable filler material (JONKERS, 2011).

Mixture of microbial agent in concrete

According to Seifan *et al*. (2016), the microbial agent (bacteria and nutrients) can be inserted into the concrete matrix through a vascular network, already incorporated in the production phase, or it can be mixed directly during its preparation, by separate application of bacteria and nutrients or by encapsulation.

In the first process, bacterial endospores and the biological precursor of minerals (source of calcium, nitrogen, and phosphorus) are added separately to the concrete. In this process, alkaliphilic bacteria are the most attractive species for self-healing concrete as it tolerates its extreme environment (SEIFAN *et al*., 2016).

In the second process, bacterial endospores and the biological precursor are placed inside expanded clay capsules or other protective materials (diatomaceous earth, hydrogel, silica gel, and granular activated carbon) (WANG *et al*., 2012). The healing process begins with the rupture of the capsule with the formation of cracks in the concrete.

Bacterial protection by encapsulation has been the objective of some articles.

Alazhari *et al*. (2018) used coated expanded perlite to immobilize *Bacillus pseudofirmus* bacterial endospores and evaluated the self-healing performance through imaging and initial surface water absorption. The results indicated that healing could be achieved when coated expanded perlite containing self-healing agents was used as a 20% fine aggregate replacement and if an adequate ratio of spore to calcium acetate was provided.

Wiktor & Jonkers (2011) encapsulated calcium lactate (6% aggregate mass) and yeast extract (less than 0.1% aggregate mass) with *Bacillus alkalinitriculus* endospores in expanded clay (1 to 4 mm) to eliminate the effects of concrete properties. The result showed that, upon cracking, these encapsulated particles could cure mortars.

Zhang *et al*. (2017) used expanded perlite to immobilize *Bacillus cohnii* endospores. The volumes used were 3.6×10^9cells/ml. Calcium lactate (8 g/l) and yeast extract (1 g/l) were sprayed onto the surface of the particles but were not encapsulated or prevented from interfering with hydration reactions.

Assessment of bacterial concrete

The influence of biological healing agents on the strength and water absorption of concrete has been reported by several studies. Abo-El-Enein *et al*. (2013) incorporated different concentrations of *Sporosarcina pasteurii* with the mixing water of the concrete, resulting in a 33% increase, in 28 days, in the compressive strength of the cement mortar. The improvement in strength and water absorption was related to the growth of calcite crystals in the pores of the mortar matrix.

Achal *et al*. (2011) found that the application of *Bacillus sphaericus* caused an improvement in concrete permeability. The permeability test showed that the water absorption coefficient in the treated samples was six times lower than in the control samples, during 168 hours.

Krishnapriya *et al*. (2015) studied the influence of Bacillus megaterium, *Bacillus licheniformis*, and *Bacillus flexus* bacteria on compressive strength and crack healing in concrete. The compressive strength of the bacterial concrete samples increased compared to the control.

De Belie & De Muynck (2008) evaluated the ability of *B. sphaericus* to repair cracks in concrete by precipitating calcium carbonate via urea hydrolysis. As a result, $CaCO_3$ crystals were formed inside the pores of the studied material, sealing the crack. One of the consequences of this effect was the decrease in water permeability.

Bang *et al*. (2010) studied the effect of *Sporosarcina pasteurii* on the compressive strength of mortar samples for 7 and 28 days. The authors noticed that the highest concentration of *S. pasteurii* immobilized on porous glass beads could substantially increase the compressive strength of the mortar sample by 24%.

B - Soil stabilization through biocementation

The presence of bacteria on the land surface, about 10^{12} microorganisms per kg of soil, is considered as a resource for in-situ cementation applications in soil strengthening (DHAMI *et al*., 2017; DEJONG *et al*., 2013; DE MUYNCK *et al*., 2010; MITCHELL & SANTAMARINA, 2005). Furthermore, the prevalence of ureolytic communities has been widely seen in different soils, regardless of type, mineralogy, and environmental conditions (GAT *et al*., 2016; ZHU & DITTRICH, 2016), such as the genera *Bacillus*, *Sporolactobacillus, Clostridium,* and *Desulfotomaculum* (KUCHARSKI *et al*., 2006).

Consequently, calcium carbonate biomineralization becomes promising in soil stabilization, as it aims to change its properties, stimulating natural biochemical processes in situ to produce calcite precipitate (DHAMI *et al*., 2013; DEJONG *et al*., 2010; HARKES *et al*., 2010).

MICP promotes cementing bonds of non-cohesive soil particles, allowing contact between them, modifying the mechanical strength of the material resulting in a decrease in hydraulic conductivity, an increase in stiffness at small deformations, and an increase in resistance to large deformations (DEJONG *et al*., 2014; VAN DER STAR *et al*., 2011; VAN PAASSEN *et al*., 2010).

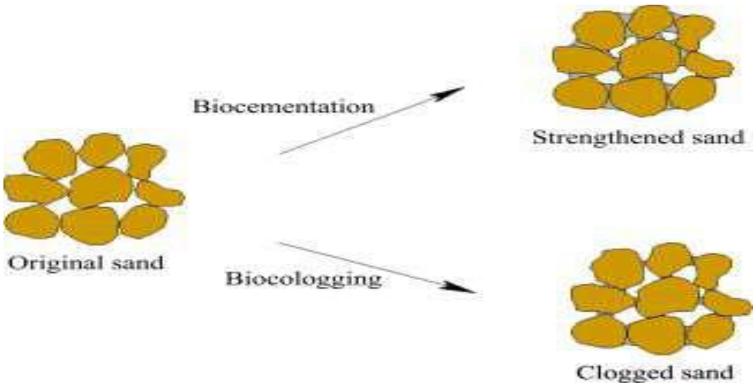
Biogeochemical processes can favor changes in soil physical properties (density, porosity, granulometric distribution), conductivity (hydraulic, electrical, thermal), mechanical (rigidity, compressibility, expansion, contraction, cohesion, cementation, friction angle, erodibility, and soil-water retention curve), and chemical compositions (reactivity, cation exchange capacity) (DEJONG *et al*., 2013).

There are two processes to increase shear strength and reduce soil permeability, biocementation and bioclogging, respectively.

Biocementation, or microbial cementation, improves the strength and stiffness properties of soil and rocks through microbial activity or microbial products. Forming, therefore, binding material between soil particles after the introduction of microorganisms and specific additives into it.

Bioclogging consists of reducing the hydraulic conductivity of soils and porous rocks, from the activity of microorganisms and their products, with the formation of carbonates that clog the pores and bind soil particles (IVANOV & CHU, 2008).

Figure 3. Biocementation and Bioclogging processes



Source: Chu *et al.* (2015)

The bacterial incorporation that forms calcium carbonate in building materials provides a sustainable solution for bioengineering. For example, the strengthening liquefied sand deposits, stabilizing slopes and slopes, reinforcing subgrades (AL QABANY & SOGA, 2013; CHENG *et al*., 2013; WHIFFIN *et al*., 2007), erosion control, and ease of excavation in the soil without cohesion (IVANOV & CHU, 2008).

The techniques used for MICP in soil applications are biostimulation and bioaugmentation. In biostimulation, indigenous calcifying bacteria are stimulated by adding nutrients and carbon sources. In the case of bioaugmentation, the system is supplemented with non-native bacteria and nutrient sources (GAT *et al*., 2016).

The introduction of bacteria into the soil can be through direct injection or pre-mixed. In the first method, the bacterial solution stream is injected into the soil from top to bottom, but the solution must have a retention period, allowing bacteria to attach to the grains before the nutrient solution injection. In the second method, the bacteria are mechanically mixed with the soil before introducing the nutrient solution (MUJAH *et al*., 2017).

Evaluation of shear strength and soil permeability

Several studies have evaluated soil biostabilization through shear strength and soil permeability, reducing hydraulic conductivity and water absorption (CHU *et al*., 2012; AL QABANY & SOGA, 2013; CANAKCI *et al*. 2015).

The calcium carbonate precipitated in the soil gaps increases the bond between the grains, improving soil strength, as shown by Soon *et al*. (2013), whose results proved a shear strength of 96% at a concentration of 0.5 M of cementing reagents.

Dejong *et al*. (2006) demonstrated that microbial cementation promoted an increase in the resistance of sandy soil and that the behavior of specimens cemented with bacteria was similar to that of soil cemented with gypsum.

Studies by Whiffin *et al*. (2007) in a 5-meter sand column, to simulate field conditions, showed that soil porosity, strength and stiffness were significantly affected by calcium carbonate content. They also reported that the increase in soil strength is directly proportional to the increase in calcium carbonate content produced, requiring a minimum calcium carbonate content of 60 kg/m³ for a measurable improvement in strength in the material.

Chu *et al*. (2012) used *Sporosarcina pasteurii* ureolytic bacteria and observed a substantial reduction in permeability and an improvement in soil shear strength at 35.9 Mpa, comparable to limestone.

Canakci *et al*. (2015) studied bacterial calcium carbonate precipitation and its effect on the compressibility and strength of organic soil. They found that the amount of calcium carbonate precipitated in the organic soil increased by about 20% in the treated samples compared to the untreated.

MICP promotes the reduction of the volume of gaps caused by the precipitation of calcium carbonate, reducing the permeability of the soil, as in the research by Whiffin *et al*. (2007), who reported a reduction from 22% to 75% of the initial permeability of the soil after microbial treatment.

Lee *et al*. (2013) studied the effect of MICP on hydraulic conductivity and shear strength of sandy soils and residual soils. As a result, there was a reduction in hydraulic conductivity (1,14–1,25 times) and an increase in shear strength (1,41–2,64 times) for both soil types.

Soon *et al*. (2013) evaluated the biotreatment of residual tropical soil and sand, through the MICP, with different densities. The results showed more reductions in hydraulic conductivity in the sand samples than in the residual soil samples. In the sand samples, the hydraulic conductivity decreased by approximately an order of $3{,}5\times10^{-3}$m/s to $3{,}2\times10^{-4}$m/s.

Al Qabany & Soga (2013) conducted permeability and unconfined compressive strength tests on sand samples treated with 0,1, 0,25, 0,5, and 1 M solutions of urea and calcium chloride. The treatment with a low concentration of the solution increased the strength of the treated samples. The use of a solution with a high concentration of calcium chloride and urea resulted in a rapid drop in permeability in the initial stage, while a low concentration resulted in a gradual and uniform decrease in permeability.

2.4 MICP challenges

The biomineralization field has potential in several sectors. However, it is necessary to make several efforts, with experts from different areas, to address the main research and development questions needed for commercial-scale applications (DHAMI *et al*., 2013).

MICP is a more complex and slower process than chemical methods, as microbial activity depends on many environmental factors, including temperature, pH, concentrations of electron donors and acceptors, concentrations and diffusion rates of nutrients and metabolites (DHAMI *et al*. al., 2013).

The choice of microorganisms in the biomineralization process must consider their resistance to alkalines (alkaliphilic) environments such as concrete, their ability to form spores and to survive adverse environmental conditions, in addition to not belonging to the group of pathogenic microorganisms, that pose risks to people's and environment's health (JONKERS *et al*., 2010).

The production of ammonium ions through ureolytic activity results in the emission of nitrogen oxides into the atmosphere. Furthermore, the excess of ammonium in the concrete matrix increases the risk of salt damage by conversion to nitric acid. Therefore, an optimization to find the required amount of urea is necessary to avoid excess ammonium emission (DE MUYNCK *et al*., 2010).

Likewise, the ammonia production in urea hydrolysis, where two moles of ammonia are generated for each mole of urea, can form ammonium chloride or nitrate in the soil. High ammonia concentrations are toxic to most organisms (VAN PAASSEN *et al*., 2010). Therefore, it is necessary to avoid leaching ammonia into the groundwater, treating the ammonia-rich effluent by biocementing (MUJAH *et al*., 2017).

The activity of microorganisms may be limited in clayey soils, as the bacteria have a size of 0.5 to 5 µm and cannot move in fine-grained soils (VAN PAASSEN *et al*., 2010; MITCHELL & SANTAMARINA, 2005). It occurs possibly due to limited pore space, which could hamper the transport and survival of microorganisms because of the interaction of the cell with the sediment and, therefore, could cause puncture failure or traction of the cell membrane (REBATA-LANDA & SANTAMARINA, 2006).

Detailed studies of soil microbial ecology are essential to determine the effects of introducing new bacteria in native communities, as well as the short, medium, and long term development of communities and the eventual secondary colonization of heterotrophic microorganisms (DHAMI *et al*., 2013).

3 FINAL CONSIDERATIONS

Biomineralization has brought a solution to different engineering applications, integrating nature-based systems into construction methods, reducing anthropogenic carbon dioxide emissions, minimizing environmental impacts, and promoting sustainable production systems with low energy consumption.

In addition, it is an effective technique compared to conventional treatment approaches due to its compatibility and efficient bonding in the soil-cement matrices, promoting increased resistance of materials to compressive strength and impermeability.

4 ACKNOWLEDGMENTS

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