

FACTORS AFFECTING BIOCEMENTATION PROCESS BY MICP IN SOILS

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Abstract

The aim of the paper is to present factors affecting the biocementation process using the Microbially Induced Calcium Carbonate Precipitation (MICP) method in soils. The MICP process is the one where microorganisms are used to precipitate calcium carbonate to improve soil parameters through the bridges formed between soil particles. The most common bacterial strain, with the ability to hydrolyse urease, and in the presence of calcium ions, promotes the precipitation of calcium carbonate, is *Sporosarcina pasteurii*. The paper focuses on the factors dependent on the soil, treatment method, and the bacteria density determined on the basis of OD₆₀₀. Research on factors influencing MICP in soil is essential for establishing optimal conditions required for the occurrence and effectiveness of biocementation.

Keywords: Biocementation of soil; MICP; Soil improvement; Soil parameters; Ureolytic bacteria.

1. INTRODUCTION

Rapid economic development in recent years has forced engineers to seek sustainable and environmentally friendly methods of improving soils and building materials. That is the reason for the emergence of new methods. One of such methods of soil improvement is biocementation, extensively investigated in the laboratories [1–4] to be applied in geotechnical and environmental engineering [5–7] as ensuring environmental sustainability [8].

Biocementation is a type of biomineralisation. It is a process whereby minerals, such as calcium carbonate, are formed through biological activity. Biomineralisation is the process by which living organisms influence the formation of mineral materials [9]. The phenomenon occurs in nature, including soil, geological sediments and water [10,11]. In soil improvement, biocementation is the process by which microorganisms create bonds between soil particles [12,13], and it is called Microbially Induced Calcium Carbonate Precipitation (MICP). MICP, based on urea hydrolysis reaction, facilitated by the enzyme ure-

ase, is the most common and efficient bio-mediated technique. Many soil and groundwater bacteria can hydrolyse urea, producing ammonia and carbon dioxide [14]. Ureolytic bacteria are microorganisms that produce high levels of urease, resulting in an alkaline environment that promotes the precipitation of calcium carbonate deposits [15]. This mineral precipitation occurs as positively charged calcium ions (Ca^{2+}) bind to the negatively charged cell walls of the microorganisms [16]. As illustrated schematically in Figure 1, calcium carbonate (CaCO_3) crystals form on the external surfaces of the cells through successive layering, eventually encasing the cells within the mineral deposits.

Calcite precipitation is described by the following overall equilibrium reaction [18, 19]:



In urea- CaCl_2 medium, microorganisms produce ammonia as a result of enzymatic urea hydrolysis to create an alkaline micro-environment around the cell. Biochemical reactions at the cell surface, which is the nucleation site, are as follows [16,19]:

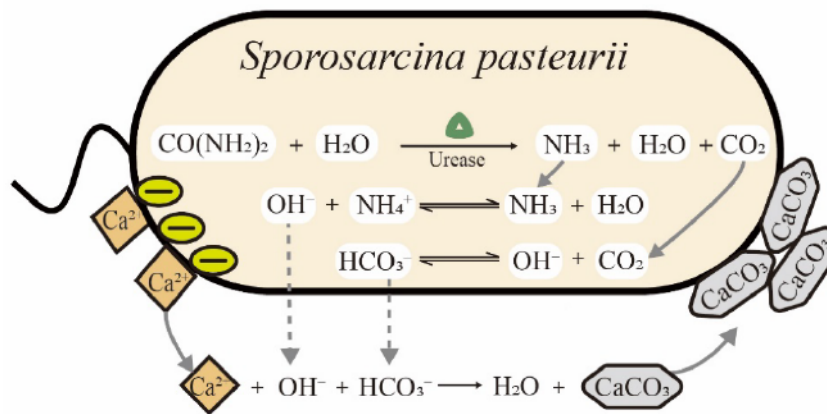
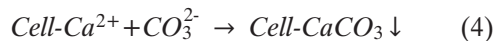
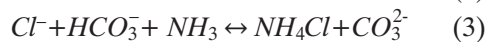


Figure 1.
Schematic diagram of microbially induced calcium carbonate precipitation on the bacteria *Sporosarcina pasteurii* cell [17]



As some research has proved, urease-aided $CaCO_3$ mineralisation is a quick process that can produce large quantities of calcium carbonate in a short time compared to the process under natural conditions. Despite numerous publications on the topic, the process remains the subject of ongoing research to better understand and implement it successfully from the laboratory to the field [21–24].

The process of initiating the Microbially Induced Calcium Carbonate Precipitation (MICP) in soil relies on three key components [25]: ureolytic bacteria, urea and calcium ions Ca^{2+} . The overall procedure consists of several steps: first, a microbial solution containing the bacteria is added to the soil to introduce the bacteria. This is followed by the addition of urea and a calcium source, forming what is known as the mineralisation or cementation solution. The desired outcome of biocementation can be achieved by varying the order and method of introducing these mixtures.

Ferris et al. [14] distinguished three stages of urea hydrolysis and calcium carbonate deposition. The concentration of the ion in the solution gradually increases, and as the ion concentration reaches the saturation critical point, calcium carbonate precipitates. Then, spontaneous $CaCO_3$ crystals grew on the nucleation site. Various process conditions can result in different crystal morphologies and types of precipitated calcium carbonate (vaterite, aragonite, and calcite), thereby affecting the strength of biocemented soil [15,20].

Microbial cementation is more complex than chemical methods since microbial activity depends on many environmental factors, such as temperature, pH, concentrations of electron donors and acceptors, concentrations and diffusion rates of nutrients and metabolites [15]. The paper aims to describe biological, chemical and physical factors influencing ureolytic MICP in soils based on the literature review.

2. FACTORS INFLUENCING MICP IN SOILS

Microbial Induced Calcium Carbonate Precipitation (MICP) is a soil improvement technique considered as one of the environmentally friendly methods compared to chemical soil improvement [25]. The additional advantage of biocementation is that it has a positive effect on the contaminated environment, such as: remediation of groundwater and soil [15,20,26,27], treatment of heavy metals [21–23].

In most of the research MICP method is used to improve the strength of the soil, which is assessed based on the unconfined compressive strength (UCS) parameter. However, other soil properties are also tested to determine the impact of MICP: shear strength [28, 29], permeability [30–32], unconfined compressive strength [33–35], compressibility [36–38], liquefaction resistance [39–41], erosion control [42,43].

In the research conducted by Cheng et al. [32] and Zhao et al. [33] found that the unconfined compressive strength (UCS) of samples treated with microbial-induced calcite precipitation (MICP) was improved. According to tests by Harkes et al. [2], the biocementation method stabilised loose sands to a

range of strength levels, from loosely cemented sand to moderately strong rock, with a UCS value between 0.2 and 20 MPa.

The MICP method is complex due to the numerous parameters and variables that influence the process. These include the bacterial strain and density, the composition and concentration of the cementation medium, and various environmental conditions such as pH value, temperature, and oxygen availability [44]. These factors can significantly affect the overall effectiveness of the process, the speed of precipitation, and the type of crystal formation. Additionally, the MICP method can enhance soil quality by filling pore voids with precipitated CaCO_3 , particularly in cases of sandy organic silt [35].

The hydraulic conductivity of materials subjected to biocementation decreases with an increase in the amount of cementation solution used and the duration of treatment. Conversely, a decrease in the duration of the treatment cycle also impacts hydraulic conductivity. Prolonged treatment time improves hydraulic conductivity reduction due to the continuous injection of the cementation solution, which leads to the formation of calcite within the pore spaces and results in reduced porosity [40]. Additionally, a higher content of CaCO_3 contributes to a more significant decrease in hydraulic conductivity. Whiffin et al. [8] mentioned the permeability reduction only up to 30% after the biocementation, with considerable improvement in strength. The lesser reduction in permeability was observed as an advantage of the MICP method, which allows more treatment injection for strength improvement, if needed, to increase liquefaction resistance.

2.1. Injection method

Effective calcium carbonate accumulation often requires multiple treatments (using cementation solution). A flow rate that is too fast may wash out bacteria, while a flow rate that is too slow can decrease the efficiency of the method. Common treatment methods for samples undergoing biocementation include injection using the grouting method, soaking, percolation or spraying, premixing, or a combination of these approaches.

The most common method of injection is the use of the of the grouting method to incorporate bacteria solution and cementation solution into the soil sample [3,4,8]. The method improved tested soils – the UCS reached a value of about 20 MPa [1], and the permeability was reduced [1,32]. However, the distri-

bution of precipitated calcium carbonate was uneven in the tested samples. One-phase injection is to mix the bacteria solution and the cementation solution, where urea and calcium ions Ca^{2+} are present. Mixed solutions are injected into the soil (biodeposition). In most cases, this way of injection causes immediate flocculation of bacteria and crystal precipitation. Krajewska [25] stated that it results in clogging pores, so the mixture would not reach deeper layers. However, surface biocementation in coarse-grained soils is an adequate technique.

In the literature [2, 8], the most common is two-phase injection. At first, bacterial solution is injected into the soil to ensure deeper penetration and uniform attachment of bacteria to the soil grains. Then the urea- Ca^{2+} solution is injected, so the precipitation process of calcium carbonate can be initiated.

Percolation through the soil surface is a widely used method of treatment [45,46], also referred to as spraying [42]. In this process, solutions are either poured or sprayed onto the soil, with capillary action and gravitational flow ensuring their distribution throughout the soil. In the sample treated using the surface spraying method [47], a noticeable level of cementation was achieved on the soil surface. However, this level of cementation decreased with depth due to an insufficient infiltration rate, which led to clogging at the sprayed surface. Achal et al. [48] conducted tests on sand columns that were injected with a combination of bacteria and a cementation solution using a gravimetric free-flow method. The results revealed that the samples exhibited inhomogeneity, with calcium carbonate predominantly accumulating in the upper layer of the column. Cheng and Cord-Ruwisch [49] employed a surface percolation method for injecting the MICP solution into dry and partially saturated samples. This approach led to greater relative homogeneity among the samples. However, the unconfined compressive strength (UCS) test results for coarse materials varied significantly, ranging from 850 kPa to 2067 kPa. As noted by Konstantinou et al. [50], achieving uniformity and homogeneity in MICP-treated samples is crucial for ensuring the repeatability of test results.

In the submerging or soaking method, the cementation solution penetrates the samples and relies on soil permeability. Zhao et al. [33] conducted tests on sand samples placed in geotextile moulds submerged in cementation media. The additional equipment used included a magnetic stirrer to maintain a uniform solution and an air pump to provide oxygen for bacteria. According to the injection/treatment method

employed, the results indicated that samples submerged in cementation media exhibited homogeneity in the biocemented samples, which is a crucial factor in the test of soil geotechnical parameters.

The premixing method (also called mixing) is another way of sample preparation. In this method, the bacteria solution or urease is mixed with the soil, so the uneven distribution of bacteria is avoided. The soil and solutions are mixed before placing material in the mould. Cheng et al. [51] claimed that this method of soil treatment can improve the MICP process. In the research of [52], sand samples prepared by the pre-mixing method reached the UCS values from 400kPa to 1.6 MPa. The pre-mixing method aims to ensure a more uniform distribution of bacteria within the soil. It should lead to effective calcium carbonate precipitation. Zhao et al. [33] found that 83% of CaCO_3 was evenly distributed in the sand column treated by the premixing method. In some of the cases, mixing method is a one-shot treatment (when it is not subjected to the cementation solution treatment after sample preparation) [53].

Researchers are exploring various methods for soil treatment, one of which is the pretreatment-mixing method proposed by [54]. In this approach, soil is first mixed with a cementation solution and then dried. After this preparation, the soil is combined with a bacterial solution. According to [54], the results indicate that the pretreatment-mixing MICP method shows greater improvement over the standard mixing method, enhancing the reinforcement effects of the mixing process.

2.2. Microbial factors

Several microbial factors influence MICP, including the bacterial strain, concentration, and viability. Various bacterial strains have been tested for their ability to initiate calcium carbonate precipitation. The ureolytic mechanism of MICP is the most commonly employed due to its low cultivation costs in complex media and its easily controllable metabolism [15,55].

The microbial factors that can affect the speed, the efficiency of the process, the uniformity of the cementation (homogeneity) through the sample, the forms of CaCO_3 – stable or unstable, shapes and sizes of crystals. Different bacterial strains exhibit varying levels of urease activity and growth characteristics. One of the most commonly used bacterial strains in the MICP method, due to its high urease activity, is *Sporosarcina pasteurii* [10,18,56,57].

Sporosarcina pasteurii is a soil bacterium known for its ability to tolerate extreme conditions [20,58]. The kinetics of urease hydrolysis, which is the enzymatic breakdown of urea, are influenced by several factors: urea concentration, cell density, ammonium (NH_4^+) concentration, and pH. Cell density can be expressed as the bacterial cell concentration (biomass concentration). Generally, higher cell concentrations lead to increased urease activity and faster CaCO_3 precipitation [59, 60]. This process typically results in the formation of smaller crystals of calcium carbonate [51,61].

The aim of biocementation in soils is to create a uniform sample in which stable calcite crystals are firmly attached to the soil grains. This process forms bonds between the soil grains, thereby enhancing the strength of the soil [33, 42, 62]. The concentration of bacteria in the bacterial solution used for biocementation is described by the optical density, which is measured at a wavelength of 600 nm (OD_{600}) using an ultraviolet-visible spectrophotometer [63].

The precipitation of calcium carbonate (CaCO_3) is influenced by four key factors: the concentration of dissolved inorganic carbon, the pH, the concentration of Ca^{2+} ions, and the presence of nucleation sites, which facilitate crystal nucleation [64]. The temperature and the salinity of the suspension also affect the precipitation process [65]. The duration and number of cementing liquid injections affect urease activity, and the amount of calcium carbonate produced.

Wang et al. [61] conducted tests to determine bacterial density based on the OD_{600} parameter. The optical density of the bacterial suspension on the microfluidic chips was measured at values of 0.2, 0.5, 1.0, 2.0, and 3.0. All other test parameters remained constant. Based on the results, the bacterial densities were found to correlate with the initial OD_{600} values of the bacterial suspensions. The OD_{600} parameter indicates bacterial concentration and activity. The formula to calculate bacterial density, based on the results of [61], is:

$$\text{Bacterial density (cells/ml)} = OD_{600} \cdot 4 \cdot 10^8 \quad (5)$$

The value of OD_{600} had an impact on the precipitated crystals' sizes, which was observed on a microscale. In the case of the lowest bacterial density (0.6×10^8 cells/ml), the crystals formed slowly and there were fewer of them. However, these crystals

were larger compared to those in the other samples on the microfluidic chip [61]. In the case of the higher bacterial density (2.0×10^8 cells/ml), the calcium carbonate crystals were small and unstable. However, the researchers concluded that, over time, unstable crystals might transform into larger and stable forms. A very high bacterial density of 5.2×10^8 cells/ml gave the less favourable results. While the rate of CaCO_3 precipitation increased, it resulted in the formation of large amounts of unstable calcium carbonate. This could lead to inhomogeneity in the improved soil, as unstable forms might become trapped, creating clogs in the pores and flow paths during the MICP treatment.

Wang et al. [61] concluded that the same optical density (OD_{600}) values in different studies can result in varying bacterial densities. This discrepancy is attributed to differences in bacterial cell sizes. In [61], the average size of the bacterial cells was $3 \mu\text{m}$, while in [66], it was approximately $10 \mu\text{m}$. These variations in cell size can lead to differences in the rate of urea hydrolysis. Consequently, even when the optical densities of the bacteria are the same, the ureolysis rates may differ due to the differences in bacterial size.

Zhao et al. [33] tested sand samples with an initial OD_{600} values of 0.3, 0.6, 0.9, 1.2, and 1.5. The results indicated that higher concentrations of bacteria, represented by OD_{600} , led to increased urease activity.

Fukue et al. [67] presented a new approach to assess optical density. The researchers highlighted that OD_{600} varies as some of the bacterial strains might be damaged with time. Additionally, a drawback of using OD_{600} is that it counts dead cells as well. Thus, the optical density does not represent the viability of bacteria and their quality. Taking this into consideration, researchers conducted a test of the relationship between the cell viability and optical density, to finally present the formula which might assess the amount of precipitated CaCO_3 based on the OD , which represents viable bacteria cell number [68]:

$$\text{CaCO}_3 = 8.46 \cdot OD \quad (6).$$

However, researchers highlighted, that the quantitative results are dependent on microbes and strains used in the biocementation method.

As Al Qabany et al. [69] indicted, precipitation of CaCO_3 is dependent on the chemical concentration used in the treatment. The most efficient condition for MICP is when all the chemical reactants (cemen-

tation solution with urea and CaCl_2) precipitate as calcium carbonate. Tests were conducted under various retention times (injection frequencies) and chemical concentrations in the liquid media (cementation solutions) from 0.1M to 1.1M. Test results showed that the precipitation pattern is dependent on the cementation solution concentration, where for the higher concentrations than 0.5M, a smaller amount of injections resulted in more hardened specimens and a tendency to clog. A treatment with a low concentration of 0.25M generally led to a uniform distribution of calcite precipitation across different levels of cementation. The crystals were distributed throughout the sand grains, with no areas of concentrated precipitation identified. Rather than accumulating around the crystals, precipitation appeared to occur on the surface of the sand grains. A more random distribution of precipitated CaCO_3 was observed when the 0.5 M concentration treatment was performed. As more CaCO_3 accumulated, the precipitation pattern became increasingly random, resulting in a less uniform distribution of crystals in some samples, while others displayed a more uniform distribution.

As Konstatinou and Wang [70] noted, environmental factors such as salinity, temperature, and nutrient availability significantly impact the overall performance of MICP by affecting the metabolic activity of bacteria.

2.3. Soil properties

The application protocol for the biocementation method may vary based on the type of soil and the desired enhancements in soil parameters. Initial MICP applications were primarily conducted on sandy soils [15,19,32,71,72] because the pore spaces in sandy soils facilitate the easy flow of bacteria and cementation solutions. The permeability of the soil is the parameter which is related to the efficiency of Microbially Induced Calcium Carbonate Precipitation. It also affects the crystal deposition [71]. Al Qabany et al. [69] conducted tests showing that well-graded and coarser sands have a higher precipitation rate than finer, poorly graded soils. A relation between grain size and CaCO_3 content was observed by Rebata-Landa [after [50], where the maximum carbonate deposition on the grain was approximately $100 \mu\text{m}$ in size.

Mitchell & Santamarina [73] presented a schematic graph with the bacteria size and types of soils, where the biocementation process might be effective. Few

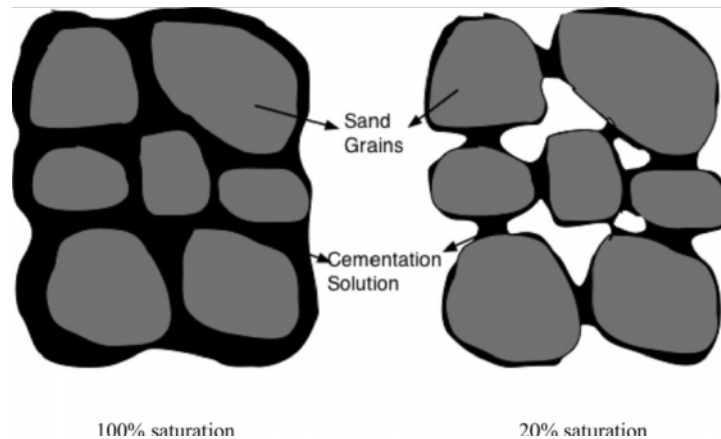


Figure 2

Conceptual illustration of pore cementation solution distributed in the sand matrix under different saturation conditions [32]

studies have tested the effect of MICP as a suitable method for fine-grained materials [74–78]. According to [47], test results conducted on sand and silt to compare effectiveness of MICP method in both types of soils. The amount of CaCO_3 precipitated for the sand specimens was about two times more than that for silt specimens, which was connected to the wider voids in non-cohesive soils. However, the researchers confirmed successful cementation in fine-grained material. tested two types of fine-grained and the results showed, that UCS of the soils after MICP treatment increased.

Cheng et al. [42] conducted research into the impact of soil grain size on the properties of biocemented soil at different degrees of saturation. Based on the test results and those of Cheng and Cord-Ruwisch [49], the researchers concluded that sand columns treated under lower saturation conditions exhibited higher strength, with a similar or identical amount of precipitated CaCO_3 . More calcite crystals formed at the points of interparticle contact (which have the greatest impact on the strength of cemented samples) in samples under a lower degree of saturation (see Fig. 2). The degree of saturation affects the location of crystal deposits. In fully saturated samples, the MICP solution occupies the pore space, and the precipitated crystals are not restricted in size or location. This leads to the formation of agglomerated crystals.

3. SUMMARY

Microbial Induced Calcite Precipitation (MICP) through urea hydrolysis is a complex biochemical process used to enhance soil properties, and it has recently undergone extensive testing. Various factors

influence MICP in soils, many of which are interdependent. The paper describes various factors that influence this process, mostly focused on sample preparation, where factors such as the soil type, bacteria concentration and treatment method are highlighted. As the literature shows, researchers are making attempts to find the best and most optimal way to apply the MICP method in the field. The multiplicity and complexity of factors and interdependence make this method challenging, but it has many advantages. It is important to emphasise that the MICP method, which utilises ureolytic bacteria, is multidisciplinary; therefore, specialists from fields such as geotechnics, chemistry, and microbiology should collaborate to achieve the best results.

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