

# Kahramanmaras Sutcu Imam University Journal of Engineering Sciences



Geliş Tarihi : 11.12.2024 Kabul Tarihi : 21.03.2025 Received Date : 11.12.2024 Accepted Date : 21.03.2025

# APPLICATIONS OF MICROBIAL CALCIUM CARBONATE PRECIPITATION FOR SOIL IMPROVEMENT: A REVIEW

# ZEMİN İYİLEŞTİRME İÇİN MİKROBİYAL KALSİYUM KARBONAT ÇÖKELİMİ UYGULAMALARI: BİR İNCELEME

Nurdan BAYKUŞ<sup>1</sup> (ORCID: 0000-0002-6199-3363)

<sup>1</sup>Kilis 7 Aralık University, Vocational School of Technical Sciences, Department of Construction, Kilis, Turkey

\*Sorumlu Yazar / Corresponding Author: Nurdan BAYKUŞ, nurdanbaykus@kilis.edu.tr

## ABSTRACT

Soil improvement is one of the significant geotechnical engineering issues in many parts of the world. It can be stated that traditional soil improvement techniques are widely used. However, since the perception of the environment and sustainability is very effective today, meeting the ever-increasing needs requires searching for innovative solutions and techniques in this direction. Recently, the improvement of soils with microbial calcium carbonate precipitation, which is known for its successful, sustainable, and environmentally friendly potential, has provided promising opportunities to provide solutions to many geotechnical problems. Microbial calcium carbonate precipitation is a naturally occurring technique used in soil improvement as part of biological and chemical metabolic activity processes, and has made significant progress with scientific research. This study aims to provide an overview of the effect of microbial calcium carbonate precipitation on the engineering properties of soils. In this study, firstly, an overview of the microbial calcium carbonate formation mechanism and the factors influencing this mechanism are presented. Secondly, the interaction between microorganisms and soil is explained. Thirdly, current studies in the literature are reviewed in detail, and their applications, advantages, and the difficulties that may be encountered in their application are presented. Finally, it contains evaluations and recommendations.

**Keywords:** Soil improvement, biocementation, microbial calcium carbonate precipitation, geotechnical engineering, soil strength

## ÖZET

Zemin iyileştirme dünyanın birçok yerinde önemli geoteknik mühendisliği konularından biridir. Geleneksel zemin iyileştirme tekniklerinin yaygın bir kullanım alanının olduğu belirtilebilir. Ancak günümüzde çevre ve sürdürülebilirlik algısının önemle etkin olması, sürekli artan ihtiyaçları karşılama da yeni çözümler ve tekniklerin aramasını zorunlu kılmaktadır. Son zamanlarda başarılı aynı zamanda sürdürülebilir ve çevre dostu potansiyele sahip mikrobiyal kalsiyum karbonat çökeltisi ile zeminler iyileştirilebilmesi birçok geoteknik sorununa çözüm sağlamak için umut vaat eden fırsatların ortaya çıkmasını sağlamıştır. Mikrobiyal kalsiyum karbonat çökeltisi doğal olarak oluşan ve biyolojik ve kimyasal metabolik aktivite süreçlerinin bir parçası olarak zeminlerin iyileştirilmesinde kullanılan ve dahası yoğun bilimsel araştırmalar ile önemli ilerlemeler kaydeden bir tekniktir. Bu çalışma mikrobiyal kalsiyum karbonat çökeltisinin zeminlerin mühendislik özelliklerine etkisine dair genel bir bakış sunmayı amaçlamaktadır. Bu çalışmada, öncelikle mikrobiyal kalsiyum karbonat oluşum mekanizmasına ve bu mekanizmayı etkileyen faktörlere genel bir bakış sunulmaktadır. İkinci olarak mikroorganizmalar ve zemin arasındaki etkileşim açıklanımaktadır. Üçüncü olarak ise literatürdeki güncel çalışmalar ayrıntılı olarak incelenmekte ve uygulamaları, avantajları ve uygulamasında karşılaşılabilecek zorlukları sunulmaktadır. Son olarak değerlendirmeleri ve önerileri içermektedir.

Anahtar Kelimeler: Zemin iyileştirme, biyoçimentolama, mikrobiyal kalsiyum karbonat çökelmesi, geoteknik mühendisliği, zemin mukavemeti

ToCite: BAYKUŞ, N., (2025). APPLICATIONS OF MICROBIAL CALCIUM CARBONATE PRECIPITATION FOR SOIL IMPROVEMENT: A REVIEW. *Kahramanmaras Sutcu Imam University, Journal of Engineering Sciences,* 28(2), 1110-1122.

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#### **INTRODUCTION**

In recent years, the rapid increase in population, urbanization, and industrialization has led to the rapid depletion of building areas in many countries. The fact that residential areas in cities have become insufficient over time has made it necessary to open new settlement and development areas (Candoğan, 2015). Many of these residential areas that have been opened are limited by the availability of soil that is not suitable for construction (DeJong et al., 2010). These areas, which have been opened for use and have weak soil characteristics, have brought many engineering problems and infrastructure investments to the agenda, especially in the last 20 years (PwC Türkiye, 2024; Candoğan, 2015).

Soil improvement is one of the oldest practices in civil engineering (Selçukhan & Ekinci, 2021). Soils can be improved by mechanical (compaction) methods, removal of water from the environment (drainage), creation of rigid columns in the soil, injection techniques, reinforced stabilization, geosynthetics and geotextiles, stabilization with additives, heat treatments, biotechnical improvement, etc (Selçukhan & Ekinci, 2021; Akyıldız, 2019; Demiröz & Karaduman, 2009). Many of these methods may require significant amounts of energy or cost to manufacture or assemble materials, or the use of artificial materials (DeJong et al., 2010). Moreover, to ensure the soil's stability, synthetic artificial materials such as cement, epoxy, silicates, phenoplasts, acrylamide, and polyurethane are applied to the soil with chemical, spraying, and permeability injection techniques (Yıldırım et al., 2016; DeJong et al., 2010). However, these practices create environmental concerns with the deterioration of the chemical properties or structure of the soil and the release of some toxic or dangerous gases such as carbon dioxide ( $CO_2$ ) during production or use (Yıldırım et al., 2016; DeJong et al., 2010; Karol, 2003). This issue has led researchers to develop environmentally friendly methods, production, and techniques.

The amount of cement consumption in the world in 2022 is 4,125 million tons (PwC Türkiye, 2024). During the cement production process, high temperatures, dust, toxic and allergenic chemicals, heavy metals, and waste gases in chimney emissions can harm all living and non-living environments day by day (Yıldırım et al., 2016). Cement production, which is an energy-intensive sector, accounts for a significant share of global CO<sub>2</sub> emissions from industrial production (PwC Türkiye, 2024). This shows the magnitude of the impact of the cement industry on carbon emissions, which is one of today's important environmental problems (Cuzman et al., 2015). As a result, research into using alternative raw materials, sustainability, and reducing environmental impacts in cement production is becoming increasingly important (PwC Türkiye, 2024; Naqi & Jang, 2019; DeJong et al., 2010)

As is known, cement is a hydraulic binding material formed by the calcination of a raw material mixture containing natural limestone and clay at high temperatures (Özer & Özgünler, 2023). The structure of limestone consists of 90% calcium carbonate (CaCO<sub>3</sub>) by weight (Ramakrishna et al., 2018). Therefore, it can be stated that approximately 65-70% of the cement consists of CaCO<sub>3</sub> (Yıldırım et al., 2016). New research aims to develop by chemical and biochemical means, cement-based composites, especially those used in repair or improvement processes, by imitating nature, thanks to the spontaneous formation of the components contained in cement (Yıldırım et al., 2016). In recent years, the process of producing CaCO<sub>3</sub> as a result of the metabolic activities of microorganisms using minerals in their environment has opened up exciting opportunities as a new and environmentally friendly approach (DeJong et al., 2010).

This study aims to provide an overview of the effect of microbial  $CaCO_3$  precipitation on soil properties. First, an overview of the microbial  $CaCO_3$  formation mechanism and the factors influencing this mechanism is presented. Secondly, the interaction between microorganisms and soil is explained. Thirdly, current studies in the literature are reviewed in detail. Its applications and advantages are presented. Then its difficulties are given. Finally, evaluations and recommendations are presented.

## MICROBIAL CaCO<sub>3</sub> PRECIPITATION MECHANISM

The mechanism of microbial CaCO<sub>3</sub> precipitation is the biological or chemical transformation of organic components into inorganic components that form them as a result of the reaction carried out by microorganisms (Bozbeyoğlu Kart, 2021; Yıldırım et al., 2016). Microbial CaCO<sub>3</sub> precipitation can occur in soil, water, and marine environments by various microorganisms. These precipitates can form through the following reactions depending on the type of microorganism (Bozbeyoğlu Kart, 2021; Anitha et al., 2018; Yıldırım et al., 2016; Dhami et al., 2013).

(i) In photosynthetic metabolism carried out by photosynthetic microorganisms (e.g. Nostoc calcicola, Oscillatoria willei, Anabaena cycadae)

(ii) In sulphate reduction metabolism by sulphate-reducing bacteria (e.g. Desulfovibrio desulfuricans, Desulfobacterium autotrophicum)

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(iii) During denitrification reactions by nitrate-reducing bacteria (e.g. Nitromonos spp., Nitrobacter spp.)

(iv) In ammonification reactions of myxobacteria (e.g. Myxococcus xanthus)

(v) Urea hydrolysis reactions carried out by urolytic bacteria (e.g. Bacillus sphaericus, Bacillus megaterium, Sporosarcina pasteurii (S.pasteurii was previously classified as Bacillus pasteurii)).



**Figure 1.** Schematic Presentation of Suggested Bacterial Calcium Metabolism and Subsequent CaCO<sub>3</sub> Precipitation under High-pH and High-Ca<sup>2+</sup> Extracellular Conditions (Hammes & Verstraete, 2002).

Of all the above, microbial CaCO<sub>3</sub> precipitation via urea hydrolysis is the simplest and most widely used method for the precipitation of carbonates in various technical applications (Dhami et al., 2013). A schematic diagram of bacterial calcium metabolism and subsequent CaCO<sub>3</sub> precipitation is presented in Figure 1. Considering the reactions mentioned, it can be stated that there are 4 important factors in CaCO<sub>3</sub> precipitation (De Muynck et al., 2010). These factors can be counted as a) Dissolved inorganic carbon concentration (to increase calcite precipitation by reducing CaCO<sub>3</sub> saturation) b) pH c) Concentration of calcium (Ca<sup>2+</sup>) ions d) Nucleation (to ensure the accumulation of Ca<sup>2+</sup> ions on the surface of the cell wall) (De Muynck et al., 2010; Hammes & Verstraete, 2002).

## FACTORS AFFECTING MICROBIAL CaCO<sub>3</sub> PRECIPITATION

Since microbial CaCO<sub>3</sub> precipitation is a biological and chemical process, it can be affected by many factors. However, the main factors can be listed as bacterial type, temperature, pH, inoculation rate, type and concentration of calcium source, and urea concentration (Bozbeyoğlu Kart, 2021; De Muynck et al., 2010; Okwadha & Li, 2010).

Bacteria are microorganisms that are active in many different processes and have different CaCO<sub>3</sub> production capabilities. The most commonly used of these microorganisms is the precipitation of  $CaCO_3$  via urea hydrolysis by ureolytic bacteria (Anbu et al., 2016; Candoğan, 2015; Okwadha & Li, 2010). Ryparova et al. (2021) compared the viability and calcite production abilities of three bacterial species commonly used in the literature under suboptimal conditions. They showed that Bacillus pseudofirmus is the most suitable bacterium due to its ability to precipitate sufficient amounts of dense calcite and its satisfactory metabolic activity. Temperature is an important parameter affecting all chemical reactions (Bozbeyoğlu Kart, 2021). It is stated that temperature is effective on bacterial activity and growth, calcite precipitation amount and content, and nucleation rate in the biological process by microorganisms (Sun et al., 2019; Kim et al., 2018). The pH value of the environment is also important for microbial CaCO<sub>3</sub> precipitation. The increase in pH can be considered as an indicator of urea hydrolysis (Okwadha & Li, 2010). In addition, the pH value of the environment can affect the urease enzyme activity of bacteria (Anbu et al., 2016). Therefore, the number of cells in the environment may also be affected. Because the number of cells is proportional to the amount of enzymes in the environment. Ali & Karkush (2021) and Martinez et al. (2013) found that yield was higher in the environment with a higher inoculation rate. For CaCO<sub>3</sub> precipitation to occur outside the cell as a result of the activities of the enzymes, Ca<sup>2+</sup> ions must be present in certain concentrations in the environment. The amount of CaCO<sub>3</sub> released at the end of the CaCO<sub>3</sub> precipitation process depends on both the type of calcium source (organic or inorganic) and its amount in the environment (Bozbeyoğlu Kart, 2021). In the study conducted by Xu et al. (2014), they stated that an organic calcium source increased the efficiency of CaCO<sub>3</sub> precipitation. De Muynck et al. (2010) and Naveed et al. (2020) revealed that bacterial CaCO<sub>3</sub> precipitation increases as the urea concentration increases.

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#### **BACTERIA AND SOIL INTERACTION**

Bacteria and soil interaction generally refers to the formation of a chemical reaction network that is managed and controlled within the soil through biological activity, which ultimately changes the soil's engineering properties by forming by-products (DeJong et al., 2010). This formation occurs through the harmony between the bacteria and the soil into which they are injected. This harmony can cover a wide size range thanks to bacteria typically having sizes of 0.5 to 3  $\mu$ m (Madigan et al., 1997), enabling them to interact with both coarse and fine-grained soil particles. As a result, bacteria or microorganisms can interact with many types of soil (DeJong et al., 2010).



Figure 2. Typical Sizes and Geometric Limitations of Microorganisms and Soil Particles (Mitchell & Santamarina, 2005).

The primary factor affecting microbial transport is pore size, which allows bacteria to move through the pore space (Mitchell & Santamarina, 2005). Figure 2 may present a limit for an in-situ soil improvement that depends on the soil particle size relative to the size of the bacteria. However, the range of soils that are not in situ and are suitable for improvement can be expanded to pure clays (DeJong et al., 2010). As it is known, clay is a natural material consisting of minerals. Due to their fine colloidal particle structure, clays can have very high specific surface areas and affinity for microorganisms. Therefore, it can be stated that there is a positive relationship between the tendency of microorganisms to stay near or together on the water and ion-rich clay surface (Bozbeyoğlu Kart, 2021).



Figure 3. Microbial CaCO<sub>3</sub> Precipitation with Urea Hydrolysis Improves Soil Structure (Naveed et al., 2020).

In recent years, the precipitation of CaCO<sub>3</sub> through the biological activity process that can occur naturally in the soil and thus the formation of a structure that strengthens the bond between soil particles has become an innovative and sustainable method for geotechnical applications (Naveed et al., 2020). This method called 'Biocementation' depending on microbial activity, generally results in the catalyzing of urease hydrolysis and thus the production of carbonate ( $CO_3^{2-}$ ) ion (Zhang et al., 2024).  $CO_3^{2-}$  ions form CaCO<sub>3</sub> precipitates in the soil in the presence of soluble Ca<sup>+2</sup> (Putra et al., 2017). As seen in Figure 3, these precipitates can improve soil properties through interparticle bonding and filling pore spaces (Meng et al., 2021).

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#### SOIL IMPROVEMENTS TREATED BY MICROBIAL CaCO3 PRECIPITATION

Many researchers worldwide are studying the microbial CaCO<sub>3</sub> precipitation process to improve the strength and mechanical properties for soil stabilization (El Mountassir et al., 2018). Since it is currently a very young and new field, it is widely used in research areas for the efficiency, limitations, advantages, disadvantages, effective methods, and techniques of the applications. In general, many researchers have reported the successful potential of microbial CaCO<sub>3</sub> precipitation for soil improvement in properties such as soil shear strength parameters, permeability, unconfined compressive strength (UCS), and binding of the soil particles (Ali & Karkush, 2021; Kalantary & Kahani, 2019; Akoğuz et al., 2018; Sharaky et al., 2018; Putra et al., 2017; Chu et al., 2015; Dhami et al., 2013). Moreover, successful results have been shown in reducing the liquefaction ability of sand (Riveros & Sadrekarimi, 2020; Xiao et al., 2019; Chu et al., 2015) and erosion prevention (Salifu et al., 2016) studies. On the contrary, the disadvantages of microbial CaCO<sub>3</sub> precipitation are also stated in the literature, such as the formation of toxic by-products such as ammonia (NH<sub>3</sub>) and ammonium (NH<sub>4</sub>) during urea hydrolysis and the uncontrolled accumulation of these by-products that may endanger human and environmental health (Zhang et al., 2024; Naveed et al., 2020).

Although microbial  $CaCO_3$  precipitation generally takes place in a complex physical environment, it can change the properties of the soil in which it is applied, including biotic and abiotic factors, at the macro or micro level (Zhang et al., 2024). When Figure 4 is examined, it is seen that permeability, hardness, compressibility, shear strength, and volumetric behavior are among the primary soil properties that can change 10 times or more (DeJong et al., 2010). However, to achieve this stabilization of the soil with microbial  $CaCO_3$  precipitation, it can be said that factors such as the bacterial concentration in the soil particles, the concentration and morphology of the cementing solution, the convection and diffusion of solute molecules, the injection method and time intervals are also important (Zhang et al., 2024; Naveed et al., 2020).



**Figure 4.** Overview of Biological Mediation Soil Improvement Systems (Ω=Resistivity, Vs=Shear Wave Velocity, Vp=Compression Wave Velocity, [–]=Chemical Concentration (DeJong et al., 2010).

The most effective organisms in CaCO<sub>3</sub> precipitation are ureolytic bacteria (Bozbeyoğlu Kart, 2021). Among ureolytic bacteria, studies in the literature mostly include bacteria of the genus Bacillus as model microorganisms. Because these bacteria are popular as the bacteria with the fastest rate of catalyzing urea due to their excellent compatibility with the soil environment (Zhang et al., 2024; Yıldırım et al., 2016). Urease catalysis in urea hydrolysis may be a critical step related to the rate of CaCO<sub>3</sub> precipitation (Zhang et al., 2024). Table 1 presents the types of bacteria and soils, tests, and brief results that some researchers in the literature have examined in the process of microbial CaCO<sub>3</sub> precipitation with urea hydrolysis.

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Table 1. Overview of Soil Improvement	t Research with Microbial CaCO <sub>3</sub> Precipitation
Types of	Evaluation

Researchers	Soil type	Types of bacteria	Evaluation procedures	Findings achieved
Ali & Karkush (2021)	Soft clay soil (CL)	Bacillus sonorensis	UCS	$\rightarrow$ UCS value increased with the microbial CaCO <sub>3</sub> precipitation technique $\rightarrow$ UCS value increased significantly with increasing treatment duration.
Kalantary & Kahani (2019)	Poorly graded sand (SP)	S. pasteurii and Arthrobacter crystallopoietes	UCS, SEM and XRD	$\rightarrow$ Depending on the temperature variable, the UCS strength increases from approximately 70 kPa to 230 kPa has been observed.
Akoğuz et al. (2018)	Coarse sand, coarse sand - gravel mixture and silica sand	Viridibacillus arenosi	UCS, SEM and XRD	<ul> <li>→Different environments can affect bioremediation.</li> <li>→Biological soil improvement can be achieved by using V. arenosi bacteria.</li> </ul>
Sharaky et al. (2018)	Sandy soil (BS classification)	Sporosarcina pasteurii	Stability in water, UCS, Shear parameters (c, $\phi$ ), Point load test, Bulk density, Slake durability index, CaCO <sub>3</sub> content, SEM, and XRD	<ul> <li>→The precipitation of CaCO<sub>3</sub> has played a significant role in increasing the compressive strength of bio-cemented samples.</li> <li>→The stiffening of the treated soil increased.</li> <li>→Calcite formation inside the sample may affect bulk density.</li> </ul>
Shan et al. (2022)	Calcareous sand (with activated carbon addition)	Sporosarcina pasteurii	Cyclic triaxial (CTX) tests, Bacterial retention tests, and SEM for liquefaction resistance	→As the activated carbon content increased, the samples' bacterial retention rate, cyclic strength, and secant modulus improved significantly. →The test using 0.75% activated carbon content best increased the resistance of calcareous sand to liquefaction. →When the activated carbon content exceeded 0.75%, the CaCO <sub>3</sub> content decreased. Excessive addition of activated carbon to the sample can reduce the effect of the microbial CaCO <sub>3</sub> precipitation process.
Bağrıaçık et al. (2021)	High plasticity clay (CH) and sandy clay (SC)	Bacillus Sp.	UCS, Swelling pressure	→Bacillus Sp. injection has a positive effect on the strength and swelling pressures of soils in freeze-thaw situations. →The increase in the SC soil's strength value was 12.60% more than the CH soil.
Whiffin et al. (2007)	Sand	Sporosarcina Pasteurii	Strength, Stiffness, Porosity, and Permeability	→A linear relationship was observed between the presence of CaCO <sub>3</sub> and porosity. →The highest strength or stiffness in the column was 570 kPa, measured at the same location as the maximum CaCO <sub>3</sub> amount, approximately 1 m away from the injection point. →Permeability decreased slightly independent of CaCO <sub>3</sub> content.
Liu et al. (2020)	Clayey soils (CL)	Sporosarcina pasteurii	Crack image analysis system (CIAS), SEM	<ul> <li>→Microbial CaCO<sub>3</sub> precipitation is effective in increasing the resistance of soil to drying cracking.</li> <li>→Microbial CaCO<sub>3</sub> precipitation reduced soil cracking by a max of 89%.</li> <li>→CaCO<sub>3</sub> precipitation improves the mechanical behavior of the soil.</li> </ul>

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Canakci et al. (2015)	Sandy organic silt (OH)	Sporosarcina pasteurii	SEM, EDX, Consolidation tests, and Shear strength (c, $\phi$ )	→Bacterial CaCO <sub>3</sub> precipitation improves the shear strength and the compressibility behavior of organic soil. →The amount of CaCO <sub>3</sub> in organic soil increased by approximately 20%.
Soon et al. (2013)	Tropical residual soil and sand	B. megateri um	Shear strength, Hydraulic conductivity, SEM, CaCO <sub>3</sub> precipitation	$\rightarrow$ The improvement rates in the shear strength of residual soil samples are higher than those of sand samples. $\rightarrow$ A decrease in hydraulic conductivity can be observed with a relative increase in the amount of precipitated calcite.
Nagy et al. (2022)	Quartz and calcareous sands.	Sporosarcina pasteurii	Uniaxial compressive strength, CaCO <sub>3</sub> precipitation, XRD, and SEM	<ul> <li>→Higher compressive strengths are obtained in calcareous sand compared to quartz sand.</li> <li>→The CaCO<sub>3</sub> precipitation rate remains nearly constant at about 97.8 - 99.3% over the tested duration.</li> </ul>
Duo et al. (2018)	Desert aeolian sand	Sporosarcina pasteurii	Density, Permeability, UCS, XRD, Micro-structure analysis, CaCO <sub>3</sub> content	<ul> <li>→The microbial CaCO<sub>3</sub> precipitation was found to increase the UCS and sand density.</li> <li>→The permeability decreased due to the increase in CaCO<sub>3</sub> content.</li> <li>→Solidification solutions at different concentrations (0.5, 1.0, 1.5, 2.0, and 2.5 mol/L) can affect sand properties.</li> </ul>
Wani & Mir (2021)	Low plasticity silt (ML)	Bacillus pasteurii	UCS and Plate load tests, CaCO <sub>3</sub> precipitate, XRD, and SEM	→The increased strength values, higher bearing capacity, and decreased vertical stresses are attributed to the CaCO <sub>3</sub> formation seen in the SEM micrographs. →The UCS values of the treated soil increased by 3.5 times and the carrying capacity by 2 times.
Akoğuz et al. (2023)	Poorly graded sand (SP)	Viridibacillus arenosi	UCS, XRD, SEM, and Line profile analysis, Permeability, Calcite content, Direct shear test, pH, and Urease activity	→Different results were obtained with the same cementation solution flow rate (0.266, 0.400, and 0.666 ml/sec) and treatment times (3, 6, and 11 days) of two soil types with different grain sizes. →Decreasing the flow rate in soils with small pore volumes or increasing the flow rate in soils with large pore volumes produces better UCS results. →Differences in strength and permeability results can be attributed to grain size distribution, flow rate, and treatment time.

In studies conducted with microbial CaCO<sub>3</sub> precipitation for soil improvement as examined in Table 1, the strength of the treated soil was a direct measure of the improvement effectiveness. Researchers usually relate the UCS values reported in literature studies to the rate of CaCO<sub>3</sub> precipitated to indicate the level of biocementation achieved (Fu et al., 2023). Moreover, They have confirmed that CaCO<sub>3</sub> is present in the soil environment and that its amount has increased or the grains have bonded together using X-ray diffraction analysis (XRD) and scanning electron microscopy (SEM) tests (Nagy et al., 2022; Wani & Mir, 2021; Duo et al., 2018; Sharaky et al., 2018; Canakci et al., 2015; Soon et al., 2013; Whiffin et al., 2007) Although the ratio of precipitated CaCO<sub>3</sub> is important in predicting and modifying the level of strength improvement, there are different correlations reported in the literature (Zhang et al., 2024; Fu et al., 2023; Duo et al., 2018). When the proportion of precipitated CaCO3 is low, the CaCO3 crystal units between soil particles are few, and thus the cementing bonds may be weak and easy to break. In this case, it can be said that the strength gain is uncertain. It can be noted that as the proportion of CaCO<sub>3</sub> increases and more CaCO<sub>3</sub> crystals accumulate between the particles, the cementation bonds can be stronger and the strength gain can increase (Fu et al., 2023). Therefore, it is also reported that UCS may increase with higher CaCO<sub>3</sub> content (Zhang et al., 2024; Wani & Mir, 2021). Additionally, significant improvements in the shear behavior (friction angle and cohesion) of soils treated with microbial CaCO<sub>3</sub> precipitation have been shown (Sharaky et al., 2018; et al., 2015; Soon et al., 2013). Behzadipour & Sadrekarimi (2023) stated that compared to untreated samples, the cohesion and friction angle

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values of samples treated with microbial  $CaCO_3$  precipitation improved by approximately 19 kPa and 5°, respectively.

There are many experimental studies in the literature where different reagent concentrations are examined or depending on other variables. For example, Soon et al. (2013) revealed that when the reagent concentration was increased from 0.25 to 0.5 mol/L, the UCS of the soil increased, but when 1 mol/L was removed, the UCS returned to its unbiocemented state. Lian et al. (2019) and Mahawish et al. (2019) demonstrated that the reagent concentration, reaction time, temperature, and cementing solution affected the microbial CaCO<sub>3</sub> precipitation process. Whiffin (2004) demonstrated changes in urease activity occurring with increasing urea and calcium concentrations. Kalantary & Kahani (2019) examined the effect of temperature on the microbial CaCO<sub>3</sub> precipitation process and stated that the temperature decrease is more effective in bacteria with high urease activity. Decreasing temperature may lead to a decrease in urease activity, reduce the cementation degree, and cause low CaCO<sub>3</sub> content. In addition, depending on the temperature conditions, the formation of CaCO<sub>3</sub> polymorphs in various particle morphologies can be observed (Tai & Chen, 1998). Therefore, it can be stated that the effect of temperature variables on microbial CaCO<sub>3</sub> precipitation-based soil improvement is complex (Fu et al., 2023). In the study conducted by Sadjadi et al. (2014), they reported that the swelling potential of the soil decreased with the increase in the bacterial ratio and application time during the microbial CaCO<sub>3</sub> precipitation process.

Researchers have shown that the application of microbial CaCO<sub>3</sub> precipitation plays an important role in the improvements in the porosity and permeability of soils (Song & Elsworth, 2024; Zhang et al., 2024; Duo et al., 2018; Soon et al., 2013; Whiffin et al., 2007; Kantzas et al., 1992). The formation of cementation that fills the voids due to the bonding between particles with microbial CaCO<sub>3</sub> precipitation optimizes the soil pore structure and reduces its permeability (Zhang et al., 2024; Wani & Mir, 2021). The conversion of loose, unconsolidated sand into well-cemented sand using B. pasteurii-type bacteria has been demonstrated by Kantzas et al. (1992). They reported that porosity decreased by up to 50% and permeability decreased by up to 90% in areas where cementation took place. Song & Elsworth (2024) observed a decrease in porosity from 19.11%-19.67% to 10.1% and a decrease in permeability of approximately 95% after microbial CaCO<sub>3</sub> precipitation cycles. Whiffin et al. (2007) found that the permeability of sand column treated with microbial CaCO<sub>3</sub> precipitation decreased from  $2 \times 10^{-5}$  m/s to  $9 \times 10^{-6}$  m/s compared to the original soil. Zhang et al. (2024) showed that permeability decreased with increasing CaCO<sub>3</sub> content.

Research has also proven that microbial  $CaCO_3$  precipitation applications have significant application value in reducing soil erosion and liquefaction (Zhang et al., 2024; Shan et al., 2022; Chittoori et al., 2020; Amin et al., 2017). Chittoori et al. (2020) showed that microbial  $CaCO_3$  precipitation successfully provides stabilization of expanding soils. They concluded that the calcite content of the soil increased from 3% to 8%, and the free swelling index decreased from 114% to 29%. Amin et al. (2017) showed that the application of microbial  $CaCO_3$  precipitation provided a 95% reduction in erodibility and a 5 times increase in shear stress compared to untreated sand.

## CHALLENGES OF SOIL IMPROVEMENTS TREATED WITH MICROBIAL CaCO<sub>3</sub> PRECIPITATION

The novel, promising, sustainable environmental potential of soil improvements treated with microbial CaCO<sub>3</sub> precipitation has been well demonstrated in many studies. However, the multiple effects on the microbial CaCO<sub>3</sub> precipitation process can be considered to be full of uncertainty before it reaches relatively practical applications (Fu et al., 2023). It can be stated that the biological, chemical, physical, and environmental effects in question are of a complex nature (Zhang et al., 2024; Fu et al., 2023; Naveed et al., 2020). The process of microbial CaCO<sub>3</sub> precipitation potential mineralization mechanism is difficult to precisely control, and the effect of by-products (such as NH<sub>4</sub> ions) formed during the mineralization mechanism may be limiting (Suresh & Uday, 2024; Zhang et al., 2024; Yu & Yang, 2023; Naveed et al., 2020). In the case of large-scale application of soil improvement by microbial CaCO<sub>3</sub> precipitation, the technique's applicability or simultaneous progress may require integrated efforts (Fu et al., 2023; Dhami et al., 2013).

Injecting bacteria and cementing solutions into the soil is an important step for the effective application of microbial CaCO<sub>3</sub> precipitation mineralization in the field. The application method can affect the mechanical properties of the soil, such as strength and permeability, by affecting the efficiency and spatial distribution of the injection (Zhang et al., 2024). In the literature, soil improvement and application methods with microbial CaCO<sub>3</sub> precipitation are included as mix and compact, injection, percolation, spraying, and immersion/soaking (Suresh & Uday, 2024; Zhang et al., 2024). The criteria for adopting these methods are the soil type and the field application where the process is performed (Suresh & Uday, 2024). For example, the percolation methods can be performed in coarse or fine sand due to the ease of flow. However, it may be appropriate to adopt the "mix and compact" method in fine-grained soils

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such as silts and clays due to their low permeability (Suresh & Uday, 2024; Oliveira et al., 2017). Efficient application of the method (such as injection pressure and rate) is as important as choosing the right method for the application area (Zhang et al., 2024). Creating cementation with homogeneously distributed CaCO<sub>3</sub> precipitation in fields treated with microbial CaCO<sub>3</sub> precipitation can allow the soil structure to develop more stably (Kalantary & Kahani, 2019). A heterogeneous CaCO<sub>3</sub> precipitation distribution may lead to uneven cementation. The result of this may lead to an uneven strength distribution throughout the soil, creating weak areas in terms of mechanical performance (Fu et al., 2023). Van Paassen et al. (2010) stated that the size of the treated soil volume, the volume of injected grouts, and the distance of grout application points can be criteria to evaluate the distribution of mechanical properties throughout the soil. It has been shown by Al Qabany et al. (2012) and Su et al. (2023) that the injection velocity affects the spatial distribution. Finally, the interaction between bacteria and soil particles during the microbial CaCO<sub>3</sub> precipitation process and sufficient retention time for chemical reactions to occur may also be attributed to the homogeneity (Su et al., 2023; Fu et al., 2023; Al Qabany et al., 2012; Van Paassen et al., 2010).

## CONCLUSION AND RECOMMENDATIONS

This study presents in detail the improvement of soils treated with microbial CaCO<sub>3</sub> precipitation, which has recently innovative, sustainable, and environmentally friendly potential. Although there are already some gaps in the existing literature, important results have been demonstrated that the engineering properties of soils can be successfully improved with microbial CaCO<sub>3</sub> precipitation both at the laboratory and field scale, and the applicability of the technique. The bonding between soil grains by microbial CaCO<sub>3</sub> precipitation can improve the internal structure of the soil by providing a more compact structure, and it can improve properties of its permeability, strength, density, shear, liquefaction resistance, etc. However, it is also stated that multiple effects, such as biological, chemical, physical, or environmental related to both the operation of the microbial CaCO<sub>3</sub> precipitation process and its success in practice have an uncertain or complex structure. It seems that additional research is needed to more rationally explain the factors that may directly or indirectly affect the microbial CaCO<sub>3</sub> precipitation process, to present its performance in long-term field application, and the environmental compatibility effect of suspected by-products. It can be stated that these researches emerging in the multidisciplinary field will provide significant contributions to the development of decision-making methods that aim to determine the best alternative by taking into account many variables and the anticipated potential and application range of microbial CaCO<sub>3</sub> precipitation.

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