





Microbial-Induced Calcite Precipitation

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Definition

Microbial-induced calcite precipitation (MICP) is a process that uses naturally occurring bacteria to bind soil particles together through calcium carbonate (CaCO_3) precipitation. It is a promising new technology in the area of Civil Engineering with the potential to become a cost-effective, environmentally friendly, and sustainable solution to many problems such as ground improvement, liquefaction remediation, enhancing properties of concrete, and so forth.

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1. Definition

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2. Introduction

With increasing population and civil infrastructure demands worldwide, the availability of suitable soil sites for construction continues to decrease and ground improvement is now an integral part of many modern development projects. The most common methods to strengthen soils use either one or a combination of several mechanisms such as compaction, preloading, vibration, and chemical grouting. These techniques have been proven to have different degrees of effectiveness in improving soil strength and other properties in different situations. However, they come at a cost of consumption of a substantial amount of energy either in their application or production of the grouting materials or both.

Microbial-induced calcite precipitation (MICP) uses naturally occurring bacteria to bind soil particles together through calcium carbonate (CaCO_3) precipitation as shown in Figure 1, thereby increasing the strength. The expected life of MICP-treated soil is more than 50 years, which is compatible with the expected service life of many geotechnical structures [1]. Therefore, biogeochemical processes in MICP offer the potential for solving many engineering issues related to ground improvement. MICP also offers advantages over other common approaches as it uses natural processes and it has the potential of being a comparatively inexpensive technique.

The effectiveness of MICP in cementing soil depends on the types of biogeochemical process chosen, type of bacteria used, their concentration, the pH and temperature, the concentration and volume of cementation solution, the soil properties (e.g., the availability of nucleation sites, degree of saturation, soil gradation, particle size, pore throat size) and so forth [2-6]. It is noteworthy to report that there are several review articles published in the past focusing on a particular aspect of MICP, e.g., optimizing protocols [7], mitigating liquefaction [8, 9], stabilization [7, 9-11], construction [12] and other aspects [13-20].

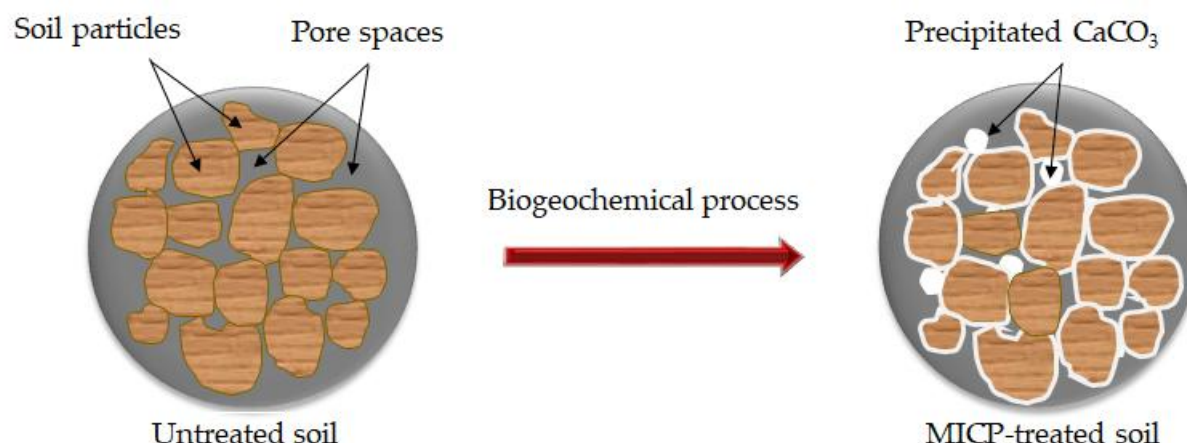


Figure 1. A schematic diagram of CaCO_3 precipitation in the pore space of the soil matrix via MICP.

3. Biogeochemical Processes

As discussed before, the effectiveness of the MICP process depends on several physical and biogeochemical factors. These factors are carefully reviewed and discussed below.

3.1. Bacteria Used in MICP

The most commonly used urease bacteria in past studies are *S. pasteurii*, *Spolactobacillus*, *Clostridium*, and *Desulfotomaculum*. Of these, *S. pasteurii*, an alkalophilic non-pathogenic bacterium with highly active urease enzymes, has been found to be one of the most effective and efficient and has been widely used [5, 21-24] even though contradictory evidence can be found in the literature [25, 26].

Venda Oliveira et al. [27] assessed the performances of two types of bacteria (*S. pasteurii* and *I. insulissae*) on the strength and stiffness of sandy soil using UCS and splitting tensile strength (STS) tests. The results from their study show that the bacterium *I. insulissae* was more efficient than *S. pasteurii* in strengthening the soil. However, the optimum environmental conditions for the growth of each bacterium was not considered in their study, which could have affected their findings.

The screening of bacteria for self-healing of concrete cracks was investigated by Zhang et al. [28]. In their study, the calcium precipitation activity (CPA) of bacterial strains was evaluated using genomic 16S rDNA sequencing and phylogenetic tree analysis. The results from their study show that the *Bacillus* species (which was designated as the H4 strain) showed the highest CPA of 94.8%. By assessing the influence of other factors on the performance of the H4 strain, they observed that lactate and nitrate were the best carbon and nitrogen sources for the H4 bacterial strain, with optimal concentrations of approximately 25 and 18 mM, respectively, at an optimum pH range of 9.5–11.0.

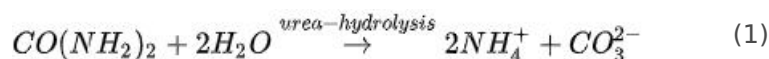
3.2. Biogeochemical Mechanisms in MICP

Biological processes involved in MICP can be broadly categorized into two groups, i.e., biostimulation and bioaugmentation. In biostimulation, indigenous microbes of the soil are stimulated with external nutrient medium, thereby inducing growth. Bioaugmentation occurs when external microbes are either injected or percolated into the soil along with nutrient medium to help their growth.

Gomez et al. [29] assessed the performance of biostimulated treatment solutions to stimulate native ureolytic bacteria in a variety of soils using soil columns (10.2 cm height x 5.1 cm diameter). The results from their study showed strength improvement and a significant reduction in the permeability of the treated soils. A maximum UCS of 5.3 MPa with an average calcite content of 13.2% was achieved in their study. A large-scale biostimulation experiment was conducted by Gomez et al. [30] using two identical 1.7 m diameter and 0.3 m thick soil layers in a tank. Cone penetration tests were conducted after treatment and they observed that biostimulation may provide good cementation improvement at that scale. Chen and Achal [31] demonstrated that a biostimulation process can be used to precipitate calcite inside a soil matrix and at the same time can effectively remediate Cu contamination by precipitating carbonates of Cu. Feng and Achal [32] used a small quantity of cement and biostimulation to improve the strength of rammed earth materials. Despite the success of implementing the biostimulation approach in some studies, this approach has its drawbacks, such as homogeneity of treatment and a longer time requirement for the stimulation and growth of microbes.

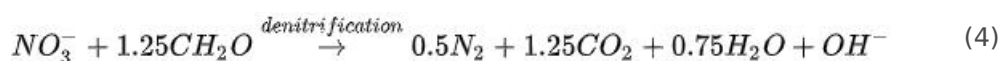
The MICP process can be achieved through many biogeochemical pathways including urea hydrolysis, denitrification, iron reduction, sulphate reduction and others [24–26]. Among these, urea hydrolysis has been most widely used due to its high CaCO_3 precipitation efficiency. On the other hand, denitrification, iron reduction and sulphate reduction have been paid less attention due to the low solubility of oxidizing substrates and as a result, require a large amount of substrate solution to obtain sufficient precipitation [33].

In urea hydrolysis, the major ingredients involved are urease enzyme, urea $\text{CO}(\text{NH}_2)_2$ and calcium chloride CaCl_2 . In general, CaCO_3 precipitation via urea hydrolysis can be divided into three main stages: (1) hydrolysis of urea into ammonium (NH_4^+) and carbonate ions (CO_3^{2-}); (2) dissociation of CaCl_2 into calcium ions (Ca^{2+}); and (3) CaCO_3 precipitation. The chemical reactions involved are presented in Equations 1 to 3.



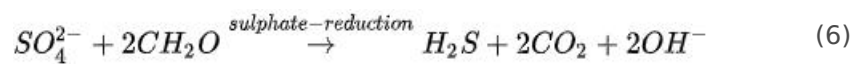
Compared to other microbial pathways, CaCO_3 precipitation via urea hydrolysis provides many advantages including a high chemical conversion efficiency up to 90% and ease of control of the process. A disadvantage of this process can be the release of undesirable NH_4^+ and can be treated as a major cause of water pollution and potent oxygen demand [26].

MICP by denitrification refers to dissimilatory reduction of nitrate (NO_3^-) to generate nitrogen gas (N_2), inorganic carbon (CO_2), and alkalinity (OH^-) using denitrifying bacteria (e.g., *Pseudomonas denitrificans*) under anaerobic conditions. The generation of CO_2 raises the carbonate content of the solution, while the consumption of NO_3^- increases the pH. The production of alkalinity favours precipitation of CaCO_3 in the presence of Ca^{2+} . The chemical reactions are presented in Equations 4 and 5.



Compared to urea hydrolysis, denitrification requires a lower concentration of the substrate to induce CaCO_3 precipitation, however, the rate of CaCO_3 precipitation is considerably lower, possibly due to the accumulation of intermediate NO_3^- . A high initial NO_3^- concentration may inhibit bacteria growth by altering the pH across the cell membrane.

In MICP by sulphate reduction (similar to iron reduction), sulphate-reducing bacteria (e.g., *Desulfovibrio* and *Desulfotomaculum*) oxidize sulphates under anaerobic conditions to produce hydrogen sulphide (H_2S), CO_2 and increased alkalinity. pH due to increased alkalinity favours $CaCO_3$ precipitation. The sulphate reduction process is presented in Equation 6.



The release of CO_2 in the presence of Ca^{2+} fosters $CaCO_3$ precipitation (see Equation 5). Anaerobic oxidation via sulphate reduction requires a large substrate quantity due to low solubility. The mechanism also results in the production of H_2S , which is an odorous and highly toxic gas even at low concentrations.

4. Engineering Properties of Treated Soil

The calcite precipitation modifies or enhances the strength of the sample and different studies have investigated this influence through various laboratory tests namely, UCS tests [24, 34–39], STS tests [27, 37, 38, 40–43], direct simple shear tests [44], triaxial tests [21, 37, 45–50], cyclic triaxial tests [51] and cone penetration tests [52]. Some of the observations are summarised below.

4.1. Unconfined Compressive Strength

Several studies conducted UCS tests on many different types of MICP-treated soil to quantify the improvement of strength. A summary of the studies is presented in Table 2. A brief overview of the literature is presented below.

Table 2. Summary of studies using UCS tests to evaluate strength improvement in MICP-treated soils.

Sand Type	MICP Process			Testing Method							
	BTM	DoS (%)	TM	D ₁₀ (mm)	D ₅₀ (mm)	C _u	C _c	Size H x D (mm)	Ave. UCS (MPa)	Ave. C _c (%)	k (m/s × 10 ^{−5})
Plaster sand [29]	BS	100	Gf	0.18	-	7	0.7	102 × 51	3.25	6.6	0.1–100
Concrete sand [29]	BS	100	Gf	0.18	-	10.1	0.6	102 × 51	3.64	9.9	0.1–100
Cushion sand [29]	BS	100	Gf	0.09	-	3.6	1.3	102 × 51	3.95	9.8	0.1–100
Russian River [29]	BS	100	Gf	0.24	-	8.7	0.7	102 × 51	1.22	6.5	0.1–100

Folsom Lake [29]	BS	100	Gf	0.24	-	6.9	0.7	102 × 51	1.07	7.4	0.1–100
Napa Bay [29]	BS	100	Gf	0.18	-	1.6	0.8	102 × 51	5.34	13.2	0.1–100
Cemex Fill [29]	BS	100	Gf	0.38	-	8.4	1.2	102 × 51	2.67	6.0	0.1–100
Granite sand [29]	BS	100	Gf	0.22	-	7.7	0.6	102 × 51	2.73	7.5	0.1–100
Coarse sand [36]	BA	30–100	I	0.54	0.70	1.27	0.1	110 × 55	0.1–2.4	4–14	8.0–42
Silica sand [35]	BA	100	I	0.25	-	-	-	1000 × 45	9–20	-	-
Pure silica sand [24]	BA	Unsat	SP	0.23	-	-	-	1000 × 45	19.61	-	-
Silica sand [39]	BA	Unsat	SP	0.35	-	-	-	2000 × 55	0.065	-	-
Ottawa silica [34]	BA	100	Sb	0.19	0.30	1.8	1.1	102 × 51	1.9–15	1.8–14	-
Fine sand [53]	BA	100	I	0.10	0.19	2.1	0.9	100 × 50	0.6–2.5	4–9	-
Medium sand [53]	BA	100	I	0.26	0.39	1.6	0.9	100 × 50	0.5–12	3–11	-
Coarse sand [54]	BA	100	I	1.45	1.60	1.4	1.0	102 × 51	0.5–15	2–23	-
Coarse sand [55]	BA	100	SP	0.61	0.72	1.2	1.0	100 × 50	0.2–2.3	3–16	0.1–66
Ottawa 20–30 [37]	BA	100	I	-	0.72	1.2	1.0	75 × 25	2.5	6.5	-

Ottawa 50–70 [37]	BA	100	I	-	0.22	1.4	0.9	75 × 25	3.04	10.8	-
Navada [37]	BA	100	I	-	0.12	1.7	1.2	75 × 25	2.6	13.9	-
Ottawa 20–30 [42]	BA	100	I	0.65	-	1.2	1.0	100 × 50	0.2–1.8	8–12	2.0–6.0
Beach sand [56]	BA	100	I	0.50	0.70	-	-	100 × 50	0.4–10	10–29	-
Ottawa 20–30 [57]	BA	100	I	0.65	-	1.2	1.0	100 × 50	0.8–1.1	6.4–7.6	4.0–6.5
Mizunami sand [58]	BS	100	Gf	-	1.50	-	-	60 × 30	0.9–10	10–32	-
Silica sand [61]	BA	100	I	0.5–0.1	-	1.2–6.3	0.9–1.1	90 × 45	0.1–2.0	1.4–10	-
Silica sand [59]	BA	100	I	-	-	-	-	180 × 50	0–0.31	1.0–4.1	4.0–10
Ottawa sand [60]	BA	100	Gf	-	0.42	-	-	100 × 50	0.2–0.5	5.2–7.7	0.1–0.6
14 soil types [61]	BA	100	I	0.07–0.38	-	2.3–10.1	0.6–1.4	-	0.1–5.4	2.6–14	-
Standard sand [62]	BA	100	Sb	-	0.42	-	-	-	0.03–2	1.4–10	-
Residual soil [63]	BA	100	I	-	-	-	-	170 × 50	0–0.2	0.6–2.8	-
Ottawa sand [34]	BA	100	Sb	0.19	0.30	-	-	102 × 51	0–2.3	1.8–15	-
Itterbeck fine [21]	BA	100	I	-	0.17	1.64	-	-	0.7–13	12–28	-

Silica sand [64]	BA	100	I	-	0.17	-	-	100/250 × 35/100	0–3.0	2.6– 10	0–13
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BTM—Bio-treatment approach, BA—Bioaugmentation; BS—Biostimulation; DoS—Degree of Saturation; Dx—Particle size at 'x'% finer; Cus—Uniformity coefficient of sand particles; Ccs—Coefficient of curvature of sand particles; TM—Treatment method; Gf—Gravity fed; I—Injection; Sb—Submerged; SP—Surface Percolation; k—Soil permeability.

4.2. Indirect (Splitting) Tensile Strength

Similar to UCS, a series of STS tests on MICP-treated soils have been reported in recent literature [27, 37, 38, 40–43]. In these studies, STS values have been assessed and compared with other parameters such as the CaCO_3 content [37, 38, 40], different soil types [27, 40], and reinforced fibre content [41–43]. A summary of the studies can be found in Table 3.

To evaluate the influence of different factors such as soil type, C_c and fibre content on the STS of MICP-treated soils, data from previous studies were compiled and critically analysed. The STS of MICP-treated soils ranges from 0.04 to 1.06 MPa, C_c ranges from 3.8 to 31.0%, while the fibre content ranges from 0 to 1.2%. STS increases exponentially with increasing C_c ; however, three distinct trends were observed possibly due to the effect of the particle size distribution not being properly captured by D_{10} as well as the grain shape.

Table 3. A summary of the literature reporting STS test results on MICP-treated soils.

Sand Type	MICP Process		D ₁₀ (mm)	D ₆₀ (mm)	C _{uS}	C _{cS}	Testing Method			
	EM	TM					Size H × D (mm)	Method	Ave.	Ave.
									STS (MPa)	C _c (%)
Calcarous sand [37]	-	I	0.15	0.35	2.33	1.01	20 × 40	STS	0.04–0.36	-
Ottawa 20–30 [40]	-	I	0.65	0.80	1.17	1.02	75 × 25	STS	0.51	4.83
Ottawa 50–70 [40]	-	I	0.18	0.25	1.40	0.90	75 × 25	STS	0.52	8.55
Nevada [40]	-	I	0.08	0.13	1.70	1.24	75 × 25	STS	0.39	14
Sand [27]	Clay (0%)	MC	0.53	1.35	2.55	0.99	20 × 70	STS	0.01	-

Sand + Kaolin [27]	Clay (28%)	MC	-	-	-	-	20 × 70	STS	0.03	-
Ottawa 20–30 [57]	-	I	0.65	-	1.2	1.0	100 × 50	STS	0.15	6.9
Ottawa 20–30 [42]	FR (0%)	I	0.65	0.80	1.17	1.02	100 × 50	STS	0.15	7.57
Ottawa 20–30 [42]	FR (0.4%)	I	0.65	0.80	1.17	1.02	100 × 50	STS	0.29	8.50
Ottawa 20–30 [42]	FR (0.8%)	I	0.65	0.80	1.17	1.02	100 × 50	STS	0.44	8.93
Ottawa 20–30 [41]	FR (0%)	I	0.65	0.80	1.17	1.02	100 × 50	STS	0.05	4.03
Ottawa 20–30 [41]	FR (0.2%)	I	0.65	0.80	1.17	1.02	100 × 50	STS	0.07	4.10
Ottawa 20–30 [41]	FR (0.4%)	I	0.65	0.80	1.17	1.02	100 × 50	STS	0.07	4.30
Ottawa 20–30 [41]	FR (0.6%)	I	0.65	0.80	1.17	1.02	100 × 50	STS	0.08	4.30
Ottawa 20–30 [41]	FR (0.8%)	I	0.65	0.80	1.17	1.02	100 × 50	STS	0.09	4.50
Silica sand [43]	BFR (0%)	I	0.21	0.28	1.3	0.98	100 × 50	STS	0.09	10.08
Silica sand [43]	BFR (0.4%)	I	0.21	0.28	1.3	0.98	100 × 50	STS	0.32	14.08
Silica sand [43]	BFR (0.6%)	I	0.21	0.28	1.3	0.98	100 × 50	STS	0.37	15.94
Silica sand [43]	BFR (0.8%)	I	0.21	0.28	1.3	0.98	100 × 50	STS	0.44	17.14

Silica sand [43]	BFR (1.0%)	I	0.21	0.28	1.3	0.98	$100 \times$ 50	STS	0.45	17.58
Silica sand [43]	BFR (1.2%)	I	0.21	0.28	1.3	0.98	$100 \times$ 50	STS	0.33	14.87

EM—Enhancement method; I—Injection; TM—Treatment method; FR—Fibre reinforcement; BFR—Basalt fibre reinforcement; MC—Mixed and compacted; STS—Splitting tensile strength; E_{50}^s —secant elastic modulus at 50% of the peak tensile stress.

5. Key Engineering Applications of MICP

5.1 MICP as Binders

Le Métayer -Levrel *et al.* [65] suggested using the bacterial ability of MICP for producing superficial protective coatings for limestones buildings, monuments and statuary. Another study by Webster and May [66] also suggested bioremediation as an additional technology for restoring stone surfaces in heritage buildings.

MICP being a non-toxic and eco-friendly process has advantages over commonly used methods for binding soil particles, such as chemical grouting. Ivanov and Chu [67] evaluated the cost of raw materials for chemical grouting to be in the range of \$2–\$72 per m³ of soil whereas for microbial grouting was in the range of \$0.5–\$9 per m³ of soil when waste materials are used as a carbon source for microbial growth.

Ramachandran *et al.* [68] concluded through microscopy investigation that MICP is an effective method for crack remediation in concrete. Jonkers *et al.* [69] established that MICP is effective as a self-healing agent to activate the process of autonomous repair of freshly formed cracks. Achal *et al.* [70] suggested MICP as an alternative high-quality concrete sealant and crack remediation method which demonstrated a 36% increase in compressive strength of cement mortar as well as six times lower water absorption in the treated samples. Amidi and Wang [71] proposed a new surface treatment method for treating concrete and similar absorbent materials to enhance their resilience and mechanical properties and achieved a 36% increase in compressive strength due to MICP.

5.2 Soil Strengthening and Stabilisation

Multiple studies have applied MICP to different types of soil and tested these under various conditions for strength enhancement and soil stability. Dejong *et al.* [3] applied MICP to improve the engineering properties of sands such as shear strength and stiffness. The results showed that the ultimate shear capacity and initial shear stiffness were both higher for treated samples compared to untreated loose specimens. Whiffin *et al.* [4] applied MICP successfully using a 5 m long sand column for ground improvement which was achieved with relatively low flow rates. This study also mentioned that balancing the rate of urea hydrolysis with the delivery of reactants aided in the uniform distribution of CaCO₃.

A study by Harkes *et al.* [5] evaluated MICP as in situ soil strengthening technique in fine-grained sand. The study reported that for a homogenous distribution of bacteria in large sand bodies, a low ionic strength solution promoted bacterial transport over longer distances. Many researchers have evaluated the strength of biotreated sands and have demonstrated improved strength, increased stiffness, liquefaction resistance and enhanced dynamic properties of the treated specimens [44, 72, 45, 73].

5.3 MICP in Bricks

Bricks constitute a significant part of construction materials and are known for their durability and sustainability. However, bricks are also prone to deterioration over time due to the presence of voids and pores resulting in cracking. MICP has proved to be a novel method of treating these cracks or strengthening bricks [74]. Raut *et al.* [75] demonstrated MICP in bricks and studied the effect of the method on compressive strength and water absorption capacity. Bricks treated with MICP showed 83.9% improvement in compressive strength and 48.9% lower water absorption capacity after 28 days as compared to the control specimen. Lambert and Randall [76] evaluated the process of MICP to produce bio-bricks using the urea from stabilized human urine. Results demonstrated higher compressive strength with an increase in the number of treatments with the highest compressive strength of 2.7 MPa.

5.4 Remediation of Contaminants from the Environment

Rapid industrial development poses a major threat in the form of heavy metals and other contaminants as a by-product of these industries which impacts our environment. In the past, conventional treatments were used to remove heavy metals from contaminated environments. However, these methods are ineffective, expensive and consume high amounts of chemicals and energy [77]. Therefore, alternative methods such as MICP are needed to effectively remove heavy metals without having much impact on the environment. Several researchers [2, 78, 79] have reported the capability of MICP for heavy metal remediation in the environment.

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Keywords

sustainable construction; Microbial-Induced Calcite Precipitation; Biocementation; biostimulation; bioaugmentation; biological process; sustainable; cementation; ground improvement; calcium carbonate



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