

MICROBIAL-INDUCED CALCITE PRECIPITATION: A MILESTONE TOWARDS SOIL IMPROVEMENT

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ABSTRACT

Microbial-induced calcite precipitation (MICP) is a natural process that offered various applications in the engineering field materials and construction industry. It has recently emerged as an innovative, eco-friendly, and economically engineered process. One of the successful MICP methods of improving the engineering properties of soils is ureolysis. The ureolysis drove the process of precipitate calcite which binds soil particles within the soil matrix thus enhance soil characteristics such as stiffness, permeability, hydraulic conductivity, shear strength, and compressive strength. This paper aimed at reviewing the concept of utilizing calcium carbonate (CaCO₃) precipitation by ureolytic bacteria towards the improvement of soil and sand engineering properties for various geotechnical applications. The potential applications of this technology in soil erosion, stability, and reinforcement were discussed. Detail overviews of the temperature, pH, bacteria cell concentration, nutrients, types of bacteria, and concentration of reactants that affect the efficiency of the process are also reviewed. This review demonstrated the potential of the MICP technology from the laboratory to a field-scale or commercial applications.

Key words: Biomineralization, Calcium carbonate, calcite, urease enzyme, ureolysis, ureolytic bacteria

INTRODUCTION

In this contemporary world, fast population growth and urbanization have made lands available for raising infrastructures relatively scarce and people are left to make use of problematic loose soils to develop earth structures (Chang *et al.*, 2016; Hiranya *et al.*, 2018). These weak and problematic soils affect the safety and stability of structures constructed on them, due to over shear stress or limitation of shear strength applied during loading onto the soil. This condition consequently fails the built structures. It is almost impossible or expensive to replace problematic soils such as soft marine clay that is commonly found at riverbanks and coastal areas as they can extend to a great depth (Saad *et al.*, 2018). This situation improves soil mechanical properties crucial. An investigation carried out in 2008 reported that over six billion US dollars were spent annually to finance over forty thousand soil improvement

projects worldwide (Kalantary & Kahani, 2015). Although, for the past few years, several methods and materials were developed to improve the engineering characteristics of soils. However, they varied in terms of environmental impact, cost, penetration depth, and treatment uniformity which portrays their merits and demerits (Khan *et al.*, 2016; Duo *et al.*, 2018). For example, the major material often use for chemical grouting is cement, but the production of cement is energy consuming and ecologically unfriendly, as it contributes to world carbon dioxide (CO₂) emissions by 7% (Hiranya *et al.*, 2018). This draws the interest of researchers towards the idea of simulating nature for the improvement of loose soils.

Microbially induced calcite precipitation (MICP) or bio-cementation is a bio-mediated, environmentally conscious soil improvement technique that can improve the engineering properties of granular soils. It refers to the process of precipitation of calcium carbonates (CaCO₃) in a microenvironment from a supersaturated solution as a result of microbial

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and biochemical activities (Omeregíe *et al.*, 2016; Omeregíe *et al.*, 2018). The MICP is a biomineralization process that involves the reaction between carbonate ions (CO_3^{2-}) produced by microbes with available calcium ions (Ca^{2+}) within the microenvironment by which results in the precipitation of CaCO_3 mineral (Omeregíe *et al.*, 2018). The precipitated CaCO_3 fills the pore spaces within the soil matrix leading to improvement of the engineering properties of the soil. MICP is highly desirable as it is contamination-free of natural microbial activity. The process offers unique functions and features that have favored high-performance applications and innovations in the engineering field materials and construction industry (Park *et al.*, 2010; Montañó *et al.*, 2017). Among the various branches of biomineralization, MICP is the most widely studied and employed in many applications such as soil improvement (DeJong *et al.*, 2010; Khamsehchian *et al.*, 2015), manufacturing of bio-bricks (Lambert & Randall, 2019), bio concrete (Cheng *et al.*, 2014; Mondal & Ghosh, 2019), compressive strength improvement in a mortar (Park *et al.*, 2010), biocementation (Zamani & Montoya, 2019), wastewater treatment (Hammes *et al.*, 2003), construction materials (Muyneck *et al.*, 2010), microbially enhanced oil recovery (Nemati *et al.*, 2005), healing of cracks in concrete structures (Ruan *et al.*, 2019; Jongvivalsakul *et al.*, 2019) and dust control (Meyer *et al.*, 2011). This current review aimed to discuss the biomineralization using calcium carbonate precipitation by ureolytic bacteria, factors affecting its efficiency, and application of MICP towards soil and sand improvement.

BIOMINERALIZATION

Biominerals are abundant in nature existing in the form of anthills, mollusks shells, beautiful corals, caves, teeth, rocks, and bones (Dhami *et al.*, 2013). Recently, researchers across the world are focusing on these biominerals to harness their technical applications in various fields. So far, about sixty biominerals such as silica, calcium phosphate, barite, magnetite, and fluorite have been described and documented (Nudelman, 2015; Chaparro-Acuña *et al.*, 2017). Among these, calcium carbonates and calcium phosphates are the main families that are particularly abundant in nature (Azañs *et al.*, 2019).

Biomineralization is the process by which microbial activity results in the chemical alteration of an environment leading to the precipitation of minerals. This process provides control over the composition, size, morphology, and structure of biominerals, given rise to complex materials with well-defined structures and distinct properties that are of great dissimilarity with geological materials and

surpass synthetic analogues (Krajewska, 2018). It is a natural phenomenon occurring in virtually all living organisms ranging from prokaryotes to higher vertebrates (Chen *et al.*, 2019). There are broadly three types of mechanisms involved in the synthesis of these biominerals;

- (i) Biologically Controlled Mineralisation (BCM) involves the synthesis of minerals inclusive of their growth, morphology, composition, and location that occurs only under certain conditions at a precise location within or on the cell. Mineral structures that are well defined for example shells, bones, fish otoliths, and teeth are formed through this process (Arias *et al.*, 2017; Chen *et al.*, 2019; Seifan & Berenjian, 2019).
- (ii) Biologically Influenced Mineralisation (BIM) refers to the passive precipitation of minerals accompanied by cell surface organic matter such as extracellular polymeric substances that are commonly associated with biofilms (Phillips *et al.*, 2013a; Arias *et al.*, 2017).
- (iii) Biologically Induced Mineralization (BIM) is the process by which the biological activity of an organism results in modification of the environment chemically leading to supersaturation and mineral precipitation. The features of the minerals induced by this process include wide particle size distribution and amorphous or poorly crystalline calcium carbonate formation (Chaparro-Acuña *et al.*, 2017; Seifan & Berenjian, 2019).

Bacteria involved in biomineralization can promote an alteration in the geological properties of soil or any component of the earth, thereby improving the soil quality by enhancing soil stiffness via the production of calcite precipitate. This is important towards soil improvement particularly in areas and towns with windy and desert-like environments such as the Gulf region due to higher temperatures and calcium content in the soil (Bibi *et al.*, 2018). Among the various applications of urease-producing bacteria, soil improvement is highly recommended (Burbank *et al.*, 2012).

SOIL AND SAND IMPROVEMENT VIA MICP

Soil improvement is a universal term for any chemical, biological, physical, and combined technique of altering the natural soil to meet up with engineering purposes. The first introduction of microbial application to biogeochemical engineering was in the year 1992 (Gunjo & Youn, 2016). The process

involves the production of sediments that can bind grains together due to chemical reactions catalyzed by microbes, which result in an alteration within the soil matrix that stiffens and strengthens the soil (Burbank *et al.*, 2011; Gunjo & Youn, 2016). As such it is seen as an eco-friendly and novel ground improvement technique (Hiranya *et al.*, 2018). It is a naturally occurring process as the microorganisms are available in nature. A large number of species ranging from autotrophic to heterotrophic microbes are involved in this process of calcium carbonate precipitation in different environments such as caves, soils, oceans, and saline/soda lakes (Sarayu *et al.*, 2014; Seifan & Berenjian, 2019). Although the largest global CaCO₃ precipitation occurs due to biotic processes that take place in the oceans, the biosynthesis of microbial carbonate precipitation may be achieved through various mechanisms as listed in Table 1.

Noteworthy, microbial precipitation of CaCO₃ through hydrolysis of urea (ureolysis) is more favorably utilized for various technical applications due to energy efficiency due to its capability of inducing a high amount of CaCO₃ in a short duration of time (Krajewska, 2018; Mukherjee *et al.*, 2019). The process involves urea hydrolysis that results in CaCO₃ precipitation through biochemical reactions governed by the production of urease enzyme by ureolytic bacteria. It is an ideal methodology in civil engineering and geotechnology because it occurs within a diverse group of microorganisms as a common microbial process (Montaño *et al.*, 2017; Fujita *et al.*, 2017). Ureolysis is distinguished from other metabolic pathways by its high amount of CaCO₃ yield and is used for MICP soil treatments, which is carried out with the aid of urease-producing bacteria (Krajewska, 2018; Seifan & Berenjian, 2019). There two common mechanisms in microbial

Table 1. An application and limitation of various MICP metabolic pathway

Metabolic Pathway	Bacteria	Precipitated crystal	Application	Limitation
Ureolysis	<i>Bacillus sphaericus</i>	Calcite	• Dispersive soils stabilization (Moravej <i>et al.</i> , 2018)	• Release of toxic ammonia gas and nitric acid production (Ganendra <i>et al.</i> , 2014)
	<i>Sporosarcina pasteurii</i>	Calcium carbonate	• Silty sands improvement (Zamani & Montoya, 2019)	
	<i>Proteus vulgaris</i>	Calcium carbonate	• Microbially enhanced oil recovery (Nemati <i>et al.</i> , 2005)	
Photosynthesis	<i>Chorella</i> sp.	Calcite	• Biological CO ₂ sequestration (Ramanan <i>et al.</i> , 2010)	• In the dark, respiration result to decalcification (Bissett <i>et al.</i> , 2008)
Sulfate reduction	<i>Desulfovibrio</i> sp.	Calcium carbonate	• Lithifying microbial mats (Braissant <i>et al.</i> , 2007)	• Ineffective in hypersaline and alkaline waters (Meister, 2013)
Ammonification	<i>Myxococcus xanthus</i>	Calcite Vaterite	• Conservation of ornamental stone (Rodriguez-Navarro <i>et al.</i> , 2003)	• Production of ammonia gas (Li <i>et al.</i> , 2015)
Denitrification	<i>Pseudomonas aeruginosa</i>	Calcite Aragonite	• Papermill wastewater treatment (Ersan <i>et al.</i> , 2015)	• Occurrence of poisonous inhibitive intermediates (nitrous oxide and nitrite) (van Paassen <i>et al.</i> , 2009)
Anaerobic sulfide	Sulfate-reducing bacteria	Dolomite	• Carbonate sedimentation oxidation (Warthmann <i>et al.</i> , 2015)	• Ineffective in hypersaline and alkaline waters (Meister, 2013)
Methano-synthesis	<i>Methylocystis parvus</i>	Calcite Vaterite	• Calcium carbonate deposition (Ganendra <i>et al.</i> , 2014)	• Depressed water potential lowers uptake of methane per unit of biomass (Schnell & King, 1996)
Utilization of organic acids to CaCO ₃	<i>Bacillus subtilis</i>	Calcium carbonate	• Crack healing in concrete (Khaliq & Ehsan, 2016)	• Adsorption affinity of organic acids decrease the precipitation rate of minerals (Kawano & Tokonami, 2014)

precipitation of CaCO_3 as shown in Figure 1. The bacteria catalyze the intracellular hydrolysis of urea ($\text{CO}(\text{NH}_2)_2$) by the urease enzyme to ammonia (NH_3) and carbamate (NH_2COOH). This carbamate decomposes simultaneously to form carbon dioxide (CO_2) and ammonia (NH_3). These products are further transformed into carbonate (HCO_3^-), ammonium (NH_4^+), and hydroxyl (OH^-) ions. This process increases the pH value due to the production of NH_4^+ . The carbonate ions react with calcium ions (Ca^{2+}) available in the surrounding environment under saturation conditions, which favors the precipitation of CaCO_3 as described in Figure 1A (Kumari & Xiang, 2017; Mahawish *et al.*, 2018). Further, CaCO_3 can be precipitated on the bacterial cell surface with a net negative charge and attract calcium ions rapidly and tightly to its cell wall. These calcium ions react with dissolved carbonate ions to precipitate CaCO_3 as shown in Figure 1B (Renner & Weibel, 2011; Helmi *et al.*, 2016).

Microbial precipitation of calcium carbonate has proven its potential as a microbial geotechnology-based method that is adapted for soil improvement. It is applied for various soil improvement projects such as controlling erosion in rivers and coastal areas, enhancing the stability of non-piled and piled foundations, treating pavement surface, decreasing dust levels on exposed surfaces by binding together the dust particles, reinforcing soil for the

enhancement of underground constructions (Wath & Pusadkar, 2016). There were several reports (Burbank *et al.*, 2011; Jiang *et al.*, 2016; Fujita *et al.*, 2017; Krajewska 2018) that had drawn our attention to the possibility of stimulating *in situ* soil urease positive bacteria in natural soils. With the presence of urease-positive bacteria and the availability of Ca^{2+} , the precipitation of CaCO_3 may be produced in adequate quantities to change the engineering properties of the soils. The coral sand solidification test has been carried out using this methodology with *Pararhodobacter* sp. as the microbial strain that shown the specimen has solidified with unconfined compressive strength (UCS) with a value reading up to 12 MPa after a curing time of 14 days (Khan *et al.*, 2016). This confirmed that MICP is an effective technique for enhancing sandy soil characteristics.

However, the major issue affecting this technique is bioclogging, which occurs due to uneven distribution and uniformity of precipitated CaCO_3 in tested sand columns (Rowshanbakht *et al.*, 2016; Omeregic *et al.*, 2018). This resulted in the retention of the cementation solution and bacterial culture at the treatment injection point. Thus, it may affect the overall stiffness and shear strength of the treated samples. Factors that influencing the transport and attachment of bacteria to soil grains are including, flow rate, cell surface properties, Vander Waals forces, ionic strength of carrier solution, and

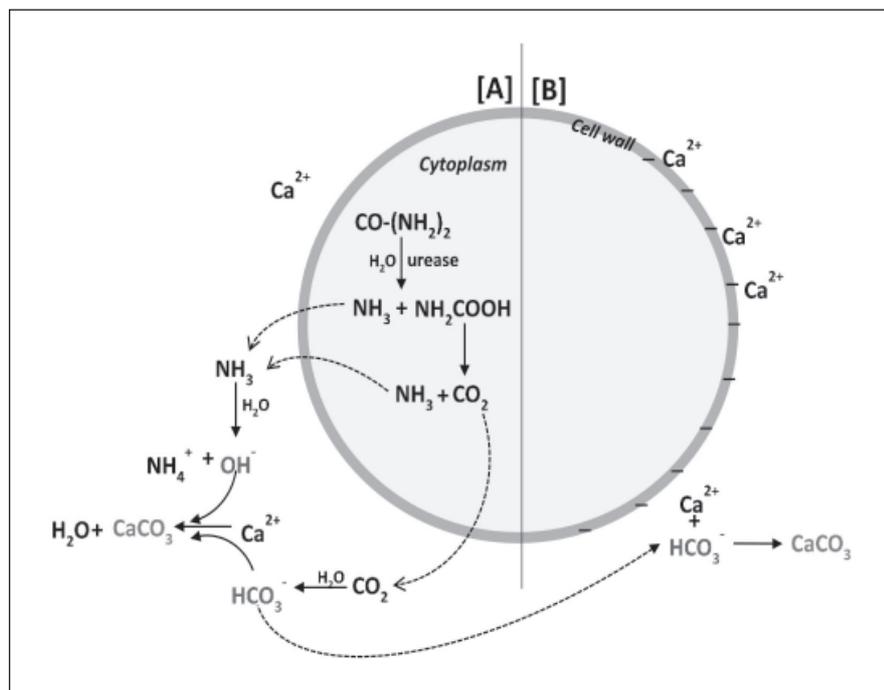


Fig. 1. Summary of calcium carbonate (CaCO_3) formation via two methods; [A] Net breakdown of urea producing NH_3 and CO_2 which later were broken to form OH^- and HCO_3^- ions, respectively and combine with Ca^{2+} from the environment; [B] Negatively charged bacteria well call attract Ca^{2+} and form a ‘nucleus’ for HCO_3^- attachment and form CaCO_3 .

straining (Burbank *et al.*, 2011). Harkes *et al.* (2010) recommend that the bacterial suspension should first be injected followed by cementation solution for uniform distribution of bacterial activity and precipitation of calcite. Kalantary and Kahani, (2015) demonstrated that the application of biological precipitation on a poorly graded sandy soil increased the unconfined compressive strength (UCS) of the soil up to a range of 100 – 400 KPa. This amount of unconfined compressive strength (UCS) satisfies the range for several soil improvement projects. This was achieved due to calcite deposition by bacterial activity (Figure 2), which enhances the physico-mechanical characteristics of the sandy soil by sticking together sand particles. The sand particles bonding further paves the way towards the stabilization of dispersive sand particles against erosion (Moravej *et al.*, 2018).

MICP technique has also been promising for *in situ* application as it enhances the cyclic strength of silty sands within the range of 0 – 35% fines content, due to its compatibility with the silty sands diminutive pore size (Zamani & Montoya, 2019). Also, to intensify the MICP technique for soil improvement, the cementation reagent and the concentration of urease-producing microorganisms are increased within the soil matrix. One of the successful MICP methods of introducing the cementation reagent and microorganisms into the soil matrix to improve its engineering characteristics such as permeability, unconfined compressive strength, shear strength, hydraulic conductivity, and

stiffness is biogrout (Kumari & Xiang, 2017). Biogrout is a sand consolidation technique achieved with the release of CO_3^{2-} from the hydrolysis of urea in the presence of Ca^{2+} to form CaCO_3 *in situ* (Omoregie *et al.*, 2018). This process is greatly enhanced using microorganisms with high urease activity. The microorganisms are introduced into the soil matrix through three main methods: (i) percolation; (ii) premixing method and (iii) injection.

The percolation method simply implies spraying or trickling cementation solution with bacterial suspension onto the soil surface which penetrates the soil matrix under the influence of gravity (Seifan & Berenjian, 2019). The method is relatively cheap as no heavy machinery is required for solution injection into the soil. However, its efficiency is limited to the treatment of fine-grained soils such as silt and clay due to low permeability and infiltration rate (Mujah *et al.*, 2017). While the premixing method is another process to introduce potent microbial strain into the treated soil matrix by the mechanical mixing of soil with bacteria. The technique is relatively costly and requires a higher source of energy when compared with the trickling method. However, its effectiveness is higher, especially in deeper levels of soil (Seifan & Berenjian, 2019).

The injection method approach is the most preferable MICP treatment technique that involves the introduction of the biological healing agent deep into the soil from top to bottom (Mujah *et al.*, 2017). This process lies on injection parameters such as flow rate and pressure which can be easily controlled and

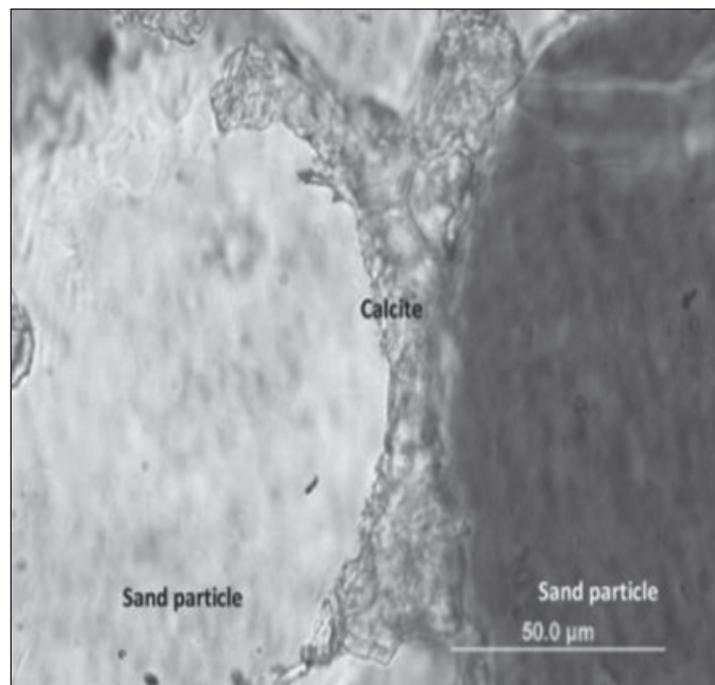


Fig. 2. Two sand particles bonded by calcite crystals precipitated by ureolytic bacteria (Al-Thawadi, 2011).

allows injection of the bacteria solution in both horizontal and vertical directions (Seifan & Berenjian, 2019). However, uneven distribution of bacteria and nonuniformity of precipitated CaCO_3 in tested sand columns are the major disadvantages of this method. An investigation on the various MICP injection scenarios was reported by Shahrokhi-Shahraki *et al.* (2015) and suggested four strategies to prevent the occurrence of excessive crystal accumulation nearby the injection point and enhance the homogeneous distribution of CaCO_3 crystal formation over a great length in the sand specimen. Those strategies are: (i) mixing the cementation solutions with bacterial cell before injecting into the soil matrix; (ii) two-phase injection involves the injection of the bacterial cell solution before cementation solution; (iii) staged injection, with or without retention periods between the injection phases and (iv) single-phase injection involves the injection of reagents simultaneously into the sands.

MICROBIAL UREASE ACTIVITY FOR MICP

The bacteria's core roles during the biochemical reactions include the generation of an alkaline environment via different physiological actions and the production of urease enzymes. The enzyme urease activity is controlled by natural environmental factors that influence the occurrence of these reactions (Okwadha & Li, 2010). If the reactions can occur within unconsolidated sand pores, a sandstone-like material is formed due to bonding together of the sand particles (Jiang *et al.*, 2016). Ureolysis that is mediated microbially occurs more rapidly than the uncatalyzed rate by approximately 10^{14} times (Tobler *et al.*, 2011). Although, there are different opinions with regards to the role of bacteria in CaCO_3 precipitation through ureolysis; the precipitation is (i) an accidental and unwanted by-product of microbial metabolism, and (ii) a specific process that ecologically benefits precipitating microbes (Seifan & Berenjian, 2019).

The production of urease enzyme by soil bacteria provides the simulative biomineralization application process via ureolysis possible and potentially useful for various soil engineering applications (Burbank *et al.*, 2012). In brief, the production of urease has been demonstrated to be inducible, constitutive, and repressible. Bacterial species with calcite precipitating potential, produce urease and these bacteria reported in literature include; *Pararhodobacter* sp., *Bacillus massiliensis*, *B. licheniformis*, *B. sphaericus*, *B. magatarium*, *Arthrobacter crystallopoietes*, *Lysinibacillus fusiformis*, *Sporosarcina pasteurii*, *S. soli*, and *Morganella morgana* (Park *et al.*, 2010;

Helmi *et al.*, 2016; Fujita *et al.*, 2017; Moravej *et al.*, 2018; Duo *et al.*, 2018; Mukherjee *et al.*, 2019).

MORPHOLOGY OF PRECIPITATED CALCIUM CARBONATE CRYSTALS

The precipitation of calcium carbonate forms various polymorphs distributed as minerals in nature (Liu *et al.*, 2017). These include the anhydrous polymorphs namely, calcite, aragonite, and vaterite with the most thermodynamically stable anhydrous CaCO_3 polymorph at ambient conditions is calcite. The formation behavior with regards to each polymorph is influenced by synthesis factors such as temperature, pH, ratio and concentration of calcium and carbonate ions, reaction time, additives, etc. (Chang *et al.*, 2017a; Chang *et al.*, 2017b). The crystal structure of calcite is rhombohedral while aragonite and vaterite are orthorhombic and hexagonal respectively (Oral & Ercan, 2018). There are also two hydrated phases which are monohydrate calcium carbonate ($\text{CaCO}_3 \cdot \text{H}_2\text{O}$) and hexahydrate calcium carbonate ($\text{CaCO}_3 \cdot 6\text{H}_2\text{O}$), in addition to different amorphous calcium carbonate (ACC) polymorphs (Krajewska, 2018).

CaCO_3 is first precipitated from supersaturated solutions as an unstable ACC, which is converted to vaterite spontaneously. This conversion of metastable vaterite to calcite takes place in the presence of water over a short duration of time, with virtually all vaterite transformed to calcite within 24 hr (Burbank *et al.*, 2011). The solubility of calcium carbonate polymorphs is in increasing order of calcite, aragonite, vaterite, and ACC (Chang *et al.*, 2017a). Lower solubility product values of aragonite and vaterite indicate the difficult nature of obtaining these polymorphs as a result of their unstable characteristics (Oral & Ercan, 2018). ACC can be converted to a more thermodynamically stable polymorph of CaCO_3 such as vaterite, aragonite, and calcite when suspended in an aqueous solution at ambient temperature (Seifan & Berenjian, 2019).

The precipitation of the polymorphs of vaterite, aragonite, and calcite depend both on bacterial strains and their growing environment (Jones, 2017). It was observed that different bacteria precipitate calcium carbonate of various types and were mainly either polyhedral or spherical crystalline forms. Emerging evidence propose that bacteria do not have a direct effect on the selection of calcium carbonate polymorph or morphology. Thus, the morphological features may be influenced by the composition of the culture medium and the chemical nature of the specific bacterial outer structures consisting of proteins, lipids, nucleic acids, and polysaccharides,

which might be essential for the process of bacterial crystallization (Wei *et al.*, 2015; Kim *et al.*, 2016).

FACTORS AFFECTING MICP EFFICIENCY

The chemical process involved in CaCO_3 precipitation is usually governed by four main parameters; pH, the concentration of dissolved inorganic carbon, calcium concentration, and availability of bacteria nucleation sites (Hammes & Verstraete, 2002; Omoregie *et al.*, 2018). The first three aforementioned factors influence the carbonate ions concentration while the nucleation site plays a vital role in the stable and continuous formation of calcium carbonate by bacteria (Rodriguez-Navarro *et al.*, 2012; Anbu *et al.*, 2016). MICP as a biological process is influenced by a large number of abiotic and biotic factors influencing its successful completion. These factors include; bacterial genotype, bacteria cell concentration, nutrients, geometric compatibility of bacteria, reactant concentration, temperature, pH, availability of moisture, level of aeration, and quality of water (Ng *et al.*, 2012; Al-salloum *et al.*, 2017).

Temperature

MICP is highly influenced by temperature as it affects the microorganisms' urease activity, solubility, nucleation rate, and growth of CaCO_3 crystals (Mujah *et al.*, 2017). The temperature may vary with altitude, latitude, moisture content, incident solar radiation, depth of soil, and type of soil (Ng *et al.*, 2012). Previous studies have demonstrated that increasing temperature from 10°C to 15°C and 15 to 20°C enhances the rate of ureolysis by twice and five times, respectively (Nemati & Voordouw, 2003; Ferris *et al.*, 2004; Umar *et al.*, 2016). Also, raising the temperature from 20°C to 50°C promotes the rate of CaCO_3 production and influences the shape and size of CaCO_3 crystals formed (Mujah *et al.*, 2017). Any rise in temperature within this temperature range increases the precipitation rate and therefore more CaCO_3 is precipitated. This might be due to the high ureolytic activity of the bacterial solution within the temperature range leading to relatively large amounts of induced CaCO_3 during the MICP treatment process (Peng & Liu, 2019). The rate of ureolysis is marginally higher at 30°C and any increase in temperature above 30°C does not promote the rate of decomposition (Nemati *et al.*, 2005). This is a clear indication that any rise in temperature within the optimum range speeds up the rate of urea hydrolysis depending on other conditions such as reactants concentration within the system and environmental conditions (Umar *et al.*, 2016). Kim *et al.* (2018) researched the effect of temperature on MICP using two microbial species *Sporosarcina pasteurii* and

Staphylococcus saprophyticus, and reported that the maximum calcite precipitation was measured at a temperature of 30°C, while the least precipitation was observed at a temperature of 50°C that caused about 30 to 40% of the precipitation is reduced than the amount precipitated at the optimum temperature of 30°C. Moreover, the solubility of CaCO_3 decreases with a rise in temperature over the optimum temperature range, thus affecting precipitation. This showing that temperature is the most critical factor influencing the formation of CaCO_3 polymorphs (Okwadha & Li, 2010). The precipitation of supersaturated solutions of Ca^{2+} and CO_3^{2-} initially formed ACC which is transformed into crystalline CaCO_3 polymorphs, mainly forming vaterite and calcite at a low-temperature range of 14 - 30°C while at a higher temperature range of 60°C to 80°C aragonite and calcite are formed (Chang *et al.*, 2017a).

pH

The pH of the bacterial medium plays a significant role in inducing microbial morphological changes, affect its stability and instigate its enzyme secretion (Omoregie *et al.*, 2017a). The presence of hydroxyl ions (OH^-) formed from the generation of ammonium ions (NH_4^+) during neurolysis, changed the pH level, thus creating an alkaline environment suitable for the precipitation of CaCO_3 (DeJong *et al.*, 2010; Mujah *et al.*, 2017). Microbial ureases' optimum pH is approximately neutral, except for a few groups of acid ureases. Urease activity of alkalo-tolerant bacteria, for example, *S. pasteurii* have an optimum pH of 8.0. However, the microbial urease could be denatured irreversibly at a pH lower than 5.0 (Ng *et al.*, 2014). The amount of CaCO_3 precipitated increases with pH and converges at a pH range of 8.7 to 9.5 (Kim *et al.*, 2018).

Bacteria cell concentration

Bacterial cells are a definite nucleation site for CaCO_3 precipitation, their concentration in surface treatment solution, bio-mortar, or inactive matrices is a significant factor for effective MICP application techniques (Al-salloum *et al.*, 2017). The availability of nucleation sites is important in governing the urease activity that determines the amount of CaCO_3 produced (Xu *et al.*, 2014). Also, when the soil matrix contains more bacterial cells, the produced CO_3^{2-} ions would be consumed mainly by nucleation of new calcium carbonate crystals, as a result of the abundant nucleation sites, rather than growing the existing CaCO_3 crystals, leading to the precipitation of more small CaCO_3 crystals (Al-Thawadi & Cord-Ruwisch, 2012; Mujah *et al.*, 2017). However, the optimum CaCO_3 precipitated can only be attained within the concentration of bacteria cells from 10^5 to 10^8 cells mL^{-1} (Okwadha & Li, 2010; Mondal & Ghosh, 2018).

Nutrients

Nutrients serve as the energy sources of bacteria; thus, it is important to provide proper and adequate nutrients for CaCO₃ producing bacteria. The common bacteria nutrients include N, CO₂, Ca, K, Fe, Mg, P, and are supplied to the bacteria throughout the culture and soil treatment stage (Ng *et al.*, 2012). Ureolytic bacteria utilize urea as a sole nitrogen source, while the calcium source may be calcium chloride, calcium chloride dihydrate, calcium nitrate, or calcium lactate (Siddique & Chahal, 2011; Navneet *et al.*, 2016). It is noteworthy that organic calcium source such as calcium lactate provides additional nutritional requirements for bacterial growth, hence more beneficial for microbial precipitation of CaCO₃ (Xu *et al.*, 2015). The dissolved inorganic carbon generated from the extracellular polysaccharide of the bacteria complexes the calcium ions, thereby reducing CaCO₃ saturation and enhancing the CaCO₃ precipitation (Sarayu *et al.*, 2014).

Types of bacteria

Bacterial type is significant to the production of urease activity. There are different types of bacteria capable of producing different amounts of urease and CaCO₃ precipitation. The bacteria suitable for MICP should be able to catalyze the hydrolysis of urea via the production of urease enzyme (Krajewska, 2018; Mondal & Ghosh 2019; Zhao *et al.*, 2019; Lapierre *et al.*, 2020; Marin *et al.*, 2021; Saneiyani *et al.*, 2021). These bacteria should neither be modified genetically nor enclose any exchangeable elements capable of enhancing the pathogenicity of other microbes within the environment (Okwadha & Li, 2010). Thus, the bacteria should be alkalophilic, non-pathogenic, and possess high ureolytic efficiency with the ability to homogeneously precipitate calcite within the substratum (Khamchayan *et al.*, 2015).

The bacterial strain plays a significant role in the crystal size, morphology, form, and biochemistry of CaCO₃, thus makes the selection of bacteria is crucial for a successful solidification due to varies of the production urease and the amount of CaCO₃ produced in MICP varied with the type of bacterial strain (Xu *et al.*, 2014; Fujita *et al.*, 2017; Seifan & Berenjian, 2019). Typical urease-producing bacteria are genera *Desulfotomaculum*, *Bacillus*, *Sporosarcina*, *Clostridium*, and *Sporosarcina* (Sarayu *et al.*, 2014). The most preferable bacteria are aerobic bacteria that capable of producing CO₂ during cell respiration. This released CO₂ is parallel to pH rise due to the production of ammonium (Ng *et al.*, 2012). The most cited bacteria for MICP is *S. pasteurii* due to its large amount of CaCO₃ precipitation, high urease activity, tolerance to high pH, and precipitation of a large amount of calcite

(Rowshanbakht *et al.*, 2016; Minto *et al.*, 2018; Ruan *et al.*, 2019).

Nature and concentration of reactants

Application of urease-producing bacteria towards attaining MICP necessitates the use of urea as a nitrogen source (Tirkolaei & Bilsel, 2015). An ideal urea and calcium chloride concentration is significant in achieving precipitation of calcite. In terms of weight, urea (CO(NH₂)₂) and calcium chloride dihydrate (CaCl₂·2H₂O) stoichiometric ratio of 2.5 is critical (Ng *et al.*, 2012). Muynck *et al.*, (2010) reported that the best concentrations of urea and CaCl₂ are 0.5 M and 0.25 M, respectively. However, in a few other studies, the efficiency of calcite precipitation and the average size of CaCO₃ crystals increases with low concentrations of urea and calcium chloride (<0.25 M) and concentration of more than 0.5 M will decrease the efficiency of calcite precipitation but depends on the species of bacteria. (Okwadha & Li, 2010; Al-Thawadi & Cord-Ruwisch, 2012; Lia *et al.*, 2018; Golavkina *et al.*, 2020; Liu *et al.*, 2021). Since enzymatic hydrolysis of urea generates ammonia which increases the pH, it is mandatory to be used with calibrated discretion to avoid inhibiting bacterial growth due to excessive alkalinity (Muynck *et al.*, 2010). Ultimately, for CO₃²⁻ to react with available Ca²⁺ in the system, there must be an equivalent amount of calcium source supplemented with urea (Siddique & Chahal, 2011). These two compounds together may generate impurities for the cement, as such should be used in minimal quantity to achieve the maximum possible advantage of calcification (Al-salloum *et al.*, 2017).

APPLICATIONS OF MICP

MICP technique is an effective and environmentally friendly technology that can be applied to solve various environmental problems, such as cementation, crack remediation in concrete, bio-consolidation, CO₂ sequestration, remediation of heavy metals and radionuclides, and other essential applications (Umar *et al.*, 2016; Al-salloum *et al.*, 2017; Arias *et al.*, 2017; Omoregie *et al.*, 2018; Mutitu *et al.*, 2019).

Remediation of cracks

In concrete, crack forms due to several mechanisms such as aging, freeze-thaw reactions, mechanical compressive, and tensile forces (Anbu *et al.*, 2016). Cracks in the concrete lead to corrosion of embedded steel via easy ingress of ions and moisture that react with reinforcements in concrete and expansive stressed which leads to spalling. There are several methods employed for crack

remediation concrete but most are polymer-based which have a severe effect on the strength of the material (Al-salloum *et al.*, 2017). In case of remediation of cracks in concrete structures, MICP has proved its efficacy in several reports. It involves the supplementation of concrete structures with bacteria and nutrients as healing agents to stimulate the remediating action of sealing cracks and is identified as a self-healing approach (Vijay *et al.*, 2017; Jongvivatsakul *et al.*, 2019; Ruan *et al.*, 2019; Liu *et al.*, 2020; Sun & Miao 2020; Fun *et al.*, 2021).

There are different methods employed to incorporate self-healing agents into the concrete matrix to activate its healing property whenever a crack appears in a structure. These mechanisms are broadly classified as vascular-based, intrinsic-based, and capsule-based (Joshi *et al.*, 2017). (i) Vascular-based self-healing mechanism involves the supplying of healing material from outside of the structure using distributed vascular channels embedded in a concrete matrix (Joshi *et al.*, 2017). As cracks appear, healing material moves through the channels due to pressure gradient between cracks position and agent source (Seifan *et al.*, 2016) (ii) In intrinsic self-healing; the embedded materials in the concrete matrix has a latent self-healing function which might be triggered by external stimulus or damage (Joshi *et al.*, 2017). (iii) Capsule-based self-healing mechanism involves the use of capsules to store healing agents which are released into the concrete matrix when the capsules are triggered by rupture or external stimulus (Xu & Wang, 2018).

An investigation carried out by Li *et al.* (2018) on the consolidation of cracks produced in masonry cement mortars revealed that the bio-consolidation results in a significant reduction in water absorption and positively influence improvement in compressive strength of mortars. A similar study by Algaifi *et al.* (2018) using *Lysinibacillus sphaericus* as a microbial agent for crack self-healing concrete demonstrated that a crack of 0.4 mm wide was completely sealed with calcite after 60 days. At time 42 days, the predicted crack healing was 58% with the healing ratio increasing with time. This was supported by Zhang *et al.* (2019) who reported a maximum value of completely self-healed crack of width 1.22 mm using open cultures of microbial consortia after 28 days of healing. Also, the microbial consortia result in a 61% decrease in the cost of production when compared to pure cultures.

CO₂ Sequestration

The increase in high concentrations of greenhouse gas emissions in the atmosphere results in environmental disasters such as global warming (Jacobs *et al.*, 2017). The most abundant emitted greenhouse gas is CO₂, which significantly affects the earth's climate (Srivastava *et al.*, 2014). CO₂

sequestration alleviates the released CO₂ from the atmosphere (Phillips *et al.*, 2013a; Krajewska, 2018). The proposed ways of reducing CO₂ emission into the atmosphere include; the use of energy sources such as solar, wind, and nuclear energy which are less carbon-intensive, or by increasing the efficiency of energy utilization, from production to end-use, but none of these options are practically possible (Dhami *et al.*, 2013).

Sequestration of CO₂ is naturally achieved by chemical fixation of CO₂ at a very slow reaction rate to form carbonate like calcite, magnesite, aragonite, and dolomite (Anbu *et al.*, 2016). MICP has been examined to be a potential technology for the sequestration of CO₂ (Achal & Mukherjee, 2015). In this technique, CO₂ is transformed into carbonate of different crystals like calcite, magnesite, aragonite, and dolomite (Srivastava *et al.*, 2014). The technique is eco-friendly and safer than conventional methods of sequestration of CO₂ (Anbu *et al.*, 2016). The biological sequestration of CO₂ into environmentally friendly and stable mineral carbonates utilizes bacterial carbonic anhydrases extracted from the pure culture of high urease-producing bacteria such as *Bacillus cereus* (Li *et al.*, 2013). This was supported in a study by Okyay *et al.*, (2016) which revealed the potential of ureolytic microbial consortia towards the sequestration of CO₂. It was observed from the study that the sequestration was higher in consortia with low diversity than in a very diverse consortium. The sequestered CO₂ can be stored permanently as an inert solid stable inorganic carbonate and has several potential industrial applications such as paints, paper, food, pharmaceuticals, cement, sealants, adhesives, and construction materials (Chang *et al.*, 2017b).

Remediation of heavy metals and radionuclides

Rapid industrialization has led to the accumulation of radionuclides and heavy metals contaminants that resulted in both environmental and human health problems. These pollutants include heavy metals such as copper, cadmium, arsenic, antimony, chromium, lead, tin, cobalt, mercury, zinc, and radionuclides such as uranium, thorium, radium, and strontium (Arias *et al.*, 2017). The major threat with radionuclides and heavy metals is that they are not biologically or chemically degradable, once used or released into the environment (He & Chen, 2014; Nigro *et al.*, 2017). Conventional methods of metal contaminant treatment in soil include Physico-chemical techniques associated with high costs, energy, and chemical consumption with possible emission of secondary pollutants (Kumari *et al.*, 2016).

The application of biological treatment of metals from contaminated sites through bioaccumulation, bioleaching, bioimmobilization, biocoagulation,

phytoremediation, and absorbents have also not been effective because they are time-consuming, expensive, and may result in the release of absorbed or immobilized heavy metals back to the environment (Anbu *et al.*, 2016). This paved way for the novel applications of urease-producing organisms in the bioremediation of heavy metals and radionuclides to form insoluble metal-containing carbonates (Kumari *et al.*, 2016). During the process, biomineralization of heavy metals and radionuclides occurs as a competitive co-precipitation reaction whereby suitable divalent cations are incorporated within the calcite lattice (Dhami *et al.*, 2013). Several authors have reported the potential application of MICP to remediate radionuclides such as strontium and heavy metals such as cadmium and lead from the environment (Achal *et al.*, 2012a; Achal *et al.*, 2012b; Mwandira *et al.*, 2017; Zhao *et al.*, 2019).

Other essential applications

MICP is a potential technology for the removal of calcium from the environment (Anbu *et al.*, 2016). Excess calcium ions present in wastewater generate serious operational problems which include scaling in reactors and pipelines due to its precipitation as phosphate, sulfate, and or carbonate salt but the application of ureolytic bacteria in the biomineralization process of calcium ions removal from wastewater is poorly described (Arias *et al.*, 2017). A study developed by Hammes *et al.* (2003) on the removal of calcium from industrial wastewater reported the removal of calcium over 90% in the period under experiment mediated by ureolytic microbiological carbonate precipitation through the use of a semi-continuous reactor system. In another study, heterotrophic bacteria isolated from submerged fixed-film bioreactor meant for urban wastewater treatment carried out the precipitation of carbonates crystals. This biological activity could be applied in calcium capture in the treatment of urban wastewater (Uad *et al.*, 2014).

Polychlorinated biphenyls (PCBs) contaminated oil is a recalcitrant contaminant comprising severe environmental concern and conventional methods available for the removal of PCBs include hydro blasting, solvent washing, or sandblasting followed by encapsulation in the epoxy coating, but all these methods are not effective because of the resurfacing of oil over time (Phillips *et al.*, 2013b). Alternatively, microbial processes are now used in the removal of these recalcitrant contaminants (Omoriegbe *et al.*, 2018). *Sporosarcina pasteurii* was used to generate a biosealant that reduced water permeability on the surface of PCB-contaminated concrete by a magnitude of 1 – 5 orders with high resistance to carbonation (Okwadha & Li, 2011).

Mortar is a workable paste usually consisting of aggregates, water, and binder for binding building

materials and filling the gaps between them (Muynck *et al.*, 2010). Specifically, a biological mortar consists of a mixture of nutritional medium containing calcium salt with bacteria and finely ground limestone, usually used to avoid the problems associated with the physical and chemical incompatibilities of the underlying material (particularly brittle materials) with commonly used repair mortars (Muynck *et al.*, 2010). Several studies have demonstrated the potential application of MICP towards improving the compressive strength of mortar (Park *et al.*, 2010; Kanta *et al.*, 2016; Choi *et al.*, 2017). In a study by Elmanama and Alhour (2013) the inclusion of ureolytic *Bacillus mycoides* in cement mortar enhances the compressive strength of the cement mortar to a maximum increase of 17% and recorded a 32% reduction in water absorption.

In addition to the applications mentioned above, MICP can be a potentially promising technique for other essential applications. While developing a green biodegradable film, red seaweed was used as a base matrix and microbially induced CaCO₃ precipitated by *Bacillus sphaericus* as a filler for enhancing the red seaweed material properties for plasticulture purpose (Abdul Khalil *et al.*, 2018). Application of MICP for the biosynthesis of fluorescent Cadmium Sulphide (CdS) nanoparticles has been demonstrated by Zhu *et al.* (2016) using a urease-producing bacterium *Citrobacter braaki* isolated from the calcium-rich shells of abalone.

LIMITATIONS OF MICP

MICP can be scored high as construction sustainability due to its scope for recycling and low energy requirement. However, the safety of MICP application for various construction purposes is of great concern (Omoriegbe *et al.*, 2017b). MICP is not perfectly eco-friendly due to the production of ammonia as a by-product and its oxidized by-product nitrate during the ureolysis-driven process (Soon, 2013). These gases are toxic to soil microorganisms and highly detrimental to human health, particularly at high concentrations (Omoriegbe *et al.*, 2017a). Hence, it is significant to avoid ammonia contaminating the environment through the development of strategies such as treatment of the MICP released ammonia before discharge and possible utilization of the ammonia as fertilizer by surrounding plants (Mujah *et al.*, 2017). The use of the facial mask is highly recommended for protection against the release of these gases by ureolytic bacteria during handling (Omoriegbe *et al.*, 2016).

MICP is considered expensive for field-scale applications or commercial implementation, especially when compared with conventional cementitious composites (Mujah *et al.*, 2017). The aseptic

production of bacteria spores, labor required, protective material and nutrients contribute to high cost thus pose a threat to bacterial technological cementitious composites application (Xu & Wang, 2018; Li *et al.*, 2019). The laboratory-grade nutrient medium for bacteria cultivation alone can cost as high as 60% of the entire production cost (Omorieg *et al.*, 2019). However, the utilization of industrial by-products or wastes as an alternative nutritional source for bacteria cultivation can limit the economic challenges of MICP (Achal & Mukherjee, 2015). Cheng *et al.* (2014) proposed the potential use of seawater rich in calcium ions as the main calcium source for calcite precipitation during the MICP technique. The experimental results revealed that the UCS of the treated sand recorded two times higher strength (with the same amount of crystal precipitated) than MICP treatment with higher concentrated solutions of calcium and urea, retaining permeability at about 30% which signifies a good drainage ability. Further studies should be carried out on the possibility of utilizing this method for a sustainable and cost-effective way of strengthening the soil.

Other challenges include a reduction in the population of the introduced bacteria into the soil, due to competition, predation, and stress arising from abiotic factors like osmotic pressure, pH, availability of suitable nutrients, and temperature (Burbank *et al.*, 2011). Proffering solutions to the above-mentioned limitations are significant for an effective field-scale application of the MICP technique.

CONCLUSION

This paper reviewed the eco-friendly ground improvement technique using bio-mineralization known as MICP. A vast range of potential applications of MICP continues to expand with the rapid development of MICP technology. However, there is uncertainty in the method of microorganism application such as injection and optimization of urease activity in the soil. Currently, biogROUT is the best application method available where it enhanced the release of CO_3^{2-} in the presence of Ca^{2+} to form CaCO_3 . MICP is much influenced by abiotic and biotic factors where the release of CO_2 from anaerobic bacteria respiration enhances the CaCO_3 formation with stabilization of pH and temperature will ensure the bacteria growth at the optimum level. Though, the CO_3^{2-} concentration is still the vital factor in the continuous formation of CaCO_3 by bacteria. Bio-mineralization plays a significant role in improving soil and sand engineering characteristics for different geotechnical applications. A vast range of potential applications of MICP continues to expand with the

rapid development of MICP technology. Still, its effectiveness is limited to several shortcomings associated with its industrial application since most of the previous studies on microbial geotechnology are at the field trial stage. This review recommends further interdisciplinary research to overcome the challenges of this technology before effective large-scale or commercial applications.

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