

The Amendments in Typical Microbial Induced Soil Stabilization by Low-Grade Chemicals, Biopolymers and Other Additives: A Review



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Abstract Soil with the required engineering properties is the first prerequisite in construction engineering, as the stability of the buildings and structures highly hinges on characteristics of soil by which they are supported. Microbial induced carbonate precipitation (MICP) is a newly developed soil improvement technique that has drawn the great deal of interest among geotechnical and geo-environmental engineers. The mechanism relies on set of biochemical reactions, utilizing bacterial enzyme to produce calcium carbonate bio-cement that could effectively bind the soil particles, leading to enhance the engineering properties of soil. Despite being a novel ground improvement technique, numerous studies have demonstrated that MICP can be a promising alternative for several geotechnical applications. Recently, researchers have started focusing on amending the typical MICP approach by incorporating preeminent materials, so that to enhance the MICP responses. This chapter presents a review on the amendments in MICP made by low-grade chemicals, biopolymers and other salient additives including magnesium ions, fly-ash and fibers. The effects and responses of the above materials in MICP are extensively described and discussed, and some research gaps are also pointed out. Although studies have suggested a wide range of advantages, there are many aspects and challenges on the above subjects are still to be addressed in future works prior to the real field applications.

Keywords Soil · Biocement · Low-grade chemicals · Biopolymers · Additives

1 Background

Soil is the key material that serves as an engineering media construction and geotechnical engineering. In most of the engineering cases, buildings and infrastructures

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are constructed on ground that requires firm soil conditions with the capability of supporting the structure. The soil with the required engineering properties is the first prerequisite in geotechnical engineering. However, soils are complex material composed of minerals and organics, largely varies from place to place, exhibiting distinct engineering characteristics and behaviors. Due to the population and urbanization, the demand for the land continues to increase at unprecedented rate, even the lands with poor engineering properties are continuously employed for constructions with required pre-treatments (Bordoloi et al. 2017; Gowthaman et al. 2018). It should be noted that a total cost of 6 billion USD being spent per year on more than 40,000 soil improvement projects all over the world (DeJong et al. 2010).

Researchers have been investigating numerous treatment techniques since a long time ago to improve the engineering properties of weaker soils. Some of the commonly used mechanical approaches are displacement, replacement, preloading, stone columns, soil nailing and reinforcing. Most of the above methods utilize synthetic materials and mechanical energy and, both required high energy for manufacturing and installation. Stabilizing the soil using chemical additives such as cement, bottom ash, silica fume, blast furnace and synthetic products (e.g. phenoplasts, polyurethane, epoxy and acrylamide) have been reported to be under the scrutiny of public policy, as they are detrimental causing hazardous and toxic effects to the environment (DeJong et al. 2010; Cheng et al. 2014a). Most of the man-made synthetic products are advised to be banned by recent regulations in some countries. It is reported that the production of cement clinker largely contributes to the environmental issue by releasing carbon dioxide via burning of fossil fuels, resulting a great impact in global warming (Jonkers et al. 2010; Kavazanjian and Hamdan 2015). It is predicted that the emission of carbon would reach to 260% in 2050, if the present rate continues in cement manufacture (Cuzman et al. 2015a). Thus, it is clearly perceived that there is a high social requirement for new mechanisms to improve the soil to be eco-friendly.

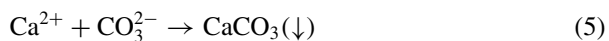
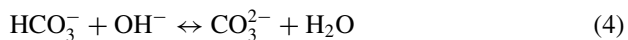
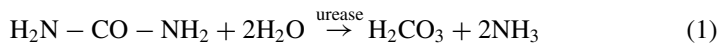
2 An Overview on MICP

The confluence of the environmental issues discussed above necessitated to practice the eco-friendly materials in conjunction with technological developments, hence biological mediations started to be questioned whether it is benefited as an alternative soil improvement technique. Many geo-scientists and engineers were fascinated to this novel subject area since not only the technique is sustainable for eliminating the shortcomings in conventional soil improvement techniques, but also the key substance: bacteria, exists abundantly in soil environments even at large depths. Markedly, the number of bacteria live in a gram soil close to the ground surface ranges between 10^5 and 10^{12} and can easily be accessible (Mitchell and Santamarina 2005; Gowthaman et al. 2019a). With the explosion of new knowledge of microbiology and biochemistry, microbial induced carbonate precipitation (MICP) has been pioneered

as a new alternative soil improvement technique in the field of geotechnical and construction engineering before couple of decades.

MICP relies on set of biochemical reactions, utilizing microbial metabolic activities (i.e. ureolytic activity) to produce calcium carbonate biocement that could alter the engineering properties of soil. Actually, the mechanism is learnt from the nature: formation of calcium carbonate is a widely occurring process involved in the formation of animal and protozoan shells, tropical sands, coral reefs, and etc. (Decho 2010; Achal and Kawasaki 2016). The above natural processes are not always biologically induced and can sometimes be the result of interactions between extracellular biopolymers and the geochemical environment. But in MICP process, microorganisms are the most involved, whose biological activity mediates chemical reactions system, leading them to proceed at unprecedented rates which is faster than that predicted based on the thermo-chemical reactions (Mitchell and Santamarina 2005). Generally, microorganisms can survive at wide range of temperatures and pH conditions, and their enzymes are more stable regarding various characteristics compared to the enzymes derived from plants and animals (Alves et al. 2014). It has been reported that bacteria cells were identified even in boiling hot springs and at temperatures than 100 °C (Madigan et al. 2000). Also, the bacteria samples obtained from Antarctic zone that had been at the frozen state for thousands of years, exhibited metabolic activity after thawing (Doran et al. 2003).

Not all the bacteria exist in the earth could contribute to the MICP, but the urease producing bacteria. Urease enzymes are protein macromolecules, which can be localized in the bacterial cell membrane, cytoplasm, or excreted outside the cell (Mobley et al. 1995). The prominent feature of urease is its active site that consists of a bi-nickel center with a functional structure (Krajewska 2018), that could exert the catalytic function: hydrolysis of urea (Eq. 1). And the process of MICP is triggered by the hydrolysis of urea. In the aqueous medium, the products equilibrate, resulting ammonium and bicarbonate ions (Eqs. 2 and 3 respectively). Production of hydroxide ions increases the pH of the reaction medium and leads to form the carbonate ions (Eq. 4). And eventually, the calcium carbonate precipitates at the presence or supply of calcium ions (Eq. 5).



Bacteria cells which are typically negatively charged, attract and bind calcium ions, leading to nucleate the formation of crystals. The precipitated calcium carbonate could coat the soil particles (grain coating), cement the soil matrix at or near the particle contacts (contact cementing), and fill the void spaces (matrix supporting), resulting enhancement in engineering properties such as strength, stiffness and dilatancy (Feng and Montoya 2016; Lin et al. 2016).

Despite of being relatively a new technique, numerous studies on MICP have been reported in the field of geotechnical engineering. Studies have focused on various applications such as soil improvement (Whiffin et al. 2007; van Paassen 2009; Feng and Montoya 2016; Lin et al. 2016; Amarakoon and Kawasaki 2018), liquefaction control (Montoya et al. 2013), dust stabilization (Li et al. 2017), slope soil stabilization (Gowthaman et al. 2019a, b), stabilization of coastal zone (Danjo and Kawasaki 2016) and erosion control (Jiang and Soga 2017). From the literature survey, it can be seen that most of the challenges regarding procedure and materials required for MICP have been studied well and demonstrated in various scales. These results have advanced the MICP towards field implementation stage.

Currently, the researchers have started focusing on amending the typical MICP approach by incorporating salient materials such as low-grade chemicals, biopolymers and other organic-inorganic additives for various reasons such as cost control, efficiency and high performance. This chapter presents a detailed review on the amendments in conventional MICP approach, done through low-grade chemicals, biopolymers and other salient additives including magnesium ions, fly-ash and fibers. The mechanical, chemical effects and responses of the above materials are extensively described and discussed, and some research gaps are also pointed out for future works.

3 Inexpensive Low-Grade Chemicals in MICP

The feasibility of MICP does not depend only on technical aspects regarding conditions of treatment, but also accompany with economic challenges. One of the greatest challenges in assessing the complete feasibility of the process is the material cost of the required substances/chemicals (Achal et al. 2010; van Paassen et al. 2010; Mujah et al. 2017; Omoregie et al. 2018; Gowthaman et al. 2019a), and that becomes enormous, particularly at the commercialization or field implementation stages that demands bulk productions. Most of the MICP studies have employed the conventional culture media (incorporates yeast extract, ammonium sulphate, tris-buffer, tryptic soy broth, nutrient broth, luria broth) to cultivate the ureolytic bacteria (Fujita et al. 2017; Omoregie et al. 2018). The researchers still supply the required resources to the cementation using laboratory-grade chemicals that consist of CaCl_2 , urea, NaHCO_3 and nutrients. The above laboratory-grade substances significantly affect the cost of the MICP process, often inhibiting the promotion of MICP towards commercialization and field implementation. The above monetary challenge necessitates the

exploration of alternative reliable growth medias and required resources to control the material cost reasonably.

3.1 *Inexpensive Media for Bacteria Cultivation*

The nutrient medium used to cultivate the bacteria is one of the major cost factors (Achal et al. 2009), that would cover a large percentage in total production cost ranging from 10 to 60% in MICP process (Kristiansen 2006; Omoregie et al. 2018). Basically, the factors governing the activity and reproduction rate of bacteria are availability of nutrients, availability of water and physicochemical factors (such as pH and temperature). Bacteria could metabolize in desorbed or dissolved phase, but hardly ever in nonaqueous phase. They require nutrients for cellular material as well as for an energy source, and lack of nutrients limits the rate of metabolism (Mitchell and Santamarina 2005). Many nutrient medias have been suggested as cheaper alternative for bacteria cultivation based on comprehensive studies, and those are discussed briefly under this section.

Few researchers have investigated the feasibility of using inexpensive nutrient sources from food industry. Beer yeast (BY) is one of the potential medias often investigated (Gowthaman et al. 2019a, b), and that has been widely used to break down sugars and to add alcohol to beer in the food industry. The results suggested that the urease activity of *Psychrobacillus* sp. cultivated in BY (pH of 7) is more than two times higher compared to that in laboratory-grade ammonium yeast extract (NH₄-YE) media, even at relatively low biomass concentration (Gowthaman et al. 2019b). Omoregie et al. (2019) have studied the feasibility of food-grade yeast (FGY) for the cultivation of *Sporosarcina pasteurii*. The urease activity, bacteria growth and carbonate precipitation in FGY were compared with those in eight various laboratory-grade media (nutrient broth, yeast extract, tryptic soy broth, luria broth, fluid thioglycollate medium, cooked meat medium, lactose broth and marine broth). The results suggested that FGY can be a better alternative media that resulted highest biomass concentration and urease activity.

Another set of researchers focused on industrial wastes and byproducts as the alternative nutrient source. Achal et al. (2009) introduced the lactose mother liquor (LML) (the effluent of the dairy industry). The growth and the urease activity of *Sporosarcina pasteurii* in LML is similar to that in standard laboratory medias, suggesting LML as a potential alternative. Some other dairy wastes such as whey and buttermilk were also suggested as a better alternative for the growth of *Sporosarcina pasteurii* (Cuzman et al. 2015b). The corn steep liquor (CSL) is the byproduct in corn wet-milling process, and that was suggested as a better alternative nutrient media for *Sporosarcina pasteurii* (Achal et al. 2010) and *Bacillus* sp. CT5 (Joshi et al. 2018). Yoosathaporn et al. (2016) have investigated a protein rich agricultural waste, the chicken manure effluent (CME), as nutrient media for *Bacillus pasteurii* KCTC3558. The results indicate that there was a significant increase in urease activity compared to that in standard medias. Recently, Fang et al. (2019) have reported that there is

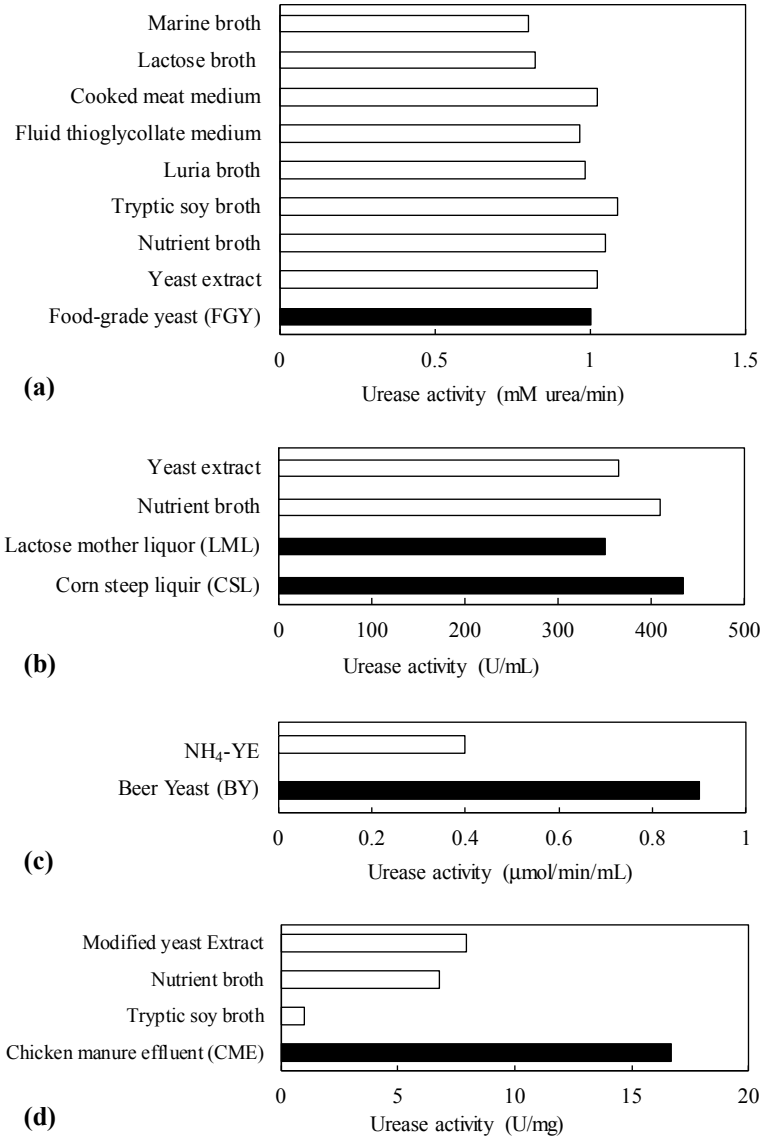


Fig. 1 Comparison of the urease activity of **a** *Sporosarcina pasteurii* NB28 (SUTS) (Omoregie et al. 2019), **b** *Sporosarcina pasteurii* (NCIM 2477) (Achal et al. 2009, 2010), **c** *Psychrobacillus* sp. (Gowthaman et al. 2019b), **d** *Bacillus pasteurii* KCTC3558 (Yoosathaporn et al. 2016) cultivated in laboratory-grade (□) and inexpensive (■) media

no significant different in growth and urease activity of *Bacillus cereus* cultivated in tofu wastewater and standard medias.

Figure 1 presents a summary: comparison of urease activities of the strains cultivated in different inexpensive and standard medias. The results (Fig. 1) suggest that the proposed inexpensive medias could be a perfect and reliable alternation. The alternative inexpensive medias are generally rich in proteins, ions and organic compounds (Hammes et al. 2003; De Muynck et al. 2008). For example, the beer yeast consists of protein (48%), carbohydrate (39%), sugar (6%), dietary fiber (33%) and ash including K, P, S, Ca, Fe and Cu (Gowthaman et al. 2019a); CME consists of crude protein (17%), organic matter, N, P, K, Na, Ca and S (Yoosathaporn et al. 2016); LML consists of Ca, Na, K, Mg and other elements (Achal et al. 2010), and the production of the bacteria could be altered by the adsorption of proteins, inorganic and organic substances, resulting enhanced ureolytic performances. However, the adsorption and nutrients requirements vary from bacteria to bacteria, thereby different bacteria may exhibit different responses in a same growth media. It is worth noting that this alternative approach in MICP could meet the requirement of green technology, as they could benefit to reuse and reduce the industrial and agricultural wastes.

3.2 *Low-Grade Chemicals as Cementation Media*

Calcium ions and urea are obligatory resources to process the biocement formation in MICP. It has been reported that the above chemicals are very expensive at the supply in large quantities, particularly the CaCl_2 (Choi et al. 2016a). Therefore, few researchers attempted to investigate some possible low-grade alternatives those are rich in calcium, some food wastes such as eggshells, seashells and oyster shells. Purity is the key difference between the laboratory-grade chemicals and low-grade chemicals (Gowthaman et al. 2019a, b). Eggshell is the mostly studied material, as it contains more than 94% of calcium carbonate (Lechtanski 2000), and the calcium ions can be easily extracted by dissolving in weak acidic medium. Chang et al. (2014) have demonstrated the feasibility of using eggshell to supply the required Ca^{2+} ions derived in vinegar. At the same time, Choi (2014) reported the formation of calcium carbonate while incorporating eggshells and oyster shell with plant extracts. After that, Choi et al. (2016) have used the calcium source extracted from the eggshell to solidify the sand. They have suggested that the finer eggshell fragments (0.85 mm or less) produced more soluble calcium compared to that of coarser, and the mixing ratio of 1:8 (eggshell and vinegar by weight) resulted the optimum precipitation.

Very recently, set of researchers have introduced the snow melting agent as a potential alternative for the calcium resource in MICP (Gowthaman et al. 2019a, b). The snow melting agent/de-icing salt is widely applied as bulk to melt the ice deposited on roads and pavements in winter seasons all over the world. Cuzman et al. (2015b) performed a pilot study on the feasibility of using manures and fertilizer as urea source. Recently, Gowthaman et al. (2019a, b) have demonstrated the feasibility of using urea fertilizer as the alternative urea resource at different application

scales. As mentioned earlier, the purity is the key difference between the laboratory-grade chemicals and low-grade chemicals, and the low-grade chemicals consist of elements other than the required substances. For example, the purity of calcium chloride in snow melting agent is 74%; the purity of nitrogen in urea fertilizer is 46%, and they additionally include sodium, potassium, copper and iron. In their comprehensive study, Gowthaman et al. (2019a) have shown that precipitated carbonate content is higher in the soil treated using laboratory-grade chemicals compared to that in the soil treated using low-grade chemicals. However, the estimated UCS was significantly high for the samples treated using low-grade chemicals. The researchers have explained the reason with the aid of scanning electron microscopy. Precipitated crystal structure in the above both cases were calcite (confirmed by XRD analysis). However, typical crystalline form of calcite (rhombohedral shape) was not identified anywhere in the soil treated by low-grade chemicals. The presence of wide range substances (organic and inorganic) in low-grade chemicals highly affects the MICP responses either chemically or mechanically. It has been reported that the precipitated calcite encapsulated with the additional substances, effectively filled the pores, merged the soil particles in a compacted manner, resulting remarkable stiffness in soil matrix (Gowthaman et al. 2019a).

Composition of the media has a profound impact on calcite crystal morphology (De Muynck et al. 2008). Omoregie et al. (2019) have observed both calcite and vaterite polymorphic forms in the precipitated carbonate while incorporating FGY, whereas no vaterite form observed while using laboratory-grade medias, suggesting that the extra salt content presented in FGY stimulated the vaterite formation. A similar crystal morphology with calcite and vaterite was reported while using CME and CSL as nutrient media (Yoosathaporn et al. 2016; Joshi et al. 2018). Vaterite is the least stable form, and its formation greatly impact in mechanical responses. Basically, the formation of vaterite is promoted by several factors such as higher urea hydrolysis rate (Hammes et al. 2003; Lin et al. 2016), high supersaturation, organic compounds and thermodynamic effects (Achal et al. 2009; Rodriguez-Navarro et al. 2007). Therefore, it is suspected that the presence of additional substances (organic and inorganic) or salts could affect the kinetics of biological and chemical reactions, often resulting the partial formation of vaterite crystals.

3.3 *Cost Effectiveness*

As mentioned earlier, the cost of laboratory-grade media and chemicals are extremely expensive. As reported, growth medium alone costed USD240 to cultivate the bacteria for a required concentration of 2–3 g/m² in order to perform the biocalcification of building material (De Muynck et al. 2010), which is not affordable. In terms of cost, low-grade materials provide higher feasibility in MICP compared to the laboratory-grade reagents. Omoregie et al. (2019) clearly tabulated and compared the cost of FGY with several laboratory-grade growth medias. They also have reported that commercially available FGY media only costs USD18/kg, where most of the

laboratory-grade growth medias cost between USD122-1073/kg, suggesting the reduced bacterial production cost ranges between 82.80% and 99.80%. Similarly, cost per liter of CME medium was around 88.2% lower than other standard medias (Yoosathaporn et al. 2016). At the same time, Gowthaman et al. (2019a) have clearly revealed the material cost of the reagents used to treat 1 m³ soil using MICP technique. It costed USD11,972 and USD468 when the laboratory-grade chemicals and low-grade materials (BY, snow melting agent, urea fertilizer) were used respectively. The material cost cut was 96%, and this would be certainly a beneficial turning point for the industrial level advancements in MICP.

4 Polymer Modified MICP

Use of organic materials to upgrade the MICP process would be a more attractive initiation. The idea is closely related to the organic matrix mediated biomineralization in which inorganic minerals are deposited upon the organic matrix, providing organic-inorganic hybrid material with excellent physical and optical properties (Kato et al. 1998; Hosoda and Kato 2001). This concept is inspired from the nature. Exoskeleton of the crustacean is a best example available in nature for the organic matrix mediated biomineralization, where crystalline CaCO₃ was deposited between the chitin protein fibers. This inorganic-organic composite material gives extremely higher strength to the cuticle (Raabe et al. 2005). Thus, incorporating the concept of organic matrix mediated biomineralization would be a more sustainable approach to improve the efficiency of the MICP process.

Organic macromolecules can be categorized mainly into three: polynucleotides, polypeptides, polysaccharides. Several factors must be considered before introducing these organic macromolecules in MICP process. Efficiency of the MICP process is mainly depend on the activity of the urease enzyme. Also, success of the MICP process depends on its capability to produce CaCO₃. Therefore, organic materials should not interfere with the enzyme activity, bacterial growth, nucleation and the growth of CaCO₃. It is a well-known fact that some organic materials inhibit CaCO₃ crystallization and could also affect the morphology and polymorphism of the CaCO₃ crystals drastically. Therefore, the selection of suitable organic material is highly important. However, only a very little number of research works have been carried out earlier to investigate the effect of organic materials, mainly biopolymer on the efficiency of the MICP process. In this section, effects of some of biopolymers on the MICP process have been discussed.

The concept of use biopolymer on the MICP process is still in the initial stage, and a limited number of published data is available under this topic. Using the available data, the effect the natural and synthetic biopolymer on the MICP process has been addressed based on the type of polymer.

4.1 Poly-L-Lysine

Poly-L-Lysine is a synthetic cationic polypeptide which is charged positively in neutral pH (Dzakula et al. 2009). It is a prevalent reagent in the bio medical field as a cell adhesion reagent to attachment of protein and cells into solid surfaces (Mazia et al. 1975). Also, it is widely used as a food preservative in Japan and Korea (Hiraki et al. 2003). Effect of poly-L-lysine on the CaCO_3 crystallization has been studied earlier by the chemical precipitation (Dzakula et al. 2017). For the first time, Nawarathna et al. (2018a) have investigated the effect of the poly-L-lysine on the CaCO_3 crystallization by MICP. They have conducted precipitation experiments with and without poly-L-lysine by hydrolysis of urea using ureolytic bacteria, *Pararhodobactor* sp. in the presence of CaCl_2 . The results suggested a higher amount of CaCO_3 precipitation while adding poly-L-lysine than that without poly-L-lysine. The positive charge of the poly-L-lysine (in neutral pH) strongly attracts the carbonate ions in the vicinity, resulting high supersaturate carbonate ions in the local region, providing favorable environment for more CaCO_3 crystals to nucleate and grow (Nawarathna et al. 2018a, b). Therefore, poly-L-lysine could produce higher amount of precipitation compared to that in conventional MICP process. In the presence of poly-L-lysine, peanut-like twin sphere-shaped calcite crystals were obtained (Fig. 2a), whereas the usual rhombohedral and polyhedron shaped calcite crystals were reported in that with no poly-L-lysine (Fig. 2b). This morphology change was primarily associated with the conformation change of the poly-L-lysine chain from random coil conformation to α -helix conformation under the alkaline conditions.

Further, more compacted and well cemented sand specimen could be obtained by adding poly-L-lysine than that in conventional MICP. By adding poly-L-lysine, the unconfined compressive strength of top of the sample was increased by 31% than the case without polymer as shown in the Fig. 3. Positively charged poly-L-lysine act as a binder to attach negatively charged bacteria cells into the negatively charged sand surface and assisted to provide better bridge between the sand particles which is an essential factor to achieve better cementation and strength.

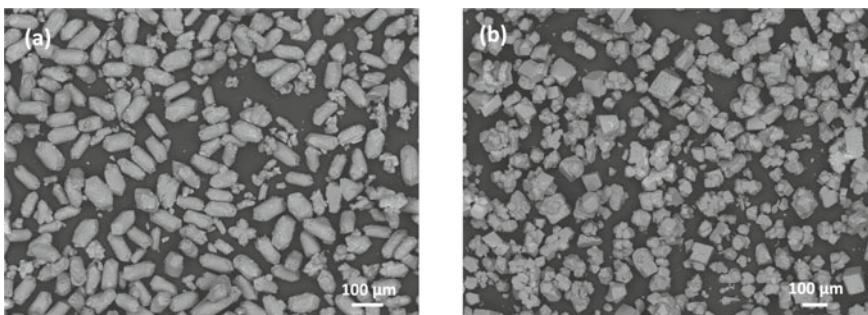
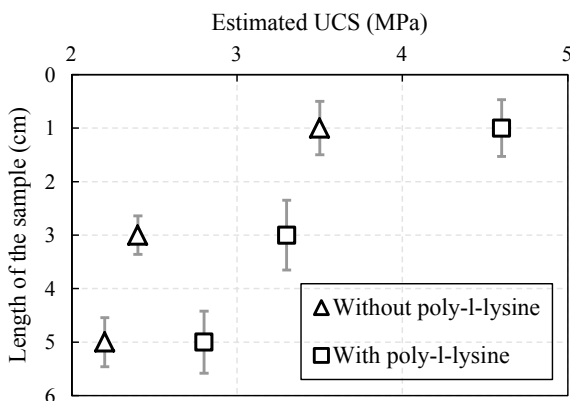


Fig. 2 SEM Images of CaCO_3 precipitate (a) with poly-L-lysine (b) without poly-L-lysine

Fig. 3 Estimated UCS values of the solidified sand samples. Error bars shows the standard deviation of three independent experiments



4.2 Sodium Alginate

Sodium alginate is a natural polysaccharide which is derived from the brown sea weeds, has number of industrial applications due to its gelling property and commonly in use as an immobilization material (Butler et al. 2006). Generally, the polysaccharides have better influence on the formation of biominerals. Nacre of the mollusk is a best example available in nature, in nacre biogenic CaCO_3 was deposited upon the chitin layer which is one of the most abundant natural cationic polysaccharides (Meyers et al. 2011). Similarly, negative charged polysaccharides have influence on the biogenic mineral formation and its effect on the calcium carbonate crystallization has been extensively studied earlier by several researchers (Butler et al. 2006; Manoli and Dalas 2002; Wang et al. 2010) by chemical precipitation of CaCO_3 .

Wu and his research group have taken an attempt to introduce sodium alginate into the MICP process for immobilization of the bacteria (Wu and Zeng 2017). By using fluorescence microscopy, they have demonstrated that the bacteria were successfully immobilized on the calcium alginate gel. The porosity of the calcium alginate gel has been provided an extra support for the bacteria immobilization. The addition of the sodium alginate modified the morphology of the CaCO_3 crystals significantly. Their results also indicate that only calcite crystals were resulted with no sodium alginate, which is similar to that observed in many MICP works (Nawarathna et al. 2018a, b, c; Hsu et al. 2018). With sodium alginate polymorphism of the calcium carbonate has changed enormously with time. After 6 h reaction, it has formed spherical vaterite crystals with rough surface, and it totally converted into dumb bell-shaped consisting with rhombohedral calcite crystals. Vaterite is the metastable phase of the CaCO_3 polymorphism and can be easily transformed to the stable form of aragonite or calcite (Kralj et al. 1997; Lopez et al. 2001). It is worth to note that the organic materials have capability to stabilize the vaterite crystals without transformation into the stable form (Trushina et al. 2014; Falini et al. 1998). However, in the case of alginate, as explained by the authors, initially it formed vaterite crystals due to the presence of the

alginate stabilizer and continued to transform into calcite due to the thermodynamic instability nature of the vaterite.

Effect of the sodium alginate concentration on the CaCO_3 crystallization was also studied by the same research group (Wu and Zeng 2017). Results indicated that there is a significant influence of sodium alginate concentration on the morphology of the crystals. Hexagonal shape crystals were obtained for the 1% of the sodium alginate with the length and thickness of 2 μm and 1 μm respectively. With the increase of the alginate concentration, hexagonal shaped crystal was converted into spherical vaterite, and further increase of the concentration led to form capsule shaped particles. Formation of the hexagonal crystals was due to the interaction of the ammonium ions with the carbonate ions in the unfavorable (001) plane of vaterite. In contrast, at higher sodium alginate concentrations resulted more negative charges, and they attached to the CaCO_3 surface efficiently. And thus, at higher alginate concentrations effect of the ammonium ions became neglected, resulting the formation of capsule-shaped crystals.

4.3 Polyvinyl Alcohol (PVA)

Polyvinyl alcohol (PVA) is a synthetic polymer which has a superb adhesive and emulsifying property and extensively used to strengthen textile yarn and papers. PVA is manufactured commercially from polyvinyl acetate and primary raw material is vinyl acetate monomer (Haweel and Ammar 2008; Alihemati and Navarchian 2007). PVA has been recognized as a CaCO_3 inhibitor which is promoted to formation of the vaterite and aragonite without transform into the stable calcite. On the other hand, it has a capability to stabilize the meta stable phase of CaCO_3 . This excellent character of PVA is due to its extensive adsorption property and its ability to make hydrogen bonds (Kim et al. 2004).

Effect of the PVA on the MICP process have been examined previously (Wang and Tao 2018). Mainly PVA have been used as an additive to control the viscosity of the MICP solution. Experiments were conducted by using both water-based cementation solution and PVA modified cementation solution. PVA modified cementation solution has been prepared by slowly adding the PVA powder to cementation solution with continuous stirring at 60 °C. Sand solidification experiments have been conducted separately for Ottawa sand in a clear plastic container by using both water-based cementation solution and polymer modified cementation solution.

The results indicated that a higher amount of CaCO_3 was obtained in the case with PVA than the case without adding PVA. PVA modified MICP process formed framboidal shaped vaterite crystals while rhombohedral calcite crystals were dominant in the case of water based MICP approach. Relatively a denser and uniform distribution of vaterite crystals between the sand particles were also reported. But calcite produced from the water based MICP approach was neither uniform nor compacted. As explained earlier, the reason behind the formation and stabilization of the vaterite crystal is higher absorption capacity of the PVA (Kim et al. 2004).

Authors also explained that the surface structure of the vaterite was changed due to the absorption of the polymer into the vaterite crystals, and it inhibits the solution mediate transformation process and stabilize vaterite crystals.

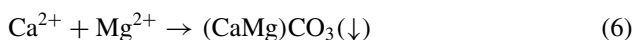
Furthermore, higher unconfined compressive strength has been obtained for the cemented sand samples by using polymer modified cementation solution than the case with water-based cementation solution. However, at lower calcium concentrations, approximately a same strength was obtained for both of the water and polymer based treated samples. It is clearly perceived that PVA resulted more uniform distribution of CaCO_3 along the sample, and that could be achieved due to the inhibition of the growth of the CaCO_3 compared to that in conventional MICP. Moreover, this positive effect of the PVA on the soil stabilization have been confirmed through the bench-scale experiments.

5 Mg^{2+} Addition in MICP

The presence of various mineral species can have a significant influence on the calcium carbonate crystallization process (Mejri et al. 2014). Among them, magnesium has identified as a key determinant in calcium carbonate mineralization as considerable amounts of Mg^{2+} in parent solution changes the polymorphism of calcium carbonate (Oomori and Kitano 1985). Higher molar ratios of magnesium to calcium concentrations (herein after referred to as $\text{Mg}^{2+}/\text{Ca}^{2+}$) favor aragonite precipitation while lower $\text{Mg}^{2+}/\text{Ca}^{2+}$ predominantly favor calcite precipitation (Declat et al. 2016). Further, previous findings suggest that addition of magnesium to the inorganic carbonation system drastically modify the size and structure of the crystals precipitated and give rise to variety of carbonate minerals including calcite, aragonite, vaterite, magnesium carbonate and dolomite (Boyd 2012; Reddy and Nancollas 2002). Few studies have shown that Mg^{2+} ions act as an inhibitor for nucleation and/or growth of calcite under abiotic conditions preferentially due to strong adsorption of hydrated magnesium ions onto the growing calcite crystals (Oomori and Kitano 1985; Rushdi et al. 1992).

Not many studies have been carried out so far to investigate the effect of magnesium on the bio inspired calcium carbonate precipitation. Nevertheless, magnesium carbonate possesses similar cementing ability as calcium carbonate which can significantly affect the desired outcomes of MICP treatment. Since the whole mechanism of microbial induced soil stabilization is dependent on the secretion and execution of urease enzyme that degrades the urea, it is important to determine whether magnesium act as an inhibitor or promoter to catalysis of urea hydrolysis. It is already addressed in literature that there is a decrease in urease activity with the increase in amount of calcium which is applicable to a range of calcium ion concentration from 0 to 0.5 M (van Paassen 2009; Whiffin 2004). In a recent study by Sun et al. (2019) revealed that increase in the concentration of calcium chloride from 0 to 1.8 M has a significant inhibitory effect on the urease activity of bacterium *Sporosarcina*

pasteurii. In comparison to that, the same researchers shown that urease activity gradually rises for a magnesium ion concentration from 0.3 to 1.5 M for bacterial solutions with OD₆₀₀ of 0.5 or 1.0. For a higher concentration of bacteria, that is when OD₆₀₀ equals to 1.5, the rise in activity is more significant and stronger, making magnesium ions better substitution for calcium ions. However, extremely high concentrations of magnesium have a negative effect on the urease activity due to the high saltiness in the reaction solution (Sun et al. 2019; Sun and Miao 2019). Although the carbonate precipitation process become more complex in the presence of both Ca²⁺ and Mg²⁺, that results various products depending on Mg²⁺/Ca²⁺ ratio. A reaction solution with equal concentrations of Ca²⁺ and Mg²⁺ will ideally react as follows (Eq. 6) (Boyd 2012).



Fukue et al. (2011) explored the effects of magnesium on bio-mediated calcium carbonate precipitation and observed that total amount of precipitation decreases with an increase in Mg²⁺/Ca²⁺ ratio from 0 to 2.3. A precipitation ratio of only 75% of the theoretical precipitation was achieved from a mixture of 0.5 M MgCl₂ and 0.5 M CaCl₂ whereas 1.0 M CaCl₂ alone resulted in a precipitation ratio of 95% suggesting a slight inhibitory effect on carbonation reaction by magnesium. The reduction in precipitated mass can be attributed to the process of crystallization and/or recrystallization of carbonate minerals until most stable form is gained which causes the solution to lose energy (Folk and Land 1975). The rhombohedral calcite crystals in the presence of magnesium were altered into needle/thorn type crystals indicating crystal growth in one direction as magnesium content increases. This is a result of the inhibition of growth of calcite crystals along a and b axes by magnesium (Fukue et al. 2011).

Sun et al. (2019) compared the productive rates for microbial induced calcium and magnesium carbonate precipitation and demonstrated that magnesium carbonate has a lower productive rate than calcium carbonate. Moreover, it was found that magnesium precipitation is insignificant within first 24 h irrespective of the testing temperature (Sun et al. 2019; Sun and Miao 2019). Two possible reasons for aforementioned phenomenon have been mentioned: (i) higher solubility of magnesium carbonate which is almost one order of magnitude higher than its calcium counterpart and (ii) possibility of partial hydrolysis of magnesium carbonate to generate magnesium hydroxide. Putra et al. (2016) substituted MgCl₂ in enzyme induced carbonate precipitation to learn that increasing MgCl₂ in the reaction solution beyond 40% drastically reduces the total carbonate precipitation. However, a maximum value of 90% of theoretical precipitation was achieved by substituting 20% of 0.5 M CaCl₂ solution with magnesium elucidating addition of small amount of magnesium can bring positive impacts on the precipitation ratio. Magnesium in calcite precipitation process exhibit potential to promote more compact and harder aragonite crystallization at pH levels below 11 and ability to delay the precipitation reactions. However, substitution of 0.10 M MgSO₄ in enzyme mediated carbonate precipitation technique can give rise precipitation ratio of more than 100% along with aragonite and

gypsum crystallization in addition to calcite (Putra et al. 2017). Relatively a similar observation was reported by Chandra and Karangat (2019) that calcite crystals were resulted when Mg^{2+}/Ca^{2+} was equal to 0.25 and 0.67, whereas aragonite and fibrous dumbbell dolomite crystals were dominant for a Mg^{2+}/Ca^{2+} ratio of 1.

It is worth noting that same specimens indicated better resistance against dissolution from acetic acid where Mg incorporated sample achieved only 40% reduction in UCS after acid washing in comparison to 66% reduction in control sample. This may be possibly due to the better inhibition of acid dissolution shown by dolomite and magnesite than calcite crystals (Fukue et al. 2011). Cheng et al. (2014b) explored the applicability of soil stabilization in marine environments using artificial seawater and presented that magnesium carbonate hydrate positively contribute to the strength enhancement by MICP. The addition of magnesium in MICP enables the bio cement to have a higher strength and lower porosity compared to control samples (Rong et al. 2013; Sun et al. 2019; Sun and Miao 2019). Magnesium encourages the precipitation of aragonite with a specific gravity and Mohr hardness higher than those of calcite (Oomori and Kitano 1985; Boyd 2012). In fact, aragonite has a more compact structure than calcite thus making the microstructure of the cementitious material denser in the presence of optimum levels of magnesium (Putra et al. 2016; Rong et al. 2013). Interestingly, one of the latest studies by Gawwad et al. (2016) presents completely opposite results where compressive strength of the bio-mortar prepared by the *Sporosarcina pasteurii* decreases with increasing Mg^{2+}/Ca^{2+} ratio. It was evidenced that in absence or lower concentrations of magnesium, rhombohedral calcite crystals that are more frequently interweaved and crosslinked at contact points gave rise to a more compact microstructure than those spherical and rod crystals formed at higher concentrations of magnesium. However, further investigation is required to draw the exact reasoning to these contrasting results.

6 Fly Ash Addition in MICP

The application of different additives such as cement, lime, gypsum, nano-chemical additives, ashes and powders to improve the weak soils is a very common approach nowadays. Among them fly ash has shown a high degree of efficacy as an additive which enhances the strength and compressibility characteristics of soils in desired manner (Cokca 2001; Kumar and Sharma 2004). Fly ash is a byproduct of coal combustion often consisting of hollow spheres of silicon, aluminum, and iron oxides and unoxidized carbon (Kumar and Sharma 2004). In the presence of moisture, fly ash which is a pozzolanic material chemically reacts to form cementitious compounds promoting improvement of problematic soils like expansive soils (Karthik et al. 2014). The underlying mechanism is that fly ash can provide an array of divalent and trivalent cations (Ca^{2+} , Al^{3+} , Fe^{3+}) under ionized conditions which helps in flocculating the dispersed clay particles by cation exchange (Cokca 2001).

Although fly ash has a long history as an engineering material in the field of geotechnical engineering it has been hardly used as an additive in microbial induced

soil stabilization. However, fly ash is known to help soil surface stabilization of sand dunes by assisting development of biocrusts (Zaady et al. 2017). In this scenario, soil matrix is bound together more efficiently as fly ash increases the number of fine particles which can be used as bridges by the microbes to grow toward and adhere to sand grains. Moreover, it has been proved that fly ash can be utilized as an economical carrier for calcifying bacterial isolates as it effectively sustains the viability of isolates while improving the efficacy of calcium carbonate biomineralization under certain favorable conditions (Dhmi et al. 2013). Also, Dhmi et al. (2012) suggested that the coupled effect of fly ash and MICP enhanced the durability of bricks by reducing permeability and water adsorption, whereas a UCS of 14.94 MPa was achieved which is a 17.4% improvement with respect to the red brick control sample.

Nevertheless, the most critical study was done by Li et al. (2018) in which different concentrations of fly ash was mixed with bio-cement precipitated by *Bacillus megaterium* to enhance strength of expansive soils. Addition of 25% of fly ash greatly reduced the swelling potential of expansive soil specimen and shown an improvement in unconfined compressive strength that is about two folds higher than the control. In general, swelling characteristics of expansive soils are higher due to presence of clay minerals that results in volumetric changes upon changes in moisture content. The use of bio-cement as admixture with fly ash can aggregate clay particles by cation exchange which leads to co-precipitation and subsequent reduction in surface area and water affinity. The ultimate improvement in strength of the specimens with optimum concentration of fly ash with bio-cement can be attributed to the coupled effects from plugging of pores in the fly ash-soil matrix via bio-cementation (Li et al. 2018) and self-cementing and pozzolanic reactions via fly ash hydration (Cokca 2001; Karthik et al. 2014). On the other hand, concentrations of fly ash as high as 50% with biocement deemed to yield serious negative impacts with regard to engineering properties of the test samples for two reasons; (i) restricted movement of bacterial cells along the soil specimen resulted by pore space clogging by fine fly ash particles and (ii) reduced bacterial metabolic activity due to non-availability of nutrients (Dhmi et al. 2012; Li et al. 2018).

7 Fiber Addition in MICP

The brittle behavior of MICP treated soils is one of the serious issues on which attention has been preferably focused nowadays (Bernardi et al. 2014). MICP treated specimens often tend to fail at low axial strain in unconfined and triaxial compression tests and eventually demonstrate a quick loss of post peak strength (Mortensen et al. 2011). Albeit in limited number of published works, it is reported that incorporation of fibers in bio-cementation process can improve the ductility of MICP treated samples and minimize the loss of post peak strength (Choi et al. 2016b; Li et al. 2016; Wen et al. 2018). Among different mechanisms of fiber reinforcement of soil, randomly distributed discrete fiber reinforcement has shown a great deal of influence

on increasing the shear strength, ductility and reducing post peak strength loss of soils (Shao et al. 2014; Li et al. 2015; Gowthaman et al. 2018).

Anderson et al. (2014) studied the effects MICP incorporated with fibers to reduce the wind erosion potential of loose soil. Four different types of fibers viz synthetic fibers made of polypropylene, cotton threads, shredded corn husks and hemp fibers were utilized to show that degree of soil stabilization against wind erosion is highly dependent on the type of the fiber. Sand specimens subjected to MICP along with fiber reinforcement has indicated the highest potential to prevent the mass loss due to wind erosion when compared with control specimens with independent treatment of MICP or fiber reinforcement. However, high moisture absorbing fibers such as bio-degradable corn husks or cotton fibers also with relatively higher surface area are ineffective in optimizing MICP process, as such fibers could absorb the cementation/growth medium which eventually limits the bioavailability of nutrients and the amount cementation medium.

Substantial amount of research that have been already performed on finding combined effect of fibers and bio-cement elucidate that fiber reinforcement is most effective for MICP treated sand when the fiber content varies in the range 0.2–0.3% by weight of sand (Anderson et al. 2014; Li et al. 2016; Wen et al. 2018). It has been reported that the failure strains of MICP treated sand with 0.2–0.3% of fiber mesh (polypropylene multifilament fiber) content shown a threefold increment than that in specimens without fiber (Li et al. 2016). Also, the flexural strength of a bio-beam reinforced with 0.3% of randomly distributed fibers of same kind gave rise to a significant improvement from 1% to 6% (Wen et al. 2018). The strength improvement effect in fiber MICP treated soil specimens is primarily due to expanded interface between fibers and sand grains which generally aids in better cohesion and internal friction in soil-fiber matrix. However, the addition of fibers results in only a slight improvement in elastic modulus, concluding that strength improved by fiber reinforcement is mainly due to extending of the elastic strain (Li et al. 2016; Wen et al. 2018). In another study using PVA fibers, the bio-cemented fiber reinforced sand revealed a 138% and 186% increment in UCS and splitting tensile strength respectively (Choi et al. 2016b). In general, MICP treated sand mixed with fibers exhibit improved shear strength parameters (cohesion and angle of friction), more ductile behaviors at which they maintain shear stress under continuous deformation at larger strains, reduced post peak strength loss and reduced permeability. Reduced permeability is a result of better pore clogging and calcium carbonate bridging among sand grains facilitated by presence of fibers in comparison to the samples without fibers.

However, too much fibers (beyond 0.3%) clogs the pores between soil particles which hinders the free passage of microbes creating a non-uniform distribution of bacteria within soil matrix (Li et al. 2016; Wen et al. 2018). This is followed by a heterogeneous distribution of calcium carbonate precipitation which ultimately affects the expected engineering properties. On the other hand, Wen et al. (2018) reported that the increased number of MICP treatment cycles boost the calcium carbonate coating on and around fiber surfaces thereby inhibiting ductility enhancement through fiber reinforcement. Considering the above facts, it can be concluded that much more extensive research need to be done with in-depth experimental work

and carefully designed parametric studies before a comprehensive understanding of fiber reinforced MICP treatment technique become apparent.

8 Limitations and Future Works

MICP is one of the eco-friendly soil improvement techniques, and the amendments discussed in the previous sections revealed the present trend of MICP, suggesting a vast potential in the future of geotechnical engineering. However, there are some associated limitations that are required to be considered at this developing stage.

There are some reminiscent drawbacks that might hinder the usage of low-grade materials for MICP applications. Some of the issues are as follows: local unavailability of materials, deficiency of material for bulk use and limitations in quality management. The waste materials (such as LML, CSL and CME) suggested for nutrient media are not available everywhere, and the frequency of accessible is also still a question. At the same time, there are lot of challenges associated regarding quality control while supplying the above nutrient medias (which are rich in various ions and proteins) into the ground during MICP applications. Therefore, more studies should introduce various inexpensive alternative nutrient sources and cementation sources that are locally available and readily accessible in bulk quantity.

The studies bear witness that biopolymers can significantly enhance the response of MICP. However, high cost is one of the major downsides of biopolymers, particularly at field applications. Also, only a very limited number of researches have focused on biopolymer modified MICP to date, suggesting very limited outcomes. Number of factors such as efficiency, bond stability, heavy metal removal, durability etc. are still required to be addressed more clearly in future works prior to the field applications. Also, MICP should widely focus on the feasibility of other biopolymers including Poly (acrylic) acid and Chitosan.

Introduction of various other additives such as magnesium ions, fly-ash and fibers have a great deal of importance in the precipitation process. However, supplying large amount of magnesium ions is harmful to the environment, contaminating the ground water, which could eventually result number of health issues in human and other living beings. Also, fly-ash is not a complete eco-friendly material, as it is rich in many toxic trace elements. Therefore, quantity control and mitigations measures are recommended while incorporating the above additions during field implementations.

9 Concluding Remarks

MICP is one of the most promising soil improvement techniques being investigated today. The versatile amendments in conventional MICP approach are often required to cater a wider range of applications with higher performance. The alternative approach in MICP by substituting low-grade materials could help significantly to

minimize the economic challenges often being faced during large-scale/field applications. Also, introducing various additives such as biopolymer, Mg^{2+} , fly-ash and fibers exhibited a great potential in MICP, and could enhance the conventional MICP outcomes either in chemically or mechanically, providing the needs of various practical applications in geotechnical and geo-environmental engineering field. However, there is mandatory need for deeper investigations, particularly on the biopolymer modified MICP, under various conditions and scales, in order to establish the results and bring them to the practical use.

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