Bio-Mediated Soil Improvement: An Introspection into Processes, Materials, Characterization, and Applications

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REVIEW PAPER

Bio-mediated soil improvement: An introspection into processes, materials, characterization and applications

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Abstract

For a long time in the practice of geotechnical engineering, soil has been viewed as an inert material, comprising only inorganic phases. However, microorganisms including bacteria, archaea and eukaryotes are ubiquitous in soil and have the capacity and capability to alter bio-geochemical processes in the local soil environment. The cumulative changes could consequently modify the physical, mechanical, conductive and chemical properties of the bulk soil matrix. In recent years, the topic of bio-mediated geotechnics has gained momentum in the scientific literature. It involves the manipulation of various bio-geochemical soil processes to improve soil engineering performance. In particular, the process of microbial-induced calcium carbonate precipitation (MICP) has received the most attention for its superior performance for soil improvement. The present work aims to shape a comprehensive understanding of recent developments in bio-mediated geotechnics, with a focus on MICP. Referring to around one hundred studies published over the past five years, this review focuses on popular and alternative MICP processes, innovative raw materials and additives for MICP, emerging tools and testing methodologies for characterizing MICP at multi-scale, and applications in emerging and/or unconventional geotechnical fields.

KEYWORDS

bio-cementation, microbially induced carbonate precipitation, soil improvement, ureolysis

Jiang and Wang equally contributed to this work. An Invited Review Paper submitted for publication in *Soil Use and Management*.

1 | INTRODUCTION

For a long time, geotechnical engineering practice has viewed soil as an inert material for construction, comprising only three phases—mineral solids, pore water and trapped air. The understanding of the fundamentals of soil behaviour is primarily based on these three inorganic phases. However, one fact that has been ignored for a long time by geotechnical engineers is that microorganisms including bacteria, archaea and eukaryotes are ubiquitous in soil (Jiang et al., 2020; Mitchell & Santamarina, 2005). The importance of microbes in soil has been well recognized by agricultural, ecological and environmental scientists, and numerous studies have been conducted to reveal the interactions between microbial activities and crop production, ecological conservation and environmental remediation (DeJong et al., 2015). Microbes have the capacity and capability to alter bio-geochemical processes in the local soil environment, and the cumulative changes could consequently modify the physical (e.g. density and porosity), mechanical (e.g. strength and compressibility), conductive (e.g. hydraulic and thermal conductivity) and chemical (e.g. buffering and ion exchange capacity) properties of the bulk soil matrix (DeJong et al., 2010; Ivanov & Chu, 2008).

While various natural microbial processes can change soil properties, an engineered process is required for soil treatment. The microbially induced carbonate precipitation (MICP) process has been the primary focus of research in bio-mediated geotechnics to date (Dejong et al., 2013; Zhu & Dittrich, 2016), though other microbial processes can also be used to change soil engineering properties, such as denitrification and other processes involving the use of iron-reducing, nitrifying and oligotrophic bacteria as discussed by Chu et al. (2009). MICP research in geotechnical engineering has experienced rapid developments in the past decade. There have been several published comprehensive review articles on MICP research in the past five years (Chu et al., 2015; He et al., 2019; Jiang et al., 2020; Osinubi et al., 2020; Shashank et al., 2016; Tang et al., 2020; Terzis & Laloui, 2019; Umar et al., 2016; Yu et al., 2020; Zhu & Dittrich, 2016). These reviews have focused on various aspects of MICP, ranging from fundamental processes, influencing factors, raw materials and multi-scale/multidisciplinary applications in various fields. In this review paper, the state of the art of MICP and more broadly bio-mediated geotechnics are reviewed based primarily on publications from the past five years. In particular, the following aspects are reviewed to add to the recently published review papers in this field. The current research frontlines, their challenges and future directions are also discussed, as follows:

1. A comparison between bio-stimulation and bio-augmentation in MICP application;
2. Some innovative raw materials and additives for MICP;
3. Emerging tools and testing methodologies for characterizing MICP at multi-scale;
4. Applications in emerging and/or unconventional geotechnical fields.

2 | MICP PROCESSES

Microbial-induced calcium carbonate precipitation can be achieved through different pathways, both autotrophic and heterotrophic, such as urea hydrolysis, sulphate reduction and denitrification. This section will provide a critical review on both popular (i.e., urea hydrolysis) and alternative MICP processes.

2.1 | Ureolysis: bio-augmentation vs. bio-stimulation

Since MICP was firstly introduced into geotechnical engineering, most studies have focused on ureolysis (Eq. (1)) because of its simplicity and high efficiency. Ureolytic bacteria can hydrolyse urea into carbon dioxide (CO_2) and ammonia (NH_3) because of the presence of microbially produced urease, where the hydrolysis rate can be 10^{14} times higher than the natural degradation reaction without urease (Estiu & Merz, 2004). The production of hydroxide ions (OH^-) because of the dissolution of ammonia in the water increases the local pH value. Meanwhile, the alkaline solution environment can increase the solubility of aqueous ${CO_3}^{2-}$. The bacterial cell walls are usually negatively charged and hence attract calcium ions in the solution and thus can be used as a suitable nuclear site for crystallization. While most researchers inject known ureolytic bacteria cultivated in the laboratory to complete bio-cementation (i.e. bio-augmentation), enriching indigenous ureolytic bacteria to achieve MICP (i.e. bio-stimulation) is an alternative approach that can potentially reduce complexity, costs and environmental risks. The comparative study between the bio-augmented and bio-stimulated MICP for soil stabilization (as illustrated in Figure 1) has become one of the frontlines of MICP research.

$$(NH_2)_2CO_{(aq)} + H_2O \rightarrow 2NH^+_{4(aq)} + CO_3^{2-} \tag{1}$$

Bio-augmentation is an approach where the exogenous bacteria cultured in the laboratory are added into the soil (Jain & Arnepalli, 2019; Mujah et al., 2019; Xiao et al., 2019a). In contrast, the bio-stimulation approach modifies the local environment by injecting nutrient media to enrich indigenous ureolytic bacteria capable of continuously producing a large quantity of urease to generate bio-cementation. There are

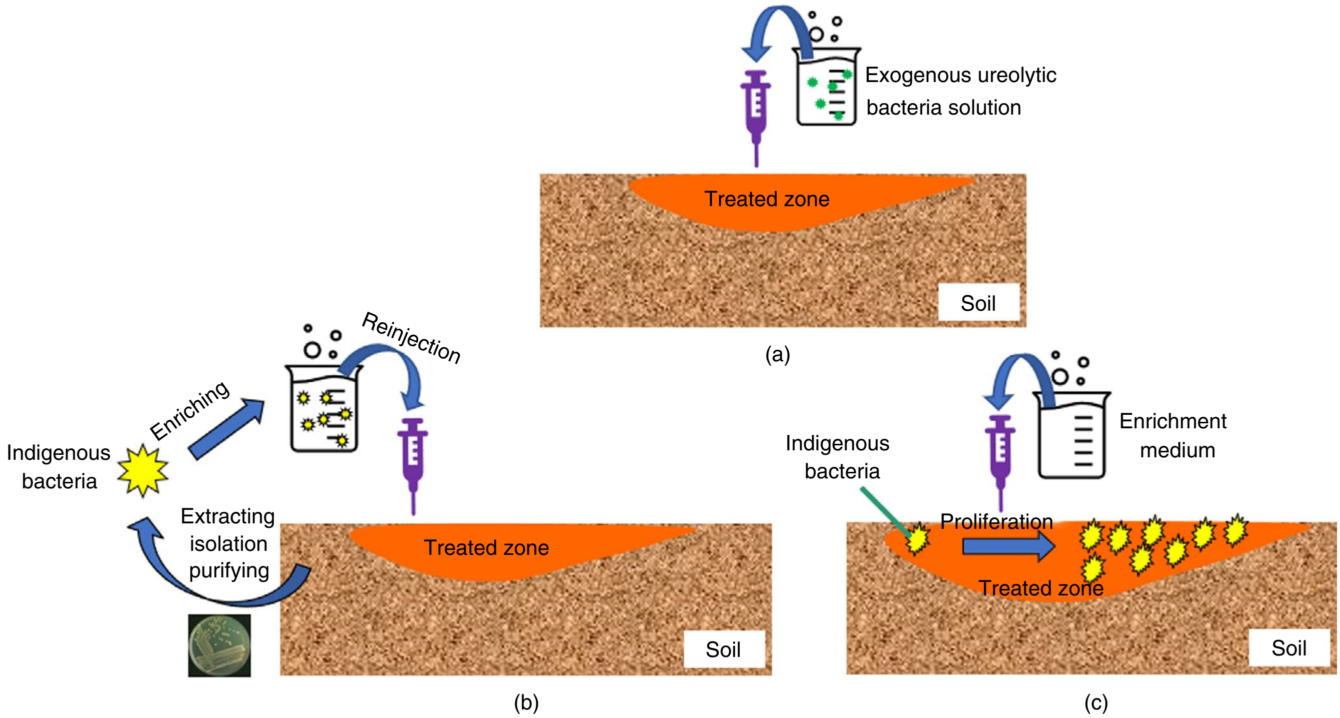


FIGURE 1 Three pathways of MICP ((a) bio-augmentation; (b) ex situ bio-stimulation; (c) in situ bio-stimulation)

TABLE 1 Comparison of bio-augmentation, ex situ bio-stimulation and in situ bio-stimulation

Aspects	Bio-augmentation	Ex situ bio-stimulation	In situ bio-stimulation
Ureolytic activity	High	High	Low
Urease loss	High	High	High
Reaction rate	High	High	Low
Environmental risks	High	Medium	Low
Estimated cost	High	Medium	Low
Required labour works	Medium	High	Low
Technical maturity	High	High	Low
Range of potential application	High	Low	Low

two ways to achieve bio-stimulation: ex situ and in situ. Ex situ bio-stimulation needs to isolate indigenous bacteria from soil and then performs isolation and purification to obtain pure, enriched ureolytic bacterial strains. The bacteria are finally reinjected into the soil to trigger MICP. In situ bio-stimulation, instead, can avoid the above-mentioned complicated and lengthy laboratory processes and simply involves directly injecting selective enrichment media into the soil to stimulate the growth of native ureolytic bacteria and thus generate bio-cementation.

Both bio-augmentation and bio-stimulation (include both in situ and ex situ ones) have advantages and drawbacks. A comparison among the three approaches from various aspects is summarized in Table 1 (Choi et al., 2020; Gomez et al., 2017; Hamed Khodadadi et al., 2017; Jiang, 2020; Kadhim & Zheng, 2016; Terzis & Laloui et al., 2019). Generally, while bio-augmentation can yield a higher reaction rate and initial ureolytic activity, the competition with other indigenous bacteria may affect the ultimate MICP efficiency (Graddy et al., 2018; Jiang, 2020). Consequently, it is required to repeatedly inject bacteria suspensions to maintain a sufficient amount and activity of urease enzyme within the soil matrix. Though bio-stimulation requires more treatment time than bio-augmentation to achieve comparable ureolytic activity or urea hydrolysis rate, it can significantly reduce costs associated with cultivating bacteria. The successful achievement of bio-stimulation is dependent on whether the ureolytic bacterial species are pre-existing in the environment or not.

While bio-augmentation and bio-stimulation are equally effective with respect to improving soil engineering properties (Gomez et al., 2017; Graddy et al., 2018), it does not mean that bio-stimulation can totally replace bio-augmentation. In specific applications, such as cracks and fracture repair or sealing in porous materials (e.g. concrete) under a harsh environment that cannot support sufficient indigenous bacteria, bio-augmentation is still a preferred approach (Alazhari et al., 2018; Ghosh et al., 2005; Jadhav et al., 2018; Marin et al., 2020; Phillips et al., 2016; Xu & Wang, 2018). In addition, the bio-stimulation might not be suitable for a sterile area, such as desert and cold region. The advantages of bio-stimulation over bio-augmentation should not be overestimated.

In recent years, the diversification of urease-positive bacterial species upon completion of MICP treatment, either through bio-augmentation or bio-stimulation, has gained more attention. Graddy et al. (2018) compared the diversity of *Sporosarcina*-like bacterial strains during bio-augmentation and bio-stimulation treatment in metre-scale tank experiments (shown in Figure 2). They found that a variety of urease-positive species was recovered at the end of the investigation in both cases, which was attributed to the numerous ecological niches created by the non-uniform cementation solution injections.

Through either bio-augmentation or bio-stimulation, the ureolytic MICP process has been demonstrated to be an effective technique for soil improvement. There have been many studies to investigate the feasibility of the ureolysis process for MICP from the perspective of microbiological behaviours, mechanical properties and microstructural characteristics (Graddy et al., 2018; Mujah et al., 2019; O'Donnell & Kavazanjian, 2015). However, there are still some challenges and research gaps that deserve further investigation. Below are a few examples:

1. Ammonium removal during ureolytic MICP;
2. Regulatory mechanisms of crystal morphology;
3. Adaptability to complex environments;
4. Bio-augmentation *v.s.* bio-stimulation in field application;
5. Protocols for up-scaling field applications in diverse or varying climatic conditions.

2.2 | Denitrification

The denitrification process (Eq. (2)) involves multiple reactions by denitrifying bacteria. Several enzymes catalyse the reduction of nitrate (NO_3^-) to nitrogen gas (N_2). Using acetate (i.e. $C_2H_3O_2^-$) as the electron donor and nitrate as the electron acceptor, carbonate ions (CO_3^{2-}) can be generated. When free calcium ions (Ca^{2+}) are available, precipitation can be induced spontaneously (O'Donnell et al., 2019; Pham et al., 2016).

$$2.6H^+_{(aq)} + 1.6NO^-_{3(aq)} + CH_3COOH^-_{(aq)} \rightarrow 0.8N_{2(aq)} + 2CO_{2(aq)} + 2.8H_2O \quad (2)$$

In recent years, although the denitrification-induced biocementation process is relatively slow to form a comparable amount of bio-cementation compared with ureolysis (O'Donnell et al., 2017), denitrification has its own advantages. Usually, denitrifying bacteria occur in wet and anaerobic soil conditions, which has the potential to improve submerged granular soils in deeper locations by forming biocementation via denitrification process (Kavazanjian et al., 2015; Pham et al., 2016). Although the peak shear strength of lightly bio-cemented sand could not be greatly improved,

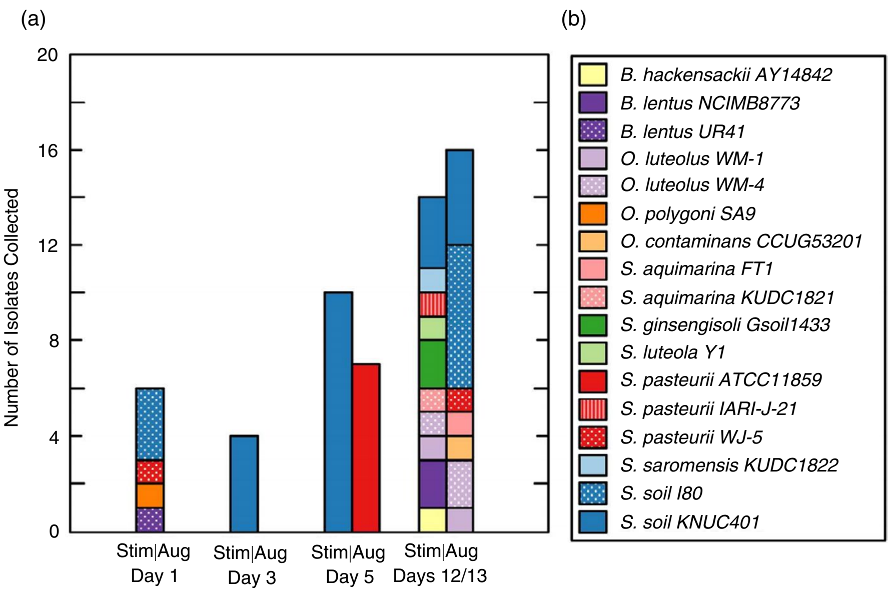


FIGURE 2 Strain assignments and abundances of isolates collected in experimental tanks treated by bio-augmentation and bio-stimulation (Graddy et al. 2018)

its stiffness and dilatancy, especially under low strain, could still be enhanced significantly (Pham et al., 2016). The other major advantage of the denitrification process for bio-cementation is that there is no ammonia production. On the other hand, the N_2 generated in this process can desaturate sand to increase liquefaction resistance. The partially saturated conditions because of the presence of N_2 gas bubbles can dampen pore pressure build-up and thus increase the undrained shear strength (Hall et al., 2018; He et al., 2013; O'Donnell et al., 2017). However, the potential negative effects of the generated gas bubbles on soil stabilization and the flow rate of various injected solutions should be considered carefully.

To develop denitrification-induced bio-cementation as a feasible and implementable soil improvement technique, further studies are needed on the following aspects:

1. Increasing bio-cementation amounts and reaction rates;
2. Developing appropriate substrate recipes to prevent the generation of toxic intermediate nitrogen compounds (i.e. NO_2^-);
3. Expanding applications under aerobic condition (i.e. surface soil);
4. Investigating the effects of regionally variable factors such as temperature, physical and chemical properties of local soils on the ultimate success of denitrification;
5. Enhancing the durability of bio-cementation by denitrification.

2.3 | Sulphate reduction

Sulphate reduction induced bio-cementation under anoxic conditions is another alternative MICP approach. Sulphate-reducing bacteria have played an important role throughout earth's 4.6 billion years history. They are predominantly anaerobic heterotrophs without oxygen involved in their metabolic activity (Baumgartner et al., 2006). Sulphate-reducing bacteria are able to reduce sulphates to sulphides while oxidizing organic carbon (Eq. 3):

$$SO_{4(aq)}^{2-} + 2[CH_2O]_{(aq)} + OH_{(aq)}^{-} \rightarrow HS_{(aq)}^{-} + 2HCO_{3(aq)}^{-} + H_2O \quad (3)$$

When sufficient calcium is present in the solution, the increased alkalinity will move the equilibrium towards calcium carbonate precipitation. However, some obvious disadvantages including the generation of toxic and combustible hydrogen sulfide (H_2S) gas may threaten the environment and human health (Gu et al., 2019). Therefore, challenges such as the treatment of toxic by-products should be resolved prior to its application in soil improvement. While sulphate-reducing bacteria are not preferred for soil improvement, they can bind with heavy metals, making sulphate reduction a promising method for the immobilization of heavy metal contaminants (Hwang & Jho, 2018; Le Pape et al., 2017).

2.4 | Iron reduction

Iron reduction (Eq. (4)), which induces ankerite and other mixed mineral precipitation, has also been investigated by some researchers as a potential bio-cementation process (Weaver et al., 2011). The redox reactions involved have been shown to be dominant in the anaerobic subsurface environment (Lovely, 1991). While iron-based precipitation is much cheaper, it is less effective, for example, in reducing the permeability of the soil, than calcium-based precipitation (i.e. by urea hydrolysis process) (Ivanov et al., 2014). Zeng and Tice (2014) found that the mineral composition was unstable and was easily affected by the concentration of other ions (e.g. Mg^{2+} and Ca^{2+}) and that cementation commonly occurred as the co-precipitation of calcite, siderite and dolomite. The studies related to iron reduction are somewhat limited currently, suggesting that iron reduction may not be a viable solution to induce bio-cementation for soil improvement.

$$4FE(OH)_3 + [CH_2O]_{(aq)} + 7H_{(aq)}^{+} \rightarrow 4Fe_{(aq)}^{2+} + HCO_{3(aq)}^{-} + 10H_2O \quad (4)$$

3 | MATERIALS FOR MICP

Materials involved in the MICP process include bacteria strains, nutrients, cementation solutions, auxiliary additives and soils. In this section, the recent developments in the research into materials involved in MICP, in particular the ureolysis process, are reviewed and discussed.

3.1 | Bacterial strains

The efficiency of bio-cementation is highly associated with the performance of either the injected exogenous or the enriched native ureolytic bacteria. A widely accepted assumption of the $CaCO_3$ precipitation process during MICP is that bacterial cells can serve as the nucleation sites for $CaCO_3$ because of the negative charge on the cell walls (EI Mountassir et al., 2014). Different bacteria have their own preferred environments, and even a slight variation of external conditions may affect their growth. Generally, ureolytic bacteria used for MICP should (1) have a reliable and constantly high enzymatic activity and (2) be harmless to humans and pose a low risk to the local ecosystem.

For the ureolysis process, various ureolytic bacterial strains isolated from the soil environment have been identified and found to be effective for MICP. These include, but are not limited to, *Bacillus* strains, *Sporosarcina* strains, *Shewanella* strains, *Pararhodobacter* strains and *Lysinibacillus* strains. Some most representative species include *Sporosarcina pasteurii* (Gomez et al., 2015; Kannan et al., 2020), *Bacillus sphaericus* (Mujah et al., 2016; Saffari et al., 2017), *Pararhodobacter* sp. (Amarakoon & Kawasaki, 2018) and *Bacillus megaterium* (Jiang et al., 2016). The bacterial strains could affect MICP efficiency in terms of (1) ureolysis rate (i.e. urease activity) and (2) nucleation and crystallization, which will be reviewed in the next two paragraphs.

On the one hand, different bacterial strains may display very different ureolysis rate. To assess the ureolysis capability of a specific bacterial strain, urease activity is usually used as the key indicator. The urease activity values that were attained by various bacteria in previous studies are summarized in Table 2. It should be noted that many factors may affect urease activity, including bacterial type, biomass density and culturing medium conditions (e.g. concentration of substrate (urea) and by-product (NH_4^+), pH and oxygen availability) (Bachmeier et al., 2002; Martin et al., 2012). From Table 2, it is clear that the microorganism type can make a significant difference to urease activity. Specifically, *Sporosarcina pasteurii* in most previous studies show a higher urease activity than other types of ureolytic bacteria, and higher biomass density results in higher urease activity.

On the other hand, bacterial cells can promote nucleation and crystallization by creating supersaturated alkaline environments and secreting extracellular polymeric materials (i.e. exopolysaccharides (EPS) and capsular polysaccharides (CPS) (Sundaram and Thakur 2018; Ercole et al., 2012). The extracellular polymeric materials produced by microorganisms might be tightly bound to the cell, loosely adherent to cells or existing in the form of free dissolved matter (Ercole et al., 2012), which can trap calcium ions at a given pH, control crystallization and influence the polymorphic development of $CaCO_3$ crystals (Dupraz et al., 2009; Bains et al., 2015). For example, in recent years, Szcześ et al. (2016) investigated *Rhodococcus opacus* cultures and found that the crystal size and polymorph could be controlled by EPS. EPS could stabilize vaterite and this effect is stronger at basic pH. Azulay et al. (2018) indicated that the EPS generated by *Bacillus subtilis* strain affected the crystal's nucleation, and the proteins (TasA and TapA) induced the aggregation of crystallites.

Most of the currently identified ureolytic bacteria are aerobes and hence are used for MICP under oxic conditions. The aerobic ureolytic bacteria target the surface soil where the oxygen is sufficient for the continuous expression of enzymatic activity. For instance, although the most widely used *Sporosarcina pasteurii* is a facultative anaerobe, it is unable to synthesize urease anaerobically. However, some ureolytic bacterial strains are reportedly able to survive and induce ureolysis under anoxic conditions. There is a controversy regarding the efficiency of ureolysis process that can be achieved in the absence of oxygen. For instance, on the one hand, Jiang et al. (2016) and Martin et al. (2012) observed considerable ureolytic activity of *B. megaterium* (ATCC 14581) and *Sporosarcina pasteurii* under anoxic conditions, which was attributed to the aerobic preculture and the urease already present in the cells. On the other hand, Mortensen et al. (2011) observed extensive ureolytic activity of *Sporosarcina pasteurii* under anoxic conditions, suggesting that the anoxic environment did not inhibit urease activity. Mitchell et al. (2019) investigated the kinetics of ureolysis and $CaCO_3$ precipitation of *Sporosarcina pasteurii* in the absence of oxygen. The results indicated that *Sporosarcina pasteurii* was capable of ureolysis in anaerobic environments; however, sustained growth over time in the absence of oxygen was not possible. Also, oxygen-free environments did not substantially affect the initial rate of ureolysis or $CaCO_3$ precipitation. The microorganisms used for other MICP processes including denitrification, sulphate reduction and iron reduction are ubiquitous in the anoxic subsurface environment. Thus, bio-stimulation of indigenous bacteria is preferred in these circumstances.

Finally, there are still research gaps in terms of the role of ureolytic bacterial strains during the MICP process. For example, it was observed that the amount of injected bacteria was diminishing with time and the diversity of bacteria was reduced at the end of bio-augmented MICP treatment

TABLE 2 Urease activity of representative ureolytic bacteria

Bacteria	Urease activity	Bacterial concentration	References
Sporosarcina pasteurii	1.8 mM urea/min	0.8–1.2 (OD_{600})	Xiao et al. (2019b)
Sporosarcina pasteurii	1.4–2.0 mM urea/min	10^7 cells/ml	Xiao et al. (2019a)
Sporosarcina pasteurii	4–5 mM urea/min	-	Hoang et al. (2019)
Sporosarcina pasteurii	2.08 mM urea/min/OD	0.22 (OD_{600})	Jiang et al. (2017)
Lysinibacillus sphaericus	0.4 μM urea/72 hr	250 mL sporulating bacterial spore solution	Kang et al. (2016b)
Bacillus sphaericus	10 μM urea/min	2–2.5 (OD_{600})	Mujah et al. (2016)
Bacillus sp.	20 ± 1 μM urea/min	4.2 ± 0.2 (OD_{600})	Cheng et al. (2019)
Bacillus sp.	15 μM urea/min	3–3.15 (OD_{600})	Hao et al. (2018)

(Graddy et al., 2018). In addition, the function of EPS to facilitate bio-cementation is still unclear for most ureolytic strains. Therefore, further research is needed on the following aspects:

1. Maintaining the dominance of injected ureolytic bacteria during bio-augmented MICP.
2. Understanding the microbiological and biochemical interactions between the exogenous and native bacterial species during the MICP process.
3. Illustrating the function of EPS to facilitate bio-cementation during the MICP process.

3.2 | Bacterial culture and enrichment media

Nutrients as the energy sources are crucial to the MICP process, in which bacteria utilize nutrients to sustain their metabolic and enzymatic activity. For the bio-augmentation approach, bacteria are cultivated using specific culture media in the laboratory to the desired concentration (i.e. bacterial culture medium). Table 3 shows some representative culture media for ureolytic bacteria that are found to be able to induce high urease activity with the presence of enzyme-substrate (i.e. urea or ammonium) (Jiang & Soga, 2017; Liu et al., 2020b; Zamani et al., 2018), suggesting that urea is not necessarily the only substrate that can induce high urease activity, and ammonium-rich media also have a comparable capability to produce similar urease activity.

For bio-stimulation, the enrichment media play a similar role to the culture media for bio-augmentation, which enriches the indigenous ureolytic bacteria and sustains the enzymatic activity (Wang et al., 2020a). Unlike bio-augmentation, the indigenous bacteria cannot reach the desired ureolytic rate immediately after injection. The primary principle for developing an effective enrichment medium is (1) to maximize the urease activity and (2) to minimize the enrichment time. Various carbon (C) sources including molasses, glucose and sodium acetate have been used to stimulate native heterotrophic ureolytic bacteria (Amini Kiasari et al., 2018), and urea is commonly added as the nitrogen source (Nassar et al., 2018). However, recent studies have shown that using carbohydrates as the only main C source could not induce sufficient urase activity. For instance, Amini Kiasari et al. (2019) investigated the effects of various C and nitrate sources, including yeast extract, sugarcane molasses, sodium acetate and glucose, on enriching the indigenous ureolytic bacteria for MICP. The results showed that the protein-based media such as yeast extract could yield a higher microbial urease activity, which was consistent with the findings of Cheng and Cord-Ruwisch (2013). Yeast extract is a complex nutrient that can provide vitamins, minerals, nucleic acid, amino acids, as well as growth factors that can increase the growth rate of microorganisms (Gat et al., 2016).

While appropriately designed sterilized culture or enrichment media have been adopted to grow ureolytic bacteria, non-sterilized conditions have also been found to be feasible. Yang et al. (2020) enriched urease-producing bacteria from activated sludge under non-sterilized conditions for MICP. Although the urease activity of the non-sterile enriched culture started to decrease after 3 days from the initial value of 15 to 7 U/ml at 10 days, it was still adequate for MICP for bio-cementation. The non-sterile enrichment method can reduce the total energy consumption and production cost by 30% (Yang, Chu, et al., 2020). A similar non-sterile method was also performed by Cheng and Cord-Ruwisch (2013). Sharaky et al. (2018) compared the bio-cementation content precipitated in sand achieved by using sterilized and non-sterilized media. It was observed that the amount of precipitation of $CaCO_3$ treated by the non-sterilized media method was equal to that of the sterilized media method.

Finally, for future large-scale field applications, the effectiveness of the culture and enrichment and the cost and environmental risks should be considered carefully. Some alternative inexpensive industrial substrates such as corn steep liquor, vegemite, torula, lactose mother liquor and food-grade yeast extract that can produce a considerable urease activity have been reported to replace the expensive laboratory-grade yeast extract (Achal et al., 2009; Chaparro-Acuña et al., 2018; Joshi et al., 2018; Omoregie et al., 2019; Babakhani et al., 2020). Moreover, some industrial wastes can also be used as the nutrients such as chicken manure effluent (Yoosathaporn et al., 2016). In these studies, the urease activity induced by the traditional laboratory-grade nutrients did not show a significant superiority over the industrial substrates. For instance, urease activity in "NB (nutrient broth)-urea medium" and "YE (yeast extract)-urea medium" was only 0.17- and 0.04-fold higher than that in LML (lactose mother liquor)-urea medium (Achal et al., 2009).

The current research gap relating to the bacterial culture and enrichment media lies in reducing the dosage, cost and environmental risk as much as possible to achieve the requirements for MICP.

3.3 | Cementation solution

For the bio-augmented ureolytic MICP process, most researchers commonly adopt a two-stage treatment strategy, in which cementation solution is injected following the cultivated bacteria suspension. Urea-calcium composite chemicals are usually adopted as the main ingredients of cementation solutions, sometimes with trace nutrients. For the urea source, researchers have not only used the traditional pure chemical, but have tried natural resources

TABLE 3 Representative media for the culture of *Sporosarcina pasteurii*

Media	Batch condition	pH	Main ingredients
NH_4^+-YE medium	Aerobic	9	20 g/L Yeast extract, 10 g/L $(NH_4)_2SO_4$
Urea-YE medium	Aerobic	7.5	20 g/L Yeast extract, 170 mM urea
Urea-rich NH_4^+-YE medium	Aerobic	-	20 g/L Yeast extract, 10 g/L $(NH_4)_2SO_4$, 0.5 M urea

such as pig and human urine (Chen et al., 2019; Lambert & Randall, 2019). For the calcium (Ca) source, common chemicals such as calcium chloride ($CaCl_2$), calcium acetate ($Ca(CH_3COO)_2$) and calcium nitrate ($Ca(NO_3)_2$) have been used. These Ca sources are chemically stable and relatively inexpensive for large-scale applications. Among them, $CaCl_2$ is the most common. However, it is worth noting that, to obtain sufficient cementation content, the amount of $CaCl_2$ required is commonly excessive and may be harmful to the environment. Also, to further minimize the environmental risk, alternative Ca sources are being developed. Choi et al. (2016b) compared $CaCl_2$ and Ca from eggshells dissolved by culinary vinegar. The results showed that the Ca produced from eggshells was as good as using $CaCl_2$. Moreover, Liu et al. (2018) utilized the soluble Ca dissolved from calcareous sand by acetic acid to improve the strength and permeability of MICP-treated calcareous sand. Choi et al. (2017a) and Casas et al. (2019) released and reused Ca from quarry limestone and Ca-rich silicate quarry, respectively, to apply to MICP.

Previous studies have suggested that the cementation solution plays a role in forming variable morphologies and sizes of $CaCO_3$ crystals. It has been reported that the type of cementation solution (i.e. Ca^{2+} source) and the time interval between cementation solution injections could largely affect the mineralogy and morphology of $CaCO_3$. The crystal types of $CaCO_3$ through MICP process usually include calcite, vaterite and aragonite, among which the calcite is the most common and stable. Zhang et al. (2014) found that the samples treated with $CaCl_2$ and $Ca(NO_3)_2$ were more likely to form calcite, whereas the samples treated with $Ca(CH_3COO)_2$ were composed of 88% aragonite and 12% calcite. Burdalski and Gomez (2020) investigated the effect of different Ca^{2+} concentrations of cementation solution on the morphology of $CaCO_3$. When the concentration was 500 mM, the sample showed a dominant calcite phase (96%) with negligible amounts (around 2%) of vaterite and aragonite. If the concentration increased to 1250 mM, calcite was still the dominant phase (75%), and the quantities of vaterite significantly increased to 23%. Wang et al. (2019b) conducted a microchip experiment and found that the size of $CaCO_3$ crystals was highly dependent on the time interval between cementation solution injections. The average size of $CaCO_3$ crystals was considerably larger when the injection interval was 23–25 hr than 3–5 hr.

In short, cementation solution is critical to achieving satisfactory MICP treatment. However, the potential secondary pollution from injecting large amounts of solution into soil remains unexplored. In addition, further reducing the cost by selecting alternative Ca and urea sources is also necessary. Future work is needed to further reduce the cost and environmental risks associated with the cementation solution.

3.4 | Auxiliary additives

In order to improve bacterial growth, urease activity and bio-cementation content, researchers have developed several auxiliary additives to supplement the culture/enrichment media and cementation solution, or to add directly into the soil matrix.

For the auxiliary additives added into the culture/enrichment media, apart from the common necessary elements (C, N, P, H, O), nickel dichloride ($NiCl_2$) has been used by many researchers, usually in very small amounts (Amini Kiasari et al., 2018, 2019; Fang et al., 2020; Gat et al., 2016; Xu et al., 2020). MICP is highly associated with the metabolic and enzymatic activity of the specific ureolytic bacteria. As a key part of the urease enzyme structure, the nickel ion has been shown to be an essential trace element for maintaining sufficiently high ureolytic activity of urease-producing bacteria (Svane et al., 2020). In addition, Shashank et al. (2018) investigated the effect of buffer solution added with the bacterial suspension on regulating the ureolysis procedure. Adding a buffer solution was found to prevent the instantaneous increase of pH which might lead to a rapid clogging near the injection port during the MICP process.

For the auxiliary additives in the cementation solution, Xu et al. (2020) proposed magnesium ions to modify carbonate crystal polymorphs. The results showed that the incorporation of 0.01–0.5 M magnesium ions with the cementation solution composed of $Ca(CH_3COO)_2$ and urea could promote the formation of acicular aragonite and inhibit the growth of rhombohedral calcite, which resulted in a 40%-200% increase in the unconfined compression strength. Nawarathna et al. (2018) used various concentrations of poly-Lys ranging from 0 to 50 mM as the additive to the cementation solution ($CaCl_2$+urea). The findings indicated that the addition of poly-Lys created stronger cemented sand specimens than those obtained by the conventional method without poly-Lys. Wang et al. (2018) modified the cementation solution by adding polyvinyl alcohol (PVA) to successfully improve the erodibility of the treated sand.

For the auxiliary additives directly added into the soil matrix, randomly distributed fibres have been used to increase the ductility of sandy soil and to prevent the loss of post-peak strength. Different types of fibres including natural plant-based and synthetic polymers have been used (Choi et al., 2016a; Imran et al., 2020; Li et al., 2016; Zhao et al., 2020). The optimum fibre content was usually between 0.2% and 0.3% by weight (Qiu et al., 2019; Li et al., 2016; Fang et al., 2020). Li et al. (2016) used homopolymer polypropylene multifilament fibres as the additive in sand and found the unconfined compression strength gradually increased when the fibre content was between 0.2% and 0.3%, which is the optimum fibre content. Fang et al. (2020) added modified-polyester fibres to improve the engineering properties of MICP-treated coral sand. The results showed that the permeability reduced by 2–3 orders of magnitude and UCS increased from 2.78 to 21.65 MPa. Besides randomly distributed fibres, researchers have also added clay particles as an auxiliary additive. Won et al. (2020) added kaolinite into sand as the extra nucleation sites for the calcite precipitation. The results indicated that the kaolinite particles could function as nucleation sites and facilitate the heterogeneous nucleation of calcite. Ma et al. (2020) introduced bentonite into coarse sand and found that the unconfined compressive strength (UCS) was substantially improved with an optimal bentonite concentration of 20 g/L. Higher bentonite concentrations (40 and 80 g/L in this study) might have a negative effect on UCS.

3.5 | Soil

Microbial-induced calcium carbonate precipitation has been widely investigated for its applicability in different types of soils. Table 4 shows a few examples reported in the past five years. Typically, the size of bacteria for MICP is between 0.5 and 3 μm, making it suitable for a broad range of soil types.

For granular soils, MICP has already been intensively studied to modify the physical (density, gradation, porosity, saturation), mechanical properties (strength, stiffness, shear behaviour, compressibility) and hydraulic properties through bio-cementation. The relatively larger pore space in coarse-grained soils allows microbes to move freely within the soil matrix. Thus, it is much easier to conduct MICP treatment in granular soils. It is also necessary to consider the effect of relative density, one of the most important parameters for coarse-grained soils, on strength improvement. The biggest difference between bio-cemented and chemically cemented soil was that soil structure remains intact by using MICP, thus the initial packing of soil governs the formation of cementation within the soil matrix (Terzis & Laloui, 2019). Rowshanbakht et al. (2016) found that increasing the initial relative density resulted in the reduction in cementation content, which was because of the decreasing pore volumes for the nucleation sites of calcite and the decreasing amounts of microbes and nutrients that could be absorbed.

In recent years, more and more studies have focused on MICP applications in soils that were traditionally regarded as unsuitable for MICP treatment (i.e. clay soil and loess). For clay soils, percolation may be difficult because of their low permeability and surface charge on the particles. Thus, premixing the bacterial solution with clay soil is one of the primary sample preparation methods (Kannan et al., 2020). Cardoso et al. (2018) suggested that MICP application in clay soils is much more challenging than in sand because of the complex chemical interactions between clay minerals and the injected solutions. For example, Sharma and Ramkrishnan (2016) conducted bio-augmented MICP in clay. A noticeable improvement (1.5–2.9 times higher than the untreated samples) in the unconfined compressive strength was observed. Saffari et al. (2017) found that MICP treatment could increase the cohesion, friction angle and shear strength in low plasticity clay. For collapsed loess, Atashgahi et al. (2020) reported that MICP could reduce the collapse potential between 24 and 54.8% by enhancing particle-particle contacts between the soil particles. Sun et al. (2020) suggested that the collapsibility of loess soils was significantly decreased at an optimum cementation solution concentration of 0.75–1 M.

Soil is a rather complex mixture consisting of organic matter, minerals, gases, liquids and organisms (i.e. solid, air, liquid and organisms). The effects of soil environment on bacterial growth and precipitation formation remain unclear. In future research, it is important to assess whether environmental conditions including pH, oxygen availability, temperature and humidity within the soil matrix can support the activity of injected microbes before application. Moreover, the sustainability and durability of induced precipitation in various soil environments after MICP treatment also need to be examined.

4 | CHARACTERIZATION OF MICP PROCESSES

As a multidisciplinary research field in the intersection of geotechnics, microbiology, biochemistry, environmental engineering and material science, the MICP process needs to be characterized using observational and experimental tools from multi-disciplines and at multi-scales (Figure 3).

TABLE 4 MICP in different types of soils in laboratory studies

Soil type	Problems	Improvement mechanism	References
Sandy soil	Very loose; low strength and stiffness	Strengthen particle bonds via bio-cementation	Whitaker et al. (2018); Cui et al. (2017)
Sandy soil	Low cohesion; low wind or water erosion resistance	Stabilize the surface layer of soil via bio-cementation	Maleki et al. (2016); Shanahan and Montoya (2016); Wang et al. (2018); Fattahi et al. (2020)
Mixed soil (gravel-sand/ sand-clay)	Internal erosion as the core materials in embankment dams and levees	Mitigate internal erosion and improve hydraulic condition via bio-cementation and bio-clogging	Jiang and Soga (2017, 2019); Jiang et al. (2017)
Liquefiable sand	Loose; saturated; insufficient cyclic shear strength	Reduce the degree of saturation via generating biogas (denitrification); Solidify and densify soil via calcite precipitation (ureolysis)	Hall et al. (2018)
Clayed soil	Formation of desiccation cracks lead to an increase in hydraulic conductivity	Remediate the desiccation cracks via bio-cementation	Liu et al. (2020a)
Marine clay	Low bearing capacity; high natural water content (close to the liquid limit); be susceptible to large consolidation settlements	Reduce liquid limit and increase strength via bio-cementation; increase strength via bioencapsulation	Kannan et al. (2020); Li, Li, et al. (2016); Ivanov et al. (2015); Li, Li, et al. (2016)
Loess soil	Potential collapsibility; insignificant adhesion; very unstable	Improve the strength and hydraulic features via bio-cementation and bio-clogging	Atashgahi et al. (2020)
Heavy metal contaminated soil	High environmental and ecological risks	Coprecipitate and then remove the heavy metals with calcite	Cheng & Shahin, (2017); Jalilvand et al. (2020); Zhu et al., (2016); Zhao et al., (2017)

This section will review the characterization techniques for the MICP process at nano, micro, meso, element, pilot and field scales.

4.1 | Nano-scale

Nano-scale material characterization techniques are used to show the interactions between bacteria and substrate (soil particles) during the MICP process, which can illustrate fundamental mechanisms between microorganisms and their nucleation surface. Atomic force microscopy (AFM) developed in 1986 is an ideal tool which can be applied to acquire the surface topography and texture information with demonstrated resolution on the nanometre scale. Shashank et al. (2020) used AFM to investigate the capability of the biosorption of bacteria on the surface of soil particles. They found that the extent of bacterial adhesion on soils depends on the available hydrophobic binding sites, and bacteria could be entrapped in the pores of formed crystals based on the surface texture parameters during the MICP treatment.

4.2 | Micro- and meso-scale

The characteristics of calcite crystals and their interactions with soil particles can affect the mechanical properties of bio-cemented soil. Various characterization micro-scale techniques including, but not limited to, scanning electron microscope (SEM) with energy-dispersive X-ray spectroscopy (EDS), X-ray diffraction (XRD), Fourier-transform infrared spectroscopy (FTIR), thermogravimetric analysis (TGA), X-ray CT and mercury intrusion porosimetry test (MIP), and meso-scale techniques, such as microfluidic chip, have been used to provide insight into the evaluation of the crystal shape, size, content, distribution pattern, structure, contact and surface fractures of bio-cemented soils.

The size of the precipitated crystals can be detected clearly using SEM images. For instance, Mujah et al. (2019) found that different size, distribution pattern and shape were observed by comparing SEM images at different concentrations of cementation solution. It showed that the lower cementation solution concentration (0.25 M) resulted in larger crystal size than the higher cementation solution concentration

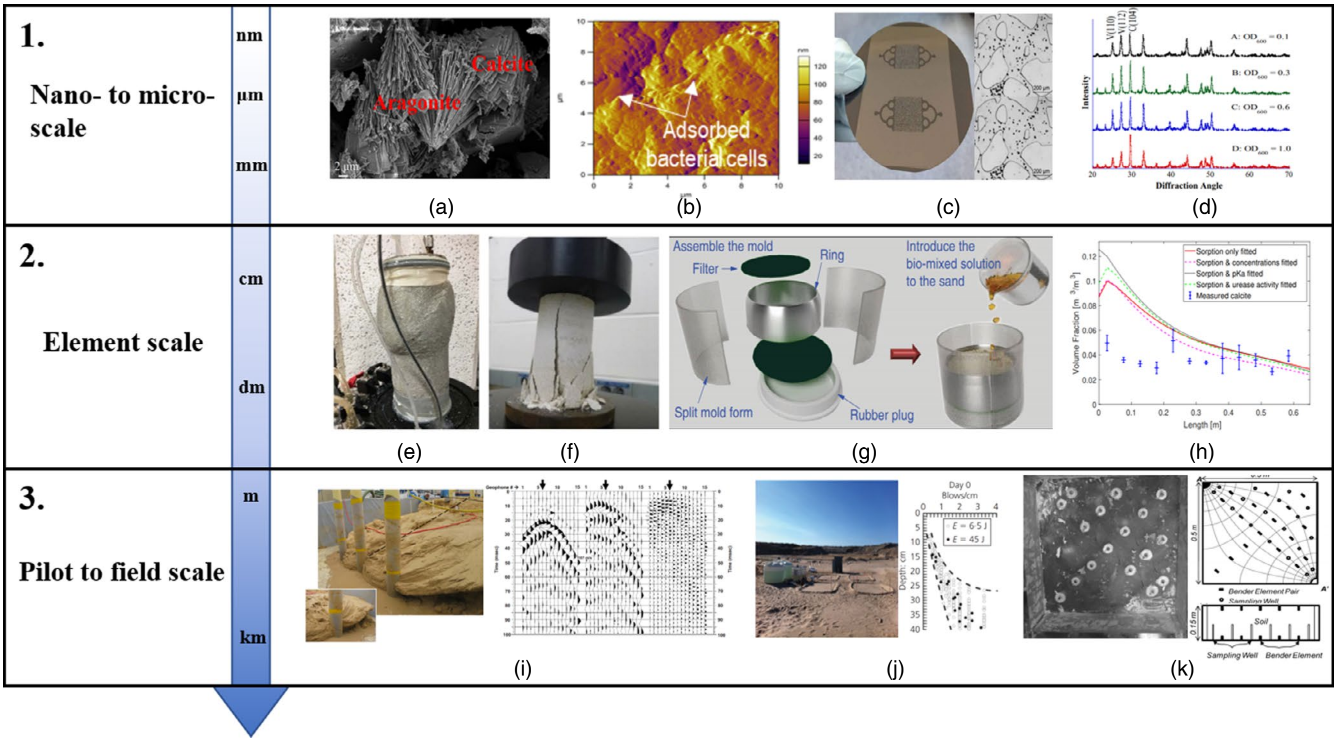
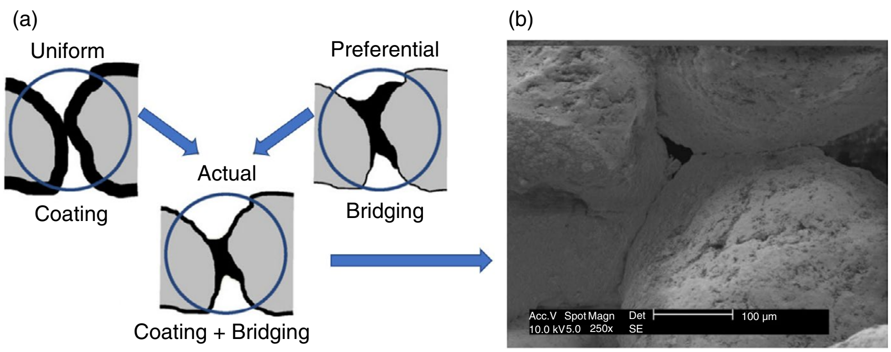


FIGURE 3 The characterization of MICP at multi-scales ((a) SEM images of precipitation (Xu et al., 2020); (b) AFM peak-force error images of substrates with bacteria cell adhesion (Shashank et al., 2020); (c) the particle-scale behaviour of calcite precipitation from a microfluidic chip (Wang et al., 2019a); (d) the XRD patterns of $CaCO_3$ crystals (Wen et al., 2020); (e) shear response of bio-cemented sand by undrained triaxial test (Nafisi et al., 2019); (f) the unconfined compress (UCS) test (Fang et al., 2020); (g) one-dimensional compression test (Xiao, Chen, et al., 2020a); (h) predicted volume fractions of final calcite by numerical modelling (Hommel et al., 2020); (i) seismic shear-wave data of 100-m^3 large-scale bio-grouting test (Van Paassen et al., 2010a; Van Paassen et al., 2010b); (j) dynamic cone penetration (DCP) data of field-scale test measuring 2.4 m × 4.9 m on loose sand (Gomez et al., 2015); (k) Five-Spot treatment model (Dejong et al., 2014))

FIGURE 4 The precipitated crystal distribution pattern within pore space of soil matrix [modified after Dejong et al. (2010) and Cheng et al. (2013)]



(1 M). Choi et al. (2020) and Fang et al. (2020) suggested that bonding at the particle-particle contacts as a bridge and coating on the particle surface are the two primary functions of precipitated crystals within bio-cemented soil. It is found that coating and bridging as the actual distribution pattern usually coexist after MICP treatment under unsaturated conditions (as shown in Figure 4).

The variation in porosity of MICP-treated soils can be determined by conducting MIP test and X-ray CT scanning. Amarakoon and Kawasaki (2018) found that the porosity of treated sand samples decreased with depth by analysing the X-CT images at the top, middle and bottom parts. Gao et al. (2019) conducted MIP test to identify the changes in the pore size distribution of bio-cemented quartz sand. The results also showed that the porosity of the treated sample was significantly reduced from 0.428 to 0.28–0.33 in the surface layer by forming a hard crust and 0.37–0.39 below the crust.

By using EDS, XRD and FTIR, the crystal morphology can be determined. The rhombohedral, hexahedral, orthorhombic, acicular, spherical and ellipsoidal crystals commonly appear in the form of calcite, aragonite and vaterite under different environmental conditions (temperature, cementation solution, pH, etc.). For instance, by analysing the data from XRD and EDS, Xu et al. (2020) found that cementation solution types had an effect on the crystal morphology. The main crystal form was aragonite when $Ca(CH_3COO)_2$ was used as the Ca source. After adding 0.5 M magnesium ions to the cementation solution, a small amount of calcite converts into low-magnesium calcite; however, the growth of aragonite is not inhibited. Wen et al. (2020) conducted XRD tests and found that the concentration of bacteria or urease did not have an apparent effect on the morphology of $CaCO_3$ crystals. Moreover, results from FTIR suggested that vaterite was the major form of $CaCO_3$ crystals within 72 hr with calcite increasing over time during the MICP process.

At the meso-scale, Wang et al. (2019a, 2019b) developed a microfluidic chip, a 2-D representation of porous media, to observe the MICP process and the behaviour of bacteria and growth of precipitated crystals under saturated condition (as shown in Figure 3c). It contains an inlet, upstream flow distribution channels, a porous medium, downstream flow distribution channels and an outlet (Wang et al., 2019a). A computer-controlled microscope is used for image collection. The observations showed that bacteria were distributed evenly after the bacterial suspension injection and $CaCO_3$ crystallized at narrow pore throats or open-pore bodies. The precipitated $CaCO_3$ crystal transformed from irregularly shaped amorphous $CaCO_3$ precipitates to spherical vaterite and then to rhombohedral calcite (Wang et al., 2019b). Marzin et al. (2020) also used a microfluidic cell to investigate the influence of the injection time and the ionic strength on the adhesion rate of *Sporosarcina pasteurii* bacteria on sandstone grains and crystals formation during MICP. The results indicated a rise of adhesion rate from 0.005 per minute to 0.03 per minute with an increase of NaCl concentration in solution from 3 to 20 g/L.

4.3 | Element-scale

A series of element-scale characterization techniques have been applied in the laboratory to assess the improvement of engineering properties including, but not limited to, permeability, unconfined compressive strength, shear behaviour, particle breakage and compressibility behaviour, cementation content, thermal conductivity and durability.

The formed bio-cementation, which serves as a clogging material between particle contacts, can reduce the size of pore throats and resist water permeation. In the laboratory, both constant head and falling head tests can be applied to a variety of bio-cemented soils. Bio-grouting using bio-slurry containing preformed urease active calcium carbonate crystals is an emerging technique for soil improvement by reducing the coefficient of permeability. Various types of soils have been studied, which widens the practical applications in terms of the bio-grouting technique in different soil conditions. For example, Peng et al. (2020) reported that the coefficient of permeability of MICP-treated fractured rock was reduced to $3–5 \times 10^{-5}$ m/s by four orders of magnitude using bio-grouting method. Lian et al. (2019) conducted bio-grouting on hydraulic fill fine sands for reclamation. The permeability coefficient was reduced by approximately three orders of magnitude using the constant head approach. Wu and Chu (2020) used bio-grouting to treat granite aggregates and found a reduction of permeability in both saturated and unsaturated cases. A larger reduction was observed in the unsaturated condition. Pan et al. (2020) successfully applied bio-grouting on sands with grain sizes ranging from 0.30 to 2.36 mm to significantly reduce the coefficient of permeability. Compared with the conventional chemically treated soil, the reduction in permeability via the MICP process is much less. The minor reduction in permeability can, on the one hand, create a good drainage passage which ensures the unhindered penetration of bacterial solution (Chu et al., 2014) and, on the other hand, can avoid the development of excess pore water pressure during loading (Mujah et al., 2017).

The unconfined compression test is another popular characterization method at element-scale, which could assess the strength of bio-cemented soils (Amini Kiasari et al., 2019; Hoang et al., 2019; Rowshanbakht et al., 2016; Terzis et al., 2018). Existing studies have primarily focused on the effect of cementation level on unconfined compression strength (UCS), and an empirical correlation between UCS and cementation content was proposed. Specifically, the UCS was found to be positively correlated with the cementation content. The UCS values could fluctuate between 50–100 kPa and 10 MPa with the cementation content varying substantially from less than 2% to 25%–30% (Amarakoon & Kawasaki, 2018). Usually, the UCS values were reported when the $CaCO_3$ content is over 3%, at which the bio-cemented samples could maintain their integrity and stand alone. However, Terzis and Laloui (2019) pointed out that the lower cementation content (<2%) could still offer the desired improvement if considering the role of confinement. Choi et al. (2020) gave an empirical correlation between UCS and cementation content (CC, in percentage) based on the compiled data as follows, where α_{UCS} and β_{UCS} are two empirical fitting parameters.

$$\mathrm{UCS[kPa]} = \alpha_{\mathrm{UCS}} \cdot \mathrm{CC}^{\beta_{\mathrm{UCS}}},\ \mathrm{when\ CC} < 30\% \quad (5)$$

Researchers have conducted various types of tests to evaluate the shear strength of bio-cemented soils, among which the triaxial compression test and direct shear test are the most common methods. More attention has been placed on the stress–strain relationship and stress–dilatancy behaviour of bio-cemented samples. The evolution of cohesion, effective friction angle and failure envelope with the variation of cementation level was the major concerns. Generally, the results suggested that the shear strength performance improves significantly compared with uncemented soils. For instance, Cui et al. (2017) conducted triaxial undrained tests on sand. It was found that the effective cohesion and effective friction angle increased with cementation level with a linear and exponential relationship, respectively. The increase in effective cohesion with cementation level has also been observed in the other study (Wu et al., 2021). However, Wu et al. (2021) found that the effective friction angle did not change when the cementation content exceeded 5%. The major effect of cementation is to increase the cohesion. Thus, the effect of cementation on the friction angle of bio-cemented soils should not be overestimated. Amini Kiasari et al. (2018) performed direct shear tests on bio-cemented sand treated using the bio-stimulation approach. The results showed an increase of 190% in cohesion and 16.79% in the friction angle compared with the uncemented sand. The bonding effect enhancing the interparticle contacts is commonly considered as the main governing role for bio-cemented soils. The cementations deposited on the surface of particles were regarded as ineffective and made little contribution to the enhanced peak shear strength of bio-cemented soils (Cui et al., 2017; Dejong et al., 2010). However, interestingly, O'Donnell and Kavazanjian (2015) reported a significant improvement in stiffness and dilatancy on bio-treated Ottawa 20–30 sand with very little cementation under isotropically undrained triaxial compression (CIUC) tests. They hypothesized that such improvement was possibly from particle roughening because of the coating effect of $CaCO_3$ on the particle surface. Current investigations also show variations of shear parameters at critical or residual state. For instance, in triaxial tests, Feng & Montoya (2016) indicated that the residual cohesion is assumed to be zero. The residual friction angle decreased with the increase of confining pressure but was larger at all levels of confinement in heavily and moderately cemented sand. In contrast, the residual friction angle of lightly cemented sand is close to that of untreated soil.

Regarding the compression and particle breakage behaviours, while many researchers have investigated them for uncemented soils, very limited studies have been conducted to explore those of bio-cemented sand treated using MICP. The particle breakage and compressibility behaviour of MICP-treated sands have been investigated mostly by oedometric compression tests. The extent of particle breakage is usually quantified by the difference in particle size distribution (PSDs) before and after loading. Generally, MICP treatment can effectively reduce the magnitude of grain crushing and compressibility. For example, Lin et al. (2016) conducted confined compression tests and found the MICP-treated specimens were less compressible than untreated specimens. The compressibility decreased with the increase of $CaCO_3$ content. For a given vertical effective stress or input work, specimens with a larger bio-cementation content exhibited smaller particle breakage and vertical strain. The formed bio-cementation serves to restrain particle breakage and compressibility mainly via three mechanisms: (1) increase the effective diameter of soil particles; (2) dissipate energy during loading; and (3) remain in the void and reduce the magnitude of particle contact forces through a cushioning effect (Xiao et al., 2020a; Xiao et al., 2020b).

Cementation content is another essential parameter that is characterized at the element-scale, which has a strong correlation with the engineering performance of bio-cemented soils. In previous studies, cementation content measurement was measured mainly using the destructive method. Dissolving samples in hydrochloric acid (HCl) is a conventional destructive method, in which HCl is added to dissolve the formed cementation and then the weight difference of samples or the pressure difference because of the generation of CO_2 is calculated before and after washing by acid (Feng & Montoya, 2015; Xu et al., 2020). Although acid washing is rather convenient, the accuracy of this method is not inferior to any other methods as compared by Choi et al., (2017b) because of the non-uniformity of treatment. In recent years, new non-destructive methods have been applied to characterize bio-cementation content. For instance, the bender element test has been conducted to obtain bio-cementation by correlating it with measured shear-wave velocity (Nafisi et al., 2018, 2020).

The thermal conductivity of MICP-treated soil has also been studied in recent years. The effect of bio-cementation within the soil matrix is an influential factor on the thermal conductivity. Venuleo et al. (2016) compared the thermal conductivity between MICP-treated and untreated soils. The results showed that the thermal conductivity of MICP-treated soils was increased by 250%. The induced $CaCO_3$ acting as "thermal bridges" among sand grains offered more effective heat transfer paths by increasing the surface contact area. Wang et al. (2020b) found that the thermal conductivity was linearly correlated with dry density, treatment cycles and $CaCO_3$ content in bio-cemented sands. Martinez et al. (2019) investigated the relationship between the degree of saturation and soil thermal conductivity for a poorly graded quartz sand, and results indicated that thermal conductivity increased with the degree of saturation especially at low saturation levels. In the future, the performance of MICP in practical applications related to thermal conductivity properties, such as energy piles and ground source heat pumps, could be assessed.

The efficacy of the engineering performance of MICP is another concern. There are many environmental conditions that could reduce durability, such as wet–dry cycles, freeze–thaw cycles and acid rain infiltration. Element-scale experiments have been conducted to characterize the durability of MICP-treated soils. For instance, Liu et al. (2019) conducted a series of unconfined compression tests to investigate the durability of bio-cemented sandy soils under various artificial environmental conditions. The results showed that nearly 80% UCS reduction after one wet–dry cycle, 58% UCS reduction after 15 freeze–thaw cycles and 83% UCS reduction after 15 days immersed in acid rain solution with a pH of 3.5. Gowthaman et al. (2020) investigated the influence of freeze–thaw cycles on slope soil treated by MICP. The results indicated that the erosion induced by freeze–thaw cycles was dependent on the cementation content. Liu et al. (2019) found that fibre-reinforced samples could reduce the strength reduction after wet–dry and freeze–thaw cycles, though their resistance to acid rain attack remained weak.

4.4 | Pilot- and field-scale

Several pilot and field trials have been conducted in recent years to treat sandy soils using MICP, in which different characterization tools were adopted. Normally, pilot-scale tests are carried out to validate the MICP concept, which are smaller in dimensions and cheaper than the field-scale ones. In addition, although the experimental conditions at pilot-scale are easier to control, the representation of experimental results is not as relevant as those from field-scale tests. Gomez et al. (2015) conducted a pilot-scale test on loose sand deposits measuring 2.4 m by 4.9 m to improve the surficial erosion resistance. Dynamic cone penetrometer (DCP) measurement was applied to the three bio-treated test plots to evaluate variations in the penetration resistance. The results showed the improvement could reach to around 28 cm depth. San Pablo et al. (2020) developed 3.7-m-long horizontal columns to investigate the spatial uniformity of bio-cementation and the removal of posttreatment ammonium by-product. The results indicated that using bio-stimulated approaches with a low ureolytic rate could reach a farther treatment distance. Do et al. (2020) developed a double wall pile delivery system in a soil box (measuring 0.91 m × 0.91 m × 0.91 m) to improve the submerged sand adjacent to a pile foundation system using MICP treatment. This system involved the cementation of the general area adjacent to the pile in an ellipsoidal shape with few plugging issues.

For the field-scale tests, Dejong et al. (2014) adopted a scaled repeated five-spot treatment model to monitor the efficiency of MICP during treatment. Bender elements were installed to capture the spatial and temporal changes in mechanical properties using shear-wave velocity, which is a good proxy for the distribution of calcite precipitation. Phillips et al. (2018) applied MICP in enhancing wellbore cement integrity with a diameter of 24.4 cm (9.625 inches). The treated region was identified using an ultrasonic imaging tool (USIT), providing a continuous image of the quality of the cement bond at the cement-casing interface. Meng et al. (2020) used the MICP technique to control the wind erosion of surface desert soil (with a depth of ~10 cm). Surface penetration tests (penetration depth ~2 cm) by a digital micro-penetrometer were conducted to evaluate soil-bearing capacity. Saneiyan et al. (2019) adopted induced polarization (IP), a geophysical method in mineral exploration, to monitor the status of soil strengthening via MICP in the field spatially and temporally. Terzis et al. (2020) applied field-scale bio-grouting to mitigate landslide risk through MICP within the targeted slip zone hit by extreme rainfall in Switzerland. The data collected by drone surveillance indicated a slower movement within the MICP-treated zone after treatment compared with other zones without MICP treatment.

Although some pilot- and field-scale experiments have been conducted, more attention has been placed on the short-term treatment efficiency. There are rather limited large-scale research studies focusing on the long-term durable behaviours. Further work is needed to conduct long-term verification of MICP treatment in the field.

4.5 | Numerical modelling

Numerical modelling is a useful tool to virtually assess the performance of MICP where the complex coupled bio-geochemical processes are involved. Numerical prediction can be conducted from different aspects, namely biology, chemistry, hydraulics and mechanics as illustrated in Figure 5. MICP is the collective result of water flow, solute transport, chemical reactions and microorganism mobilization/immobilization/growth/decay/dynamic metabolic potential (Nassar et al., 2018). Recently, much of the numerical modelling of MICP has focused on small-scale experiments in porous media (mostly sand) whereas large-scale modelling is rather limited.

On the biochemical aspect, the developed models normally consider both solid and aqueous phases and take various coupling mechanisms into account including the flow of the aqueous phase, and the transport of the chemical and bacterial components (i.e. advection, diffusion, dispersion, sorption, bacterial decay) in these two phases (Matsubara et al., 2020; Wang & Nackenhorst, 2020). Researchers may have different assumptions for the phase adsorption of diverse species and chemical components, which could either simplify or complicate models. For instance, Wang and Nackenhorst (2020) assumed that both the urea and ammonium were in the liquid phase, while Fauriel and Laloui (2012) divided them into two parts: solute ammonium/urea in the liquid phase and absorbed ammonium/urea in the solid phase. Furthermore, based on the different assumptions of distribution of various aqueous chemicals and reactants, two types of models have been applied in previous studies: macroscopic continuum and pore-scale models (Wang & Nackenhorst, 2020). Macroscopic continuum models are based on homogenization

FIGURE 5 The bio-chemo-hydro-mechanical (BCHM) mechanisms and their couplings (modified after Fauriel & Laloui, 2012)

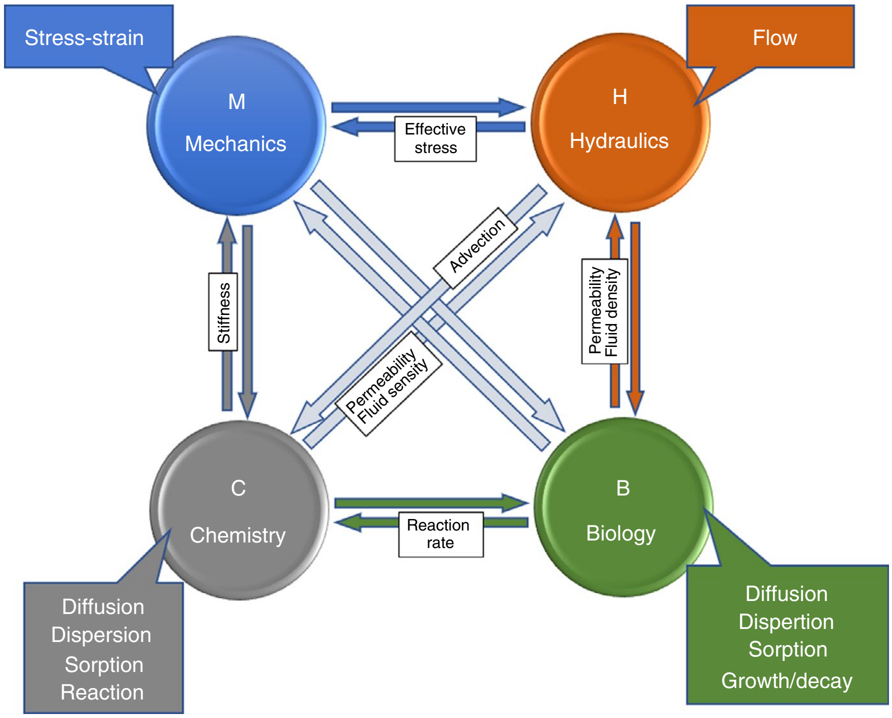
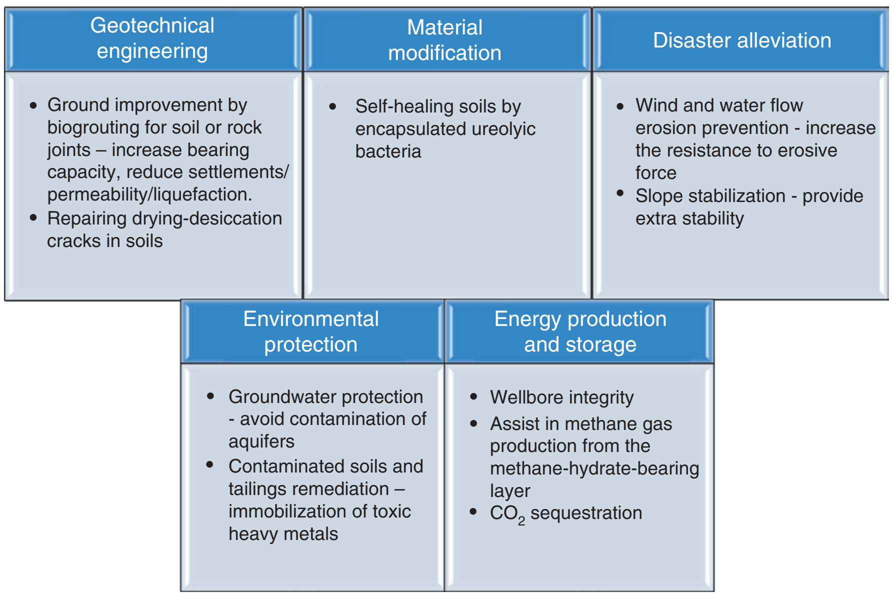


FIGURE 6 The potential applications of MICP in various fields



techniques and have been shown to have advantages in large-scale MICP applications (Cunningham et al., 2018; Fauriel & Laloui, 2012; Hommel et al., 2015). The aqueous biochemical components are assumed to be mixed completely, and the impacts of the local incomplete mixing of reactants at the pore throat are not covered. Pore-scale models focus more on the local heterogeneity; thus, the variations in porosity and permeability could be considered (Qin et al., 2016; Wang & Nackenhorst, 2020).

Various methods have been established on the mechanical aspect to predict the element-scale or pilot-scale properties such as the distribution of calcite precipitation, permeability and porosity in the solid phase. Zamani and Montoya (2016) conducted simulations in a *Seep/W* program based on the finite-element method to detect the effect of cementation level change on permeability. Feng et al. (2017) conducted three-dimensional DEM simulations using PFC^{3D}. The stiffnesses, shear behaviours, bond breakage and average void ratio were analysed. Yang et al. (2017) developed a five-parameter DEM model to simulate the behaviour of MICP-treated sands. The peak and residual friction angles predicted by the simulations were very comparable to the experimental results. So far, large-scale numerical modelling is still limited.

In general, numerical modelling is a complex system. Future work is still needed to obtain: (1) a better understating of interactions among coupled bio-chemo-hydro-mechanical processes especially during the bio-stimulation treatment method; (2) the unified rational assumptions regardless of MICP treatment procedures; (3) the more accurate inputs of various modelling parameters for numerical prediction.

In summary, the development of reliable and convenient characterization techniques at various scales is critical for the understanding and implementing of the MICP process. The nano-, micro- and meso-scale assays help to understand the fundamental mechanisms of MICP. Element-scale tests can provide available methodologies for the mechanical behaviours in the laboratory. In order to apply MICP at a large scale, pilot- and field-scale characterizations are indispensable. Moreover, reliable numerical models are beneficial for understanding fundamental mechanisms and the prediction of engineering behaviour of bio-cemented soils. Currently, most characterization techniques at nano-, micro-, element-scales are carried out in the laboratory. However, with further scaling up, there are more challenges during different stages including test setup and preparation, fluid injection, bio-grouting monitoring and efficiency assessment.

5 | PROMISING APPLICATIONS OF MICP

Since the MICP process was firstly explored for its applications in geotechnical engineering, researchers keep exploring new fields that can utilize MICP. While substantial effort is still put into applications in geotechnical engineering such as ground improvement and liquefaction control, there are an increasing number of studies now focusing on other fields, particularly material modification, disaster alleviation, environmental protection and energy production/storage (as presented in Figure 6). In this section, recent developments in MICP applications in both geotechnical engineering and other fields are reviewed. The existing studies are crucial to provide some insights on this promising technique and potentially help broaden the horizon of MICP applications.

5.1 | Emerging applications in geotechnical engineering

In recent years, the application of MICP in geotechnical engineering has expanded out of traditional areas such as soil improvement. One such promising application is bio-grouting for soil or rock joints. Studies on the use of bio-grouting for soil and rock joints have been carried out by Chu (2012). The results have shown that bio-grouting effectively reduces the seepage in soil and rock joints and increases the shear strength of sand (Pan et al., 2020; Wu & Chu, 2020; Wu et al., 2019). However, bio-grouting using the conventional MICP method does have one disadvantage. When the method is used for coarse sand or rock joints with relatively big openings, the number of treatments required to generate sufficient $CaCO_3$ to occupy the pores in order to reduce the permeability is excessive. An innovative way to overcome this problem was the use of bio-grouting containing bio-slurry (Cheng et al., 2019). By adjusting the solid content in the bio-slurry, this so-called unified bio-grouting method (Pan et al., 2020) can be used for fine to coarse sand or rock joints of different sizes of the opening.

Another promising application is repairing drying-induced cracks in the soil. Drying-induced desiccation cracking of soils is a common natural phenomenon, considerably degrading soil mechanical and hydraulic properties. In recent years, Liu, Zhu, et al. (2020a) applied MICP to remediate desiccation cracking in clayey soils. They found that soils treated with MICP present the highest resistance to cracking (Figure 7). Geometrical parameters featuring the crack pattern such as surface crack ratio, average crack width, total crack length, crack width distribution range and the most probable value of crack width decrease significantly with the increasing MICP treatment cycles. Guo et al. (2018) conducted MICP treatment to repair desiccation cracks that occurred in bentonite. It was found that the MICP-treated samples had larger polygons and smoother surface than the untreated ones after they were totally dried. Liu et al. (2020a) investigated the crack repair in tabia (a traditional artificial Chinese soil which mainly consists of clay, sand and lime) by MICP. The results indicated that the peak recovery rate of flexural and shear strength reached up to 79.92% and 88.54% when the crack width was 5 mm. Wider cracks could lead to a decrease in repair efficiency.

5.2 | Material modification

While soils, especially coarse-grained ones, are the primary targets of MICP treatment, more and more efforts are being put on the application of MICP on a variety of other materials. The concept of self-healing of materials using MICP has become popular recently. Harbottle et al. (2014) demonstrated the potential of self-healing of sand by MICP to respond to damage by vane shearing the specimens. The results showed a substantial strength increase of 300%–400% after the initial healing stage. Botusharova et al. (2020) used a spore-forming strain (*Sporosarcina ureae*) for the self-healing procedures in sand. A conceptual process of utilizing sporulated bacteria in porous media was proposed (shown in Figure 8). Self-healing via the ureolysis-based MICP process has also been applied in concrete (Alazhari et al., 2018; Jongvivatsakul et al., 2019; Xu & Wang, 2018; Zhang et al., 2019), which can help reduce the high maintenance costs of concrete in a relatively eco-friendly way. Although most ureolytic bacteria are alkali-tolerant species, which can, to a certain extent, maintain the enzymatic activity in a harsh environment in concrete (pH up to 12–13),

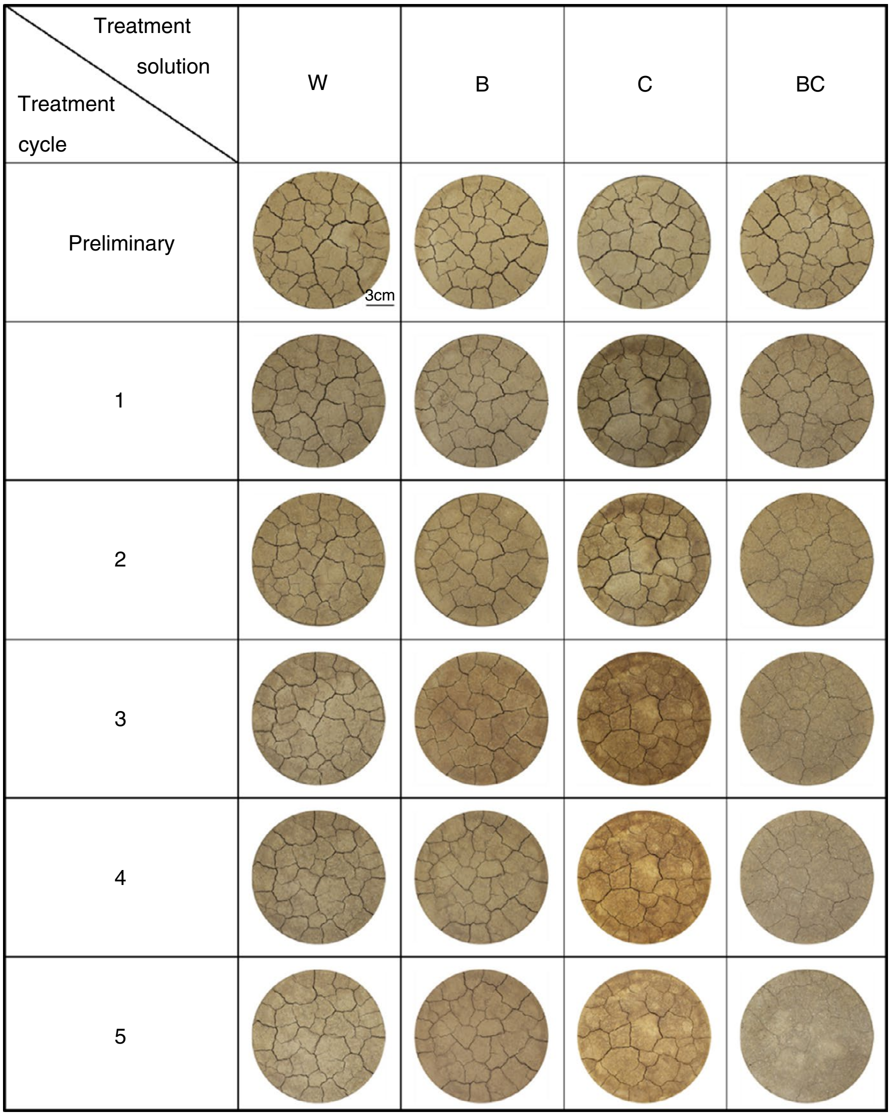


FIGURE 7 The spatiotemporal evolutions of soil crack patterns after different treatment cycles. W—treated with deionized water; B—treated with bacteria solution; C—treated with cementation solution; BC—treated with both bacteria and cementation solutions (Liu et al., 2020a)

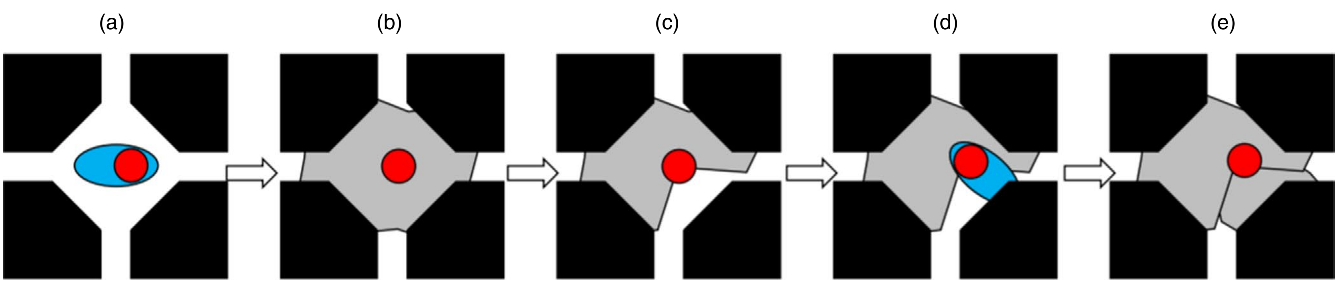


FIGURE 8 Concept of bacterial self-healing in porous media: (a) sporulating bacteria capable of biomineralization present in the pore space; (b) bacteria produce mineral products, entombing themselves with spores surviving; (c) deterioration of the mineral exposes spores; (d) germination of new cells form spores; and (e) further mineral formation caused by new cells, entombing themselves in the process once more (Botusharova et al., 2020)

encapsulation technique is necessary to further increase the survival of bacteria. Nevertheless, Lee et al. (2019) pointed out that ensuring the survival of bacteria is still the biggest challenge of self-healing concrete. Furthermore, the released by-product ammonia along with ureolysis process is highly undesirable for the people who live in the buildings with such self-healing concrete.

5.3 | Disaster mitigation

Microbial-induced calcium carbonate precipitation techniques have also been applied to mitigate the impacts of natural disasters such as coastal erosion, wind erosion in desert areas and landslides (Chu, 2013; Chu et al., 2015). Coastal erosion is mostly caused by storm wave attacks and long-term sea-level rise, which lead to the loss of beach sand dunes and the erosion process related to coastal sediments. The traditional hard structures are expensive and not eco-friendly. MICP-based foreshore sandy slope stabilization is a type of soft structural protection method which has gained increasing interest. Both bio-augmentation and bio-stimulation have been investigated in the laboratory for sandy slope stabilization (Gowthaman et al., 2019; Imran et al., 2019; Kou et al., 2020; Liu et al., 2020b; Salifu et al., 2016; Shahin et al., 2020). However, considering that human activities are sometimes intensive in the beach zones, the issues such as by-product treatment and durability should be addressed carefully before application in beaches. Alternatively, the creation of artificial beachrocks using natural materials (e.g. microbes, sand, shell, pieces of coral and seaweed) within a short time by MICP method was a milder approach (Daryono et al., 2020; Imran et al., 2019).

Wind erosion is a common problem occurring in arid regions. Fattahi et al. (2020) developed an element-scale cube sand box with a dimension of 10 × 10 × 10 cm. A uniform crust was formed and able to provide considerable protection for aeolian sand against erosion by airflow at different velocities. Meng et al. (2021) performed a field-scale test to investigate the efficiency of reducing wind erosion via MICP. Under the optimal condition, the thickness of the soil crust reached up to 12.5 mm and the bearing capacity exceeded 300 kPa. So far, the high cost is the major challenge for the use of MICP in desert areas mainly because of logistics and harsh environments (Meng et al., 2021).

Microbial-induced calcium carbonate precipitation also shows potential for slope stabilization. In order to prevent landslides, it is important to immobilize the ureolytic bacteria within the target surface zones. However, Gowthaman et al. (2019) suggested that well-immobilized bacteria could only exist within the surface zone based on their experimental observations. Factors such as the particle size distribution may limit the treatment in deeper locations. Therefore, it is recommended that the MICP technique is used to enhance the surface cover condition or prevent the instability of surface slopes based on the current knowledge and implementation method.

5.4 | Environmental protection

Heavy metal contamination in soil and groundwater has been a threat to the ecosystem and human health because of (1) the long-term accumulation in many phases (i.e. air, solid, fluid) and (2) pollution from industrial activities (Jiang et al., 2019; Xia et al., 2019). In recent years, with the development and increasing knowledge of MICP techniques, ureolysis-based MICP has been proven to be an effective approach to stabilize soils contaminated with heavy metals.

Various heavy metals including lead (Pb), cadmium (Cd), zinc (Zn), copper (Cu), mercury (Hg), cobalt (Co), strontium (Sr), barium (Ba), iron (Fe) and nickel (Ni) have been investigated for immobilization efficiency by either bio-stimulated or bio-augmented MICP processes. For example, Kang et al. (2016a) found that treatment by four isolated bacterial co-mixtures was more effective in removing mixtures of Pb, Cd and Cu in soil. Chen et al. (2019) observed that various bio-stimulated ureolytic bacteria could facilitate Cu immobilization by accelerating MICP process. It was proved that the immobilized Cu was mainly in the form of Cu carbonates. Jiang et al. (2019) applied bio-augmented MICP to immobilize Pb in aqueous conditions. The results showed that *S. pasteurii* exhibited compatible resistance to Pb toxicity. The immobilization of Pb might follow a specific sequence, and a hypothesized multi-layer precipitation structure was proposed. In order to investigate the mechanism of ureolytic bacteria in immobilizing heavy metals, Kang and So (2016) isolated six ureolytic bacteria from an abandoned mine. The maximum tolerance concentrations of isolated strains were tested for various heavy metals (Co, Cu, Fe, Cd, Ba, Pb, Sr and Zn). It was found that the heavy metal resistance of these isolates was closely associated with their resistance to antibiotics. Zhao et al. (2017) utilized *Bacillus* sp. isolated from a mine soil to immobilize Cd. Both the efficiency of biosorption and MICP were analysed. The results indicated that MICP had a greater potential to remove Cd than biosorption process under different factors (initial pH, Cd concentration, contact time). Based on existing studies, the main mechanism of the immobilization process via MICP lies in the co-precipitation of heavy metals in $CaCO_3$ or entering the interstice or defect of the crystal. The heavy metal ions are adsorbed to the bacterial cell wall as a result of its negative charge, thus resulting in the formation of crystals on the surface of the bacterial cell. Meanwhile, adsorption and redox reactions usually accompany co-precipitation.

It is worth mentioning that co-contaminants often complicate bacterial reactions. While many bacteria possess the potential for biotreatment of various contaminants in laboratory conditions, their survival and enzymatic activity under a real natural environment are vital for field implementation (Rahman et al., 2020). Thus, further research on the impacts of various inhibiting and promoting physio-chemical factors on the capacity and capability of MICP for heavy metal immobilization is needed. Moreover, though the strong absorption of heavy metals on the surface and inside the lattice of calcite occurs, they may possibly suffer under unexpected adverse environmental conditions such as the sudden variation of temperature, pH or microbial population. Thus, the durability of the formed co-precipitation needs to be further studied.

5.5 | Energy production and storage

Recently, the MICP technique has been developed to assist energy resources production procedures. During the production and extraction of some energy resources such as petroleum and methane hydrate, the disturbance and strength loss of ambient soils or facilities such as wellbores may significantly affect the efficiency of energy production. MICP, as an environmental-friendly approach, can be applied to stabilize the weak soil in these areas. For instance, Phillips et al. (2018) applied MICP in enhancing wellbore cement integrity in the field. The reduced injectivity, reduced pressure fall-off and increased solids showed a successful sealing effect of MICP. Hata et al. (2020) evaluated the feasibility of MICP for ground improvement in the deep ocean using native bacteria. The urease-producing bacteria (i.e. *Sporosarcina newyorkensis*) were isolated from the depressurized core sample in the methane hydrate-bearing zone. It was found that deep-ocean microbes can still survive in the methane hydrate stable area. The bonding and clogging effects of induced bio-cementation had the potential in preventing sand and water production. In addition, Okyay and Rodrigues (2015) and Okyay et al. (2016) investigated the potential of MICP in CO_2 sequestration to reduce atmospheric CO_2 levels. The results confirm that CO_2 can be removed from the atmosphere through two possible mechanisms: (a) sequestration by MICP biotically and (2) sequestration by increasing the environment pH (i.e. CO_2 solubility) abiotically.

6 | SUMMARY

Bio-mediated geotechnics is viewed as the "next big thing" in geotechnical engineering and has great potential to advance current soil improvement practices. In this paper, recent developments in the understanding of MICP processes, materials involved, characterization methods and emerging applications were comprehensively reviewed. The key points are summarized as follow:

1. Urea hydrolysis is the most popular MICP approach because of the simplicity of the process. It has the best efficiency towards the formation of bio-cementation. Bio-augmentation and bio-stimulation are the two approaches used for MICP. Bio-augmentation yields higher reaction rates and initial ureolytic activity than bio-stimulation, while bio-stimulation can overcome drawbacks of bio-augmentation including higher cost, unpredicted environmental risks and laborious work.
2. The materials involved in the MICP process, namely bacterial strains, culture and cementation solutions, auxiliary additives and soils, are the factors that define the success of MICP treatment. Among them, bacterial strains promote the nucleation and crystallization by creating supersaturated alkaline environments and secreting extracellular polymeric materials. For the bacterial culture and enrichment media, urea is not necessarily the only substrate that can induce high urease activity. In order to reduce the cost, alternative industrial by-products or wastes and non-sterilized media can be used. Cementation solution plays an important role in forming variable morphologies and sizes of $CaCO_3$ crystals. Several inexpensive Ca/urea sources have been proposed as alternatives for cementation solution. The auxiliary additives added either in the culture/enrichment/cementation solutions or directly into soil help to improve bacterial growth, urease activity and bio-cementation content. More and more successful attempts have been made to implement MICP in soils that traditionally are regarded unsuitable for MICP treatment (i.e. clayey soil and loess).
3. The MICP process needs to be characterized using observational and experimental tools from multiple disciplines and at multiple scales. Nano-, micro- and meso-level apparatus can evaluate the crystal shape, size, morphology, distribution pattern, structure, contact and surface fractures of bio-cemented soils. Element-scale characterization techniques are used to assess the improvement of engineering properties, such as permeability, unconfined compressive strength, shear behaviour, particle breakage and compressibility behaviour, cementation content, thermal conductivity and durability. With scaling up to pilot- and field-scale, there are more challenges during different stages including test setup and preparation, fluid injection, bio-grouting monitoring and effectiveness assessment. Numerical modelling is a useful tool to virtually assess the performance of MICP where complex coupled bio-geochemical processes are involved.
4. The application of MICP in geotechnical engineering has been greatly expanded in recent years. Bio-grouting for soil or rock joints and repairing drying-induced cracks in soil are two emerging applications in geotechnical engineering. In addition, MICP has also been trialled in new areas, such as material modification (self-healing of materials), disaster alleviation (coastal erosion, wind erosion and landslide mitigation), environmental protection (contaminant immobilization), and energy production and storage (maintaining wellbore integrity).
5. In a word, with the recent development of MICP technique, there are numerous innovations in methods and its applications, which provides us an alternative way to deal with various soil problems. While MICP has already shown its great potential in weak soil improvement and contaminated soil remediation, its interdisciplinary nature will definitely offer more possibilities in the field of biogeotechnology.

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DECLARATION OF INTEREST

None.

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