



Microbially induced carbonate precipitation (MICP) for soil strengthening: A comprehensive review

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ARTICLE INFO

Keywords:

Biogeotechnics
Microbially induced carbonate precipitation
Bio-cementation
Soil strengthening

ABSTRACT

Geotechnical research has been yearning for revolutionary innovations that could bring breakthroughs to conventional practices, especially at a time when energy efficiency and environmental sustainability are of unprecedented importance in the field. Recently, exciting opportunities emerged utilising microorganisms, the ubiquitous soil dwellers, to provide solutions to many geotechnical problems, prompting the development of the new, multidisciplinary subject of biogeotechnics. Research interest has been centred on the use of microbially induced carbonate precipitation (MICP) to improve the engineering properties of soils. The present work aims to comprehensively review the progress of more than a decade of research on the application of MICP in soil strengthening. Through elucidation of underlying mechanisms, compilation and interpretation of experimental findings, and in-depth discussion on pivotal aspects, with reference made to key published studies, a holistic picture of the state of the art of MICP-based soil strengthening is drawn. Current knowledge gaps are identified, and suggestions for future research are given, along with the opportunities and challenges that lie ahead of practically implementing this technique in real-world geotechnical applications.

1. Introduction

Soil plays a fundamental role in modern construction, constituting the foundation of civil infrastructure systems (e.g., buildings, bridges, roads, dams, etc.). However, the mechanical performance of soils can be inadequate for civil engineering applications. Thus, soil strengthening to artificially improve the strength properties of soils is commonly practiced in geotechnical engineering, with over 40,000 projects performed globally at an annual cost exceeding 6 billion dollars [59]. Owing to this significance, it is incumbent on geotechnical engineers in both academia and industry to seek better solutions, and thus there has been a wealth of research on soil strengthening with various techniques developed and implemented [113,123,214].

Conventionally, soil can be strengthened by mechanical means (e.g., compaction, pre-loading, vibration, pre-wetting, drainage, etc.) or by using chemical and synthetic additives (e.g., cement, lime, fly ash, industrial wastes, organic compounds, geosynthetics, etc.) [113,123,203,214,245,279].

Despite their proved efficacy, these conventional methods are being increasingly scrutinised due to their high cost and energy consumption as well as adverse impacts on the environment (e.g., releasing toxic substances and emitting carbon dioxide) [114,151,288,59]. It is thus imperative to develop novel techniques that, in addition to effectively strengthening the soil, are energy efficient, economically feasible and environmentally sustainable.

The tremendous advances in scientific research over the past century have led engineers to realise that nature may have already provided ideal solutions to many engineering problems. Various technologies inspired by nature have been developed to tackle engineering challenges, such as butterfly-inspired solar cells and photocatalysts [149], hierarchical nanomaterials for sensing and energy storage [302], root-inspired anchorage and foundation systems [162], and many others. One inspiration coming from microbiology and biochemistry is the potential of employing microorganisms as the '21st-century engineers' [60]. By moderating complex bio-chemo-physical interactions, microorganisms generate a wide range of substances that have

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engineering implications. Bio-mineralisation is one such process by which minerals are formed with biological involvement [130,15,150,204]. Early observations of bio-mineralisation date back to the 1970 s (e.g., [17,132,150,212,71]; among others), and, to date, more than 60 minerals have been identified that can be produced by bio-mineralisation [12,219]. Among all bio-mineralisation processes investigated, microbially induced carbonate precipitation (MICP), which harnesses microbial metabolites to promote the precipitation of calcium carbonate (CaCO_3) minerals, offers high process efficiency and versatility as well as low energy requirements and environmental impacts, and is widely regarded promising for engineering applications [114,115,2,54,59,60].

The use of MICP in soil systems is of particular interest to geotechnical engineers, as the minerals produced are capable of altering the physical, chemical and mechanical properties of the soil [120]. This has opened up new horizons for research and innovations and has become the primary focus of the fast-developing sub-discipline – biogeotechnics [60]. MICP was initially applied to soil for enhanced oil recovery (EOR) in the 1990 s with the precipitated CaCO_3 serving as the plugging agent [122,78,79,93]. Since an Australian research group successfully turned a bag of sand into sandstone columns using MICP in 2001 [133,258], this technique has gained great research momentum in the field of soil improvement worldwide. To date, MICP has been used for a wide range of soil improvement purposes, including but not limited to remediation of contaminated soil [139,4,5], enhancement of soil thermal conductivity [163,270,296,298], soil bio-clogging for permeability reduction and leakage control [237,41,49], liquefaction mitigation [177,208,293], erosion control [118,215,316,44], fugitive dust suppression [167,184], and soil strengthening [10,176,265,31,32,56].

Typically, MICP treatment of a soil is performed by adding to it bacteria and chemical reagents. The bacteria, through their metabolism, excrete specialised enzymes that interact with the chemical reagents and form a biochemical reaction network that favours the precipitation of CaCO_3 minerals, which subsequently alter the soil properties. The term bio-augmentation is used when the MICP treatment employs pre-cultured exogenous bacteria [205,256,95]. While this continues to be the preferred approach [120], other treatment approaches also emerged and are gaining research interest. For example, bio-stimulation, which refers to modifying the soil environment (e.g., providing nutrients, altering pH, etc.) to stimulate and enrich indigenous bacterial communities [216,22,23,256,86,94–96]. Extracted enzymes, instead of whole bacterial cells, have also been found capable of precipitating CaCO_3 minerals in soils, which is termed enzymatically induced carbonate precipitation (EICP) [103,189–191,202,269,307,7]. The principal difference between these approaches is the microbial source utilised. Each approach has its own merits and shortcomings, and the selection is case-specific [58]. It should be noted that the present paper exclusively focuses on the first approach (i.e., bio-augmentation using active bacteria).

More than a decade of research on MICP has yielded remarkable insight. A number of review papers have been published to summarise the research progress (e.g., [114,59,183,279,247,245,35,120]; among others). These review papers provide an overview of the use of MICP in geotechnical engineering, epitomising the fundamentals of MICP, its advantages and limitations, the characterisation of the ensuing materials and modelling methods for simulating the bio-chemo-physical interactions involved, as well as the wide range of potential applications. Rather than reviewing MICP from a general perspective, the present work exclusively focuses on its application in soil strengthening and, through a comprehensive compilation and interpretation of up-to-date findings, draws a holistic picture of the state-of-the-art of MICP for soil strengthening (readers interested in other application scenarios and numerical modelling of MICP are referred to the review papers listed above and the references therein). By discussing pivotal points in depth, comments and suggestions on current research gaps and potential future steps are provided.

2. MICP in soil environment

As described above, MICP-based soil strengthening refers to the construction of a biochemical reaction network within the soil using bacteria and chemical reagents, which subsequently produces CaCO_3 that strengthens the soil matrix. Therefore, two fundamental questions need to be answered: (i) how the bacteria and reagents mediate the reactions; and (ii) how the produced CaCO_3 creates the strengthening effect. This section will answer both questions by elucidating several key mechanisms of MICP in the soil environment.

2.1. Bacterial transport and adsorption in soil

Bacteria and reagents are normally introduced into soil in aqueous solutions. When added to soil, bacteria will undergo two processes simultaneously: bacterial transport in soil pores through advection and diffusion, and bacterial adsorption onto soil particles. A number of factors can influence these two processes, including the geometry of soil pores [1,171,254,56], features of bacterial cells such as shape, surface charge, hydrophobicity and appendages [117,261,40,90], characteristics of soil particles such as surface roughness and mineralogy [107,220,81], and properties of pore fluids such as temperature, chemistry and flow regime [1,109,171,181,204,238,254].

Bacterial transport is primarily governed by the geometric compatibility between bacterial cells and soil pore throats [56,59]. Bacterial cells are known to have diameters ranging from 0.5 to 3 μm , which enables them to travel freely in soils with relatively large pores (e.g., sands), while their movement is likely to be inhibited in fine soils with pore throats smaller than the cell size [171,207,233]. According to DeJong et al. [59], when assessing the feasibility of a soil for MICP treatment via injection, 20% of the effective particle size (D_{10}), which is an estimate of the size of the pore throat, can be used as a lower bound limit. Updegraff [259] also suggested that the physical blocking of bacterial movement becomes more pronounced when the cell size is greater than the D_5 size of 5% of the soil.

The adsorption of bacteria onto soil particles can be explained by the Derjaguin-Landau-Verwey-Overbeek (DLVO) theory, originally developed by Derjaguin and Landau [61] and Verwey and Overbeek [271]. It suggests that bacterial adsorption is a result of the combined effect of the attractive van der Waals force and the repulsive electrostatic force, whose balance enables bacterial cells to be fixed onto soil particles at a short distance (a few nanometres) [110,160,165,220,238,262]. The attraction between a soil particle and a bacterial cell is assumed to exist over a short distance ($< 1 \text{ nm}$), namely the primary minimum, and a larger distance (5–10 nm), namely the secondary minimum. Between them is a zone of maximum electrostatic repulsion [160,238]. The adsorption process has two steps, reversible adsorption, which occurs when the bacterial cell is located in the secondary minimum and overcomes the secondary repulsive force, and irreversible adsorption, which occurs when bacterial polymers form bridges between the cell and the soil particle [102,159,263]. The reversible adsorption is weak and easy to break, while the irreversible adsorption is permanent with a large amount of energy involved [238]. Reducing the size of soil particles and increasing their surface roughness can respectively result in more available adsorption sites and smaller shear forces, enhancing bacterial adsorption [238,244,80]. Changes in the properties of pore fluids, such as elevated temperature, increased ionic strength and reduced flow velocity, can also aid bacterial adsorption [109,112,160,195,220,228].

2.2. Bio-chemical reaction network

In abiotic environments, the rate of CaCO_3 precipitation could be extremely low due to the presence of an energy barrier for crystal nucleation [15,55]. In MICP, bacteria can help undermine this energy barrier and catalyse the reaction by a factor of 10^{20} by excreting specialised enzymes [171].

MICP can be achieved through a number of pathways. Castanier et al. [27] categorised these pathways into two main groups, autotrophic and heterotrophic, based on the nutrition source used. Non-methylotrophic methanogenesis, anoxygenic photosynthesis and oxygenic photosynthesis belong to autotrophic pathways, which use dissolved carbon dioxide as the carbon source, whilst heterotrophic pathways include dissimilatory reduction of sulphates, ammonification of amino acids, denitrification and hydrolysis of urea [105,27,59]. Detailed descriptions of these pathways and their comparisons can be found in Zhu and Dittrich [315], Seifan and Berenjian [222], Jiang et al. [120], and Bu et al. [21]. Among these pathways, hydrolysis of urea (also referred to as ureolysis) is considered to be the most straight-forward, productive, controllable and energy-efficient, making it the most booming research area of MICP [130,205,245,279,309,59,64]. The enzyme involved in ureolysis, urease (urea amidohydrolases, a nickel-dependent metalloenzyme), is widely found in microorganisms [104,14,204], of which *Sporosarcina pasteurii* (*S. pasteurii*, formerly classified as *Bacillus pasteurii*), a non-pathogenic, gram-positive, alkalophilic and halophilic strain, has been the most commonly employed due to its high urease activity and tolerance to harsh conditions [145,153,247,44,56,79].

Ureolysis-driven MICP contains six interdependent steps. First, urease excreted by bacteria catalyses the hydrolysis of urea into ammonia and carbamic acid (Step 1). The latter spontaneously hydrolyses to produce carbon dioxide and additional ammonia (Step 2). The carbon dioxide equilibrates with water into carbonic acid, bicarbonate ions and carbonate ions in a pH-dependent manner (Step 3). The ammonia also equilibrates with water, forming ammonium and hydroxide ions (Step 4), which increases the pH of the environment and shifts the bicarbonate equilibrium into the formation of excessive carbonate ions (Step 5). In the presence of a soluble calcium source, CaCO_3 precipitation is favoured in the created alkaline environment (Step 6) [104,239,27,294,59]. In addition to providing urease, another observed role of bacteria is acting as nucleation sites for crystallisation [206,286,56,72,89,9,93]. This has been attributed to the high surface-to-volume ratio of the bacterial cell and the functional groups (e.g., carboxyl, phosphate and amine) on the cell surface. These functional groups enable the cell to be negatively charged, prompting the attachment of calcium ions and thereby favouring crystal nucleation [104,221,315,67,68,72]. A schematic of the bio-chemical reaction network in ureolysis-driven MICP is shown in Fig. 1.

2.3. Strengthening mechanisms

Bio-cementation is the most used term to describe the strengthening mechanism, and MICP-strengthened soils are commonly called bio-cemented soils. Bio-cementation refers to the preferential deposition of CaCO_3 crystals at soil inter-particle contacts (crystals circled by red line in Fig. 1) forming cementing bonds between the particles [114]. This pattern of selective cementation differs from conventional soil cementation techniques in which soil pores are filled with the cementing agent in a uniform manner. This preferential deposition of CaCO_3 crystals at soil inter-particle contacts results from the combination of bacterial adsorption and crystal filtering [59]. Bacteria have a general tendency to stay away from the exposed surfaces of soil particles and remain at inter-particle contacts where flow-induced shear stress is lower and nutrients are more readily available. This results in a higher bacterial concentration at these contact points, leading to more CaCO_3 crystals being precipitated and able to form cementing bonds. Meanwhile, crystals precipitated in soil pores or detached from soil particles tend to be filtered by pore throats and accumulate at inter-particle contacts as the pore fluid flows. These crystals may get re-attached as they grow in size and contribute to cementation [279,51,59]. The formed cementing bonds create an optimised structural configuration and improve the strength and stiffness properties of the soil.

CaCO_3 crystals coating soil particles (crystals circled by blue line in Fig. 1) and suspended in soil pores (crystals circled by yellow line in

Fig. 1), which are not involved in forming cementation, may still contribute to strengthening the soil. These crystals can increase the roughness of the soil particles and act as extra fines that densify the soil, enhancing the frictional capacity and inter-locking of the soil particles and thereby causing strength improvement [310,56,59,85]. On one hand, the roughening and densification effects are considered to be less efficient than bio-cementation in improving peak strength [146,275,28,47,51,59], while, on the other hand, they tend to remain effective at the residual state when soil cohesion has vanished and strength is predominantly determined by frictional resistance [287].

3. Characteristics of bio-cemented soils

Numerous studies have characterised bio-cemented soils using various techniques and testing methods at multiple scales. The observed properties and behavioural changes, their significance and implications, and how macro responses can be linked to micro characteristics are discussed in this section.

3.1. Strength

For soil strengthening techniques, the strength of the treated soil is undoubtedly a direct measure of strengthening efficacy. The unconfined compression test has been the most employed strength test for bio-cemented soils. Fig. 2a plots the unconfined compressive strength (UCS) values reported in literature works against the corresponding mass contents of precipitated CaCO_3 (hereinafter referred to as C_{cc}), which is normally used to denote the level of cementation achieved. It can be seen that, with C_{cc} ranging from below 1% to over 35%, UCS varies from below 50 kPa to over 18 MPa. This shows the effectiveness of MICP-based soil strengthening and its versatility of enabling varying degrees of strength improvement to cater for different applications [247]. The increase in UCS with C_{cc} is nonlinear, being slow when C_{cc} is low and significantly accelerating at higher C_{cc} , a phenomenon well captured in many previous studies (e.g., [265,31,146]). This non-linearity is intimately linked to the microstructural evolution at the particle scale. As described in Section 2.3, the mechanism by which soil strength is improved depends on the location of CaCO_3 crystals with respect to soil particles. Cementation at inter-particle contacts is more effective than roughening and densification. When C_{cc} is low, the volume of CaCO_3 crystals at inter-particle contacts is small, and the number of cemented contacts is few. Even for these cemented contacts, the formed cementing bonds are weak and easy to break, and failure can bypass the few strong contacts. A robust network of cemented soil particles has not established. Strength gain at this stage is obscure and probably more provided by the roughening and densification mechanisms. With C_{cc} further increasing and more crystals accumulated, cementing bonds grow in number and size, and cementation becomes the dominating strengthening mechanism, manifested by the faster strength gain.

Mathematically correlating the UCS and C_{cc} of bio-cemented soils has been a focus of research, as it enables the level of strength improvement to be predicted and manipulated, which is crucial for engineering design. However, the correlations reported in different works are diverse, including linear [233,314,40], polynomial [69,251], and exponential ones [146,182,205,31]. Indeed, the massive data discrepancy in Fig. 2a shows how difficult and unreliable it is to describe the relation with a single trendline. This is because the MICP process itself involves many variables (e.g., types and concentrations of bacteria and reagents), which, together with the variety of intrinsic soil characteristics and differences in treatment schemes and environments, create a complex network of influencing factors that affect the final result [11,245,258]. A detailed discussion on these factors and how they affect MICP-based soil strengthening is presented in Section 4. Given this great number of influencing factors, it seems inappropriate to use C_{cc} as the sole measure to denote the behaviour of bio-cemented

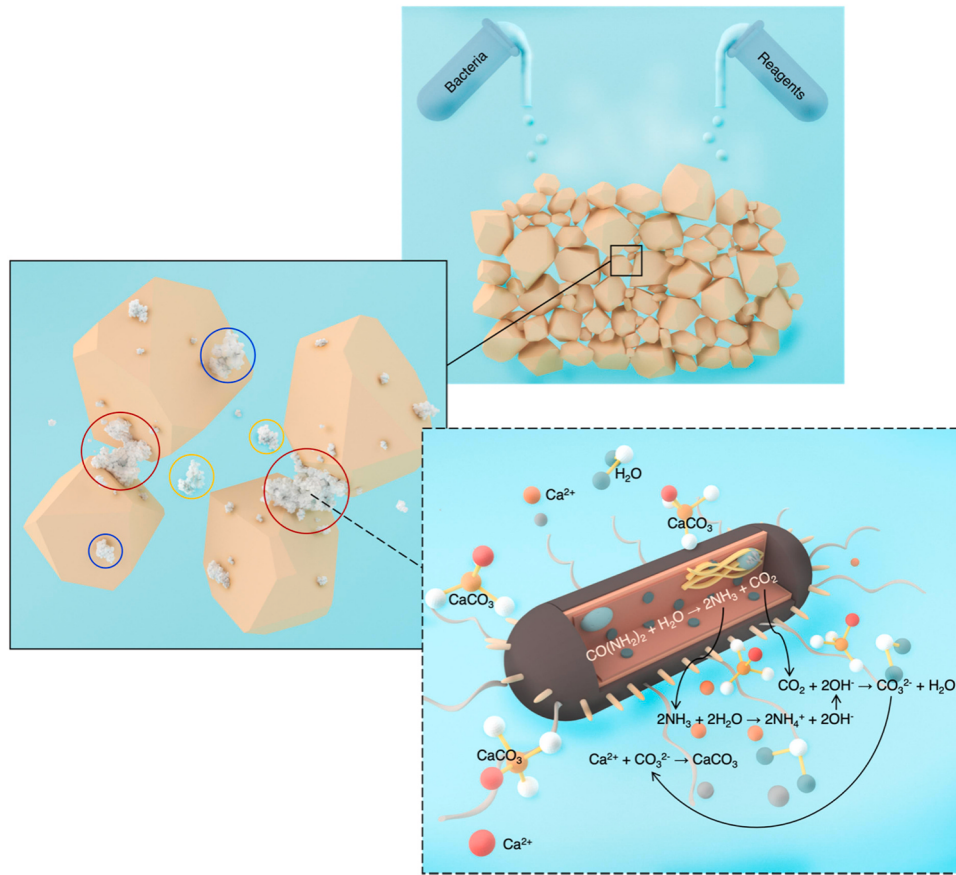


Fig. 1. Schematic of MICP-based soil strengthening via ureolysis.

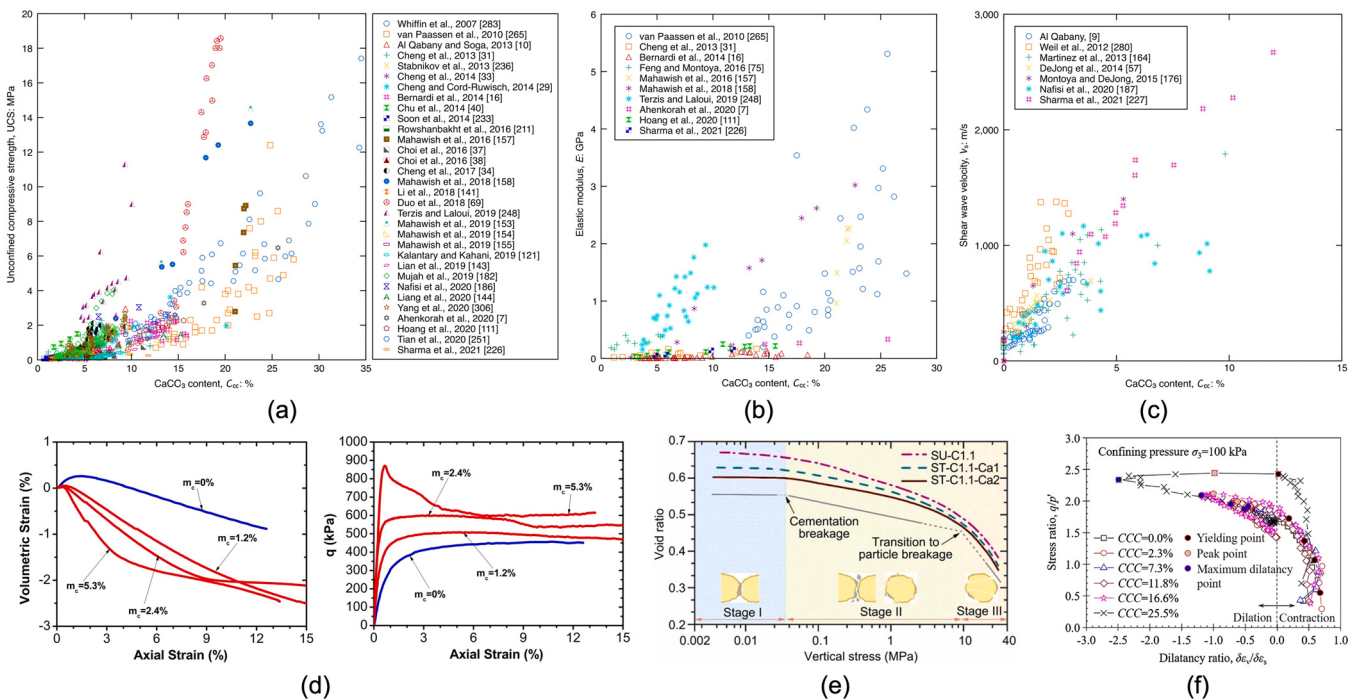


Fig. 2. Characteristics of bio-cemented soils: variation of **a** UCS, **b** E , and **c** V_s with C_{cc} ; **d** shear responses of bio-cemented sand under drained triaxial compression (m_c equals to C_{cc} used herein) [75]; **e** compression behaviour of bio-cemented sand (SU-C1.1 refers to untreated specimen, and ST-C1.1-Ca1 and ST-C1.1-Ca2 refer to specimens treated with reagent concentrations of 1 and 2 mol/L, respectively) [301]; and **f** stress-dilatancy relationship of bio-cemented sand (CCC equals to C_{cc} used herein) [48].

soils, as also pointed out by Terzis and Laloui [247] and Clarà Saracho et al. [44]. At a same C_{cc} , bio-cemented soil samples produced under different experimental settings could have distinctly different properties [276]. At the moment, to come up with some form of metric that takes into account all the influencing factors seems intimidating, if not impossible. It therefore becomes vital to conduct preliminary investigation for any application of MICP-based soil strengthening so that case-specific information can be obtained to provide accurate reference and improve controllability.

For a bio-cemented soil specimen to be tested for UCS, it has to gain enough self-integrity to stand alone without confinement, which requires a minimum C_{cc} of circa 3% to achieve [120,247]. Given this fact, high C_{cc} values (over 10%) are needed in order to capture the trend of strength improvement over a range of cementation levels, which normally lead to UCS values in the order of megapascals (Fig. 2). However, many geotechnical applications may not require turning the soil into a rock-like material. In fact, when the role of confinement is accounted for, C_{cc} values of less than 2% can bring the required strength improvement in most cases [193,247].

Triaxial compression tests and direct shear tests have been used to study the shear behaviour of bio-cemented soils under confined conditions. Generally, the test results show (i) increased peak strength and stress ratio (i.e., the ratio of deviator stress to mean effective stress), (ii) enhanced initial stiffness, (iii) a transition from strain hardening to strain softening, (iv) marginally increased residual strength, and (v) aggravated strain localisation, with increasing C_{cc} and decreasing confining pressures, as shown in Fig. 2d as an example [101,145,176,187,193,227,299,300,310,39,56,75,85]. These responses are, again, related to the microstructural changes that occur during the different stages of loading. Cementation formed by CaCO_3 crystals enables soil particles as a whole to attain some form of structure, analogous to the so-called ‘bonded structure’ used for other naturally and artificially cemented soils [138,278,46]. This structure makes the soil matrix much stronger and stiffer than the uncemented counterpart, manifested by increased peak strength and reduced strain under initial loading. However, due to the brittleness of CaCO_3 cementing bonds, this structure is brittle by nature. With the load increasing further, forces in the cementing bonds gradually build up and eventually exceed the bond strength, causing bond breakage that disintegrates the structure. As a result, strength drops rapidly, displaying strain softening, and shear banding occurs where bond breakage is the most prominent. The degradation of cementing bonds and loss of structure under continuous shearing progressively turn the soil matrix back towards its uncemented state. By contrast, the roughening and densification effects of CaCO_3 crystals are not sensitive to loading, as the enhanced roughness and inter-locking of soil particles serve to maintain a certain level of improved strength at the residual stage [101,145,176,187,310,56,75,85].

In conventional soil mechanics, strength is considered to consist of two components, friction and cohesion, which are respectively described by the friction angle and cohesion intercept under the framework of the Mohr-Coulomb criterion [172]. By calculating these two strength parameters from the results of shearing of bio-cemented soils, researchers have attempted to quantify the respective contributions of bio-cementation to the strength components. However, controversy exists in this regard. Chou et al. [39] and Feng and Montoya [75] observed considerable increases in the friction angle of bio-cemented sands with minor changes in the cohesion intercept. By contrast, Lin et al. [145], Hataf and Jamali [108], and Liu et al. [146] reported increases in the cohesion intercept with the friction angle almost unaffected. Cui et al. [48] found the friction angle to decrease as C_{cc} increased. In other studies, both parameters were improved by MICP [101,227,246,31,47,85]. In general, values of friction angle and cohesion intercept are obtained by plotting data points of peak deviatoric stress against mean effective stress or of peak shear stress against normal stress and drawing a straight failure envelope of best fit through these points. For bio-cemented soils, the strength parameters for a

certain cementation level are determined based on the test results of several samples (usually three or four). It should be noted that, for highly cemented samples whose peak stresses are high, the data points are far from the origin, and thus a slight change in the slope of the envelope (i.e., friction angle), which may still give a reasonable fit, may cause a substantial change in the cohesion intercept. Furthermore, while the enhanced dilatancy of bio-cemented soils also leads to improved strength, dilation is increasingly suppressed as confining pressure increases, which will in turn lead to an underestimated friction angle and an overestimated cohesion intercept. Indeed, the contribution of bio-cementation to different components of soil strength not only depends on the cementation level, but also on the confining pressure and the loading stage due to microstructural variations.

3.2. Stiffness

The bio-cemented structure can improve soil stiffness. This stiffness improvement can be measured from stress-strain curves obtained from strength tests. Fig. 2b plots literature data for the elastic moduli (E) and corresponding C_{cc} of bio-cemented soils. Clearly, the evolution of E with increasing C_{cc} follows the same trend as that of UCS and can thus also be attributed to microstructural changes. In terms of the role of confinement, Feng and Montoya [75] studied the effect of confining pressure on the initial moduli of bio-cemented sands in their triaxial tests and found that the confining pressure becomes less influential as C_{cc} increased. This agrees with the conclusions drawn by Lin et al. [145] and Terzis and Laloui [248] that bio-cementation reduces the sensitivity of soil stiffness to variations in confinement.

Although strength tests enable direct determination of elastic moduli, they are incapable of characterising the evolution of soil stiffness as MICP treatment proceeds and cementation grows, which can only be captured using non-destructive means such as seismic wave measurements. Shear wave velocity (V_s) has been used as an indirect measure for soil shear stiffness [172,218]. V_s is the velocity of small-strain elastic waves that propagate only in the direction perpendicular to that of soil particle movement, and thus it directly relates to the shear stiffness of the soil and is not affected by the properties of the pore fluid [218,280,59]. Owing to the fact that V_s can be measured non-destructively, it has been employed to monitor changes in soil stiffness during MICP treatment [145,164,280,56,57]. It informs the trend of V_s evolution throughout the treatment course, reflecting the pattern of cementation growth and its impact on soil stiffness. This capability of monitoring the bio-cementation process in real time and relating to soil mechanical properties allows for quantitative analyses and optimisation of treatment parameters. Fig. 2c shows the V_s of bio-cemented soils reported in several studies with the corresponding C_{cc} . It can be seen that MICP treatment can increase the V_s of soil to over 2500 m/s, which is comparable to that of hard rock [280], from around 200 m/s at the untreated state. Again, achieving such a level of stiffness improvement is useful for the demonstration of bio-cementation, but might not be instructive for practical applications. An advantage of using V_s to represent the level of improvement in bio-cemented soils is its ability to automatically weigh different CaCO_3 crystals based on their contributions to stiffness enhancement [59,187]. Crystals actively forming cementation and effectively improving stiffness are more appreciated than the less effective ones (i.e., those suspended in soil pores and attached to exposed particle surfaces), while, in the case of using C_{cc} , all crystals are treated equally. V_s measurement has also been used in strength tests on bio-cemented soils to capture cementation degradation at different stages of loading. Montoya and DeJong [176] and Nafisi et al. [187] observed in their triaxial tests that the V_s of bio-cemented sand samples underwent rapid decreases after peak strength was reached, a result of bond breakage and loss of cemented structure. It should be noted that V_s values obtained at large strains might be overestimated. This is due to V_s being a bulk measurement across the full dimension of the soil sample, whereas bond breakage and structural

failure occur predominately within the localised shear band, which only makes up a small portion of the sample. As a result, the measured V_s is an average over failed and intact regions [145,176,59].

3.3. Volumetric responses

Soil volumetric responses to various loading conditions, including isotropic or anisotropic compression and shearing, are of great significance in geotechnical problems that concern deformation. Common agreement is found among studies on the compression behaviour of bio-cemented soils, that the compressibility is reduced as C_{cc} increases [13,136,145,301]. More precisely, Lee et al. [136] reported reductions of up to 23% in the total settlement of bio-cemented residual soil samples during one-dimensional compression with respect to uncemented samples. A more detailed inspection into the test results revealed that the recompression index, C_r , measured before yielding, decreased markedly, but the compression index, C_c , calculated at the post-yielding stage, had no noticeable change, suggesting that the enhanced rigidity endowed by cementation was most notable at low stresses and diminishes at high stresses due to breakage of cementing bonds. A similar conclusion was reached by Xiao et al. [301], who observed a clear bilinear feature in the compression response of bio-cemented sand denoting the onset of bond breakage (Fig. 2d). Arbolada-Monsalve et al. [13] performed compression tests in a triaxial cell where pseudo K_0 conditions were simulated through the use of on-sample instrumentation. The K_0 values obtained from bio-cemented sand samples were far lower than those of uncemented samples, but gradually increased and converged towards the values of uncemented samples as the applied stress increased.

The volumetric responses of bio-cemented soils to shearing have been well captured in triaxial tests, that initial changes in volume are inhibited, followed by significantly increased dilation after yielding [187,248,272,291,48,75]. Quantitative analyses of the experimental results further revealed that the stress-dilatancy relationship of bio-cemented soils display a unique pattern when the dilatancy ratio (i.e., the ratio of incremental volumetric strain to incremental deviatoric strain) is plotted against the corresponding stress ratio (Fig. 2e): in contrast to the typical pattern for uncemented soils, where the variation of the dilatancy ratio follows a smooth, defined curve with the stress ratio, the dilatancy ratio of bio-cemented soils is relatively constant with increasing stress ratio initially, showing inhibited volumetric change; at the yielding point, the curve suddenly deviates, with a sharp increase in the dilatancy ratio at an almost constant stress ratio; and finally, the behaviour of the bio-cemented soil degenerated to that of the uncemented soil, following the same curve towards the critical state [248,272,291,48]. Clearly, the structural evolution of cementation at the particle scale plays a primary role in causing these behavioural changes. The initially inhibited volumetric change can be readily explained by the cemented structure restricting relative movements of soil particles, analogous to its effects on soil stiffness and compressibility. The enhanced dilatancy has been attributed to the roughening and densification effects of CaCO_3 crystals assisting volumetric expansion when soil particles start to rearrange [291], although no direct evidence has been provided. Since volumetric behaviour affects soil strength, more research efforts are needed to fully uncover the underlying mechanism causing the observed responses.

3.4. Homogeneity of CaCO_3 distribution

The homogeneity of the distribution of precipitated CaCO_3 has long been considered a major problem of MICP-based soil strengthening [183,279,59]. A heterogeneous distribution will cause non-uniform cementation, which will in turn lead to an uneven strength distribution throughout the soil matrix, with mechanical performance primarily governed by the weaker regions. Normally, the distribution of CaCO_3 in a bio-cemented soil sample is characterised using the spatial profile of

C_{cc} or V_s across the sample. Commonly found is a higher amount of CaCO_3 near the inlet where bacteria and reagents are introduced [145,164,253,265,283,292,57,75]. Furthermore, it was observed that, the larger the soil body treated, the more heterogeneous the CaCO_3 distribution would be. For example, van Paassen et al. [265] performed MICP treatment on 100 m³ of sand and found that the cemented sand body had a volume of only 40 m³, of which the measured UCS ranged from 0.7 to 12.4 MPa. A widely accepted explanation attributes this heterogeneity to the interactions between the bacteria and the soil particles during MICP treatment. Most previous studies introduced bacteria into the soil through the injection of a bacterial suspension, with the hope that the bacteria will transport with the flow and form a uniform population profile along the injection path. However, bacterial cells tend to be filtered at the pore throats as they travel through the soil matrix, leading to more cells accumulating at the vicinity of the injection point and creating a log-linear profile of decreasing bacterial density along the injection path [58,91,92]. Upon subsequent injection of reagents into the soil, the presence of gradients in the bacterial density will cause gradients in the reaction rate, and hence, in the amount of CaCO_3 produced and the level of cementation achieved [311,58,75]. In the study conducted by Martinez et al. [164], bender elements and pore fluid sampling ports were installed at four equidistant points along the height of half-metre-high sand columns to monitor V_s and OD_{600} changes during MICP treatment. The results showed decreasing bacterial concentrations and ureolysis rates along the injection path, which subsequently resulted in non-uniform distributions of C_{cc} and V_s . Given this filtration phenomenon, it can be inferred that the size of soil particles can make a difference to the homogeneity of bio-cementation – smaller particles with narrower pore throats can cause more severe bacterial filtration. This was confirmed in the tests performed by Cheng and Cord-Ruwisch [29] and Zamani et al. [311], where they compared the C_{cc} distributions in bio-cemented samples of fine and coarse sands. To alleviate this heterogeneity issue, a number of new treatment methods have been proposed and showed some effectiveness (discussed later).

Certainly, achieving uniform cementation is critical for applications in which the soil to be strengthened is equally stressed throughout its matrix when loaded. However, for many geotechnical problems, stress distribution in the soil region of interest is inherently non-uniform, and thus the homogeneity of cementation becomes less important. In road foundations, for example, the stress caused by traffic loading gradually decreases in the vertical, downward direction. If it was to be strengthened by MICP, heterogeneous cementation would not be problematic as long as the gradient in cementation level only exists vertically and aligns with the change in stress. Strong, stiff zones within the soil may also preferentially carry stresses even if the soil is non-uniform.

3.5. Micro characteristics

Previous sections have elucidated the significance of micro characteristics in determining the macro behaviour of bio-cemented soils. Various techniques have been employed to characterise the precipitated CaCO_3 crystals and the way in which they interact with soil particles at micro scales. Scanning electron microscope (SEM) has been commonly used to visualise CaCO_3 crystals and their locations with respect to soil particles. The abundant SEM images in the literature show clear evidence of crystals precipitating within soil pores, on particle surfaces and at inter-particle contacts, contributing to densification, roughening and cementation, respectively [11,145,146,253,301,39,56,75,85]. More recently, micro-computed tomography (micro-CT) has been utilised to facilitate more robust and quantitative analyses of the micro characteristics [248,249,43,51,52]. For example, Dadda et al. [51] quantified the evolution of contact properties with increasing C_{cc} by analysing 3D images of bio-cemented sand samples obtained using X-ray micro-CT. The results suggest that, at small C_{cc} of under 3%, the majority of inter-particle contacts remain uncemented, and they progressively turn

into cemented as C_{cc} increases. This validates the hypothesis adopted earlier to explain the nonlinear strength improvement with increasing C_{cc} (see Section 3.1).

To monitor the behaviour of bacteria and capture the growth pattern of CaCO_3 crystals in real time, some researchers have performed MICP experiments in microfluidic chips that represent the soil environments at the particle scale ([275,276], 2021b, 2022; [296]). Wang et al. [275] found that, after injection, bacteria evenly distribute within the pore space and continue multiplying. Subsequent reagent injections can wash out a significant amount of the bacterial cells and cause the cells to gradually aggregate due to the introduction of calcium ions (Fig. 3a). Irregularly shaped, small CaCO_3 crystals that randomly coat soil particles precipitate first, and subsequently dissolve and reprecipitate as regularly shaped, larger crystals that are more conducive to the formation of cementing bonds (Fig. 3b). This process is time-dependent, and thus the interval between injections has a strong influence on the crystal properties, which in turn affects the macro behaviour of the soil [276,296].

Regarding the crystallisation mechanism, Tobler et al. [253] observed a layered texture in the CaCO_3 crystals from backscattered electron (BSE) imaging of bio-cemented sand samples, with the number of crystal layers corresponding to the number of reagent injections performed. This hierarchical pattern of crystal growth was further confirmed by Terzis and Laloui [248], who used time-lapse video microscopy to monitor the crystallisation process. These findings reveal the mechanism of crystal growth in MICP, that newly formed crystal layers incrementally accumulate on existing ones upon the introduction of fresh reagents [249]. Theoretically, in CaCO_3 precipitation, there is a competition between the nucleation of new crystals and the growth of existing crystals. Which of the two prevails depends on the supersaturation level of calcium and carbonate ions as well as the availability of nucleation sites [84]. At a high level of supersaturation with limited nucleation sites, there is a tendency for crystals to grow in size, while the formation of new crystal nuclei prevails when the supersaturation level is low and nucleation sites are abundant [11,34]. As mentioned previously, in MICP, bacterial cells serve as nucleation sites for CaCO_3 crystallisation, and thus a high bacterial concentration promotes crystal nucleation and vice versa [183]. Meanwhile, the supersaturation level is affected by a number of parameters such as urease activity (which is also a function of bacterial concentration), reagent concentration, pH and temperature. It is the interplay between all these factors that determines the eventual number and size of CaCO_3 crystals, and therefore they need to be elaborately controlled in order to achieve the desired treatment result. Researchers have reported that large crystals are more effective for soil strengthening, because they are more conducive to forming cementing bonds and reducing interparticle stresses [245,248,249,277,34]. By contrast, the formation of large crystals resulting from high reagent concentrations can lead to localised clogging of soil pores, aggravating the cementation heterogeneity [10].

Using X-ray diffraction (XRD) and Fourier-transform infrared spectroscopy (FTIR), different CaCO_3 polymorphs have been identified in bio-cemented soils [10,147,195,44,62]. CaCO_3 is known to have three anhydrous polymorphs, namely calcite, vaterite and aragonite, as well as some hydrated phases such as monohydrocalcite, ikaite and amorphous calcium carbonate (ACC) [179,243,6]. These polymorphs differ in solubility and stability, with calcite being the least soluble and most stable [125,129,276,44]. In abiotic environments, the kinetics of CaCO_3 precipitation involves a series of phase transformations according to the Ostwald's step rule [267]: ACC phases form first and then transform into vaterite, the metastable phase, and eventually into calcite, stepwise increasing stability and changing shape and size [125,131,209]. These phase transformation sequences can be achieved through multiple reaction pathways and are sensitive to environmental changes [44,62]. The supersaturation level, for example, is crucial in determining the prevailing polymorph: high supersaturation levels favour metastable vaterite and aragonite while low supersaturation levels promote the

formation of calcite [170,18,194,264]. In MICP, the presence of bacteria poses further uncertainties, such as the altered energy barrier, reaction rate and supersaturation, the secretion of specific macromolecules and kinetic inhibitors, and many others, which have marked effects on CaCO_3 crystallisation [124,170,18,20,55,72]. The selection of CaCO_3 polymorph and morphology also depends on the bacterial strain and reagent composition used [104,44,98], which will be discussed in later sections. It is commonly agreed that calcite is the preferred polymorph for soil strengthening purposes due to its great stability, binding effect and mechanical performance [245,264,277,62]. Nevertheless, direct evidence that compares the strengthening effects of different CaCO_3 polymorphs is still lacking, necessitating further investigation.

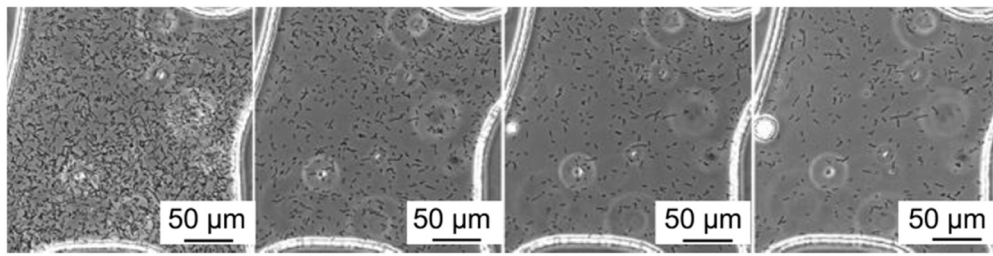
4. Factors influencing MICP-based soil strengthening

From previous sections, it is clearly recognised that MICP-based soil strengthening, albeit promising, is rather complex with substantial uncertainty that tampers the level of controllability. This complexity is due largely to the variety of influencing factors involved, which can be biological, chemical, physical, environmental or operational [21,245,309]. This section therefore focuses on some critical factors that are known to impact the behaviour of bio-cemented soils.

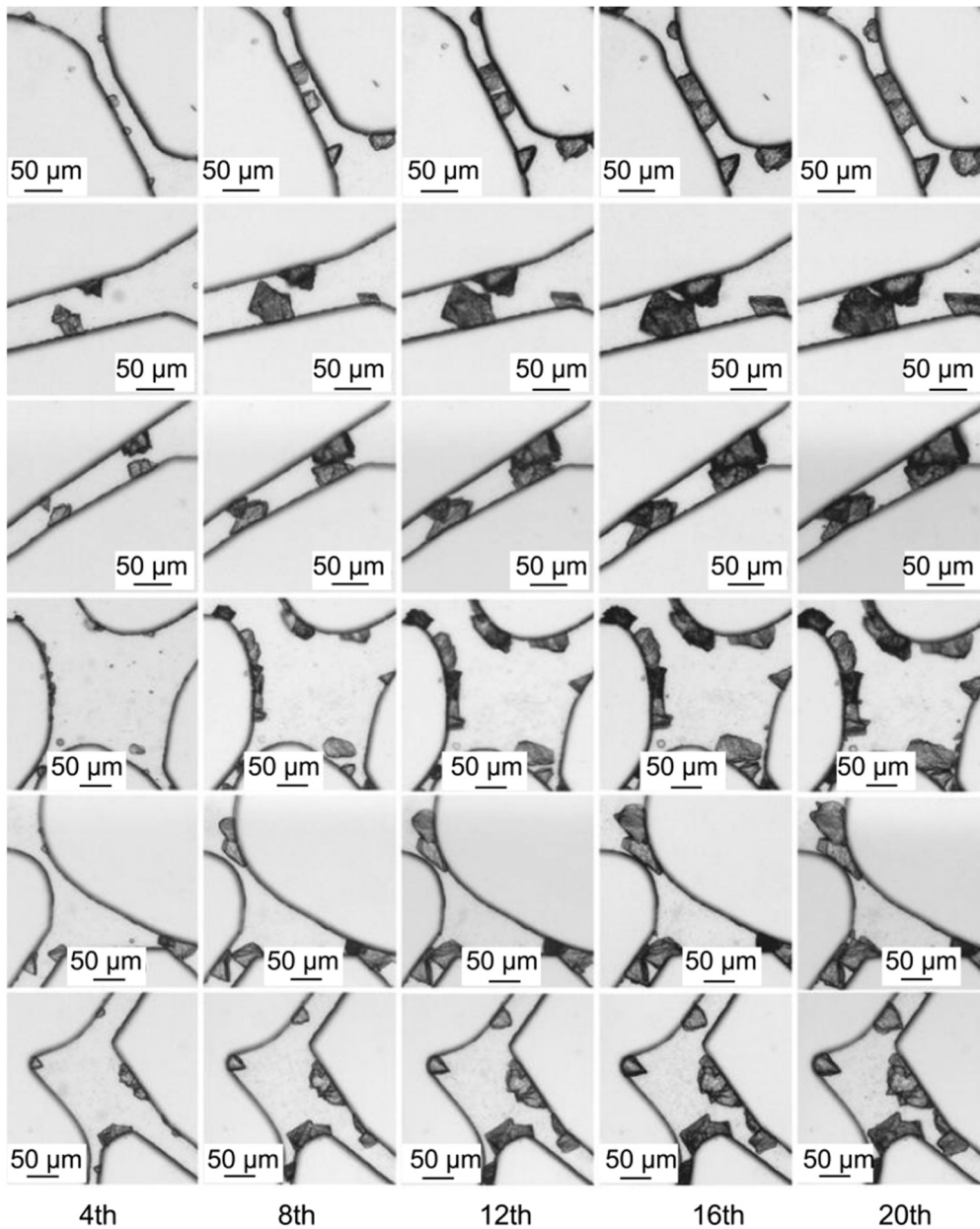
4.1. Bacterial strain and concentration

Although *S. pasteurii* has been the most used bacterial strain for MICP so far, researchers have been investigating other strains as alternatives. It was shown that using different bacterial strains could yield different results in terms of reaction rate and CaCO_3 crystal features such as size, polymorph and morphology [120,245].

Of particular interest is whether the polymorph and morphology of precipitated CaCO_3 crystals have strain specificity, as suggested by some early studies [73,76]. Under the same cultivation conditions, Hammes et al. [104] observed clear morphological differences in the CaCO_3 crystals formed by 12 different isolated strains and suggested two possible explanations: (i) different colony growth rates and urease activities might cause variations in the rates of crystal growth along different crystallographic planes; and (ii) crystal growth could be inhibited or altered by strain-specific proteins, organic matter or inorganic components [124]. Similar findings were reported by Park et al. [201], who analysed CaCO_3 crystals produced by *S. pasteurii* and other four strains. Dhami et al. [63] isolated five strains from calcareous soils for MICP experiments and also observed great morphological variations in the precipitated crystals. Further, three strains formed calcite as the major polymorph, while the other two mainly produced vaterite. Likewise, Sondi and Salopek-Sondi [229] showed that urease enzymes extracted from *S. pasteurii* and *Canavalia ensiformis* induced the formation of vaterite and calcite, respectively. Clarà Saracho et al. [44], based on the results of their study involving three strains from the *Sporosarcina* family, suggested that varying urease activities of different bacterial strains could cause supersaturation and alkalinity to increase at different rates, resulting in different crystallisation kinetics and nucleation rates. The subsequent phase transformations and polymorph stabilisation are highly dependent on pH and the timing of precursor dissolution: high pH and delayed dissolution following fast nucleation prompt stabilisation of ACC, while near-neutral pH and early dissolution following slow nucleation promote transformation of ACC into calcite. They also found that vaterite tends to be stabilised when water molecules and strain-specific amino acids are present in the crystal structure, which is consistent with the conclusions in previous studies [229,281]. Bacterial extracellular polymeric substances (EPS) also play an important role in CaCO_3 crystallisation. Strain-specific proteins and other organic macromolecules in EPS can alter surface properties of bacterial cells, trap and complex calcium ions, release dissolved organic carbon, and change supersaturation conditions, which all affect crystal



(a)



(b)

Fig. 3. Microscopic observation of MICP treatment in microfluidic chip: **a** images of bacterial cells in pore space (from left to right are images taken after bacterial injection, after 1st reagent injection, after 3rd reagent injection, and after 12th reagent injection), and **b** images of CaCO_3 crystals taken upon completion of 4th, 8th, 12th, 16th and 20th reagent injections [275].

nucleation and growth [124,142,18,20,210,255,63,72]. Overall, it is clear that bacteria and associated macromolecules exert a high level of control over the precipitation kinetics in MICP, with evident strain-specific crystal morphological and polymorphic features. However, the exact mechanisms behind these observations and the ways in which desired crystal properties can be selectively achieved are still largely unknown.

Ureolysis is the first and also the rate-limiting step in MICP [204]. Theoretically, using bacterial strains of varying urease activities would, when other conditions are analogous, results in different ureolysis rates and therefore different amounts of CaCO_3 produced within a given period of time. Most relevant studies indicated that *S. pasteurii* has higher urease activity than other ureolytic strains under similar growth conditions [104,120,121,222], which justifies its extensive use in previous works. Some studies, on the other hand, identified strains that show comparable or even higher urease activities under certain environmental conditions. For example, *Bacillus megaterium* (*B. megaterium*, another gram-positive strain commonly found in soils) has been found to have a similar level of urease activity compared with *S. pasteurii* [62,241], and is capable of forming endospores that are resistant to a wider range of temperatures than *S. pasteurii* [119,233,241]. Clarà Saracho et al. [44] found that *S. newyorkensis* and *S. aquimarina*, which were isolated from the deep sea, display optimal urease activity at 4 and 15 °C, respectively, while *S. pasteurii* is inactive at those temperatures. These findings are useful as they provide alternatives to *S. pasteurii* that are more conducive to extreme environments, broadening the application scenarios of MICP-based soil strengthening.

Another determinant factor for urease activity and ureolysis rate is bacterial concentration [120,195,204,281]. This can be easily understood – more bacteria simply produce more urease for reaction. Okwadha and Li [195] captured a positive, linear correlation between ureolysis rate and log-scale bacterial concentration when the concentration varied between 10^6 and 10^8 cells/mL. It is thus prone to infer that using higher bacterial concentrations could be beneficial for MICP-based soil strengthening. Experimental data obtained in many studies agree well with this inference. Higher strength values of bio-cemented soils have been reported when higher bacterial concentrations were used [225,233,314,39]. However, opposite results are also found in the literature. Shahrokhi-Shahraki et al. [223] and Cheng et al. [34] showed that the strength and stiffness of bio-cemented sands reduced with increasing bacterial concentration. Explaining this controversy is challenging because of the multiple impacts of bacterial concentration on the MICP process and their coupling with the effects of other influencing factors. More specifically, it has been elucidated in Section 3.5 that the number and size of precipitated CaCO_3 crystals are determined by the competition between crystal nucleation and growth. Increasing the number of bacterial cells, on one hand, provides more nucleation sites for crystal nucleation, promoting the formation of a large number of small crystals [34,183]. On the other hand, if sufficient reagents are present, elevated urease activity due to increased bacterial concentration accelerates ureolysis, raising the supersaturation level and prompting crystals to increase in size [11]. In addition, as also described in Section 3.5, different supersaturation levels would cause the predominance of different CaCO_3 polymorphs that might be different in strengthening performance. Further uncertainty is posed by the interplay with other influencing factors such as reagent concentration and soil intrinsic characteristics. For example, soils of different particle sizes may require different crystal numbers and sizes to achieve the optimal strengthening effect [43,277]. It is therefore difficult to consistently predict changes in the mechanical behaviour of bio-cemented soils with variations in the bacterial concentration used, and the conclusions drawn from one study may not hold true if different experimental settings are used. It should be noted that using a very high bacterial concentration would lead to serious bacterial aggregation during injection, which would intensify the non-uniformity of bacterial distribution within the soil matrix and eventually aggravate cementation heterogeneity [223,277].

One point worth mentioning is that the predetermined bacterial concentration in the injected bacterial solution usually does not represent the actual number of bacterial cells engaging in MICP. This is because, after being injected, bacteria can keep multiplying in the soil, especially when nutrients are injected together [275,277]. Further, each subsequent reagent injections can flush out a significant portion of the bacterial cells [275,277]. Finally, upon CaCO_3 nucleation on bacterial cells, the formed crystals can encapsulate the cell as precipitation proceeds, blocking access to reagents and causing cell lysis [11,169,170,204,252,50]. Further, when it comes to in situ applications where indigenous microorganisms are present, injected exogenous bacteria may face nutrient competition and predation [148,152,204,268,88]. Relevant works have shown that the survival and activity of *S. pasteurii* and the precipitation efficiency were significantly lower under natural soil conditions than those under sterile soil conditions [148,152].

4.2. Reagent composition and concentration

As can be seen from the reactions involved in MICP (Section 2.2), two reagents are essentially required, urea and a soluble calcium source, the reagent solution used in most previous studies being a mixture of urea and a calcium salt, with some having a small amount of nutrients. Most works investigating the reagent composition therefore focus on the effects of different calcium sources. Gorospe et al. [98] compared seven calcium salts and found that all of them reduced the urease activity of *S. pasteurii* at a concentration of 50 mmol/L, with calcium chloride causing the largest activity drop. Conversely, the study conducted by Achal and Pan [3] which involved four calcium salts showed that the use of calcium chloride led to the highest urease activity and CaCO_3 production, although a different bacterial strain, *Bacillus sp.* CR2, was used. Zhang et al. [312] reported that sand samples treated with calcium acetate displayed higher UCS and had aragonite as the dominant polymorph, compared with samples treated with calcium chloride and calcium nitrate that mainly produced calcite. The production of different CaCO_3 polymorphs and crystal morphologies as a result of using different calcium salts have been well observed irrespective of the bacterial strain used [120,3,304,312,54,98]. Yet, the underlying mechanisms remain underexplored. Undoubtedly, calcium chloride remains the mainstream calcium source adopted for MICP. Considering its high price and potential environmental impacts when used in large quantities, researchers have attempted to produce soluble calcium in a cheaper and more sustainable way. To date, calcium has been produced and used for MICP from a variety of natural and recycled sources, including shells of eggs, oysters and scallops [38,144], calcareous sand [147], limestone [36], dolerite [26], and seawater [33,180], many of which show comparable or even better performance in soil strengthening than the use of calcium chloride.

The concentrations of urea and calcium reported in the literature cover a wide range. Numerous studies examining the influence of reagent concentration have come to a commonly agreed conclusion that, for a given experimental setting, there exists an optimum concentration range that gives the best strengthening outcome, although it may vary greatly with changes in other variables. For example, Soon et al. [233] increased the UCS of a bio-cemented residual soil by increasing the reagent concentration from 0.25 to 0.5 mol/L, while a further increase to 1 mol/L returned the UCS to the value for the uncemented state. Similarly, Mahawish et al. [154] performed MICP treatment on a coarse sand and found that a reagent concentration of 1 mol/L led to the greatest strength gain compared with 0.75 and 1.5 mol/L. A reagent concentration of 0.75 mol/L was reported by Lian et al. [143] to provide the highest chemical conversion efficiency as well as best cementation homogeneity in a fine sand. The different optimum concentrations obtained in these studies are probably due to the different soil properties (e.g., particle size). Since the soil samples were compared based on a fixed treatment duration or injection number, the poor

performance of low reagent concentrations is readily explained – insufficient reagent supply causes low CaCO_3 production and limits cementation development, while the reasons for the negative effects of high concentrations are more complicated. One possible explanation is that high reagent concentrations inhibit bacterial urease activity. Whiffin [282] recorded changes in the urease activity of *S. pasteurii* with increasing concentrations of urea and calcium and found that the presence of urea boosts the activity up to a urea concentration of 1.5 mol/L, while increases in calcium concentration cause monotonic activity reductions. The promoting effect of urea up to a certain concentration and retarding effect of calcium on urease activity have been confirmed in many studies [134,189,233,236,242,308]. Urea is an energy source for many ureolytic bacteria [104,280,56], and its presence is conducive for bacteria to secrete urease [242,282]. However, excessive urea can lead to high reaction rates and rapid pH increases, creating an overly alkaline environment that represses bacterial activity [135,239,282]. With respect to calcium, its greater inhibition on urease activity at increased concentrations is primarily due to the increased salinity that poses osmotic stresses to bacterial cells as well as calcium ions coating bacterial cells and affecting nutrient transport [104,107,143,258,33,98]. These findings urged the use of non-equimolar concentrations (i.e., molarity ratio of urea to calcium greater than one) in the reagent solution, which has shown to be beneficial for MICP [154,164,213,223,282,307]. The presence of an extra portion of urea not only enhances urease activity, but also helps maintain the pH level for continuous CaCO_3 precipitation [164].

Another possible explanation for the inferior treatment effects of high reagent concentrations lies in their impacts on the precipitation kinetics and crystal properties. A high reagent concentration results in a high reaction rate, producing abundant precipitation precursors that bring the supersaturation of CaCO_3 to a high level [135,252]. This resultant high supersaturation level leads to (i) the prevailing of crystal growth over nucleation, which gives rise to large crystals being produced, and (ii) preferential formation of metastable CaCO_3 phases with irregular morphologies [11,134,154,264,303,44]. These large, irregularly shaped crystals tend to easily detach from soil particles and occupy pore spaces, especially in fine soils [309]. As a consequence, the crystals do not contribute to forming cementation and improving soil strength but induce localised clogging. As shown by Al Qabany and Soga [10], using a reagent concentration of 1 mol/L caused early reductions in the permeability of sand samples, compared with the gradual reductions observed when lower concentrations (0.25 and 0.5 mol/L) were used. Localised clogging, once formed in the soil matrix, is problematic as it could create preferential flow paths that cause non-uniform delivery of reagents in subsequent injections or even block the inlet and hinder further reagent transport, compromising cementation homogeneity [154,183,195,300,99]. In addition, the tendency of forming large crystals may result in a more rapid decline in urease activity as bacterial cells are more likely to be encased in large crystals [11]. Nonetheless, although using low reagent concentrations can be more effective for MICP-based soil strengthening, it is not desirable from a practical and economic perspective, because the volume of reagent solution, the number of injections and the duration of treatment have to be increased in order to reach the target cementation level [266,274].

4.3. Environmental factors

Environmental conditions during treatment have all-round influences on various aspects of MICP, from bacterial activity to precipitation kinetics to yield and properties of CaCO_3 crystals. Here, three key environmental factors are discussed, temperature, pH and oxygen availability.

Although most experimental studies were conducted at constant temperatures of around 20 °C, the importance of temperature in MICP-based soil strengthening should never be overlooked, particularly under

the context of geotechnical applications where in situ temperature varies greatly by location and depth. Commonly agreed is that the growth and activity of most ureolytic strains are positively related to temperature in the range of 0–30 °C [196,241,264,308,77]. More precisely, Ferris et al. [77] performed MICP experiments using *S. pasteurii* and adopted a first-order kinetic expression to describe ureolysis rate. The results showed that the rate constant at 20 °C was 5 times greater than that at 15 °C and 10 times greater than that at 10 °C. In the study conducted by Sun et al. [241], both *S. pasteurii* and *B. megaterium* showed steadily increasing urease activity when the temperature increased from 15° to 30 °C. The optimum temperature for *S. pasteurii* to grow and express urease activity lies between 25 and 30 °C [196,308]. Kim et al. [127] reported that the amount of CaCO_3 precipitation induced by *S. pasteurii* was the highest at 30 °C and almost halved when the temperature was raised to 50 °C. Mahawish et al. [154] demonstrated that, while the greatest amount of precipitation was detected in coarse sand samples treated at 40 °C, samples treated at 20 °C showed much higher strength, indicating the lower strengthening efficacy of CaCO_3 crystals produced at high temperatures. This is consistent with the findings reported by Cheng et al. [34], who also showed different microstructures of CaCO_3 crystals precipitated at different temperatures. Crystals precipitated at 50 °C were small and uniformly distributed across the entire surface of the sand particle as a coating layer, incapable of forming effective cementation. At 25 °C, the average size of crystals increased by 10 times, enabling them to fill up the gaps at sand inter-particle contacts and create strong cementing bonds. This difference was attributed to the impact of temperature on the crystallisation kinetics. High temperatures can lower the energy barrier for the nucleation of CaCO_3 crystals and thus interfere with the competition between crystal nucleation and growth, promoting the former over the latter and causing the formation of excess small crystals [34]. Temperature variation has also been found to cause the formation of different CaCO_3 polymorphs [243]. In short, the influences of temperature on MICP-based soil strengthening are rather complex and still poorly understood, although the reported optimum temperature range for bacterial activity and strengthening effectiveness is encouraging as it is compatible with site conditions for most inland geotechnical applications. Wherever temperatures out of this range are encountered, some specific strains with different temperature-dependent behaviour may be considered. *S. newyorkensis* and *S. aquimarina*, for example, display optimum urease activity at 4 and 15 °C, respectively [44].

pH also exerts complicated influences on MICP-based soil strengthening. Further complexity arises due to the fact that MICP itself contains many pH-moderating processes, such as ammonia generation and volatilisation, carbon dioxide dissolution and degassing, and CaCO_3 precipitation and redissolution, which regulate any pH change [233,70,87]. As a result, most relevant works could only investigate the effects of initial pH set before addition of reagents. The growth and activity of most ureolytic bacteria used for MICP are favoured in moderately alkaline environments. For *S. pasteurii*, the maximum growth has been reported to occur at pH values around 9 [282,285], while the urease activity is expected to be the highest at nearly neutral pH [173,196,239,42]. Acidic or strongly alkaline conditions would undermine the metabolism of *S. pasteurii* and its urease production or even irreversibly denature the produced enzyme [173,233,42]. Lauchnor et al. [135] conducted a series of batch experiments where the initial pH of the culture medium containing *S. pasteurii* and urea was varied between 5 and 10 by adding buffer. The results showed only a slight dependence of ureolysis rate on the initial pH, particularly in the pH range of 6–9. This implies that, in a complete ureolysis system, initial pH within a proper range may have minor significance, because the reactions could self-regulate the pH to reach and stay at a level that maintains sufficiently high urease activity. This is beneficial because the pH level in MICP, once the reactions start, is determined through the dynamic trade-off between the numerous biochemical elements and hardly manipulatable in reality.

Commonly used ureolytic strains for MICP (e.g., *S. pasteurii* and *B. megaterium*) are considered aerobes or facultative anaerobes. It is generally assumed that their proliferation and metabolic functions are inhibited in the absence of oxygen, and MICP is not applicable for anoxic conditions. However, there are discrepant points of view in this regard. Mortensen et al. [180] monitored the urease activity of aerobically grown *S. pasteurii* under oxic and anoxic conditions and found that, surprisingly, the anoxic group showed an equal or higher level of urease activity compared with the oxic group. Likewise, Tobler et al. [252] observed no difference in ammonium production and calcium consumption induced by *S. pasteurii* transferred from oxygenated culture media into oxic or anoxic groundwater. Similar results were obtained for *B. megaterium* [119]. Although these findings give positive indications for the possibility of using MICP in oxygen-limited circumstances, several researchers have pointed out that, at least for *S. pasteurii*, cell growth and de novo synthesis of urease are not possible in the absence of oxygen and the observed urease activity under anoxic conditions is caused by the urease produced during previous aerobic cultivation [161,169]. Since the urease enzyme tends to degrade over time, the use of MICP in strengthening deep subsurface soils should be treated with cautions as urease activity and CaCO_3 precipitation are unlikely to persist, which might be fine if light cementation is intended but could be detrimental for high cementation levels. As demonstrated by Li et al. [141], subjected to a same treatment duration of 7 days, sand samples treated under air-restricted conditions were substantially weaker than those treated under aerated conditions.

4.4. Treatment method

A key part of MICP-based soil strengthening is the treatment method by which bacterial and reagent solutions are introduced into the soil. It is important not only for its massive influences on the MICP process and final soil properties, but also because it implies to what extent samples fabricated in laboratory resemble treated soils in field. In general, two methods have been mostly used in previous studies, mixing and injection, with various specific implementation procedures prescribed for each method.

The mixing method stands for direct blending of bacterial solution or mixed solution of bacteria and reagents with soil particles prior to sample moulding [126,136,178,199,233]. It is typically adopted for fine soils containing a significant portion of silty and clayey particles that make the permeability very low and penetration of treatment solutions difficult [21]. The most prominent advantage of the mixing method is that it avoids the abovementioned filtering issue when using the injection method and allows for an even distribution of bacteria throughout the soil matrix, which is critical for achieving homogeneous cementation [183,200,21]. Nevertheless, several distinct drawbacks have rendered this method less preferred. Considering the normal scale of geotechnical applications, mixing a large volume of soil with treatment solutions presents practical difficulties. The mechanical mixing process also inevitably causes disturbance to the natural soil structure and may leave pseudo stresses in the soil that complicate the stress history [183], which contradicts the favoured feature of MICP that soil structure remains intact throughout treatment [247].

The injection method has been the most extensively used method for performing MICP treatment on laboratory soil samples. In this method, bacterial and reagent solutions are pumped through the soil sample from one end to the other at a predetermined hydraulic pressure or flow rate. Initially, bacteria and reagents were mixed together to form one solution to be injected. This was found to cause rapid clogging at the injection point and considerable cementation heterogeneity when used on sand samples as a result of instant bacterial flocculation and CaCO_3 precipitation upon mixing [107,223,239]. This so-called one-phase injection strategy is thus more suitable for coarse soils with large pores and high permeability, or applications requiring only shallow treatment depths [107,309]. In response to this clogging issue of one-phase

injection, Whiffin et al. [283] proposed a two-phase injection strategy in which bacterial solution is injected first, followed by the injection of reagent solution, and in between a fixation solution of high salinity, whose high ionic strength reduces the repulsive electrostatic force between bacterial cells and soil particles and thus promotes bacterial adsorption (Section 2.1), is injected to enhance bacterial retention within the soil sample [107,283]. Using this strategy, Whiffin et al. [283] successfully treated a 5-m long sand column with CaCO_3 detected across the entire length, although the cementation was highly non-uniform. Chu et al. [40] reported that the use of fixation solutions increased bacterial retention in sand samples by an average of 31%. Further changes made to two-phase injection mainly include the addition of a retention period between bacterial and reagent placements, and repeating reagent injections for different cementation levels, with a reaction period between each two adjacent injections. This discrete injection mode with predetermined intervals is then referred to as the staged injection strategy [21,223,253] and is now the most commonly practiced in MICP-based soil strengthening. Shahrokhi-Shahraki et al. [223] tested and compared all these injection strategies and showed staged injection to be the most effective. Undeniable is that the improved effectiveness of MICP treatment brought by the iteration of the injection strategy from one-phase to two-phase and then to staged comes at the expense of progressively longer treatment durations, which is not ideal but seems to currently be the best option. More recently, some researchers have attempted to optimise the injection parameters to retain the simplicity of one-phase injection while tackling the clogging and heterogeneity problems. Examples include low-pH injection [32,290] and low-temperature injection [121,299,300], which at their core aim to suppress bacterial activity and insert a time window for injection and bacterial transport between solution mixing and start of MICP reactions.

Although pressurised injection is generally used because of its controllability of hydraulic gradients and flow rates, it implies the use of complex injection machinery which could be costly considering large-scale field implementations. This method also assumes fully saturated soil conditions throughout the course of treatment, which is true for deep soils but not the case for soils above the groundwater table. In this context, gravimetric injection, more commonly known as surface percolation, emerged. Originally developed by Cheng and Cord-Ruwisch [28], this method allows bacterial and reagent solutions to be simply sprayed onto the soil surface whereby their infiltration is autonomously driven by gravimetric and capillary forces, thereby discarding injection equipment and enabling treatment to be performed at different degrees of saturation [183,28,31]. A fascinating finding is that sand samples treated using gravimetric injection at unsaturated states showed comparable strength to saturated samples treated by pressurised injection but had a significantly lower C_{cc} (i.e., improved strengthening efficiency of precipitated CaCO_3 crystals) [28,31]. This is because, under unsaturated conditions, any solution retained in the soil forms menisci at inter-particle contacts due to capillary tension, and hence CaCO_3 crystals are predominately precipitated therein, effectively contributing to cementation [28,31]. This feature renders gravimetric injection advantageous over the most commonly used pressurised injection, since improved performance of MICP-based soil strengthening could be achieved with simpler operations and lower costs. It therefore has been adopted in many subsequent experimental works [111,155–157,16,99]. However, the strong dependency of solution penetration on soil permeability hampers the viability of applying gravimetric injection to treat fine soils as the attainable treatment depth is limited [183,28,29].

For high cementation levels, multiple injections of reagent solution are needed. This highlights the importance of a treatment parameter, injection interval. From their microfluidic experiments, Wang et al. [276] observed that the use of a long injection interval of 24 h resulted in large, rhombohedral calcite CaCO_3 crystals being produced, while numerous small, spherical crystals were precipitated with short

intervals (3–5 h), which led to the hypothesis that a short interval gives insufficient time for the growth and phase transformation of CaCO_3 crystals. In their subsequent study [274], it was demonstrated that the use of longer injection intervals improved the chemical efficiency of injected reagents being converted into precipitates and the strength of treated sand samples, which is in line with the findings reported by Soon et al. [233] and Mahawish et al. [154]. Al Qabany et al. [11] suggested a more holistic approach in which reagent concentration and injection interval are combined as input rate for interpretation. They found that, for bacterial optical density (OD_{600}) between 0.8 and 1.2, the input rate of 0.042 mol/L (i.e., an injection interval of 6, 12 or 24 h for a reagent concentration of 0.25, 0.5 or 1 mol/L, respectively) formed an upper threshold for high chemical efficiency exceeding 80%. Apparently, long injection intervals are favoured for better chemical efficiency and strengthening effectiveness, but, again, these enhancements come at the price of prolonged treatment durations and increased treatment costs.

4.5. Soil intrinsic characteristics

Formed through natural deposition and weathering, soils intrinsically vary in terms of particle size distribution, particle shape, density, mineral composition, and many other characteristics, all of which have a certain extent of influence on the effect of MICP treatment. In the parametric analysis performed by [213], the type of soil showed to be the most dominant influencing factor for MICP-based soil strengthening.

With respect to other soil characteristics, particle size is considered the most influential and has received enormous research interest. As elucidated in Sections 2.1 and 3.4, bacterial transport in soil is governed by the geometric compatibility between bacterial cells and soil pore throats, with poor compatibility in fine soils causing severe bacterial filtration and non-uniform bacterial distribution that eventually leads to heterogeneous cementation and strength improvement. Evidence for this is abundant in the literature. Cheng and Cord-Ruwisch [29] treated 2-m columns of fine (0.05–0.6 mm) and coarse (0.3–1.18 mm) sands and found that clogging near the injection end was prominent in the fine sand column but not observed in the coarse sand column, which resulted in a more uniform UCS profile in the latter. Terzis and Laloui [249] also tested two sands of different particle sizes ($D_{50} = 0.19$ and 0.39 mm) but with identical strength properties at the untreated state. The results showed that the coarser one yielded more pronounced strength and stiffness improvement. Further micro-structural characterisation revealed that the cementing bonds formed in the coarser sand had larger diameters, which were postulated to lower contact stresses and enhance particle interlocking, resulting in higher overall resistance. Similar results relating larger particle sizes to better efficacy of MICP treatment were reported by Zhao et al. [314]. However, some studies came to the opposite conclusion that smaller particle sizes are more conducive for effective cementation and strength gain [34,290]. Meanwhile, the test results obtained by Terzis and Laloui [248] showed that, among the three sands investigated ($D_{50} = 0.19$, 0.39 and 0.6 mm), the sand with the intermediate particle size yielded the most significant strength improvement. Obviously, the influence of soil particle size on the final performance of MICP-based soil strengthening is not straightforward and should be interpreted in a more comprehensive way. In finer soils, bacterial filtration during injection is more pronounced and cementation tends to be less homogeneous, but the number of inter-particle contacts per unit volume is higher, which leads to two simultaneous consequences: (i) more sites are available for cementing bonds to form [234,290,34]; and (ii) a smaller amount of CaCO_3 can be allocated to each contact at a given C_{cc} [187]. Coarser soils possess fewer inter-particle contacts that allow for cementation, but they have less problem with bacterial filtration and cementation heterogeneity and each contact can receive more CaCO_3 . On the other hand, the amount of CaCO_3 required at each contact for

effective particle cementation also depends on particle size – the bigger gaps between and greater masses of larger particles necessitate a higher amount of CaCO_3 to form strong bonding and vice versa [187,206,289,31].

Notably, most previous works were carried out using uniformly sized soils with a narrow particle size distribution, while studies involving well graded soils, which are more commonly encountered in situ, have been sporadic. This is somewhat surprising as a few preliminary works have revealed that well graded soils might be a better candidate for MICP-based soil strengthening. Through monitoring MICP treatment on soils with a variety of particle size distributions, Mortensen et al. [180] demonstrated that well graded soils typically had faster V_s increases compared with poorly graded soils. Mahawish et al. [156] treated aggregate mixtures having five different particle size distributions produced by blending coarse and fine aggregates in varying proportions. It was found that, despite having lower C_{cc} , the mixtures containing both coarse and fine aggregates yielded higher UCS than those solely made of coarse or fine aggregate. Cardoso et al. [25] also reported MICP treatment to be more efficient in improving the strength of more well graded sand. It is normally hypothesised that the better performance of MICP-based soil strengthening in well graded soils is due to their higher number of inter-particle contacts and better particle packing [156,180]. Well graded soils represent matrices of in-contact particles of a wide size range, and hence the respective effects of differently sized particles on bacterial transport and the formation and relative strength of cementing bonds fuse together in a sophisticated way. Clearly, more research efforts are needed in this regard.

In addition to particle size distribution, the number of inter-particle contacts in a soil and the particle packing state in a soil are also related to the relative density (D_r), which is therefore another significant influencing factor for MICP-based soil strengthening. It has been commonly observed that increasing the initial D_r is beneficial for strength gain of bio-cemented soils. More precisely, Chou et al. [39] recorded a considerable increase in the California bearing ratio (CBR) of bio-cemented sand samples when the initial D_r increased from 35% to 85%. Several studies also reported increased UCS of bio-cemented soils at higher initial D_r [10,211,234]. Triaxial test results showed that greater increases in peak strength and dilatancy were obtained at higher initial D_r [85,257]. Indeed, a higher D_r creates a denser packing in the soil matrix that brings soil particles closer to each other with an increased number of inter-particle contacts, enabling a more resistant and robust cementation network to be formed under MICP treatment [211,232,247,39]. A hypothesis made here is that a sufficient amount of CaCO_3 precipitation is needed to fulfil this advantage. At a low C_{cc} , increasing D_r could cause reductions in the amount of CaCO_3 allocated to each contact point and thus weaken the cementing bond formed. Further, a denser state could result in soil pore throats being squeezed, intensifying bacterial filtration and cementation heterogeneity. As observed by Mahawish et al. [158], a drop in the strength and stiffness of bio-cemented coarse sand occurred when the initial D_r reached 100% due to heterogeneous cementation.

Other soil characteristics, such as particle shape, mineral composition and organic content, can also influence MICP-based soil strengthening. Nafisi et al. [185] investigated the shear behaviour of bio-cemented sands with an angular or round particle shape and found that the angular sand yielded greater strength improvement. Xiao et al. [297] mixed varying percentages of angular and round glass beads to resemble natural sands with different particle shapes. The strength and stiffness of these mixtures after MICP treatment was shown to increase with increasing percentage of angular glass beads. Similar results were obtained by Song et al. [231], who demonstrated that, after receiving identical MICP treatment, angular sands obtained greater UCS values than round sands, despite the higher amount of precipitation in the latter. It has been postulated that contacts between angular soil particles are mostly in a planar or cone-to-plane form, which is more conducive for forming strong and stable cementing bonds than sphere-to-

sphere contacts for round particles [185,230,231], although such postulation remains to be validated. Difference in soil mineral composition may also cause variation in the outcome of MICP treatment. Mortensen et al. [180] treated several sands that were respectively rich in silica, calcite, feldspar and iron oxide, and monitored the V_s changes during treatment. The calcite-based sand experienced the fastest V_s increase, which might be because sand particles rich in calcite provide ideal surfaces for additional CaCO_3 deposition. Some researchers also observed marked differences in the mechanical behaviour of bio-cemented silica and calcareous sands [48,146], which might stem from the differences in the hardness and surface roughness of silica and calcareous sand particles which result in different particle-scale failure mechanisms when bio-cemented [146]. Again, this hypothesis requires further confirmation. The presence of organic matter in soil may impair the effectiveness of MICP treatment. Canakci et al. [24] performed MICP treatment on an organic soil with an organic content of 60%. The results showed that the obtained amount of CaCO_3 precipitation and shear strength were lower than literature values reported for inorganic soils. They therefore suggested that the organic matter present in soil could not only inhibit the precipitation and growth of CaCO_3 crystals, but also governs the strength of the soil matrix. Overall, possibly due to their secondary significance, the influences of other soil characteristics on MICP-based soil strengthening have received far less research interest compared with those related to particle size and density state, and most observations are in lack of adequate theoretical explanation.

Through the discussion in the above sections, it can be seen that the factors influencing MICP-based soil strengthening are complex and inter-dependent, and for many of them the exact mechanism of influence on the MICP process remains unclear. A number of studies have conducted parametric analyses on these factors and have attempted to optimise MICP-based soil strengthening by looking for the optimal treatment parameters (e.g., [180,232,233,154,308]). However, due to the complexity and uncertainty related to these influencing factors, experimental studies are inevitably limited in terms of the number of factors included and the range investigated, and the results tend to be only applicable to the unique experimental setting adopted. At the moment, modelling of MICP and its interactions with soil remains the only tool for predicting and optimising performance at scale, uniquely informing local and time-dependent properties and their changes, as elucidated by some preliminary works (e.g., [168,188]). Yet, the mathematical description of MICP processes beyond a simple biological flask remains a challenging task, because of the multi-physics (chemistry, mechanics, thermodynamics, fluid, and ionic effects), multi-scale (from bacteria, through sample scale, to large-scale applications), and multi-phase (liquid, solid, bacterial films) phenomena.

5. New developments in MICP-based soil strengthening

In parallel with the continuous research on conventional MICP-based soil strengthening, a number of recent works have brought new insight into advancing this technique and broadening the application scenarios. In the following, two main streams of developments are discussed. One is the use of additives, and the other is the incorporation of additional engineered functions.

5.1. Additives

Broadly speaking, the main elements involved in MICP-based soil strengthening are the bacterial and reagent solutions and the soil. Notable is that the formula and composition of these elements adopted in most previous studies, albeit having some divergence, share principal commonalities. In this context, researchers attempted to identify additives for these elements that could ultimately lead to enhanced performance.

Certain metal ions were studied for use in the reagent solution with the aim to produce stronger cementation. In this regard, the use of

magnesium has gained great interest. Fukue et al. [83] found that increasing the concentration ratio of magnesium to calcium resulted in the precipitated crystals changing from pure calcite to magnesium-calcite, then to dolomite and calcium-magnesite, and finally to pure magnesite. Sand samples treated with an equimolar concentration of calcium and magnesium at 0.5 mol/L yielded UCS values three times those of samples treated with 1 mol/L of calcium. Similar results were reported by Xu et al. [305] who used a reagent solution containing 0.5 mol/L of urea and 0.5 mol/L of calcium acetate and added magnesium chloride at varying concentrations and showed that the strength of bio-cemented sand samples increased as the concentration of magnesium chloride increased. Attempts were also made to use various water-soluble polymers as MICP additives. Wang and Tao [273] suggested that the addition of polyvinyl alcohol (PVA) could increase the viscosity of MICP solutions, which slows their infiltration and improves their retention in highly permeable soils, enabling localised and targeted treatment. The experimental results indicated that the incorporation of PVA markedly enhanced the chemical efficiency and strengthening effect. Another studied polymer is guar gum, a polysaccharide-based polymer. Relevant works show that the supplement of guar gum could extend bacterial activity as a result of biodegradation of guar gum into free sugar and eventually escalate the production of CaCO_3 and the strength of bio-cemented soils [65,66].

Regarding additives mixed with soil prior to MICP treatment, fibres are the most commonly studied, both natural and synthetic [120,313]. The addition of fibres shows to improve the strength of bio-cemented soils while also reducing the brittleness [140,295,37,74,8]. In the study conducted by Xiao et al. [295], bio-cemented sand samples containing randomly distributed basalt fibres displayed higher peak strength as well as failure strains compared with samples with no fibre. Fang et al. [74] reported that the addition of polyester fibres caused a change in the failure mode of bio-cemented sand samples from brittle into plastic with significantly increased residual strength. Likewise, Al Imran et al. [8] observed a slowed strain-softening response in the stress-strain behaviour of bio-cemented sand as a result of adding jute fibres, which were also found to help persist bacterial survival for longer. This capability of added fibres in improving ductility gives great promises for their use in combination with MICP. Meanwhile, given the numerous types of fibres investigated, comparative studies exist. For example, Lei et al. [137] compared three types of fibres, glass fibre, basalt fibre and carbon fibre, and concluded that carbon fibre was the best for assisting strength improvement for bio-cemented calcareous sand, providing an increase in UCS by as much as 1133%. Zhao et al. [313] adopted a similar approach and demonstrated polyester fibre to perform better than glass fibre and hemp fibre. Natural fibres, such as jute fibre, which tend to be more affordable, recyclable and readily available than synthetic fibres, are superior in terms of cost-effectiveness and environmental sustainability [8,235]. Nonetheless, it should be noted that the requirement of pre-mixing soil with fibres poses a disadvantage for their wide use in MICP-based soil strengthening because of the associated practical challenges and soil disturbance (Section 4.4).

5.2. Engineered functions

For bio-cemented soils, damage to the cementing bonds resulting from external loading or chemical deterioration will irreversibly impair or even eliminate any achieved mechanical improvement. In other words, MICP treatment endows soil with one-off cementation of which post-damage restoration is only possible through re-treatment. It is thus desirable to incorporate some form of 'self-healing' capabilities, similar to those developed in cementitious materials [53,284], so that bio-cemented soils can become engineered living materials that respond to damage and repair themselves autonomously [192]. A few studies have been conducted to realise this vision. In the experiment performed by [175]), bio-cemented sand samples were fabricated and tested under triaxial compression following standard procedures, and, immediately

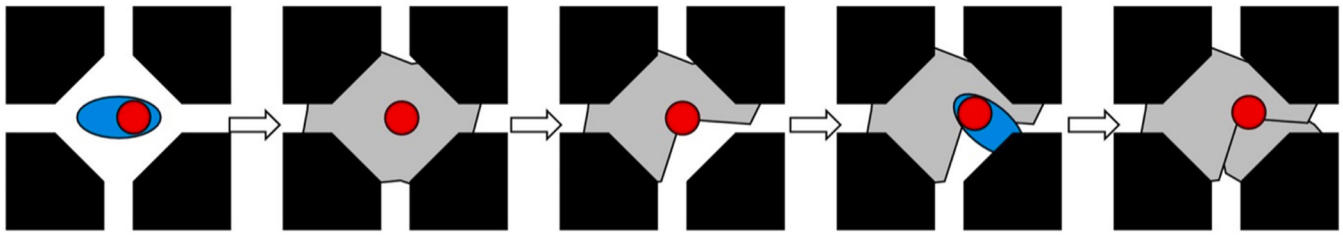


Fig. 4. Concept of self-healing of bio-cemented soil [19].

after the sample reached an axial strain of 10%, testing was paused for re-injection of the reagent solution and then resumed. It was found that healing of the broken cementing bonds was possible with the sample showing identical strength and stiffness with respect to the virgin one. A subsequent study [106] adopted a similar approach and showed that post-shearing replenishment of reagents successfully repaired degraded cementation and enabled the damaged sand sample to re-gain a strength value equivalent to or greater than the initial value. However, both studies achieved healing of cementation and strength restoration through supplying additional reagents shortly after damage occurred, which cannot be considered fully representative of the envisioned self-healing function due to the short-term feasibility (healing is only possible when the previously injected bacteria are still active) and required human intervention (the procedure for reagent replenishment is no other than a new round of treatment but without new bacteria). A more recent study [19] attempted to address these limitations by showing that dormant spores encased in CaCO_3 crystals remain viable for long periods of time and can germinate into functioning cells and re-initiate MICP if reagents are re-introduced following cementation damage that breaks the crystal (Fig. 4). *Sporosarcina ureae*, which was deemed to have a better spore-forming capability than *S. pasteurii*, was used. After being damaged, bio-cemented sand samples were flushed with a 0.5% hydrogen peroxide solution to remove active cells, followed by re-injection of the reagent solution. The results showed cell growth, ureolysis, and CaCO_3 precipitation during the healing phase, as well as strength re-gain. However, human intervention is still required to provide additional reagents, which renders the healing process not truly autonomous.

The importance of spatially uniform bacterial distribution on cementation homogeneity is highlighted throughout the present review, so is that of CaCO_3 microstructure on the final soil properties obtained. Yet, current limited control over these two elements hampers further optimisation of MICP-based soil strengthening. Further, the standard practice of utilising vegetative bacteria that have a short shelf-life means that treatment can only be administered on an as-needed basis, and it is not possible to pre-embed bacteria in soil and activate the MICP process when required. Recently, a study conducted by Clarà Saracho et al. [45] revealed that encapsulation of bacteria prior to their addition into soil might be a potential solution to these shortcomings. In their demonstration (Fig. 5), *S. pasteurii* in the freeze-dried form was encapsulated in alginate beads. The release of bacteria was based on a competitive ligand exchange mechanism: when yeast extract is added and a high-pH environment is created, yeast extract with a higher calcium affinity will seize the calcium ions used to cross-link the alginate, thereby dissolving the hydrogel structure and releasing the encapsulated bacteria. This mechanism also controls CaCO_3 precipitation. When CaCO_3 supersaturation is high, precipitation is fast and pH will decrease, which will reduce the calcium affinity of yeast extract and cause some calcium ions to be returned to alginate. This synergistic competition for calcium ions enables tuning of the supersaturation level directly related to the yield, polymorph and morphology of CaCO_3 crystals. Therefore, the use of bacterial encapsulation could simultaneously enable spatiotemporal regulation of bacteria and controlled precipitation of CaCO_3 [45].

6. Upscaling of MICP-based soil strengthening and its challenges

Over ten years of intensive research on MICP-based soil strengthening is surely not just to develop a ‘technique in laboratory’. The will to ultimately incorporate this unconventional, bio-inspired technique into normal geotechnical practice has never stopped swelling. Limitations and challenges, however, are real and remain to be addressed.

Several pilot-scale experiments have been conducted. The first one was performed by van Paassen et al. [265] who treated 100 m^3 of a poorly graded, fine to medium sand prepared in a concrete container (Fig. 6a). Three pairs of injection and extraction wells were inserted below the sand surface, pumping tons of bacterial and reagent solutions through the sand body over a period of 16 days. Post-treatment excavation revealed a cemented sand body of 40 m^3 , which was found to have significant heterogeneity in CaCO_3 distribution and strength improvement (C_{cc} varied from 0.8% to 24% and UCS ranged from 0.7 to 12.4 MPa) due primarily to the injection method causing preferential flow paths. DeJong et al. [57] devised a five-spot treatment model that enabled investigation into MICP treatment in a three-dimensional flow regime, with bender elements and fluid sampling tubes configured to monitoring the treatment process. The results revealed the spatial and temporal evolution of bacterial density and urease activity during treatment as well as the gradual increases in V_s as cementation developed, providing valuable insights into the design and optimisation of field treatment schemes adopting similar setups. San Pablo et al. [216] fabricated sand columns using steel beams with dimensions of $0.2 \times 0.2 \times 3.7 \text{ m}$. The columns were placed horizontally with tubes and valves installed at both ends for performing injection, simulating the one-direction flow condition in the well-to-well half-space. Bender elements and fluid sampling ports were also used to monitor MICP treatment. Post-treatment removal of the reaction by-product, ammonium, using a rinse solution containing 0.2 mol/L calcium chloride at a pH of 9 was examined and showed satisfactory results. These pilot-scale studies provide the proof of concept for field implementation of MICP-based soil strengthening in a more realistic context than centimetre-scale laboratory settings. Yet, the meticulous, artificial control of soil conditions and experimental parameters inevitably compromises the representativeness of the results.

Field trials offer direct and crucial evidence of the feasibility of MICP-based soil strengthening at scales of geotechnical interest. van der Star et al. [260] treated an area of natural gravel to improve its stability in preparation for subsequent horizontal directional drilling and installation of a gas pipeline (Fig. 6b). The volume of gravel to be treated reached 1000 m^3 , which required a number of injection and extraction wells to be employed. The treatment took 7 days in total with the last 3 days used for removal of the residual chemicals. During the following drilling process, the treated gravel showed improved stability, confirming the treatment effectiveness, although no further quantitative analysis was conducted. Another field trial using gravimetric injection for surficial treatment was presented by Gomez et al. [97]. Four test plots (Fig. 6c) each measuring $2.4 \times 4.9 \text{ m}$ were established at a mine site in Saskatchewan, Canada, three of which received MICP treatment and the other one was treated with water as a control. Post-treatment

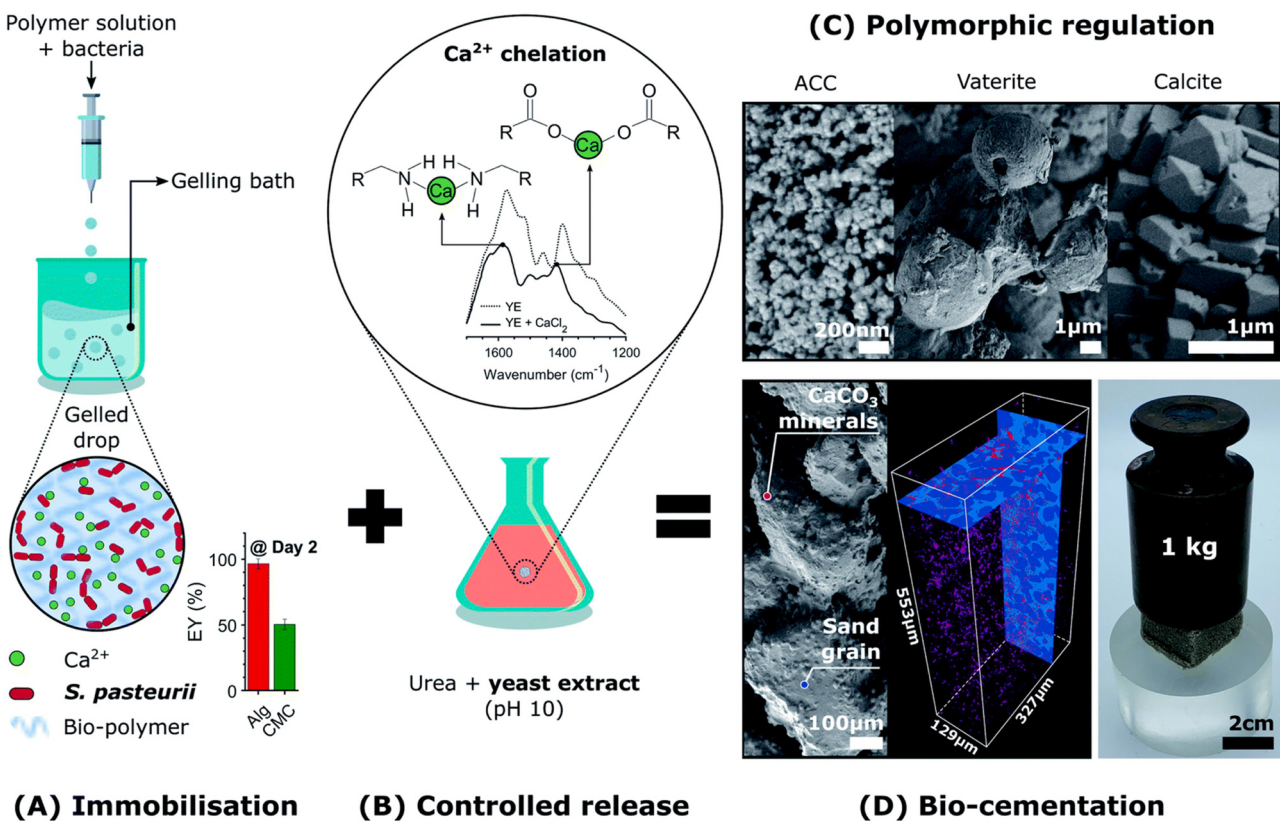


Fig. 5. Schematic of spatiotemporal regulation of bacteria and controlled precipitation of CaCO_3 through use of bacterial encapsulation [45].

observation showed that a cemented crust up to a depth of 2.54 cm was formed, and the penetration resistance measured using a dynamic cone penetrometer was improved with respect to the control plot to depths of near 30 cm. A similar investigation was conducted by Meng et al. [166], who performed MICP treatment on 4- m^2 test plots in Ulan Buh Desert, China. The concentration and applied volume of the reagent solution were varied between 0.1 and 1 mol/L and 1 and 4 L/m^2 , respectively. Test results (Fig. 6d) showed that the bearing capacity of the soil was greatly improved with the measured C_{cc} below 1%, and a significant part of this improvement was maintained 180 days after treatment while the soil had been constantly exposed to the natural environment. Terzis et al. [250] treated a slope in Switzerland with MICP to mitigate the landslide risk (Fig. 6e). Half of the slope was treated via injection under a hydraulic head of 2 m through 51 drilled boreholes, while the other half was left untreated for comparison. After the 4-day treatment period, increases in the C_{cc} ranging from 0.8% to 2.8% were detected from samples cored in the vicinity of the boreholes. The following long-term aerial surveillance and 3D reconstruction of the slope revealed that the treated half had slower movement compared with the untreated half.

From the extensive laboratory studies to these pioneering trials at larger scales, the great potential of MICP-based soil strengthening is well demonstrated, and so are the major challenges to be overcome before practical applications are manageable. The first, and probably the most formidable challenge is to acquire a sound understanding of the bio-chemo-physical processes involved and how control can be exerted in a flexible, reproducible and relatively simple way. One difficulty, as elaborated in Section 4, is the current insufficient knowledge about the numerous process-influencing factors that are highly inter-related and whose variation can cause the final behaviour of treated soil to be highly variable. Field implementation would bring further uncertainties as in-situ conditions in terms of soil type, groundwater chemistry and biodiversity vary site by site. At the moment, there is apparent need for case-specific pre-investigation based on laboratory

testing so that the feasibility of MICP treatment can be assessed and treatment strategies can be tailored. However, such an approach is not ideal for geotechnical practitioners especially if major amendments in design are frequently required. Clearly, a widely applicable and repeatable standard of execution needs to be established, which still seems intimidating. As pointed out by many [120,204,247,59,60], the development of MICP and its widespread use in soil systems represent a multidisciplinary problem for which advancement can be made only through integrated efforts from relevant fields of expertise.

The lack of understanding and control necessitates precise, real-time monitoring of the progress of the MICP process and changes in soil properties of interest if MICP-based soil strengthening is to be implemented at large scales, which imposes another technical challenge. Preferably, this monitoring is done by non-invasive means so that the natural soil structure can be preserved, which discourages the use of direct testing methods such as strength tests and microscopic analysis that are mostly based on cored soil samples [186,217,280]. Analysis of biochemical factors (e.g., pH, ion concentrations, bacterial density and activity, etc.) using extracted pore fluids is suggested as a viable method for monitoring the state and efficiency of MICP reactions, although it still requires discrete sampling that is labour-intensive, costly and potentially destructive (due to installation of sampling device), particularly in cases involving treatment of large volumes of soil [59,217]. For monitoring the evolution of bio-cementation and soil mechanical properties, geophysical methods such as real-time V_s measurement are considered to be the most viable and have been examined in many laboratory studies [145,176,180,187,280,56]. Combination of biochemical and geophysical measurements as the ultimate monitoring tool was typically adopted in the abovementioned pilot-scale tests, while its use in field trials has not been documented. There are also sporadic studies that proposed other non-invasive monitoring techniques, such as nuclear magnetic resonance [128,224,82] and induced polarisation [217], but their usefulness is in question due to the limited data series reported. Collectively, with current methods proving

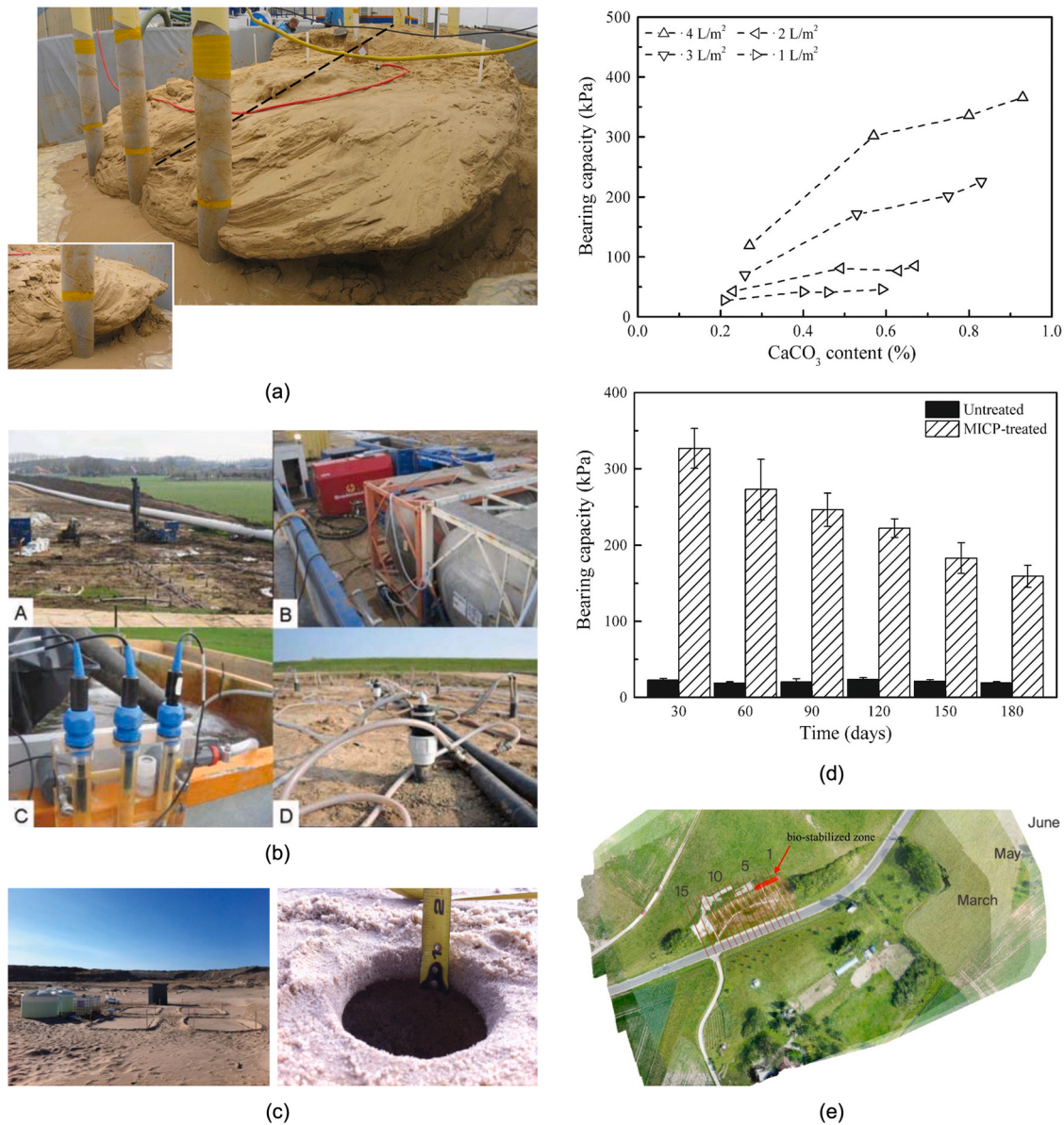


Fig. 6. Upscaled trials of MICP-based soil strengthening: **a** treatment of 100 m³ of sand in a concrete container [265]; **b** treatment of an area of natural gravel for horizontal directional drilling and pipeline installation [260]; **c** surficial treatment of loose sand at a mine site and crust formation after treatment [97]; **d** bearing capacity and durability of treated desert sand [166]; and **e** slope treatment for landslide mitigation [250].

feasible but having certain shortcomings, in-situ monitoring of MICP-based soil strengthening still requires further development.

Ureolysis-driven MICP generates ammonia (or dissolved ammonium) as a by-product, which is known to cause various environmental issues (e.g., eutrophication, depletion of dissolved oxygen, increased toxicity, etc.) when released to the air and groundwater in excess amounts. This has long been regarded as a major limitation for wide applications of MICP-based soil strengthening and has sparked debate over whether this technique is truly environmentally friendly as always claimed [183,204,315,64]. An estimation made by Ivanov et al. [116] revealed that MICP treatment of 1000 m³ of soil, assuming that 62 kg of CaCO₃ is yielded per cubic metre of soil, could pollute over 10¹⁰ km³ of the air or 4.5 × 10⁶ m³ of drinkable water. In light of this dispute, researchers are actively seeking solutions for ammonia/ammonium removal, with several options proposed. One option is based on capture and recycling of generated ammonium. It involves extracting from soil the treatment effluent and adding to it magnesium and phosphate ions which consume ammonium through the precipitation of struvite, a common fertiliser

[100,174]. On-site recirculation facilities are needed, incurring additional costs. Alternatively, zeolites, which are microporous aluminosilicate minerals, can be used as adsorbents for ammonium so as to control its release. A preliminary study conducted by Su et al. [240] showed that pre-treating sand with a zeolite suspension prior to MICP treatment fixed up to 43% of generated ammonium and also improved the strengthening effect. However, as suggested by the authors therein, the relatively large particle sizes of zeolites limit their use in fine soils.

Large-scale implementation implies low costs. The use of sterile bacterial cultivation and high-grade reagents, which has been common in previous laboratory studies, is certainly not pragmatic, yet understandable as, at the initial stage of development, the priority of research has been to explore the core mechanisms and demonstrate performance in a way as controlled and precise as possible. Recently, researchers have been actively working on cost optimisation, providing various solutions including non-sterile enrichment of bacteria [30,197,226,227,306] and treatment with low-grade reagents [99,198] or recycled materials (see Section 4.2).

7. Conclusions

Inspired by nature, MICP is an emerging technique that has been gaining research momentum in various engineering fields. To geotechnical engineers, particularly appealing is the potential of this technique in improving the most fundamental yet intractable geomaterial, soil. This paper presents a comprehensive review specifically focusing on the use of MICP in soil strengthening, which has been under intensive research for over a decade and is in need for a holistic compilation and interpretation of the findings.

Literature works characterising bio-cemented soils show promising results, with the efficacy of MICP-based soil strengthening clearly proved. Experimental observations combined with theoretical explanations highlight the role of micro characteristics in macro-behavioural changes, in particular the number, size and microstructural features of precipitated CaCO_3 crystals which are directly related to the dynamics of supersaturation and nucleation, as well as the spatial arrangement of crystals with respect to soil particles, which determines the strengthening mechanism and efficiency. Achieving homogeneous CaCO_3 distribution, which is important for laboratory characterisation, has been considered a major difficulty, but the present authors claim that, depending on the specific application, heterogeneity in cementation may not be so much of an issue.

Previous studies commonly correlate the degree of mechanical enhancement in relation to the mass content of precipitated CaCO_3 . This, while intuitive and simple, may be misleading, as reflected by the discrepancies in the conclusions reported. A more robust and inclusive method specified to bio-cemented soil for evaluating mechanical improvement is to be established, and until then treatment prediction and manipulation have to be based on case-specific experimental data. Also identified is the common tendency to produce highly cemented samples as well as the use of very similar soil types. Reaching high strength values certainly aids demonstration, but turning soil into rock or concrete may not be required in most geotechnical problems. Hence, designing experimental parameters under the context of a defined application is recommended.

The immense complexity of MICP-based soil strengthening is revealed from the various influencing factors and their inter-relations. Although it is generally comprehended that the biochemical and environmental factors influence the MICP process through their conjunct effects on urease activity, reaction rate and supersaturation level, current understanding of the underlying mechanisms is insufficient and far from enabling process control. This difficulty builds up when accounting for the diversity of treatment methods and variation in the intrinsic characteristics of soils. Optimisation and tailoring of the technique are possible only when a sound appreciation of and effective control over these factors are obtained.

Research on MICP-based soil strengthening to date has been encouraging, demonstrating its great potential, but simultaneously dispiriting, revealing various issues that remain to be addressed. While several pilot-scale and field trials have shown preliminary success and recent attempts have led to new ideas for improved performance, the major challenges that lie ahead of real-world applications are clear. To what extent these challenges are addressed determines whether this technique will truly bring a transformative change in geotechnical practice. Here, the authors suggest several focuses for future research: (i) to derive a unified and widely applicable way of describing the mechanical properties of bio-cemented soil; (ii) to fully correlate the characteristic and behavioural changes in bio-cemented soil at different scales; (iii) to completely understand the respective effects of the influencing factors and how they interplay; (iv) to optimise and standardise the treatment formula and protocol to enable economic and efficient implementation in different application scenarios; and (v) to develop robust techniques that facilitate treatment monitoring and by-product removal as well as long-term performance surveillance.

Statements and Declarations

The authors declare that they have no competing financial or non-financial interests that are relevant to the present work.

Acknowledgements

This work was supported by the UK Engineering and Physical Sciences Research Council (EPSRC) grant (reference number: EP/S02302X/1) for the University of Cambridge Centre for Doctoral Training in Future Infrastructure and Built Environment.

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