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European Journal of Environmental and Civil Engineering

ISSN: 1964-8189 (Print) 2116-7214 (Online) Journal homepage: https://www.tandfonline.com/loi/tece20

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To cite this article: Tong Yu, Hanène Souli, Yoan Péchaud & Jean-Marie Fleureau (2020): Optimizing protocols for microbial induced calcite precipitation (MICP) for soil improvement–a review, European Journal of Environmental and Civil Engineering, DOI: 10.1080/19648189.2020.1755370

To link to this article: https://doi.org/10.1080/19648189.2020.1755370

Published online: 23 Apr 2020.

EUROPEAN JOURNAL OF ENVIRONMENTAL AND CIVIL ENGINEERING
https://doi.org/10.1080/19648189.2020.1755370

Optimizing protocols for microbial induced calcite precipitation (MICP) for soil improvement–a review

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ABSTRACT

With the fast-developing bioengineering techniques in recent decades, researchers have started to try to apply bio-techniques to geotechnical engineering. Microbial induced calcite precipitation (MICP) has known a mushroom growth, due to its sustainability and feasibility. In order to achieve lower cost, higher efficiency and higher operational feasibility, many studies have been carried out to optimise the protocols. It is crucial to review the existing literature to give a synthetic summary of the optimised conditions in the various protocols. This article assembled, analysed and summarised the results of studies on the optimisation of protocols in state-of-the-art literature. The main factors incorporating biological, physical, chemical and operational aspects, were presented in this article. It can provide a clear insight in how these factors are acting on the process. Up-to-date instructions on the selection of parameters can inspire further studies.

ARTICLE HISTORY
Received 8 October 2019
Accepted 8 April 2020

KEYWORDS
MICP; soil improvement; optimisation of protocol; efficiency; influence factors

1. Introduction

Biotechnology (including environmental microorganisms, related products-enzymes, biosensors …) has been extensively used in depollution, detection and monitoring of the environment, which has resulted in tremendous advance in soil science and soil remediation. As for geotechnical applications, the majority of traditional soil improvement techniques consume substantial amounts of energy in producing materials and on-site operation, which also gives rise to potential danger (toxic chemicals, massive carbon dioxide emissions) to the environment. Producing concrete accounts for the major source of man-made global CO_2 emission (around 6%) (Achal & Mukherjee, 2015). For expanding applications and ecological concerns, researchers have started to find sustainable biogenic alternatives for ground improvement with minimal carbon footprint (Ashraf et al., 2017; Chang et al., 2016).

Microbial induced calcite precipitation (MICP), commonly realised by injecting ureolytic bacteria and reagents (urea and Ca^{2+}), makes use of bioactivity to cement sand by precipitating calcium carbonates (Al Qabany et al., 2012). This technique has been used to enhance mechanical properties of soil by taking advantage of the energy-conserving microbial metabolic processes, which can remarkably reduce carbon footprint compared to other traditional techniques. It has gained more and more attention from researchers and companies in the last 10 years (DeJong et al., 2013; Whiffin et al., 2007). Lots of research have been carried out using this technique, the majority of which at the laboratory scale, in columns (several centimetres to metres) (Mirmohammad Sadeghi et al., 2015; Qabany & Soga, 2013;

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Rowshanbakht et al., 2016; Zhao et al., 2014). A few researchers carried out large-scale in-situ tests (Esnault Filet et al., 2019; Gomez et al., 2015, 2017; van Paassen et al., 2010); some others set up comprehensive models (Barkouki et al., 2011; Fauriel & Laloui, 2012; Gai & Sánchez, 2019; Mahanty et al., 2014) or carried out microscopic visualisation of the fabric of cemented soils (Li et al., 2017; Terzis & Laloui, 2019). This research on MICP in geotechnical field mainly concerned the following aspects: (1) exploring the mechanisms to optimize the effectiveness of soil bio-cementation through the study of different factors, (2) measuring the properties (especially the mechanical properties) of bio-cemented soils. Though there are still unsolved problems, results from these studies give a comprehensive view of the process and of the resulting soil properties from the microscopic scale (a few micrometers) to the macroscopic scale (tens of meters).

MICP has shown a huge potential in geotechnical applications (Achal & Mukherjee, 2015; DeJong et al., 2013; Ivanov & Chu, 2008), such as liquefaction mitigation (Montoya et al., 2013), suffusion control (Jiang et al., 2017; Sibille et al., 2015), crack repair (Choi et al., 2017; Son et al., 2018), dust reduction, stabilisation of dams, slopes, and offshore structures (Cheng et al., 2014; Salifu et al., 2016), etc. It is worth noting that there are still problems regarding this technique for future applications. For instance, the left ammonium produced by urea hydrolysis might bring about the pollution of subsurface environment. In a long-term study, the ammonia volatilisation can also lower the pH in the liquid and cause dissolution of a portion of the precipitated calcite (Gat et al., 2017). Hence there are still barriers for using MICP in real practical works.

Attempts have been made to optimise the effectiveness of MICP process under various conditions. For instance, Al Qabany et al. (2012) investigated the injection mode and cementation reagent concentration. Soon et al. (2014) studied the influence of bacteria concentration, cementation reagent concentration, treatment duration and reagent flow pressure. Among these studies, monitoring and evaluation of MICP often includes biological analyses (bacterial concentration through optical density measurements), physical analyses (temperature), chemical analyses (pH, concentrations of urea and calcium, ammonium, $CaCO_3$ content…) and geotechnical analyses (strength, stiffness, porosity, permeability…) (Martinez et al., 2013).

In consideration of the huge potential and the high feasibility of MICP method in the field of soil improvement, it is of great significance to give a clear view of the whole cementation process, especially for geotechnicians, and to try to establish a practical protocol that can be scaled-up to real site applications. The previous reviews were mainly focussed on the description of MICP method, on the comparison of the effectiveness of MICP method with other soil-improving methods, and on the engineering properties of MICP-treated soils and potential applications in various fields. Because it is unpractical to draw conclusions among different strains of bacteria, this article is focussed on the results of different studies aiming to optimize the protocols mainly based on the widely used strain called *Sporosarcina pasteurii*. In this review, results obtained by researchers are presented, analysed, summarised and compared. At the end of the article, some helpful suggestions and reference values for designing experiments are given. It aims to help prospective researchers to choose their own parameters in the framework of their own studies.

2. MICP process and μ-organisms involved

MICP is an ubiquitous natural phenomenon (Stocks-Fischer et al., 1999) that occurs with a wide range of microbial species in various environments (soils, oceans, freshwaters, saline lakes, etc.) (Hammes et al., 2003; Wei et al., 2015). There are three groups of microorganisms that can be involved in the precipitation of calcium carbonate. One group is that of photosynthetic microorganisms (such as cyanobacteria and microalgae), which is photoautotrophic. The other two are heterotrophic, and are related to sulphate cycle (sulphate-reducing bacteria) and nitrogen cycle (such as nitrate reducing bacteria and ureolytic bacteria), separately (Al-Salloum et al., 2017; De Muynck et al., 2010).

Urea is an important organic nitrogen carrier, and large quantities of urea are released in the environment through urine and biodegradation. In soil and water environments, urease (urea aminohydrolase E.C.3.5.1.5) produced by bacteria, fungi, plants and animals, plays an important role in global nitrogen cycle through urea hydrolysis (Kafarski & Talma, 2018). Urea hydrolysis, catalysed by urease, which releases ammonium and carbonate ions in the environment, is a rapid process compared to urea degradation without urease (10^{14} times) and the reaction can be controlled easily. With the presence of Ca^{2+}

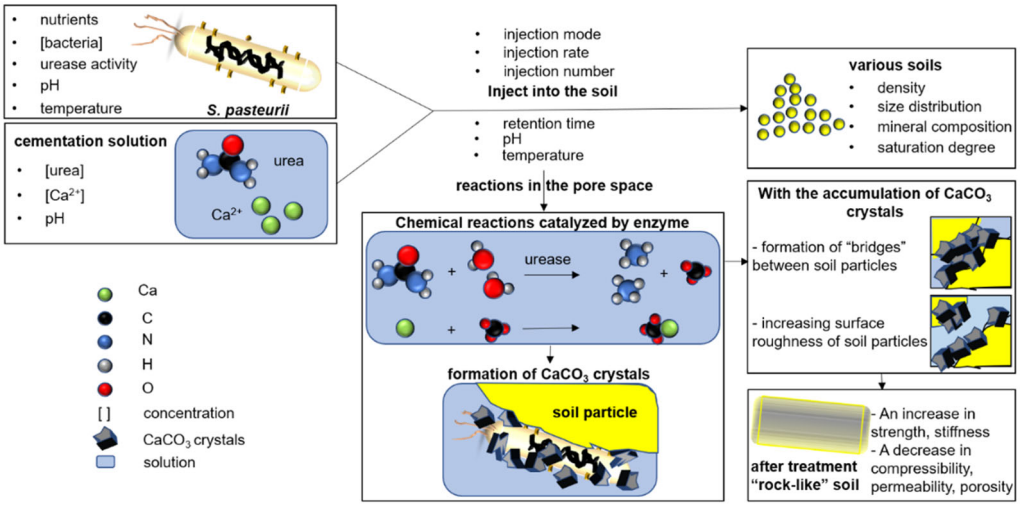

Figure 1. MICP process (using *S. pasteurii*) and factors influencing the process.

ions, calcium carbonate can be formed. To date, one of the most commonly used systems of MICP is based on the urea hydrolysis catalysed by ureolytic bacteria that can produce urease. Figure 1 gives out a schematic representation of the processes involved in MICP. During the process, urea is degraded, the pH of the ambient environment increases due to the production of ammonia, which favours calcite formation on the surface of particles as well as at particle contacts in the presence of calcium ions. The role of bacteria can be described as follows, (i) it produces urease by hydrolysing urea, (ii) it increases pH by generating alkalinity, (iii) it provides nucleation sites to produce precipitation (van Paassen, 2009). Chemical equations are as follows,

$$CO(NH_2)_2 + 2H_2O \xrightarrow{\text{urease}} 2NH_4^+ + CO_3^{2-}$$
$$Ca^{2+} + CO_3^{2-} \text{–} CaCO_3 \downarrow$$

Sporosarcina pasteurii (*S. pasteurii*, also known as *Bacillus pasteurii*, *B. pasteurii*) is extensively used as model microorganism in MICP, due to its high urease activity (giving a high efficiency in MICP process), high adaptability to the ambient environment with no pathogenicity. *S. pasteurii* is a gram-positive, aerobic, alkalophilic bacteria (DeJong et al., 2006; Zhao et al., 2014), classified as risk group 1 (unlikely to cause human disease) (Venda Oliveira et al., 2015). It is either round, rod-like or spiral, and its cell diameter is usually in the range of 0.5–3 μm. Thus the free passage of this bacteria is inhibited usually when pore throat is smaller than 0.4 μm (DeJong et al., 2006). Bacteria used in MICP studies is either bought from companies or isolated locally from water, soils or sludge samples (Omoregie et al., 2017). For type strains, such as ATCC 11859, the growth condition is cultivated aerobically (inadequate oxygen limits growth) at ambient temperature (optimal temperature is around 30 °C) in a pH range from 6 to 9. Early stationary phase can be achieved after around 40 hours cultivation.

To carry out MICP, injection of bacteria solution and injection of chemical reagents are needed. As for bacterial injection, bio-augmentation method (addition of pre-grown microbial cultures) and bio-stimulation method (addition of nutrients to stimulate the growth of specific indigenous bacteria) are used by researchers to enhance the performance of bacteria. In most lab-based studies, bio-augmentation is used to inject bacteria into artificially prepared soils. For field trials, Gomez et al. (2018) have already completed a successful trial of a 12 m bio-stimulation treatment in the field. Although there are few studies about bio-stimulation at field scale, it is an effective method that uses indigenous bacteria, which lowers the ecological risk and the cost of cultivation and transportation.

For injecting cementation solution, commercial chemical reagents (urea and Ca^{2+} ions) are used. For sustainable, environmental and cost-effective consideration, researchers used alternatives to replace pure chemical reagents in specific regions. According to Danjo and Kawasaki (2016), urea available in coastal regions, resulting for instance from biodegradation of dead fish as well as urine from animals, can be used as carbon source. In a limited-resource-region like Sahel in Sahara desert (Bernardi, 2012), urea from

urine and calcium possibly from bones and milk are used to produce bricks together with sand and soil bacteria as building material. Using urine is under debate because of the related sanitary problems (water pollution, health risk). Chemical reagents (like calcium chloride, calcium acetate) are used as calcium source. Cheng et al. (2014) successfully used seawater as calcium source. Liang et al. (2019) proposed to use kitchen waste (oyster shells, scallop shells and eggshells) instead of pure reagents. Though these usages of waste are promising, attention should be paid to the problems like sanitary problems or secondary pollution.

In recent years, some researchers started to use enzymes directly instead of bacterial solutions to achieve the process of bio-cementation. Some studies indicate that the growing cells give better results in enhancing soil properties than dead and resting cells (Chou et al., 2011). However, many aspects related to this no-cell method should be considered, like high cost, relatively sensitive enzymes compared to live cells, etc.

3. Factors influencing the fabrication of bio-cemented soils

In order to better understand the mechanisms and maximise the efficiency of MICP, a large amount of experiments has been designed considering various factors that influence the cementing process. In Figure 1, comprehensive factors involved in different steps of the process were given. In short, these factors can be summarised as (i) factors related to bacteria and cementation solution (strain source and type, nutrient, cell concentration, oxygen availability, aqueous environment, pH, temperature), (ii) factors related to soil (size distribution, density, saturation degree), (iii) factors related to the fabrication of bio-cemented soils (injection rate and mode, retention time, number of cycles). Since urea hydrolysis is not notably inhibited by the concentration of ammonium within the range of mostly used concentrations of cementation solution in various studies (Lauchnor et al., 2015), the ammonium concentration does not appear as a main factor in this review. In this part, only the major factors in each aspect were chosen to clarify their effects on MICP.

3.1. Factors influencing microbial activity

Controlling biological activity provides a way to control the timing, rate and spatial distribution of chemical reactions (DeJong et al., 2010). Obtaining the maximum biomass and enzyme activity and fixing the bacteria at the desired place is vital to assure the final success of MICP.

3.1.1. Bacteria concentration

Usually, the late exponential phase (early steady phase, when the number of bacteria becomes stable) of bacteria growth is adopted by most researchers. Hindered by organic matters and continuously formed precipitates, it is unpractical to monitor the number of bacteria during MICP reactions in porous medium. Knowing the input number of bacteria in the system is necessary. OD_{600} is the optical density of the biomass measured at 600 nm wavelength using ultraviolet-visible spectrophotometer. The OD_{600} of the bacteria solution is usually used to characterise the input biomass in MICP studies. Sometimes, the value of OD_{600} is converted into cells/mL by the following equation for *S. pasteurii* (Okwadha & Li, 2010),

$$C_{(cells.mL^{-1})} = 8.59 \times 10^7 \times (OD_{600})^{1.3627}$$

Some authors also use other microbiological methods to quantify bacteria concentration, like the plate count method, using cfu/mL (colony forming units per mL) to represent bacteria concentration (Soon et al., 2014).

Van Paassen et al. (2009) used an initial $OD_{600} = 1.583$ to achieve cementation of a 5 m column. Mirmohammad Sadeghi et al. (2015) used four OD_{600} values (0.75, 1.5, 2.5 and 4) to conduct experiments. A huge difference was seen between 0.75 and 1.5 and small differences between 1.5, 2.5 and 4. Therefore, these authors recommended a value of 1.5 for large-scale applications. Zhao et al. (2014) used OD_{600} ranging from 0.3 to 1.5 (0.3, 0.6, 0.9, 1.2, 1.5), and observed increases in unconfined compression strength (UCS), from 100% (0.44 MPa) for 0.3 to 300% for 0.6, 337% for 0.9, 424% for 1.2 and 478% for

1.5. Okwadha and Li (2010) used several concentrations of bacteria (10^6–10^8 cells/mL) and found that the 10^8 cells/mL concentration was optimal, with a 30% $CaCO_3$ increment.

During the cementation process, a greater influence on the efficiency of MICP was seen when increasing the amount of cells (8.5×106, 7.5×107, 2.3×108) rather than the initial concentration of urea (333 mM and 666 mM) (Okwadha & Li, 2010). This means that injecting more bacteria to increase the rate of ureolysis is more efficient than providing more urea to the system during MICP. Similar results were obtained by Mirmohammad Sadeghi et al. (2015). Nonetheless, a high concentration of bacteria (OD_{600} over 2) does not provide a significant improvement compared to a relatively lower concentration.

3.1.2. Urease and its activity

Enzyme content is not always proportional to biomass (Whiffin, 2004). Bacteria will release their enzymes when confronted with depletion of nutrients (van Paassen, 2009) and diluted in saline solution (9 g/L NaCl) (Harkes et al., 2010). Therefore, biomass concentration is not the appropriate parameter to quantify urease activity. Thus, to achieve repeatability, urease activity must be controlled before injection. It is obvious that, with a higher urease activity, more precipitation can be obtained if other conditions are favourable. In the majority of the studies, urease activity is always measured and calculated according to Whiffin's method before injection (Whiffin, 2004). Urease activity is equal to the slope of the conductivity change curve according to time in the first five minutes of measurement. And the specific urease activity is calculated as follows,

$$specific\ urease\ activity = \frac{urease\ activity\left(mM\ urease\ hydrolysed.\ min^{-1}\right)}{biomass(OD_{600})}$$

A certain amount of biomass can provide sufficient urease for MICP process. Zhao et al. (2014), using a bacteria solution with $OD_{600} = 0.6$, concluded that a urease activity equal to 5.5 mM hydrolysed urea/min/OD_{600} was efficient. Al Qabany et al. (2012), using a bacteria solution with OD_{600} ranging from 0.8 to 1.2, found that this guaranteed a high urease activity (5–20 mM urea/h).

Urease activity drops quickly (from 90 mM urea/h in the first 24 hours to 30 mM urea/h between 24 and 48 h), possibly because of the increasing amount of precipitation and the reduction of bacteria and pore space (Whiffin et al., 2007). van Paassen (2009) found that urease activity dropped to less than 5 mM urea/h (for an initial $OD_{600} = 1.583$, without nutrients injection) after 20 days due to hydraulic constraints (encapsulation of bacteria in small pore spaces or generated precipitation, smaller available volume of cementation solution) and starvation (less biomass). A re-injection of bacteria can help to maintain the activity for another 20 days. After six to eight steps of injection, a drop in the pH of the effluent (from 9 to 8) was observed, indicating a decreasing activity of bacteria. Feng and Montoya (2016) also re-injected a small dose of bacteria suspension (2 mL) to maintain urease activity. Urease activity can also influence the crystal type and shape of $CaCO_3$.Van Paassen prepared MICP samples for XRD and SEM analysis. Results showed that, for urease activity increasing from 9 to 36 mM urea/h, vaterite content increased from 5 to 90%. With urease activity higher than 30 mM urea/h or lower than 10 mM urea/h, spherical crystals of vaterite or rhomboidal crystals of calcite were formed separately (van Paassen, 2009). However, there is much more that needs to be understood of this aspect.

3.1.3. pH and temperature

pH and temperature have a direct bearing on the growth and urease activity of the bacteria. *S. pasteurii* is sensitive to pH and temperature during the cementation process as some studies have shown (Kim et al., 2018; Sun et al., 2019). pH and temperature also have impacts on the equilibria of dissolution and precipitation during MICP process. Here we mainly talk about the influence of microbial activity caused by these parameters. pH has a crucial biochemical effect on the activity of urease produced by *S. pasteurii* (Whiffin, 2004). Optimal pH for bacteria growth and urease activity are not the same. For cultivation of the bacteria, the optimal pH is around 9, while the optimum pH for urease activity is usually near neutral for *S. pasteurii* (Mobley et al., 1995). According to Whiffin (2004), pH in the range of 6.25–7.7 gives a urease activity higher than 40 mM urea/min and the maximum (around 43 mM urea/min) occurs around pH = 7. Cheng et al. (2014) found that pH lower than 3.5 and higher than 9.5 is adverse to the cementation process. The experimental results of Omoregie et al. (2017) showed that the pH range 7.5–8 was the

optimal one for the urease activity of five *S. pasteurii strains*. During MICP process, Stocks-Fischer et al. (1999) determined that MICP starts at pH = 8.3 and its rate increases up to pH 9. Kim et al. (2018) studied the effect of the pH (in the range of 6–10) of an urea-$CaCl_2$ solution and found that pH = 7 was the optimal condition for biocementation.

Temperature affects microbial growth and urease activity. Bahmani et al. (2017) studied the urease activity of *S. pasteurii* at different temperatures (10, 15, 21, 35, 50, 60 and 80 °C), and found that urease activity increased with temperature up to an optimum temperature of 60 °C. During the process of cultivation of bacteria, the optimal temperature for different strains of *S. pasteurii* to reach the maximum specific urease activity is 25 °C or 30 °C, e.g. for DSMZ 33 the optimal temperature is 30 °C (Omoregie et al., 2017). Cheng et al. (2014) found that increasing temperature could increase the production of calcite; however, the strength was smaller than that obtained at room temperature. For the cementation of relatively coarse materials (1–3 mm), a moderate temperature of 20 °C was optimal (Mahawish et al., 2018). In Sun et al. (2019) study, 30 °C resulted in the highest rate of $CaCO_3$ precipitation. Kim et al. (2018) studied the influence of temperatures between 20 and 50 °C and found that 20, 25, 30 °C were the optimal temperatures for different strains of *S. pasteurii*.

3.2. Soil properties

Soil properties, such as density, grading, saturation, have a vital impact on bio-treatment efficiency. Studying soils with different characteristics are beneficial to understand the use of MICP in various sites.Soil samples preparation should consider the aim of the research. Studying the effect of soil characteristics makes the protocol more feasible and efficient in varying conditions of geological sites. Some of these parameters are considered below.

3.2.1. Soil density

Density of sand has a great impact on its mechanical behaviour. The density state is also characterised by the relative density D_r, calculated by:

$$D_r(\%) = \frac{e_{max} - e}{e_{max} - e_{min}}\%$$

where e_{max}, e_{min} represent the standardised maximum and minimum void ratios, and e, the actual void ratio of the sand. For similar MICP treatments, increasing density (40%, 70%, 80%) resulted in a reduction of $CaCO_3$ production and an increase in strength (Rowshanbakht et al., 2016). Bahmani et al. (2017) conducted a series of experiments with various soil densities (1.86, 1.93, 2.11, 2.23, 2.36 gr/cm^3, corresponding to relative densities of 0%, 17%, 56%, 78%, 100%). Results indicated that the treated soil sample with a density of 2.11 gr/cm^3 had the highest value of stiffness and compressive strength. It shows that the highest density does not necessarily lead to the highest strength. Rowshanbakht et al. (2016) used poorly graded silica sand (D_{max} = 0.4 mm, C_u = 1.46, C_c = 0.83, D_{50} = 0.2 mm) with no shape description, Bahmani et al. (2017) used poorly graded angular to sub-angular quartz grains (D_{max} = 1 mm, C_u = 2.2, C_c = 0.77, D_{50} = 0.18 mm). Both of them used ASTM Standards. The results obtained by Rowshanbakht et al. (2016) and Bahmani et al. (2017) are conflicting, maybe because Bahmani used a sand with a higher fines content (20% < 0.1 mm) whereas, in the study of Rowshanbakht et al., the fines content (<0.1 mm) was 1%. When the relative density increases from 56% to higher values, the smaller pore throats inhibited the transport of bacteria, thereby decreasing the efficiency of MICP.

Gao et al. (2019) used Ottawa sand (ASTM poorly graded round quartz sand), with grain sizes ranging from 0.2 to 0.5 mm, and a mean size of 0.36 mm. For loose (D_r = 30%) and medium dense (D_r = 50%) sands, a light bio-treatment gave a strength improvement comparable to, or exceeding, that of untreated dense sand (D_r = 90%) (Gao et al., 2019). Xiao et al. (2019) applied cyclic loadings to MICP-treated calcareous sand (angular, with no fines, D_{10} = 0.19 mm, D_{50} = 0.38 mm) and untreated sand with different relative densities (10%, 50%, 80%) and different degrees of bio-cementation. Comparing treated and untreated sands, with the same increment in dry unit weight, they showed that treated sand samples had gained a larger increase in cyclic resistance, which indicates that the MICP treatment method is more efficient in promoting cyclic resistance of calcareous sand than densification.

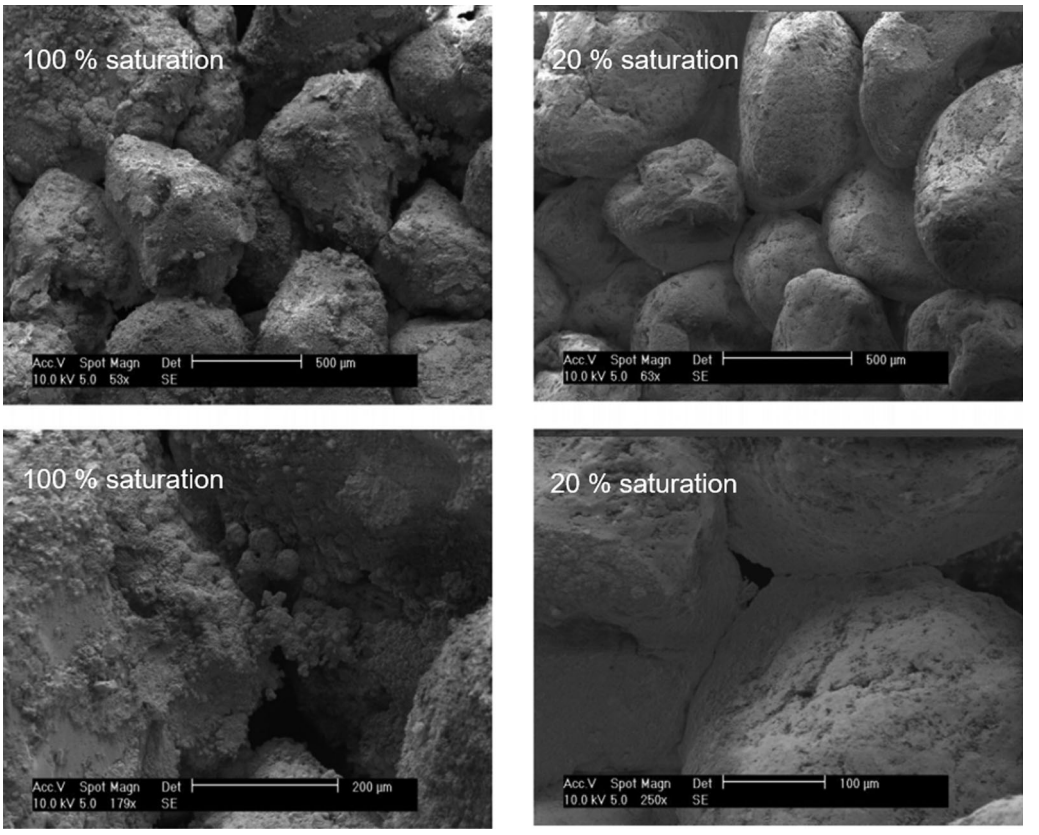

Figure 2. Scanning electron microscopy images of soil at different degrees of saturation after MICP treatment (Cheng et al., 2013).

3.2.2. Particle size

Many studies have been carried out using sands (e.g. Ottawa silica sand, Fontainebleau sand) with grain diameters smaller than 2 mm (Choi et al., 2016; Gao et al., 2019; Hamdan et al., 2013; O'Donnell & Kavazanjian, 2015; Zhao et al., 2014). Under the consideration of free passage of bacteria, as well as limit of injectability in-situ, very fine grains are usually not used. For example, DeJong et al. (2006) used Ottawa 50–70 sand to represent loose natural deposit, which is sufficient for the bacteria in the size range of 1–3 µm. Bahmani et al. (2017) used a soil with a particle size ranged between 50 and 400 µm, which was sufficient for the transportation of bacteria. Hataf and Jamali (2018) tried to determine the maximum fine content (i.e. in a clay with low plasticity) that did not influence the effect of MICP. For that, a fine-grained soil (100% finer than 75 µm, 25.6% finer than 2 µm) and a coarse-grained soil (0.4 mm–5 mm) were mixed at different percentages, and consolidated drained direct shear tests were carried out before and after the MICP treatment. Results showed that the higher the fine content , the lower the strength increase due to MICP. A fine content up to 20% did not affect the efficiency of MICP. Few studies include larger grains in soil preparation. However, in the study of Mahawish et al. (2018), Pakenham Blue Metal (Old Basalt) coarse grain (2.36–16 mm) and relatively fine grains (0.075–9.5 mm) were mixed at different percentages to conduct column cementation experiments. As a result, in comparison with other groups of materials, materials with 25% fine grains resulted in a better distribution of $CaCO_3$ and a relatively higher value of unconfined compressive strength.

3.2.3. Saturation degree

In the literature, it has been proved that a decrease in the saturation degree (by a few percents) can dramatically increase the effect of MICP on small strain stiffness (He et al., 2014) during undrained loading, with a marked increase in cyclic resistance. He et al. (2014) used a denitrifying bacteria (i.e. that produces

N_2 gas) to lower the saturation degree (100%–87.5%) in undrained soil samples (100 or 140 mm long, $\Phi = 50$ or 70 mm, $D_r = 9$–10%). They used Ottawa sand (round poorly-graded quartz sand) with 0.4 mm mean diameter. As a result, a considerable increase in undrained liquefaction resistance and a substantial reduction in pore water pressure was observed after MICP treatment, compared to saturated samples. The authors concluded that gas bubbles acted as pressure buffer to abate the increasing pore water pressure and thus enhanced liquefaction resistance. As the gas was produced, MICP concentration increased due to the decreasing liquid volume, which might also lift the efficiency of precipitation and enhance liquefaction resistance. Cheng et al. (2013) achieved different saturation degrees in a MICP-treated soil column (160 mm long, $\Phi = 55$ mm) by using a vacuum pump to control the volume of solution remaining in the sample. It came out that, for a certain amount of $CaCO_3$ produced, a higher strength was obtained with a decrease in the saturation degree (from 100% to 80%, 40% and 20%). To obtain similar strength, MICP-treated samples with a 20% degree of saturation needed around 1/3 $CaCO_3$ content with respect to MICP-treated saturated samples. Figure 2 shows that $CaCO_3$ bonds mainly occurred at soil particle contact because of the restricted meniscus- shaped distribution of MICP solution on the basis of unsaturated soil mechanics theory (Cheng & Cord-Ruwisch, 2012) in the sample at 20% degree of saturation. This means that the efficiently distributed $CaCO_3$bonds gave a significant strength enhancement for a smaller quantity of calcium carbonate produced. By contrast, in the fully saturated MICP-treated sample, most of the $CaCO_3$ bonds were located on the surface of the soil particles.

3.3. Cementation solution

In the MICP protocols, the cementation solution (CS) certainly provides the basic chemicals for MICP process (as urea and Ca^{2+} source). Sometimes, it also includes components like pH stabiliser ($NaHCO_3$), a carbon source or nutrients (nutrient broth, yeast) to maintain the bacteria. Concentration of CS refers to the concentration of urea and Ca^{2+} in the CS. It is an important parameter when designing a MICP protocol (DeJong et al., 2013). Many authors have conducted laboratory experiments by using different concentrations of CS (either equimolar or non-equimolar concentrations of urea and Ca^{2+}, usually < 2 M)to different soils, and tried to find out the optimal concentration for their experimental conditions.

3.3.1. Equimolar CS

Many researchers have used CS with equimolar urea and Ca^{2+} to conduct experiments. Lee et al. (2012) concluded that the MICP process was improved with an increasing concentration of CS up to 0.5 M, whereas the improvement was less important for the concentration of 1 M. De Muynck et al. (2010) concluded from their study that 0.425 M was the upper limit dosage for the improvement of MICP. Higher dosage had an inhibiting effect. Zhao et al. (2014) came to the conclusion that, for concentrations ranging from 0.25 to 0.5 M, the unconfined compression strength (UCS) increased 10 times compared to a 2-times increase from 0.5 M to 1.5 M. Al Qabany et al. (2012) compared two series of SEM images of treated samples with 0.5 M and 0.25 M CS, with the same injection rate. Thicker, larger and more heterogeneous distributions of precipitation crystals were produced by using 0.5 M CS. They also found that a higher concentration (1 M) could change the calcite precipitation pattern. New calcite precipitates preferentially on existing crystals instead of forming nucleation in new sites, which gives bigger crystals. These bigger crystals occupy the pore space and hamper the metabolic process of bacteria when the soil is relatively fine, resulting in higher risks of partial clogging and presenting an inhibiting effect on MICP. Reasons for the inhibiting effect of higher CS concentration can be attributed to the enzyme amount that gives a limited urea hydrolysis rate, which influences the MICP efficiency (Whiffin, 2004). Mahawish et al. (2018) successfully used higher concentrations of CS (1 M) to cement coarse materials (1–3 mm) that require larger size and amount of precipitates to attain good results.

3.3.2. Non-equimolar CS

Some authors tried to improve MICP effects by using non-equimolar CS. Mahawish et al. (2018) found that non-equimolar CS (e.g. 0.5 M urea and 0.25 M Ca) promised higher amount, larger crystals and more homogenous distribution of $CaCO_3$, and also larger compressive strength, while using much more concentrated solutions (2.0 M urea, 1.0 M Ca or 1.5 M urea, 1.5 M Ca^{2+}) produced a larger amount of $CaCO_3$,

but a lower compressive strength (Mahawish et al., 2018). Increasing only the urea concentration of the CS can also increase the efficiency of MICP. However, if the urea content in the CS is increasing more than the amount that is sufficient for precipitation process, the efficiency stops to grow. Only increasing the Ca^{2+} concentration of the CS from 0.025 M to 0.25 M can provide more than 100% of the amount of $CaCO_3$ (Okwadha & Li, 2010).

3.4. Injection mode and rates

3.4.1. Injection methods

Injection methods are quite different from one study to another. The main injection methods are presented below,

3.4.1.1. Mixing before injection. Mixing the bacteria and CS before injection gives rise to an instant reaction, producing $CaCO_3$ precipitation and bacteria flocculation immediately. This injection method is appropriate for the treatment of coarse materials (van Paassen, 2009) that need higher reaction rates and larger amounts of precipitates. And it is also used in surface stabilisation, because it only needs to cement the soil to a limited depth. Because this method needs less injection time, it makes the process easier and reduces cost in real works. In recent studies, this method has been improved to prevent the occurrence of an immediate reaction by prolonging the lag period of the reaction. It has been applied successfully to lab column experiments, either by lowering concentration of bacteria and adjusting the initial pH of the mixture to pH4 (Cheng et al., 2019), or by refrigerating the bacteria and CS at low temperature (4 °C) before mixing (Xiao et al., 2019).

3.4.1.2. Percolation. This method is easy to perform and suitable for stabilisation of the soil surface. A limited depth can be reached by using this method. Cheng and Cord-Ruwisch (2012) achieved the treatment down to 1 m depth with a reasonable degree of homogeneity by using the percolation method.

3.4.1.3. Two-phase injection (by first injecting the bacterial cell solution followed by the CS). This method is expected to prevent clogging and give a more homogeneous distribution of $CaCO_3$ crystals (Whiffin et al., 2007). It is widely used by many researchers.

3.4.2. Retention time

The time intervals during the different phases of a test must be long enough to ensure sufficient reaction process, but not too long to guarantee substantial bacterial activity. Usually there are two retention times that are used during the MICP process, one between the injection of bacteria and the injection of the CS, and the other one after the injection of the CS to allow cementation to occur.

After injection of bacteria, a retention time is needed before injecting the CS, so that the bacteria in the column will have time to distribute and fix on the surface of the soil. Retention time for bacteria solution should be decided by the results of preliminary experiments. When the injection concentration of bacteria is low, a longer retention time will be needed for the bacteria to grow to a certain amount (i.e. providing a sufficient urease activity of 5–20 mM/h) in the column.

Retention time after injection of the CS requires the accomplishment of cementation process. It depends on the reaction time of the chemicals and can be estimated according to the concentration of the CS. Al Qabany et al. (2012) found that either a 1 M CS with 24 h retention time, or a 0.5 M CS with 12 h retention time, or a 0.25 M CS with 6 h retention time, representing the same CS content injected, were equivalent to obtain high efficiency of the MICP process. These three concentrations of CS with corresponding retention times can all give a significantly large efficiency (over 80%, and up to 100% injected chemicals precipitating as $CaCO_3$) in producing $CaCO_3$.

3.4.3. Injection rate

Injection rate plays an important role in the distribution of bacteria and precipitates, thus influencing the homogeneity of the treatment. Dynamic interactions among the rate of urea hydrolysis, retention time

and the flow rate of CS need to be considered to achieve homogeneity and required strength. Pulse injection (i.e. injecting a certain amount of CS into the soil and giving a rest time for the reactions) has been proved to be more efficient than continuous injection (Al Qabany et al., 2012). Many studies used this injection-retention process repetitively for MICP treatment.

For strengthening soil surface, injection is usually realised by surface percolation. For ground improvement, Whiffin et al. (2007) used 0.35 L/h (for a column 5 m long, $\Phi = 66$ mm), Mortensen et al. (2011) used 10 mL/min (column: 100/50 mm long, $\Phi = 50$ mm), Cheng et al. (2013) used1 L/h (column: 160 mm long, $\Phi = 55$ mm). They all obtained good cementation results in their samples of various sizes (<1 mm) with the mentioned injection rates. To make the results clearer, seepage velocity (the velocity through the bulk of the porous medium) is calculated using the following equation to unify the units, $v = Q/A$, where v is the seepage velocity (m/day), Q is the total volume flowing through the corresponding cross-sectional area per time unit (m^3/day), A is the cross-section area of the flow (m^2). The results are 2.5 m/day, 7.3 m/day and 10.1 m/day for Whiffin et al. (2007), Mortensen et al. (2011) and Cheng et al. (2013), respectively. Whiffin et al. (2007) concluded that relatively low flow rates (<10 m/day) were desirable. However, if the urea hydrolysis is quite fast, to prevent clogging near the inlet, a higher injection rate is expected to deliver precipitates to further locations.

3.4.4. Numbers of injections

Feng and Montoya (2016) defined the cementation level by the mass percentage of precipitate: a value below 1.5% represents light cementation, a value between 1.5 and 3.5% represents moderate cementation and above 3.5% represents heavy cementation. They achieved these different levels of cementation by injecting a solution (333 mM urea, 374 mM NH_4Cl, 50 mM $CaCl_2$) around 10 times, 20 times and 40 times for light, moderate and heavy cementation of samples (145 mm long, $\Phi = 72$ mm), respectively. In practice, it should be noted that a larger number of injections might cause higher risks of clogging, and also increase costs of operation. In the next study, Feng and Montoya (2017) found that, for a similar cementation content, the samples behaved differently under cyclic loading, indicating that this parameter alone (for a given concentration of reagents) is not sufficient to choose the number of injections to characterize the cementation level. Another parameter is necessary, such as the shear wave velocity derived from bender elements measurements. Montoya et al. (2013) set target shear wave velocities of 300 m/s, 650 m/s and 1200 m/s to represent light, moderate and heavy cemented samples, respectively. This is a range of values going from soil-like behaviour to rock-like behaviour.

3.4.5. Injection of fixation solution

The adsorption rate of the input biomass on the pore surface and the movement of bacterial cells in pores affect MICP efficiency and homogeneity. Factors of transportation and adsorption of bacteria on the soil surface have been studied a lot, including physiological characters of microorganisms (size, surface charge, hydrophobicity …), physical and chemical properties of pore water (pH, salinity, etc.) and properties of the porous medium itself (pH, water content, mineral composition, texture and particle size distribution, etc.) (Abu-Ashour et al., 1994).

Whiffin et al. (2007) achieved the consolidation of a 5 m column by injecting a 50 mM $CaCl_2$ solution to immobilise the bacteria after bacterial injection. Harkes et al. (2010) compared the injection of different compositions of the fixation solution (50 mM $CaCl_2$ solution, deionised water, fresh surface water, 9 g/L NaCl solution and cementation fluid), right after the injection of the bacterial suspension or maintained for 2 hours before injection of the cementation solution. The size of the soil column was 6.6 cm in diameter and 18 cm in length. Results showed that transportation of bacteria was enhanced, i.e. large amounts of bacteria were removed from the soil, when injecting a fixation fluid with low ionic strength (deionized water or fresh surface water). On the contrary, with the injection of a high ionic strength solution (50 mM $CaCl_2$, NaCl solution and cementation solution), adsorption of bacteria on the soil was enhanced. The aim of injecting a fixation solution is to enhance adsorption of bacteria and to distribute bacteria evenly in a desired way. It can both mobilise (enhance transport) or immobilise (enhance absorption) the bacteria in the soil. This could be partly explained by the classical Derjaguin–Landau–Verwey–Overbeek (DLVO) theory, i.e. that the stability of colloids (bacteria are bio-colloids) depends on the electrostatic repulsive forces (caused by the electrical double layer) and attractive van der Waals forces. High concentration of

fixation solution will provide a high ionic strength, which compresses the electrical double layer and lowers the repulsive electrostatic force. At that time, the attractive forces (Van Der Waals forces) are the primary forces, resulting in enhancement of adsorption and adhesion of bacteria to the porous media (Adamczyk & Weroński, 1999; Okwadha & Li, 2010). Chu et al. (2014) injected fixation solutions with different valences (Ca^{2+}, Fe^{3+}, Al^{3+}) before injecting bacteria (isolated from tropical beach sand, representative of genus *Bacillus*). The adsorption of bacteria was obviously enhanced by injecting different fixation solutions (20–30% increment), compared to only injecting water. The increasing effect among the three fixation solutions is similar. The authors suggested that the increase in the number of positively charged sites on soil surface enhanced adsorption, in spite of the strength of the bonds. It is known that iron is essential to microbial metabolism, while the interactions between the bacteria and the ferric ions were not taken into account by the authors. In Mortensen's study (2011), similar size ($D_{50} = 0.12$ mm) of quartz sand and sand rich in iron oxide treated by the same MICP process led to similar shear wave velocity increases. The results showed that the presence of iron oxide might have little influence on MICP process. For another biocementation system using ion-reducing bacteria, ferric ions can be reduced to ferrous ions, and precipitates like undissolved ferrous compounds are generated (Ivanov & Chu, 2008).

The above-mentioned results show that using a fixation solution can help to enhance the efficiency of MICP. Considering the different bacteria strains used and the various soil environments, preliminary experiments are required to obtain better results.

4. Conclusion and future expectation

The optimisation of the MICP protocol is of much concern to promote efficiency, economise reagents and simplify operations. In the light of all the results summarised from various research teams, the following conclusions can be drawn,

- For the cultivation of *S. pasteurii*, under the conditions of a pH equal to 9 and an ambient temperature equal to 30 °C, a large quantity of biomass can be obtained. And for the cementation process, a pH ranging from 7 to 8, and a temperature around 25 ± 5 °C are optimal conditions for high urease activity and precipitate production.
- A wide range of values of concentration of bacteria (corresponding to OD_{600} between 0.1 and 4) has been successfully used in various studies. A concentration range of OD_{600} values from 0.6 to 1.5, which promises a urease activity value over 5 mM urea/h, can yield a reasonable amount of cementation. It has been proved to be efficient in samples scaling from several tens of centimetres to several meters.
- To achieve a more homogeneous bacterial distribution and enhancing bacteria adsorption, a predesigned fixation solution can be used.
- In real applications, the soil is imposed, and the treatment must be adapted to the soil. Loose and medium dense soils can behave like a dense soil after treatment. Less than 20% of fine particles (<75 μm) in 0.4–5 mm soils and less than 25% medium-fine grains (75 μm–9.5 mm) in 2.36–16 mm soils were found to have no influence on bio-treatment.
- When the soil is not saturated, the bio-cementation method can give a more efficient precipitation distribution by precipitating mainly at particle contacts, which promises a larger gain in strength with a lighter treatment.
- To find an appropriate concentration that can be used in the field, beside the soil characteristics, not only efficiency (higher conversion ratio) but also cost balance (injection operation, CS and bacteria concentration) should be taken into account. Low concentration (0.2 M) of CS solution may give high efficiency in using reagents but it needs larger injection times, which does not sound cost-effective for large scale use. A concentration of 0.5 M can give a high efficiency of the calcification process and requires less injection time. For bio-cementation of soils with a relatively high content of large size grains, concentration can be raised up to 1 M to improve efficiency.
- Factors of injection depend on the value of the above parameters, and also on the site and expected mechanical properties. In practice, reducing the number of injections can be more feasible and reduce significantly the cost.

This article discussed the factors separately to understand the effect of each factor. It should be noted that MICP is a comprehensive process affected by the combined effects of all these factors. For establishing a high-efficiency and low-cost protocol, it is hard to give a unified solution in the variety of possible conditions. Nevertheless, the above indications can help to choose values for designing experiments. All the parameters and their interactions should be taken into account and it seems necessary to carry out preliminary tests to choose specific values for operational conditions and purposes.

Acknowledgements

The authors would like to thank the support of China Scholarship Council (CSC). They are also grateful for assistance from Solétanche-Bachy.

Disclosure statement

No potential conflict of interest was reported by the authors.

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