

# Review of the Factors That Influence on the Microbial Induced Calcite Precipitation

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## Abstract

Microbial Induced Calcite Precipitates (MICP) is a new and sustainable technology used to improve the properties of construction materials. This technique works by introducing bacteria solution (e.g., *Sporosarcina pasteurii*, *B. megaterium*, *Spolooactobacilus*, *Clostridium* and *Desulfotomaculum*) into the soil matrix, and then injection of a chemical solution consisting of urea and one of calcium salts (e.g., calcium chloride and calcium acetate) into the soil matrix several times. A number of factors must be considered to enable the use and control of the MICP process in field applications, including the concentrations of bacteria solution, the concentrations of the chemical solutions, in addition to methods to introduce the bacteria and these chemical solutions to the soil. The main aim of this research is to provide an overview of the various factors affecting the MICP within the soil, where the research studied the effect of bacteria, soil particle size, nutrients, chemical solutions, pH, temperature and injection strategies on the efficiency of MICP as a method to improve the chemical and mechanical properties of the soil.

**Keywords:** MICP, Bacteria, Nutrients, Chemical Solutions, pH, Temperature, Injection Strategies, Soil Particle Size

## 1. Introduction

In the last two decades, new and sustainable technology appeared to improve the mechanical and physical properties of construction materials, this technique is called Microbial Induced Calcite Precipitates (MICP). MICP has great potential for use in many engineering applications. The researchers suggested use MICP in biomineralized concrete ( i.e. biological mortar, remediation of cracks in concrete and Self Healing) (Castanier, Levrel et al. 1995, Bang, Galinat et al. 2001, Oriol, Vieweger et al. 2002, Ghosh, Mandal et al. 2005, Jonkers and Schlangen 2007, Ramakrishnan 2007, De Belie and De Muynck 2008, De Muynck, Cox et al. 2008, De Muynck, Debrouwer et al. 2008, Achal, Mukherjee et al. 2010, Achal, Mukherjee et al. 2010), reductions in foundation settlement (DeJong et al. 2010), soil stabilization prior to tunnelling construction (J.T. DeJong et al. 2006, J.T.Waller et al. 2009), wastewater treatment (Hammes et al. 2003), liquefaction mitigation (DeJong et al. 2006, Montoya et al. 2012), improvement in the stiffness/strength of sandy soil (Rong et al. 2012, Van Paassen 2009, Whiffin et al. 2007), reduction in soil permeability (Nemati et al. 2005, Dennis and Turner 1998, Seki et al. 1998 ), dust control (Meyer et al. 2011), slope stabilization (J.T.DeJong et al. 2009), piping prevention for dams, levees (J.T.DeJong et al. 2009 ).

Since MICP is a process that rely on the chemical and biological reactions there will be numerous factors influence it. Kile et al. (2000) reported that MICP process is regulated mainly by four key factors: (1)concentration of calcium ion; (2) availability of nucleation sites; (3) concentration of dissolved inorganic carbon; and (4) pH. Hammes (2003) reported that calcite creation by bacteria is ruled by four factors;

(1) the concentration of calcium ions, (2) the concentration of carbonate ions, (3) the pH of the environment and (4) the existence of nucleation sites. Also, some researchers have focused on the other factors affecting on the MICP process. Al Qabany et al. (2013) focused on the input rates, retention times, and chemical concentrations , while De Muynck et al. (2010) focused on the temperature . From the above mentioned, there are several factors that influence MICP including those related to the bacteria itself , those related to the environmental parameters, those related to chemical solutions and those related to treatment way spotted.

The primary goal of this research is to provide an overview of the various factors affecting the (MICP) within the soil, where the research studied the effect of bacteria, soil particle size, nutrients, chemical solutions, PH, temperature and injection strategies on the efficiency of (MICP) as a method to improve the chemical and mechanical properties of the soil.

## 2. Factors influencing MICP

### 2.1 Bacteria

Microbial activity is considered as a major player in the creation of soil carbonate deposits and since bacteria is the only living organism found in the MICP system, so it is considered as one of the most influential factors in the precipitation process . It could affect through different parameters, and also affect different parameters.

#### 2.1.1 Bacteria types

The type of bacteria could affect the urease activity of the bacteria and consequently control the efficiency of

MICP (Okwadha and Li 2010). Most research on microbially induced calcium carbonate precipitation on limestone focused on the microbial aspects, i.e, the type of microorganism and metabolic pathway to improve the efficiency of the biodeposition treatment (De Muyncka et al. 2010). The bacteria types that are suitable for MICP application should be able to catalyze urea hydrolysis, and they are usually urease positive bacteria. The typical urease bacteria are genera *Bacillus*, *Sporosarcina*, *Sporosarcina*, *Sporosarcina*, *Clostridium* and *Desulfotomaculum* (Kucharski et al. 2012). The aerobic bacteria are preferable as they release CO<sub>2</sub> from cell respiration, and CO<sub>2</sub> production is paralleled by the pH rise due to ammonium production. *Bacillus Sporosarcina* is a more common type of bacteria used to precipitate calcium carbonate in the soil through transformation of urea to ammonia and carbon dioxide (Le Métayer-Levrel et al. 1999, Hammes 2003). Animesh and Ramkrishnan (2016) made study intends to experimentally analyse the effectiveness of use of Microbial Induced Calcite Precipitates for improving the shear strength parameters of two different types of fine soils. For this process, they used *Bacillus Sporosarcina* to catalyze the calcite precipitation. Other types of *Bacilli* used in MICP for instance *B. pasteurii* in concrete and soil improvement (Whiffin et al. 2007). Lee (2014) investigated the performances of bio-mediated soil improvement by using *B. megaterium* to trigger calcite precipitation on different types of soils. Also, N. J. Jiang et al. (2016) used *B. megaterium* to quantify the ureolytic efficiency of urease-producing bacterium and purified urease enzyme in the oxic and anoxic conditions

### 2.1.2 Bacteria Concentration

A high bacterial cell concentration supplied to the soil sample would certainly increase the amount of calcite precipitated from MICP process (Okwadha and Li 2010). The rate of urea hydrolysis is directly proportional to the concentration of bacteria. A high concentration of bacteria produces more urease per unit volume to commence the urea hydrolysis. Van Paassen (2009) reported that a high concentration of bacteria close to the injection well could result consequently a limited injection distance, especially in fine sands. It has been reported that bacterial cells are excellent sites for growing minerals throughout the creation of rock. Lian, Hu et al. (2006) identified from 30 SEM images that nucleation of calcite takes place at bacteria cell walls. Montoya (2012) reported that the greater concentration of microbes near the particle-particle contacts results in increased calcite precipitation in the soil. DeJong et al. (2006) concluded that the concentration of microorganisms is an important factor for the success of this application.

### 2.1.3 Size and shape of Bacteria

The size of bacteria potentially influences bacterial calcification. Bacteria size typically ranges between 0.5 and 3.0 μm (Mitchell and Santamarina 2005), but the length of microbial cellular filaments can be up to 100 μm, which can be an obstacle in penetration of filamentous microorganisms into soil. In the case of saturation of the pore fluid (1.0 μm size bacteria such as *S. pasteurii* could reach approximately 10<sup>8</sup> bacteria cell/mL, Mitchell and Santamarina (2005) noticed it could also cause space limitation. Transportation of microbial cells into soil depends on cell size, cell surface properties, and cell physiological state (Murphy and Ginn 2000).

## 2.2 Soil Particle Size

The size of soil pores should be sufficient to allow the transportation of bacteria with size of 0.5–3.0 μm in length (Mitchell and Santamarina 2005). The most favorable soil particle size range for bacterial activity in the pores is reported as 50 – 400 μm (Rebata-Landa 2007). The compatibility between the grain size of soil and bacteria size is an important factor for MICP treatment. The soil pores should be has adequate size to allow the bacteria movement (Mitchell and Santamarina 2005). The effectiveness of MICP on a soil can be attributed to the ability of the bacteria to move freely through the pore space and the adequate particle-particle contacts per unit volume. These situations need a balance relationship between the pore structure characteristics and the bacteria size, specifically the pore throats. Compatibility relationship between bacteria-soil type illustrate the dimensional boundaries of compatibility is presented in fig. (1). Jawad and Zheng (2016) explored the effectiveness of the MICP technique for improving the engineering properties of the poor graded fine sandy soil. They found that MICP has the ability to improve this type of soil.

## 2.3 Nutrients

The bacteria are the only organism in MICP system, which in turn needs to energy source to continue to proceed metabolic processes and thus the continuation of the precipitation. Nutrients are the energy sources for bacteria, and which provide to bacteria through both culture stage and soil treatment stage. For better precipitation of carbonates Experimental studies on the reduction of soil hydraulic conductivity of improved biomass growth in soil with dextrose-nutrient solution have appeared a positive correlation between attached microbial biomass and the soil hydraulic conductivity (Wu et al. 1997). Common nutrients for bacteria include CO<sub>2</sub>, N, P, K, Mg, Ca, Fe, etc. (Mitchell and Santamarina 2005), but these nutrients have high cost. The nutritional profile of bacterial cultures indicates a high preference for protein based media as for *S. Pasteur* (Morsdorf & Kaltwasser 1989). Other alternative nutrients to reduce the cost and protect the environment have been proposed. Lactose mother liquor and corn steep liquor are two industry waste studied by Achal et al. (2009), Achal et al. (2010)

respectively. Numerous previous reported studies have included 3 g/l of nutrient broth into the treatment solution to keep the growth and feasibility of urease producing bacteria (DeJong et al. 2006, Qabany et al. 2011).

## 2.4 Chemical Solutions

To induce urea hydrolysis and calcium carbonate precipitation in the soil in addition to bacteria, chemical solutions need to be injected to the location where improvement is required. The chemical solutions and additives used in the experiments included calcium sulfate, calcium chloride, sodium carbonate, sodium chloride, ammonia, alcohol, and sodium acetate. Most of the studies adopted Urea-calcium (urea-  $\text{CaCl}_2$ ) based cementation media were used to influence on analytic- driven calcium carbonate precipitation. The quantity of the added chemical solutions determines the difference between bioclogging and biocementation. So, at low quantity of the added chemical solutions the precipitation of calcium carbonate is going mainly in the sites of particle contacts, which is sealing the micro channels while at higher quantity of added chemical solutions precipitation will be in the pores creating high strength. De Muynck et al. (2010) examined the influence of the concentration of calcium salts and urea, on the efficiency of the biodeposition treatment. They observed the waterproofing effect for a calcium dosage of  $17 \text{ g Ca}^{2+}\text{m}^{-2}$  the water absorption was similar to that of untreated specimens, concentrations of  $67 \text{ g Ca}^{2+}\text{m}^{-2}$  resulted in a 50% decrease of the rate of water absorption. In the same vein Nemati et al. (2005) reported that the repeated injection of chemical solutions containing urea or a mixture of urea and calcium chloride increased the extent of plugging in porous media, while increases in reactant concentrations up to a certain level (urea and  $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$  concentrations: 36 and 90 g/L, respectively) increased the quantity of produced  $\text{CaCO}_3$ . Y. Zhang et al. (2014) studied the Effects of calcium sources on microbially induced carbonate precipitation, the results illustrate that the samples using  $\text{Ca}(\text{CH}_3\text{COO})_2$  as the calcium source have a higher strength and a more distribution in the pore of soil than those using  $\text{CaCl}_2$  or  $\text{Ca}(\text{NO}_3)_2$ . The crystal type of the MICP of the samples treated with  $\text{Ca}(\text{CH}_3\text{COO})_2$  is chiefly aragonite, while that of the others is chiefly calcite, fig. (2). they believed that  $\text{Ca}(\text{CH}_3\text{COO})_2$  is an appropriate alternative calcium source to replace  $\text{CaCl}_2$  for the MICP technology applied in the reinforced concrete structures.

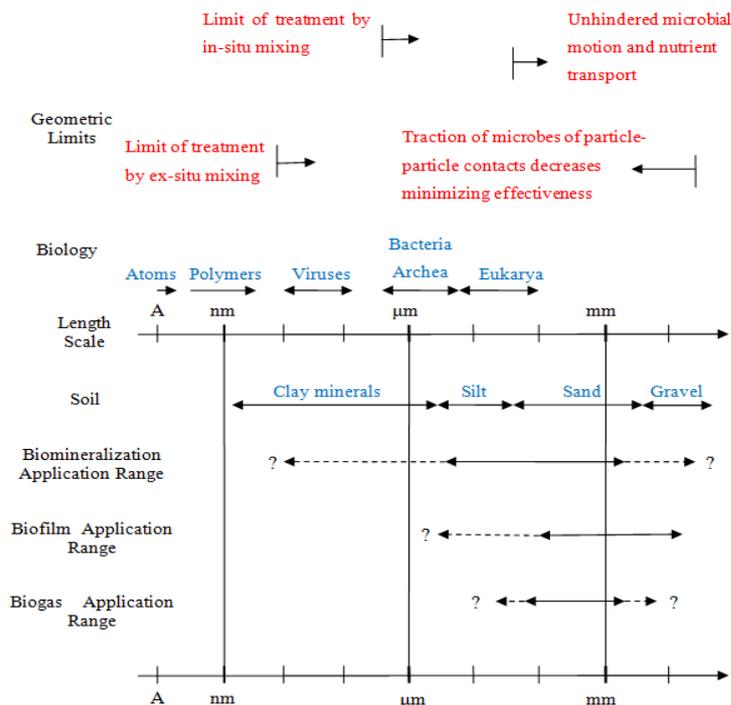


Figure 1. Comparison of typical sizes of soil particles and bacteria, geometric limitations, and approximate limits of various treatment methods (DeJong et al. 2010)

YU XiaoNiu et al. (2015) examined the influence of using barium hydrogen phosphate on the MICP treatment. Their results show that the cementing mechanism of the bio-phosphate cement is that barium hydrogen phosphate particles by microbial precipitation can form large agglomerates with each other and interact with quartz sand to produce van der Waals bonds in sandstones. H.A. Abdel Gawwad et al. (2016) studied the effect of different  $\text{MgCl}_2$  concentrations on the mechanical properties of bio-mortar. They concluded that the presence of  $\text{MgCl}_2$  in cementation solution leads to change in the crystal type and morphology of microbial precipitated mineral. Also, the presence of  $\text{MgCl}_2$  leads to the retardation of microbial precipitation

rate, producing little content of carbonate containing phases. Other researchers studied the Effect of Magnesium as Substitute Material in MICP, they used magnesium chloride as added to the chemical solutions to delay the reaction rate and to enhance the amount of carbonate precipitation (Yasuhara, Hayashi et al. 2011, Yasuhara, Neupane et al. 2012, Neupane, Yasuhara et al. 2013, Neupane, Yasuhara et al. 2015, Putra, Yasuhara et al. 2016).

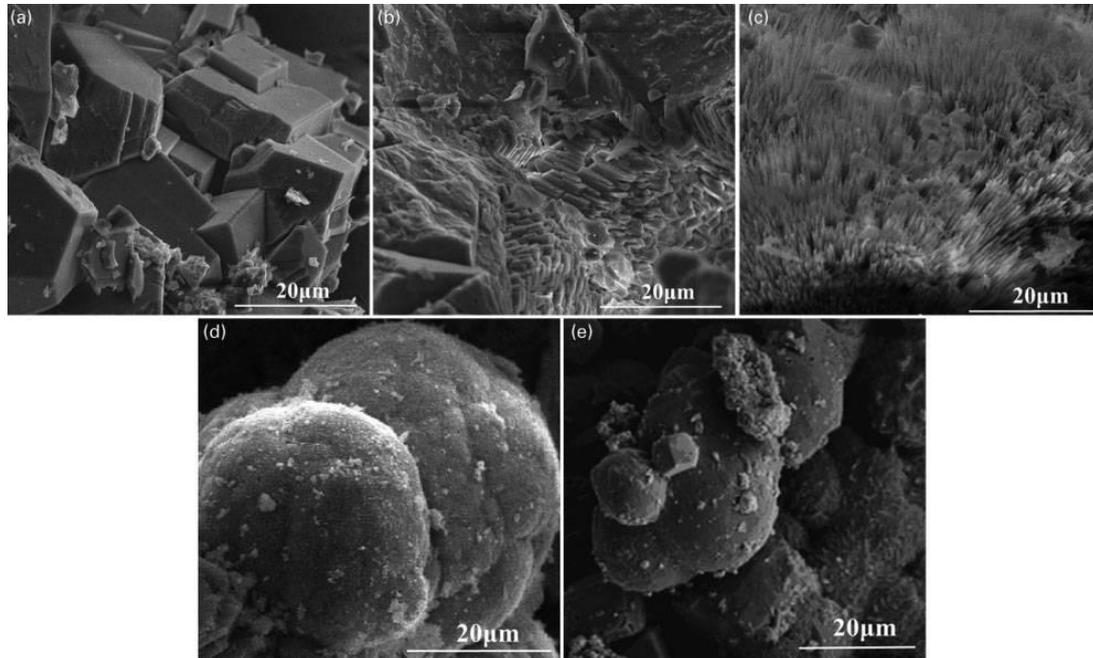


Figure 2. Images (SEM) of biogrouted samples of different calcium sources: (a) calcite (chloride sample); (b) calcite (nitrate sample); (c) aragonite (acetate sample); (d) vaterite; (e) vaterite. (Y. Zhang et al. 2014 )

## 2.5 pH

The carbonate ions concentration is concerning to the concentration of pH at MICP process. A microbial process which leads to an increase of both pH and the concentration of dissolved inorganic carbon is the utilization of organic acids (Braissant et al. 2002). Urea hydrolysis, by the urease activity of some bacteria is studied more than the other biological systems for calcite precipitation (Whiffin et al. 2007). This subsurface bacterial ureolytic activity could produce  $\text{NH}_4^+$  and bicarbonate ions and thus increase the pH, which results in  $\text{CaCO}_3$  production. Urease catalyzes the hydrolysis of urea to  $\text{CO}_2$  and ammonia, resulting in an increase of pH and carbonate concentration in the bacterial environment. Microbiologically induced carbonate mineral precipitation occurs for a pH range of 8.3–9.0, for which urease activity remains high (Stocks- Fischer et al. 1999). When ammonia is used to form calcium carbonate precipitate, the pH is controlled between 8 and 11 (Popescu et al. 2014). Stocks-Fischer et al. (1999); Ferris et al. (2004); Fujita et al. (2004); Harkes et al. (2010) performed studies using *S. pasteurii* investigated the series of events happening during ureolytic calcification asserting the importance of pH.

## 2.6 Temperature

The ideal temperatures have a good effect on precipitation of calcite by bacteria and increasing the ability of the strain to form crystals. Urease- catalyzed ureolysis temperature dependent and the optimum temperature ranges from 20 °C to 37°C. Actually, it has been reported that an increase in temperature will result in an increase in urease activity up to a temperature of 60°C (Whiffin 2004). Van Paassen (2009) indicated that if the temperature rises of 10°C over the range 5–35°C this will causes the urease activity to increase by a factor of 24. Van Paassen (2009) also referenced that no urease activity was observed for a soil temperature below 5°C. most of the studies are investigated at an ambient laboratory temperature of 20 ±2°C. Calcium carbonate of calcite type can stay stable at room temperature. Nemati et al. (2003) and Nemati et al. (2005) study the effect of temperature in MICP. They observed enhancing in both, the production rate of  $\text{CaCO}_3$  and the extent of conversion in the batch system with a low concentration of enzyme (0.02 g / l), an increase in temperature (from 20 to 50°C). With (0.03 g / l) urease, an increase of temperature (from 22 to 30°C) they observed that the extent of plugging is enhanced simultaneously the permeability ratio calculated at 30°C was 26% lower than that achieved at 22°C. In contrast, bacterial production of  $\text{CaCO}_3$  was not sensitive to temperature.

## 2.7 Injection Strategies

A suitable injection method is needed to assure Successful MICP treatment. Several ways of injection in chemical grouting can be used also for microbial grouting. The most important factor in achieving an even calcite precipitation throughout the soil mass is the uniform distribution and fixation of the bacterial cells. Several MICP injection strategies have been investigated. Mixing of the bacterial cell and cementation solutions together before injection led to immediate flocculation of bacteria and crystal growth. while this path may be taken into account for treatment of surfaces, very coarse grained materials and mixed in place applications (Le Métayer-Levrel et al. 1999) . This could lead to rapid clogging of injection point and surrounding areas pore space for many of the fine or medium sand (Whiffin et al. 2007). The Two-phase injection is another Strategy has been conducted, in this Strategy the bacterial cell solution is injected first, followed by the cementation solution (Whiffin et al. 2007). This strategy applied to prevent crystal accumulation around the injection point and led to a more homogeneous distribution of  $\text{CaCO}_3$ . Else scenario which is Staged injection has been applied by Tobler et al. (2012) to prevent excessive crystal accumulation close to the injection point from taking place and achieved a more uniform distribution of calcite crystal formation over a greater distance in the sand specimen. A fourth Strategy that was examined by Shahraki et al. (2015) which is Single-phase injection achieved improvements in stiffness and strength, but not significantly affect the drainage capacity.

## 3. Summary

Microbial Induced Calcite Precipitates (MICP) is new and sustainable technology appeared to improve the mechanical and physical properties of construction materials. Since MICP is a process that depend on the chemical and biological reactions there will be many factors influence it.

1. The type of bacteria could affect the urease activity of the bacteria and consequently control the efficiency of MICP. The bacteria types that are suitable for MICP application should be able to catalyst urea hydrolysis, and they are usually urease positive bacteria.
2. The rate of urea hydrolysis is directly proportional to the concentration of bacteria. A high concentration of bacteria produces more urease per unit volume to commence the urea hydrolysis.
3. The compatibility between the grain size of soil and bacteria size is an important factor for MICP treatment. The soil pores should be has adequate size to allow the bacteria movement.
4. Nutrients are the energy sources for bacteria, and which provide to bacteria through both culture stage and soil treatment stage.
5. To induce urea hydrolysis and calcium carbonate precipitation in the soil in addition to bacteria, chemical solutions need to be injected. The chemical solutions and additives may Consists of calcium sulfate, calcium chloride, sodium carbonate, sodium chloride, ammonia, alcohol, and sodium acetate.
6. The concentration of carbonate ions is related to the concentration of pH of a given aquatic system.
7. The ideal temperatures have a good effect on precipitation of calcite by bacteria and increasing the ability of the strain to form crystals. Urease- catalyzed ureolysis temperature dependent and the optimum temperature ranges from 20 °C to 37°C.
8. A suitable injection method is needed to assure Successful MICP treatment. There are four injection strategies. The first strategy is mixing of the bacterial cell and cementation solutions together before injection, the second strategy is the two-phase injection , single-phase injection is another strategy has been conducted and finally the staged injection strategy.

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