A critical review on soil stabilization using bacteria

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Abstract. To gratify the escalating need for land, it is vital to improve the lands with loose weak soils. These problematic loose soils can be optimized by nurturing the shear strength, lessening the time and potential for settlement and also reducing the hydraulic conductivity of soil, by using Microbial induced calcite precipitation (MICP) process. This process of MICP is carried out by adding bacterial solution into soil specimen which is continued with inoculation of cementation reagents having urea and one calcium salt (CaCl₂) for enormous times. As a result, calcite precipitate (CaCO₃) is formed in the soil and stabilize the soil. In this study, a number of factors that are responsible for better formation of calcite precipitate are examined. This process mainly depends upon soil type, bacteria species, concentration of cementation solution, injection methods and also on pH, temperature conditions and curing period. The aim of this paper is to review the main factors that influence the MICP process, such as soil properties, bacterial species, nutrients, concentration of cementation solution, pH, temperature and injection methods.

Keywords. MICP, Soil properties, Bacterial species, Concentration of cementation solution, pH, Temperature, Injection methods.

1. Introduction
In recent years, it is unassailable to commence construction work on weak soils. The weak soils are normally having less shear strength and high settlement. In order to overcome such problems, the engineering characteristics of weak soils can be enhanced. To correct this problems, it is necessary to develop the shear strength, diminish settlement and also to minimize the permeability of weak soils. The method of chemical admixtures is commonly used to enhance the engineering characteristics of weak soils by the use of different admixtures (including lime, cement, fly ash, bitumen, bottom ash and sodium hydroxide). By implementing this method, chemical admixture is either injected into the pore spaces of weak soil or mixed manually or mechanically to enhance the shear strength and minimize the settlement and permeability of weak soils.[1]

Now a day, the construction engineer's give more importance route to green and sustainable methodologies, these chemical admixtures are considered as toxic and hazardous for environment. Chemical methods are economical techniques but it pollutes the soil and ground water. Rather, soil
stabilization is carried out in a bio-technology way which is called as MICP (Microbial Induced Calcite Precipitation) since it is environmental friendly technique. This MICP technique is developed by creating a large population of urease-producing bacteria and cementation materials into the weak soil medium, whereby a bio-cement compound is produced to enhance the mechanical characteristics of the soil. This assessment study, examines the effect of different factors, in enhancing shear strength and minimizing the permeability of weak soil using MICP technology.

2. Methodology

2.1. Microbial Induced Calcite Precipitation (MICP)

Inspite of contributing large shear strength in weak soils, MICP method is still growing green and sustainable technology in enhancing other construction materials like concrete, brick and mortar. Plenty of researches and studies pertained on this topic include Soon et al. (2013), Baveye et al. (1998), Castainer et al. (1999), Mitchell and Santamarina (2005), Lian et al. (2006), Ivanov and Chu (2008), Dejong et al. (2010), Okwadha and Li (2010), Harke et al. (2010) and, Lu et al. (2010). Dejong et al. (2006) were executed as small scale laboratory studies to examine MICP on loose, weak soil samples. The conclusions show a non-collapsing strain-shear behavior with initial shear stress and ultimate shear strength than untreated samples. Basic two applications of MICP technology is, bio-clogging and bio-cementation, has been examined. Bio-clogging is the generation of void-filling gels through bacterial means so that the void space and permeability of soil sample can be minimized. Bio-cementation is the formation of soil-binding gels through bacterial biological processes in situ so that the strength characteristics of weak soil can be improved.

The most appropriate bacteria for bacteria stabilization, are aerobic and anaerobic bacteria, anaerobic urease producing bacteria, anaerobic urease producing bacteria, and obligate aerobic bacteria to be used in microbial geotechnology (Ivanov and Chu; 2008). In most of the previous studies use B. pasteurii (Bacillus sp.) bacteria is used to generate CaCO\(_3\) precipitate. Based on the previous studies made on MICP technology Lee et al., (2012), the urease enzyme developed by urease-producing bacteria (UPB), hydrolysis urea (CO\((NH_2)_2\)) through the following reaction:

\[
CO(NH_2)_2 + H_2O \rightarrow NH_4^+ + HCO_3^-
\]

The ammonium \((NH_4^+)\) ions generated in above equation will rise the pH of the soil, which creates adoptable situation for calcite precipitation \((CaCO_3)\) with the external supply of calcium ions in the form of calcium chloride and eggshell solution.

\[
Ca^{2+} + HCO_3^- + OH^- \rightarrow CaCO_3(\downarrow) + H_2O
\]

The developed end product of calcite precipitation \((CaCO_3)\) is important for the above said bio-cementation and bio-clogging technologies.

The MICP technology improves the engineering properties of weak soils through two phenomenon's known as bio-cementation and bio-clogging. The mechanisms that contribute to bio-cementation or bio-clogging in soils are (i) filling the void spaces in soil matrix with inorganic bio-cement due to the application of bacteria, (ii) bonding soil materials with inorganic bio-cement which is arbitrated by bacteria, (iii) filling in the pores and channels with bacterial biomass, (iv) forming salt bridges among soil materials arbitrated by bacteria.

2.1.1. Bio-cementation

Bio-cementation process enhance the shear strength properties of weak soils through soil binding materials which is generated by the inclusion of bacteria and cementing materials to the soil matrix.
Ivanov and Chu 2008 listed the most commonly used soil bonding compounds are carbonates, silicates, phosphates, sulphides and hydroxides.[7] Due to the availability of urease positive bacteria in nature calcite formation is most commonly adopted in bio-cementation process in BCCP technology. Dejonget al. (2010) studied the phenomenon of strength enhancement granted by CaCO₃ precipitation.[8] The calcium carbonate development results in reduction in pore space and consequently brings attention into a revision in index and engineering properties. In addition to that the precipitation of calcium carbonate within the soil matrix plays a crucial role in strength improvement of soil. DeJong et al. 2010 classified the calcite precipitation within the soil matrix into two categories namely, uniform distribution and preferential distribution.[8] Uniform distribution reveals the calcite precipitated on the surface of soil grains constantly, at an equivalent thickness. As a result, the binding created by calcite to join two soil grains is comparatively poor, and therefore negligible improvement in soil properties may be forecasted. Preferential distribution attributes to a precondition in which the calcite precipitated only at particle to particle contacts. This is the favored spatial distribution as all calcite precipitation shares directly to the improvement in soil properties.

2.1.2. Bio-clogging
Bio-clogging is a biological process which reduces the permeability of soils through bacterial processes. In bio-clogging process the soil void spaces are reduced by bio-cements which are generated by bacterial calcium carbonate precipitation processes.[13] Choi et al. (2016) studied that through the consistent mechanisms of bio-cementation and bio-clogging the calcium carbonate precipitation is generated in the soil void spaces which in turn improve the shear strength and reduce the permeability of soil.[15]

3. Factors influencing MICP
The main purpose of MICP process is to improve the shear capacity of soil matrix by production of Calcite precipitation. This production rate is greatly influenced by soil properties, Bacterial species, concentration of cementation solutions and nutrients. The external factors like pH, temperature and injection methods also have some influences on calcite production.[16]

3.1. Soil properties
The soil properties considered by utmost of the studies were grain size of soil matrix. The size of soil should be more efficient for the penetration of microbes of size ranges 0.5 μm – 3.0 μm. The size of pores in soil depends greatly upon the grain size.[5] The effectiveness of the MICP process improves when the movement of bacteria becomes frequent inside the soil pores. The most preferable grain size of soil for free movement of bacteria is specified as 50 – 400 μm. But, the compatibility relationship between bacteria size and grain size of soil is essential for efficient MICP process. The studies made by Kadhim and Zheng, 2016, Baveye et al. (1998), Castainer et al. (1999), Mitchell and Santamarina (2005), Lian et al. (2006), Ivanov and Chu (2008) shows that this process is effective for strengthening loose sandy soils.[3, 4, 5, 6, 7, 16]

3.2. Bacterial species
The MICP process is carried over primarily by microbial activity (i.e, bacterial activity), hence bacteria is stared as a significant parameter in soil stabilization. Bacteria influence this process through various parameters as follows.

3.2.1. Bacteria type
The bacteria used for MICP process should be capable of catalyzing the urea hydrolysis which favors the calcite production; hence they are commonly urease positive bacteria. The aerobic bacteria are mostly preferred for this purpose, as they release CO₂ from cell respiration, which is paralleled by pH rise due to ammonium production. The most of the urease positive bacteria belongs to the following
genera *Bacillus*, *Sporosarcina*, *Spoloactobacilus*, *Clostridium* and *Desulfotomaculum*. [17] Most common type of bacteria used for calcite precipitation by conversion of urea into ammonia and carbon dioxide belongs to *Bacillus* sp. [4, 18] The majority of the previous studies uses *B. megaterium*, *B. pasteurii* and *B. sphaericus*. [2, 14, 19, 20, 21, 22] The amount of calcite precipitation depends mainly upon type of *Bacillus* strain. [23] Kulanthaivel et al. (2020) studied that the unconfined compressive strength of low compressible clay soil (CL) and intermediate compressible clay soil (CI) treated with L.fusiformis and S.pasteurii bacteria. [24] The test results showed that S.pasteurii bacteria treated clay soils gives more strength than L.fusiformis bacteria treated soils due to high urease producing ability of S.pasteurii bacteria.

### 3.2.2. Geometry of bacteria

The geometry of bacteria greatly influences the calcite formation. The size of bacteria for this process is usually 0.5 µm – 3.0 µm [5] and the length of filament can reach up to 100 µm, which becomes a limitation for movement of bacteria inside soil matrix. But the size and geometry of bacteria depends on the soil particle size. [2]

#### 3.2.3. Bacteria concentration

A large microbial cell combination applied to the weak soil may surely hike the extent of CaCO$_3$ precipitated from MICP technology. [9] The urea hydrolysis amount is directly proportional to the cell combination of microbe. A large cell combination of microbe generates further urease to initiate the urea hydrolysis Kadhim and Zheng (2016), Li et al. (2011) and Stocks-Fischer et al. (1999) proposed that microbe cell offered as nucleation station for calcium carbonate to accelerate in biological-chemical reaction. [16, 25, 26] Lian et al. (2006) reviewed the solidification by *Bacillus megaterium*. [6] They concluded from SEM photograph that nucleation of calcium carbonate takes place at bacteria cell walls. The opportunity of nucleation spot is one of the important factors for calcium carbonate precipitation. Stocks-Fischer et al. (1999) also examined that calcium carbonate precipitation is correlated with the concentration of urease producing *Bacillus pasteurii*. [26]

#### 3.2.4 Type of cementing solution

The cementing material used in MICP technology plays a crucial role in calcium carbonate precipitation. Choi et al. (2016) studied that excess usage of calcium chloride in soil is harmful so they use eggshell as cementing solution and compare the results with calcium chloride. [15] The test data suggested that eggshell cementing solution gives higher calcium carbonate precipitation than calcium chloride solution. Liu et al. (2011) concluded that the excess calcium chloride solution in MICP technology reduces the urease activity. [25]

### 3.3. Concentration of cementation solution

From the equations of MICP reactions, it was known that, the calcite was formed by the products from 1 mole of urea and 1 mole of calcium chloride. A solution containing equimolar of both reactants would provide better conversion to calcite. [27] Other chemical additives used from enhance of MICP process are calcium sulphate, calcium chloride, sodium carbonate, sodium chloride, ammonia, alcohol, and sodium acetate. Though, most of the researchers use urea- calcium chloride as cementation medium. Higher concentrations of urea and calcium chloride (0.5 – 1.0 M) can provide eminent amount of calcite precipitate. De Muynck et al. (2010) demonstrated the weight gain of limestone specimen due to carbonate precipitation increased with increased concentration of reagent. [28] The weight gain increased from 0.33 g to 0.56 g and 0.66 g when reagent concentration increased from 0.25 M to 0.5 M and 1.0 M, respectively. vein Nemati et al. (2005) examined that the continuous injection of urea
and calcium chloride mixture increased the extent of plugging in porous media, while increases in reactant concentrations up to a certain level (urea and CaCl₂•2H₂O concentrations: 36 and 90 g/L, respectively) increased the quantity of produced CaCO₃.[27] In spite of these studies, the study of Thawadi, (2008) exhibits that highest microbial activity was at 0.5 M concentration of cementation solution than at 1M and 2M concentrations.[29] Dilrukshi et al. (2018) demonstrated that the concentration of cementing solutions CaCl₂-urea in the range of 0.3 M to 0.7 M gives higher unconfined compressive strength. Beyond 0.7 M of cementing solution concentration reduces the UC strength of soil.[30]

3.4. Nutrients

All MICP processes use only bacteria as microbe for calcite precipitation, since they have non-pathogenic species. These bacteria require an energy source to proceed its metabolisms in culture and soil matrix, to produce the desired calcite precipitate. Nutrients are those energy sources for bacteria, hence they should be provided at an ample quantity in both culture and soil treatment stages.[2] Common nutrients for bacteria include CO₂, N, P, K, Mg, Ca, Fe, etc. [5] Nutrients supply for soil specimen is extremely vital, since the soil lacks organic constituents which limit the bacterial growth. From theenamours previous reports, its known that 3 g/l of nutrient broth into the treatment solution to sustain the growth and viability of urease producing bacteria.[2, 12, 26, 31]

3.5. pH

The pH is an indirect parameter that influences the production of calcite by affecting the urease enzyme activity. The urease enzyme produce from bacterial metabolism is responsible for the urea hydrolysis, which inturn produces calcite. As like other enzymes, urease also has some pH limit for its occurrence. With the omission of a low group of acid urease, bacterial ureasenormallyoccupies an optimum pH of near neutrality and also at pH below 5, the bacterial urease can be irrevocably denatured.[32] Many studies made on the bond between pH and calcite formation, by using B. pasteurii illustrate that the MICP is superior at a pH range of 8.7 – 9.3 (i.e 9.5 [26]; 9.3 [33]; 9.1 [34]; and 8.7 - 9.5 [35]). Arunachalam et al. (2010) performed MICP treatment using B. sphaericus reported that the calcite precipitation peaked at pH 8.[36] Van Elsas and Penido (1982) found that B. megaterium phage was stable between pH 6-8.[37] Only 19% and 59% of the bacteriophage survived at pH 5 and 9 respectively. The ammonium ion (NH₄⁺) released during urea hydrolysis increases the pH of the soil, which is controlled by the buffer i.e., bicarbonate, formed in the same reaction. Canakci et al. (2015) studied that the calcite precipitation was higher when the peat soil treated with bacteria and the highest value of pH achieved was 9.3. The pH value was increase from 7.5 to 9.3 for the time interval of 0 hour to 16 hours and beyond 16 hours the pH value remains almost constant 9.3.[38]

3.6. Temperature

The temperature is vital parameter for the activity of urease enzyme. The temperature for MICP starts from 5 °C and exists up to 60 °C.[14, 39] Sahrawat (1984) suggested that the most favorable temperature for urease activity libel at relatively 60 °C.[40] Urease activity enhanced with enhancing temperature from 10 °C and attained the high value at 60 °C. The activity was constrained at 100 °C when temperature is increased further. The most favorable temperature suggested by Sahrawat (1984) is reliable with the decisions from Liang et al. (2005) and Chen et al. (1996).[41, 42] This most favorable temperature for urease activity, however, is unfeasible to be practiced for soil improvement either on site or in laboratory. Therefore, it’s suggested to adopt urease producing microbe that survive in typical soil temperature. Temperature of the soil differs with latitude, solar radiation, water content, conduction, soil type, soil depth and etc.[43, 44, 45] The study performed by Nik et al. (1986) on soil temperature in Malaysia at open areas and forest areas (from depth 0 to 30 cm) wereroughly 30°C.[46]
It is found that the urease activity is maximum at this optimum temperature for \textit{B.megaterium}. Gowthaman et al. (2017) investigated that comparing the unconfined compressive strength of Mikawa sand and Natural soil treated with Psychrobacillus sp. and low grade chemicals at a temperature of 20 °C and 30 °C.[47] The test results shows that at 30 °C temperature the maximum unconfined compressive strength was achieved due to the treated soil samples producing higher urease activity when temperature is increased.

3.7. Injection methods
The process of injecting microbe in the void spaces of soil sample shows a important parameter in generation and circulation of calcium carbonateinterior of the void spaces, which conclusivelyenhances the shear strength of soil. Most of the methods used for microbial injection and cementation fluid were similar to the methods used for chemical grouting. Harkes et al. (2010) examined that two-phase injection technique cancommit to identical circulation of \textit{B. pasteurii} in sand column.[10] The two-phase injection technique was carried out by first, injection of \textit{B. pasteurii} bacteria and secondly, injection of a cementation solution. This process has stronglyed 100% of urease enzyme in the sand column. The second was, adding of the microbesolution and cementation materials and to form bacteria mixed cementation solution before injection in to the soil sample, which results in sudden flocculation of microbe and crystal expansion.[48] This could be responsible for sudden clogging of injection point and neighboring areas void space for many of the fine to medium sand.[14] Thus, use of two phase injection is recommended for effective and uniform distribution of calcite. This can be carried by repeated injection of cementation fluid to the soil mass for increased formation of crystals.

4. Conclusion
Thus, the MICP process uses urease enzyme for urea hydrolysis, which results in the formation of calcite precipitate, a desired substance that increases the shear capacity of soil. Though there are many factors that affect MICP progress, this paper examined some of those such as, soil properties, bacteria species, concentration of cementation solution, nutrients, pH, temperature and injection methods. From this cram the forthcoming results have been obtained.

- MICP technique is more suitable for cohesion-less soils when compared to cohesive soils due to the permeating ability of microbes through the soils.
- Strength of the weak soil increased when the size of the microbes is as small as possible.
- For better MICP treatment the cementing medium concentration should be maintained at equal moraleity of the solutions.
- The optimum temperature and pH of MICP treatment is approximately 30 °C and 5 to 9 respectively.
- Two phase injection method is preferable one for increased production of calcite crystals in soil pores.
- Eggshell cementing solution is more effective in MICP technology than calcium chloride cementing solution.

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