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# Soil Improvement using Microbial Induced Calcium Precipitate: A Review

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**ABSTRACT**
This paper represents a review on the use of Microbial Induced Calcium Precipitate (MICP) as a method of soil improvement, its metabolic processes and biomineralization. Soil improvement is targeted mainly at increasing the engineering properties of any soil. Various methods of soil improvement has evolved as a result of increasing demand of infrastructural development occasioned by rapid industrialization and population growth. Most of the known soil improvement methods rely greatly on mechanical or man-made materials which require ample amount of energy for production, installation, and are often costly, coupled with environmental as well as health issues. MICP is a bioactivity which involves the introduction of calcite forming microorganism (bacteria) and cementitious reagent into soil matrix which forms a cement compound within the soil, thus improving its engineering properties. The reviewed studies showed that MICP can significantly improve strength properties of deficient soils.

**Keywords:** *Microbial induced calcite precipitate, Microbial metabolic process, Soil cementation, Soil improvement, Stabilization.*

## 1. INTRODUCTION

Soil is a heterogeneous material which contains a mixture of gravel, sand, silt and organic matter (Aasimnaeem, 2015). There are soils that are good for construction in their natural state and there are also others that require improvement before they can be used for civil engineering works. Soil improvement can be achieved either by modification or stabilization or a combination of both. While modification is defined as the improvement of soil by addition of a modifier such as cement or lime to improve its index properties, stabilization is the treatment of soil to enable improvement of their strength and durability such that it is suitable for construction (Alhassan and Alhaji, 2017). Various methods of soil improvement have evolved as a result of the increasing demand of infrastructural development, occasioned by rapid industrialization and population growth. Some of these methods use conventional soil improving additives (such as cement, lime and natural pozzolanas), others use agricultural waste products (such as Rice Husk ash, Bagasse ash, Groundnut shell ash).

Biological processes have also recently been introduced into the process of soil improvement. One of these biological processes of soil improvement is Microbial Induced Calcite Precipitation (MICP). Microbial induced calcite precipitation can be applied to ground improvement and bioremediation. It is referred to as a multi-field of study which combines engineering, chemistry and microbiology (Whiffin *et al.*, 2007; DeJong *et al.*, 2010; Rajasekar *et al.*, 2017). Microbial induced calcite precipitation is a bioactivity which involves the introduction of calcite forming microorganism and cementing reagent into a soil matrix which form a cement compound that improves the engineering properties of soil (Ahmad, 2019). Fatima and Benoit (2018) defined microbial induced calcite precipitation (or calcification) as a biochemical process governed by microbial activity to induce the precipitation of calcite between soil particles. Geotechnical applications of microbial induced calcite precipitation include the cementation of sands to enhance the bearing capacity and liquefaction resistance, soil erosion control, crack healing in concrete and masonry and remediation of radionuclide and metal contaminated soils (Mark *et al.*, 2019). Introduction of bacteria into soil can be achieved by either injection or by premixing methods. Injection method involves the flushing of the bacteria solution top down and a waiting period is observed before the inoculation of cementitious reagent while in premixing method, the bacteria is mechanically mixed with soil before the introduction of cementitious reagent, (Donovan *et al.*, 2016).

Generally, conventional ground improvement techniques are either by mechanical compaction which involves high energy consumption or by injection of cement or other chemicals which involve the use of synthetic materials which could be risky to the natural environment and human health. Chemical grout is under research due to the detrimental effect it has on the environment (DeJong *et al.*, 2010). Example is a case in 1974 where acrylamide grout was leached into surrounding water sources in Japan leading to five cases of water poisoning (Karol, 2003). Certain enterprises in countries such as United States proposed the ban of most synthetic grouting material (DeJong *et al.,* 2010). Ordinary Portland cement which is easy to use in stabilization and compactible to most soil type but contributes up to 7 percent of world carbondioxide emission (Ariyanti *et al.,* 2011), therefore, there is the need to development an alternative soil improvement technique which is economically feasible, environmentally suitable and can achieve optimum performance.

**2. Microbial metabolic processes involved in microbial induced calcite precipitation**

Microbial metabolic process in microbial induced calcite precipitation include autotrophic metabolic process which are photosynthesis and methane oxidation and heterotrophic metabolic process which are urea hydrolysis (Ureolysis), ammonification of amino acid, dissimilatory sulphate reduction and denitrification. Microbial induced calcite precipitation is generally achieved by the following six processes although hydrolysis of urea is said to be the highest calcite conversation compared to other processes (Paassen *et al.,* 2010).

**a). Urea hydrolysis:** Urea hydrolysis refers to a chemical reaction where urea $\{CO\ (NH_2)_2\}$ is decomposed by urease enzyme that can be either supplied externally or produced in-situ by urease-producing microorganisms (Greene *et al.,* 2003; DeJong *et al.,* 2006). Hommen *et al.,* (2016), In the presence of urease-positive microoragnisms, the reaction for urea hydrolysis is

$$CO(NH_2)_2+H_2O+Ca^{2+}+cell \longrightarrow 2NH4+cell+CaCO3 \quad (1)$$

**b). Ammonification of amino acid:** When ammonia is hydrolyzed, it produces ammonium and hydroxide ion which result in super-saturation which favours precipitation of calcium carbonate (Zhu and Dittrich, 2016).

$$NH_3+H_2O \longrightarrow NH_4+OH^- \quad (2)$$
$$CO_2+OH \longrightarrow HCO_3^- \quad (3)$$
$$Ca^{2+}+HCO_3^- \longrightarrow CaCO_3+H^- \quad (4)$$

**c). Dissimilatory sulfate reduction:** In anaerobic environment, the calcium present induces the formation of calcium carbonate minerals by sulphate reducing bacteria *(Desulfovibrio species)* indirectly.

$$6CaSO_4+4H_2O+6CO_2 \longrightarrow CaCO_3+4H_2S+11O_2 \quad (5)$$

**d). Denitrification**: Microbial induced calcite precipitation results from the oxidation of organic matter using $NO^{3+}$ as the final electron acceptor. The bacteria create an alkaline microenvironment by the consumption of $H^+$ in the presence of soluble calcium ion (Zhu and Dittrich, 2016). Denitrification process is limited by the accumulation of toxic by product generated such as nitrate and nitrous oxide.

$$(CH_3COOH)_2+Ca+NO_3 \longrightarrow CO_3 + 45NO_2 +3CO_2+3H_2O+OH \quad (6)$$

**e). Photosynthesis**

In aquatic environment, cyanobacteria and microalgae are the main microorganism responsible for microbial induced calcite precipitation. Calcium carbonate precipitations by photosynthetic microorganism occur due to bicarbonate and carbon trioxide exchange.

$$Ca^{2+}+ 2HCO_3^- \longrightarrow CaCO_3 + CO_2 +H_2O \quad (7)$$

**f). Methane oxidation**

Carbondioxide produced from the conversion of methane to methanol by methane mono-oxygenase activity in the presence of oxygen form carbon trioxide and calcium carbonate in the presence of calcium ion.

$$Ca^{2+}+CO_2+2OH^- \longrightarrow CO3+H2O \quad (8)$$

**3. Biomineralization, biocementation and bioclogging**

**A). Biomineralization:** is a natural process aided by living organisms. It is referred to as a process whereby living organisms produced minerals which ultimately hardens the existing tissues. Examples of minerals obtained from these organisms are silicates in algae and diatoms, carbonates in invertebrates, calcium, phosphate and carbonates from vertebrates etc. Achal *et al.,* (2015), biomineralization is divided into three different mechanisms: biologically controlled mineralization (BCM), biological induced mineralization (BIM) and biological mediated mineralization (BMM).

In biologically controlled mineralization, metabolic activity of the microorganism controls growth, morphology and location for deposition of the mineral. Furthermore, this mechanism could be intracellular, intercellular or extracellular with the precipitation of organic macromolecule exopolysaccharides or vesicles. Biological induced mineralization involves the modification of chemical environment like change in pH to result in mineralization. The reaction between metabolic byproducts of microorganisms and ions present in the environment results in mineral precipitation. Minerals generated in biological induced mineralization are characterized by wide range in particle size, poor crystallinity and morphology. In biologically mediated mineralization, mineral formation is due to the interaction between an organic matrix and an organic and/or inorganic compound without the necessity of extracellular or intracellular biological activity, (Wikipedia).

It was stated that microbial induced calcite precipitation occurs through extracellular means though studies have shown intracellular precipitation of calcium carbonates in cyanobacteria, (Cam *et al.,* 2015). In an investigation carried out by (Xu *et al.*, 2019) on the precipitation of calcite and aragonite using virus induced lysis of cyanobacteria cells, it was concluded that this is a new mechanism in expanding the calcium carbonate bio-mineralization process.

**B). Biocementation and Bioclogging**

Biocementation is one of the common processes of achieving microbial induced calcite precipitation. It is a branch of geotechnical engineering that deals with the application of microbial activity to improve the engineering properties of soils. Biocementation improves shear strength of soil through the production of soil-particle binding material with the use of a bacteria and cementing reagent (mostly carbonates, silicate, phosphate, sulphides and hydroxides) in the soil, (Ivanov and Chu, 2008). Bioclogging on the other hand, is a process where soil voids is filled by the product from microbial induced biochemical process. Bioclogging of soil restrict water flow through soil leading to a reduction in hydraulic conductivity.

**4. Factors affecting microbial induced calcite precipitation process**

**i). Reagent concentration:** There is a decrease in the efficiency of microbial induced calcite precipitation at higher concentration of reagent. DeMuynck *et al.,* (2010) concluded from one of his research that the efficiency of calcite formation ratio dropped from 0.66 to 0.56 and to 0.33 as the concentration of urea calcium chloride increased from 0.25M to 0.5M and 1.0M respectively.

**ii). Potential of hydrogen (pH):** Urease enzyme is active at a certain pH. The change in pH level, which is due to the formation of the hydroxyl ions ($OH^-$) generated from the production of ammonium ions ($NH_4^+$) helps to create an alkaline environment suitable for calcite precipitation (DeJong *et al.,* 2010). In soil bio-cementation, variability of the pH values can influence the bacterial transport and adhesion, which is an important factor affecting homogeneity in the distribution of calcite crystals precipitation. *Bacillus megaterium* had an optimum pH of 7-9 and urease activity peaked at pH of 7 (Khan *et al.*, 2011).

**iii). Bacteria cell concentration:** A high bacterial concentration supplied to the soil sample increase the amount of calcite precipitation from microbial induced calcite precipitation process (Okwadha and Li, 2010). The rate of urea hydrolysis has a direct relationship with the bacterial cell concentration as long as there is adequate amount of cementitious reagent. High concentration of bacteria produced more urease per unit volume to commence the urea hydrolysis.

**iv). Nutrients:** Bacteria obtain energy from nutrients therefore, it is important to provide sufficient and adequate nutrient of urease-producing bacteria. Common bacteria nutrient are carbondioxide, nitrogen, potassium, phosphorus, magnesium, calcium, lead etc. Bacteria need nutrient to sustain long calcite precipitation process in order to achieve the desired improvement level.

**v). Type of bacteria:** Bacteria types are usually urease positive bacteria. Bacteria used in microbial induced calcite precipitation must be able to catalyse urea hydrolysis. Aerobic bacteria are preferable as they release carbondioxide from cell respiration. Carbondioxide production is paralleled by the pH rise due to ammonium production.

**vi). Temperature:** The rate of microbial induced calcite precipitation is affected by urease activity in which temperature is of significant influence. The effect of temperature on microbial induced calcite precipitation is complex as it affects the urease activity of microorganisms, growth and nucleation rate of calcite crystals and solubility of calcite.

**vii). Degree of saturation:** Lower degree of saturation gives higher strength at even lower calcite precipitation within a soil matrix.

## 3. Soil improvement using microbial induced calcite precipitation process

Osinubi *et al.,(*2019) investigated the strength of compacted lateritic soil improved with microbial induced calcite precipitate and established that the research showed an increase in unconfined compressive strength value with increasing *Sporosarcina pasteurii* suspension density and moulding water content in relative to optimum for the compactive effort used. For a suspension density of above $1.2x10^9$cells/ml, the unconfined compressive strength decreased with an increase in suspension density. The study suggested that for the construction of liners and covers in municipal solid waste containment systems, a suspension density of $1.20x10^9$ cells/ml can be used improve lateritic soils. The suspension density used for this study did not exceed 8 McFarland standards which is equivalent to $2.4x10^9$cells/ml and the study also did not evaluate the strength for other engineering structures aside covers and liner for waste containment.

A research was conducted (Ahmad, 2019) on mid expansive soil treated with *Sporosarcina pasteurii* using microbial induced calcite precipitation as a method of soil improvement. The soil was investigated and classified as A-7-6 (21) and CL according to American Association of State Highway and Transportation Officials classification system and Unified Soil Classification System respectively. The soil was compacted using British Standard light energy level at an optimum moisture content of 15.80%. The soil was observed to have a CBR value of about 40% greater when compared with the natural soil. The treated soil was only considered for road bases. The effects on other geotechnical structures were not considered.

Kaninathan and Shashank (2015) carried out a research on poorly graded sand (SP), clayey sand (SC) and silty sand (SM) using *Sporosarcina pasteurii* bacteria to treat the soils. Unconfined compressive strength, hydraulic conductivity and shear parameters were evaluated. The study showed an inversely proportional relationship between the reduction in hydraulic conductivity and increase in strength. For shear parameters, SP and SM soil increased by more than 90% while SC increased by roughly 50% with increasing treatment days. However from the result presented, only the durability of cohensionless soils was investigated and treatment days showed were from days 6-15 (figure 1)

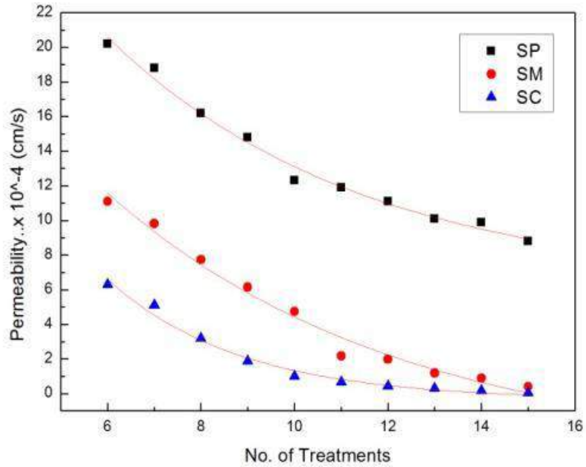


Figure 1:Hydraulic conductivity of treated soil

Soon *et al.*, (2013) studied the optimum condition for improving engineering properties and concluded that, improvement was achieved for undrained shear strength when residual soil was treated using microbial induced calcite precipitation process. The research assumed the residual soil to be homogeneous whereas in reality, residual soil are often characterized by high variability in physical and geotechnical properties which can greatly affect microbial induced calcite precipitation treatment of the soil. Lee *et al.*, (2012) researched on the effect of microbial induced calcite precipitation on shear strength and hydraulic conductivity of sandy and residual soils (sandy silt). The study concluded that there was an increase in shear strength and a decrease in hydraulic conductivity for both soils.

Osinubi *et al.*, (2018) studied the strength of tropical residual lateritic soil treated with *Sporosarcina pasteurii*. It was cured in a polythene bag for 12hours at a temperature of 25 $\pm 2^0$C. It was proved that at $1.2x10^9$ cell/ml of bacteria suspensions, a peak unconfined compressive strength value of 2232kN/m$^2$ was obtained as an optimal treatment for the lateritic soil under study. This investigation only considered British standard light compaction energy level. Compaction energy level such as West African standard may have resulted in lower permeation values. In addition, the injection method was used in introducing the bacteria into the soil which may hinder uneven distribution of bacteria resulting in inhomogeneity of calcite distribution in the treated soil.

Eberemu *et al.*, (2017) obtained soil sample from an erosion prone zone in Anambra, Nigeria which was improved by using *B.pumilus* suspension densities at varying concentration. The sample was left for complete saturation of solution before the introduction of the cementitious reagent (which contains 3g of nutrient broth, 20g of urea, 10g of $NH_4Cl$, 2.12g of $NaHCO_3$ and 2.8g of $CaCl_2$ per litre of distilled water) which initiate the MICP process. The unconfined compressive strength was increased from 66.74kN/m$^2$ in its natural state to 2507.29kN/m$^2$ after treatment at a suspension density of 18x10$^8$/ml concentration. The durability of the material assessed through its resistance to loss in strength with an increasing bacteria concentration increased by more than 60% (that is from 3.64% to 17.38%). The treatment with the bacteria was done at an interval of 6 hours for 2 days. The effect of longer treatment duration (days) and other compaction energy level was not considered.

Sanchita *et al.,* (2019) carried out an investigation on soil stabilization using microbial induced calcite precipitate. The soil composed of 70% black cotton soil, 8% sand and 22% silt. The shear strength of the black cotton soil was increased while hydraulic conductivity was reduced with increasing treatment day (6 days and 14 days respectively). The urease producing bacteria was injected into the soil and shear strength of the soil was observed to increase by 42.80% and the hydraulic conductivity decreased by 70.83% compared with the natural soil. The method of compaction was not stated. Also, the treatment days result presented for the hydraulic conductivity test was for 3 days only (that is days 0, 7th and 14th).

Osinubi *et al.,* (2019) investigated the plasticity characteristics of lateritic soil treated with *Sporosarcina pasteurii* suspension density in MICP application. The study involved the use of lateritic soil obtained by undisturbed sampling. In this investigation, the soil sample was in three portions, A, B and C (A-11%:33% representing 25% bacteria and 75% cementation reagent, B-22%:22% representing 50% bacteria and 50% cementation reagent, C-33%:11% representing 75% bacteria and 25% cementation reagent). The suspension density according to McFarland standard used to initiate the MICP process was 0, 0.5, 2.0, 4.0, 6.0 and 8.0. Liquid limit decreased from 44%-38%, plastic limit from 21.6%-16% and plasticity index from 22.5%-17.8 at a suspension density of 8.0 (equivalent to 24x10$^8$/ml). The decrease in Plasticity index is one of the key features to be considered in soil improvement. The soil was compacted using two compaction energy levels Reduced British Standard light (2.5kg rammer falling through a height of 30 cm, 3 layers, 15 blows) and British Standard light(2.5kg rammer falling through a height of 30 cm, 3 layers, 27 blows) . The peak unconfined compressive strength values was observed to be 2011kN/m$^2$ and 2232kN/m$^2$ for RBSL and BSL respectively. This study did not evaluate the strength for other engineering structures aside covers and liner for waste containment. The compressive strength using other compactive energy level was not evaluated.

## 4. CONCLUSION

This paper generally reviewed the utilization of microbial induced calcium precipitate in soil improvement. The study showed that microbial induced calcite precipitate can be used for improving soil the geotechnical properties of soil supporting new or existing structures. It stands as a favourable technique in modifying engineering properties of soil.

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