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Effects of cementation reagent on the precipitation of calcium carbonate induced by *Bacillus Megaterium*

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Abstract. A laboratory experiment was carried out to determine the concentration of cementation reagent that will produce the maximal amount of calcium carbonate induced by *Bacillus Megaterium.* The optimum condition for calcium carbonate precipitation was evaluated for its application in improving the geotechnical properties of soil. The process was studied using the test-tube experiment and evaluating the amount of calcium carbonate precipitated and subsequently verified using X-ray diffraction test. Five different concentrations of cementation reagent (0.25, 0.5, 0.75, 1.0 and 1.5M) were used in the study. Results showed calcium carbonate was higher with increase in concentration of cementation reagent irrespective of the curing period. Furthermore, the XRD scan confirmed the precipitate formed was calcium carbonate. Calcite formed acts ad bio-cement which is responsible for improving the geotechnical properties of various soil.

1. Introduction

Microbial induced calcium carbonate precipitation (MICP) is one of the most important bio-mineralization process that is receiving much attention in recent years because of their applications in the field of biotechnology, geotechnology and civil engineering [1]. More ever MICP was shown to be effective in increasing shear strength and stiffness of soil through bio-cement formation [2, 3, 4]; decreasing the permeability of soil through formation of bio-grouts [5, 6, 7]. Formation of microbial calcite precipitate ($CaCO_3$) can occur through various biochemical pathways. So far, the identified bio-chemical processes capable of inducing calcium carbonates are ureolysis, denitrification, sulphate reduction and iron reduction [8]. MICP via ureolysis is the most efficient and easily controlled mechanism that can produce 3.8 - 7.4% precipitate

$CaCO_3$ within 24 – 48 hours [9], thus it has become the most studied and utilized process of inducing calcium carbonate precipitates. In a simple form, MICP via ureolysis is accomplished in two stages. The first stage involves hydrolysis of urea by the action of urease enzyme secreted by urease producing bacteria. Equation 1 – 2 describe how urea is reduced to ammonium (NH_4^+) and carbonate ion (CO_3^{2-}) [10].

$$CO(NH_2)_2 + H_2O \rightarrow NH_3 + CO_2 \quad (1)$$

$$NH_3 + CO_2 + H_2O \rightarrow {NH_4}^+ + {CO_2}^{2-} + OH^- \, pH \uparrow \quad (2)$$

The second stage is the formation of calcium carbonate which is accomplished by the association of calcium ions and carbonates ion and the reaction is reversible but it is shifted to the right to form calcium carbonate as shown in equation 3 when the environmental condition is favourably alkaline – with pH between 7.5 – 9.5 [6] [11]. The source of calcium ions during the ureolysis reaction is normally calcium chloride otherwise known as cementation reagent when it is combined with urea.

$$Ca^{2+} + CO_3^{2-} \leftrightarrow CaCO_3 \quad (3)$$

MICP controlled ureolysis as catalytic reaction is influenced by many environmental factors such as temperature, pH of the medium, bacterial concentration and cementation reagent. Many studies had been conducted to determine the optimum parameters that will give rise to maximum calcium carbonate precipitates via ureolysis [12, 13, 14]. Among those mentioned factors influencing MICP, initial concentration of cementation reagent remains the unverified result. For instance, [6] had conducted laboratory studied on the variation of cementation reagent to produce calcite precipitates in residual soil using *Klebsiella pneumoniae* UM123 urease producing bacteria strain. From the studies it was determine that the optimum cementation reagent was 0.5 M. [15] had studied the effect of cementation reagent on the pH, ammonium content, shear strength and the amount of calcite formed. It was determined that high amount calcite precipitates and maximum strength were determine at 0.5 M. But, [16] determined that optimum concentration of cementation reagent when *Bacillus sp.* strain was used to induce calcium carbonate precipitates was 0.25 M. In another experimental study [13] found that the amount of calcite formed when sporosarcina pasteurii was used for MICP increased by 100% when the initial concentration of Ca^{2+} increases by 10-fold from 0.025 M to 0.25 M. They also determined that the optimum concentration of cementation reagent solution for best MICP performance were at 0.666 M urea and 0.25 M initial Ca^{2+} concentrations. [17] argues that efficient ureolysis and calcite formation can be obtained when equal concentration of calcium chloride and urea are used in the formation of cementation reagent. Similarly, authors like [18] reported that the amount of calcium carbonate formed is proportional concentration of cementation reagent, while other authors like [19],[14] reported efficient urea hydrolysis and calcite formation at lower of cementation reagent. Thus, with thus contrasting results on the optimum concentration of cementation reagent there is need to conduct laboratory investigation to ascertain the actual concentration of cementation that will produce maximum amount of calcite precipitates

2. Materials and methods

2.1 Bacillus Megaterium

The urease-producing microorganism used in this study was *B. megaterium* (strain ATCC 14581). *B. megaterium* is a Gram-positive bacterium that can be found in a broad range of habitat; however, it is mainly found in soil [20]. *B. megaterium* has been proven to have the ability to induce calcite precipitation in natural soils [21]. The bacteria cells required for the experiment were initially grown on a solid nutrient medium (tryptic soy agar) and a single colony was then transferred to liquid growth medium (nutrient broth). The liquid growth media containing the bacteria colony was incubated at 30 ^{0}C under agitation for a period of 24 hours to attain the stationary stage of the cell growth curve.

The concentration of cells suspended in the stock culture was estimated by the expression shown in equation 1, after [22].

$$Y = 8.59 \times 10^7 . Z^{1.3627} \quad (4)$$

Where Z is reading at OD_{600}, and Y is the concentration of cells mL^{-1}.

2.2 Cementation Reagents

Cementation reagents serves as important ingredients for promoting calcite precipitation. The cementation reagents used consists of urea, calcium chloride at different concentrations in addition to other supplements such as nutrient broth, ammonium chloride and sodium bicarbonate. The concentrations were varied to evaluate their influence on ureolytic-driven calcium carbonate precipitations. Table 1 shows the components of the cementation reagents and their masses.

Table 1: Chemical composition of cementation reagents.

Concentration (M)	**0.25**	**0.5**	**0.75**	**1.0**	**1.5**
Urea (g/l)	15	30	45	60	90
$CaCl_2$ (g/l)	27.8	55.5	83.3	111	165.5
Nutrient broth (g/l)	3	3	3	3	3
Ammonium Chloride (g/l)	10	10	10	10	10
Sodium Bicarbonate(g/l)	2.12	2.12	2.12	2.12	2.12

2.3 Test-tube experiments

In this work, the precipitation of carbonate was evaluated directly in polypropylene (PP) tubes. The experimental procedures developed by [23, 24] were adopted in this work. Predetermined masses of the cementation reagents were dissolved into distilled water based on the different equimolar requirements (Table 1), the solution was thoroughly mixed to ensure the chemical products were completely dissolved. Then, 15ml of bacterial solution and cementation regents each were mixed in the test tubes, resulting in a total solution volume of 30 ml. The test tubes were then placed in a temperature-controlled room (30± 2°C) for a specified curing time of 3days. At the end of the curing period, the solution was filtered, the particles deposited on the filter paper and remaining in the tubes were dried, and their amounts are measured. The total amount of calcium carbonate was determined as the sum of the material deposited on the filter paper and on the bottom of the test tube. Each stage of the test was repeated two times to guarantee the reliability of the procedure.

X-ray diffraction test was carried out on the precipitate to verify the calcium carbonate formed during the process. The outout data of the XRD test was analysed and compared using the standard calcite (PDF 01-080-9775) and Vaterite (PDF 01-080-4618) Phases collected from the Power Diffraction File Database of the International Centre for Diffraction Data (ICDD).

3. Results and discussions

Figure 1 show the amounts of calcium carbonate precipitation by *B. Megaterium* over the range of cementation reagent concentration. Results showed a general increase in carbonate precipitates with increase in concentration of cementation reagent irrespective of the curing period. For the 3 days curing period, the calcite precipitate increased from 60 – 490mg for concentration of cementation reagent of 0.25 – 1.5M having a percentage increase of 87.8%.

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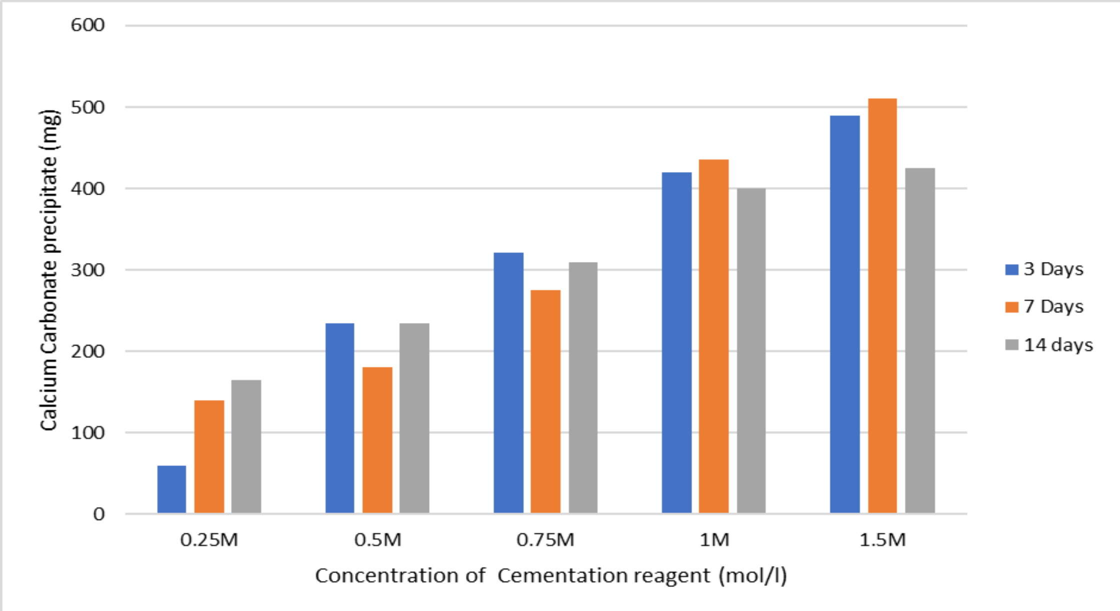

Figure 1: Variations of calcium carbonate precipitate with different concentration of concentration reagent.

Similarly, for the curing periods of 7 and 14 days, the percentage increase in the precipitate over the same range cementation reagent concentration are 72.5 % and 61% respectively. The increase in calcite precipitate with higher concentration of cementation reagent could be attributed to the fact that higher urea concentration gave rise to more localized rise in pH around the bacterial cells as more urea molecules are available, and thus making the precipitate higher [25].

Similar trend was observed by [26] when *S. aquimarina* and *S. pasteurii* where used to optimize the calcium carbonate precipitation at different combination, urea and calcium. [13] investigated various non-equimolar cementation solution (CS) and concluded that a higher amount of deposited calcium carbonate was associated with a higher CS concentration.

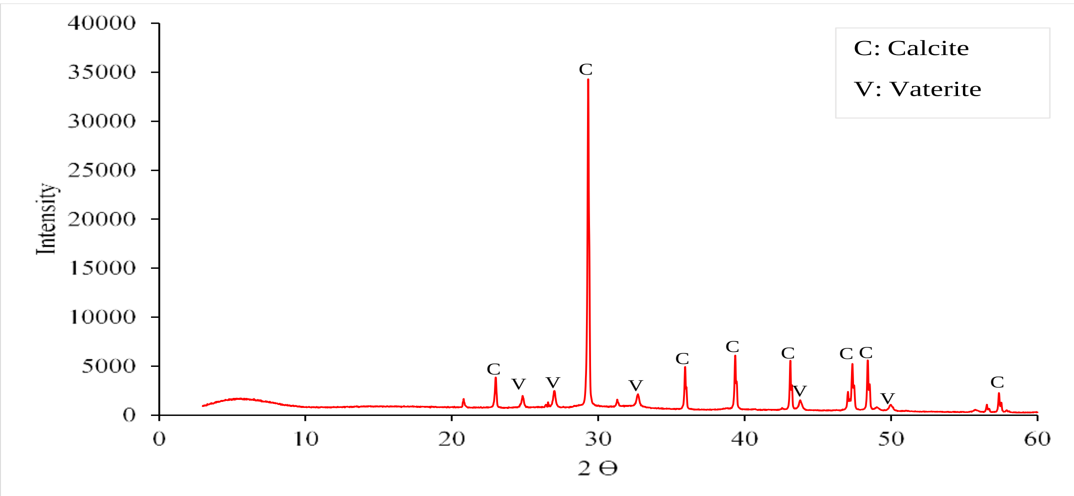

Figure 2: XRD analysis of the material precipitated in the test-tube experiment.

The formation of calcium carbonate was confirmed by the results of XRD shown in figure 2. According to ICCD, the major peak for calcium carbonate is usually found at 29^o while other lower peaks were found 36^o, 39^o and 48^o which corresponds to the peaks on the diffractogram for the precipitate. Traces of Vaterite were also detected as part of the minerals contained in the precipitate formed.

4. Conclusions

From the results of the test - tube experiments carried out to study the efficacy of calcium carbonate precipitation for various concentration of cementation reagent (urea - calcium chloride), the following conclusion were drawn.

1. *Bacillus Megaterium* was able to produce urease enzyme that was required for the process of calcium carbonate precipitation through urea hydrolysis.
2. Higher calcium carbonate precipitation was observed with higher concentration of cementation reagent.
3. Precipitate formed from the experiment was able to be confirmed that calcium carbonate was formed.

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