

EFFECT OF CEMENTATION REAGENTS CONCENTRATIONS ON MICROBIAL CALCITE PRECIPITATION IN RESIDUAL SOIL

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Abstract: Microbially induced calcite precipitation has been under investigation since early 1990s for its potential application in improving the strength and durability of construction materials such as limestone and cementitious materials. However, excellent results demonstrated by this technique in the studies so far conducted have shown greater potential of exploring its wider applications in geotechnical engineering. This study examines the microbial carbonate precipitations in tropical residual soil via urea hydrolysis. An isolate of urease active strain of *Klebsiella pneumoniae* UM123 was used to precipitates calcite into the soil with the aim of improving the strength and reducing the hydraulic conductivity of the soil. Cementation reagents concentrations of 0.25, 0.5, 0.75, 1.0 and 1.5M were used to evaluate the strength and hydraulic conductivity of the soil using treatment durations of 24, 48 and 60 hours. Meanwhile, bacteria concentrations of 1.5×10^4 cfu/ml and 2.9×10^6 cfu/ml were used to assess the effect of microbial concentrations in the study. The results obtained indicated a general increase in the strength of the treated soil as the bacteria concentrations increases. Likewise, the strength also increases with the increase in reagents concentrations up to 0.5M after which the strength declined. The hydraulic conductivity also decreased by 50% and 65% after 48 hours for 1.5×10^4 cfu/ml and 2.9×10^6 cfu/ml bacteria concentrations. The results so far obtained revealed that the optimum reagent concentration for this particular microorganism for efficient soil improvement is 0.5M.

Keywords: Residual soil, microorganism, calcite, cementation reagents, soil improvement

1.0 Introduction

Microbially induced calcite precipitation (MICP) and its potential applications in soil improvement and other civil engineering applications have been reported in many studies in recent years (DeJong *et al.*, 2010; Ivanov and Chu, 2008; Mitchell and Santamarina, 2005; van Paassen *et al.*, 2010). Therefore, MICP processes sometimes take place naturally in situ cementing sand into sandstone; on the other hand, the technique can be harnessed and utilized as an environmentally friendly soil improvement process (Konhauser, 2009). Generally, biomediated soil improvement is achieved through a biochemical reaction that takes place within the soil matrix and

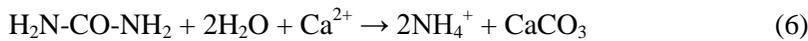
modifies the engineering properties of the soil; such as strength, stiffness and hydraulic conductivity. Hence, majority of the studies conducted on bio-mediated ground improvement were based on urea hydrolysis. This is because urea hydrolysis is regarded as the most easily controlled carbonate generating reactions; and has the capability of generating high concentrations of carbonate within a short period of time. Hydrolysis of urea is an irreversible reaction in which urea reacts with water to form ammonium and carbonate and subsequently increases the alkalinity of the pore fluid and induce calcite precipitation (Fujita *et al.*, 2008; Stocks-Fischer *et al.*, 1999).

Microbial cementation can also be achieved through the utilization of indigenous bacteria to induce calcite cementation into the soil (Burbank *et al.*, 2011; Fujita *et al.*, 2010). Hence, recent studies on biocementation attracted numerous industrial applications such as bioremediation (Ferris *et al.*, 2004; Fujita *et al.*, 2000; Warren *et al.*, 2001), treatment of wastewater (Hammes *et al.*, 2003) and refurbishment of calcareous stone materials (Castanier *et al.*, 2000; Stocks-Fischer, *et al.*, 1999; Tai and Chen, 1995). Biocementation was also used to improve the strength and stiffness of loose, saturated soil susceptible to liquefaction (DeJong *et al.*, 2006; Montoya, 2012; Mortensen and DeJong, 2011). Similarly, the technique has been utilized in binding soil particles together and increasing the soil's resistance against wind erosion (Bang and Bang, 2011; Kavazanjian Jr *et al.*, 2009). In all these conditions, bio-mediated soil improvement has successfully created bonds between soil grains and improved the mechanical properties of the soil.

Micro-organisms containing the enzyme urease are mainly used in this technique. The main function of bacteria in the biomineralization process is to consume urea and decompose it into ammonia (NH_3) and carbon dioxide (CO_2). These chemicals disperse into the surrounding solutions via the cell wall of the bacteria. The released ammonia converts to ammonium ions (NH_4^+) and carbon dioxide depending on the pH, converts into carbonic acid, carbonate and bicarbonate ions. Hence, in the process of converting ammonia to ammonium, hydroxyl ions (OH^-) are generated which are in excess of the available Ca^{2+} for calcite precipitation; as such resulted in the net increase in the pH. Thus, providing the alkaline environment and carbonate required for the precipitation of calcite (CaCO_3) (DeJong, *et al.*, 2010).

According to Burne and Chen (2000) urea hydrolysis generally follows a series of chemical reactions that leads to the formation of ammonia (NH_3) and carbon dioxide (CO_2). The chemical reaction is presented in Equation 1. The hydroxyl ions (OH^-) generated from the conversion of ammonia to ammonium resulted in the increase in the local pH that leads to the decomposition of bicarbonate to carbonate ions (Eq. 2). The carbon dioxide quickly decomposed in the presence of water into bicarbonate (HCO_3^-) and the bicarbonate reacts with the hydroxyl ions to form carbonate ions (Eqs. 3 and 4). Hence, in the presence of calcium ions (Ca^{2+}), the calcium carbonate (CaCO_3) is precipitated (Eq. 5) (Burne and Chen, 2000; Castanier *et al.*, 1999). Therefore, (Eq. 6)

described the summary of the overall process of urea hydrolysis and formation of calcium carbonate.



The amount of calcite precipitated during the MICP process directly influence the engineering properties such as strength, stiffness and hydraulic conductivity of the soil treated. Similarly, the distribution of the calcite crystals within the soil pores and amount precipitated are also influenced by some properties of the soil such as particle sizes, mineralogy, insitu saturation, density, shape and texture (Ismail *et al.*, 2002; Mitchell and Ferris, 2006). Hence, during MICP treatment for biocementation, the distribution of the injected bacteria into the subsurface is affected by many factors that influenced the flow and movement of the bacteria into the pores of the soil. The actual rate of ureolysis when used for biocementation is a function of many parameters as given in Equation 7 (Lioliou *et al.*, 2007).

$$r_h = \frac{dC_{urea}}{dt} = f(C_{bacteria}, C_{urea}, pH, C_{Ca^{2+}}, T, t, S_{CaCO_3}, \dots) \quad (7)$$

Where:

r_h	= actual hydrolysis rate
$C_{bacteria}$	= concentration of bacteria
C_{urea}	= concentrations of urea
$C_{Ca^{2+}}$	= concentrations of calcium ions
T	= Temperature
t	= time
S_{CaCO_3}	= supersaturation of calcium carbonate

Therefore, experimental factors such as bacteria concentrations, temperature and cementation reagents concentrations (urea and calcium chloride) also have direct impact on the amount and distribution of the calcite that would be precipitated during the MICP experiment. Therefore, for effective MICP treatment, appropriate concentrations of these parameters need to be assessed. This is because the concentrations of the cementation reagents which increase the alkalinity of the environment directly affect the

activity of the microorganisms. Therefore, this paper intends to evaluate the effects of cementation reagents concentrations and bacteria concentrations on the calcite formation within the soil matrix structure.

2.0 Materials and Methods

2.1 Soil Sample

Tropical residual soil was collected at Universiti Teknologi Malaysia, Johor Campus and used for the study. The soil was classified as Gravelly clay of high plasticity (CHG) based on British Soil Classification System (BSCS). Table 1 present the Index properties of the soil.

Table 1: Index properties of the soil sample

Properties	Description
Gravel (%)	31
Sand (%)	14
Silt (%)	32
Clay (%)	23
Liquid limit (%)	63.54
Plastic limit (%)	46.79
Specific gravity	2.62
MDD (Mg/m ³)	1.395
OMC (%)	31.2
Classification(BSCS)	CHG
UCS (kPa)	30.4
Hydraulic conductivity(m/s)	1.35×10^{-6}

2.2 Microorganism and Growth Conditions

A urease active strain of *Klebsiella pneumoniae* UM123 was isolated from the soil sample used in the study. After the isolation and identification processes the microorganisms was grown to its exponential growth phase in a yeast extract-based medium containing 20 g yeast extract, 10 g ammonium sulphate in a 1 Litre 0.3 M Tris buffer solution of pH 9.0. After 24 hours incubation at 30 °C, the culture was harvested and stored at 4 °C prior to use. Bacteria concentrations of 1.5×10^4 cfu/ml and 2.9×10^6 cfu/ml were obtained and used for the study.

2.3 Cementation Reagents

The cementation reagents used consists of urea and calcium chloride at different concentrations. The concentrations were varied to evaluate their influence on ureolytic-driven calcium carbonate precipitations. Table 2 presents the components of the cementation reagents and their concentration.

Table 2: Chemical composition for cementation reagents

Concentrations (M)	0.25	0.5	0.75	1.0	1.5
Urea (g/L)	15	30	45	60	90
CaCl ₂ (g/L)	36.5	73.0	109.5	146	219.5
Nutrient broth (g/L)	3	3	3	3	3

2.4 Ammonium Ions Determination

The ammonium ions concentrations were determined using phenate method as described by the American Public Health Association/American Water Works Association/Water Environment Federation 2005. The method is used for measuring ammonium ions within the range of 0.02 – 2 mg NH₄⁺/l. Therefore, samples with concentrations outside the range were first diluted with distilled water before testing.

2.5 Calcium Carbonate Contents Determination

Calcium carbonate contents in the treated soil were determined using gravimetric analysis of acidified samples. The samples were prepared after treatment and 20g of the powdered samples were oven dried. Hydrochloric acid (2 M) was then added to the powdered sample and the subsequent effervescence from the reaction between calcium carbonate and acid indicated that carbon dioxide was liberated from the calcite. The residue was collected on a filter paper and oven dried again. The weight loss was used to estimate the percentage of calcium carbonate content in the soil specimen. The calcium carbonate content was expressed on a dry-weight basis (percentage of the dry weight of soil, i.e., 20 g). It was assumed that the increment of carbonate content in the soil after the MICP treatment was purely caused by the formation of the calcium carbonate.

3.0 Results and Discussions

3.1 Effects of Cementation Reagent Concentrations on Calcite Precipitations

Higher concentrations of calcium ions and other salts as may be supplied during MICP process affects the urease activity of the microorganisms involved. The extent to which the urease activity is going to be inhibited or repressed depends on the type of

microorganisms (Whiffin, 2004). Figure 1 and 2 present the UCS results of residual soil after bio-treatment using various cementation reagents concentrations. The strength of the treated soil increases proportionally with the increase in the reagent concentrations up to 0.5M after which the strength decreases continuously as the concentrations increases. This indicated that for the microorganism used in this study, reagent concentration of 0.5M is the optimum for efficient MICP treatment as any concentration beyond that resulted in the decline of the bacterial activity. This findings corresponds with the findings of (Soon *et al.*, 2013). Figure 3 also shows the scanning electron microscope images of the calcite formation after treatment of the residual soil with 0.25 and 0.5M reagents concentrations. The calcites are more densely and evenly precipitated in the image with 0.5M than 0.25M concentrations.

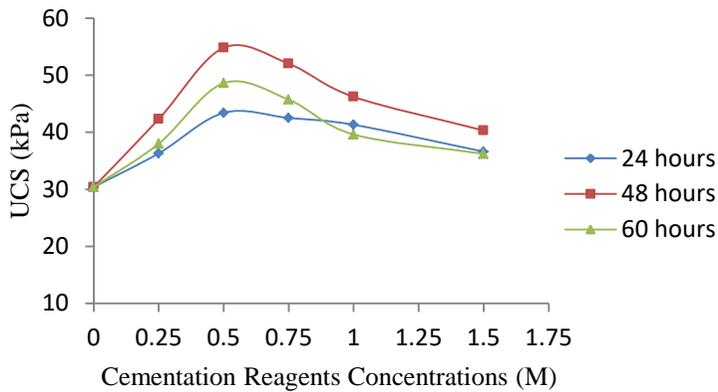


Figure 1: Unconfined Compressive Strength (UCS) at varying concentrations of cementation reagents for 1.5×10^6 cfu/ml bacteria concentrations

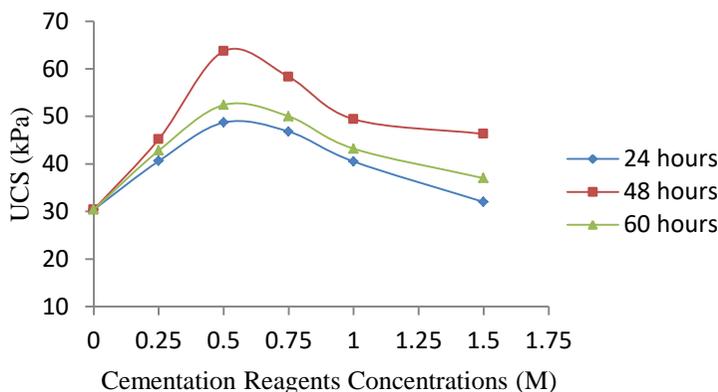
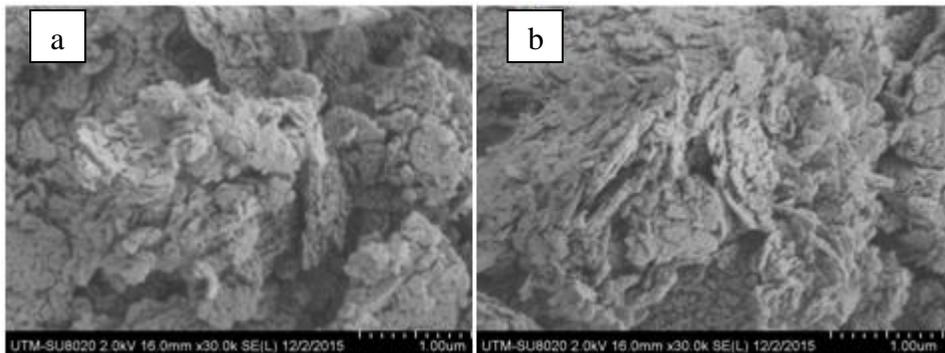


Figure 2: Unconfined Compressive Strength (UCS) at varying concentrations of cementation reagents for 2.9×10^6 cfu/ml bacteria concentrations



Figures 3: FESEM Images of MICP treated soil with (a) 0.25M and (b) 0.5M cementation reagents concentrations

3.2 Bacteria Concentrations and Strength Improvement

Bacteria cells concentration is another factor that influence MICP process, the higher the cells concentrations the more calcites are likely to be precipitated (Okwadha and Li, 2010). Figure 1 and 2 also present the relationship between bacteria concentrations and strength improvement. The result shows a general increase in strength as the bacteria concentrations increases for all the treatment durations up to 48 hours beyond which the strength decreases. At 48 hours treatment duration strength improvement of 80% and 110% were recorded relative to untreated soil for 1.5×10^4 cfu/ml and 2.9×10^6 cfu/ml bacteria concentration at 0.5M reagent concentration respectively. This suggests that higher bacterial concentrations should be used to precipitates more calcite for effective MICP treatment.

3.3 Bacteria Concentrations and Hydraulic Conductivity Reduction

The calcium carbonate precipitate generated microbially is responsible for clogging the soil pore spaces, thereby restricting flow of water and decreasing the permeability of the soil. Therefore, the higher the microbial concentrations the more calcite precipitates are expected during MICP treatment (Ferris and Stehmeier, 1992; Nemati and Voordouw, 2003). Figure 4 presents the relationship between bacteria concentrations and hydraulic conductivity reductions at 0.5M reagents concentrations for different treatment durations. The results are comparable with the shear strength results. The hydraulic conductivity decreased by 50% and 65% after 48 hours for 1.5×10^4 cfu/ml and 2.9×10^6 cfu/ml bacteria concentrations which is an indication that higher bacteria concentrations precipitates more calcite for clogging the soil particles and subsequent reduction in permeability. This finding is in agreement with the findings of (Soon *et al.*, 2014). It was also observed that the permeability decreases with treatment durations up to 48 hours after which the trend changes. This findings conforms to the strength improvement obtained

that provided the maximum strength at 48 hours; indicating that MICP in residual soil is most effective within 48 hours.

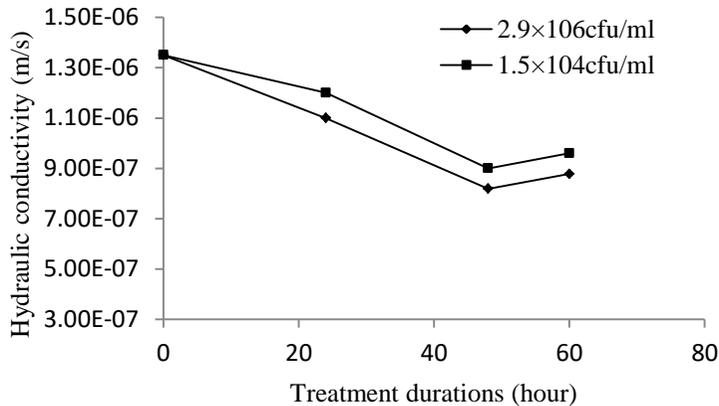


Figure 4: Relationship between bacteria concentrations and hydraulic conductivity reduction at 0.5 M reagent concentrations

3.4 Cementation Reagents Concentrations and Calcite Contents

Bacterial activity in urea hydrolysis reaction leads to the precipitation of calcite into the soil matrix. However, the bacterial urease activity is repressed when the environment is too alkaline. Therefore, the effects of reagents concentrations on the calcite precipitation and the subsequent increase in the strength of the residual soil have been evaluated. Figure 5 presents the relationship between reagents concentrations and calcite contents for 2.9×10^6 cfu/ml bacteria concentrations at 48 hours. Similarly, the relationship between ammonium ions concentrations and calcite contents under the same conditions were presented in the same Figure. It was observed that calcite contents increases as the cementation reagents concentrations increases up to 0.5 M. This shows that the bacterial urease activity was active until when the reagents concentration exceeded 0.5M thereby repressing its activity due to high salinity. The same trend and mechanism follows for ammonium ion concentrations.

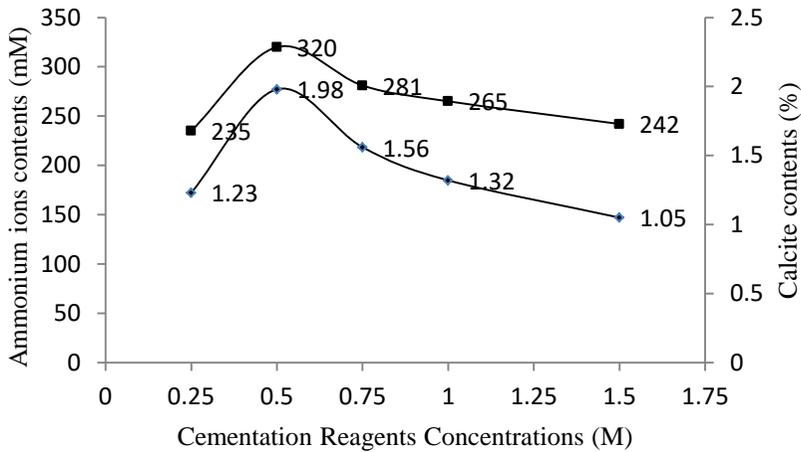


Figure 5: Correlation between calcite contents, ammonium ions contents and cementation reagents concentrations

3.5 Variation of pH with Cementation Reagents Concentrations

Figure 6 presents the variations of pH with the reagents concentrations. The first measured pH was 7.5, slightly above the pH of the untreated soil; which is an indication of very little ammonium ions productions at the initial stage of the treatment. The urea hydrolysis induced by the bacteria results in the production of ammonium ions that subsequently increased the pH of the soil environment. The results indicated an increase in the pH for 0.25 and 0.5 M concentrations which is an indication of microbial activity at these concentrations. On the other hand, the pH decreases as the concentrations were increased beyond 0.5M, which is a confirmation of the decline in the bacterial urease activity.

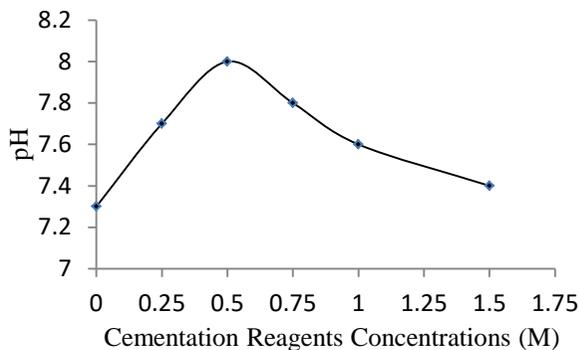


Figure 6: Variation of pH with cementation reagents concentrations

4.0 Conclusions

This study revealed the viability of using MICP in improving the engineering properties of tropical residual soil. It can be deduced that lower concentrations of cementation reagents provided better strength improvement. This is because the microorganisms involved are more active at concentrations up to 0.5M, beyond which their activity declined. The longer the treatment durations the more the calcite contents increase up to 48 hours causing more binding effects between the soils particles; thereby increasing the strength. However, at 60 hours treatment duration the calcite content decreases due the decline in the bacterial activity as the pH reached 8.0. Hence, using 0.5M cementation reagent concentrations at 48 hours treatment duration which produces up to 80% and 110% strength improvement and 50% and 65% hydraulic conductivity reductions relative to untreated samples for 1.5×10^4 cfu/ml and 2.9×10^6 cfu/ml bacteria concentrations was found to be the most effective.

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