EFFECT OF CURING TIME ON MICROBIALLY STABILIZED RESIDUAL SOIL

Murtala Umar

Department of Civil Engineering, Faculty of Engineering, Bayero University Kano, P.M.B. 3011, Kano Nigeria

ABSTRACT

Microbial Calcite Precipitation (MCP) is a technique that utilizes the concept of microbial involvements in calcium carbonate precipitation within the soil matrix structure. This leads to the cementation of the soil particles and consequently improving the strength and stiffness of the soil. In this study microbial carbonate precipitations were induced in tropical residual soil via urea hydrolysis and effects of curing time on the strength improvement after treatment was determined. An isolate of urease active strain named Klebsiella pneumoniae was used to precipitates calcite into the soil with the aim of improving the engineering properties of the soil. Bacteria concentrations of $1.5 \times 10^5$ cfu/ml and $2.9 \times 10^6$ cfu/ml and 0.5 M cementation reagents concentrations were used to evaluate the shear strength of the soil. Treatment durations of 24, 36, 48 and 60 hours were used in the study and biotreated specimens were cured for 1, 3, 7, 14 and 21 days. The results obtained indicated that higher bacteria concentrations of $2.9 \times 10^6$ cfu/ml provided better strength improvement than the lower concentrations of $1.5 \times 10^5$ cfu/ml. Likewise, the strength also increases proportionally with the increase in curing time up to 14 days. Hence, the optimum curing period of biotreated residual soil was found to be 14 days. The results obtained revealed that the higher the amount of calcite precipitated the more the strength improvement up to 48 hours treatment duration.

Keywords: Include 5 keywords or phrases, separated by semi columns to distinguish them.

1.0 INTRODUCTION

Residual soil has been considered as one of the most important civil construction materials in civil engineering. However, some of these soils encountered as foundation or construction material often do not satisfy the expected requirements in terms of their index and engineering properties. As such, buildings and other civil infrastructures founded on these loose, weak or soft sediments may require some preventive measures to avoid structural damage (Van Paassen, 2011). Hence, many approaches have been adopted by geotechnical engineers to modify and improve the engineering properties of the soil in order to serve an intended purpose. According to (Gunaratne, 2013), some of these approaches that are practically utilized include soil replacement, compaction, reinforcement and fixation. Fixation involves binding the soil particles to improve their strength and decrease compressibility; jet and permeation grouting falls under this category. Though, most of these techniques have proved successful in improving the engineering properties of soil, application of some of these methods usually require high amounts of energy, costs, have limitations with regards to treatment range and require materials which have considerable impact on the environment (Karol, 2003).

Chemical grouting has commonly been used as a soil improvement technique due to its economic benefits. It is usually achieved with a variety of additives including cement, lime, asphalt, sodium silicate, lignin, urethane and resins. Though many of these additives were found to be successful in improving the engineering properties of the soils, they may often contaminate the soil and groundwater (DeJong et al., 2006). These approaches create environmental concerns over
their field application and are increasingly under the scrutiny of public policy and opinion; in fact all chemical grouts except sodium silicate are toxic and/or hazardous (DeJong et al., 2010; Karol, 2003). Hence, the need for new and sustainable methods of soil improvement is inevitable.

Hence, new technique that utilizes the interdisciplinary knowledge of microbiology, geochemistry and civil engineering to modify the engineering properties of soils in the subsurface have recently emerged and is referred to as Microbially Induced Calcite Precipitation (Cheng et al., 2013; DeJong, et al., 2006; DeJong, et al., 2010; Ivanov and Chu, 2008; Lee et al., 2012; Mitchell and Santamarina, 2005; Whiffin et al., 2007).

Microbially Induced Calcite Precipitation (MICP) method of soil improvement refers to the biochemical reaction that take place within a soil mass to produce calcite precipitates to alter the engineering properties of the soil (DeJong, et al., 2010). The technique takes the advantage of native biological process to produce calcite precipitates into the soil matrix. The calcites generated are responsible for cementing and clogging the soils, and hence improve the strength and reduce the hydraulic conductivity of the soils. MICP can be a viable alternative technique that improve soil supporting new and existing structures and in many geotechnical engineering applications such as liquefiable sand deposits, slope stabilization, and subgrade reinforcement (Cheng, et al., 2013; DeJong, et al., 2006).

Microbial carbonate precipitation has gained interest in the last twenty years, especially with regard to the excellent result demonstrated by this technique in sealing leakages in water retaining structures and reducing the permeability of some soils by means of bioclogging (Ivanov and Chu, 2008; Van Paassen, 2011; Whiffin, et al., 2007). Meanwhile, the technique of using microorganisms for improving the strength of granular soil which is referred to as biocementation started in 2001 in Australia (Kucharski et al., 2006). Hence, considerable increase in unconfined compressive strength and limited reduction in permeability of treated samples are the basic qualities that make biocementation treatment attractive (Harkes et al., 2010; Cheng and Cord-Ruwisch, 2012; Soon et al., 2014).

The prevailing organisms available in soils are bacteria. They are usually available at various depths of the soil profile, but in smaller numbers at large depths in the earth’s lithosphere (Bartlett, 1998). A number of bacteria species are capable of producing urease enzyme and are used in bio-mediated soil improvement technique. Since the bacteria are innate to the earth, they are likely not posed any environmental hazard in future (Fritzges, 2005). Some of the species include genera Bacillus, Sporosarcina, Spoloactobacilus, Clostridium and Desulfitomaculum(Kucharski, et al., 2006).

The main function of bacteria in the biomineralization process is to consume urea and decompose it into ammonia (NH₃) and carbon dioxide (CO₂). These chemicals disperse into the surrounding solutions via the cell wall of the bacteria. The released ammonia converts to ammonium ions (NH₄⁺) 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 Ca²⁺ 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 (CaCO₃) (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 (NH₃) and carbon dioxide (CO₂). The chemical reaction is presented in Equations 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 (Equation 2). The carbon dioxide quickly decomposed in the presence of water into bicarbonate (HCO₃⁻) and the bicarbonate reacts with the hydroxyl ions to form carbonate ions (Equations 3 and 4). Hence, in the presence of calcium ions (Ca²⁺), the calcium carbonate (CaCO₃) is precipitated (Equation 5) (Castanier et al., 1999; Burne and Chen, 2000). Therefore, the summary of the overall process of urea hydrolysis and formation of calcium carbonate is presented in Equation 6.

\[
CO(NH₂)₂ + H₂O → 2NH₃ + CO₂
\]  

*(Equation 1)*
Hence, this study intends to evaluate the effects of bacteria concentrations and curing time on the microbially induced calcite precipitations in residual soil.

2. MATERIALS AND METHOD

2.1. Bacteria Isolation, Cultivation and Cementation Reagents

The microorganism used in the study was isolated from the residual soil sample and was identified and named as *Klebsiella pneumoniae* strain UM123. The isolated strain was further tested for urea hydrolysis reaction by inoculating it into urea broth containing phenol red that indicates a pH change associated with ammonium production from urea hydrolys is reaction. The colour changed from yellow-orange to pink was an indication that urea hydrolysis occurred. The strain was then cultivated in American Type Culture Collection (ATCC) specific yeast extract–based medium containing of 20g yeast extract, 10g ammonium sulphate in 1 Litre 0.3 M Tris buffer solution at pH 9.0. After 24 hours incubation at 30 °C, the culture was then harvested and stored at 4°C prior to use. The microorganism was grown to its late exponential growth phase and desired concentrations of $1.5 \times 10^4$ cfu/ml and $2.9 \times 10^6$ cfu/ml at optical density (OD$_{600}$) were obtained and used in the study. The cementation reagents used throughout the study consist of 3g nutrient broth and 0.5 M concentrations of urea and calcium chloride.

2.2. Index Properties of Soil Sample

Index properties are basically used for classification and determination of engineering properties of the soils. In this study, tests conducted were particle specific gravity, Atterberg limits and particle size distribution. The tests were conducted based on the procedures outlined in the British Standard, BS 1377 Part 2: 1990.

2.3. Standard Proctor Compaction

Compaction test generally provides the moisture content for the most efficient compaction at which the maximum dry density is achieved under same compactive effort. The tests were performed to obtain the maximum dry density of a soil and its corresponding optimum moisture content. Hence, it was conducted in accordance with the provision of BS 1377 Part 4: 1990.

2.4. Unconfined Compressive Strength

The Unconfined Compressive Strength (UCS) test is a special form of unconsolidated undrained triaxial test in which no confining pressure is applied to the specimen. Hence, axial stress was applied to the soil specimen and gradually increased until it fails. The test was conducted.
using the load frame method in accordance with the procedure outlined in BS 1377 Part 7: 1990.

2.5. Sample Preparations and Curing Conditions

The microbial cementation experiments were conducted in two stages; the first stage involved mixing the residual soil specimens with a liquid medium containing the microorganism (*Klebsiella pneumoniae* strain UM123) at concentrations of 1.5×10^5 and 2.9×10^6 cfu/ml. The quantity of the medium was determined to correspond to the optimum moisture content of the soil of 31.2%. The cementation reagents (urea, calcium chloride and nutrient broth) at 0.5M concentrations were then sprayed to the soil-bacteria mixture at 6 hours interval for 24 hours; while curing at atmospheric temperatures. This was to allow for fixation and uniform distribution of the bacteria into the soil. The second stage involves compacting the soil-bacteria mixture into a 50mm diameter and 100mm height prefabricated steel mould to a maximum dry density of 1.390 Mg/m^3^. The compacted soil-bacteria mixtures were then placed between two clean gravel layers to serve as filter layers to avoid turbulent inflow and clogging at the inlet. The specimens were treated for 24, 36, 48 and 60 hours durations. After treatment, the specimens were extruded and cured at atmospheric temperatures for 3, 7, 14 and 21 days before the Unconfined Compressive Strength tests. However, some specimens were tested for the strength improvement immediately after the treatment. Hence, after strength determinations, the calcite contents of each treated sample were determined using gravimetric analysis of acidified samples.

2.6. Calcite contents determination

The calcite contents of each treated sample were determined using gravimetric analysis of acidified samples. 10g of the powder sample was used after oven drying at 105°C for 24 hours. Hydrochloric acid 2 M was added to the prepared powdered sample and carbon dioxide was liberated due to the reaction between calcite and hydrochloric acid. The residue was collected and oven dried again and the loss in weight was used to estimate the percentage of calcite contents in the specimen. The method was based on the assumption that the increment of carbonate content in the soil after the MICP treatment was purely caused by the formation of calcium carbonate. This method was also adopted by Soon *et al.*(2014).

### 3.0 RESULTS AND DISCUSSION

#### 3.1. Residual Soil

The tropical residual soil used in the study was collected from a site at Universiti Teknologi Malaysia, UTM. The soil sample was classified as Gravelly silt of high plasticity (MHG) based on British Soil Classification System (BSCS). Table 1 and Figure 1 present the Index / engineering properties and particle size distribution curve of the soil sample respectively.

#### 3.2. Effect of Bacteria Concentrations

To ascertain the effects of *Klebsiella pneumoniae strain UM123* concentrations on the strength improvement of the MICP treated residual soil, two bacteria concentrations of 1.5×10^5 cfu/ml to 2.9×10^6 cfu/ml were used in the biocementation process. The two concentrations were obtained from exponential growth phase of the microorganism. Figure 2 presents the correlation between bacteria cell concentrations and strength improvement at atmospheric temperature curing conditions using reagents (urea and calcium chloride) concentrations of 0.5M.
Figure 1: Particle size distribution curve of the soil

Table 1: Index and Engineering Properties of the Soil

<table>
<thead>
<tr>
<th>Properties</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gravel (%)</td>
<td>32</td>
</tr>
<tr>
<td>Sand (%)</td>
<td>10</td>
</tr>
<tr>
<td>Silt (%)</td>
<td>32</td>
</tr>
<tr>
<td>Clay (%)</td>
<td>26</td>
</tr>
<tr>
<td>Moisture content (%)</td>
<td>36</td>
</tr>
<tr>
<td>Liquid limit (%)</td>
<td>71</td>
</tr>
<tr>
<td>Plastic limit (%)</td>
<td>47</td>
</tr>
<tr>
<td>Plasticity Index (%)</td>
<td>24</td>
</tr>
<tr>
<td>Specific gravity</td>
<td>2.62</td>
</tr>
<tr>
<td>MDD (Mg/m³)</td>
<td>1.390</td>
</tr>
<tr>
<td>OMC (%)</td>
<td>31.2</td>
</tr>
<tr>
<td>Classification(BSCS)</td>
<td>MHG</td>
</tr>
<tr>
<td>UCS (kPa)</td>
<td>30.4</td>
</tr>
</tbody>
</table>

The result shows a general increase in strength as the bacteria concentrations increases for all the treatment durations up to 48 hours. It was observed that when the concentrations of the bacteria were increased from 1.5×10⁵ cfu/ml to 2.9×10⁶ cfu/ml, the strength improvement also increased for the treatment durations of 24, 36 and 48 hours. The possible explanations to this observable fact is that, as the bacteria concentrations were increased from 1.5×10⁵ cfu/ml to 2.9×10⁶ cfu/ml, more viable cells were provided that released more urease enzymes and catalyzed the urea hydrolysis reactions. This consequently resulted in the release of more carbonate ions from the decomposition of urea that reacts with the calcium ions from the supplied calcium chloride to precipitates more calcite. The precipitated calcite then fill in the soil voids and cement the soil particles together to improve the strength. Hence, the strength improvement was found to be directly proportional to the bacteria concentrations. Though, uncatalyzed urea hydrolysis is a very slow process; the release of an enzyme urease by the introduced microorganisms catalyzes the reaction significantly. As it was reported by (Benini et al., 1999) that urease enzyme catalyses urea hydrolysis reactions by up to 10¹⁴ times faster. Figure 3 present the FESEM images of the MICP treated specimens using 1.5×10⁵ cfu/ml and 2.9×10⁶ cfu/ml bacteria concentrations. The calcites precipitated at higher bacteria concentrations of 2.9×10⁶ cfu/ml are more densely and evenly distributed than the one of lower concentrations.

Figure 3: FESEM Images of MICP treated soil (a) 1.5×10⁵ cfu/ml (b) 2.9×10⁶ cfu/ml bacteria cell concentrations
However, it was observed that beyond 48 hours treatment duration, the strength generally declined. This may be attributed to the decays in urease activity with time, due to cell lysis, porosity reduction due to calcite precipitations, wash-out of urease from the soil or due to encapsulation in the calcium carbonate crystals. Experiments performed by Booster et al. (2008) showed that the cumulative effect of enzyme excretion, cell decay, wash out, encapsulation and porosity reduction caused a decay in urease activity by a factor of 3 in about 80 hours of continuous flushing with 1M cementation reagents.

3.3. Bacteria Concentrations and Calcite Contents

Bacterial activity plays an important role in urea hydrolysis reaction that leads to the precipitation of calcite into the soil matrix and the subsequent strength improvement of the soil. Figure 4 shows the calcite content and ratio of strength improvement relative to untreated samples for 1.5x10^5 cfu/ml and 2.9x10^6 cfu/ml concentrations of Klebsiella pneumoniae. It was found that more calcites were precipitated at higher bacteria concentrations of 2.9x10^6 cfu/ml than at lower concentrations of 1.5x10^5 cfu/ml. Highest calcite content of 2.65% were recorded at 48 hours treatment using 2.9x10^6 cfu/ml that provided the highest strength improvement. Similarly, the ratio of strength improvement relative to the untreated samples were found to be higher at bacteria concentrations of 2.9x10^6 cfu/ml; with the highest ratio of 2.1 reported at 48 hours treatment durations.

3.4. Effect of Curing Time on the Ureolysis Reaction

To study the effects of curing time on the biotreated soil; specimens were cured for 1, 3, 7, 14 and 21 days at atmospheric temperatures. The microbial treatment was conducted using the two bacteria concentrations of 1.5x10^5 cfu/ml and 2.9x10^6 cfu/ml for 48 hours at 0.5M reagent concentrations. Figure 5 presents the shear strength of the treated soil samples cured for 1, 3, 7, 14 and 21 days. The shear strengths of the treated specimens increases with increase in curing time up to 14 days for the two bacteria concentrations. However, the strength remained unchanged when cured for 21 days. This suggest that the strength improvement of biotreated residual soil was peaked at 14 days curing. Similar observations were made by (Kenny, 2016); the author reported peak strength improvement of 28.6% when the MICP treated residual soil was cured for 14 days. Likewise, Sadjadi et al, (2014) reported the peak strength improvement of MICP treated soil on the third day of curing. Thereafter, no further improvement was observed for samples cured at 7 and 14 days.

Likewise, it was observed that the calcite contents of the specimens had also increased with curing period up to 14 days. The possible explanation is that Klebsiella pneumoniae strain UM123 continue to utilise the remaining nutrients and cementation reagents to precipitates calcites up to 14 days; thereby improving the strength of the treated soil. However, after 14 days curing the available nutrients and reagents might have been exhausted;
as such making the survival and activity of the bacteria very difficult and the strength remained constant. Though, most of the microorganisms utilized in the MICP process are capable of surviving harsh environmental conditions without nutrients; they tend to excrete their enzymes which degrade more easily outside than inside the cells. This may also results into microbial attack; which is caused by the formation of acidic substance as a by-product of microbial activity and may dissolve the precipitated calcites (Bin et al., 2008; Gadd, 2010).

4. CONCLUSIONS

This study revealed the viability of using MICP in improving the engineering properties of tropical residual soil. It can be deduced that higher concentrations of bacteria provided better strength improvement. This is because the higher the concentration of the microorganisms the more calcite would be precipitates that subsequently bind the soil particles together and improve the strength. 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 a result of high salinity. Hence, 14 days curing of biotreated residual soil at ambient temperatures provided the highest strength improvement of up to 184% and 212% relative to untreated samples for 1.5×10⁵ cfu/ml and 2.9×10⁶ cfu/ml bacteria concentrations respectively. As such 14 days were found to be the optimum curing period of the biotreated residual soil.

REFERENCES


