



## Performance Evaluation of Microbial Cementation in Soil Improvement Using Different Ureolytic Bacteria Species

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### ABSTRACT

The process of using microorganisms to improve the strength of residual soil is referred to as biocementation process. This technique utilizes the concept of microbial calcite precipitation to precipitates calcium carbonate into the soil matrix structure leading to the cementation of the soil particles and subsequent improvement in the strength and reduction of hydraulic conductivity. This research studied the performance of two bacteria species in the stabilization of residual soil via urea hydrolysis. The bacteria species used were *Sporosarcina pasteurii* and *Klebsiella pneumoniae* UM123 at concentrations of  $1.20 \times 10^6$  cfu/ml and  $1.80 \times 10^6$  cfu/ml and  $1.5 \times 10^5$  cfu/ml and  $2.9 \times 10^6$  cfu/ml respectively to evaluate the strength improvement of the residual soil. Similarly, cementation reagents used consists of urea and calcium chloride at 0.5M concentration. The study also employed treatment durations of 24, 36, 48, and 60 hours. The findings obtained demonstrated that higher concentrations of bacteria of  $1.80 \times 10^6$  cfu/ml and  $2.9 \times 10^6$  cfu/ml provided better strength improvement compared to lower concentrations of  $1.20 \times 10^6$  cfu/ml and  $1.5 \times 10^5$  cfu/ml for *Sporosarcina pasteurii* and *Klebsiella pneumoniae* UM123 species respectively. Thus, it was also found that as the bacteria cells concentrations are increased during biocementation process more calcite are precipitated leading to higher strength improvement with 2.2% being the highest strength improvement ratio obtained at 48 hours treatment duration for *Sporosarcina pasteurii* specie. Similar observations were made with *Klebsiella pneumoniae* UM123 with 2.1% being the highest strength improvement ratio recorded at 48 hours treatment duration. Hence, the higher the amount of calcite precipitated the more the strength improvement up to 48 hours treatment duration for the two bacteria species. Therefore, at 48-hours of biocementation process, the strength of the soil had improved by 120% and 110% for *Sporosarcina pasteurii* and *Klebsiella pneumoniae* UM123 species respectively; compared to the untreated sample, representing the highest recorded strength improvement. Consequently, *Sporosarcina pasteurii* was found to performed better than *Klebsiella pneumoniae* UM123 in urea hydrolysis reaction and subsequent biocementation.

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### INTRODUCTION

The demand for new and sustainable methods of soil improvement continue to increase,

with more than 40,000 soil improvement projects being performed per year at a total cost exceeding 6 billion US Dollar/year worldwide (DeJong et al,

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2010). The majority of these soil improvement techniques utilize mechanical energy and/or man-made materials, both of which require substantial energy for material production and/or installation. A common approach is to inject synthetic man-made materials, such as micro-fine cement, epoxy, phenoplasts, silicates, and polyurethane into the pore space to bind soil particles together (Karol, 2003). This is accomplished using a variety of chemical jetting and permeation grouting techniques. These approaches create environmental concerns and are increasingly under the scrutiny of public policy and opinion. Hence, it was revealed that all chemical grouts except sodium silicate are toxic and/or hazardous (Karol, 2003).

Hence, new technique that utilizes the interdisciplinary knowledge of microbiology and geochemistry to modify the engineering properties of soils in the subsurface have recently emerged and is referred to as bio-cementation or Microbially Induced Calcite Precipitation (MICP) (Cheng et al., 2013; DeJong, et al., 2006; DeJong, et al., 2010; Ivanov and Chu, 2015; Lee et al., 2012; Whiffin et al., 2007).

Bio-cementation is an emerging soil improvement technology in which inorganic compounds are induced by bacteria or free enzymes to cement geotechnical materials. The effective products of the bio-cementation process are usually precipitates with cementing properties, including carbonate, phosphate, and iron hydroxide etc. Among them, ureolysis-based carbonate precipitation is the most widely studied process to achieve bio-cementation. During this process, the hydrolysis of urea is catalyzed by the bacteria or urease enzyme, releasing a large amount of free carbonate ions into the reaction environment (Cardoso, et al, 2020). With free calcium ions in the reaction system, calcium carbonate can precipitate in soil pores. The reaction process is shown as follows:

The ureolysis is an important step in the bio-cementation process. This step usually relies on urea-hydrolyzing bacteria or free urease. Depending on the type of selected catalyst, the two common processes are named Microbially-Induced Carbonate Precipitation (MICP) and

Enzyme-Induced Carbonate Precipitation (EICP) respectively. In recent years, the use of soybean urease for carbonate precipitation and bio-cementation has attracted many research studies. Urea-hydrolyzing bacteria are widely used in the bio-cementation process. It can adapt to various reaction environments, effectively promote carbonate precipitation, and provide nucleation sites for mineral growth (Cardoso, et al, 2020).

The introduction of bacteria or urease can accelerate the hydrolysis of urea, promote the precipitation of carbonates, and regulate their cementation patterns. The produced carbonate interconnects soil particles and fill soil voids, thereby improving the strength and reducing the permeability of geotechnical materials. In recent years, many scholars have successfully applied bio-cementation technology in several geotechnical engineering fields, such as ground soil strengthening (Cardoso, et al, 2020), erosion control (He, et al, 2022) and conservation of historic relics (Gao, et al, 2022).

The ultimate effect of bio-cementation is determined by a series of factors, such as properties of soils, the concentration of the cementation reagents and treatment methods. These factors lead to the differences in the bio-treated materials such as the amount, uniformity and cementation pattern of calcium carbonate. According to previous studies, there is a significant positive correlation between the calcium carbonate content and the strength of the specimens and treated specimens with higher calcium carbonate content tend to exhibit higher strength (Liu, et al, 2023; Qi, et al, 2022). Thus, this study intends to evaluate the performance of bio-cementation process in residual soil improvement using two ureolytic bacteria species.

## EXPERIMENTAL PROCEDURE

### Residual Soil

The residual soil utilized in this study was sourced and collected within the premises of University of Technology Malaysia (UTM). Laboratory tests were conducted to determine the index and engineering properties of the soil sample. Additionally, the soil's mineralogical



compositions were analyzed through X-Ray Diffraction Analysis (XRD).

### Bacteria Cultivation and Cementation Reagents

The *Sporosarcina pasteurii* (ATCC® 11859™) strain was cultured in a yeast extract-based medium as specified in American Type Culture Collection, 2021. The medium consists of 20 g of yeast extract and 10 g of ammonium sulphate dissolved in a 1000 ml Tris buffer solution with a pH of 9.0 and a concentration of 0.3 molar. The culture was then incubated for 48 hours at a temperature of 30 °C. After incubation, the culture was harvested and stored at 4 °C prior to use. To obtain the desired concentrations of  $1.20 \times 10^6$  cfu/ml and  $1.8 \times 10^6$  cfu/ml, the plate count method was adopted from Soon, et al, 2014. Similarly, 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 microorganism was grown to its exponential growth phase in the same medium as specified in American Type Culture Collection, 2021. Hence, concentrations of  $1.5 \times 10^4$  cfu/ml and  $2.9 \times 10^6$  cfu/ml were obtained and used for the study. These concentrations were then utilized in the study to assess the impact of different bacteria concentrations on the bio-cementation process. Hence, cementation reagents (calcium chloride and urea) of 0.5 M having established to be the optimum concentrations were used in this study.

### Preparation of Sample and Biocementation Process

The soil specimens were thoroughly mixed with the bacteria species of *Sporosarcina pasteurii* and *Klebsiella pneumoniae* UM123 which are prepared and contained in a liquid medium at the desired concentrations. The volume of the liquid medium containing the bacteria used in the sample preparations was found to be equivalent to the soil's OMC, which was measured at 26.5%. The soil samples were subsequently compacted into the pre-fabricated mold until reaching a maximum dry density of  $1.402 \text{ Mg/m}^3$ . The specimens were sandwiched between clean gravels layers to function as filters

in order to evade blockage and turbulent inflow. Cementation reagents were injected into the mould containing the compacted soil-bacteria mixtures at a pressure of 0.2 bars from a compressed vessel. Treatments were performed for the durations of 24, 36, 48 and 60 hours after which the samples were extruded for curing and testing. The preparation method was adopted from Soon, et al, 2014.

### 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.

## RESULTS AND DISCUSSION

### Index properties of the residual soil

The summary of the index and engineering properties of the residual soil samples are presented in Table 2. The results are in agreement with those obtained by Yunusa, et al., (2015) on residual soil from the study area. The particle size distribution of the soil is presented in Figure 1 and the soil sample was classified based on British Soil Classification System as Gravelly Silt of intermediate plasticity (MIG). Similarly, the X-Ray Diffraction Analysis revealed that Kaolinite is the dominant clay mineral present in the soil.

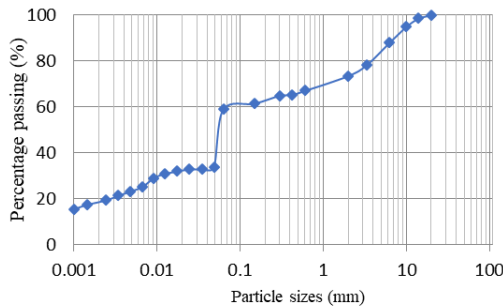
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**Figure 1:** Particle size distribution curve of the soil

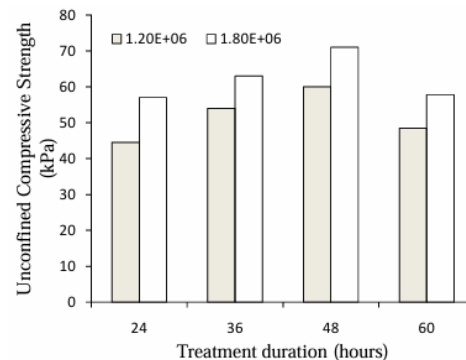
**Table 1:** Index and Engineering properties of the soil sample

Properties	Description
Gravel (%)	27
Sand (%)	21
Silt (%)	34
Clay (%)	18
Moisture content (%)	33
Liquid limit (%)	64
Plastic limit (%)	43
Plasticity Index (%)	21
Specific gravity	2.60
MDD (Mg/m <sup>3</sup> )	1.402
OMC (%)	26.5
Classification (BSCS)	MIG
UCS (kPa)	32.3
Hydraulic conductivity (m/s)	3.2E-06
Dominant clay mineral	Kaolinite

### Effect of Bacteria Concentrations on Strength Improvement

Effects of *Sporosarcina pasteurii* concentrations on the strength improvement of MICP treated residual soil were determined. Two bacteria concentrations of  $1.2 \times 10^6$  cfu/ml and  $1.8 \times 10^6$  cfu/ml obtained from exponential growth phase were used to conduct the biocementation treatment on the residual soil used in the study. Figure 2 presents the correlation between bacteria cell concentrations and strength improvement. The results shows a general increase in strength as the bacteria concentrations increases for all the treatment durations up to 48 hours. This is because as the concentration of the bacteria increases, more active and viable cells would be available for the urea hydrolysis reaction. 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 catalyzes urea hydrolysis reactions by up to  $10^{14}$  times faster.



**Figure 2:** Correlation between bacteria cell concentrations and strength improvement of *Sporosarcina pasteurii*

It was observed that when the concentrations of *Sporosarcina pasteurii* were increased from  $1.2 \times 10^6$  cfu/ml to  $1.8 \times 10^6$  cfu/ml the strength also increased for the treatment durations of 24, 36 and 48 hours. Hence, 18% increment in the strength at 48 hours treatment duration was recorded when the concentrations of *Sporosarcina pasteurii* were increased. Hence, findings from the studies of Soon, et al., 2014 using *B. megaterium* and Whiffin, et al., 2007 using *Sporosarcina pasteurii* also confirmed the role bacteria concentrations plays in obtaining efficient improvement of MICP treated soil.

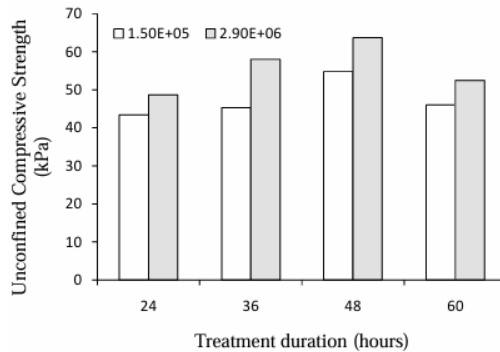
Similarly, to ascertain the effects of *Klebsiella pneumoniae* strain UM123 concentrations on the strength improvement of the MICP treated residual soil, two bacteria concentrations of  $1.5 \times 10^5$  cfu/ml to  $2.9 \times 10^6$  cfu/ml were used in the biocementation process. Figure 3 presents the correlation between bacteria cell concentrations and strength improvement.

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**Figure 3:** Correlation between bacteria cell concentrations and strength improvement of *Klebsiella pneumoniae* strain UM123

Likewise, as the strength of the residual soil was reported to improved when biotreated with *Sporosarcina pasteurii*; similar observations were made when the concentrations of *Klebsiella pneumoniae* strain UM123 were increased from  $1.5 \times 10^5$  cfu/ml to  $2.9 \times 10^6$  cfu/ml. Consequently, it was observed that 16% increment in the strength at 48 hours treatment duration was recorded when the bacteria concentrations were increased from  $1.5 \times 10^5$  cfu/ml to  $2.9 \times 10^6$  cfu/ml.

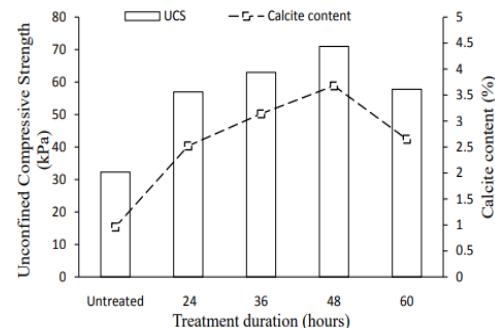
Thus, it can be deduced that, as the bacteria concentrations are increased, more viable cells were provided that released more urease enzymes and catalyzed the urea hydrolysis reactions. This consequently resulted in the release of more carbonate ions from the decomposition of urea that reacts with the calcium ions from the supplied calcium chloride to precipitates more calcite. The precipitated calcite then fills in the soil voids and cements the soil particles together to improve the strength. 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.

### Effect of Treatment Duration on the MICP Treatment Process

To assess the effects of treatment duration on the MICP process using *Sporosarcina*

*pasteurii*, bacteria concentrations of  $1.8 \times 10^6$  cfu/ml were used to conduct the treatment at durations of 24, 36, 48 and 60 hours. Figure 4 presents the correlation between unconfined compressive strength, calcite content and treatment durations using 0.5M reagent concentration.

It was observed that the unconfined compressive strength of the treated residual soil increases with increase in treatment duration up to 48 hours; attaining the highest UCS of 71kPa. However, at longer treatment duration beyond 48 hours; specifically at 60 hours the strength of the treated soil did not further improve. It was therefore deduced that beyond 48 hours treatment duration, the strength generally declined. This may be attributed to the decays in urease activity with time, cell lysis, porosity reduction due to calcite precipitations, wash out of urease from the soil or due to encapsulation in the calcium carbonate crystals (Rodriguez-Navarro, et al., 2012).



**Figure 4:** Correlation between UCS, calcite content and treatment durations for MICP treatment using *Sporosarcina pasteurii*

Figure 5 also presents the calcite content distributions at the top, middle and bottom parts of the MICP treated specimens. It appears that all the specimens treated for 36 and 48 hours exhibited identical distribution of calcites. More calcites were found at the top part of the specimens, while the calcite precipitated at the middle and bottom part of the specimens appears to be less and almost uniform. However, for 24 and 60 hours treatments the distributions of calcite

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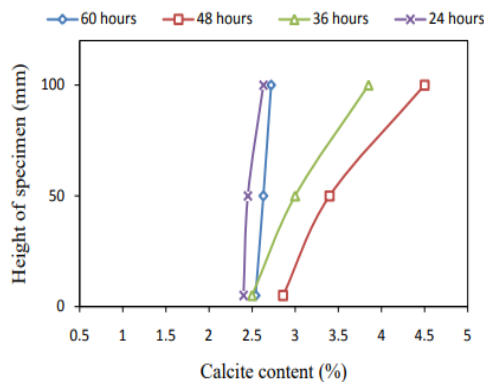
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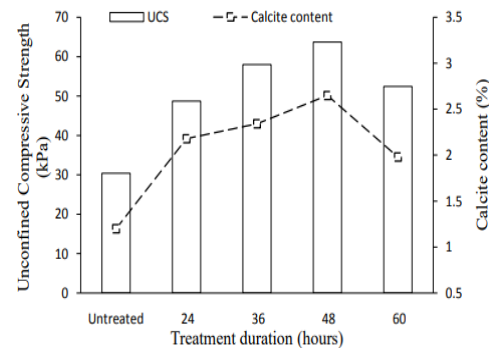
contents were almost identical across the length of the sample. Hence, having almost uniform calcite distribution at 60 hours; may possibly be connected to the wash out of the urease enzyme and calcites at the soil pores from the top to the bottom of the sample due to prolonged treatment.



**Figure 5:** Calcite content distributions at the top, middle and bottom of the treated sample using *Sporosarcina pasteurii*

Apparently, it can be deduced that uniformity of calcite precipitations plays an important role in the shear strength development. This is because the 24 hours treatment that has almost uniform calcite contents across the length of the sample; reported the highest percentage of strength improvement of 76% relative to untreated sample. During the first 24 hours of treatment, the shear strength improved by 76%, while only 44% improvement was recorded in the second 24 hours (120% at 48 hours). Likewise, it was also observed that samples with more calcite contents had higher resistance to the shear stress before failure. Similar observation was reported by Whiffin, et al., 2007.

Similarly, effect of treatment duration on the MICP process using *Klebsiella pneumoniae* strain UM123 concentration of  $2.9 \times 10^6$  cfu/ml and treatment durations of 24, 36, 48 and 60 hours was evaluated. Figure 6 presents the correlation between unconfined compressive strength, calcite content and treatment duration using 0.5M reagent concentration.



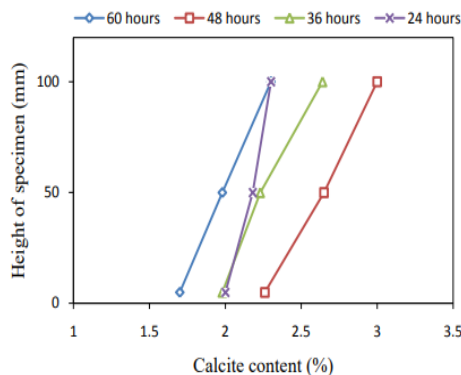
**Figure 6:** Correlation between UCS, calcite content and treatment durations for MICP treatment using *Klebsiella pneumoniae* strain UM123

The unconfined compressive strength of the residual soil increases with increase in treatment duration when the biocementation experiment was conducted using *Klebsiella pneumoniae* strain UM123. Similarly, the unconfined compressive strength attained its highest value of 63.7 kPa at 48 hours treatment duration. It was therefore observed that beyond 48 hours treatment duration, the strength generally declined.

Study conducted by Cheng, et al., (2013) on the effect of saturation on the geotechnical properties of MICP treated sand revealed that samples treated under lower degree of saturation had higher strength and more calcite are formed at the inter-particle contact. The authors also suggested that longer treatment duration of more than three days could have adverse effect on the microbial activity and strength development. Meanwhile, De Muynck, et al. (2010) also revealed that longer treatment duration could further increase the pH to create an extremely alkaline environment, which is not good for bacterial activity and urea degradation. It also results in local supersaturation and calcite precipitation on the bacteria cell wall and this may finally lead to cell death and weaken the efficiency of the MICP process.

Figure 7 also presents the calcite content distributions at the top, middle and bottom parts of the MICP treated specimens using *Klebsiella pneumoniae* strain UM123. It was

observed that all the specimens treated for 36, 48 and 60 hours exhibited identical distribution of calcites. More calcites were formed at the top part than the middle and bottom part of the specimens; with the bottom having the least calcite precipitations.



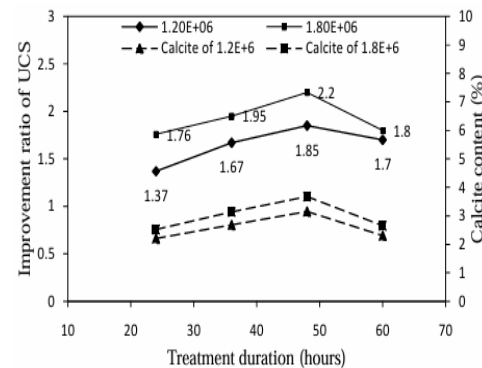
**Figure 7:** Calcite content distributions at the top, middle and bottom of the treated sample using *Klebsiella pneumoniae* strain UM123

This is because the cementation reagents are pumped into the soil from top part of the sample. Therefore, more calcites are formed at the top and middle part of the specimen filling the void spaces of the soil at the early part of the treatment. Thus, preventing further percolation of the reagents through the sample; as such more calcites accumulate at the top than at the middle and bottom of the specimen. However, for 24 hours treatments the distributions of calcite contents were almost uniform across the length of the sample.

#### Bacteria Concentrations and Calcite Contents

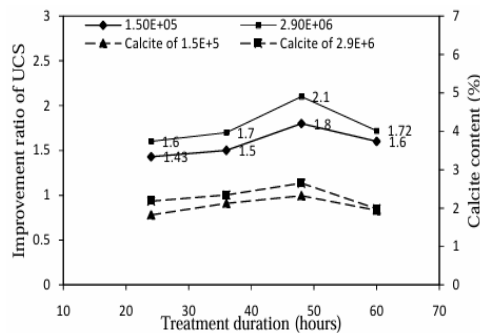
Bacterial activity plays an important role in urea hydrolysis reaction (together with the available reagents) that leads to the precipitation of calcite into the soil matrix and the subsequent strength improvement of the soil. Therefore, it is revealed that the more active bacteria cells are available during MICP process the more calcite are likely to be precipitated. As such the effects of bacteria cells concentrations on the calcite precipitation and the subsequent increase in the strength of the residual soil have been evaluated.

Figures 8 and 9 present the relationship between bacteria concentrations and calcite contents for the two microorganisms used in the study. Figure 8 shows the calcite content and ratio of strength improvement relative to untreated samples for  $1.2 \times 10^6$  cfu/ml and  $1.8 \times 10^6$  cfu/ml concentrations of *Sporosarcina pasteurii*.



**Figure 8:** Relationship between bacteria concentration, strength improvement ratios and calcite content of *Sporosarcina pasteurii*

The calcite contents were determined using acid wash gravimetric analysis and it was found that more calcites are precipitated at higher bacteria concentrations than at lower. Similarly, the ratio of strength improvement in relation to the untreated samples are found to be higher at bacteria concentration of  $1.8 \times 10^6$  cfu/ml; with the highest ratio of 2.2 reported at 48 hours treatment durations. Similar observations were made on the calcite contents and strength improvement ratios of *Klebsiella pneumoniae* strain UM123 at  $1.5 \times 10^5$  cfu/ml and  $2.9 \times 10^6$  cfu/ml concentrations as presented in Figure 9. Thus, highest ratio of 2.1 was also observed at 48 hours treatment durations with  $2.9 \times 10^6$  cfu/ml bacteria concentration.



**Figure 9:** Relationship between bacteria concentration, strength improvement ratios and calcite content of *Klebsiella pneumoniae* strain UM123

Bacterial activity plays an important role in urea hydrolysis reaction (together with the available reagents) that leads to the precipitation of calcite into the soil matrix and the subsequent strength improvement of the soil. Therefore, it is revealed that the more active bacteria cells are available during MICP process the more calcite are likely to be precipitated. Thus, calcium carbonate precipitation involves many phases such as nucleation, crystal growth and secondary changes in the crystal lattice. These processes take place in sequential order and sometimes concurrently (Loliou, et al., 2007).

## CONCLUSIONS

The residual soil used in the study was classified as Gravelly silt of intermediate plasticity (MIG) according to the British Soil Classification System (BSCS) with kaolinite being identified as the dominant clay mineral present in the soil. 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. The longer the treatment durations the more calcite precipitates increased up to 48 hours; causing more binding effects between the soil's particles, as such increasing the strength. However, at 60 hours treatment duration the calcite content decreased due to the decline in bacterial activity as a result of high salinity. Therefore, at 48-hours of biocementation process,

the strength of the soil had improved by 120% and 110% for *Sporosarcina pasteurii* and *Klebsiella pneumoniae* UM123 species respectively; compared to the untreated sample, representing the highest recorded strength improvement. Consequently, *Sporosarcina pasteurii* was found to performed better than *Klebsiella pneumoniae* UM123 in urea hydrolysis reaction and subsequent biocementation.

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