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RESIDUAL SOIL IMPROVEMENT USING MICROBIALLY INDUCED CEMENTATION OF UREOLYTIC BACTERIA

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ABSTRACT

The process of improving the strength of granular soil by utilising microorganisms is known as the biocementation process. This method involves the precipitation of calcium carbonate into the soil matrix structure through microbial calcite precipitation, resulting in the cementation of soil particles and subsequent improvement in strength and reduced permeability. This study investigated the potential of using Sporosarcina pasteurii, a microorganism strain with urease activity, to stabilise residual soil through urea hydrolysis. The cementation reagents used consisted of urea and calcium chloride at various concentrations ranging from 0.25, 0.5, 0.75, and 1.0 to 1.5 M. These concentrations were varied to assess their impact on the precipitation of calcium carbonate driven by ureolysis. Bacterial concentrations of 1.20×10^6 cfu/ml and 1.80×10^6 cfu/ml were employed to evaluate the shear strength of the soil. The study also employed treatment durations of 24, 36, 48, and 60 hours. The findings demonstrated that higher concentrations of bacteria of 1.80 × 106 cfu/ml yielded superior strength compared to lower concentrations of 1.20×10^6 cfu/ml. The optimal concentration of cementation reagents for effective treatment was determined to be 0.5 M, as any concentration exceeding this value resulted in a decline in bacterial activity, leading to a decrease in strength improvement. Furthermore, it was discovered that a more significant number of active bacteria cells during the biocementation process resulted in higher precipitation of calcite, with the highest percentage of 2.2% achieved after 48 hours of treatment. Consequently, the greater the amount of calcite precipitated, the more significant the strength improvement up to a treatment duration of 48 hours. Similarly, the Unconfined Compressive Strength (UCS) of the treated soil exhibited an increase with longer treatment durations, reaching a peak UCS of 71 kPa at 48 hours. Therefore, at the 48-hour mark, the strength of the soil had improved by 120% compared to the untreated sample, representing the highest recorded strength improvement.

Keywords: *Biocementation; reagents; urease active strain; microorganism; calcium carbonate*

INTRODUCTION

Many approaches, such as compaction, chemical stabilisation, and reinforcement, have traditionally been adopted to improve the engineering properties of some residual soils and meet the standard requirements for some engineering constructions. However, some techniques are sometimes unsuitable for large-volume treatments [1]. This issue arises because a considerable amount of energy may be needed during the production and application of these materials, which may, in turn, be detrimental to our environment.

Due to its economic viability and sustainability, chemical grouting has been considered one of the most common approaches in soil improvement. However, some of the additives used in the process may cause contamination of groundwater and the soil [2]-[3]. Though a significant number of these additives have shown greater potential for the soil improvement process regarding strength improvement and reduction of hydraulic conductivity, there are some concerns regarding environmental safety as a result of their field applications. Hence, according to Karol [1], Umar et al. [4], and Dejong et al. [5],

all chemical grouts, except for sodium silicate, are harmful and detrimental to environmental safety. Thus, a sustainable and environmentally friendly process to improve the engineering properties of soils for construction purposes is evident.

However, new interesting opportunities for utilising the available innate bacteria present in the soil to enhance the desired engineering properties of the soil have recently emerged [3],[6]-[9]. This novel technique, commonly known as microbially induced calcite precipitation, harnesses the biochemical interactions between indigenous soil bacteria and the soil to initiate the formation of calcium carbonate. This resultant calcium carbonate binds the soil particles together, enhancing the soil's strength and durability [5]. The observed improvement in strength and stiffness, coupled with the reduction in hydraulic conductivity of the soil, can be attributed to the cementation effects of the calcium carbonate precipitates on the soil particles. Consequently, the process of biocementation exhibits promising potential as an effective alternative for soil improvement and subsequent applications in geotechnical engineering [3],[9].

The largest microorganisms in soils are bacteria located at different depths in the earth's crust, and their availability decreases as depth increases [10]. They also vary in shape from nearly round, rod-like to spiral. Some bacteria species have the capability to produce an enzyme known as urease, which is believed to be a by-product of microbial metabolism. These bacteria are used in the biocementation process to stabilise soil for engineering. Since these bacteria naturally occur in the soil, they can artificially be introduced into the soil to increase their availability and are not likely to present any environmental danger in the future [11]. During the biocementation process, bacteria take in urea (which is usually introduced into the soil as reagents) and decompose it into ammonia (NH_3) and carbon dioxide (CO_2). These newly formed chemicals subsequently permeate across the cellular membrane of the bacteria.

Generally, three stages are involved in the biocementation process:

1. Urease-producing bacteria catalyse urea hydrolysis, resulting in the formation of ammonium and carbonate ions.
2. Subsequently, the carbonate ions react with the dissolved calcium derived from the provided calcium chloride, creating calcium carbonate crystals.
3. These newly formed calcite crystals effectively cement the soil particles together, improving their strength and durability and reducing permeability.

Therefore, this research aims to utilise a urease-producing bacterium commonly found in soil known as *Sporosarcina pasteurii* to induce calcite precipitation within the soil to improve or stabilise it. *Sporosarcina pasteurii* can typically be found in soil, sewage, and urinal deposits [12].

EXPERIMENTAL PROCEDURE

Residual Soil

The residual soil utilised in this study was sourced and collected within the premises of Universiti Teknologi Malaysia (UTM). Laboratory tests were conducted to determine the index and engineering properties of the soil sample. The soil's mineralogical compositions were also analysed 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 [12]. 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 molars. 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 until further use. To obtain the desired concentrations of 1.20×10^6 cfu/ml and 1.8×10^6 cfu/ml, the plate count method was adopted from Soon et al. [13]. This method allowed for the accurate determination of bacterial concentrations. The study then utilised these concentrations to assess the impact of different bacteria and reagent concentrations on the biocementation process. Similarly, the concentrations of the cementation reagents, comprising calcium chloride and urea, also varied. This is to evaluate the effect of different bacteria and reagent concentrations on the biocementation process. The components and concentrations of the cementation reagents are presented in Table 1.

Table 1 Chemical compositions 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	146.0	219.5
Nutrient broth (g/L)	3	3	3	3	3

Preparation of Sample and Biocementation Process

The soil specimens were thoroughly mixed with the bacteria (*Sporosarcina pasteurii*), which was prepared and contained in a liquid medium at the desired concentration. The volume of the liquid medium containing the bacteria used in the sample preparations was equivalent to the soil's OMC, which was measured at 26.5%. The soil samples were subsequently compacted into the pre-fabricated mould until reaching a maximum dry density of 1.402 Mg/m^3. The specimens were sandwiched between clean gravel layers to function as filters to evade blockage and turbulent inflow. Cementation reagents were injected into the mould containing the compacted soil-bacteria mixture at a pressure of 0.2 bars from a compressed vessel. Treatments were performed for 24, 36, 48, and 60 hours, after which the samples were extruded for curing and testing. The preparation method was adopted by Kong et al. [14]. Figure 1 presents the diagram of the experimental setup.

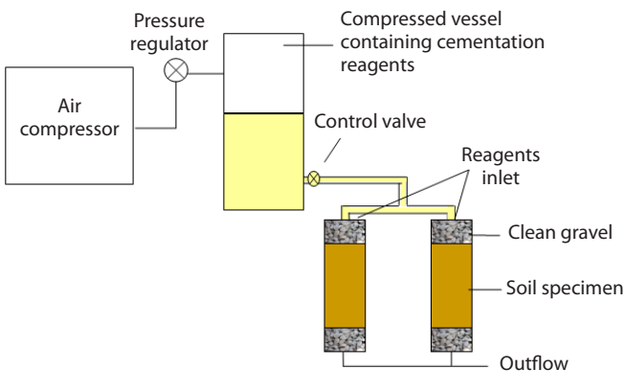

Figure 1 Diagram of the experimental setup

RESULTS AND DISCUSSIONS

Index and Engineering Properties of Soil

The residual soil sample exhibited a diverse range of particle sizes that could potentially be traced to sedimentary rock origins. According to BSCS, this soil was classified as Gravelly silt of intermediate plasticity (MIG). The engineering properties of the soil are presented in Table 2, while the gradation curve is shown in Figure 2.

Table 2 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/m3)	1.402
OMC (%)	26.5
Classification(BSCS)	MIG
UCS (kPa)	32.3
Hydraulic conductivity (m/s)	3.2E-06
Dominant clay mineral	Kaolinite

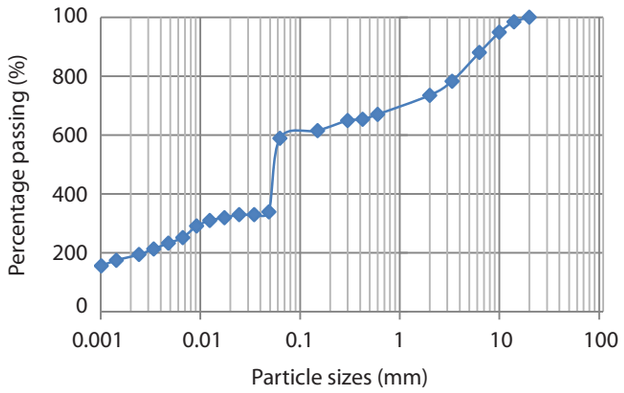

Figure 2 Gradation curve of the soil

Effect of Cementation Reagents Concentration

The results of the UCS tests on residual soil after biocementation treatment with different concentrations of reagents are illustrated in Figures 3 and 4. It could be deduced that the treated soil's strength improved with the increase in the concentration of the cementation reagents up to 0.5 M. However, beyond this value, the strength of the soil begins to decline as the reagent concentrations continue to increase.

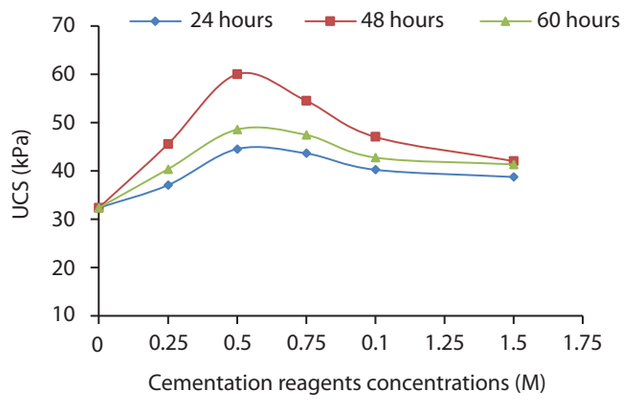

Figure 3 UCS of the soil at various reagents concentration for 1.2 × 10^6 cfu/ml bacteria concentration

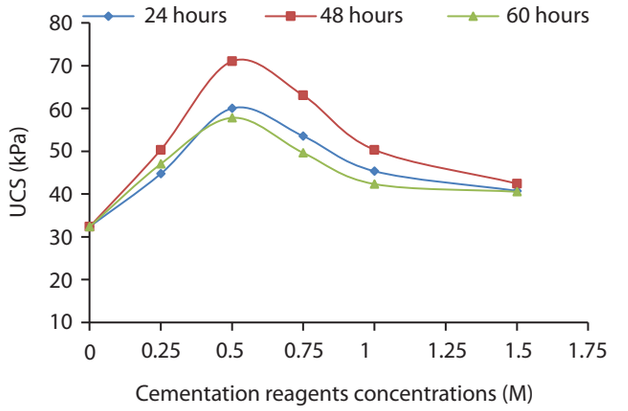

Figure 4 UCS of the soil at various reagents concentrations for 1.8 × 10^6 cfu/ml bacteria concentration

It can be deduced that there is an evident correlation between the concentration of cementation reagents and the production of urease enzyme by *Sporosarcina pasteurii*, with the latter being limited by its tolerance to high salinity. Notably, the soil's strength exhibited an increase when treated with reagent concentrations of 0.25 M and 0.5 M in conjunction with higher bacteria concentrations. However, a decline in strength was observed when the reagent concentrations surpassed 0.5 M, with the strength at 1.5 M nearly equaling that of the untreated specimen. This observation suggests that, for this particular bacteria, a reagent concentration of 0.5 M is optimal for an effective biocementation treatment. Any concentration exceeding this threshold resulted in a decline in bacterial activity, consequently decreasing strength improvement. These findings are in agreement with Soon et al. [15].

Effect of Bacteria Concentrations on the Strength Improvement

The impact of varying bacteria concentrations on the enhancement of lateritic soil strength was investigated in this study. Figure 5 illustrates the correlation between strength enhancement and bacteria cell concentrations using reagent concentrations 0.5 M. The results indicated that as soon as the concentrations of bacteria rose from 1.2 × 10^6 cfu/ml to 1.8 × 10^6 cfu/ml, the strength improvement also showed an increase across treatment durations of 24, 36, and 48 hours.

The reason behind the enhanced urea hydrolysis reactions with increasing bacteria concentration lies in the availability of more active and viable cells. While the natural urea hydrolysis process is sluggish, introducing microorganisms that secrete the enzyme urease significantly accelerates the reaction. According to Benini [16], the urease enzyme can boost the rate of urea hydrolysis reactions by a staggering 1014 times. Hence, this led to a notable 18% increase in strength after 48 hours of treatment with elevated bacteria concentrations. Studies by Soon et al. [17], Whiffin et al. [18], and Sharaky et al. [19] further support the pivotal role of bacteria concentrations in achieving optimal enhancements in the biocementation process.

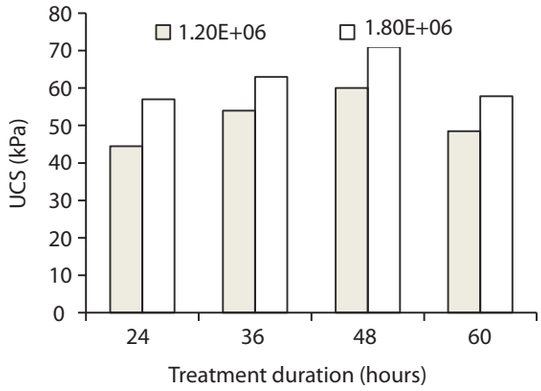

Figure 5 Correlation between bacteria cell concentrations and strength improvement

In Figure 6, the data illustrates the relationship between calcite content and the ratio of strength improvement compared to untreated samples for different bacteria concentrations. The results indicate that more calcites are formed as the bacteria concentration increases. Additionally, the ratio of strength improvement relative to the untreated samples is higher at a bacteria

concentration of 1.8×10^6 cfu/ml. Notably, the highest ratio of 2.2 was observed after a treatment duration of 48 hours.

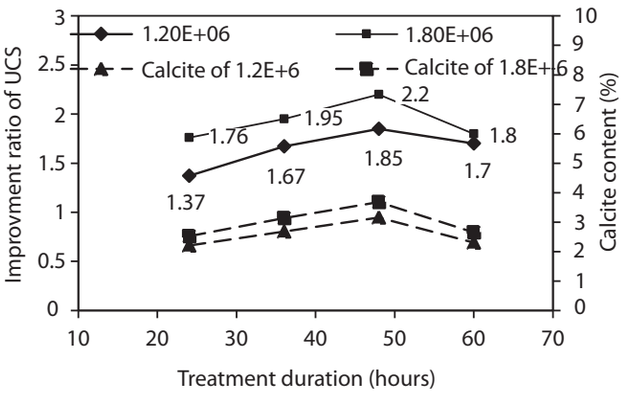

Figure 6 Relationship between bacteria concentration, the ratio of strength improvement, and calcite content

Bacterial activity is a crucial factor in the process of urea hydrolysis, along with the presence of reagents. This process leads to calcite formation in the soil matrix, resulting in the strengthening of the soil. It has been discovered that the availability of active bacterial cells during the biocementation process directly correlates with the amount of calcite to be precipitated.

Effect of Treatment Durations on the Biocementation Process

The impact of treatment durations on the biocementation process was evaluated by utilising a bacteria concentration of 1.8×10^6 cfu/ml for treatments conducted over 24, 36, 48, and 60 hours. Figure 7 illustrates the relationship between

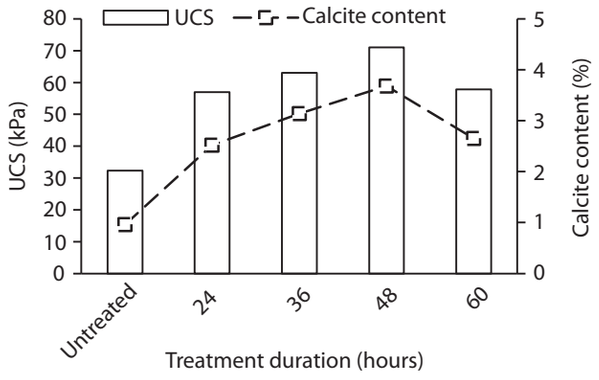

Figure 7 Correlation between UCS, calcite content, and treatment durations

Unconfined Compressive Strength (UCS), calcite content, and the various treatment durations.

The UCS of the lateritic soil subjected to treatment increased as the treatment duration increased to 48 hours, reaching a peak UCS of 71kPa. However, beyond 48 hours of treatment, specifically at 60 hours, there was no further enhancement in the strength of the treated soil. It was deduced that after 48 hours of treatment, the strength of the soil generally decreased. An improvement in shear strength of 76% compared to the untreated sample was noted after 24 hours of treatment, followed by an additional 11% increase in strength after another 12 hours (totalling 87% at 36 hours). Consequently, at 48 hours of treatment, the strength of the residual soil showed a remarkable improvement of 120% relative to the untreated sample, marking the highest strength achieved. Furthermore, it was observed that the calcite content continued to rise with the increase in treatment durations up to 48 hours, after which it started to decline. This suggests a correlation between treatment duration and the calcite content in the soil, with a peak value reached at 48 hours.

The decline in strength observed in the treatment process lasting over 48 hours can be ascribed to various factors such as the decrease in urease activity over time, the reduction in porosity caused by calcite precipitations, the washout of urease from the soil, or the encapsulation within calcium carbonate crystals. Research conducted by Harkes et al. [20] demonstrated that the combined impact of enzyme excretion, cell deterioration, washout, encapsulation, and porosity reduction led to a threefold decrease in urease activity after approximately 80 hours of continuous flushing with 1 M cementation reagents.

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). The dominant clay mineral present in the soil was identified as kaolinite, while gibbsite and quartz were also found as constituent minerals. In the study, a strain of microorganisms known for its urease activity, commonly found in soil deposits, was employed and proved highly effective in biocementation.

The investigation determined that the optimal concentration of cementation reagents for efficient treatment was 0.5 M. Concentrations exceeding this value resulted in a decline in bacterial activity, consequently decreasing strength improvement. Furthermore, the study revealed that a treatment duration of 48 hours produced the maximum strength improvement. Higher concentrations of bacteria yielded more calcite precipitates, which subsequently bound the soil particles together and enhanced the overall strength.

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