Microbial Induced Cementation on Tropical Residual Soil

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Abstract. Microbial-induced calcite precipitation (MICP) is a green and sustainable soil stabilization technique, which utilizes biochemical process that occurs naturally in soil to improve engineering properties of soils. This study of soil stabilization by MICP was focused on sandy and silty clay by using Bacillus Pasteurii. A species of bacillus named Bacillus pasteurii was used to trigger the production of soil particle-binding materials, calcite precipitate. Several variables was considered in this study including the concentration of cementation reagent (0.15M, 0.25M, 0.35M and 0.45M) and treatment condition (untreated, treated with nutrient only, treated with Bacillus pasteurii and nutrient only and treated with cementation reagent and nutrient only). The result of unconfined compression test, pH test and calcite content show that the experiment factors (cementation media concentration and treatment condition) have significant impact on MICP treatment process. The most preferable MICP treatment condition obtained are concentration of cementation reagent of 0.25M with the presence of bacteria and nutrient. Using this combination of treatment parameter, the shear strength and calcite content of soil sample had increased 65% and 61.3% respectively. However, the non-uniform distributions of calcite precipitate, high pH of treatment medium and high concentration of cementation reagent will impair the MICP improvement.

Introduction

Background of the Study Microbial-induced calcite precipitation (MICP) method is a new green and sustainable technique in soil stabilization. Bio-mediated soil stabilization method is a new and sustainable method to improve the ground condition [1]. Soil stabilization by MICP can meet the green construction requirement because the treatment cause minimal disturbance to soil environment [2].Introducing bacteria and cementation reagent into soil trigger calcite precipitation which help in strengthen the soil. [3] stated that MICP treatment by bacteria can improve engineering properties of soil. This application of these techniques such as improved concrete strength and durability, improve brick durability, increased soil strength and sand impermeability [4].

Problem Statement In the past few years before MICP technique was introduced, chemical grouting method was applied to stabilize soil. The process of chemical grouting technique is achieved by adding variety of additives which is very toxic such as Portland cement, lime, asphalt, sodium silicate, acrylate, lignin, urethane, and resins in order to strengthen the soil [5]. Many researchers had proved that these additives will modify the pH of soil and contaminate the groundwater and soil ([6], [7], [8] and [9].Chemical grouting is becoming very popular due to its economic benefits but nowadays with the increasing awareness of environmental issues, it is likely to introduce a more sustainable method in stabilization of soil such as MICP method [5].

Several problems of tropical residual soil condition such as low strength, high compressibility, softening due to infiltration on raining season had caused many geotechnical engineering problems such as settlement of embankment or foundation, debris flow, landslide and others [10]. Natural

disaster such as landslides has increased and become the major concerns of engineering geologists and geotechnical engineers [11].The conventional soil stabilization method such as grouting method had applied in order to strengthen the soil. [5] stated that chemical used in grouting method such as Portland cement, lime, asphalt and others are toxic and hazardous. Apply this method in strengthen the soil are not environmental friendly because chemical insert in the soil will change the pH of the soil and contaminate groundwater [6], [7] and [8].Due to environmental concerned, previous study about soil stabilization by using chemical grouting method are not suitable because it may cause soil and water pollution. MICP is a relatively green and sustainable soil stabilization technique which utilizes biochemical process that exists naturally in soil to improve the engineering properties of soil [4]. Hence, there is a need of study on new green technologies, MICP method which is more environmental friendly to stabilize soil.

ObjectivesThere are three objectives covered in current study included:

1. To study the feasibility of Bacillus pasteurii in MICP treatment of tropical residual soil.
2. To study the relationship of bacteria, cementation reagent and nutrient broth in MICP treatment of tropical residual soil.
3. To determine the optimum concentration of cementation reagent in MICP treatment of tropical residual soil by using Bacillus pasteurii.

Scope of Study In current study, the soil sample adopted was tropical residual soil had covered more than 80% of country land area. The tropical residual soil was taken from a site at Faculty of Electrical Engineering, University of Technology Malaysia. The urease producing bacterium used in this study was Bacillus pasteurii which is purchased from ATCC biomaterial. The Bacillus pasteurii was cultivated in a sterile culture medium consisted of 20g of yeast extract, 10g of ammonium sulfate and 15.75g (0.13M) of tris (hydroxymethyl) aminomethane.The cementation reagent required to trigger the precipitation included urea (CH_4N_2O) and calcium chloride ($CaCl_2$).

There are two variables included this study which are the concentration of cementation reagent and treatment condition. Experiments are conducted in order to determine the effects of MICP process on engineering properties of soil.The study is focused on the correlation between the shear strength of soil, pH of treatment medium and calcite content in soil. The shear strength, pH and calcite content of the sample is determined by unconfined compression tests-Load Frame Method [12] and [13], electrometric method [14] and acid wash respectively.

Previous Studies

History of related research MICP is a relatively new soil stabilization technique in geotechnical field. This soil stabilization method is likely to be the new practice in geotechnical field [15]. This chapter will further discussed about some historical MICP study that was done by other researchers and the mechanism of bio cementation that happened in soil.

Table 1: History of MICP treatment technique

Materials	Soil type	Method	Findings	References
Bacteria-*Proteus vulgaris* and Enzyme urease	Porous media	MEOR	Both bacterial and enzymic options are efficient in plugging of porous media but contribution of bacteria formed is low	[17]
Bacteria-*Bacillus pasteurii*	Sand	MICP	Treated soil specimen showed a no collapse strain softening shear behaviour, with improved in shear stiffness and ultimate shear capacity as compared with untreated soil specimen	[18]

Bacteria-*Bacillus sphaericus*	Lime-stone	MICP	Waterproofing effect increase with increase of calcium dosage	[19]
Bacteria-*Bacillus pasteurii*	Sand	MICP	Fixation and distribution of bacterial cells can be enhancing by two phase injection: bacteria injection on sand followed by fixation fluid before introduce cementation fluid.	[16]
Substrates-Calcium nitrate and calcium acetate	Sand	Biological denitrification-alternative MICP method	Calcium carbonate forms by biological denitrification are relatively low as compared with calcite precipitate form by urease process	[20]
Enzyme urease	Sand	Grouting	Unconfined compressive strength and impermeability of soil had improved	[21]
Bacteria-*Bacillus megaterium*	Sand	MICP	Shear strength and impermeability of soil had improved	[10]
Bacteria-*Bacillus pasteurii*	Sand	MICP	Lower chemical concentration and lower cementation level provide better distribution of calcite precipitation	[22]
Bacteria-*Bacillus megaterium*	Silty clay	MICP	Stiffness, peak strength, stress-deformation and compressibility reached most favourable result at 0.2 bar cementation reagent flow	[23]
Bacteria-*Bacillus megaterium*	Fine sand	MICP	Shear strength and hydraulic conductivity of soil had improved	[2]

From Table 1, it was clearly that the study of MICP treatments were focused on sand and only a few studies were conducted in other soil types. [2] stated that the fine grained soil limit the free passage of bacteria while coarse soils require a large amount of calcite precipitate in order to improve the engineering properties of coarse soil. Hence, it is a particular interest to many geotechnical researchers to study the performance of MICP in natural soil contained fine and coarse soil.

The *bacillus* species is the well-known urease positive bacteria. [24] reported that most bacillus species can produce urease enzyme to trigger urea hydrolysis. Bacillus pasteurii is considered as well-known urease-producing bacteria according to the researchers such as [4], [23], [19], [16] and others. From Table 1, most of the researchers had conducted MICP study by using bacillus species such as B. *pasteurii*, B. *sphaericus* and B. *megaterium*. However, the studies of alternate bacteria species are very limited.

For assessing the effectiveness of MICP process in improving the engineering process of soil, it is still preferable to measure shear strength and hydraulic conductivity by using unconfined compression test or direct shear test and constant or falling head test respectively. The unconfined compressive test and constant or falling head test was done by [21], [4], [23] and others in determination the effectiveness of MICP process.

Besides that, it was found that the use of urease enzyme instead of bacteria in MICP process is more direct and convenient method. Some complicated process such as cultivation and incubation process for bacteria growth can be eliminated and ease the MICP process. There is some of the researcher such as [21], [17] utilizing urease enzyme which extracted from bacteria to hydrolyze

urea. According to both researchers, the use of urease enzyme is more straight forward method as compared with bacteria. Plugging of porous media due to enzymic option are more effective as compared with than bacterial option because the plugging of porous media in bacterial option more due to death bacteria and not by calcite precipitate [17].

Distribution of calcite precipitate in soil sample is an important factor to improve the engineering properties of soil. Various MICP treatment methods were done by researcher in order to obtain a uniform distribution of calcite precipitate in soil sample. Research done by [16] had shown that the method of fixation and distribution of bacterial are very important because a uniform distribution of calcite precipitation will obtained better result.

Table 2 shows the optimum results obtained by others researchers in MICP treatment. Many of the researchers was varied several factors that will affect the performance of MICP treatment in soil. These factors included the concentration of cementation reagent, flow pressure of cementation reagent, treatment period, curing period and others. The study of these factors is to investigate the influence of various factors on the engineering properties of treated soil and to find out the most preferable condition of MICP treatment. The increment in shear strength and reduction in hydraulic conductivity are parameter to measure the efficiency of MICP treatment.

Table 2 showed that at lower concentration of cementation reagent (0.25-0.5M), utilization of bacteria to treat residual soil is more efficient. However, high concentration of cementation reagent (0.5-1.5M) will perform better if apply on sand. The MICP treatment on residual soil and sand was showed a significant improvement in shear strength and hydraulic conductivity of soil.

Table 2: The optimum performance of MICP treatment

Type of soil	Bacteria	Concentration of cementation reagent	Pressure	Treatment period (days)	Curng period (days)	Shear strength increment	Hydraulic conductivity reduction	Calcite content	References
Residual soil and sand	*Bacillus megaterium*	0.25M	NA (flow:1.7 x10^5 m/s)	2	No curing	Residual soil: 140-264%, sand: 114-125%	Residual soil: 26-46%, sand: 9- 15%	Residual soil: 1.080-1.889%, sand: 2.661%-6.102%	[4]
Residual soil	*Bacillus megaterium*	0.5M	0.2 bar	2	No curing	100%	NA	2.31%	[23]
Sand	*Bacillus pasteurii*	NA	NA	-	3	Max: 317kPa	NA	Max: 6.58%	[25]
Residual soil	*Bacillus megaterium*	0.5M	1.1 bar	2	-	69%	90%	2.6%	[2]
Sand	*Urease enzyme*	0.5 & 1.0M	50kPa		1	From ~400kPa to 1.6Mpa	60- 70%	Range from 30- 60% against max	[21]
Sand	*Bacillus pasteurii& urease*	1.5M	No pressure	7	7	*B. pasteurii*from 204-213Mpa, *urease:* from 0.79-0.81Mpa	NA	*B. pasteurii:* from 12.14-13.30%, *urease:* from 7.39%-8.90%	[24]
Sandly soil	*Idiomarina Insulisalsae*	18g/l	No pressure	No treatment	180	158%	NA	NA	[27]

Biocementation Biocementation is a process produce soil particles binding materials when introducing bacteria and cementation reagents in to soil [4]. Biocementation can help to improve the shear strength of soil due to formation of calcite precipitate. At the initial stages of the process, hydrolysis of urea provides an alkaline environment for precipitation of calcium carbonate [22]. The formation of calcite precipitation will cause the pH of the medium back to neutral and the final pH of the medium is depend on the rates of reaction and substrate concentration [20]. Below are some chemical equation showed the process of hydrolysis of urea.

$$(CO(NH_2)_2+, 3H_2O \longrightarrow 2NH_4^+$$

$$HCO_3^- \longrightarrow CO_3^{2-} + H^+$$

$$Ca^{2+} + CO_3^{2-} \longrightarrow CaCO_3$$

Figure 1 showed the overview of bio-mediated carbonate precipitate using hydrolysis. Biological activity of bacteria cell, Bacillus pasteurii which is a well-known urease-producing bacteria will decompose urea, $(CO(NH_2)_2$into ammonia and carbon dioxide [9]. In the presence of water, the ammonia will convert into ammonium ions, NH_4^+meanwhile carbon dioxide will combine with hydroxide ions, OH^- to form bicarbonate, HCO_3^-.The increase of pH is due to present of hydroxide ions, OH^- in water and alkaline environment provide a suitable condition for precipitation [9].

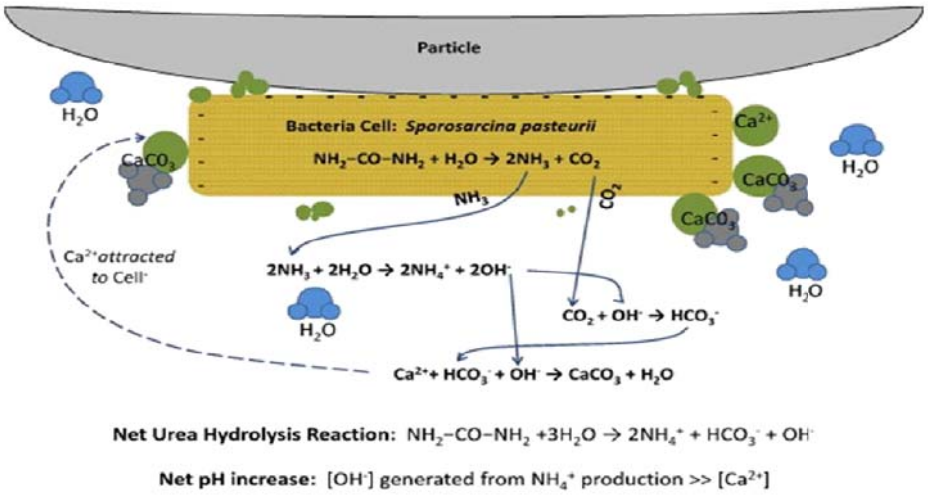

Figure 1: Overview of bio-mediated calcite precipitation using ureolysis [9]

Methodology

Laboratory study is conducted to find out the effect of microbiological mechanism on engineering properties of tropical residual soil sample. The entire laboratory test is based on the British standard except the acid wash. The acid wash method is to determine the calcite content of the treated soilsample. The procedure of the acid wash test is according to method that had been done by a few researchers such as [2], [23] and others.

Laboratory setupThe setup included a steel mold of 50 mm in diameter and 150 mm height, pressure tank, air compressor, effluent pipes, and effluent collector are needed. The steel mold by made up by stainless steel which will not rust when exposed to water or air. Before the soil sample

is compacted into the steel mold, a thin layer of lubricant will be applied at the inner surface of the steel mold so that extrusion process of the soil sample will become easier. After all the laboratory setup is complete, the next step is to ensure that no leakage of air in pressure tank and cementation reagent in the steel mold. Schematic diagram below showed the laboratory setup of the experiment.

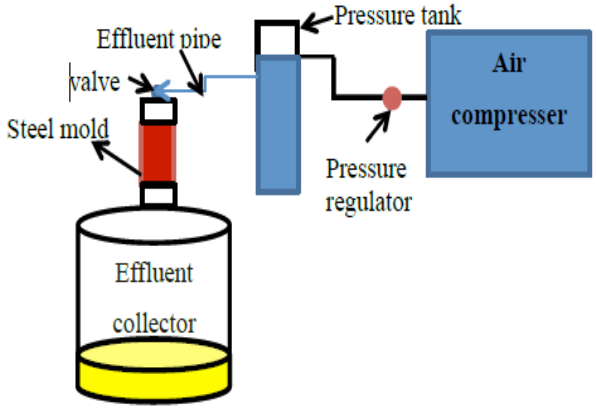

Figure 1(a): Schematic diagram of the laboratory set up

Figure 1(b) showed the schematic diagram of test soil column showed set up of each layer in the stainless steel fabrication mold. The 100 mm of soil specimen will then sandwich between two layers of sand stone with thickness about 20 mm. The plastic netting is used to separate each layer and to protect the top and bottom surface of the soil specimen

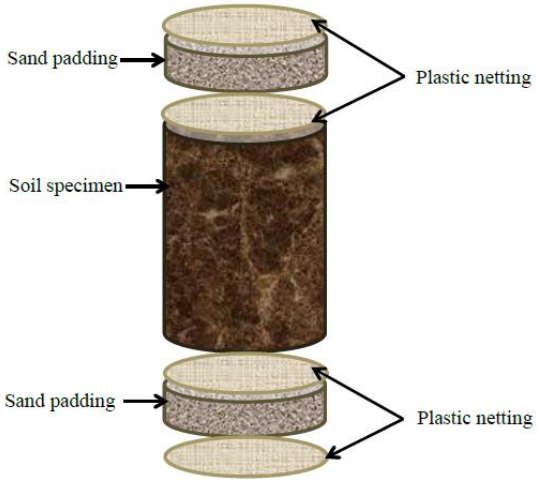

Figure 1(b): Set up of the stainless steel mold

Selection and Cultivation Process of Microorganism The bacterium used in this study was Bacillus pasteurii which able to trigger urea hydrolysis. The cell is obtained from ATCC biomaterial and the bacteria inside the media plate have to be sealed to prevent contamination and stored at low temperature to minimize the activation of the bacteria.

The selected bacteria have to incubate at 30˚C. The ingredients for bacteria cultivation included 20 g of yeast extract, 10 g of ammonium sulphate, $(NH_4)_2SO_4$, 0.13 mol/L tris buffer . All the apparatus have to be sterilized separately at very high temperature and pressure condition by using autoclave before mixing. After the bacteria growth in the plate, the bacteria will harvested and inoculated in NH_4-YE liquid media consisted of yeast extract and ammonium sulphate, $(NH_4)_2SO_4$and let it grown inside the incubator for 24 to 28 hours.

Cementation reagentThe cementation reagent contained urea, calcium calcite and also 3g/L of nutrient broth. The nutrient broth is to provide enough nutrients for bacterial growth. Table 3 shows the chemical compositions of cementation reagent for MICP treatment.

Soil specimenThe soil material used in current study was tropical residual soil extracted from a site at Faculty of Electrical Engineering, University of technology Malaysia. The fresh soil taken from site will let to air dry for few days, then the residual soil will sieve to passing 2mm sizes which is particle sizes ranging from clay fraction.

The density of residual soil specimens prepared is 98-100% of maximum dry density. In prior MICP treatment, the desired densities is achieved by mix the soil specimen with calculated amount of cultivated bacteria solution (about 87 ml). The soil sample will be spray and mix evenly with the bacteria solution until entire soil specimen saturated uniformly.

Experimental variableThere are two variables included in this study were concentration of cementation reagent and treatment condition. All soil specimens were treated for 2 days with cementation flow pressure of 0.2 bars. Table 3 shows the chemical compositions of cementation reagent for MICP treatment.

Table 3: MICP treatment variables (N: nutrient only, BN: bacteria and nutrient only, CN: cementation reagent and nutrient only)

Chemical	**Molarity of Cementation Reagent (M)**				**Treatment Condition**		
	0.15	**0.25**	**0.35**	**0.45**	**N**	**BN**	**CN**
Urea (g)	9.0	15.0	21.0	27.0	-	-	15.0
Nutrient broth (g)	3.0	3.0	3.0	3.0	3.0	3.0	3.0
$CaCl_2.2H_2O$	22.0	36.8	51.5	66.2	-	-	36.8

Data analysis

A few set of laboratory testing was conducted on residual soil sample. This chapter will further discussed about the results obtained from laboratory testing.

Soil properties testThe physical and chemical properties of the residual soil will be tested by using standard soil properties test according to British standard. Table 4 tabulates the tests conducted on tropical residual soil.

Table 4: Test conducted on tropical residual soil

Property	**Test designation**	**Abbreviated reference**	**Value/Index**
Particle density	Density bottle (small pyknometer)	BS 1377: Part 2:1990: 8.3	2.81 mg/m^3
Soil particles distribution	Wett sieving- Fine non-cohesive soils	BS 1377: Part 2:1990: 9.2	Sandy and silty clay
	Hydrometer analysis	BS 1377: Part 2:1990: 9.5 and ASTM D 422	
Liquid limit	Cone penetration method	BS 1377: Part 2:1990: 4.3	80.80%
Plastic limit	Plastic limit test	BS 1377: Part 2:1990: 5.3 and ASTM D 4318, 15	31.85%
pH	Electronic method	BS 1377: Part 3:1990: 9	4.25

Unconfined compressive strength	Unconfined compression tests- Load frame Method	BS 1377: Part 7:1990: 7.2 and ASTM D 2166	48.5 kPa

Effect of cementation reagent concentrationTo study the effect of molarity of cementation reagent on treated soil, four standard samples with same concentration of bacteria (1 x 10^8 cfu / mL) but different cementation media concentration was prepared. All samples prepared had treated for duration of 48 hours with a low cementation reagent flow pressure of 0.2 bars.

Besides, a control specimen had been prepared and tested to find out the original shear strength of soil. Figure 3 below showed the effect of cementation media concentration (0.15, 0.25, 0.35, 0.45M) on shear strength and calcite content.

Figure 3 presents the shear strength and calcite content of treated residual soil specimen. The untreated specimen (sample No. 5 in figure 4.1) served as a control. An increment of 12.9% in calcite content in the specimen treated with 0.15M of cementation reagent was observed. At the same time, the shear strength of the same specimen (0.15M) also increased by 8.2%.

At higher concentration of cementation reagent (0.25M) tend to generate more significant amount of calcite if compared with the lower concentration of cementation reagent (0.15M). The increment in calcite content of soil specimen with 0.25M of cementation reagent was observed to be increased by 61.3%. In addition, significant increase in shear strength (65%) was observed in the same soil specimen (0.25M).

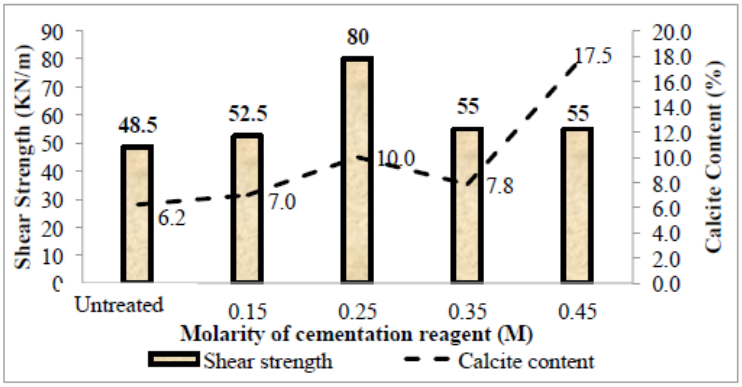

Figure 3: Shear strength and calcite content under different cementation reagent concentration of MICP- treated samples treated by S. pasteurii.

The shear strength of soil specimen seems to be increased with the increased of calcite content. The similar observation was reported by [24] that the shear strength and calcite content increased with the increased of cementation concentration during MICP treatment. The effects of reagents (urea and calcium chloride) concentration on calcite precipitation were studied by [28]. To increase the strength of the soil, a particular amount of calcite for soil particles bonding had to be produce during the urease activity [28].

The increment in shear strength is most probably due to the presence of cementation materials (Calcite) which generated from MICP process bind the soil particles together and subsequently improved the shear strength of soil. This process is known as bio cementation. Biocementation is a process produce soil particles binding materials when introducing bacteria and cementation reagents in to soil [4]. The Link or bridges between the soil particles result in increases in the soil's strength [29].

As shown in Figure 3, the calcite content of the soil sample was increased with the increased of concentration of cementation reagent from 0.15 to 0.25M, which also resulting in significantly

improved soil shear strength. The optimum concentration of cementation reagent was 0.25M which has the greatest increased in shear strength and calcite content. [30] had reported that at 0.25 M of cementation reagent, the efficient of treatment reached 95% by using the same bacterium (S. pasteurii) in MICP study. [2] also found that the formation of calcite at lower concentration (0.05M to 0.25M) is more efficient.

The result obtained showed a significant reduction of calcite content and shear strength in soil specimen treated with 0.35M cementation reagent. When the cementation reagent concentration is 0.25 M, the result shown a significant increment in calcite content (61.3%) and shear strength (65%). However, when the cementation reagent concentration increased to 0.35 M, the increment in the production of calcite content and shear strength was only 25.8% and 13.4% respectively.

This observation suggests that urea and calcium ions introduced into the soil sample have not been fully utilized. This observation is most probably due to the local fall in pH of treatment medium. A pH test was conducted for original soil sample. It was found that the pH for the soil medium is quite acidic (pH 4.12). The acidity medium of the soil will cause the urease enzymeproduced by bacteria become inactive and could not hydrolyze the urea. [4] stated that the urease enzyme only active at certain pH. [31] was also found that the urease activity increased rapidly when pH of the reaction medium increase from 6.0 to 10.0.

When the molarity of the cementation increased again to 0.45M, The calcite content of the soil sample had increased to 17.5% which is the highest among soil specimens. The calcite content produced by sample treated with 0.45M of cementation reagent is 75% higher than that produced by sample with 0.25M of cementation reagent. The calcite content result obtained from sample treated with 0.45M of cementation reagent were within expectation, in which at higher cementation reagent concentration (0.45M) can generated a higher calcite content than that of 0.25M cementation reagent.

However, although the calcite content increased, but the shear strength of the soil sample treated with 0.45M of cementation reagent reduced and become lower than that of sample treated with 0.35M of cementation reagent. The calcite content of the soil sample treated with 0.45M of cementation reagent is 124% higher than soil sample treated with 0.35 cementation reagent but the shear strength of soil sample treated with 0.45M of cementation reagent is same as soil sample treated with 0.35 of cementation reagent. This observation can be explained by the distribution of the cementation reagent. No improvement in shear strength of soil sample treated with 0.45M of cementation reagent was observed, this observation is most probably due to the uneven distribution of calcite content along soil sample.

Amount of calcite content obtained from the top, medium and bottom of soil column. It was found that the calcite distribution of soil sample treated with 0.45M of cementation reagent is not uniform in whole sample. From Table 6, highest percentage of calcite (31.3%) obtained at the top part of the treated soil column, followed by 12.1% of calcite at medium of soil column while the lowest percentage of calcite (9.1%) observed at the bottom part of soil column. The result had shown that the calcite content was not homogenously distributed over the soil sample which is most probably the reason that cause low shear strength in soil sample treated with 0.45Mof cementation reagent.

In current study, the chemical was percolated from top to the bottom part of the sample with the aid of the pressure. High cementation reagent concentration (0.45M) will expedited the formation of calcite content. This is due to available of more urea molecules available around the bacteria cells and cause localized rise in pH. Raise in pH around bacteria cell will provide a favorable condition for calcite precipitation. Furthermore, the top part of the soil column receives continuous injection of cementation reagent. Hence, the calcite might accumulate at top part of the soil column. This consequent clogging at the top of soil column and the cementation reagent might be hindered to flow to the other part of the sample.

Similar findings were reported happened from the numerical modeling developed by [32]. [4] stated that a continuous injection method might cause the formation of large amount of calcite

precipitation near the injection point and cause clogging near the inlet of soil specimen. Hence, the calcite content will decrease over a distance from injection point [4].

Effect of different treatment conditionCurrent study of MICP soil improvement also focused on effect of different treatment condition (untreated, treated with nutrient only, treated with bacillus pasteurii and nutrient only and treatedwith cementation reagent and nutrient only) on the shear strength and improvement in calcite content of soil sample. Four samples were prepared and tested to investigate the effect of different treatment method.

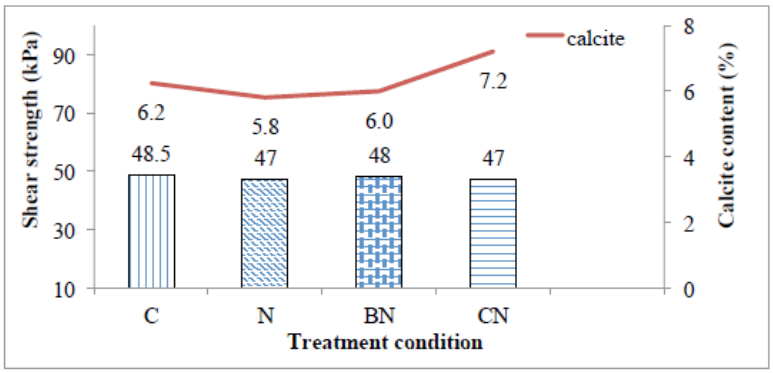

Figure 4: Shear strength and calcite content of soil sample under different treatment condition (C: untreated, N: nutrient only, BN: bacteria and nutrient only and CN: cementation reagent and nutrient only)

The untreated soil sample (C) is use as the benchmark for comparing the shear strength and calcite content of the soil sample. From Figure 4, the shear strength of soil sample treated with nutrient only, N (47kPa) and soil sample treated with bacteria and nutrient only, BN (48kPa) had no improvement as compared with the shear strength of untreated soil sample, C (48.5kPa). The calcite content for both sample N (5.8%) and sample BN (6.0%) also do not have any improvement as compared with untreated sample.

There are not improvements in shear strength and calcite content was observed for soil sample treated with nutrient only, N. Although nutrient was provided into the sample, but nutrient has no effect on shear strength of the treated soil. Nutrient is act as the energy sources for bacteria in the soil. [4] reported that the supply of nutrient during MICP treatment was to ensure the bacteria can survive for long period to support calcite precipitation.

The result for the specimens treated with bacteria and nutrient only, BN has no improvement in shear strength and calcite content. The same observation was obtained by [5] where sample treated with microorganism only has no visible improvement in shear strength of the soil sample. The result implied that biomass (bacteria) was not effective in improving the shear strength of soil.

The observations obtained from both sample N and BN most probably due to the absent of cementation reagent. Calcite precipitation process will not happen without cementation reagent (urea and calcium ion). Therefore, no formation of calcite precipitate happened in sample treated with nutrient only, N. Calcite precipitation happened in the present of cementation reagent (urea and calcium carbonate) and urease enzyme [18], and [10].

The soil sample treated with cementation reagent and nutrient only, CN happened increased in calcite content (16.1%) as compared with calcite content of untreated sample (C). This result implied that in the presence of cementation reagent, the MICP was triggered by urease positivebacteria that are naturally present in the soil sample. The calcite precipitating microorganisms naturally exist in residual soil [5]. [5] stated that when introduced cementation

reagent into the soil deposit, MICP will be triggered by microorganisms inhabiting naturally in the soil and exhibited increased in shear strength.

However, the improvement of calcite content for sample CN was lower than that in sample treated with Bacillus pasteurii, nutrient and cementation reagent of 0.25M with average calcite content of 10.0%. This is most properly due to inclusion of bacteria in sample treated with 0.25Mof cementation reagent resulted in higher production of urease enzyme which will triggered more calcite to enhance the shear strength of soil sample.

There is no any improvement in the shear strength of soil sample CN although its calcite content increased. This observation may be probably due to the microorganism originally present in the soil sample are not homogenously distributed throughout the soil. From Table 5, highest percentage of calcite (9.8%) obtained at the top part of the treated soil column, followed by 7.0% of calcite at medium of soil column while the lowest percentage of calcite (4.8%) observed at the bottom part of soil column. The result shows that the calcite content was not homogenously distributed over the soil column.

This situation happened most probably due to non-homogeneous distribution of microorganism that originally present in soil sample, CN. Calcite precipitation only happened on area where microorganisms are available.

The result obtained from sample CN shows that a uniform distribution of microorganism throughout the sample is very important because this factor will affect the shear strength of soil sample. Therefore, several studies of the fixation methods and distribution of bacteria in soil sample were done by many researchers such as [23], [16], [33] and others.

Table 5: Carbonate content distribution at top, medium and bottom of soil sample treated with cementation reagent and nutrient only, CN

	Experiment abbreviation	**CN**
Carbonate content	Top	9.8
	Medium	7.0
	Bottom	4.8

Effect of Distribution of calcite content in soil sample Distribution of calcite content along soil column is an important factor to be studied because it might affect the shear strength of soil sample. [24] stated that slightly different in calcite precipitation distributed within sample may cause fluctuation in shear strength of soil during the test. Table 6 below showed the calcite content obtained from the top, medium and bottom of the soil sample treated with different molarity of cementation reagent (0.15M, 0.25M, 0.35M and 0.45M).

The previous studies had been done to study the method of MICP treatment to obtain more evenly distributed of calcite precipitation in the sample. Zhao et al. (2014) utilized full contact flexible mold to prepare soil specimen while Ng et al. (2012) suggested that stopped-flow injection can distributed the cementation reagent evenly in soil column before calcite composition.

Table 6: Carbonate content distribution at top, medium and bottom of soil sample treated with different molarity of cementation reagent

	Sample no	1	2	3	4
	Molarity of the cementation reagent (M)	**0.15**	**0.25**	**0.35**	**0.45**
Calcite content	Top	6.7	10.4	7.5	31.3
	Medium	6.7	10.5	7.9	12.1
	Bottom	7.5	9.1	8.1	9.1

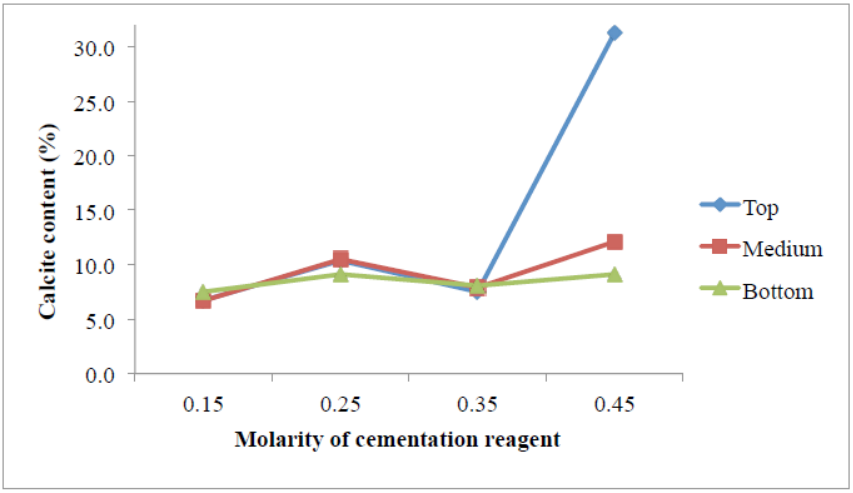

Figure 5: Carbonate content distribution at top medium and bottom of soil sample treated with different molarity of cementation reagent.

Figure 5shows the distribution of carbonate content at top, medium and bottom of soil sample treated with different molarity of cementation reagent. A low-concentration treatment (0.15M-0.35M) was found to generate more uniform distribution of calcite precipitation. The same observation obtained by [34] by conducting SEM imaging.

By conducted scanning electron microscopy (SEM) imaging, [22] found that at low concentration (0.25M) of treatment will result in a uniform distribution of calcite precipitation at different level of cementation. From SEM imaging conducted by [22] showed that calcite crystal with similar sizes were well distributed and covered the soil contact area uniformly.

However, when the soil sample is treated with high molarity of cementation reagent (0.45M), it was found that the calcite distribution of soil sample is not uniform in whole sample. From table4.2, highest percentage of calcite (31.3%) obtained at the top part of the treated soil column, followed by 12.1% of calcite at medium of soil column while the lowest percentage of calcite (9.1%) observed at the bottom part of soil column.

The different in the amount of calcite at top, medium and bottom is quite large is due to uneven distribution of calcite along the soil column. [22] stated that at high treatment concentration (0.5M), the crystals are not well distributed and the crystals formed had different sizes. Besides, it will formed larger crystal rather than uniformly distributed over soil grains [22].

The variation in the calcite precipitation distribution may be due to the distribution of urea molecules with respect to the bacterial cells. At high urea concentration, due to available of more urea molecules caused a localized rise in pH around the bacteria cell. Hence, the condition will cause production of larger calcite crystal. The second mechanism for precipitation in MICP is urea hydrolysis, which rose in pH around the bacterial cell will provide favorite condition for precipitation [18].

The top part of the soil column receives continuous injection of cementation reagent. Hence, the calcite had accumulated at top part of the soil column and formed cementation bond that bind the soil particles. This consequent clogging at the top of soil column and the cementation reagent is hard to flow down the soil. This is the reason of causing a decreasing in amount of calcite precipitation down the soil column.

Effect of pH in MICP treatment. In current study, it is worthwhile to investigate the effect of different cementation reagent concentration and different treatment condition on pH of reactant medium. Besides, the effect of pH of reactant medium on the calcite content of soil sample was studied.

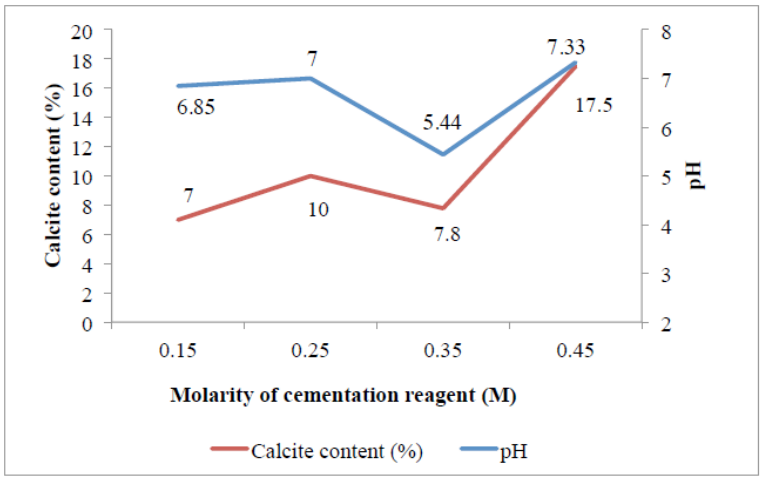

Figure 6: pH and calcite content of soil treated with different molarity of cementation reagent

Figure 6 shows pH and calcite content of soil treated with different molarity of cementation reagent. The result showed the calcite content and pH of reactant medium followed similar trend. Initially, the pH of reactant medium increased gradually from pH 6.85 to pH 7 when the cementation reagent molarity increases from 0.15M to 0.25M. At the same time, the calcite content also increase from 7% to 10%. This observation implied that the chemical efficiency increased with the increase of pH of the reactant medium. [31] found that the urease activity will increased rapidly from pH 6 until pH 8. Hence, the urease enzyme will stimulate more calcite when pH rises.

However, the calcite content and pH experienced sudden drop when the molarity of the cementation reagent increased to 0.35M. This happened may be due to the acidity of the soil medium. Acidic soil will cause the urease enzyme produced by bacteria become inactive and cannot hydrolyze the urea. Hence, the calcite content of sample treated with 0.35M of cementation reagent become low.

The calcite content and pH of reactant medium jump up to 17.5% and pH 7.33 when molarity of cementation reagent increased to 0.45M. The calcite content of soil sample treated with 0.45M of cementation reagent is higher because the treatment medium had become alkaline. At high urea concentration, due to available of more urea molecules caused a localized rise in pH around the

bacteria cell. [10] stated that high pH will increases the tendency for bacteria itself to serve as nucleation site for calcite crystallization.

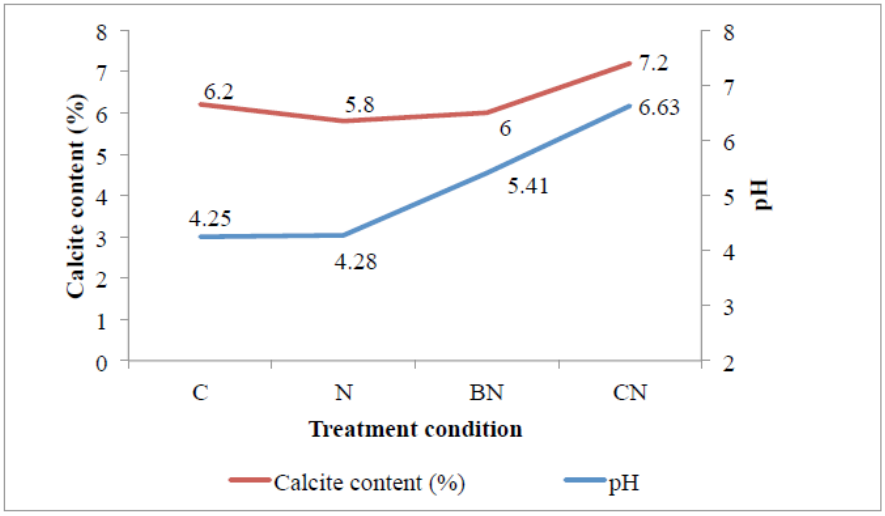

Figure 7: pH and calcite content of soil with different treatment condition (C: untreated, N: nutrient only, BN: bacteria and nutrient only and CN: cementation reagent and nutrient only)

Figure 7 shows the pH and calcite content of soil with different treatment condition. The pH of the untreated soil sample and soil sample treated with nutrient only, N are almost the same which is pH 4.25 and pH 4.28 respectively. This observation suggests that the additional of nutrient will not affect the pH of reactant medium.

However the pH changed when the soil sample treated with Bacillus pasteurii and nutrient only, CN. The pH of the reactant medium rose to pH 5.41. This result most properly due to the alkaline bacteria medium added in to the treatment medium. Dejong et al. (2006) reported that Bacillus pasteurii is a well-known alkalophilic soil bacterium; production of ammonia through urea hydrolysis will cause a localized rise in pH.

The pH of the reactant medium increase to pH 6.63 and the calcite content had been increased to 7.2% when the soil sample treated with cementation reagent and nutrient only, CN. Available of large amount of urea molecules in the reactant medium will cause ureolytic bacteria that originally present in soil medium undergone ureolysis. Formation of ammonia through urea hydrolysis will increase pH in proximal environment. This statement is further support by Ng et al. (2012), who stated that production of ammonia by urea hydrolysis will increase the pH of the medium. Hence, the pH rise will trigger the production of calcite precipitate. Stock- Fischer et al. (1990) also reported that urease activity is more efficient in the pH range of 6 to 8. In sample CN, the pH 6.63 is within the favorable pH range for urease activity. Hence, production of calcite content will be more aggressive due in pH 6.63.

Conclusion

Utilization of Bacillus pasteurii in MICP treatment of tropical residual soil showed significant improvement time in shear strength about 1.6 times as compared with untreated counterpart. This positive improvement had proven the feasibility of Bacillus pasteurii in MICP treatment of tropical residual soil. In MICP treatment, all ingredients such as cementation reagent, nutrient and microorganism play an important role in the formation of calcium precipitate. No precipitation occurs if any of the ingredient not available. Besides that, the optimum cementation reagent concentration for MICP treatment of Bacillus pasteurii in this study was 0.25M.

It was found that MICP treatment in this study perform better in low cementation reagent concentration (0.15M-0.25M) as it will form better distribution of calcite precipitation. However, at high concentration of cementation reagent, efficiency of MICP treatment reduced due to available of more urea molecules, a thick layer of precipitation take place at top of soil column and clogging the flow of cementation reagent down the soil.

Besides that, it was found that the calcite production is depended on the pH of the reactant medium. Formation of calcite will become more efficient in high pH. Under favorable pH range (pH 6- 8), the MICP process will be more efficient. Besides, different treatment condition will effect on the pH changes in reactant medium. Inclusion of bacteria and cementation will cause the rise of pH. Inclusion of bacteria and cementation will cause the rise of pH of reactant medium because of ureolysis produce ammonia molecules.

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