

BIOREMEDIATION OF COAL CONTAMINATED SOIL AS THE ROAD FOUNDATIONS LAYER

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ABSTRACT: Waste area ex-coal mining land is left without proper management and utilization. After analysis, ex-mining land material can be reused as road construction material by increasing mechanical properties. Microbial-induced calcite precipitation (MICP) is a soil improvement technique using microorganisms capable of altering and enhancing their mechanical and physical properties. In this study, an unconfined compressive test was used to see the effect of calcite precipitation on the behavior of the unconfined compressive strength of sand contaminated with coal. Variations in the concentration of *Bacillus subtilis* were applied as much as 3%, 4.5%, and 6% on sand contaminated with coal. The bacteria used were 3 days of culture was still in the stationary phase and 6 days of culture in the dead phase. After 28 days of curing, there is a significant increase in the UCS values of the MICP-stabilized soil compared to the untreated soil. The use of 3 days of bacterial culture was more effective in increasing the UCS value than 6 days of culture. At optimum conditions, the UCS value increased up to 15 times after the 28-day curing period. As a conclusion the ex-mining land material after treated with MICP using *Bacillus subtilis*, it can be reused and qualifies as a road construction material.

Keywords: Microbially Induced Calcite Precipitation (MICP), Coal, Bacillus subtilis, Unconfined Compressive Strength (UCS)

1. INTRODUCTION

Waste areas of ex-coal mining land were abandoned by the mining company without proper management. The large size and the high cost of reclamation have caused the former coal mining land to be left unattended. In general, ex-coal mining areas still contain residual coal that cannot be transported during the mining process. Although the amount is not large, this certainly has an impact on the environment. Coal that contains sulfur in the open air will experience oxidation which when mixed with water will cause acid mine drainage or what is known as acid rock drainage (ARD). ARD will provide a series of interrelated impacts, namely a decrease in pH, availability, and balance of nutrients in disturbed soils, as well as an increase in the solubility of microelements which are generally metal elements[1].

Soil pollution urgently requires applying a series of physic, chemical and biological techniques and treatments to minimize the extent of the damage. Bioremediation appeared to be an alternative that can offer an economically viable way to restore polluted areas. Due to the difficulty in choosing the best bioremediation technique for each type of pollutant and the lack of literature on soil bioremediation enhanced by the use of specific additives, review of the main in situ and ex situ methods, their current properties and applications are required. In this study, the authors aim to carry out soil bioremediation to improve its mechanical properties. The soil to be the subject is soil

contaminated with coal with the subject of stabilization in the form *Bacillus subtilis* bacteria. The results of this research are expected to help provide solutions for mining areas with coal contamination to restore the soil to its original condition physically.

Various attempts to bioremediate coal-contaminated soil have been carried out to reduce lead levels and reduce environmental pollution by using the *bacillus subtilis* bacteria. Another effect of bioremediation using the *bacillus subtilis* bacteria is bio-cementation in which *bacillus subtilis* produces calcite in the metabolic process so that it is useful for improving soil engineering properties[2].

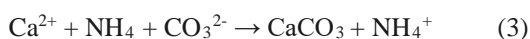
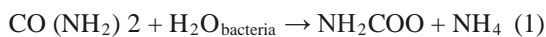
Soil improvement can be made using mechanical and chemical means. Mechanical repairs can use soil-fiber[3] to replace soil materials that do not meet standards, while chemically can be done with the addition of lime[4]. But the newest method that can be done is bio-chemical which is more environmentally friendly or known as microbial induced carbonate precipitation (MICP) which can increase soil strength[5] and increase slope stability[6]. The results of previous research indicate that coal contaminated soil can be used as a material for civil engineering construction but stabilization efforts are needed to improve the mechanical characteristics of the soil so that it can be utilized. MICP stabilization using *bacillus subtilis* bacteria is expected to enhance the mechanical characteristics of the soil so that it meets

the standards as a material for construction. But the effect of coal content on calcite precipitation needs further investigation.

Biogeotechnological is a technology currently being developed[7] to convert soil into materials such as cemented soil using bacteria (bio-cementation). Microbial Induced Calcite Precipitation (MICP) has recently gained a great deal of attention from geotechnical engineering researchers worldwide because of its flexibility and continuous use. MICP is a naturally driven biological method that makes an in situ cementing agent known as calcium carbonate or calcite using bacterial metabolism[8]. The role of bacteria or microorganisms in the precipitation of calcium carbonate in the MICP process is correlated with: producing carbonates, (increasing ambient pH and nucleation sites in saturated solutions[9]. The soil modification technology based on MICP is more superficial than other soil modification technology and has low environmental effects. The bacterial and cementation solution used in this technique are easy to inflate into geotechnical materials than conventional chemical grouting[10].

Several previous studies using bacteria as stabilizing agents revealed the advantages of bacteria as stabilizing agents compared to other materials. The mechanical performance of the MICP-stabilized soil is heavily dependent on the precipitated CaCO₃ crystalline microstructure, which is affected by different chemical, environmental and physical parameters. The use of a higher bacterial concentration (CB) and a lower bacterial culture (BC) is suggested to result in better distribution of precipitation of CaCO₃[11].

The ureolysis mechanism is that bacteria absorb Ca²⁺ from the surrounding environment on the cell surface, while urea can be broken down by urease secreted from the cell into CO₃²⁻ and NH₄⁺. Calcium carbonate crystals can be created on the cell surface when Ca²⁺ binds to CO₃²⁻, which can bind the granular particles and fill the internal pores of geomaterials[10]. The main reaction equations are as follows :



Under sterile conditions, bacteria are usually cultivated ex-situ to ensure constant production of urease activity. The higher the activity of bacterial urease, the faster calcium carbonate formation, and more calcium carbonate produced to be[12]. High urease activity attained unconfined compressive strength (UCS) twice or more than low urease

activity. MICP could be carried out after the cultivation of high urease activity bacteria by inducing high concentrations and put in the soil before the cementation solution was inserted. With the addition of nutrients to the soil, the loss of ureolytic activity during the bio-cementation process can also be reversed so that further cementation is possible[13].

The most common urease-producing bacteria are *Bacillus* and *Sporosarcina*. These species have good adaptability to the environment compared to other bacteria[14]. Also, urea can be used as a source of energy by respiration, in which CO₂ is converted into CO₃²⁻ in an alkaline environment, thereby metabolizing and depositing calcium carbonate[5]. Besides, calcium carbonate could be produced by *Bacillus* and *Sporosarcina* more fastly and with a high yield. Therefore, they are primarily used for bio-cementation geomaterial[9].

Bacteria have two important roles in the formation of calcite crystals in the MICP process. First, in the formation of calcite crystals, bacteria function as nucleation sites. Bacteria release a significant amount of HCO₃⁻, CO₃²⁻ and OH⁻ by decomposing the urea in the solution, providing the requisite ionic components and alkaline atmosphere for calcite formation[9]. Second, on the surface of bacterial cells, extracellular polymeric substances (EPS) and negative ion groups could also serve as nucleation sites for calcite crystals and control the crystal form and appearance[12]. As a result, the concentration of bacteria may affect crystal appearance, the production of calcium carbonate, and the cementation effect of geomaterials it was found that the rate of urea decomposition and the amount of calcite precipitation were correlated with the concentration of bacterial cells[16].

When the concentration of urea and calcium ion reached a definite level, the concentration of bacterial cells became the primary factor. It was observed that the morphology of the crystals are transformed with an increasing concentration of bacterial cells it was found that in low concentration the calcium carbonate crystal was a rhombus and a cube, while the calcium carbonate crystal was spherical and overlapped with agglomeration at a high concentration of bacterial cells[17].

In general, this study aims to carry out soil bioremediation to improve its mechanical properties can use as construction material especially road foundation material, obtain the best bioremediation method on polluted soil. In detail, this study aims to analyze the mechanical characteristics of coal-contaminated soil stabilized by MICP used *Bacillus subtilis* bacteria with variations in concentration and culture.

MATERIALS AND METHODS

2.1 Preparation of Bacteria and Cementation Solution

In this experiment, the microbe used to trigger ureolysis-induced calcification is *Bacillus subtilis*. It is a rod-shaped bacteria measuring 0.5-2.5 x 1.2-10 microns, arranged in pairs or chains, where silica covers the entire surface of the cell. In critical condition, it can form spores. The antagonistic bacteria *Bacillus subtilis* can survive in extreme environmental conditions, namely at temperatures of -5° C to 75° C, with an acidity level (pH) between 2-8. *Bacillus subtilis* were cultured in B4 medium, which contained Urea (20 g/L), Nutrient Broth (3 g/L), NaHCO₃ (2.12 g/L), CaCl₂.2H₂O (4.14 g/L), and NH₄Cl (10 g/L) dissolved in distilled water. The cultivated bacteria were collected at the stationary phase of the culture growth after 3 days and the death phase of culture growth after 6 days of incubation at 30°C. The optical density (OD) of the harvested area in 0.6 for 3 days and 0.4 for 6 days culture.

Three variations in the concentration of bacteria were applied to see the effect of the concentration on calcite precipitation. A series of different culture and *Bacillus subtilis* concentrations, as listed in Table 1, were used in the experiment. As much as 1% cementation solution used to trigger calcium carbonate precipitation consisted of urea and calcium chloride (CaCl₂). The urea and CaCl₂ had an equal molar concentration i.e. 0.25 M.

Table 1. Combination of culture and concentration

Bacteria	Culture (day)	Concentration (%)
<i>Bacillus subtilis</i>	3	3
		4.5
		6
	6	3
		4.5
		6

2.2 Soil Type

Sand contaminated with coal obtained from Balikpapan city, East Kalimantan Province, Indonesia.

Table 2. Variation of soil

Soil Type	Sand (%)	Coal (%)
1	95	5
2	90	10
3	85	15

3 variations of coal contaminated soil were prepared each with a comparison of sand and coal as shown in Table 2, to know the effect of coal content on calcite deposition. The sand used is predominantly 0.234 mm in size while the mixed coal has a size of ≤ 0.149 mm. The physical properties and mechanical properties of coal-contaminated sand are shown in Table 3.

From the XRD analysis, the material contains quartz (SiO₂) and Ilmenite (FeTiO₃) minerals. The percentage of mineralogical content is shown in Table 4. Mineral Quartz (SiO₂) dominates the mineralogy of coal contaminated soil by more than 90%.

Table 3. Soil characteristics

Test	Test Results			Unit
	Soil Type			
	1	2	3	
Basic Properties of Sample Soil				
Specific Gravity (Gs)	2.60	2.59	2.52	
Sieve Analysis				
a Uniformity Coef. (Cu)	2.58	2.82	2.86	
b Gradation Coef. (Cc)	1.47	1.69	1.80	
Engineering Properties of Sample Soil :				
Standard Proctor				
a Maximum dry density, (γ _d)	1.66	1.74	1.77	g/cm ³
b Optimum moisture content (OMC)	6.65	6.30	6.07	%
Unconfined Compression Test (UCT)				
UCS	1.0	1.6	3.5	kPa
Direct Shear				
a Cohesion (c) Internal	9.8	10.1	11	kPa
b friction angle (°)	31	33	36	°
California Bearing Ratio (CBR)				
a Unsoaked	16	24	34	%
b Soaked	7	12	13	%

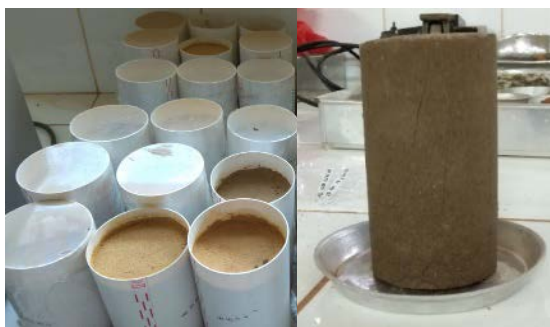
Table 4. Soil mineralogy

Mineralogy	Content (%)		
	Soil Type		
	1	2	3
Quartz (SiO ₂)	92.6	93.3	92.5
Ilmenite (FeTiO ₃)	7.4	6.7	7.5
Unidentified Peak Area	3.6	4.9	10.2

2.3 Soil Treatment Procedures

Generally, in soil stabilization using bacterial calcite urease deposition using the injection method. Some studies even inject two to seven times to make the soil look like cemented soil. However, this method is less effective because the injection method will cause the cementation solution to accumulate on the surface of the sample only and the soil pores that are already filled with calcite due to the first injection which will cause the solution injected further will not be able to enter the soil pores. Therefore, in this study, the bacteria solution and cementation solution were mixed in the soil with a mixer so that they were evenly mixed in each soil grain. Sand and coal are first mixed with a predetermined percentage ratio. Then add the bacterial solution and cementation solution then stirred again until well blended. The concentration of the added bacterial solution reduces the volume of water to achieve optimum moisture content (OMC), for example if the OMC is 6.65% and the concentration of the bacteria solution is 3%, the volume of water that must be added to reach OMC = 6.65% - 3% = 3.65%. After the material is ready then it is printed in a mold.

Sand contaminated with coal is mixed with bacteria and cementation solution using a mixer then printed on a PVC column with a diameter of 55 mm and a height of 110 mm. Three consecutive layers of sand were packed into the column, ensuring that each layer was compacted uniformly to achieve the optimum dry density to maintain consistency of experiments. The curing times applied were 14 days and 28 days as shown in Fig. 1.



(a) Curing (b) After curing
Fig. 1 UCT Sample

3. RESULTS AND DISCUSSION

Differences in concentration, culture, and coal content have different effects on the UCS value. All

MICP-stabilized samples significantly increased the UCS values compared to untreated samples. Changes in the UCS value can be observed in Fig 2.

Fig. 2 shows a comparison of changes in the UCS value between the MICP process using a 3-day culture and a 6-day culture. It turns out that there is a very significant difference where the UCS value sand contaminated with coal stabilized by MICP using the 3-day *Bacillus subtilis* culture gives better results compared to culture 6 days. This is because the 3-day culture of *Bacillus subtilis* bacteria is in a stationary phase and the optical density (OD) is in 0.6, while in 6 days of culture the bacteria are in the death phase, the optical density (OD) is in 0.4 so the MICP process is no longer effective. The UCS value of soil type 1 stabilized by MICP using 3 days of *Bacillus subtilis* culture gave better results, which increased 5 times compared to 6 days of culture.

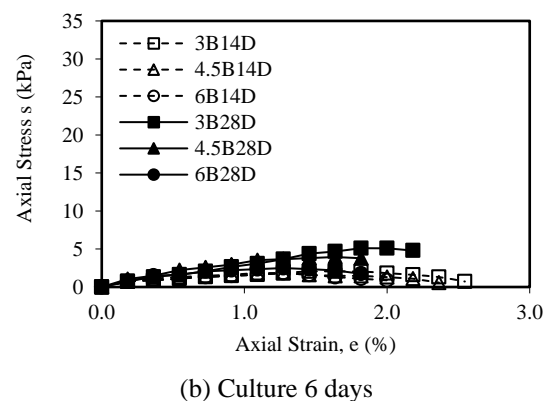
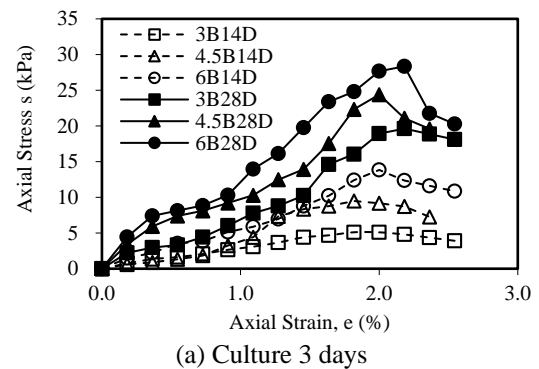


Fig. 2 Relationship between stress and strain for soil Type 1

Note:

B = Bacteria concentration (%)

D = Curing time (day)

Culture of *Bacillus subtilis* on B4 media, at the age of 3 days the culture was at the peak of the exponential phase and early stationary, so that in this phase the number of bacterial cells that could be produced was the most, meaning that more CaCO_3 would also be deposited. Meanwhile, at the

age of 6 days of culture, the bacteria are in the death phase. The death phase is the phase in which most of the bacterial cells die which can be caused by a lack of nutrients or because of poisoning due to an unfavorable growth environment. In the death phase, the number of living bacterial cells is no longer as much as from the stationary phase so that less CaCO_3 will be deposited.

concentrations. Okwadha et al[12] discovered that the rate of urea decomposition and the volume of calcium carbonate precipitation correlated positively with the concentration of bacterial cells. Soon et al[18] found that increasing bacterial solution concentrations could significantly increase the calcium carbonate content, thereby improving strength and reducing permeability. [19]found that

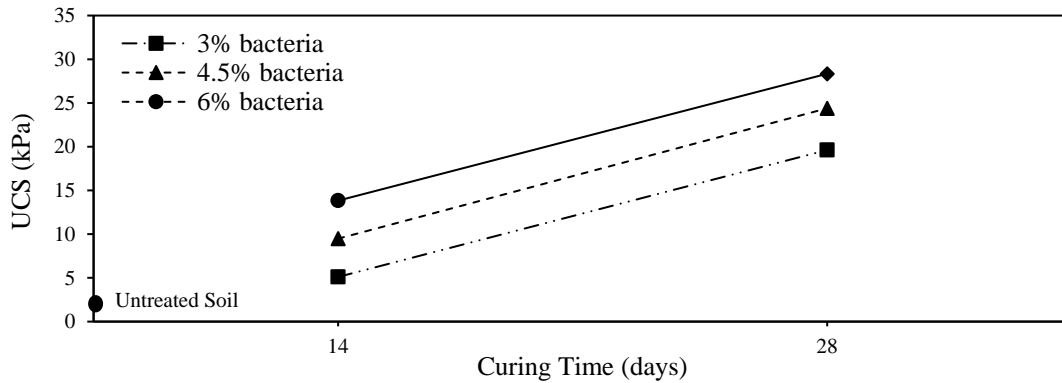


Fig. 3 Relationship between UCS and curing time, stabilization MICP culture 3 days, soil Type 1

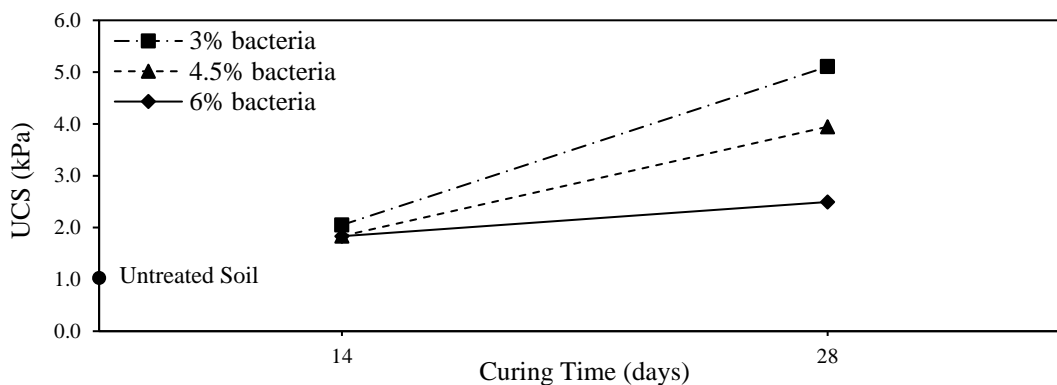


Fig. 4 Relationship between UCS and curing time, stabilization MICP culture 6 days, soil Type 1

Apart from bacterial culture, the concentration of bacteria also plays an important role in the soil stabilization process using the MICP method. The addition bacterial of 6% concentration, culture 3 days, increased the UCS value higher than the 3% and 4.5% concentrations. This shows that the higher the concentration used, the more CaCO_3 is deposited as shown in Fig. 3.

This is consistent with the results of previous studies conducted. according to which states that the addition of bacterial concentrations increases the value of soil UCS due to the increase in CaCO_3 produced. The concentration of bacteria can be related to the number of bacterial cells present in the bacterial solution The UCS value of soil Type 1 stabilized with MICP with *Bacillus subtilis*, 3 days culture gave better results, which increased 28 times compared to untreated soil.

Therefore, crystal morphology, calcium carbonate production, and the cementing effect of geomaterials can be affected by bacterial

the UCS of soils increased with elevated bacterial cell concentration. [20]found that treated quartz sand with high concentrations had a higher shear strength and lower volumetric strain. It can be seen from the analysis of previous studies that higher concentrations of bacterial solutions are useful for improving the engineering properties with MICP treated.

A different thing is shown in the results of stabilization using bacteria with a culture age of 6 days where the addition of the concentration of bacteria made the UCS value decrease. Provision of a bacterial solution with a concentration of 3% showed better results than a concentration of 4.5% and 6% as shown in Fig. 4. In the death phase, besides the number of bacterial cells, the urease activity has also decreased. A few possible reasons for this reduction of enzyme production occurred during the incubation period might be due to the reduction of essential nutrients, insufficient sugar contents in the medium, due to inhibitory

metabolites[21], or absence of sufficient hydrolyzed urea as nitrogen for energy or might be due to saturated active sites of the microbial enzyme by the substrate molecules which occurs when ureolysis has been completely used up.

Fig. 4 shows that after the 14-day curing period, the UCS value was almost the same for all variations in the concentration of bacteria, which was around 2.0 kPa. But after 28 days of curing, there is a significant difference. The addition of a 3% concentration of *Bacillus subtilis* bacteria increases the UCS value up to 5.0 kPa, meaning that there is a 5 times increase compared to unstabilized soil. While the addition of 4.5% bacteria, the UCS value was only 3.9 kPa and the addition of 6% bacteria only increased the UCS value by 2.5 kPa. This means that in the death phase, the addition of the bacterial concentration is not effective in increased the UCS value.

The comparison of the effectiveness of using a 3-day bacterial culture and a 6-day bacterial culture for soil Type 1 can be seen in Fig. 5(a). Of all the culture variations and concentrations of *Bacillus subtilis* bacteria, it turns out that using a 3-day bacterial culture with a concentration of 6% increases the highest UCS value compared to other culture variations and concentrations. The effectiveness of this optimum variation can increase the UCS value up to 28 times compared to unstabilized soil. MICP stabilization using a 3-day bacterial culture with a concentration of 6% was able to increase the UCS value up to 5 times compared to soil stabilized using a 6-day bacterial culture with a concentration of 3%.

Soil Type 2 and Type 3 stabilized by MICP also showed the same behavior as soil Type 1. MICP stabilized soil had an increase in UCS values when compared to soil that was not stabilized, the results can be seen in Figure 5(b,c). The use of 3-day bacterial culture gave a greater increase better than using a 6-day culture. variation3 days culture and 6% concentration gave the highest increase in UCS values compared to other variations.

Soil Type 2, after a 14-day curing period, the UCS values increased approximately 12-14 times in soil treated with a 3-day culture but only around 1-1.5-fold with a 6-day culture. After 28 days of treatment, UCS values increased approximately 18-25-fold in the soil treated with 3-day cultures but only increased 1.8-2-fold with 6-day cultures.

In Type 3 soils, after a 14-day curing period, the UCS values increased approximately 8-9 times in the soil treated with 3-day culture but only increased 1-2-fold with 6-day culture. After 28 days of treatment, the UCS values increased approximately 10-14 times in the soil treated with a 3-day culture but only increased 2-7-fold with a 6-day culture.

In addition to microorganism factors, coal content also has an important role in the process of CaCO_3 deposition. Fig. 5 shows that increasing the percentage of coal also increases the UCS value. For the optimum bacterial variation, the 3-day culture variation, 6% concentration in soil Type 1 has a UCS value of 28.3 kPa, Type 2 has a UCS value of 42.5 kPa and Type 3 is 51.6 kPa. This means that coal particle size has an important role in the MICP stabilization process.

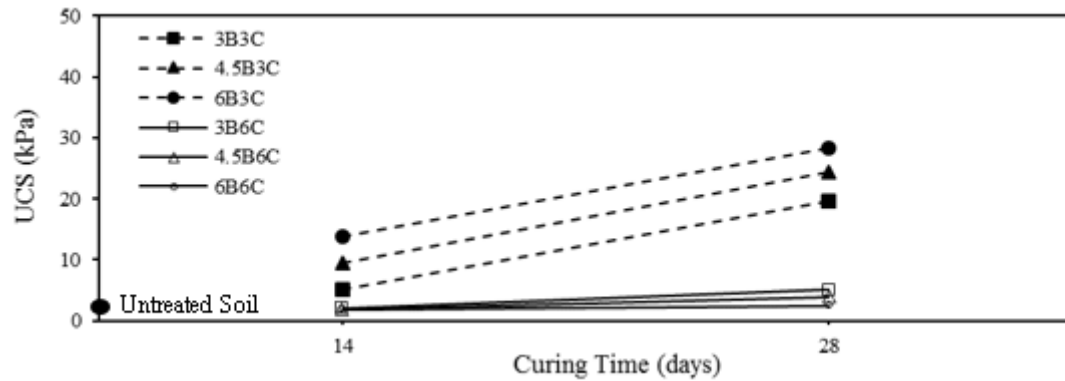
Another significant soil property that regulates MICP efficiency is soil particle size. Soil particle size is directly related to the soil pore size, which controls whether bacteria can flow without restraint and disperse uniformly in the soil matrix. Soils containing particles smaller than bacterial size could therefore prevent the bacteria movement in the soil matrix, leading to limited and heterogeneous precipitation of calcium carbonate. But larger particles have fewer intergranular contacts and larger inter-granular distances. Thus, the majority of calcium carbonate coats rather on the surface of coarse particles than the contact points, which may weaken the overall cementation quality. The addition of coal particles with grain size ≤ 0.149 improves Uniformity Coef. (Cu) and Gradation Coefficient. (Cc) so that the contact distance is not too far. Higher relative density and well-graded distribution of particle size contribute to better soil cementation and binding. Compaction or improving the degree of soil gradation are effective ways of improving the impact of MICP on cementation[9].

Table 5. Soil mineralogi after MICP

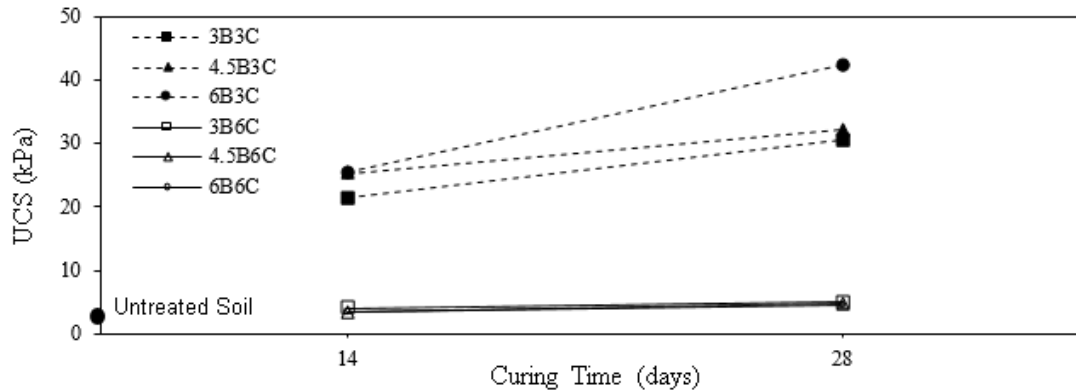
Mineralogy	Content (%)	
	Soil Type 3	
	Before	After
Quartz (SiO_2)	92.5	83.2
Ilmenite (FeTiO_3)	7.5	12.3
Calcite (CaCO_3)	-	1
Unidentified Peak Area	10.2	7

To determine changes in soil mineralogy after MICP is carried out, it can be seen in the Table. 5. XRD test results on Type 3 soil found a calcite deposit of 1%. Stabilization with MICP is a continuous stabilization so it is important to pay attention to the curing period. Fig. 5 shows that all soil types experienced a significant increase in UCS values with the longer the curing time.. The bacterium *Bacillus subtilis* can survive in nutrient-poor soil by turning into endospores and can last for 6 years and can divide again if nutrients are available again, this means that CaCO_3 deposition can continue and the increase in soil strength will

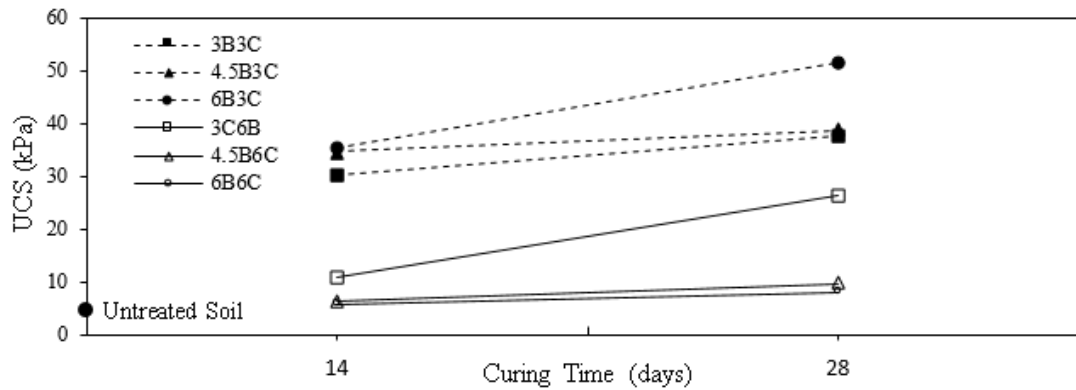
continue as long as the nutrients needed by the stabilizers is aim reduces a series of impacts that



(a) Soil Type 1



(b) Soil Type 2



(c) Soil Type 3

Fig. 5 Relationship between UCS values and the effect of coal content, culture, concentration, and curing time

bacteria are still available in the soil.

Note:

B = Bacteria concentration (%)

C = Culture (day)

4. CONCLUSIONS

This research is aim to overcome environmental damage and reuse coal mining waste as construction material especially road foundation material. The use of microorganisms as environmentally friendly

often arise due to soil stabilization processes using dangerous addictive substances. A series of tests were carried out to analyze the mechanical characteristics of coal-contaminated soil stabilized by MICP using the bacterium *Bacillus subtilis*. Stabilization with MICP increased the UCS values for all soil types compared to unstabilized soils. The addition of bacterial concentration, culture for 3 days, increased the UCS value but on the other hand, the addition of bacterial concentration, culture for 6 days would decrease the compressive strength of

sandy soil contaminated with coal. The optimum variation in concentration and bacterial culture for use was a concentration of 6% and culture for 3 days because these variations gave the highest increase in UCS values

5. ACKNOWLEDGEMENTS

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