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Reducing soil permeability for TPA (Final Disposal Site) using *Bacillus subtilis* mycobacteria

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Abstrack This study evaluates the effectiveness of using *Bacillus subtilis* bacteria in reducing soil permeability rates in landfill areas. The primary issue faced at landfills is groundwater contamination caused by leachate seepage due to high soil permeability. Conventional approaches, such as using synthetic liners, have limitations, necessitating the development of more sustainable alternative methods. *Bacillus subtilis* microbacteria were used in this study. The soil inoculated with bacteria underwent an incubation period of 3 days. This study aimed to determine the reduction in permeability values by comparing bacteria-affected soil with untreated soil as a control. SEM testing was conducted to observe the soil structure affected by bacteria after a 3-day incubation period with 15% cementation solution and 5% sand. *Bacillus subtilis* bacteria were found to reduce soil permeability rates, achieving a result of 1.612×10^{-7} for swamp soil with a 15% cementation solution (bacterial reagent) and 5% sand, as well as 9.791×10^{-8} for laterite soil with the same cementation solution and sand composition.

Keywords: *Bacillus subtilis*; Soil Permeability; Landfill; SEM.

INTRODUCTION

Final Disposal Sites (TPA) serve as the main location for municipal waste management, where waste is processed and deposited in the long term. However, TPA often poses significant environmental challenges, especially related to the risk of groundwater pollution due to liquid waste or leachate that seeps from the waste pile. Soil permeability in TPA is a crucial factor, because soil with high permeability will allow leachate to move into deeper soil layers and pollute groundwater sources and the surrounding ecosystem (Rahardjo et al., 2018).

Reducing soil permeability in landfills is necessary to slow or stop the movement of leachate. Conventional approaches, such as the use of synthetic liners and clay layers, have limitations, especially in terms of high cost and long-term durability. As more environmentally friendly and sustainable technologies develop, research is beginning to focus on the use of microorganisms that can naturally strengthen soil structure and reduce soil permeability.

Bacillus subtilis is a bacterium known to be able to carry out the bioprecipitation process, which is the formation of mineral deposits in the form of calcium carbonate through the activity of the urease enzyme produced by the bacteria. This deposit plays a role in clogging soil pores and significantly reducing permeability levels (DeJong et al., 2006). This method is known as bioclogging and is one of the innovative solutions in soil engineering because it is able to increase soil density and reduce permeability rates by utilizing natural biological processes (Mitchell & Santamarina, 2005).

Sand is used as a research medium to represent coarse-grained soil with large particle sizes that have a high level of permeability. Sand is chosen because its large pores allow leachate to seep easily, so it is necessary effective method to block the pores. With its homogeneous nature and minimal organic content, sand also facilitates the analysis of the direct effects of bioprecipitation by *Bacillus subtilis* on its permeability.

The use of swamp soil in this study is as a research medium to represent soil conditions with high water saturation levels and dominant organic content. This soil is often found in landfill areas located in lowlands or near swampy areas. High organic and groundwater content can affect the activity of *Bacillus subtilis* in the bioprecipitation process. This study will evaluate whether *Bacillus subtilis* remains effective in partial anaerobic conditions or high saturation such as in swamp soil.

Laterite soil is used to test the effectiveness of *Bacillus subtilis* on soil types with high iron and aluminum oxide mineral content. This type of soil is commonly found in tropical areas, including Indonesia, and has moderate to low permeability, but with non-uniform porosity characteristics. With a complex soil chemical composition, this study will see to what extent *Bacillus subtilis* is able to form calcium carbonate in laterite soil and affect the reduction in its permeability.

MATERIALS AND METHODS

Material Used

Soil samples were collected from two distinct locations, Banjarmasin and Banjarbaru, in Kalimantan Selatan. This diversity in soil types was essential for evaluating the effectiveness of *Bacillus subtilis* across different conditions. *Bacillus subtilis* was sourced from the MIPA Laboratory at Lambung Mangkurat University. The inoculation process involved treating the soil samples with the bacteria and allowing them to incubate for three days. This incubation period was crucial for the bacteria to interact with the soil and potentially alter its properties. Cementation Solution a 15% solution used in conjunction with the bacteria to enhance soil stabilization. Sand: 5% sand was mixed with the cementation solution to improve the soil structure during the treatment process.

Methods and Procedures

The research methodology employed in this study is experimental in nature, focusing on the application of *Bacillus subtilis* microbacteria to reduce soil permeability rates in landfill areas. The methodology encompasses several critical stages, including sample collection, bacterial preparation, soil treatment, and permeability testing. Each stage is designed to systematically evaluate the effectiveness of the bacterial treatment on different soil types. Soil samples were collected from two distinct locations: swamp soil and laterite soil, specifically from Banjarmasin and Banjarbaru in Kalimantan Selatan. This selection was made to ensure a diverse representation of soil types, which is crucial for understanding the treatment's effectiveness across different conditions.

Bacillus subtilis was cultured in a controlled laboratory environment to prepare for inoculation. This step is vital to ensure that the bacteria are healthy and active, maximizing their potential to influence soil properties. The preparation involved growing the bacteria in nutrient-rich media until they reached an optimal concentration for treatment.

The prepared *Bacillus subtilis* was then inoculated into the soil samples. The treatment involved mixing the inoculated soil with a 15% cementation solution and 5% sand. This combination was designed to enhance the binding of soil particles and improve the overall structure of the treated soil. After mixing, the soil samples were incubated for a period of three days. This incubation allowed the bacteria to interact with the soil, promoting bioprecipitation processes that lead to the formation of calcium carbonate, which helps to clog soil pores and reduce permeability.

Following the incubation period, the permeability rates of both treated and untreated soil samples were measured. The method used for this testing was the falling head permeability test, which is suitable for soils with fine pores, such as clay or very fine sand. This technique involves measuring the rate at which water flows through the soil sample under a specific hydraulic gradient. The results from the permeability tests were then compared between the treated and untreated samples to assess the effectiveness of the *Bacillus subtilis* treatment in reducing soil permeability.

RESULT AND DISCUSSIONS

Preliminary Testing

Before conducting the Permeability test using the falling head method, a preliminary test was first carried out. The tests carried out included: testing the original soil water content (swamp soil and laterite soil) and specific gravity (Gs).

Water content testing was carried out according to the procedure in ASTM D2216-98. The results of the water content test carried out on swamp soil obtained a water content value of 100.65%. This is because the original soil tested consists of organic fiber content (swamp) which can absorb a lot of water so that it contains a high water content, according to (Mochtar 2002) the water content of swamp soil ranges from 100% to 600%.

Water content testing for laterite soil obtained a water content value of 39.75%. This is because laterite soil is dry and barren. According to (Rusdiansyah 2023) Laterite soil is old soil that has experienced advanced weathering, so that its nutrient content is lost due to erosion or continuous rain.

Specific Gravity testing was conducted according to ASTM D 854-02. From the tests that have been conducted on the original soil, the specific gravity (Gs) value of the soil used was 2.4 on swamp soil and 2.65 on laterite soil. The specific gravity (Gs) value is caused by the presence of wood fibers and other organic content found in swamp soil and laterite soil.

Permeability Test Results Using the Falling Head Method

Table 1. Results of Reading the Permeability Test Using the Falling Head Method on Swamp Land

No	Sampel Name	Fermentation period (days)	Permeability Value (cm/sec)	Average Permeability
1	Native Land	0	3,688E-07	2,040E-07
			9,496E-08	
			1,482E-07	
2	Soil + 5% Bacteria	3	2,964E-07	3,390E-07
			3,683E-07	
			3,522E-07	
3	Soil + 10% Bacteria	3	4,101E-07	4,302E-07
			5,268E-07	
			3,539E-07	
4	Soil + 15% Bacteria	3	4,305E-07	5,368E-07
			6,431E-07	
			5,368E-07	
5	Soil + 5% Bacteria + 5% Sand	3	1,749E-07	1,977E-07
			1,801E-07	
			2,381E-07	
6	Soil + 10% Bacteria + 5% Sand	3	1,549E-07	1,743E-07
			1,911E-07	
			1,770E-07	
7	Soil + 15% Bacteria + 5% Sand	3	1,409E-07	1,612E-07
			1,655E-07	
			1,771E-07	

The following is a graph of the permeability test readings using the falling head method which can be seen in Figure 1.

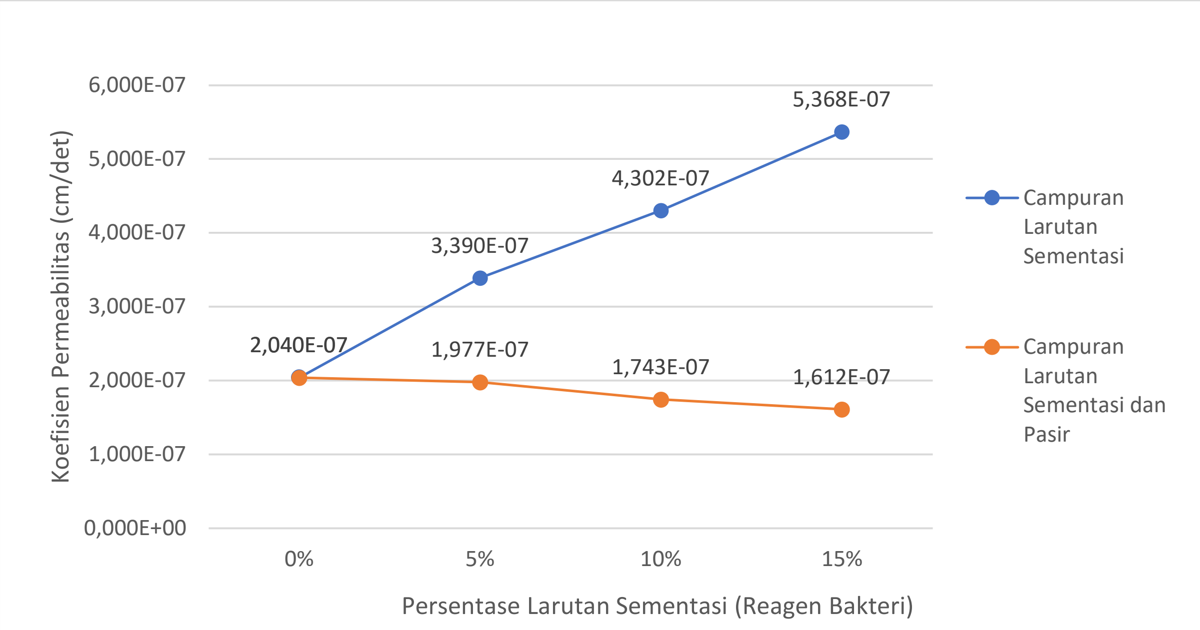


Figure 1. Permeability Testing Graph using the Falling Head Method on Swamp Land

In Table 1 and Figure 1 above, the permeability test of swamp soil with a mixture of cementation solution (bacterial reagent) and 5% sand is depicted. In the mixture of soil with cementation solution (bacterial reagent) the highest permeability figure is a mixture of 15% with a permeability coefficient of 5.368E-07 cm/sec and the lowest permeability figure is a mixture of 5% with a permeability coefficient of 3.390E-07 cm/sec. While for the mixture of cementation solution (bacterial reagent) and sand, the highest permeability figure is a mixture of 5% with a permeability coefficient of 1.977E-07 cm/sec and the lowest permeability figure is 15% with a permeability coefficient of 1.612E-07 cm/sec. The graph of the relationship between the permeability coefficient and the percentage of cementation solution (bacterial reagent) and sand addition of 5%, shows a tendency to decrease the permeability value. The calculation results will show that the permeability value reaches the minimum limit if the percentage of cementation solution (bacterial reagent) is 15% and mixed with 5% sand, so that the minimum permeability value is 1.612E-07 cm/sec.

Table 2. Results of Permeability Test Readings Using the Falling Head Method on Laterite Soil

No	Sampel Name	Fermentation period (days)	Permeability Value (cm/sec)	Average Permeability
1	Native Land	0	2,204E-07	2,163E-07
			2,213E-07	
			2,072E-07	
2	Soil + 5% Bacteria	3	2,444E-07	2,319E-07
			2,267E-07	
			2,246E-07	
3	Soil + 10% Bacteria	3	1,990E-07	1,867E-07
			1,672E-07	
			1,940E-07	
4	Soil + 15% Bacteria	3	1,739E-07	1,617E-07
			1,576E-07	
			1,536E-07	
5	Soil + 5% Bacteria + 5% Sand	3	1,238E-07	1,422E-07
			1,635E-07	
			1,394E-07	
6	Soil + 10% Bacteria + 5% Sand	3	1,635E-07	1,417E-07
			1,482E-07	
			1,132E-07	
7	Soil + 15% Bacteria + 5% Sand	3	8,896E-08	9,791E-08
			1,107E-07	
			9,411E-08	

The following is a graph of the permeability test readings using the falling head method which can be seen in Figure 2.

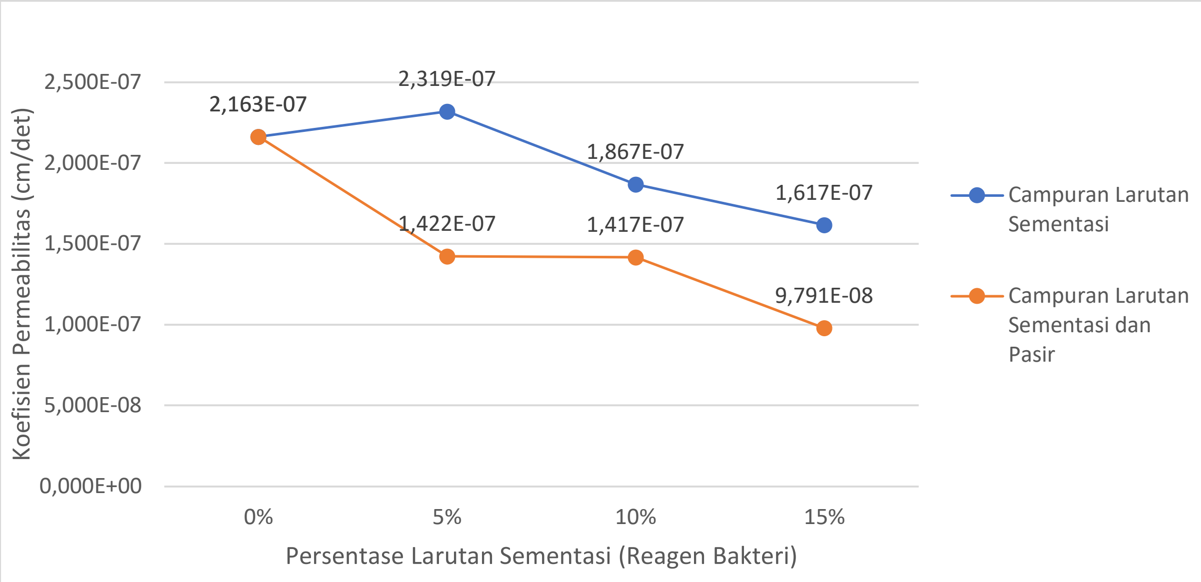
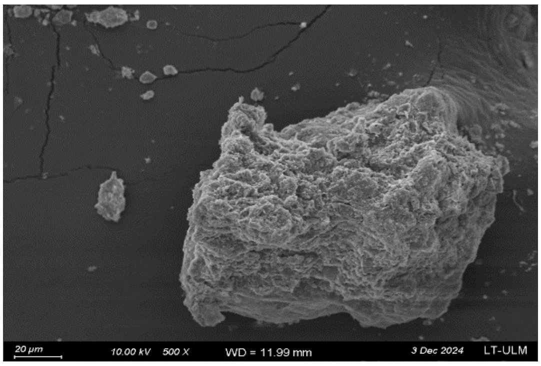


Figure 2. Permeability Testing Graph using the Falling Head Method on Laterite Soil

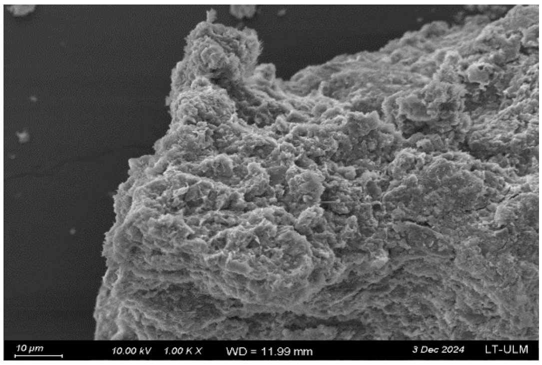
In Table 2 and Figure 2 above, the permeability test of swamp soil with a mixture of cementation solution (bacterial reagent) and 5% sand is depicted. In the mixture of soil with cementation solution (bacterial reagent) the highest permeability figure is a mixture of 5% with a permeability coefficient of 2.163E-07 cm/sec and the lowest permeability figure is a mixture of 15% with a permeability coefficient of 1.617E-07 cm/sec. While for the mixture of cementation solution (bacterial reagent) and sand, the highest permeability figure is a mixture of 5% with a permeability coefficient of 1.422E-07 cm/sec and the lowest permeability figure is 15% with a permeability coefficient of 9.791E-08 cm/sec. The graph of the relationship between the permeability coefficient and the percentage of cementation solution (bacterial reagent) and sand addition of 5%, shows a tendency to decrease the permeability value. The calculation results will show that the permeability value reaches the minimum limit if the percentage of cementation solution (bacterial reagent) is 15% and mixed with 5% sand, so that the minimum permeability value is 9.791E-08 cm/sec.

In each variation of cementation solution, there is a decrease in permeability as the mixture of cementation solution (bacterial reagent) mixed into the soil increases. Permeability decreases, which means that the soil pores are getting smaller. The pore space shrinks due to the partial filling effect of the crystals, causing a decrease in permeability and porosity.

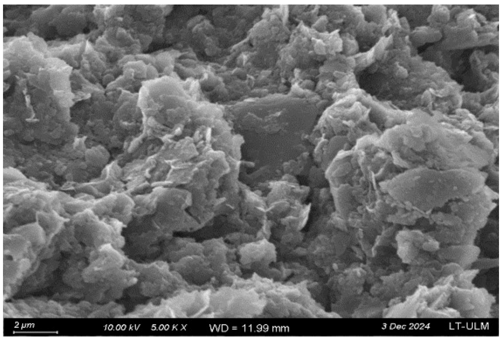
The SEM test in this study aims to visually determine the presence of extracellular polysaccharides formed in samples that have been inoculated with certain types of microorganisms within a specified time, as can be seen in Figure 3.



(a) 500 magnification



(b) 1000 times magnification

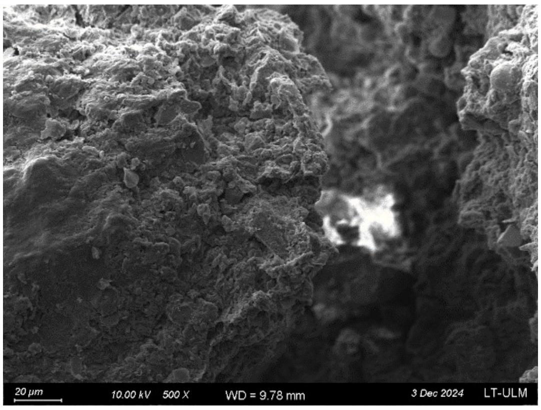


(c) 5000 times magnification

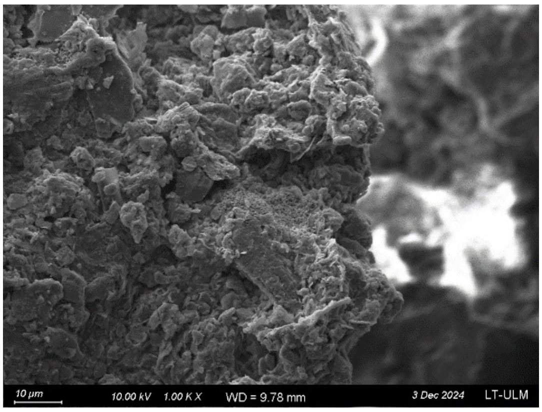
Figure 3. SEM Test Results of Swamp Soil with *Bacillus Subtilis* Bacteria During 3 Days of Fermentation

Figure (a) shows a swamp soil particle enlarged at 500 times magnification, then enlarged at 1000 times magnification as in Figure (b) and then enlarged again at 5000 times magnification as in Figure (c). Figure (b) shows only a few Bacillus subtillis colonies found on the walls of silt soil particles. Figure (c) shows Bacillus subtillis colonies are only partially visible on the walls of soil particles. This shows that for soil with high organic fiber content, *Bacillus Subtilis* microbacteria decompose faster with organic content of swamp soil.

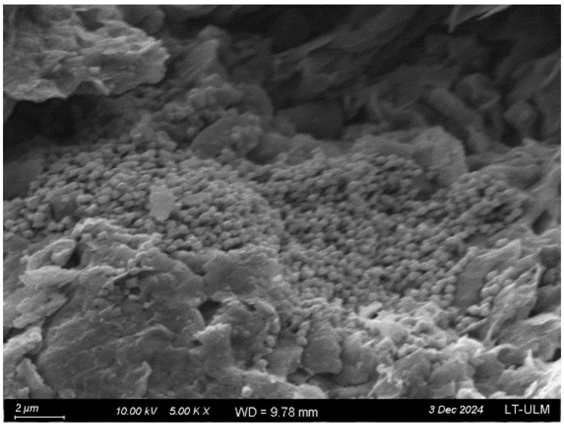
Next, the SEM test results for laterite soil are displayed, as can be seen in Figure 4.



(a) 500 magnification



(b) 1000 times magnification



(c) 5000 times magnification

Figure 4. SEM Test Results of Laterite Soil with *Bacillus Subtilis* Bacteria During 3 Days of Fermentation

Figure (a) shows laterite soil particles enlarged at 500 magnification and then enlarged at 5000 magnification as in Figure (c). Figure (c) shows Bacillus subtillis colonies found on the walls of silt soil particles. Figure (c) shows Bacillus subtillis colonies seen scattered on the walls of soil particles and experiencing solitary growth and producing exopolysaccharides of calcium carbonate compounds as adhesives between soil particles.

CONCLUSIONS

he graph of the relationship between the permeability coefficient and the percentage of cementation solution (bacterial reagent) and sand addition of 5%, shows a tendency to decrease the permeability value. The calculation results will show that the permeability value reaches the minimum limit if the percentage of cementation solution (bacterial reagent) is 15% and mixed with 5% sand.

The use of *Bacillus Subtilis* microbacteria for Final Disposal Site (TPA) soil is sufficient for swampy soil and laterite soil, this is because the standard in Indonesia is that the maximum permeability is 1×10-7 cm/second (or 10-9 m/second) for the soil-resistant layer (clay liner).

To determine the optimal concentration of bacteria on swamp soil and laterite soil, further research is needed using variations in the dosage of microbacteria that will be mixed into the soil. It is hoped that further research will estimate the amount of Calcium Carbonate (CaCO3) in each sample to be able to determine the optimum value of the reagents commonly used in the stabilization method. Further research is needed to further examine the influence of *Bacillus Subtilis* bacteria using longer or shorter time variations than the time used by the researcher, namely 3 days.

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