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Effect of Exogenous *Bacillus* sp. Addition on Bioremediation of Soil Contaminated by Used Cooking Oil

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ABSTRACT

Used cooking oil waste can be carcinogenic and toxic to living creatures in soil. Bioremediation is a way to treat used cooking oil-contaminated soil by using microorganisms that are safe for the environment. This research aimed to determine the effect of adding exogenous bacteria *Bacillus sp*. on the bioremediation process of soil contaminated with used cooking oil waste. Total Petroleum Hydrocarbons (TPH) measurement and effectiveness of *Bacillus sp*. in degrading hydrocarbons was calculated in this study. The results showed that *Bacillus sp*. has the ability to rapidly degrade used cooking oil TPH and increased the effectiveness of bioremediation in soil.

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1. INTRODUCTION

Cooking oil is defined as an oil derived from plants that maintains a liquid state at room temperature (Widayat, 2009). Cooking oil is typically utilized for three to four frying cycles before being discarded. This discarded oil is often referred to as used cooking oil (Rifqi *et al*., 2012). The quantity of used cooking oil generated on an annual basis from domestic sources is estimated at 305,000 tonnes, while that produced by the food processing industry is approximately 2 million tonnes, and 1.5 million tonnes is attributed to hotels and restaurants (Kayun, 2007, as cited in Widodo, 2011). The rising utilisation of cooking oil in the food industry, hospitality sector, food distribution networks and food trading has led to a concomitant increase in the disposal of cooking oil waste (used cooking oil) into the environment. The unprocessed disposal of cooking oil waste into the environment can give rise to a range of environmental issues (Syabanu and Cahyatri, in Zulfa, 2010).

The disposal of cooking oil waste as organic waste, a long-chain carbon compound, has been identified as a potential source of environmental concern. If disposed of directly into the environment, such waste has the potential to increase the acidity of the environment, cause unpleasant odours and damage the environment (Setyawan, 2005). Pollutants derived from used cooking water can impede the opening and closing of soil pores, thereby disrupting the natural airflow within the soil. This can lead to the growth of stunted plants and a decline in soil fertility. Furthermore, pollutants from used cooking water have the potential to cause carcinogenic and toxic effects on living organisms within the soil (Wyuliandari, 2008).

One strategy for anticipating environmental contamination resulting from the disposal of cooking oil waste is through the implementation of bioremediation techniques. Bioremediation represents a strategy for the remediation of oil-polluted land through the activity of microorganisms, with the objective of ensuring the safety of the environment and the feasibility of implementation at a low cost (Ni' matuzahroh *et al*., 2009). The objective of this research was to determine the impact of the addition of exogenous bacteria, specifically *Bacillus sp*., on the bioremediation process of soil contaminated with used cooking oil waste.

2. METHODS

This study adopted a randomised group design, comprising two factors: the concentration of *Bacillus* sp. and soil. The combination treatments in this study comprised soil and used cooking oil. These were designated as the control treatment ('P0') and the bacterial treatment ('P1'). The control treatment consisted of soil and used cooking oil waste alone, while the bacterial treatment comprised soil, used cooking oil waste and *Bacillus* sp. The soil sample used for this study was 0.294 kg of soil for each treatment. The soil were collected from Lembang, Bandung, Indonesia. The quantity of *Bacillus* sp. bacteria utilised was 1 gram, which was homogenised with 9 grams of distilled water for each treatment (Aliyanta *et al*., 2012). The quantity of cooking oil utilised was 11.04 g for each treatment. Soil degradation was conducted over a three-day period, with TPH measurements recorded on a daily basis. Subsequent to this, an analysis was conducted to ascertain the effectiveness of the degradation process based on observed changes in TPH levels. The treatments were designated as P0 and P1, with each treatment conducted in triplicate.

2.1. The Measurement of TPH

The procedure for measuring TPH is as follows: A quantity of 5 grams of the sample is transferred to an Erlenmeyer flask and 100 ml of 96% alcohol is added. Subsequently, the sample is homogenised for 30 minutes at a speed of 150 ppm using a shaker. The remaining supernatant in the Erlenmeyer is then transferred to a glass beaker and evaporated. The evaporation process leaves a residue, which is the remaining oil, and this is weighed. This is done to determine the amount of oil remaining in the sample after it has evaporated. The total hydrocarbon degradation was measured by equation 1 (Ijah and Upke, 1992 in Aliyanta et al., 2012).

$$\%TPH = \frac{(m_1 - m_0)}{m_2} \times 100\% \quad [1]$$

In this experiment, three essential measurements had been conducted for the analysis of residue and dry soil weight. The initial measurement, designated as M0, had represented the weight of the empty beaker glass in grams (g). Subsequently, M1 had documented the weight of the beaker containing the residue derived from the experimental outcomes, also expressed in grams. Finally, M2 had denoted the weight of the dry soil employed in the experimental procedure, which had been ascertained after the soil was completely desiccated to a moisture-free state. These measurements had been used to calculate the percentage of residue remaining after the separation or analysis process had been completed.

2.2 The Measurement of Bioremediation Effectivity

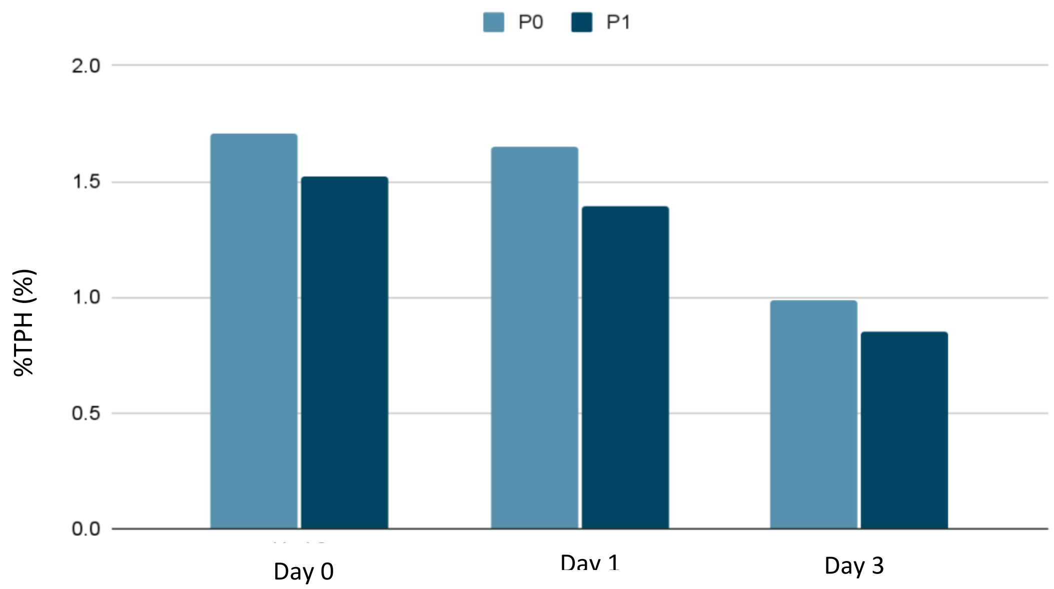
The effectiveness of exogenous bacteria, particularly *Bacillus* sp., in soil can be quantified by calculating the percentage difference between the initial oil weight and the residue weight relative to the initial oil weight. The objective of this method is to evaluate the capacity of *Bacillus* sp. to decompose organic compounds, which serves as an indicator of bacterial performance in enhancing soil quality. The bioremediation effectivity measured (Monteiro *et al*., 2006; Soenartiningsih *et al*., 2011). The measurement of bioremediation effectivity was measured by equation 2.

$$Bioremediation\ Effectivity = \frac{initial\ oil\ weight(g) - residue\ weight(g)}{initial\ oil\ weight\ (g)} \times 100\% \quad [2]$$

In the context of this study, the calculation of the percentage difference in oil weight provides a clear picture of the effectiveness of *Bacillus* sp. in the biodegradation process. A comparison of the initial weight of oil with the weight of the residue following treatment allows the contribution of this bacteria to the reduction of oil contaminants in the soil to be determined.

3. RESULTS AND DISCUSSION

The findings of this study demonstrate that soil culture contaminated with used cooking oil that received additional treatment with *Bacillus* sp. bacteria exhibited superior outcomes in comparison to the control culture that did not undergo bacterial treatment. The result shown in **Figures 1**. These findings align with those of Brown (1987), who demonstrated that each bacterial strain has a limited capacity to degrade hydrocarbons, particularly in the presence of diverse hydrocarbon mixtures, such as those found in oil waste. Additionally, Brown (1987) highlighted that *Bacillus* sp. bacteria are among the most effective biosurfactant-producing bacteria. Based on existing research, the bacterial species *Bacillus* sp. has been identified as one of the most effective biosurfactant producers. Biosurfactants are macromolecules produced by microorganisms that possess the capacity to reduce surface tension, rendering them highly efficacious in bioremediation applications, particularly in the remediation of oil



pollution. In one study, *Bacillus* spp. demonstrated favourable outcomes in emulsification activity testing, exhibiting a high emulsification index. *Bacillus* sp. 48, in particular, produced an emulsification index (E24) value of 99.5% and demonstrated the capacity to reduce the surface tension of lubricating oil to 19.7 dyne/cm (Zia et al., 2023; Mardiah et al., 2022).

Figure 1. Comparison of TPH concentration in contaminated soil between control and addition of Bacillus sp.

The results of this experiment comply with the standards set forth by the State Minister for the Environment No. 6 of 2021, which stipulates that land or land can be reused if the TPH value has reached 1% or 10,000 mg/kg or less. Despite the fact that used cooking oil waste does not fall within the category of petroleum as defined in the regulations, it does contain TPH, which has the potential to act as a source of pollutants that could prove detrimental to the environment. The findings of this study indicate that Bacillus sp. has significant potential for utilisation in bioremediation, particularly in the processing of used cooking oil waste, where these bacteria are capable of rapid degradation of TPH. These findings align with those of Oudah (2024), who posited that Bacillus sp. represents a robust biological solution for the remediation of TPH-polluted soil. These microorganisms consume hydrocarbons as a carbon source, thereby producing the energy necessary for their survival. This process generates metabolites such as CO2, H2O, and biomass, which contribute to the restoration of the quality of TPH-contaminated soil. Further research is required to elucidate the specific mechanisms and optimal conditions for the degradation activity of these bacteria in various types of hydrocarbon contaminants.

The results of the bioremediation effectiveness test conducted with Bacillus sp. demonstrated that the average effectiveness of the untreated soil was 0.9925% on day 1 and 0.9937% on day 3. In contrast, the subjects who received the treatment exhibited a concentration of 0.9955% on day 1 and 0.9961% on day 3. Following a three-day period, the effectiveness of the bioremediation test exhibited an increase; however, the observed difference was not statistically significant.

As illustrated in **Table 3.1**, the difference in percentage effectiveness is marginal, with the treated soil exhibiting a slight advantage. These findings indicate that the soil treated with Bacillus sp. bacteria exhibited only slight differences from the untreated control. This is due to the fact that the indigenous bacteria that are already present in the soil are responsible for the breakdown of pollutants. Solihin and Fitriatin (2017) have stated that the soil in Lembang contains a diverse range of fungal and bacterial species, including Actinomycetes, Azotobacter, phosphate-solubilising bacteria and soil bacteria. Therefore, the addition of *Bacillus sp* bacteria had only a slight additional effect on Lembang soil that had been contaminated with used cooking oil, in comparison to the untreated soil.

Table 3.1 The measurement of Bioremediation effectiveness by *Bacillus* sp.

Day	P0 (%)	P1 (%)
0	0	0
1	0.9925	0.9955
3	0.9937	0.9961

4. CONCLUSION

The TPH test results, in conjunction with the effectiveness of the *Bacillus* sp., indicate that the exogenous bacteria *Bacillus* sp. has the capacity to degrade used cooking oil TPH and enhance the efficacy of bioremediation in soil.

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6. AUTHORS' NOTE

The authors declare that there is no conflict of interest regarding the publication of this article. Authors confirmed that the paper was free of plagiarism.

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