

Trichoderma sp. Mushroom Optimization with the Addition of Biostimulants to Reduce TPH (Total Petroleum Hydrocarbons) Levels in Soil Polluted by Palm Oil Mill Waste

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Manuscript received: 29 October, 2025. Revision accepted: 07 March, 2026. Published: 10 March, 2026.

Abstract

Benzene and toluene compounds in aromatic hydrocarbons can be found from the results of the liquid waste treatment process of palm oil mills that have the potential to pollute the environment in the soil ecosystem. This study aimed to optimize the fungus *Trichoderma sp.* by adding biostimulants to reduce TPH levels in soil contaminated with palm oil mill waste. The method used in reducing TPH levels was carried out by gravimetric analysis with the addition of biostimulant concentrations in the form of P0 (control), P1 (*Trichoderma sp.* 15%), P2 (*Trichoderma sp.* 15% + 35 gr rice husks), P3 (*Trichoderma sp.* 15% + 100 gr cow dung), P4 (*Trichoderma sp.* 15% + 35 gr rice husks + 100 gr cow dung) on 300 gr of soil contaminated with oil palm mill liquid waste, nitrogen content test using the Kjeldahl method, phosphorus content test using Spectrophotometry method, potassium content test using AAS method, and pH test. The results showed that the optimization of the fungus *Trichoderma sp.* with the addition of 35 grams of rice husk stimulant degraded TPH levels by 64.11%, the addition of 100 grams of cow manure stimulant degraded TPH levels by 66.92%, the combined addition of 35 grams of rice husk stimulant and 100 grams of cow manure showed a degradation of TPH levels by 76.02%, and treatment without stimulants degraded TPH levels by 44.90%. Optimization of the fungus *Trichoderma sp.* with the addition of stimulants in the form of rice husks and cow manure has a significant impact on the reduction of TPH levels.

Keywords: *Trichoderma sp.*; rice husks; cow dung; total petroleum hydrocarbons (TPH).

INTRODUCTION

The high productivity of palm oil also has an impact on the increase in palm oil mill waste production (Susila Arita et al., 2020). Waste produced from palm oil mills is divided into four types, including solid waste, liquid waste, gas waste and also B3 waste (hazardous and toxic materials). Liquid waste from palm oil mills has a high content of organic matter and can potentially pollute the environment (Sisnayati et al., 2021).

Benzene and toluene compounds in aromatic hydrocarbons can also be found from the results of the processing process in the liquid waste of palm oil mills. Hydrocarbon compounds can cause environmental hazards to soil ecosystems, including disrupted plant growth, damage to soil structure, and quality groundwater will be damaged (Kurniawan et al., 2018). This is in accordance with the quality standard of TPH that can be returned to the soil in accordance with the Decree of the Minister of Environment of the Republic of Indonesia Number 128 of 2003, which is 1%.

One of the microorganisms that can decompose hydrocarbon pollutants is the fungus *Trichoderma sp.*,

this fungus is able to survive various agricultural chemicals and aromatic hydrocarbons (Jumadi et al., 2021). The biostimulation method is one of the bioremediation methods that can be used to overcome this problem, namely by adding nutrients to microorganisms (Rahayu & Mangkoedihardjo, 2022). Rice husks are organic materials that can act as additional nutrients in stimulating the growth of microorganisms and improving soil aeration and moisture retention (Wagiono et al., 2022). Cow manure is rich in nitrogen, phosphorus and potassium (NPK) which play a role as nutrients in stimulating the metabolism of hydrocarbon-degrading microorganisms and increasing the ability of the bioremediation process in decomposing hydrocarbons (Marlina, 2021).

In a study conducted by Rizky et al. (2024) in bioremediation of hydrocarbon-contaminated land with the addition of rice husk biostimulation, it was able to reduce TPH levels in the initial analysis of 89.10% to 27.64%. Marlina's (2021) research in bioremediation of hydrocarbon-contaminated soil using the composting

method with the addition of cow manure stimulant effectively reduced TPH levels by 86.66%.

This study aims to optimize the fungus *Trichoderma sp.* with the addition of rice husk biostimulants and cow manure to reduce TPH levels in contaminated soil from palm oil mill waste. The results of the research are expected to be an alternative in bioremediation of contaminated soil from palm oil mill waste.

MATERIALS AND METHODS

Research on the optimization of *trichoderma sp.* in degrading tph levels in soil contaminated with palm oil mill waste with the addition of rice husk biostimulants and cow manure, using the complete random design method (RAL) consisting of five treatments with three repeats consisting of p0: 300 gr of soil contaminated with palm oil mill waste (control), p1: 300 gr of soil contaminated with palm oil mill waste + *trichoderma sp.* 15%, P2: 300 gr Soil Contaminated Palm Mill Waste + *Trichoderma Sp.* 15% + Rice Husk 35 gr, P3: 300 gr Soil Contaminated Oil Mill Waste + *Trichoderma Sp.* 15% + Cow Manure 100 gr, P4: 300 gr Soil Contaminated Oil Mill Waste + *Trichoderma Sp.* 15% + Rice Husk 35 gr + Cow Manure 100 gr. To assess tph levels in this study, gravimetric analysis was carried out on samples that had been treated and before treatment. According to (rizky et al., 2024) tph measurement was carried out by weighing 5 grams of soil samples, then the sample was extracted using a 25 ml n-hexane solution, then the mixture was whisked for 30 minutes on a separator funnel and then let it sit until it separated with n-hexane. After the mixture is separated, 3 layers can be found consisting of water, n-hexane and oil, then the water layer can be removed and then the oil and n-hexane layers are filtered using filter paper. Erlenmeyer 50 ml is taken and then weighed to determine the weight first, then on the oil layer and n-hexane that has been filtered, then put into the 50 ml erlenmeyer which has been known to weigh and evaporated at a temperature of 70oc until the n-hexane has evaporated and only the oil layer remains. The weight of the remaining oil layer is weighed and then the degraded oil content is calculated. The analysis of the data for the calculation of the percentage of tph uses the following equation (sasmita, 2021):

$$Tph\ level = \frac{a - b}{sample\ weight} \times 100\%$$

Description:

a = final erlenmeyer weight (with oil residue result)

b = initial erlenmeyer weight (empty erlenmeyer)

To find out the %degradation, calculations can be done with the equation:

$$\%degradation = \frac{tph0 - tphn}{tph0} \times 100\%$$

Description:

Tph0 = 0-day control tph

Tphn = n-day control tph

Inspection of npk levels in contaminated soil was carried out at the testing laboratory of the North Sumatra Agricultural Instrument Standard Application Center (BRMP). This inspection is carried out to see the quality and structure of the contaminated soil at the time of the initial condition and after treatment. In testing the nitrogen content (n) of the soil, it is carried out using the kjeldahl procedure method which consists of three stages, namely in the form of destruction, distillation and titration. Where at the destruction stage the sample will be put into a digestion tube then heated at a temperature of 400oc for 2 hours with a foss digester unit. In the distillation stage where the destroyed sample will be put into the KJELTEC analyzer unit, then in the last stage, namely titration, it is characterized by a change in color in the solution which changes from blue to pink.

The testing of soil phosphorus (p) content was carried out using the spectrophotometry test method, where the absorbance of the sample solution was measured using a spectrophotometer at a wavelength of 639 nm. Meanwhile, in testing the potassium content (k) of the soil is carried out using the aas method, the sample will be carried out a stirring process by adding h2so4 and hno3 which are heated on a hot plate, then aquadest is added and beaten, then filtered and the potassium content will be determined using atomic absorption spectrophotometry.

Data analysis was carried out in a quantitative descriptive manner that presented data with tables and graphs, data acquisition in the form of percentage degradation of tph levels, and ph changes were analyzed by statistical test.

RESULTS AND DISCUSSION

Decrease in Total Petroleum Hydrocarbon (TPH) levels

The measurement of TPH levels is to determine the percentage of hydrocarbon levels remaining in polluted soil after the bioremediation process is carried out. According to Alkatiri (2017), hydrocarbon degradation involves microorganisms utilizing hydrocarbon compounds as a source of carbon and non-hydrocarbon compounds as an additional source of nutrients, this process is known as petroleum hydrocarbon biodegradation. From the results of the TPH reduction test that has been carried out with 3 repeats, overall in each treatment showing different results in reducing TPH levels can be seen in the table.

Table 1. The result of reducing TPH levels in soil contaminated by palm oil mill waste.

Time (Days)	P0	P1	P2	P3	P4	Quality Standards
0	18,40±0,20 ^c	18,53±0,30 ^c	17,47±0,30 ^c	17,13±0,64 ^d	15,73±0,70 ^c	Ministry of Environment and Forestry of the Republic of Indonesia No.128 Thn 2003 (1%)
7	16,20±0,69 ^d	13,06±0,75 ^d	11,00±0,80 ^d	9,40±1,21 ^c	6,73±0,50 ^b	
14	14,13±0,61 ^c	11,06±0,61 ^c	9,40±1,00 ^c	8,20±0,52 ^{bc}	6,33±0,41 ^b	
21	12,20±0,69 ^b	9,60±0,34 ^b	7,53±0,80 ^b	7,53±0,64 ^b	4,80±0,52 ^a	
28	10,20±0,52 ^a	8,20±0,40 ^a	6,26±0,11 ^a	5,66±0,30 ^a	3,80±1,11 ^a	

Based on the data table above, it shows that the value of TPH levels continues to decrease along with the length of the remediation time. It can be seen that the decrease in TPH on day 7 in the P0, P1 and P2 treatment has a high level of value, in the P3 treatment and P4 treatment shows that the potential for a decrease in TPH value levels has begun to increase. In P4 treatment, the optimization of *the fungus Trichoderma sp.* With the addition of a combined 35 grams of rice husk stimulant

and 100 grams of cow manure as a biostimulant, with a remediation time of 28 days has a TPH value of 3.80%. This shows that if the optimization of *the fungus Trichoderma sp.* In treatment with the addition of a combination of rice husk biostimulants and cow manure, it has reached the optimal limit in reducing TPH levels contained in samples of contaminated soil from palm oil mill waste

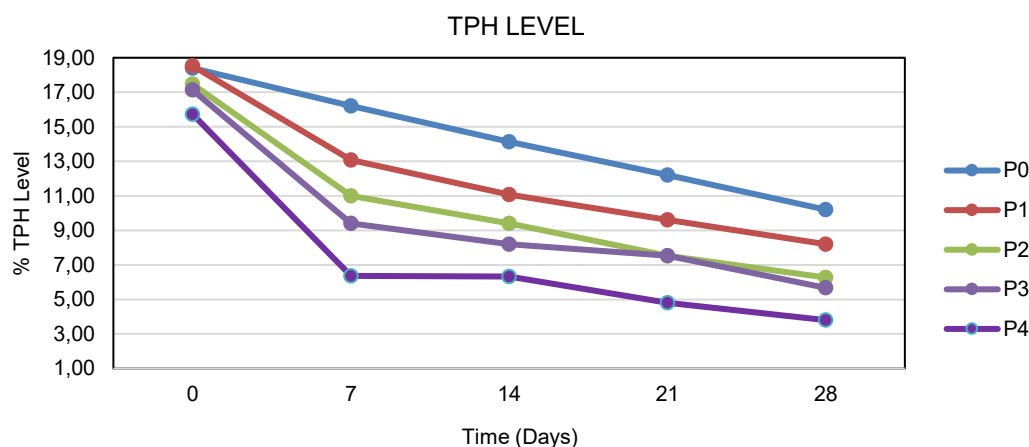


Figure 1. Graph of the percentage value of TPH levels.

Microorganisms not only play a role as nutrients in the degradation of hydrocarbon chains, but they can also produce enzymes that degrade them. All microorganisms carry out metabolism throughout their life cycle, which is always related to enzyme activity. This is in line with the view of Susanti (2017), who explains that hydrocarbon reduction results from the degradation of hydrocarbon chains through bacterial enzymatic reactions. Most bacteria produce the enzyme oxygenase that degrades hydrocarbons and converts them into water (H₂O) and carbon dioxide (CO₂), utilizing them as electron donors. Based on research conducted by Marlina (2021), TPH levels continue to decline as the composting time increases. However, the decrease in TPH levels during

the first two days indicates early adaptation to the new environment and is not significant.

Effect of adding biostimulants on the effectiveness of reducing TPH levels

The increase in the effectiveness of TPH degradation in each treatment is due to the biological response of the fungus, which is characterized by the use of substrates and the addition of biostimulants in the form of cow manure which acts as an increase in porosity and also rice husks which function as aeration. In contrast, the effectiveness of TPH degradation in control treatments is very low, due to the natural decrease in TPH concentrations caused by the biological response of soil microorganisms.

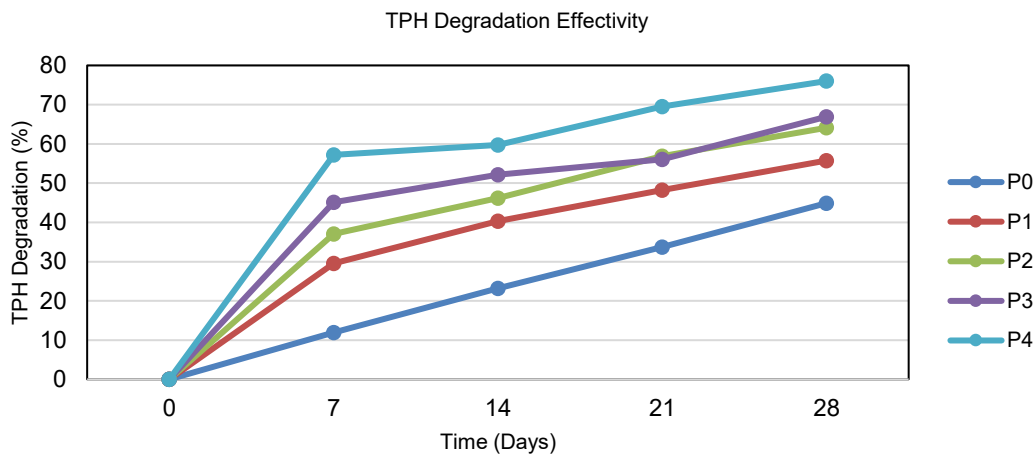
Table 2. The effectiveness of TPH degradation on contaminated soil from palm oil mill waste.

Time (Days)	P0	P1	P2	P3	P4
0	00,00±0,00 ^a	00,00±0,00 ^a	00,00±0,00 ^a	00,00±0,00 ^a	00,00±0,00 ^a
7	11,97±2,99 ^b	29,52±3,11 ^b	37,05±3,50 ^b	45,13±6,68 ^b	57,20±2,40 ^b
14	23,18±3,15 ^c	40,30±2,61 ^c	46,23±4,80 ^c	52,16±1,54 ^c	59,71±2,62 ^b
21	33,71±3,18 ^d	48,21±1,10 ^d	56,86±4,57 ^d	56,08±2,14 ^c	69,53±2,46 ^c
28	44,90±2,05 ^c	55,76±1,77 ^c	64,11±0,21 ^c	66,92±1,34 ^d	76,02±6,07 ^d

In the data table above, observations on day 7 can be seen a decrease in TPH in each treatment, with the percentage of degradation continuing to increase with the increase in time and the administration of stimulants in each treatment. Degraded TPH that is less than optimal during the bioremediation process takes place on contaminated soils can be caused by a lack of nutrients in the soil and several other factors.

The effectiveness of TPH degradation in each treatment shows the potential of each one in reducing TPH levels. The P0 treatment, which is a control

treatment, showed a decrease in TPH levels in polluted soil with TPH degradation of 44.90% and final TPH levels of 10.20%. This can occur because it is suspected that in the soil polluted by oil palm mill waste there are microorganisms that have the potential to degrade hydrocarbons, so that the bioremediation process in the P0 treatment can occur. In Marlina's (2021) study, indigenous microbes in oil-contaminated soil have the ability to degrade TPH levels even without the application of animal manure.

**Figure 2.** TPH degradation percentage graph.

Based on the graph of the percentage degradation of TPH levels, it shows the potential degradation rate of each treatment for each treatment. In each treatment, there was an increase in the degradation rate of TPH levels, it can be seen that the percentage of degradation of TPH levels in soil contaminated by oil palm mill waste. In the P1 treatment without the addition of biostimulants, the percentage of TPH degradation was 55.76% with a TPH level of 8.20%. In the P2 treatment with the concentration of 35 grams of rice husk stimulant supplement, it showed a percentage of TPH degradation of 64.11% with a TPH level of 6.26%. In the P3 treatment with the concentration of 100 grams of cow manure stimulant, it showed a percentage of TPH

degradation of 66.92% with a TPH level of 5.66%. P4 treatment with a concentration in the form of adding a combined stimulant of 35 grams of rice husk and 100 grams of cow manure had a percentage of TPH degradation of 76.02% with a TPH level of 3.80%.

Optimization of the fungus *Trichoderma* sp. With the addition of biostimulants to each treatment, it shows the potential to reduce TPH levels. Based on research that has been conducted by Rizky (2024), namely by adding 35 grams of rice husk as a stimulant, and previous research conducted by Marlina (2021) by adding 100 grams of cow manure as a stimulant, then by adding the two amounts of stimulants in optimizing the *Trichoderma* fungus Sp. The decrease in TPH levels in

polluted soils from palm oil mills showed a TPH degradation of 76.02% with a final TPH level of 3.80%. It is suspected that by adding these two stimulants it is able to support higher activity of the fungus *Trichoderma* sp. in its metabolism so that during the bioremediation process, the TPH levels contained in the contaminated soil of oil palm mill waste can be degraded for the better.

Rice husks contain fiber, lignin and several nutrients that serve as a source of carbon in microorganisms. According to Wagiono (2022), the use of rice husks functions as an organic material to improve aeration and soil moisture retention as well as a container for decomposing microbes, microorganisms stimulated by rice husks will use hydrocarbon compounds as an energy source and break down TPH into simpler and non-toxic compounds.

While cow dung is also very useful as a stimulant, the addition of cow manure can provide a source of nutrients for *Trichoderma* sp. because of its nitrogen and phosphorus content. Increased nutrient availability promotes the stimulation of fungal metabolism. Therefore, the more cow manure is added, the higher the nitrogen and phosphorus levels.

Soil NPK test results

NPK level testing in the research sample was carried out on day 0 or the initial condition of the soil sample before being treated and the 28th day, which is the condition after the bioremediation process was carried out. The NPK level examination was carried out at the Testing Laboratory of the North Sumatra Agricultural Instrument Standard Implementation Center.

Table 3. Results of soil NPK level analysis.

Time (Days)	Sample Code	TPH Level (%)	Types of Analysis		
			N-total (%)	P-Bray I (ppm P)	K-dd (me/100g)
0	Kondisi awal	18,40	1,17	8,37	3,14
28	P0	10,20	1,16	39,35	7,39
	P1	8,20	1,36	38,68	5,66
	P2	6,26	1,43	36,73	5,81
	P3	5,66	1,35	40,55	9,04
	P4	3,80	1,24	38,71	9,51
Test method			IK 0.1. 6.0 (Kjeldahl)	IK 0.1. 7.0 (Spectrofotometry)	IK 0.1. 8.0 (AAS)

The N-total analysis carried out by the kjeldahl test method aims to see the organic nitrogen content and nitrogen contained in ammonia. Based on SNI 6250:2021, nitrogen levels with very high criteria are >0.75%. It can be seen in the data table that the N-total level at the beginning of the observation has a level of around 1.17%. After going through the bioremediation process, the N-total level experienced a different increase in each treatment, the highest N-total level increased occurred in the P2 treatment of 1.43% which fell into the very high criteria, namely where this treatment was a treatment with the addition of rice husk stimulants. It is suspected that the increase in nitrogen levels occurs due to the activity of microorganisms that convert ammonium into nitrate and are able to increase the nitrogen element or can be referred to as the nitrification process. The increase in nitrogen nutrients in the soil is caused by the decomposition of soil organic matter caused by the increase in the development of microorganisms (Indis, 2022).

Based on SNI 6250:2021, the phosphorus level with a very high criterion is >15 ppm. In the data table, it can be seen that all treatments have very high phosphorus levels after being treated, and the highest phosphorus level is

found in the P3 treatment, which is 40.55 ppm, which in this treatment is a treatment with the addition of stimulants in the form of cow dung. Cow manure rich in nitrogen, phosphorus and potassium which is an important element for *the fungus Trichoderma* sp. in stimulating the growth metabolism of fungi. According to Naim (2024), phosphorus is an essential nutrient for the growth of microbial cells and plays an important role in the decomposition of organic matter and phosphorus absorption. The decomposition of organic matter and the absorption of phosphorus are driven by phosphatase enzymes produced by certain microorganisms. In polluted soils that experience a decrease in TPH levels and an increase in phosphorus nutrient levels, it can increase soil fertility and make the soil able to encourage plant growth and root development in the soil (Maulidan, 2024).

In the data table, it can be seen that the highest potassium level is found in the P4 treatment, which is around 9.51 me/100g which in this treatment is a treatment with the addition of stimulants in the form of rice husks and cow dung. Rice husk which acts as organic matter and cow manure which has a high nutrient content, one of which is in the form of potassium

nutrients can stimulate the metabolism of *the fungus Trichoderma* sp. and makes the nutrient content of potassium increase. Based on research conducted by Naim (2024), Potassium is available as a catalyst for microbes during various stages of composting. Its properties can affect the rate of decomposition. Once decomposition continues, potassium is available again. The occurrence of a decrease in TPH levels and an increase in potassium nutrients in polluted soil can restore soil quality to physiological processes, nitrogen metabolism, protein synthesis and can activate various enzymes (Trisnawati, 2022).

Soil pH test results

Based on the Food Security and Agriculture Service, fertile or productive soil has a neutral optimal ph of 6.5

to 7.5. In neutral ph conditions in the soil, the nutrient content will be high and the soil fertility will be in good condition (Purba, 2021). Meanwhile, the optimal ph in reducing TPH levels in the soil is close to neutral. Based on research conducted by Dewi (2023), the ph in reducing TPH levels is in the optimal range of 7.5 to 8.

It can be seen in table 4 at the initial pH, which is 7 where the pH is neutral, then after treatment and given the addition of stimulants and going through the bioremediation process, there is a decrease, where on the 7th day of each treatment the pH decreases. In the following days the soil pH continued to decline albeit less significantly, indicating that microbes began to adapt to their environment and began to work to break the hydrocarbon chain.

Table 4. pH value in contaminated soil from palm oil mill waste.

Time (Days)	pH				
	P0	P1	P2	P3	P4
0	7	7	7	7	7
7	6,87	6,80	6,73	6,27	6,13
14	6,73	6,47	6,33	5,73	5,60
21	6,67	6,27	6,13	5,33	5,13
28	6,47	6,13	5,87	5,07	5

Growth of the fungus *Trichoderma* sp. is influenced by several factors, one of which is ph, based on research conducted by Singh (2014) shows that the optimal growth of *Trichoderma* sp. It occurs in the ph range of 5.0 to 7.0. As time goes on, the pH continues to decrease where there is a significant decrease in P4 treatment, namely with the addition of rice husk stimulants and cow dung, this is in accordance with the optimal ph of *Trichoderma* sp growth. Which shows that the growth of the fungus *Trichoderma* sp. The P4 treatment has reached the optimal limit.

CONCLUSIONS

Based on the results of research that has been carried out in reducing tph (total petroleum hydrocarbons) levels for 28 days on soil contaminated by palm oil mill waste, it can be concluded that the optimization of the fungus *trichoderma* sp. in each treatment showed a decrease in tph levels. In the treatment without the addition of stimulants, it reduced tph levels by 55.76%, in the treatment of adding 35 grams of rice husk stimulant reduced tph levels by 64.11%, the treatment of adding 100 grams of cow manure stimulant reduced tph levels by 66.92%, then in the second treatment the amount of stimulants in the form of 35 grams of rice husks and 100

grams of cow manure showed a decrease in tph levels of 76.02% with a final tph level value of 3.80%. Follow-up research is recommended to add a longer duration in observing a decrease in tph levels, test other parameters in lowering tph levels, use the same concentrations in previous studies, and the need to use other concentrations as biostimulants.

Competing Interests: The authors declare that there are no competing interests.

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