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Hydrocarbons Bioremediation Soil Using Eschericia Coli (E. Coli) And Klebsiella SP Bacteria With The Addition Of Inorganic Fertilizers As Nutrients

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Abstract: This research studies the effectiveness of TPH bioremediation in oil-exposed soils using E. coli and Klebsiella sp with the addition of urea fertilizer, NPK-Mg 12-12-17 + 2 (N; P; K; Mg = 12%; 12%; 12 %; 2%) and NPK 15-15-15 (N; P; K = 15%; 15%; 15%). The bioremediation process lasts for 3-27 days and during the bioremediation process the TPH level is checked every 72 hours using the InfraCal TPH Analyzer. The results showed that there was an influence on the use of inorganic fertilizers on the length of the bioremediation process, this is because inorganic fertilizers such as urea fertilizer, NPK 12-12-17 + 2 and NPK 15-15-15 contain nutrients needed by bacteria during the bioremediation process so that the bacteria grows optimally. Inorganic fertilizers also affect soil conditions which can cause the bioremediation process to take place faster. The data of this study indicated that the optimal bioremediation conditions were achieved at the highest TPH concentration compared to other TPH concentration variations. This was because pollutants are also a source of carbon for bacterial growth, if the composition of carbon, nitrogen, phosphorus and potassium is available in the appropriate amount for bacterial growth, then bacteria can optimally conduct TPH bioremediation. However, for certain conditions the achievement of bioremediation was achieved in a short time, such as in the bioremediation of 2.21% TPH using E. coli bacteria and NPK 15-15-15 as much as 0.50 grams, the process of decreasing the lowest TPH levels on the 6th day, as well as the use of Klebsiella sp and NPK 12-12-17 + 2 as much as 0.5 grams, a decrease in TPH concentration of 2.21% was reached on the 6th day. This shows that under given conditions, bioremediation is only efficient during the first week. The results of this experiment show the importance of maintaining bacterial biomass by maintaining soil pH and humidity conditions to ensure high bioremediation efficiency. Kinetic form of TPH bioremediation rate in soil concentrations of 8.85%at optimal conditions using E. coli bacteria and the addition of NPK 15-15-15 as much as 0.50 grams, giving a bioremediation rate constant of 0.0560 days^{-1} with a half-life of 12 days, whereas if using the Klebsiella sp bacteria obtained optimal conditions by using NPK fertilizer 12-12-17 + 2 as much as 0.50 grams and the value of the bioremediation rate constant given is 0.0420 days^{-1} with a half-life of 17 days.

Index Terms: Bioremediation, TPH, E.coli, Klebsiella sp, Kinetics Bioremediation, Urea Fertilizers, NPK.

1. INTRODUCTION

Soil pollution by oil spills become a hot topic nowadays. Land contaminated with oil / petroleum has serious hazards to human health, causing organic pollution from ground water that limits its use, causes economic losses, environmental problems, and decreases the productivity of soil agriculture [1]. The content of oil pollution in the environment is commonly referred to as Total Petroleum Hydrocarbons (TPH)).Total petroleum hydrocarbons (TPH) are the measured amounts of hydrocarbons in petroleum in the environment. Petroleum contains various different types of chemicals. Chemicals included in TPH include hexane, benzene, toluene, xylene, naphthalene, and fluorene, other elements of gasoline, jet fuel, and other oil products. The Government of Indonesia provides opportunities for the wider community to manage the TPH waste [2]. The basis of TPH management in Indonesia, RI concentration of TPH is less than 15%. However, if the T PH concentration exceeds 15%, it must first be made use of existing technology.

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Land pollution can be overcome by various remediation efforts, including chemical, physical and biological methods. At present, the biological method is the most preferred method of handling polluted soils because the process is safer, efficient and environmentally friendly. The tainted soil processing technique used in this study is a biology method with bioremediation techniques [3].The principle of hydrocarbon bioremediation is the use of various types of microorganisms that have an enzymatic ability to degrade petroleum hydrocarbons.Bacteria are the best agents in the bioremediation process of petroleum in the environment when compared to fungi, algae and yeast. Crude oil will be used as a source of carbon and energy by bacteria needed for its growth. The bacteria used in this study are E. coli and Klebsiella sp. Bacteria are hydrocarbon clastic or petrorilic bacteria which have the ability to degrade hydrocarbon compounds for their metabolic and developmental needs. These bacteria work to degrade TPH in the soil under aerobic conditions. The main principle of the hydrocarbon bioremediation mechanism by bacteria aerobically can be seen in Figure 1 [4]. Microorganisms commonly used to degrade hydrocarbons are the types of bacteria Klebsiella, Proteus, Bacillus, Escherichia, Pseudomonas, Streptomyces, Nocardia, Seratia, Xanthomonas, Micrococcus and fungi from Rhizopus, Fusarium, Pencillium, Pseudomonas, Streptomyces, Nocardia, Seratia, Xanthomonas, Micrococcus and fungi from Rhizopus, Fusarium, Pencillium, Cladosporium and Aspergillus. Nutrients needed by microorganisms for growth are carbon, nitrogen, non-metallic elements (sulfur, phosphorus), metal elements (Ca^{2+}, Zn^{2+}, Na^{+}, K^{+}, Cu^{2+}, Mg^{2+}, $Mn^{2+}$and $Fe^{2+/3+}$), water, energy and vitamins. Bacteria need food sources that contain C, H, O and N which are useful for making protoplasm [4].Several studies have succeeded in bioremediation of hydrocarbon-contaminated soils using Spirillum, Basilus, and Micrococcus bacteria stimulated with compost. Obtained the results of levels of hydrocarbon pollutants in the soil can be reduced by 84% for 30 days [5]. In other studies a bioremediation process for hydrocarbon polluted soils has been carried out by biostimulation in which organic components such as animal waste (goats, cattle) are used to increase the number of native microbes in the soil that directly degrade contaminants. TPH reduction in soil by 26% with the addition of cow dung, while with the addition of goat manure TPH decreased by 23% [6].The content of compost is the same as inorganic fertilizers such as urea and NPK. The content of macro elements such as nitrogen, phosphorus and potassium in inorganic fertilizers is more than in organic fertilizer, but the types of elements that become bacterial nutrients are more diverse in organic fertilizers than in inorganic fertilizers. This study will compare the effectiveness of Escherichia coli (E. coli) and Klebsiella sp to degrade pollutant hydrocarbons in the soil by adding 3 types of inorganic fertilizers with different nutrient composition, namely urea fertilizer (containing 46% Nitrogen), NPK-Mg fertilizer (containing 12 % Nitrogen, 12% Phosphorus, 17% Potassium and 2% Magnesium) and NPK fertilizer 15-15-15 (containing 15% Nitrogen, 15% Phosphorus and 15% Potassium) as bulk agents and additional sources of nutrients. This research uses kinetic analysis to understand the bioremediation process, bioremediation speed measurement and the development of hydrocarbon bioremediation methods in the soil to make it more efficient in its implementation.

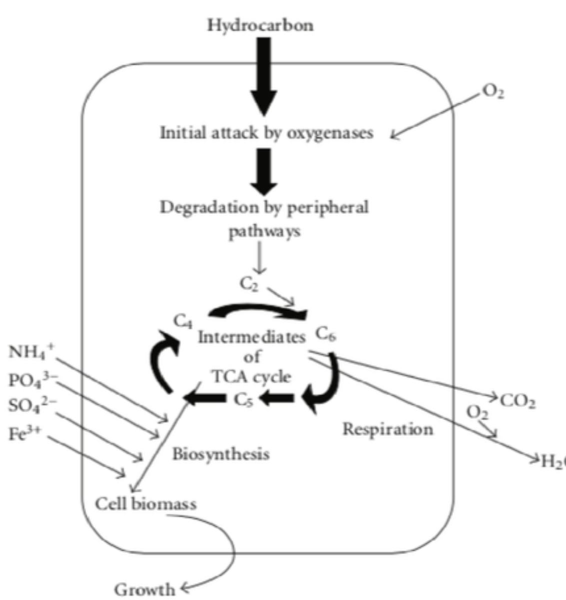

***Figure 1**. Aerobic mechanism of hydrocarbon bioremediation by bacteria*

2 MATERIAL AND METHODS

2.1 Sampling Technique of Soil

The soil is taken from a depth of 30 cm, then filtered using a 2 mm diameter sieve mesh to remove rocks, wood particles and other impurities. The soil is sterilized in a special container and stored at 10 ° C that the humidity is maintained.

2.2 Making Bacteria Media

The bacterial cultures used in this study were E. coli and Klebsiella sp. The nutrient broth (NB) powder was weighed as much as 8 grams, dissolved in 1 L of demin water. The solution was stirred using a magnetic stirer while heated until all NB powder was completely dissolved and the color of the solution turned to clear. The media was sterilized in an autoclave at 121 ° C for 20 minutes.

2.3 Bacterial Rejuvenation

Bacterial rejuvenation was carried out aiming to get active bacteria, because previously the bacteria were in the refrigerator with inactive bacterial conditions. The method included 100 mL of NB solution poured into 250 mL erlenmeyer flask with 2 pumpkins, then 1 ose of organism was added to each of the erlenmeyer flask (pumpkin 1 added 1 ose of E. coli bacteria and 2 pumpkin added 1 ounce of Klebsiella sp bacteria). The mixture was stirred slowly then incubated at 37 ° C and measured the number of bacteria after 24 hours.

2.4 Calculation of the Total of Bacteria (Total Plate Count/TPC Method)

The bacterial solution was piped as much as 0.4 mL dissolved in 1000 mL of demin water in a measuring flask, stirring slowly. The solution was poured as much as 1 mL into 20 mL of PCA medium in a petri dish by spread technique, then flattened and incubated at 37^{o}C for 24 hours. The number of colonies growing on the PCA medium was observed and counted using a colony counter haemocytometer with a Colony Forming Unit or colony forming unit. (CFU/mL).

2.5 TPH Bioremediation in the Soil

Soil weighed as much as 10 grams into 60 cups, oil was added with variations (0.25; 0.50; 1.00) mL, stirred until homogeneous, then from each variation of oil added a bacterial solution of 0.4 mL (variation of E coli and Klebsiella sp bacteria). To know the effect of variations of inorganic fertilizers, from each variation 3 different types of fertilizers are given, namely urea fertilizer variations (0.25; 0.50; 1.00) grams, NPK 12-12-17 + 2 variations (0.25 ; 0.50; 1.00) grams and NPK 15-15-15 variations (0.25; 0.50; 1.00) grams. TPH testing was carried out every 72 hours using InfraCal TPH analyzer.

2.6 Determination of TPH

Infra Red Method - Calibration curves were made from standard b-heavy oil solutions with concentrations (0; 20; 100; 250; 500; 1000) mg / L using n-hexane solvent. Absorbance readings were carried out with InfraCal TPH Analyzer, and linear regression curves were made to obtain the curve equation.Sample was weighted± 0,1 g into a closed test tube, then added 20 mL n-hexane, shook for 2 minutes, then filtered with whatman filter paper No. 40 containing Na2SO4 powder to remove water particles in the sample. The solution that passed the filter paper was put into a vial for analysis. The prepared sample was injected on a cell plate with a 50 µL syringe. Pressing the "RUN" button, waited 3 minutes until the screen showed the results of the TPH content in the sample. For the next sample analysis, the cell plate was cleaned, then the sample analysis was done as above.

2.7 Determination of Bioremediation Half-Life

The first order kinetic equation model mounted to the bioremediation data was used to determine the bioremediation rate of TPH in the soil.

$$\ln c = a + k_1 t \qquad (1)$$

Determination of the constant value was obtained from the linear curve equation of the relationship between the value of ln (C) and the bioremediation time, where C is the substrate concentration. Then the half-life of bioremediation TPH in the soil was calculated using Equation below. Bioremediation rate (k) and half-life ($t_{1/2}$) constants for different bioremediation treatments were determined.

$$t_{1/2} = \frac{\ln 2}{k} \qquad (2)$$

Where the bioremediation constant (per day). The half-life model is based on the assumption that the bioremediation level of hydrocarbons correlates positively with the size of the hydrocarbon area in the soil [7].

3. RESULT AND DISCUSSION

3.1 Determination of the Total of Bacteria (TPS Method)

Rejuvenated bacteria in the nutrient broth medium contain a number of bacteria which was determined using the TPC method. The results of bacterial calculations showed that in 0.4 mL the E. coli bacterial solution contained 3.8 x 105 CFU / mL, whereas in 0.4 mL the bacterial solution Klebsiella sp contained 6,0 x 10^5 CFU/mL

3.2The Results of TPH Analysis in Bioremediated Soil Using E. coli Bateria

The results of the analysis obtained showed that the magnitude of the reduction in TPH levels in the bioremediation process of TPH in the soil by E. coli bacteria with the addition of different inorganic fertilizers in each variation, in the land exposed to oil by 2.21% gave the most TPH reduction with the addition of urea fertilizer as much as 1 gram, where TPH bioremediation was obtained at 78.73% with the result of a decrease in TPH levels from 2.21% to 0.47%. The TPH bioremediation process in oil-exposed soils by 4.43% gave the most TPH reduction by adding 1 gram of urea fertilizer, where TPH bioremediation was obtained by 55.76% with the result of a decrease in TPH content from 4.43% to 1.96% . The TPH bioremediation process on oil-exposed soils by 8.85% gave the most TPH reduction with the addition of 0.5 grams of NPK 15-15-15 fertilizer, where TPH bioremediation was obtained at 84.86% with the result of a decrease in TPH content of 8, 85% to 1.34%.

3.3 The Results of TPH Analysis in Bioremediated Soil Using Klebsiella sp Bacteria

The analysis results obtained showed that the reduction in TPH levels in the bioremediation process of TPH in the soil by Klebsiella sp bacteria with the addition of different inorganic fertilizers in each variation, in the land exposed to oil by 2.21% gave the most TPH reduction with the addition of urea fertilizer as much as 1 gram, where TPH bioremediation was obtained by 41.63% with the result of a decrease in TPH levels from 2.21% to 1.29%. The TPH bioremediation process in oil-exposed soils by 4.43% gave the most TPH reduction with the addition of 1 gram of NPK fertilizer 12-12-17 + 2, where TPH bioremediation was obtained by 66.32% with the result of a decrease in TPH content of 4, 43% to 1.49%. The TPH bioremediation process on oil-exposed soils by 8.85% gave the most TPH reduction with the addition of NP0 fertilizer 12-12-17 + 2 by 0.50 grams, where TPH bioremediation was obtained by 82.71% with the result of a decrease in TPH levels from 8.85% to 1.53%.

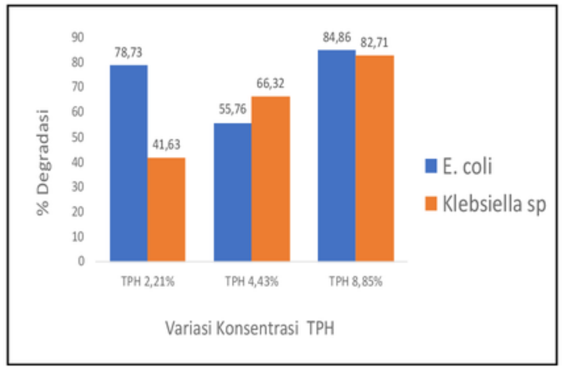

Figure 2. *Optimal Conditions for Bioremediation of TPH in Soil*

The most optimal conditions of the bioremediation process of TPH in the soil can provide a decrease in TPH levels reaching 84.86%, using E. coli bacteria and the addition of NPK 15-15-15 fertilizer

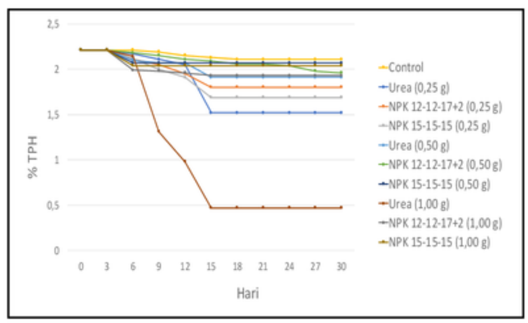

Figure3 . *TPH bioremediation of 2,21% In Soils With E. coli Bacteria With the Addition of Inorganic Fertilizers*

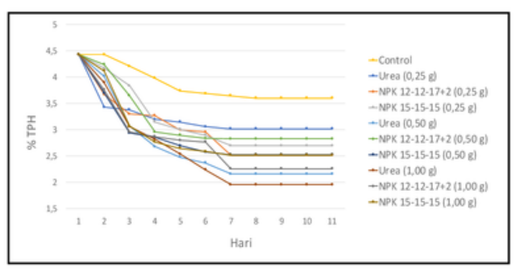

Figure4. *TPH Bioremediation 4.43% In Soil With E. coli Bacteria With Addition of Inorganic Fertilizers*

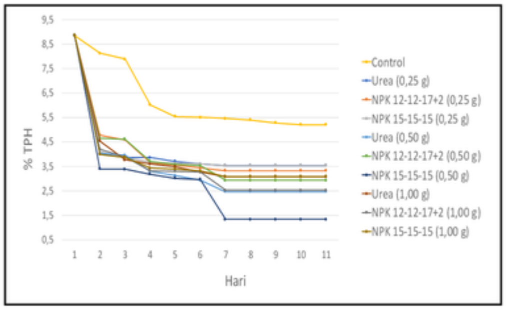

Figure5. *Bioremediation TPH of 8.85% In Soil With E. coliBacteria With the Addition of Inorganic Fertilizer*

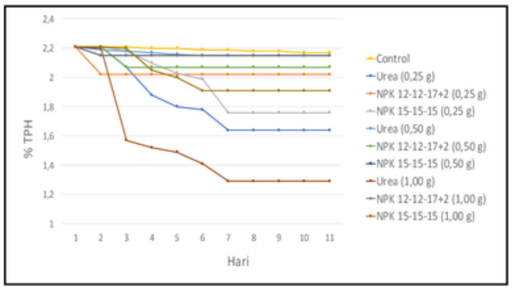

Figure6. *Bioremediation TPH of 2.21% In Soil With Klebsiella spBacteria With the Addition of Inorganic Fertilizer*

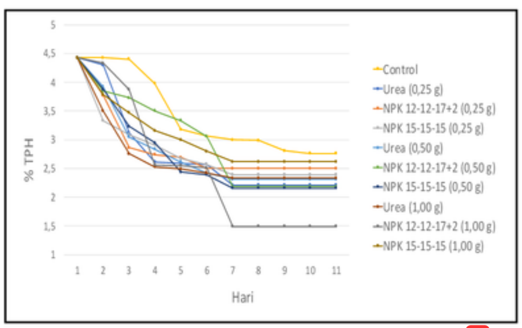

Figure7. *TPH Bioremediation of 4.43% in Soil with Klebsiella sp Bacteria with the Addition of Inorganic Fertilizers*

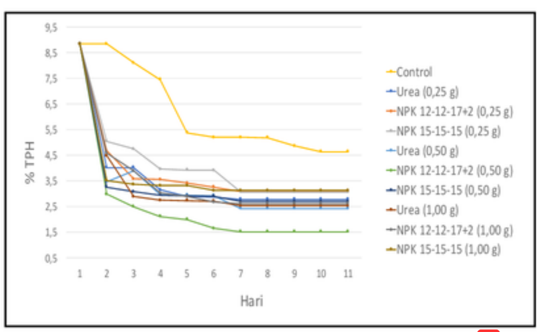

Figure8. *TPH Bioremediation of 8.85% in Soil With Klebsiella sp Bacteria With the Addition of Inorganic Fertilizer*

The addition of inorganic fertilizers, both urea and NPK fertilizers as bulk agents for bacteria, can have a major influence on the bioremediation process, both to speed up the time of bioremediation and to reduce high TPH concentrations. This can be seen in the results of TPH reduction in the control sample for the highest TPH concentration variation (8.85%) which only reached 41.13% in the use of E. coli bacteria and 47.46% in the use of Klebsiella sp. Bacteria, but if added with NPK 15-15-15 fertilizer as much as 0.5 grams reduction in TPH reached 84.86% in the use of E. coli bacteria and 82.71% in the use of Klebsiella sp with the addition of NPK 12-12-17 + 2 as much as 0.5 gram. The bioremediation time was also slower in the control sample which reached 27 days, whereas with the addition of NPK inorganic fertilizers it reached 15 days.Inorganic fertilizers used have various contents. At low TPH concentrations the use of urea fertilizer gave good results in the bioremediation process. Whereas the high TPH concentration of NPK fertilizer was more influential to reduce the TPH concentration in the soil and the most influential type of NPK fertilizer was NPK fertilizer with a balanced ratio between nitrogen, potassium and phosphorus content which were NPK 15-15-15. NPK 15-15-15 fertilizer with a balanced macro content of nitrogen, potassium and phosphorus of 15% each can be an additional nutrient for bacteria to breed. Nutrition is a very important ingredient for the success of bioremediation of hydrocarbon pollutants especially nitrogen, phosphorus, and in some cases requires iron. The addition of NPK fertilizer can trigger the growth and activation of bacterial enzymes [4].The research data showed that the optimal bioremediation conditions were achieved at the highest TPH concentration compared to other TPH concentration variations. This was because pollutants are also a source of carbon for bacterial growth, if the composition of carbon, nitrogen, phosphorus and potassium is available in an amount suitable for bacterial growth, then bacteria can optimally conduct TPH bioremediation. But for certain conditions the achievement of bioremediation was achieved in a short time, such as in the bioremediation of 2.21% TPH using E. coli bacteria and NPK 15-15-15 as much as 0.50 grams, the process of decreasing the lowest TPH levels on the 6th day, as well as the use of Klebsiella sp and NPK 12-12-17 + 2 as much as 0.5 gram, a decrease in TPH concentration of 2.21% was reached on the 6th day. This showed that under given conditions, bioremediation is only efficient during the first week. The results of this experiment show the importance of maintaining bacterial biomass by maintaining soil pH and humidity conditions to ensure high bioremediation efficiency.

3.4 Calculation of TPH Bioremediation Half-life in Soil Using E.coilu Bacteria

The results of the analysis showed that the TPH bioremediation kinetics in oil-exposed soils was 2.21% with the addition of 1 gram of urea fertilizer giving the highest constant value of 0.0644 days^{-1} with the lowest $t_{1/2}$ of 11 days, for TPH concentrations in oil-exposed soil of 4.43% with the addition of 1 gram of urea fertilizer gave the highest constant value of 0.0277 days^{-1} with the lowest $t_{1/2}$ of 25 days, whereas at TPH concentrations in oil-exposed soil of 8, 85% with the addition of NPK 15-15-15 as much as 0.50 grams gave the highest constant value of 0.0560 days-1 with the lowest $t_{1/2}$ of 12 days.

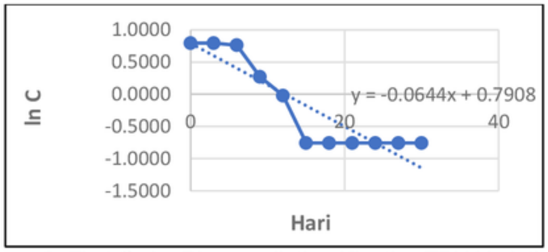

***Figure 9**. First Order Bioremediation Kinetics TPH Variation of 2.21% Using E. coli and urea 1 g*

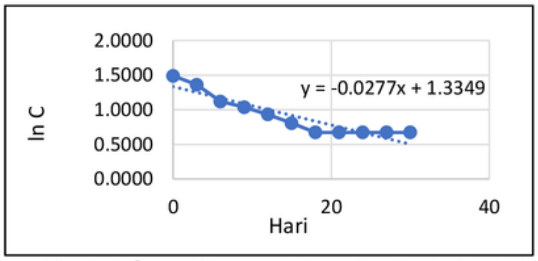

***Figure10**. First Order Bioremediation Kinetics TPH Variation of 4.43%* Using E.coli and urea 1 g

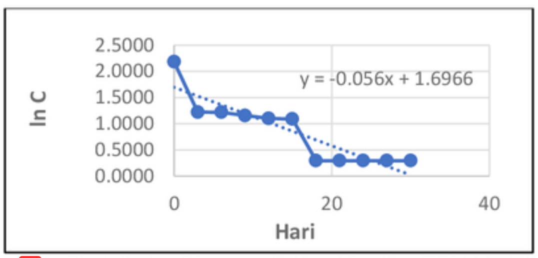

***Figure11**. First Order Bioremediation Kinetics TPH Variation of 8.8%* Using E.coli and NPK 15-15-15 bacteria as much as 0.5 g

3.5Calculation of Contants and Bioremediation Half-life of TPH in Soil Using Klebsiella sp Bacteria

The results of the analysis showed that the kinetics of TPH bioremediation in soil exposed to oil by 2.21% with the addition of 1 gram of urea fertilizer gave the highest constant value of 0.0178 days^{-1}with the lowest $t_{1/2}$of 39 days, for the TPH concentration in the soil exposed to oil by 4.43% with the addition of NPK fertilizer 12-12-17 + 2 as much as 1 gram gave the highest constant value of 0.0430 days $^{-1}$with the lowest $t_{1/2}$of 16 days, while the TPH concentration in the soil exposed to oil was 8.85% with the addition of NPK 12-12-17 + 2 as much as 0.50 grams gave the highest constant value of 0.0420 days $^{-1}$with the lowest $t_{1/2}$of 17 days

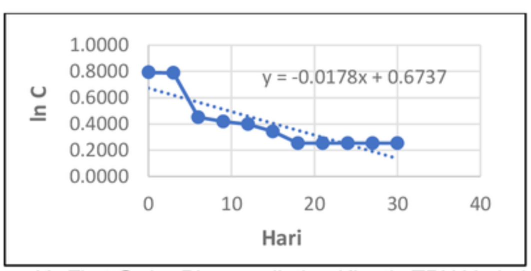

***Figure11**. First Order Bioremediation Kinetic TPH Variation of 2.21%* Using Klebsiella sp Bacteria and Urea 1 g

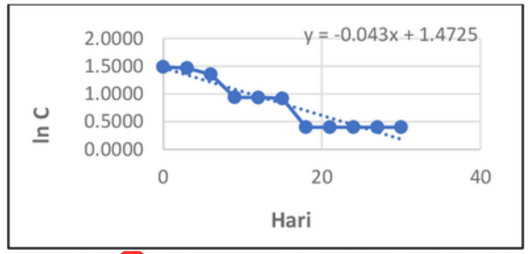

***Figure12**. First Order Bioremediation Kinetics TPH Variation of 4.43%* Using Klebsiella sp Bacteria and NPK 12-12-17 + 2 as much as 1 g

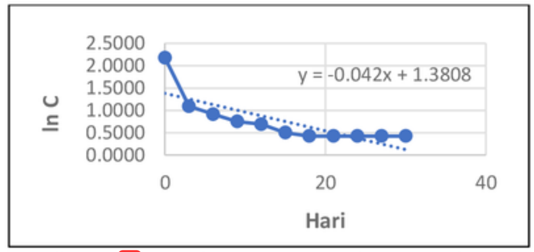

***Figure13**. First Order Bioremediation Kinetics TPH Variation of 8.8%* Using Klebsiella sp Bacteria and NPK 12-12-17 + 2 0.5 g

3.6Soild Conditions Before and After Bioremediation

The results of testing the soil exposed to oil, before and after the bioremediation process are as follows:

Soil Chemical Properties	Unit	Oil Exposed Soil Test Results Before Bioremediation	Evaluation of Quality Standards*	Oil Exposed Soil Test Results After Bioremediation	Evaluation of Quality Standards*
pH (H_2O)	-	4,10	Very sour	7,84	Rather alkalis
pH (KCl)	-	3,36	Sour	6,59	Alkalis
Nitrogen	%	0,11	Low	1,32	Very high
Carbon	%	1,73	Low	5,00	High
P_2O_5available	%	6,28	Low	239,93	Very high
Base Saturation (KB)	%	26,70	Low	82,76	Very high
Soil Texture	-	SL (sandy loam)	-	SL (sandy loam)	-

**source of quality standards (Hardjowigeno, 2015)*
***Table 1.** Evaluation of Soil Test Results*

The data above shows that the condition of the land exposed to oil changes its chemical properties after going through a bioremediation process using E. coli bacteria and the addition of 0.5 grams of NPK 15-15-15 fertilizer, this can be seen from the value of the increase in pH (H_2O), pH (KCl), nitrogen, phosphorus and carbon content in the soil after the bioremediation process. This increase is mainly the nutrient content in the soil due to the addition of NPK 15-15-15 inorganic fertilizers during the bioremediation process. The Base Saturation Value (KB) also increased, indicating that the soil after the bioremediation process became more fertile, because the higher the KB value, the fertility rate also increases. Land with a KB value of 50-80% means it has moderate fertility, if a KB value> 80% means it has a high fertility rate [8], besides the results of the soil test also show that the bioremediation process does not change the soil texture

4.CONCLUSION

E. coli and Klebsiella sp bacteria are bacteria that can degrade TPH in oil-exposed soils. E. coli strain is more effective in degrading TPH in soil concentrations of 8.85% with bioremediation percent reaching 84.86%.Inorganic fertilizers that are most suitable for the TPH bioremediation process in soils that are exposed to oil by 8.85% using E. coli bacteria are NPK 15-15-15 as much as 0.5 grams, while inorganic fertilizers are most suitable for the nutrition of Klebsiella sp bacteria on TPH bioremediation process in the soil with the same concentration of 8.85% is NPK 12-12-17 + 2 fertilizer of 0.5 grams.The kinetics of TPH bioremediation in soil concentrations of 8.85% at optimal conditions using E. coli bacteria and the addition of NPK 15-15-15 fertilizer at 0.50 grams, gave a bioremediation rate constant of 0.0560 $days^{-1}$ with a half-life of 12 days, whereas if using the Klebsiella sp bacteria obtained optimal conditions using NPK 12-12-17 + 2 fertilizer of 0.50 grams and the value of the bioremediation rate constant given at 0.0420 $days^{-1}$ with a half-life of 17 days.The TPH bioremediation process in oil-exposed soils at 8.85% under optimal conditions affects changes in soil chemical composition, an increase in pH (H_2O), pH (KCl), Nitrogen, Phosphorus and Carbon content in the soil after bioremediation processes, but does not change texture on the ground.

5.CONFLICTS OF INTEREST

The authors declare that there are no conflicts of interest regarding the publication of this article.

6.ACKNOWLEDGMENT

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