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Monitoring of Microbial Growth on Crude Oil Remediation using Processed Palm Fruit Fermented Waste Water and NKP Fertilizer

Ukpaka, C.P.[1,]*, Adaobi Stephenie Nwosi-Anele[2]

Abstract

The bacteria count was done to ascertain the microbial growth rate in each reactor and the research work demonstrates the integrity of fermented palm oil processed waste water obtained the available nutrients for the restoration of microbial activity in terms of growth as well as remediation of polluted clay soil environment for optimum utilization in terms of agricultural purposes. This study is based on the overview on the ability of the microbes to degradation the petroleum hydrocarbon and the effect of bio-stimulation on the growth of the bacteria and the fungi inoculated in each bio-reactor. This investigation demonstrates high microbial population of bacteria than fungi as well as revealed that physicochemical parameters played less role in terms of inhibiting characteristics. The growth of the bacteria was more significant in bio-stimulants of NPK fertilizer than the fermented palm oil processed waste water and this trend of characteristics was experienced for fungi as well.

Keywords: Monitoring, microbial growth, crude oil, remediation, processed palm fruit fermented waste water, NKP fertilizer

INTRODUCTION

The degradation of crude oil in soil environment has been investigated by various researchers and no conclusive result on the best bio-stimulant for effective degradation of crude oil has been reported [1–5]. However, continuous research on useful bio-stimulants that less cost effective to achieve the aim and objectives of bioremediation of contaminated soil environment is the interest of engineers and environmentalist [6–8]**.** Having this mind, there is need for continuous studies on other raw material that contains useful nutrient that can enhance microbial growth in crude oil degradation as well as possess the characteristics of withstanding the factors that influences bioremediation process [9–12].

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The application of bio-stimulation in enhancing environmental clean-up is increasing day by day and the technology is classified to the best approach, since the end result yields substance that less harmful to ecosystem [13–15]. Indeed, the technology is welcome today by the great scientists as the best in handling clean-up programmes as well as less cost effective [16, 17]. The restoration of the contaminated clay soil can be useful for agricultural purposes as well as reduce their effect on the underground water pollution in the case of diffusion [18, 19].

The remediation of crude oil using organic and inorganic material has been in use in world to enhance environmental clean-up as well as safe the ecosystem [20]. The action of microbes in the remediation of polluted environment either soil or water has been researched as well as the inhibiting factors [21]. However, there is need for continuous research to improve on the areas of deficiency in order to profound solution and cost reduction while carrying bioremediation program in polluted environment [22]. Research carried on crude oil reveals the significance of organic and inorganic substance useful in enhancing treatment of contaminants [23]. In the treatment process if adequate measures are not taken the active site of the organisms are always inhibited hereby reducing the performance of microorganisms [24]. Inhibition of the active site of the organisms by the action of the environment as well as the toxicity of the contaminant in terms of the possible factors can cause un-competitive, competitive and non-competitive inhibition during bioremediation processes [25]. This research work will address the factors, which include pH, electrical conductivity, moisture content, chemical oxygen demand (COD), biochemical oxygen demand (BOD) etc.

MATERIALS AND METHODS

Microbial Strains the Concept of Isolation and Identification

Sample of crude oil was tested for microbial strain using the methodology of isolation as well as identification: 5g samples of soil are inoculated into 100ml of minimal sact medium containing steam sterilized as carbon source and incubated at 37^{o}C on a rotary shaker for 7 days. The procedures were repeated for 5 times with the aim of 5ml of the nutrient to enhance the media in terms of enriched culture and furthermore, culture of 1ml was diluted to achieve 10^{8}fold. The dilution procedures continuous by taking 100ul and placing it on plated material containing medium of salt media with agar plates in the present of the crude oil using glycerol of 25% at 70^{o}C.

Microbial Strains in Degradation of Petroleum

The procedures entailed the use of 250ml of substrate in flask subjected into 100ml of medium salt media in triplicate in weight of the substrate of 1% as the main source of carbon and the mixture is then introduced into rotary shaker for 24hours and the density of the cell evaluated. Furthermore, the evaluated cell of 109cell ml-1 obtained was inoculated in a medium weight of 5% as inoculum**.** Residual crude is extracted from the cultures by using solvent extraction.

Consortium in terms of Microbial Selection

This concept deals with application of identifying the possible microbes capable of degradation the substrate as the strain characteristics as related to bioremediation programmers.

Total Bacterial Count

Total bacteria count in the soil samples were examined throughout the period of the experiment using serial dilution method.

RESULTS AND DISCUSSION

Results of Microbial Growth

The growth rate of the microbes as isolated and identified are presented in Figures 1 to 20 as demonstrated below in terms of total heterotrophic bacteria and total heterotrophic fungi in the various dosage of the bio-stimulant (Fermented Palm Oil Processed Waste Water and NPK Media).

Figure 1 shows the growth characteristic of THB for 30g dosage of NPK fertilizer in the bioreactor 1 with increase in period of contact. Increase in the population of TPH was experienced with increase in time. However, the increase in microbial population enhanced the TPH degradation in bioreactor 1. The result illustrates that no lag phase was observed, but progressive, stationary and decline phase was experienced.

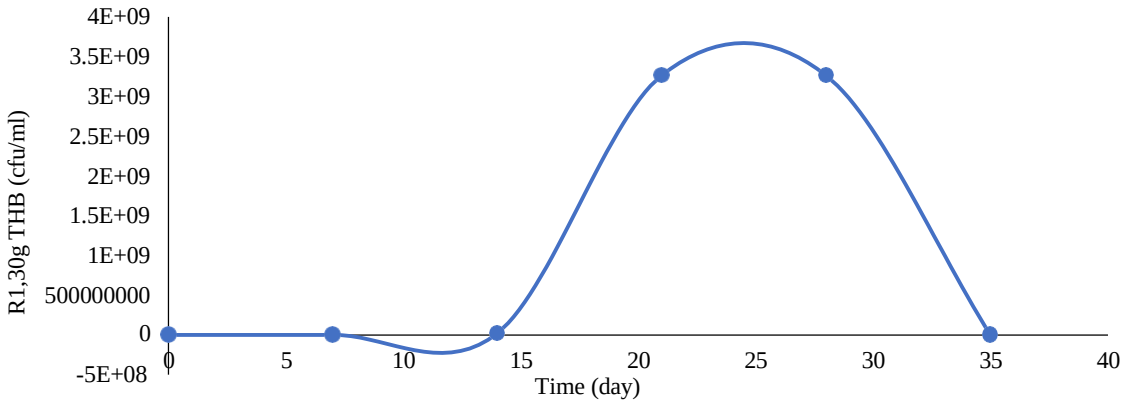

Figure 1. THB concentration against Time for 30g dosage of NPK fertilizer in Bioreactor 1 (R_1).

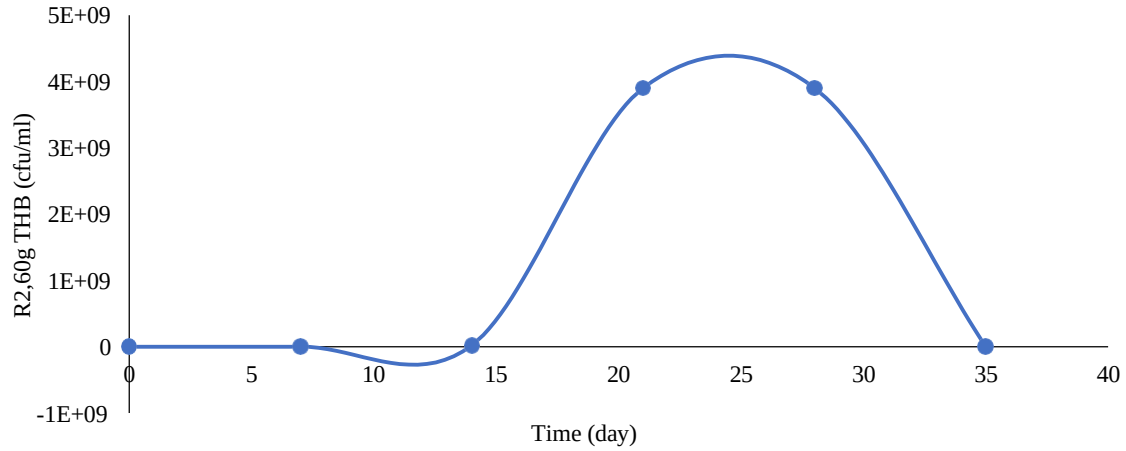

Figure 2. THB Concentration against time for 60g Dosage NPK fertilizer in Bioreactor 2 (R_2).

Figure 2 illustrates the concentration of THB on the action of 60g dosage of NPK fertilizer with increase in the contact time or period of exposure. The THB concentration increased due to increase in the nutrient (NPK) added to the bioreactor. The treatment growth revealed no lag phase but increase in microbial population which described the exponential phase as well as stationary phase and decline. The TPH degradation in the bioreactor (R_2) is faster when compared with bioreactor (R_1) because of special or available nutrient for the THB utilization.

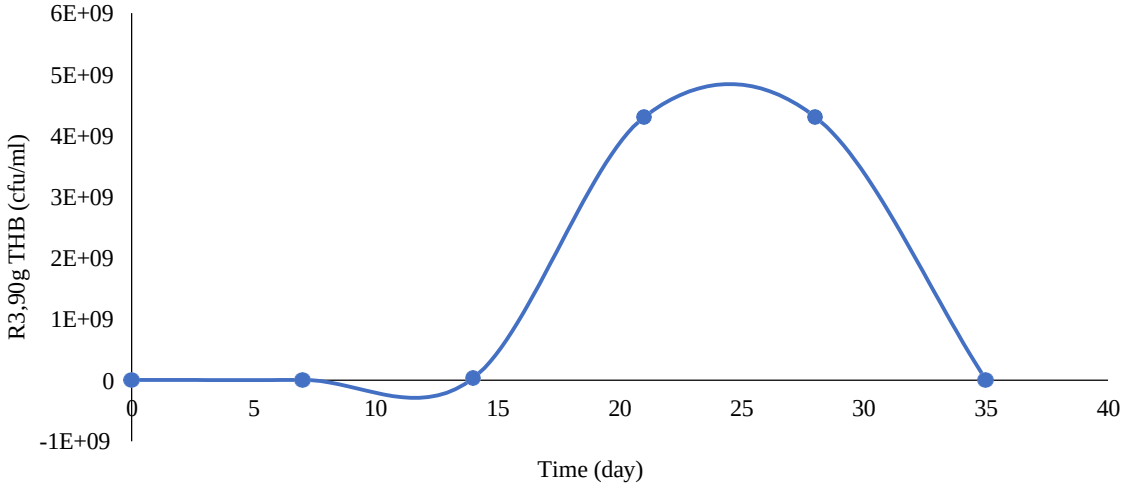

Figure 3. THB concentration against time for 90g Dosage of NPK Fertilizer in Bioreactor 3 (R_3).

Figure 3 shows the effect of contact time or the period of exposure on the THB concentration for 90g dosage of NPK fertilizer. The result obtained demonstrated that the nutrient added aided the growth of the microbes in the bioreactor (R_3) as well as speed up the TPH degradation or remediation in rapid increase in the progressive phase as stationary and death phase also experienced.

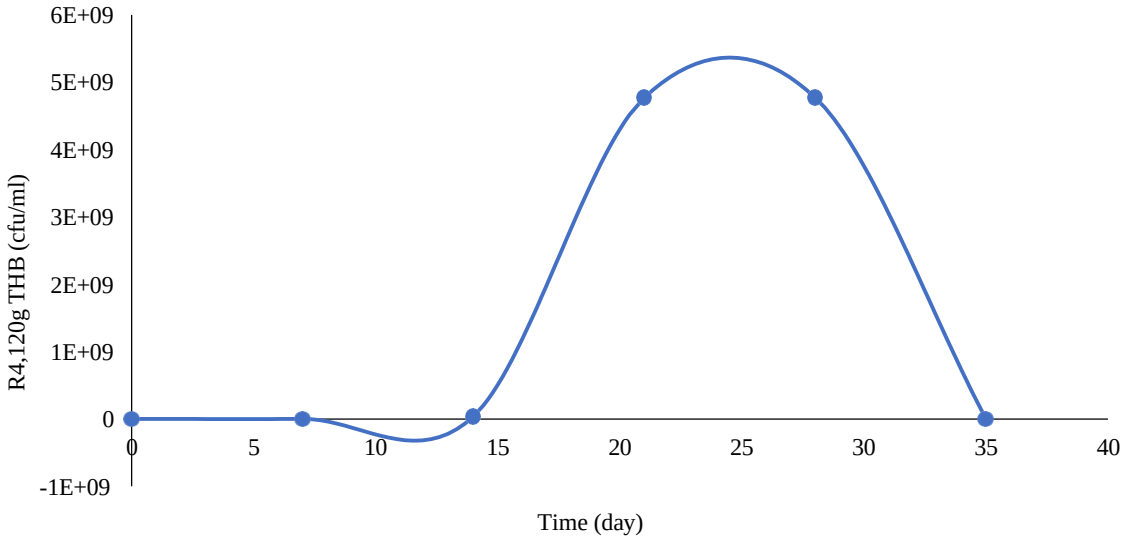

Figure 4. THB Concentration against Time for 120g Dosage of NPK Fertilizer in Bioreactor 4 (R_4)

Figure 4 shows THB concentration with effect of time contact of period of exposure for 120g dosage of NPK fertilizer in the bioreactor (R_4). Indeed, the THB concentration increase rapidly as a result of increase in the available nutrient added. The bioreactor as well as increase in the period of contact. No lag phase experienced but rapid increase in THB concentration at the progressive phase until the stationary and decline phase was experienced.

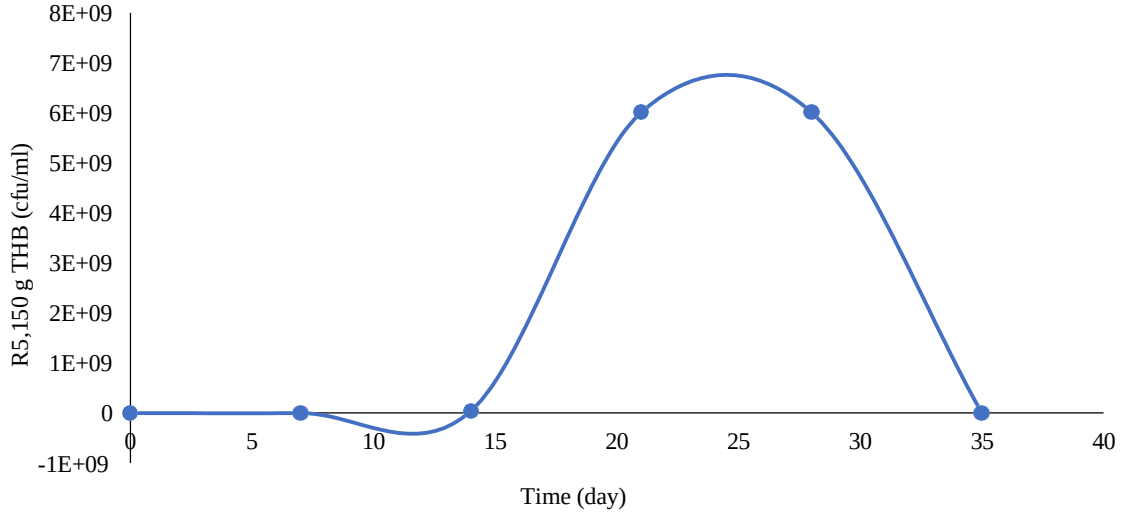

Figure 5. THB Concentration against Time for 150g Dosage of NPK Fertilizer in Bioreactor 5 (R_5)

Figure 5 illustrates the effect of period of exposure and the addition of 150g dosage of NPK fertilizer in the bioreactor (R_5) for crude oil remediation. Increase in THB concentration was observed with increase in period of contact. No lag phase observed rather exponential phase, stationary and decline phase was experienced. The increase of microbial growth of THB as well as degradation of the TPH.

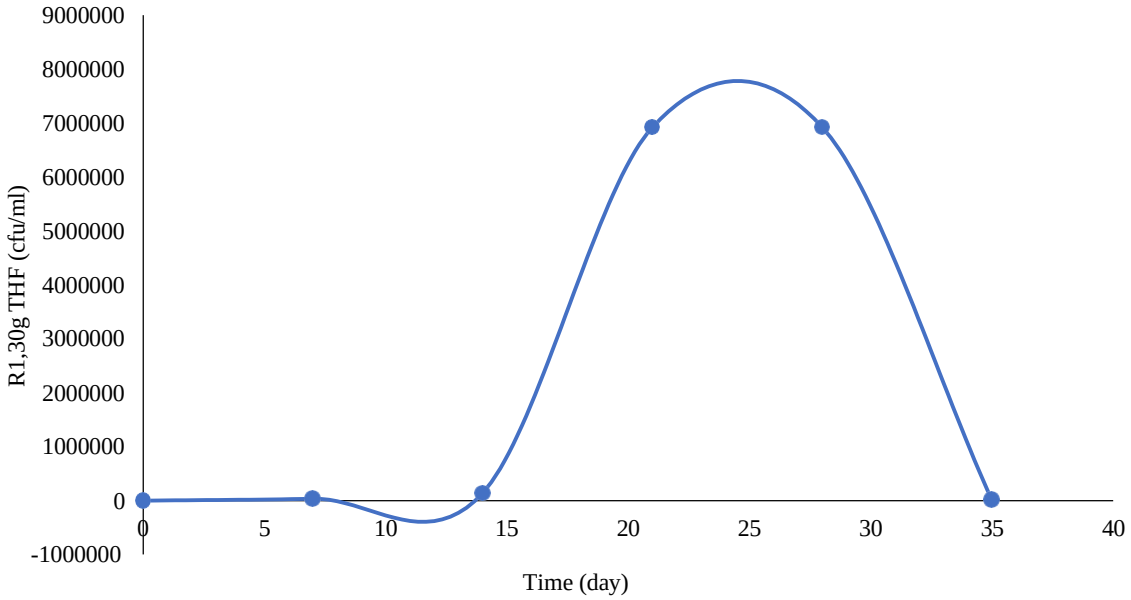

Figure 6. THF Concentration against Time for 30g Dosage of FPFPWW in bioreactor 1 (R_1).

Figure 6 shows the THF growth concentration upon the effect of nutrient dosage 30g of fermented palm fruit processed waste water in bioreactor (R_1). Increase in THF concentration was experienced with increase in period of contact. No lag phase experienced but increase in exponential phase as well as stationary and decline phase observed.

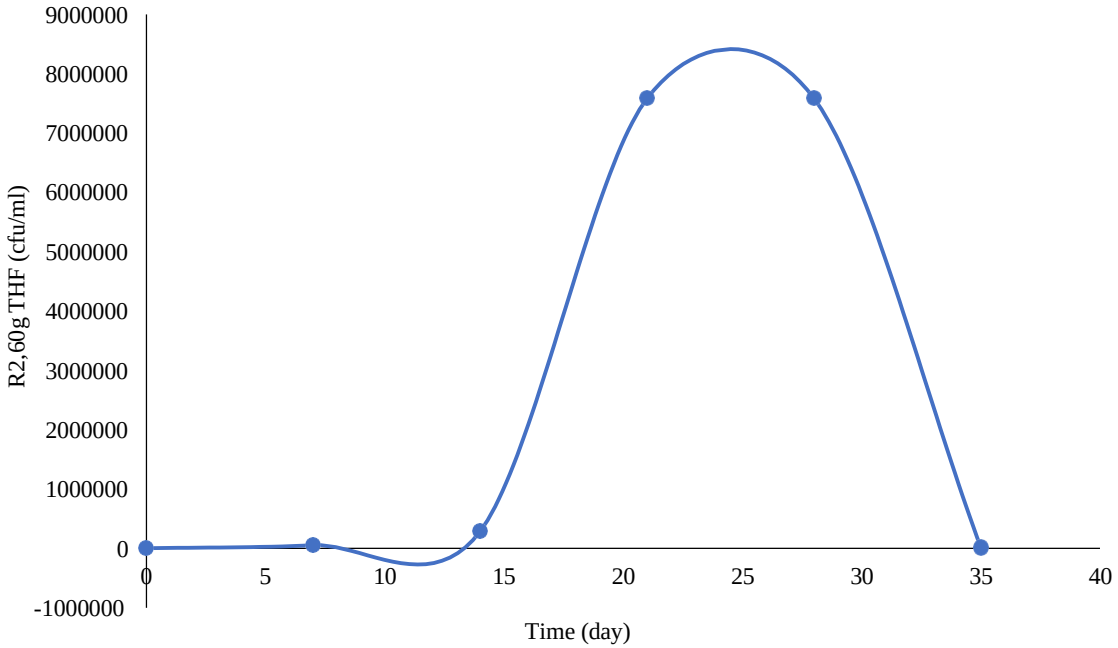

Figure 7. THF concentration against time for 30g dosage of FPFPWW in bioreactor 2 (R_2).

Figure 7 illustrates the effect of contact time on the growth of the THF concentration in the bioreactor (R_2) with the addition of FPFPWW. The microbes used the available nutrient in FPFPWW to facilitate the growth characteristics of the THF in the bioreactor as well as enhanced the TPH degradation for 60g dosage of the biostimulant. Rapid progressive phase experienced with no lag phase and stationary as well as death phase experienced.

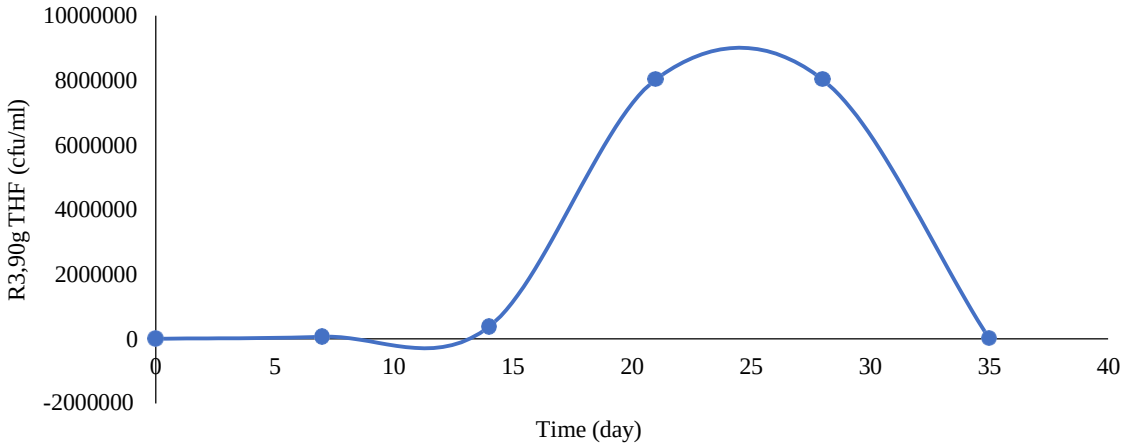

Figure 8. THF Concentration against Time for 90g Dosage of FPFPWW in Bioreactor 3 (R_3).

Figure 8 demonstrated the THF concentration upon the effect of 90g dosage of the FPFPWW and contact time for crude oil degradation in bioreactor (R_3). Increase in THF was experienced with increase in contact time.

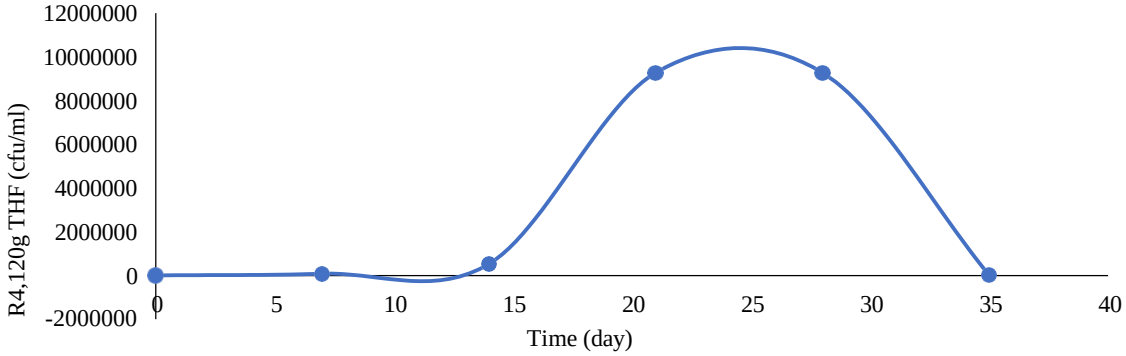

Figure 9. THF concentration against time for 120 dosage of FPFPWW in bioreactor 4 (R_4).

Figure 9 shows the effect of the available nutrient and the contact line on the crude oil remediation using FPFWW. However, the result revealed increase in THF concentration with increase in period of exposure. No lag phase experienced, but progressive, stationary and decline phase was experienced in this research for 120g dosage of the bio-stimulant.

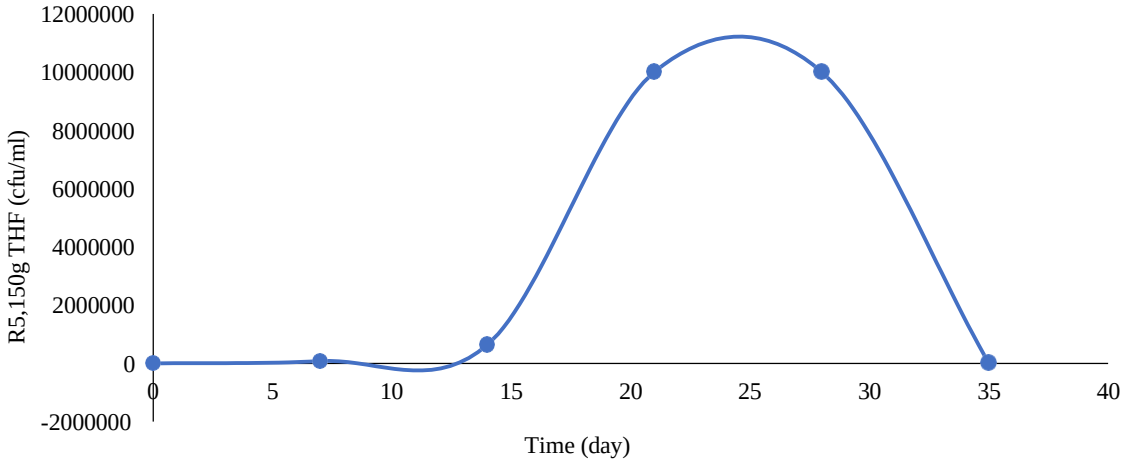

Figure 10. THF concentration against Time for 150 Dosage of FPFPWW in Bioreactor 5 (R_5).

Figure 10 shows the role played by 150g dosage of FPFWW in bioreactor (R_5) in the rapid growth of the THF with increase in time. Indeed, the FPFPWW played the role of biostimulant in enhancing the crude oil degradation as well as the increase in the microbial population of THF in the bioreactor.

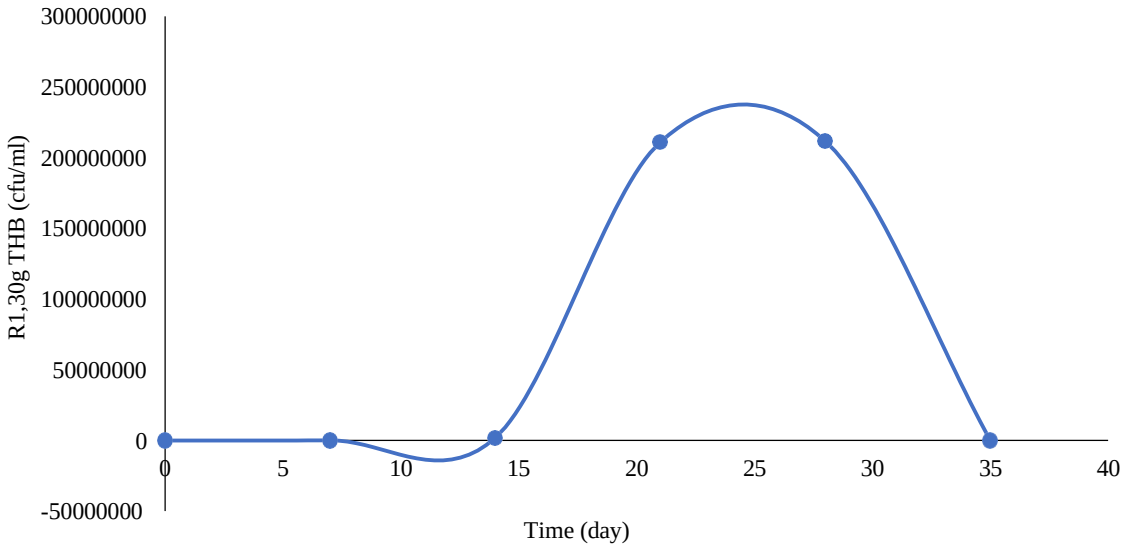

Figure 11. THB Concentration against time for 30g dosage of FPFPWW in bioreactor 1 (R_1).

Figure 11 shows the THB concentration against time for 30g dosage of FPFWW in bioreactor 1 (R_1). No lag phase was experienced rather progressive, stationary and decline phase was observed. This demonstrated that the condition of the environment favours the THB growth rate in the bioreactor as well as speed up the remediation of the TPH. However, increase in THB was observed with increase in time.

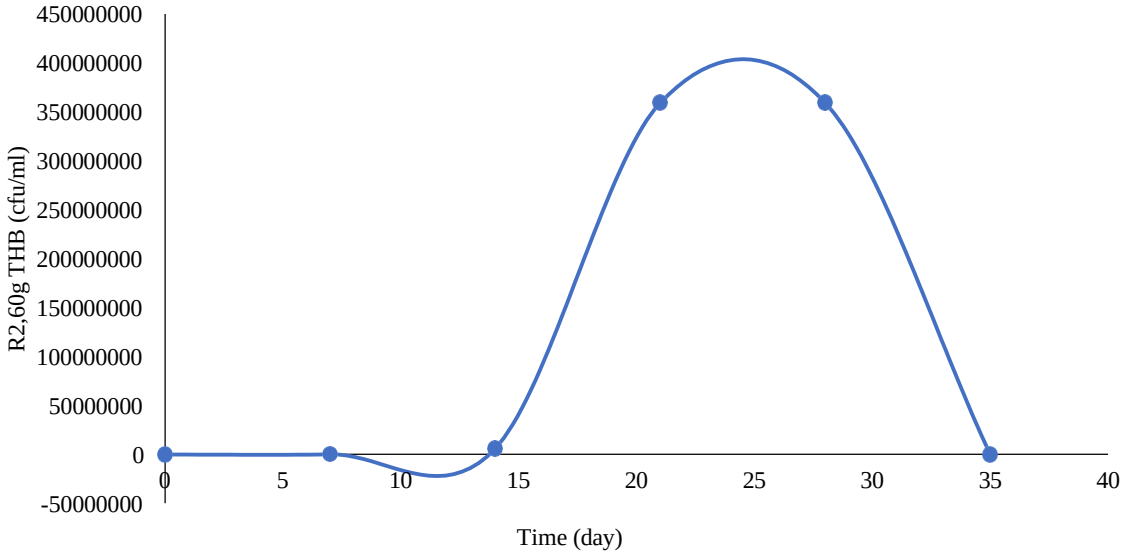

Figure 12. THB Concentration against Time for 60g Dosage of FPFPWW in Bioreactor 2 (R_2).

Figure 12 illustrates the activities of the THB concentration on the action of 60g dosage of FPFPWW in a bioreactor 2 (R_2). Indeed, increase in the THB growth rate was experienced with increase in time as well as the characteristics of the environment environment enhanced the growth of the organisms isolated and identified in the bioreactor 2.

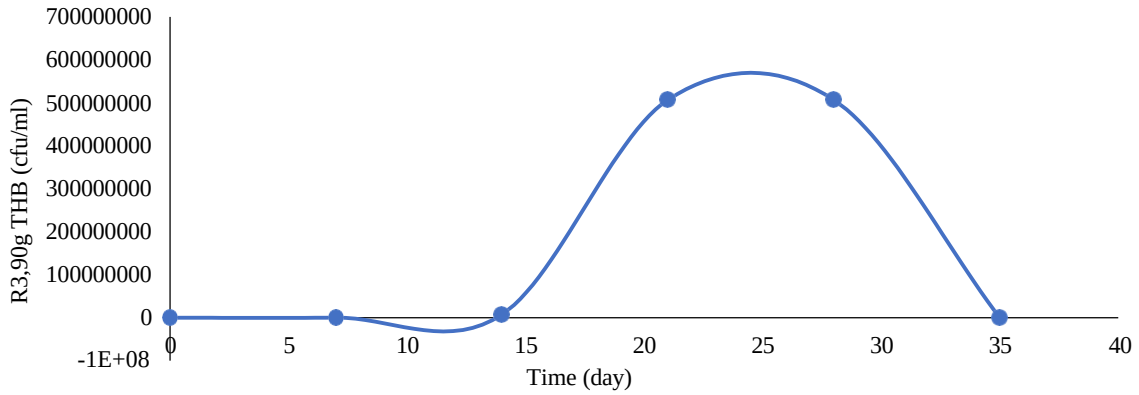

Figure 13. THB Concentration against Time for 90g Dosage of FPFPWW in Bioreactor 3 (R_3).

Figure 13 shows the rapid growth of THB for 90g dosage in the bioreactor 3 (R_3) with increase in time. The degradation of the TPH in the bioreactor 3 (R_3) can be intergrated to the favourable condition experienced by the THB, which reveals less impact due to changes in the physicochemical parameters monitored.

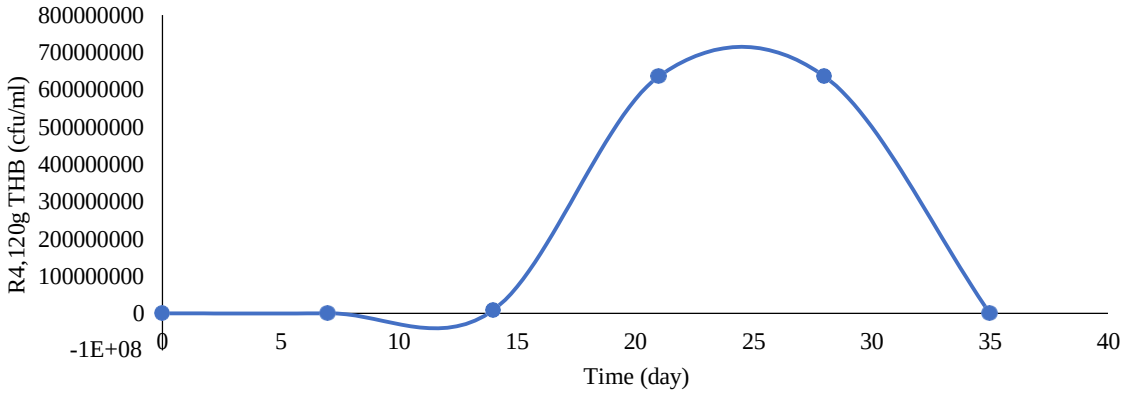

Figure 14. THB Concentration against Time for 120g Dosage of FPFPWW in Bioreactor 4 (R_4).

Figure 14 shows the THB concentration on the action of 120g dosage of FPFPWW in bioreactor 4 (R_4). However, increase in the rapid growth of THB was experienced with increase in time. The variation in the physicochemical parameters monitored favours the THB growth rate as well as enhance TPH degradation. The available nutrient in FPFPWW was useful for the effective utilization by organism. Increases in THB concentration enhanced increase in the rate of TPH remediation in bioreactor 4.

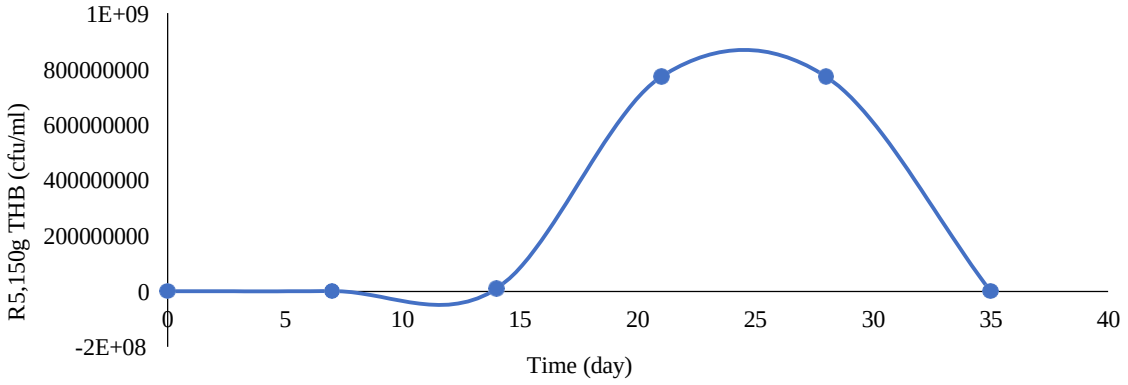

Figure 15. THB Concentration against Time for 150g Dosage of FPFPWW in Bioreactor 5 (R_5).

Figure 15 shows the THB concentration on the action of 150g dosage of FPFPWW in bioreactor 5 (R_5). However, increase in the rapid growth of THB was experienced with increase in time. The variation in the physicochemical parameters monitored favours the THB growth rate as well as enhance TPH degradation. The available nutrient in FPFPWW was useful for the effective utilization by organism. Increases in THB concentration enhanced increase in the rate of TPH remediation in bioreactor 5.

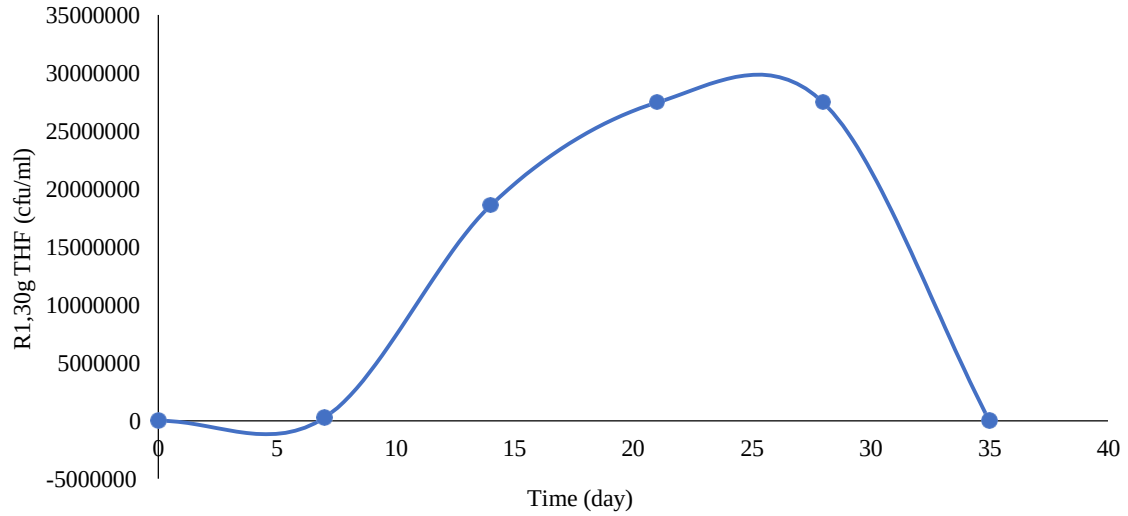

Figure 16. THF Concentration against time for 30g dosage of NPK in bioreactor 1 (R_1).

Figure 16 shows the growth rate of Fungi (THF Concentration) versus increase in time for 30g dosage of NPK fertilizer in bioreactor 1 (R_1). However, no lag phase was experienced rather rapid progressive phase encounted as well as stationary and decline phase. The concentration of the NPK fertilizer as the available nutrient enhanced the microbial growth as well as mitigate the TPH concentration in the bioreactor.

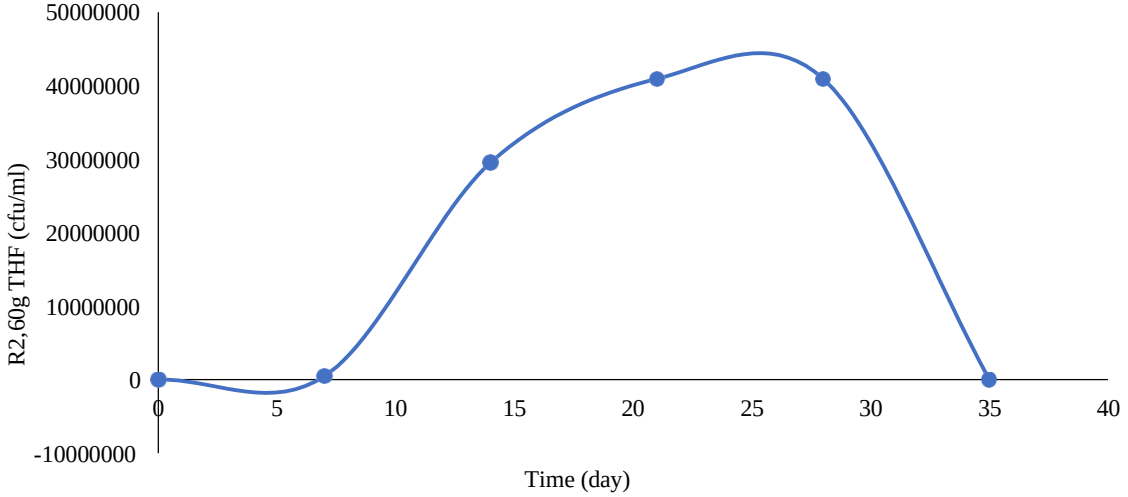

Figure 17. THF Concentration against Time for 60g Dosage of NPK in Bioreactor 2 (R_2).

Figure 17 show the concentration of THF for 60g dosage of NPK fertilizer in TPH mitigation in bioreactor 2 (R_2) upon the effect of time. Indeed, the physicochemical parameters monitored was observed not to have influence the TPH remediation negatively, but the available nutrient present in the NPK favours the microbial growth. Increase in TPH was experienced with increase in time.

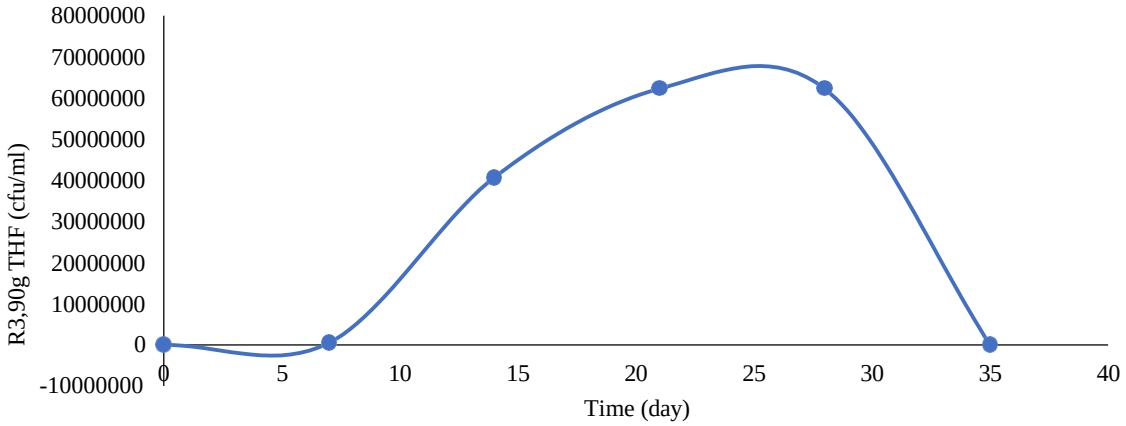

Figure 18. THF Concentration against Time for 90g Dosage of NPK in Bioreactor 3 (R_3).

Figure 18 show the effect of 90g dosage of NPK fertilizer on the microbial growth (THF concentration) in bioreactor with increase in time. The growth rate of THF was favours with the changes in the physicochemical parameters due to the available nutrient present in the nutrient used in catalysing the reactions, which is the NPK fertilizer. Increase in the THF was experienced with increase in time.

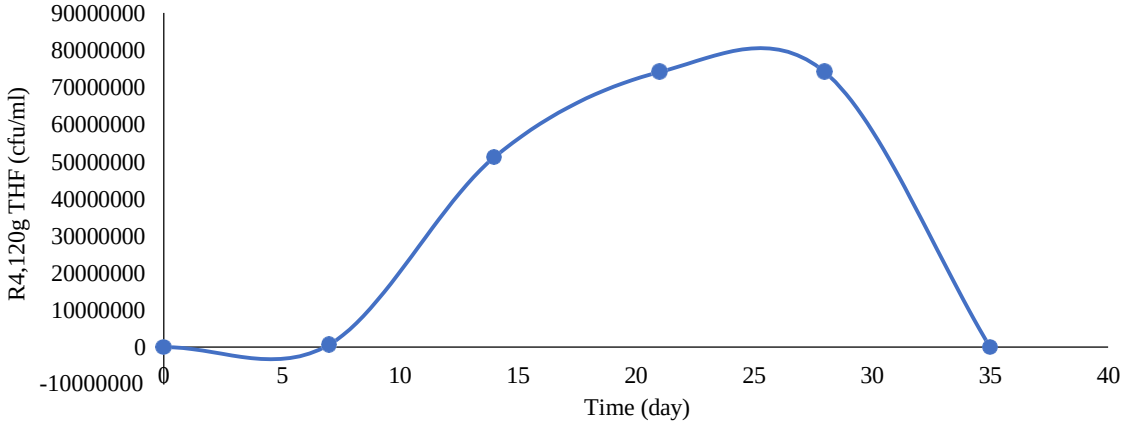

Figure 19. THF Concentration against time for 120g dosage of NPK in bioreactor 4 (R_4).

Figure 19 shows the increase in THF concentration with increase in time for 120g dosage of NPK fertilizer on TPH remediation in bioreactor 4 (R_4). The rapid growth of the THF was favourable with the available nutrient added to enhance microbial growth and TPH mitigation.

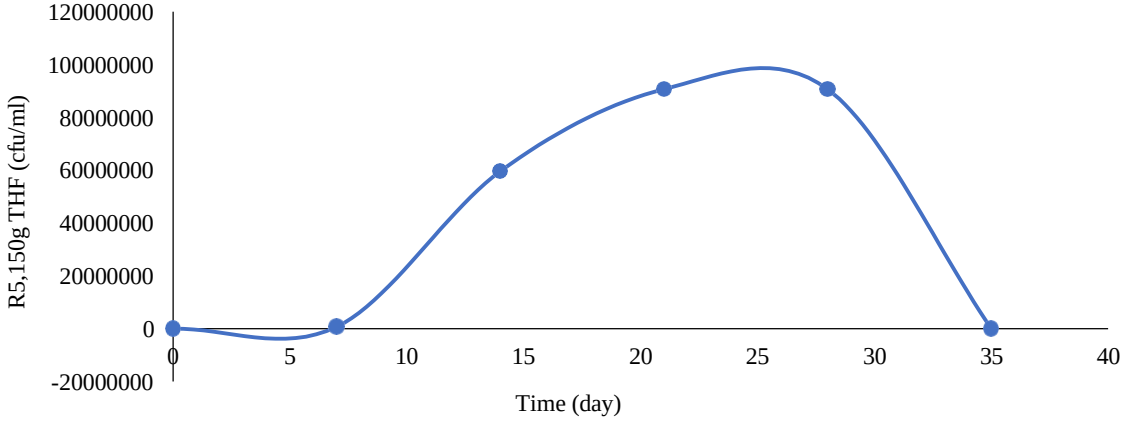

Figure 20. THF Concentration against Time for 150g Dosage of NPK in Bioreactor 5 (R_5).

Figure 20 shows the significance of NPK fertilizer in enhancing THF concentration for 150g dosage in bioreactor 5 (R_5) with increase in period of exposure (time). However, the THF growth rate was experienced with less inhibiting factors as well as increase in TPH consumption or mitigation.

CONCLUSION

The research showcases the following conclusion as demonstrated below:

i. Microbial growth rate was monitored and the obtained result reveals that the change in the physicochemical parameters does not inhibit the active site of the organism rather the physicochemical parameters variation catalyzed the reaction.
ii. The available nutrient in the biostimulants were found used by the organism to catalyze the process and increase the rate of substrate (TPH) degradation in each reactor.
iii. In conclusion I am recommending that the palm oil processed waste water can be managed by providing a storage region for further utilization in clean-up process of polluted soil environment due to its enhancement in microbial growth.

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APPENDIX

The results presented in the appendix demonstrates the experimental obtained data, which illustrates the microbial activities upon petroleum hydrocarbon degradation.

Table 1. THF Concentration for FPoPWW.

Time (day)	**THF** (cfu/ml)				
	$R_1 30g$	$R_2 60g$	$R_3 90g$	$R_4 120g$	$R_5 150g$
0	105.0	105.0	105.0	105.0	105.0
7	361.1×10^2	522.4×10^2	630.7×10^2	774.2×10^2	816.2×10^2
14	140.3×10^3	286.0×10^3	371.2×10^3	528.1×10^3	638.1×10^3
21	692.6×10^4	758.5×10^4	803.0×10^4	927.0×10^4	1001.3×10^4
28	692.6×10^4	758.5×10^4	803.0×10^4	927.0×10^4	1001.3×10^4
35	103.1×10^2	114.7×10^2	109×10^2	98.5×10^2	110.4×10^2

Table 2. THB Concentration for FPoPWW.

Time (day)	**THB** (cfu/ml)				
	$R_1 30g$	$R_2 60g$	$R_3 90g$	$R_4 120g$	$R_5 150g$

0	105.0	105.0	105.0	105.0	105.0
7	110.1 ×10³	253.7 × 10³	290.4 × 10³	401.6 × 10³	572.8 × 10³
14	183.5 ×10⁴	601.1 ×10⁴	730 ×10⁴	881.3 ×10⁴	984.0 ×10⁴
21	211 ×10⁶	359.4 ×10⁶	507.1 ×10⁶	636.0 ×10⁶	771.3 ×10⁶
28	211.8 ×10⁶	359.4 ×10⁶	507.1 ×10⁶	636.0 ×10⁶	771.3 ×10⁶
35	125 ×10³	96 ×10³	217 ×10³	84.2 ×10³	106 ×10³

Table 3. THF Concentration for NPK.

Time (day)	**THF** (cfu/ml)				
	$R_1 30g$	$R_2 60g$	$R_3 90g$	$R_4 120g$	$R_5 150g$
0	105.0	105.0	105.0	105.0	105.0
7	274.3 ×10³	470.2 ×10³	529.0 ×10³	681.4 ×10³	714.1 ×10³
14	186.0 ×10⁵	295.5 ×10⁵	407.1 ×10⁵	511.7 ×10⁵	596.0 ×10⁵
21	274.6 ×10⁵	409.1 ×10⁵	622.7 ×10⁵	741.0 ×10⁵	906.2 ×10⁵
28	274.6 ×10⁵	409.1 ×10⁵	622.7 ×10⁵	741.0 ×10⁵	906.2 ×10⁵
35	113.1 ×10²	96.2 ×10²	120.6 ×10²	87.5 ×10²	136.1 ×10²

Table 4. Remediation result of TPH concentration of clay soil subjected into NPK fertilizer of 30g to 150g with Control.

$Time$	$Control$	$30g$ $R1NPK$	$60g$ $R2NPK$	$90g$ $R3NPK$	$120g$ $R4NPK$	$150g$ $R5NPK$
0	46270.14	46270.14	46270.14	46270.14	46270.14	46270.14
7	40313.82	37906.21	36216.00	34822.62	32774.91	30833.57
14	39641.07	30530.05	24901.58	21158.03	20631.25	20001.38
21	32725.91	20373.27	18368.11	16406.74	13852.40	11052.08
28	30816.57	13566.02	12005.47	10852.50	9171.36	7360.88
35	29508.25	7134.39	7001.63	6281.36	5293.72	3081.92

Table 5. THB Concentration for NPK

Time (day)	**THB** (cfu/ml)				
	$R_1 30g$	$R_2 60g$	$R_3 90g$	$R_4 120g$	$R_5 150g$
0	105.0	105.0	105.0	105.0	105.0
7	427.3 ×10³	558.1 ×10³	613.3 ×10³	685.3 ×10³	747.0 ×10³
14	186.0 ×10⁵	207.1 ×10⁵	316.2 ×10⁵	383.6 ×10⁵	403.7 ×10⁵
21	326.4 ×10⁷	390.5 ×10⁷	430.1× ×10⁷	477.2 ×10⁷	601.3 ×10⁷
28	326.4 ×10⁷	390.5 ×10⁷	430.1× ×10⁷	477.2 ×10⁷	601.3 ×10⁷
35	219.3 ×10²	107.0 ×10²	241.6 ×10²	80.3 ×10²	160.7 ×10²

