



Optimization of Nutrients Concentration Required for the Bioremediation of Petroleum Contaminated Soil

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Abstract

There is a growing public concern as petroleum hydrocarbons are being introduced inadvertently or deliberately in large volumes into the environment thus causing a long term threat to all forms of life. Thus remediation of these is of utmost importance. The bioremediation technology is being employed for the degradation of crude oil in soil matrix through microorganisms which can transform petroleum hydrocarbons into less toxic compounds. In this study, optimum nutrients (C: N: P) concentration required for the microbial growth was investigated. The petroleum oily sludge, contaminated soil, soil rich in native microorganisms was collected and the same was used to prepare simulated contaminated soil which was filled up to 3/4th of the reactors volume. All the parameters except the C: N: P ratio in the reactors were maintained constant throughout the study period. The C: N: P ratios varies from 100:2.5:0.25 to 100:20:2 in the twenty reactors, N and P were amended to make up the required ratios. N and P were not added into the control reactor. Treatability studies on TPH contaminated soil was conducted in all the reactors for a period of six weeks. The results of the study reveals that among the different C:N:P ratios, C:N:P ratio of 100:10:1 gave the maximum TPH removal of 59.57 % with biodegradation rate of 0.022 day⁻¹, R² obtained was 0.968 which suggest that there exists a strong relationship between biodegradation and time. The results thereby imply that nutrients concentration is the most important factor that affects the biodegradation of petroleum-hydrocarbons in tropical soils.

Keywords: Microorganism; Optimum Nutrients; Simulated Contaminated Soil; TPH.

1. Introduction

The development of petroleum industry into new frontiers, the apparent inevitable spillages that occur during routine operations, and records of acute accidents during transportation has called for more studies into oil pollution problems [1], which have been recognized as the most significant contamination problem on the continent. Also, the extensive use of petroleum products leads to the contamination of almost all compartments of the environment. Crude oil can be accidentally or deliberately released into the environment leading to serious pollution problems [2]. Even small release of petroleum hydrocarbons into aquifers can lead to concentrations of dissolved hydrocarbons far in

excess of regulatory limits [3]. These pollution problems often result in huge disturbances of both the biotic and abiotic components of the ecosystems [4], more so that some hydrocarbon components have been known to belong to a family of carcinogenic and neurotoxic pollutants [5]. The processes leading to the eventual removal of hydrocarbon pollutants from the environment has been extensively documented and involves the trio of physical, chemical and biological alternatives. The currently accepted disposal methods of incineration or burial in secure landfills can become prohibitively expensive when the amounts of contaminants are large. This often results in

clean-up delays while the contaminated soil continues to pollute groundwater resources if on land, and death of aquatic life if on waterways [6], thus necessitating speedy removal of the contaminants. The biodegradation of oil pollutants is not a new concept as it has been intensively studied in controlled conditions and in open field experiments. Bioremediation, which employs the bio degradative potentials of organisms or their attributes, is an effective technology that can be used to accomplish both effective detoxification and volume reduction. It is useful in the recovery of sites contaminated with oil and hazardous wastes [7]. Besides, bioremediation technology is believed to be non-invasive and relatively cost effective [8]. In some cases, it may not require more than the addition of some degradation enhancers to the polluted system. It could end up being the most reliable and probably least expensive option for exploitation in solving some chemical pollution problems.

1.1. Objectives of the Study

- To study the initial physio-chemical and biological characteristics of both fresh and simulated contaminated soil.
- To study the influence of nutrients on bioremediation process.
- To optimize or to define a better nutrients ratio (C: N: P).
- To study the efficiency of TPH reduction in petroleum contaminated soil.
- To study the growth rate of microorganisms in bioremediation process.

2. Materials and Methodology

2.1. General

Development of design parameters for the analysis of TPH degradation in the simulated contaminated soil for optimizing the conditions is based on the laboratory investigations. Hence the scheme of experimental methodology was formulated to investigate the influence of nutrient concentration on the rate of reduction of oily sludge in the simulated contaminated soil for a period of six weeks in the Environmental Engineering laboratory, Department of Civil Engineering, U.V.C.E, Jnana Bharathi Campus, Bangalore University, and Bangalore.

They were suitably amended for the respective ratios using Ammonium nitrate and Dipotassium hydrogen orthophosphate

2.2. Materials

The materials that were used in this study are as follows:

Oily sludge and contaminated soil- They were collected from VRL Logistics Ltd located at a distance of about 4 KMs from Department of Civil Engineering.

Fresh soil- The Initial Physio-Chemical and Biological characteristics of the fresh soil are shown in Table 2.

Reactors (PVC containers) - A total number of 21 PVC Containers were selected as the bioreactors for the study.

Hydrogen Peroxide- Oxygen was supplied by means of chemical method for which Hydrogen Peroxide of 30% (w/v) was used as an additional source of oxygen [9].

Water- The distilled water was used for maintaining the moisture content in the bioreactors.

Nutrient source- Ammonium nitrate and Dipotassium hydrogen orthophosphate was used as sources for Nitrogen and Phosphorus to make up for the different C:N:P ratios.

Table 1 Parameter Analyzed on a Weekly Basis

Sl. No	Parameter	Method of analysis
1	pH	Water Quality Analyzer
2	Temperature	Water Quality Analyzer
3	Moisture content	Gravimetric Method
4	Total Organic Carbon	Walkely Black Method
5	TPH	Soxhlet Extraction Method
6	Nitrogen	Spectrophotometric Method
7	Phosphorus	Olsen's Extraction Method
8	Microbial Count	Plate Count Method

Table 2 Initial Physico-Chemical and Biological Characteristics of Fresh and Simulated Soil

Sl. No	Parameters	Unit	Fresh Soil Concentrations	Simulated Soil Concentrations
1	Type of soil	---	Sandy (well graded)	Sandy (well graded)
2	Porosity	%	37	-
3	Co-efficient of uniformity, Cu	---	6.5	-
4	Co-efficient of curvature, Cc	---	1.10	-
5	pH	---	7.0	6.8
6	Temperature	⁰ C	24.4	24.3
7	Moisture content	%	2.325	3.836
8	Total organic carbon	mg/gm of soil	31.12	80
9	TPH	mg/kg of soil	0	129000
10	Nitrogen	mg/gm of soil	0.112	0.104*
11	Phosphorous	mg/gm of soil	0.03488	0.03051*
12	Microbial count	CFU/gm of soil	32x10 ⁵	27x10 ⁶

2.3. Methodology

In order to proceed with the research and fulfil the objectives, the research investigations were organized as follows:

- The fresh soil which was excavated from Jnana Bharathi campus, near civil engineering department, at a depth of 50cm from the ground surface. The soil was air dried, pulverized and sieved through 4.75 mm sieve. The soil passing 4.75mm sieve and retained on 75micron sieve was taken for research work. The fresh soil was tested for its physio-chemical and Biological characteristics. The physio-chemical and biological characteristics of fresh soil are tabulated in the Table 2.
- Oily sludge along with contaminated soil was added to the fresh soil in the ratio 10(fresh soil):

2(oily sludge): 1(contaminated soil) to make the simulated contaminated soil.

- The simulated contaminated soil was tested in the laboratory for the different parameters mentioned in the Table 1 at regular intervals of time (once in a week) for a period of six weeks, to determine the degradation rate of TPH under various nutrient ratios(C:N:P) in laboratory conditions.
- Biodegradation of TPH with respect to the growth of microbial organisms was studied with the limiting factors such as oxygen, temperature, moisture, pH etc,

3. Results

Selection of soil for the remediation is one of the major factors that govern the efficiency of the process remediating pollutants. The soil was tested

for various parameters Laboratory and the results are tabulated below. Sieve analysis has been done for the soil to know the grain size distribution and results are as shown in Table 3 and Figure 1 shows the Particle size distribution curve (S-curve) for the sieve analysis. The initial physio-chemical and biological characteristics of fresh and simulated soil are shown in Table 2.

Table 3 Sieve Analysis Result

Sieve No.	Diameter in mm	Mass retained in g	% mass retained	Cumulative % retained	Cumulative % finer (N)
4	4.75	0	-----	-----	100
8	2.36	97	9.7	9.7	90.3
12	1.7	134	13.4	23.1	76.9
18	1	96	9.6	32.7	67.3
30	0.6	83	8.3	41	59
50	0.3	230	23.0	64	36
100	0.15	195	19.5	83.5	16.5
200	0.075	88	8.8	92.3	7.7
Pan		77	7.7	100	0
Total		1000			

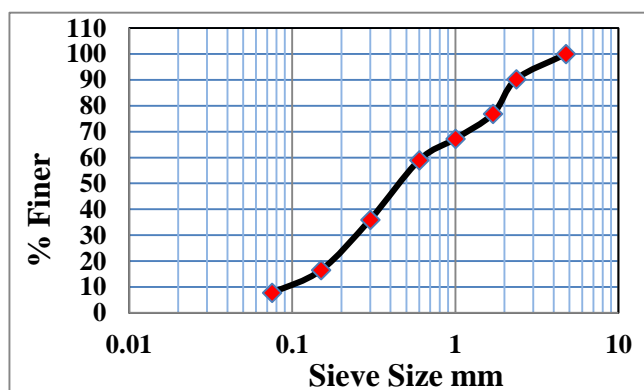


Figure 1 Particle Size Distribution Curve (S-curve)

The result of the soil test shows (Table 2) that soil had a porosity of 38% which lies within the range of 37.5

to 39.5 which is the required standard soil characteristic for bioremediation process. It is quoted in the literature that if Co-efficient of Curvature (C_c) of the soil is between 1 and 3 and Co-efficient of Uniformity (C_u) of the soil is greater than 6 then that type of is said to be sandy well graded type [10]. Thus the soil taken for analysis meets the requirements for the bioremediation process. From the Table 2 it can be said that the pH of fresh soil was 7.0 which decreased to 6.8 after the addition of oily sludge. There was sharp increase in total organic carbon content from 31.12 to 80 mg/gm of soil after the addition of oil and contaminated soil to fresh soil. The later value was considered as 100 for C during C: N: P ratio setup for the study. The nitrogen and phosphorus content was very low and hence does not meet the required N and P for the microbial activity. Thus N and P were artificially amended to make up the different C: N: P ratios required for the experimental setup. The microbial count was 32×10^5 CFU/gm of soil, the number was little lower than the required count for effective bioremediation, as the literature quotes that when the population of indigenous microorganisms capable of degrading the target contaminants is less than 10^5 cells/gm of soil, bioremediation would not occur at a significant rate [11]. Thus the soil contaminated with TPH at the site was collected as it already contained the acclimatized microorganisms that can easily degrade TPH thereby eliminating the lag phase of microbes. The addition of contaminated soil thus increased the microbial count to 27×10^6 CFU/gm of soil. The results (average values) of analysis of TPH contaminated soil samples for different parameters at an interval of one-week duration has been tabulated in the following different tables Table 4 to Table 9 for a period of six weeks.

pH: pH is an important parameter for the microbial metabolism. The pH must be maintained between 6 and 8 to maximize the rate of biodegradation [12]. During the study period the pH values ranged between 6.3 and 7.6 which is the optimum value for oil degradation. In the present study, the initial pH value of the simulated contaminated soil was

6.8 as shown in Table 2. The pH value for the first week significantly decreased to 6.3 (Table 4 to Table 9) which was due to the simultaneous production and accumulation of acidic metabolic products during the

microbial degradation of hydrocarbons. The pH value of the control reactor fluctuated in a very small range and was 7.2 at the end of remediation period.

Table 4 Variation of Physio-Chemical and Biological Characteristics of TPH Contaminated Soil in Reactor R₁ having a C: N: P ratio of 100:2.5:0.25

Bioreactor R ₁ with C:N:P ratio 100:2.5:0.25									
Sl. No	Parameter	Unit	Characteristics of Parameters Tested at an Time Interval of One Week						
			0	1	2	3	4	5	6
1.	pH	-	6.8	6.3	6.9	7.1	7.54	7.4	7.38
2.	Moisture Content	%	30.76	50.76	51.1	50.8	50.2	50.92	51.2
3.	Temperature	°C	24.3	24.1	24.3	24.9	25.4	24.5	23.5
4.	TOC	mg/gm of soil	80.04	60.16	57.23	52.25	48.4	47.18	43.26
5.	TPH	mg/kg of soil	129556	124161	120871	117615	100865	93615	81615
6.	Microbial Count	×10 ⁶ CFU/ gm of soil	27.4	31	38	47	42	33.2	25

Table 5 Variation of Physio-Chemical and Biological Characteristics of TPH Contaminated Soil in Reactor R₂ having a C: N: P ratio of 100:5:0.5

Bioreactor R ₂ with C:N:P ratio 100:5:0.5 555									
Sl. No	Parameter	Unit	Characteristics of Parameters Tested at an Time Interval of One Week						
			0	1	2	3	4	5	6
1.	pH	-	6.8	6.275	6.87	7.165	7.45	7.4	7.45
2.	Moisture Content	%	31	50.5	51.25	50.57	50.67	51.3	50.7
3.	Temperature	°C	24.3	25.5	24.6	24.4	25.6	25.4	23.5
4.	TOC	mg/gm of soil	79.665	67	61.1	50.35	46.85	45.85	41.65
5.	TPH	mg/kg of soil	129627	120430	118998	101248	90499	78998	68998
6.	Microbial Count	×10 ⁶ CFU/ gm of soil	27.7	30	38	52	43	35	26



Temperature: Microbial growth and activity are readily affected by temperature. The temperature of the reactors was in the mesophilic range of 23.4°C to 25.8°C (Table 4 to Table 9). At the end of the study period the temperature decreased to around 23°C in all the reactors.

Table 6 Variation of Physio-Chemical and Biological Characteristics of TPH Contaminated Soil in Reactor R3 having a C: N: P ratio of 100:10:1.5

Bioreactor R3 with C:N:P ratio 100:10:1 6666									
Sl. No	Parameter	Unit	Characteristics of Parameters Tested at an Time Interval of One Week						
			0	1	2	3	4	5	6
1.	pH	-	6.8	6.3	6.75	7.195	7.2	7.4	7.46
2.	Moisture Content	%	31.46	51.5	50.3	50.5	51.2	50.9	51.2
3.	Temperature	°C	24.3	24.6	24.2	24.7	26.2	25.12	23.4
4.	TOC	mg/gm of soil	80.87	61.1	53.39	50.79	42.15	45.33	35.4
5.	TPH	mg/kg of soil	128866	118338	110690	92405	79155	65905	52155
6.	Microbial Count	$\times 10^6$ CFU/ gm of soil	28.2	33	55	89	63	40.5	34.5

Table 7 Variation of Physio-Chemical and Biological Characteristics of TPH Contaminated Soil in Reactor R4 having a C: N: P ratio of 100:15:1.5

Bioreactor R4 with C:N:P ratio 100:15:1.5 777									
Sl. No	Parameter	Unit	Characteristics of Parameters Tested at an Time Interval of One Week						
			0	1	2	3	4	5	6
1.	pH	-	6.8	6.47	6.95	7.2	7.46	7.5	7.44
2.	Moisture Content	%	30.85	50.85	51.3	50.2	51.2	51.2	50.5
3.	Temperature	°C	24.3	25.2	24.1	24.3	25.4	25.15	23.9
4.	TOC	mg/gm of soil	78.99	68.14	54.6	50.72	51.6	45.33	45.61
5.	TPH	mg/kg of soil	129486	121545	112950	95450	86700	78450	74700
6.	Microbial Count	$\times 10^6$ CFU/ gm of soil	27.7	31.75	48	64	55.5	35.5	22

Table 8 Variation of Physio-Chemical and Biological Characteristics of TPH Contaminated Soil in Reactor R₅ having a C: N: P ratio of 100:20:2

Bioreactor R ₅ with C:N:P ratio 100:20:2 888									
Sl. No	Parameter	Unit	Characteristics of Parameters Tested at an Time Interval of One Week						
			0	1	2	3	4	5	6
1.	pH	-	6.8	6.32	6.9	7.16	7.2	7.34	7.48
2.	Moisture Content	%	30.67	51.7	51.1	50.67	50.67	52.1	49.95
3.	Temperature	°C	24.3	24.9	24.6	24.02	25.1	25.85	23.7
4.	TOC	mg/gm of soil	79.81	70.4	57.11	58.89	55.14	51.27	48.65
5.	TPH	mg/kg of soil	129556	125249	119598	115598	111098	94598	86598
6.	Microbial Count	×10 ⁶ CFU/gm of soil	27.7	33	38	48	42	31	24

Table 9 Variation of Physio-Chemical and Biological Characteristics of TPH Contaminated Soil in Control Reactor

Control Reactor									
Sl. No	Parameter	Unit	Characteristics of Parameters Tested at an Time Interval of One Week						
			0	1	2	3	4	5	6
1.	pH	-	6.8	6.8	7.01	7.2	7.54	7.2	7.2
2.	Moisture Content	%	24.3	49.6	51.3	50.8	50.5	51.1	50.4
3.	Temperature	°C	31.3	25.0	24.3	24.7	25.2	25.7	23.7
4.	TOC	mg/gm of soil	80.5	60.27	57.23	60.4	55.1	45.33	50.4
5.	TPH	mg/kg of soil	129350	121004	110095	110811	105811	93680	92880
6.	Microbial Count	×10 ⁶ CFU/gm of soil	27.3	31	38	29	20	14	11

Moisture Content: Soil microorganisms need water to support their metabolic processes. Too much water will hinder the supply of oxygen and as a result will

decrease the rate of biodegradation. On the contrary, too little water will inhibit microbial activities. Degradation is typically not restricted by moisture if

the soil moisture content is maintained above a certain minimum (usually between 30% and 90% of the water-holding capacity of the soil) [12]. In the present study soil moisture content in the reactors was maintained 60(+/-) 10% of maximum water holding capacity. The soil used in this study had a WHC of 48.7%. Thereby, 60% of WHC implied 29.22% of moisture content, which was maintained throughout the study.

Total Organic Carbon: The carbon content of simulated contaminated soil was sufficient to supply carbon source for the hydrocarbon utilizing microorganisms. TOC of the fresh soil and simulated contaminated soil were 31.12mg/gm and 80mg/gm of soil respectively and decreased significantly to 35.4mg/gm of soil (at the end of the 6th week) in the reactor R₃ resulting in 55.75% of TOC reduction

Nutrient Concentration: While hydrocarbons are an excellent source of carbon and energy for microbes, they are incomplete foods since they do not contain significant concentrations of other nutrients (such as nitrogen and phosphorus) required for microbial growth [13]. The input of large quantities of organic carbon sources tends to result in a rapid depletion of available inorganic nutrients [14], limiting the amount of biodegradation. Adjustment of carbon/nitrogen/phosphorous (C: N: P) ratios by the addition of ammonium and phosphate salts was done to five different ratios as given in the Table 10 below to optimize C: N: P ratio required for maximum degradation of TPH in soil. The nutrients were not added into the control reactor.

Table 10 Nutrient Ratio Setup for different Reactors

Sl. No	Name of the Bioreactors	C:N:P Ratios
1	R ₁	C:N:P=100:2.5:0.25
2	R ₂	C:N:P=100:5:0.5
3	R ₃	C:N:P=100:10:1
4	R ₄	C:N:P=100:15:1.5
5	R ₅	C:N:P=100:20:2
6	Control Reactor	C:N:P=100:0:0

Microbial Activity: Microorganisms play a major role in bioremediation and their absolute numbers can determine the overall degradative ability. The results of bacterial counts showed that the profiles of all the bioreactors followed a typical microbial growth pattern. There was no initial lag phase observed in the growth pattern due to the addition of acclimatized microbes which was achieved by the addition of contaminated soil. This eliminated the adaptation period of the microbes to the new environment.

The initial population of bacteria in the simulated contaminated soil was 27x10⁶ CFU/gm of soil (Table 4). The microbial counts varied from 29x10⁶ to 89x10⁶ CFU/gm of soil in the third week. Thus, increase in bacterial counts had a profound influence on the rate of TPH reduction. At the end of 6th week the microbial count gradually decreased to 34.5x10⁶ in bioreactor R₃ (Table 6). In the control reactor, bacterial counts decreased constantly and were 29x10⁶ at the end of third week and decreased to 11x10⁶ at the end of the sixth week (Table 9).

TPH Reduction: The percentage of TPH reduction ranged between a minimum of 32.34% in reactor R₅ to a maximum of 59.25% in the bioreactor R₃ during six weeks of treatment as shown in Table 11 and represented in Figure 2. In the reactors R₁, R₂, R₄ and R₅ the biodegradation was only slightly greater than the control (27.44%), which received no mineral nutrients (N and P). Since all other environmental conditions were kept same in all the bioreactors, the C: N: P ratio of 100:10:1 in reactor R₃ seems ideal for the indigenous microorganisms to grow and thereby cause maximum degradation of TPH.

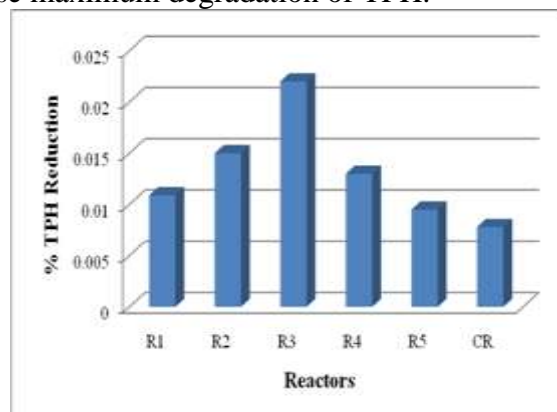


Figure 2 Percentage TPH Reduction for the all the Reactors

Table 11 Percentage of TPH Reduction in all the Reactors

Sl No	Bioreactors	Initial	Final	% reduct ion
1	R ₁ =C:N:P=100:2.5:0.25	129000	81615	36.24
2	R ₂ =C:N:P=100:5:0.5	129000	68998	46.09
3	R ₃ =C:N:P=100:10:1	129000	52155	59.57
4	R ₄ =C:N:P=100:15:1.5	129000	74700	41.64
5	R ₅ =C:N:P=100:20:2	129000	86598	32.34
6	Control Reactor	129000	92880	27.44

Biodegradation Rate: Bacterial growths are best described based on the Monod Model. The biodegradation of hydrocarbons in contaminated soil is assumed to follow the first order degradation and the calculated values are tabulated in Table 12 and represented in Figure 3.

Table 12 Degradation Rate Constant of Reactors R₁ To R₅ and Control Reactor

Sl.No.	Reactors	Degradation rate constant (k) d ⁻¹
1	R ₁ =C:N:P=100:2.5:0.25	0.0109
2	R ₂ =C:N:P=100:5:0.5	0.015
3	R ₃ =C:N:P=100:10:1	0.022
4	R ₄ =C:N:P=100:15:1.5	0.013
5	R ₅ =C:N:P=100:20:2	0.0095
6	Control Reactor	0.0078

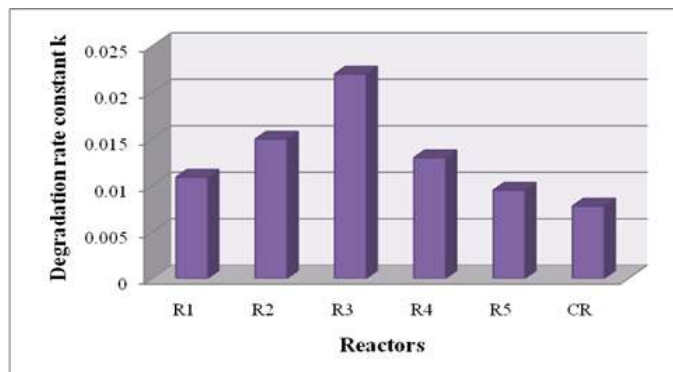


Figure 3 Degradation Rate Constant (k) of Bioreactors R₁ to R₅ and Control Reactor (CR)

The order of the bioremediation reaction was investigated and results are shown in Table 13 and represented in Figures 4. Once the degradation commences, the amount of TPH disappearing with time and the shape of the disappearance curve is a function of the compound in question, its concentration, the organisms responsible and a variety of environmental factors. In the first order reactions (unimolecular reactions), a plot of ln (TPH) against time (t) is a straight line and the slope gives the value of the rate constant. The regression coefficients (R²) obtained from the linear plot was 0.968 for the reactor R₃ which had maximum degradation efficiency. This suggests that there exists a very strong relationship between the rates of biodegradation of TPH with time (Figure 4). This suggests that the bioremediation process was very efficient and effective in the clean-up of soil contaminated with petroleum

Table 13 Data for Kinetic Studies of Bioremediation for the Reactor R₃

Time in Weeks	TPH left	ln TPH	% Removal
0	129000	11.76	0
1	118338	11.68	8.26
2	110690	11.61	14.19
3	92405	11.43	28.36
4	79155	11.28	38.63
5	65905	11.09	48.91
6	52155	10.86	59.57

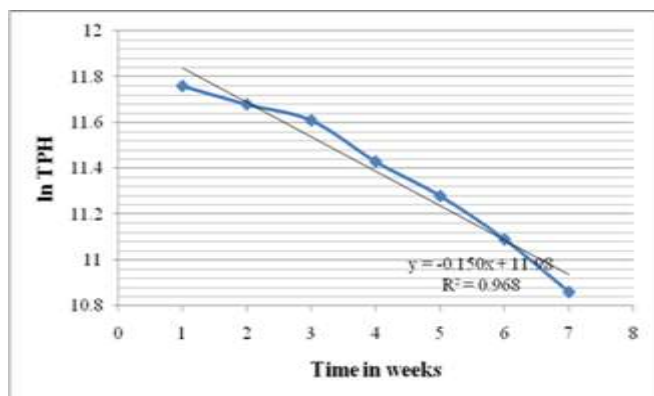


Figure 4 First Order Graph showing ln TPH against Time for the Reactor R₃

Conclusion

- Based on the results obtained from the studies, the following conclusions can be drawn.
- The initial physic-chemical and biological characteristics of the fresh soil and simulated contaminated soil were satisfying the requirements of soil characteristics for bioremediation process.
- The selected soil had sufficient indigenous microorganisms in the native soil which could degrade the TPH in the contaminated soil. Indigenous microorganisms had good potential for the bioremediation process.
- Amidst a range of conditions and inherent experimental limitations, such as differences in soil composition and other uncontrollable factors that may have some effects on the results, it is reasonable to conclude from the findings of this study that nutrients concentration is the most important factor affecting oil degradation. The C: N: P ratio in petroleum contaminated soil has influence on bioremediation, since bioremediation efficiency varies with different C: N: P concentration.
- From the study it is found that the optimal conditions for better degradation of TPH was found at a C:N:P ratio of 100:10:1 with biodegradation rate of 0.022 day⁻¹ and TPH removal efficiency of 59.57% within a period of six weeks.
- The order of reaction was investigated and was found to follow first order reaction and the

regression co-efficient (R²) obtained from the linear plot was 0.968. This suggests that there exists a very strong relationship between the rates of biodegradation of TPH with time.

- The findings of this study show that the bioremediation under optimum conditions is a very promising and efficient technology in the clean-up of soil contaminated with petroleum

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