

## **PETROLEUM-CONTAMINATED SOIL LONG DISTANCE BIOREMEDIATION WITH VARIABLE MOISTURE CONTENT**

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### **Abstract**

Oil hydrocarbon spills cause defilement of soils, surface springs and groundwater supplies, along these lines negatively affecting the earth. Advances in science and innovation have empowered us to apply the capability of natural assorted variety for contamination decrease which is named as bioremediation. Bioremediation is an inventive innovation for the treatment of wide assortment of contaminants. The current examination was thus centered around treating oil tainted soils using the capability of bioremediation.

The examination underscores on hydrocarbon debasement during the drawn out bioremediation of oil polluted soil. Dampness substance of the dirt was considered for enhancement, so as to assess its impact on the biodegradation procedure. Biodegradation of Total Petroleum Hydrocarbons (TPH) was inspected for 28 bioreactors with differing dampness content (30% - 90% field limit) and one reactor was taken as control bioreactor. The physico-substance and natural qualities of the dirt were tried on a week after week reason for a time of 23 weeks to decide the TPH debasement rate. It was seen that at dampness substance of 60% field limit, most extreme TPH expulsion of 78.21% was recorded and the debasement rate constants for fast and moderate period of

debasement were 0.0250 d<sup>-1</sup> and 0.00267 d<sup>-1</sup> respectively. Since the primary (fast) phase of degradation was overwhelming, endeavors to upgrade natural movement ought to be coordinated towards the principal period of biodegradation.

**Keywords:** Long Term Bioremediation, Total Petroleum Hydrocarbons, Moisture Content.

### **Introduction**

Petroleum hydrocarbons are widely used in our daily life as fuel and chemical compounds. As a result of this massive use, petroleum has become the most common contaminant of large soil surfaces, and eventually is considered as a major environmental problem [1]. The increase in public awareness towards the conservation of the environment has led to the development of various physicochemical techniques for cleaning up sites. Although most of the physicochemical methods can be efficient for treating a wide range of pollutants, they are extremely expensive [2]. Consequently, bioremediation has become a valuable alternative technology to many physicochemical methods as it is a cost effective and environmental friendly treatment [3]. Hence, this research work was focused on studying the biodegradation of hydrocarbons during long term treatment of petroleum hydrocarbon contaminated soils.

#### **1. Literature Review**

Bioremediation is an invaluable tool box for wider application in the realm of environmental protection. Biological agents, mainly microorganisms i.e. yeast; fungi or bacteria are used to clean up contaminated soil and water [4]. Bioremediation has been successfully applied for clean-up of soil, surface water, groundwater, sediments and ecosystem restoration. It has been unequivocally demonstrated that a number of xenobiotics can be cleaned up through bioremediation [5].

However, there are a wide range of factors known to reduce the ability of soil microbes to breakdown contaminants. These factors include nutrients, pH, temperature, moisture, oxygen, soil characteristics and contaminant bioavailability [6]. Optimizing these environmental conditions could enhance contaminants biodegradation in the soil [7]. Biosurfactants can be used to improve contaminant bioavailability to soil microbial degraders through reducing contaminant viscosity and thus increasing hydrocarbon solubility [3].

Over the years, lot of studies has been reported on petroleum hydrocarbon degraders [8]. But, there is no comprehensive and conclusive report on the kinetics of biodegradation of crude oil [9]. Few works have been dedicated to investigate the kinetic of soil bioremediation [10, 11, 12]. Information on kinetics is extremely important because it characterizes the concentration of the chemical remaining at any time and permits prediction of the levels likely to be present at some future time [13]. Thus, information on degradation kinetics and resulting residual concentrations is necessary to understand the behavior of pollutants in soils and to assess the prospects of remediation. However, data on kinetics and resulting residual concentrations from the degradation of TPH in long-term polluted field soils are scarce [14, 15]. Henceforth, this research was geared towards studying the biodegradation of hydrocarbons during the long term treatment of TPH contaminated soils and to describe the two consecutive first-order kinetic reactions. It was also focused on the optimization of moisture content required for effective bioremediation.

## MATERIALS AND METHODS

The fresh soil was excavated from an open field near Civil Engineering Department, JB Campus, Bangalore, at a depth of 50 cm from the ground surface. It was then air dried, pulverized and sieved through 4.75mm. The soil passed through 4.75mm and retained on 75 micron was taken for the experimental work. The fresh soil was analyzed for the various physico-chemical and biological characteristics in order to ascertain its suitability for bioremediation process (Table 1).

The waste oil (or oily sludge) was collected from VRL Logistics located at a distance of 4kms from JB Campus. The fresh soil was mixed with waste oil and acclimatized soil in the ratio 10:2:1 (i.e. 4kg of fresh soil: 800gm of waste oil: 400gm of acclimatized soil). Biosurfactant

rhamnolipid produced in Environmental Engineering laboratory, Civil Engineering Department, JB Campus, Bangalore was added to the bioreactors in 1:4 ratio (i.e. 1gm of biosurfactant per 4gm of soil) to increase the contaminant bioavailability to soil microbes. The soil was then mixed uniformly and filled into PVC reactors up to 75% working volume and remaining 25% was free board for efficient degradation of contaminated soil.

28 bioreactors along with one control bioreactor were used for single batch experiments. The environmental parameters affecting bioremediation process were maintained at optimum conditions in all the bioreactors throughout the study period. The pH was maintained within a range of 6.5-8.5, temperature at 20°C to 30°C and C:N:P ratio at 100:10:1 [16]. The bioreactors were maintained at a different moisture content of 30%, 40%, 50%, 60%, 70%, 80% and 90% field capacity and were represented as M1, M2, M3, M4, M5, M6 and M7 respectively. These seven bioreactors (with different moisture content) had four replicates each (number of bioreactors for the study was selected based on statistical analysis). One bioreactor was kept as Control Reactor (MC). The control reactor had no alterations done to its moisture content throughout the study period.

The bioreactors were monitored regularly and were analyzed on a weekly basis for various physico-chemical and biological characteristics for a period of 23 weeks. The weekly reduction in TPH concentrations in the bioreactors was evaluated to optimize the moisture content required for efficient bioremediation and to understand the degradation kinetics.

## RESULTS AND DISCUSSION

### 3.1 Physico-chemical and biological characteristics of fresh soil and simulated soil

The initial physico-chemical and biological characteristics of the fresh soil and simulated soil are shown in Table 1. The results of the various physico-chemical and biological characteristics of the simulated soil in the bioreactors after seven and twenty three weeks of treatment (i.e. after I and II stage) is tabulated in Table 2 and Table 3 respectively.

**Table 1: Initial physico-chemical and biological characteristics of fresh soil and simulated soil**

Table 2: chemical	Parameters	Unit	Fresh Soil Concentrations	Simulated Soil Concentrations	Physico- and
	Type of Soil	---	Sandy	Sandy	
	Porosity	%	37	---	
	Texture		Well Graded	Well Graded	
	Co-efficient of Uniformity, Cu	---	6.5	---	
	Co-efficient of Curvature, Cc	---	1.10	---	
	pH	---	6.9	7.49	
	Temperature	°C	28	26.9	
	Moisture Content	%	3.8	4.14	
	Total Organic Carbon	mg/gm of soil	54.6	78.2	
	TPH	mg/kg of soil	0	128000	
	Nitrogen	mg/gm of soil	2.62	6.28	
	Phosphorous	mg/gm of soil	0.24	0.64	
	Microbial Count	CFU/gm of soil	47x10 <sup>5</sup>	19x10 <sup>6</sup>	

**biological characteristics of simulated soil after 7 weeks of treatment (Stage I)**

Bioreactors	M1	M2	M3	M4	M5	M6	M7	MC
Parameters								
pH	7.15	7.21	7.24	7.42	7.41	7.47	7.28	7.44
Temperature ( $^{\circ}$ C)	24.5	25.1	24.5	24.5	25.3	25.1	24.5	25.1
TOC (mg/gm)	37.55	36.18	34.06	32.55	34.43	39.82	38.20	39.90
TPH (mg/kg)	91050	67991	53012	37620	42216	61110	83001	102923
Nitrogen (mg/gm)	3.50	3.42	3.28	2.90	3.13	3.65	3.76	3.40
Phosphorus (mg/gm)	0.35	0.35	0.31	0.27	0.29	0.35	0.34	0.35
Microbial Count (CFU/gm) $\times 10^6$	17.4	26.1	46.4	48.2	43	30.1	17.1	15

\* Concentrations depicted are the average values obtained by considering all four replicates of each bioreactor.\

**Table 3: Physico-chemical and biological characteristics of simulated soil after 23 weeks of treatment (Stage II)**

Bioreactors	M1	M2	M3	M4	M5	M6	M7	MC
Parameters								
pH	7.42	7.20	7.34	7.40	7.28	7.40	7.47	7.12
Temperature ( $^{\circ}$ C)	28.6	28.6	28.6	28.6	28.6	28.6	28.6	28.7
TOC (mg/gm)	18.33	15.99	15.43	13.23	14.02	17.11	17.80	22.01
TPH (mg/kg)	84203	60268	44869	27883	32864	53120	75500	99206
Nitrogen (mg/gm)	1.61	1.50	1.38	1.13	1.28	1.53	1.81	2.77
Phosphorus (mg/gm)	0.12	0.11	0.10	0.08	0.09	0.10	0.12	0.20
Microbial Count (CFU/gm) $\times 10^6$	7.2	14.2	21.3	29.2	20.4	13	8.2	7

\* Concentrations depicted are the average values obtained by considering all four replicates of each bioreactor.

### 3.2 Analysis of Data and Interpretation

**3.2.1 pH:** The initial pH of the simulated contaminated soil for all the bioreactors was 7.49. The pH values fluctuated in a very small range in all bioreactors. The final pH of all the bioreactors was within the pH range of 6.5 to 8.0 which is considered as optimum value for oil degradation. [16,17].

**3.2.2 Temperature:** The temperature ranged from 20.7 $^{\circ}$ C to 28.9 $^{\circ}$ C in all bioreactors during the study. The temperature variations did not follow a definite pattern with time. However, the temperature fell within the optimum range required for effective bioremediation process [16,18]. This facilitated optimal growth of microbial populations which in turn was responsible for biodegradation of petroleum products.

**3.2.3 Moisture Content:** The moisture content were varied like 30%, 40%, 50%, 60%, 70%, 80% and 90% of field capacity (increments of 10%) for the bioreactors M1, M2, M3, M4, M5, M6 and M7 respectively and were maintained as such throughout the study. The moisture content of the control reactor (MC) was not maintained throughout the study period, i.e. all other parameters other than moisture content was monitored for the control reactor during the study period.

**3.2.4 Total Organic Carbon:** TOC in the simulated contaminated soil was initially 78.2 mg/gm of soil and was finally reduced to 13.23 mg/gm of soil in bioreactor M4 (60% Moisture Content) owing to a maximum carbon utilization of 83.08% by the microorganisms. Maximum carbon utilization was observed in the bioreactor (M4), which also had a maximum bacterial count of  $91.5 \times 10^6$  CFU/gm of soil.

**3.2.5 Nutrient Concentration:** C: N: P ratio of 100:10:1 is considered optimal for bioremediation [18]. The nutrients when in the optimal range allow microbes to create necessary enzymes to break down the contaminants.

Hence, bioreactors were supplemented with Urea as a source of Nitrogen and Super Phosphate as the source of Phosphorus to bring the nutrient concentrations to the required levels.

**3.2.6 Microbial Activity:** Microorganisms play a major role in bioremediation and their absolute numbers can determine the overall degradative ability [19]. The results of bacterial counts showed that the profiles of all the bioreactors followed a typical microbial growth pattern. The microbial counts varied from  $19 \times 10^6$  to  $91.5 \times 10^6$  CFU/gm of soil in the third week and decreased to  $48.2 \times 10^6$  CFU/gm of soil in the seventh week and further down to  $29.2 \times 10^6$  CFU/gm of soil at the end of 23 weeks in bioreactor M4 (60% Moisture Content). Thus, increase in bacterial counts had a profound influence on the rate of TPH reduction.

**3.2.7 Total Petroleum Hydrocarbons:** The concentrations of TPH in all the bioreactors taken for the study had a decreasing trend with increasing bioremediation time which is typical of any degradation process. The percentage of TPH reduction ranged between a minimum of 22.49% in bioreactor MC to a maximum of 78.21% in the bioreactor M4 during 23 weeks of treatment. Since all other environmental conditions were kept same in all the bioreactors, the moisture content of 60% of field capacity in reactor M4 seems advantageous for the indigenous microorganisms to grow and thereby cause maximum degradation of TPH. Table 4 and Figure 1 show percentage reduction of TPH in the bioreactors.

**Table 4: TPH Reduction in the Bioreactors**

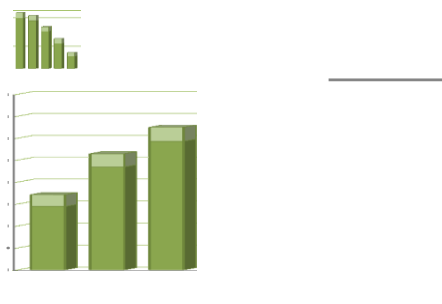
Bioreactors	Initial TPH (mg/Kg)	Final TPH (mg/Kg)		Reduction (%)		
		I Stage (7 Weeks)	II Stage (23 Weeks)	I Stage	II Stage	Total
M1=30%	128000	91050	84203	28.86	5.36	34.21
M2=40%	128000	67991	60268	46.88	6.03	52.91
M3=50%	128000	53012	44869	58.58	6.36	64.94
M4=60%	128000	37620	27883	70.60	7.61	78.21
M5=70%	128000	42216	32864	67.01	7.31	74.32
M6=80%	128000	61110	53120	52.25	6.25	58.50
M7=90%	128000	83001	75500	35.15	6.86	41.01
MC=Control	128000	105923	99206	17.24	5.25	22.49

#### 4.3 Kinetics of Biodegradation

Kinetic analysis performed on the TPH degradation revealed a degradation pattern characterized by two consecutive first order reactions (biphasic process) in most of the experimental settings. The degradation process was characterized by a period of fast decrease (Stage I) in the hydrocarbon concentrations during the first seven weeks followed by a period of slower activity (Stage 2) in the subsequent weeks of treatment.

After seven weeks of treatment the TPH concentrations were degraded down to 91050, 67991, 53012, 37620, 42216, 61110 and 83001 mg/kg of soil which resulted in 28.86, 46.88, 58.58, 70.60, 67.01, 52.25 and 35.15 percent TPH reduction in bioreactors M1 to M7 respectively (Table 4). After the initial rapid degradation phase, the biodegradation rate slowly decreased in the latter weeks of treatment. The final concentrations of TPH in the simulated contaminated soil at the end of treatment period, i.e. twenty three weeks of treatment were 84203, 60268, 44869, 27883, 32864, 53120 and 75500 mg/kg of soil which resulted in 34.21, 52.91, 64.94, 78.21, 74.32, 58.50 and 41.01 percent TPH reduction in bioreactors M1 to M7 respectively (Table 4).

The initial fast degradation phase is mediated by bacterial utilization of bioavailable



compounds and is governed by enzyme kinetics. It was also benefited by adequate nutrients present in the initial weeks. In contrast, slow phase may be governed by the rate of petroleum dissolution from soil particles.

#### **4.4 Degradation Rate Constant (k)**

The biodegradation of hydrocarbons in contaminated soil is assumed to follow the first order degradation, as such the first order degradation rate for various environmental conditions are calculated as follows using first order degradation kinetic equation. The degradation rate constants obtained for the bioreactors are tabulated in the Table 5.

**Table 5: Degradation rate constants for the bioreactors M1 to M7**

Bioreactors	Degradation rate constant (k) d <sup>-1</sup>	
	I Stage (k1)	II Stage (k2)
M1=30% Moisture Content	0.0069	0.00070
M2=40% Moisture Content	0.0129	0.00108
M3=50% Moisture Content	0.0180	0.00149
M4=60% Moisture Content	0.0250	0.00267
M5=70% Moisture Content	0.0226	0.00223
M6=80% Moisture Content	0.0151	0.00125
M7=90% Moisture Content	0.0088	0.00084

The results clearly reflect two distinct phases of biodegradation. The k1 constants were responsible for the first stage of fast degradation and k2 was responsible for second stage degradation. It is thus concluded that the extent of residual concentration in the soil was determined by the biodegradation efficiency during the first stage of treatment when the biological processes dominated. During the following period, abiotic processes leading to reduced bioavailability of TPH were limiting the biodegradation rate.

## **II. CONCLUSIONS**

Moisture content of the petroleum contaminated soil had profound influence on bioremediation, since bioremediation efficiency varies with different moisture content. The study revealed that percentage reduction of TPH concentration in bioreactors having 30%, 40% and 50% moisture content was 34.21%, 52.91% and 64.94% respectively. Maximum degradation of 78.21% was observed for 60% moisture content. There after the percentage reduction of TPH gradually decreased for 70%, 80% and 90% moisture content as 74.32 %, 58.50% and 58.50 % respectively. The control bioreactor MC showed a TPH reduction of 22.49%. Therefore it is concluded that the optimal conditions for better degradation of TPH is moisture content of 60% field capacity under the C:N:P ratio of 100:10:1.

Two distinct phases of biodegradation were observed during the long term treatment of the TPH contaminated soil. Thereby, there were two degradation rate constants (k1 and k2) obtained for the study period of 23 weeks. The k1 constants were responsible for the first stage of fast degradation and k2 was responsible for second stage degradation. The degradation rate constants of the rapid phase (k1) ranged from 0.0069 to 0.0250, whereas, for the slow degradation phase the degradation rate (k2) ranged from 0.00070 to 0.00267 for bioreactors M1 to M7. It is thus concluded that the extent of residual concentration in the soil was determined by the biodegradation efficiency during the first stage (seven weeks) of treatment when the biological processes dominated. During the second stage, abiotic processes leading to reduced bioavailability of TPH were limiting the biodegradation rate. Therefore, as the first few weeks of treatment determine its efficiency, efforts to enhance the biological activity should be directed to that period.

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