OPTIMIZATION OF TPH CONCENTRATION FOR THE BIOREMEDIATION OF DIESEL CONTAMINATED SOIL

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ABSTRACT

Extensive use of diesel and other petroleum products has caused their intrusion into soil environment and threatens the quality of soil and groundwater. Bioremediation techniques are used to destroy/render harmless various contaminants using natural biological activity; hence they have been extensively used for cleanup of petroleum contaminated sites. The present study focuses on biodegradation of diesel contaminated soil in order to optimize Total Petroleum Hydrocarbons (TPH) concentration required for efficient degradation of contaminant. 28 bioreactors was setup for the study, which involved seven bioreactors with different TPH concentrations, each having four replicates. The bioreactors were filled with fresh soil to which commercial diesel was added to attain different TPH concentrations of 1%, 2%, 4%, 6%, 8%, 10% and 12% by weight of soil. The bioreactors were maintained at optimum environmental conditions throughout the study period of ten weeks. The contaminated soil was analyzed for various physico-chemical and biological characteristics regularly on weekly basis. From the study, it was concluded that maximum percentage of TPH removal of 90.03% was observed in the bioreactor having an initial TPH concentration of 8%. A reduction from 80020 mg/kg of weight of soil to 7963 mg/kg of weight of soil was witnessed with a maximum degradation rate of $0.0377d^{-1}$*. The other bioreactors did not exhibit efficient removal of TPH as compared to bioreactor having 8% initial TPH concentration. This indicated that biological activity was greatly influenced by contaminant concentration, which in turn affected the degradation of hydrocarbons.*

Keywords: *Bioremediation, degradation rate, diesel, total petroleum hydrocarbons.*

1. INTRODUCTION

Contaminated lands generally resulted from past industrial activities when awareness of health and environmental effects connected with the extraction, use and disposal of hazardous substances were less recognized than today. This problem is worldwide, and the estimated number of contaminated sites is significantly high. These contaminated lands are recognized as potential threats to human health, which led to international efforts to remediate many of these sites, either as a response to the risk of adverse health or environmental effects caused by contamination or to enable the site to be redeveloped for use.

In response to a growing need to address environmental contamination, the biological, physical, and chemical technologies are currently available for dealing with soil and groundwater contaminated by petroleum and related products. Bioremediation of contaminated soils offers a number of advantages over the conventional treatment methods on the basis of its environmental friendliness and low costs by harnessing the degradative potential of biological systems [1]. The present study was hence focused on the biodegradation of diesel contaminated soil. Efforts were also made to optimize the TPH concentration required for better and efficient degradation of the contaminant.

2. LITERATURE REVIEW

2.1 Total petroleum hydrocarbons

Petroleum means rock oil. Petroleum is a natural organic material principally composed of hydrocarbons (83-87% Carbon and 11-15% Hydrogen) and small quantities of non-hydrocarbons such as Nitrogen (0-0.5%), Sulphur (0-6%) and Oxygen (0-0.5%). Petroleum hydrocarbons are classified into several major categories such as aliphatic compounds, aromatic compounds and polycyclic aromatic compounds based on their composition.

Total Petroleum Hydrocarbons (TPH) is a broad family of several hundred chemical compounds that originally come from crude oil. TPH is defined as the measurable amount of petroleum-based hydrocarbons in an environmental media. It is thus dependent on analysis of the medium in which it is found. Since it is a measured, gross quantity without identification of its constituents, the TPH "value" still represents a mixture.

2.2 Diesel

Diesel is one of the most important petroleum refinery products. Diesel like all fossil fuels primarily consists of complex mixture of hydrocarbons molecules with 15-18 carbon atoms and boiling point within the range of approximately 175°C-370°C [2]. The composition of diesel is approximately 30% alkanes, 45% cyclic alkanes, and 24% aromatics [3]. It contains low molecular weight compounds that are usually more toxic than long-chained hydrocarbons, because the long-chained hydrocarbons are less soluble and less bioavailable. Whereas, the light oils contain relatively high proportion of saturated hydrocarbons which can be more toxic than heavy oils.

2.3 Bioremediation

Bioremediation is the use of living organisms, primarily microorganisms, to degrade the environmental contaminants into less toxic forms. Naturally occurring bacteria and fungi are used to degrade or detoxify substances that are hazardous to human health and/or the environment. The prefix "bio" in the word bioremediation defines the process as biological that is carried out by living organisms. The noun "remediation" defines the process as one that results in the cleaning or remediation of the environment via complete degradation, sequestration, or removal of the toxic pollutants as the result of microbial activity.

2.4 Factors influencing bioremediation process

2.4.1 pH: Petroleum consuming microorganisms grow best at a pH range of 6.5 to 8.0. For soils having high concentrations of volatile compounds and low alkalinity (pH < 7), limiting becomes necessary.

2.4.2 Temperature: Most of the soil microorganisms will be active at a temperature of 20°C to 45°C. Heat generated from biodegradation reactions allows operation of bioremediation process successfully.

2.4.3 Moisture Content: The moisture content should be maintained at 35-60% of the water holding capacity, which means that the soil should not be too dry or too wet.

2.4.4 Nutrient Requirements: Optimum nutritional requirement of C:N:P is 100:10:1 for bioremediation of hydrocarbons. These nutrients i.e. carbon, nitrogen and phosphorous are the basic building blocks of life and allow microbes to create enzymes to break down contaminants.

2.4.5 Microbes: Degradation of TPH depends on having sufficient microbes to degrade the oil through their metabolic pathways. Throughout the world there are over 70 genera of microbes that are known to degrade hydrocarbons. If microbes are not present in a system they can be added (bioaugmentation) to promote bioremediation.

2.4.6 Oxygen (Electron Acceptor): Biodegradation is predominantly an oxidation process. Bacterial enzymes will catalyze insertion of oxygen into hydrocarbons so that molecule can be consumed by metabolism. Primary source of oxygen for biodegradation is the atmospheric oxygen.

2.4.7 Concentration of pollutants: High concentration of TPH will reduce the amount of oxygen, water and nutrients that are available to microbes. This creates an environment where in the microbes are stressed reducing their ability to breakdown the oil.

3. MATERIALS AND METHODS

Soil was excavated from an open field near the Civil Engineering Department, UVCE, JB Campus, Bangalore at a depth of 50m from the ground surface. The soil was air dried, pulverized and sieved through 4.75mm sieve. The soil passing through 4.75mm sieve and retained on 75micron sieve was taken for the study. The physico-chemical and biological analysis was carried in order to test the suitability of the soil for bioremediation (Table 1). Commercial diesel was collected from a petrol station located 3 km away from JB Campus. A total of 28 bioreactors was setup for the study. This comprised of seven bioreactors with different TPH concentrations, each having four replicates. Each bioreactor was filled with 5kg of fresh soil to which commercial diesel was added to attain different TPH concentrations of 1%, 2%, 4%, 6%, 8%, 10% and 12% by weight of soil and were represented as bioreactor T1, T2, T3, T4, T5, T6 and T7 respectively.

The study was carried out in the Environmental Engineering laboratory, Civil Engineering Department, UVCE by maintaining the environmental parameters affecting bioremediation process at optimum conditions.

The pH was maintained within a range of 6.5-8.0, temperature was laboratory temperature i.e. 20-30ºC, moisture content at 60% (+/-) 10% of field capacity and C:N:P at 100:10:1 in all the bioreactors. The bioreactors were analyzed regularly on a weekly basis for various physico-chemical and biological characteristics for a period of 10 weeks. The weekly concentration of TPH in the bioreactors was also enumerated simultaneously to estimate the reduction of TPH.

4. RESULTS AND DISCUSSION

Test results of fresh soil and contaminated soil obtained for various test parameters (average values considering all the replicates have been depicted) for all the bioreactors during the course of the study are shown in Table 1 and 2 respectively.

Table 1: Physico – chemical and Biological Characteristics of Fresh Soil

Parameters	Unit	Fresh Soil Concentrations
Type of Soil	---	Sandy (well graded)
Porosity	%	32
Co-efficient of Uniformity, Cu	---	5.2
Co-efficient of Curvature, Cc	---	1.2
Water Holding Capacity	%	33
pH	---	7.83
Temperature	°C	28
Moisture Content	%	19.27
Total Organic Carbon	mg/gm of soil	24.6
Total Petroleum Hydrocarbons	mg/kg of soil	0
Nitrogen	mg/gm of soil	2.62
Phosphorous	mg/gm of soil	0.24
Microbial Count	CFU/gm of soil	$50x10^5$
Chromium	mg/kg of soil	0.66
Zinc	mg/kg of soil	0.08
Manganese	mg/kg of soil	0.28
Iron	mg/kg of soil	1.27

Table 2: Test Results for the various Physico – chemical and Biological Characteristics of Contaminated Soil in the Bioreactors

Bioreactor	Initial TPH Concentration	Concentration/ Value	Parameters						
			pH	Temperature (°C)	TOC (mg/gm of soil)	TPH (mg/kg of soil)	Nitrogen (mg/gm of soil)	Phosphorus (mg/gm of soil)	Bacterial Count (CFU/gm×10^6)
T1	1%	IR	7.90	24.2	23.21	10090	2.98	0.31	28
		FR	7.60	24.1	11.14	5675	0.98	0.09	22
T2	2%	IR	7.87	24.1	35.29	20290	3.61	0.40	35
		FR	7.15	23.0	12.61	7429	1.31	0.12	41
T3	4%	IR	7.81	24.8	65.85	39899	6.64	0.73	47
		FR	7.18	22.7	14.37	8642	1.52	0.14	38
T4	6%	IR	7.75	24.9	82.09	60420	8.39	0.85	44
		FR	6.86	22.1	14.37	9645	0.95	0.10	42
T5	8%	IR	7.73	24.4	101.45	80020	10.30	1.00	67
		FR	6.91	22.4	11.75	7963	1.08	0.10	56
T6	10%	IR	7.68	24.5	107.75	100000	10.85	1.12	21
		FR	6.93	22.4	22.13	23861	2.61	0.25	28
T7	12%	IR	7.56	24.4	122.86	120000	13.12	1.41	16
		FR	6.89	22.5	58.38	58020	5.66	0.54	28

* IR= Initial reading (0^{th} day), FR= Final reading (70^{th} day/end of 10^{th} week)

* The concentrations depicted in the tables are the averaged values obtained by considering all the four replicates of each bioreactor.

4.1 Analysis of Data and Interpretation

4.1.1 pH

Adverse pH conditions affect the microbial growth and modify the soil chemistry. In the present study, pH decreased with time. The pH in all the bioreactors was well within the optimum pH range from 6.5 to 8.0 during study period.

4.1.2 Temperature

Petroleum contaminants in the soil form a dark surface coating, which increases the capacity of soil to absorb heat thus raising the subsurface soil temperature [4]. The temperature in the present study varied in the rangeof 22.1°C to 24.9°C during the study period in all the bioreactors. Hence, the observed values fall within the optimum temperature range that is suggested to be ideal for bioremediation i.e. 15°C – 45°C.

4.1.3 Moisture Content

During the study period the soil moisture level was maintained at 60 (+/-) 10% of field capacity. Water was added to obtain the desired level of moisture content.

4.1.4 Total Organic Carbon

Organic compounds serve as sources of carbon and can be estimated based on concentrations of total organic carbon. On comparing the TOC reduction, a maximum reduction from 101.45 mg/gm to 11.75 mg/gm of soil was observed in the bioreactor with 8% initial TPH concentration. Thus, maximum carbon utilization was observed in the bioreactor which showed the highest percentage of TPH reduction.

4.1.5 Nutrient Concentration

For effective degradation, microbes must be supplemented with sufficient nutrients since they produce necessary enzymes to break down the contaminants. Nutritional requirement of carbon to nitrogen ratio is 10:1 and carbon to phosphorous is 30:1 [5]. During the study calculated amounts of nitrogen (Ammonium nitrate) and phosphorus (Super phosphate) were added to obtain C:N:P ratio of 100:10:1.

4.1.6 Microbial activity

Microbial populations in the bioreactors increased from the third week of study and significantly decreased in the later weeks. In bioreactor T5 (initial 8% TPH) the microbial counts varied from 67×10^6 CFU/gm to 126×10^6 CFU/gm of soil till sixth week and then decreased to 56×10^6 CFU/gm of soil at the end of 10^{th} week. Thus, increase in bacterial counts had a profound influence on the rate of TPH reduction.

4.1.7 Total Petroleum Hydrocarbons

TPH reduction in the bioreactors ranged between a minimum of 44% in bioreactor T1 to a maximum of 90.03 % in bioreactor T5 during the ten weeks of treatment. The weekly TPH concentrations in different bioreactors during the study period are tabulated in Table 3. Substrate concentration of 8% TPH in bioreactor T5 seems better for the indigenous microorganism to grow and thereby cause increase in degradation of TPH.

Maximum reduction of TPH was observed during 4th and 5th week of the study period, which could be correlated to the microbial growth pattern. The percentage reduction of TPH in the bioreactors during the study period is tabulated in Table 4 and depicted in Fig.1.

Table 3: Weekly TPH Concentrations of the Bioreactors having different Initial TPH Concentrations

Week	Bioreactors(TPH concentration, mg/kg of soil)						
	T1 1% TPH	T2 2% TPH	T3 4% TPH	T4 6% TPH	T5 8% TPH	T6 10% TPH	T7 12% TPH
Week 0	10090	20290	39899	60420	80020	100000	120000
Week1	9642	17824	36895	56247	72154	99707	106893
Week 2	8726	13845	30527	52394	63182	92756	95842
Week 3	7618	10723	23859	47621	43258	62475	71947
Week 4	7190	9257	17685	38954	36364	52961	68618
Week 5	6275	8567	13846	31567	19645	33860	62834
Week 6	6054	8156	12941	28440	15213	29576	61297
Week 7	5932	8029	11864	19462	13802	27239	61008
Week 8	5882	7824	10964	16852	9562	26635	59152
Week 9	5795	7506	9654	14675	8234	24835	58020
Week 10	5675	7429	8642	9645	7963	23861	58483

Table 4: Overall TPH Reduction in the Bioreactors

Bioreactor	Initial TPH (mg/kg)	Final TPH (mg/kg)	TPH Reduction (mg/kg)	TPH Reduction (%)	Degradation rate constant (k) d^{-1}
T1 (1% TPH)	10090	5675	4415	44.00	0.0088
T2 (2% TPH)	20290	7429	12861	63.40	0.0142
T3 (4% TPH)	39899	8642	31257	78.34	0.0231
T4 (6% TPH)	60420	9645	50775	84.04	0.0262
T5 (8% TPH)	80020	7963	72057	90.03	0.0377
T6 (10% TPH)	100000	23861	76139	76.14	0.0242
T7 (12% TPH)	120000	58483	61517	51.26	0.0103

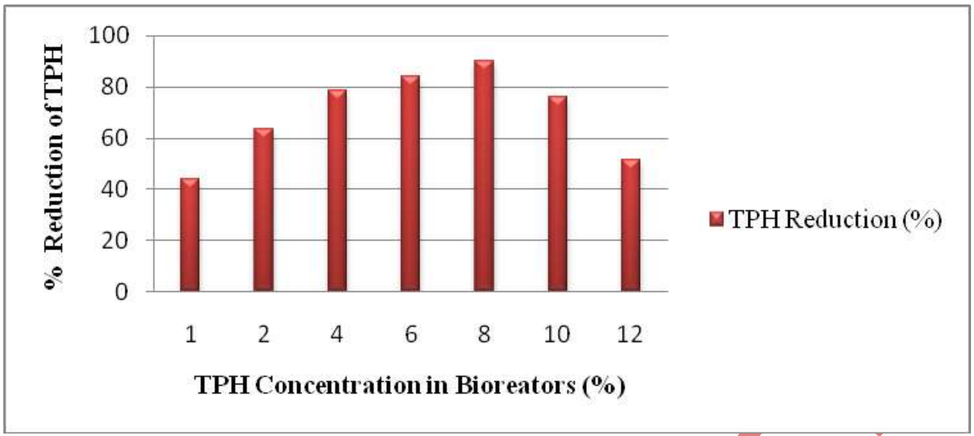

Figure 1: TPH Reduction in the Bioreactors T1 to T7

4.2 Biodegradation Rate

Maximum degradation rate was observed in bioreactor T5 (Fig. 2) having an initial TPH concentration of 8% with a percentage reduction of 90.03%. The maximum degradation rate was 0.0377 d^{-1}.

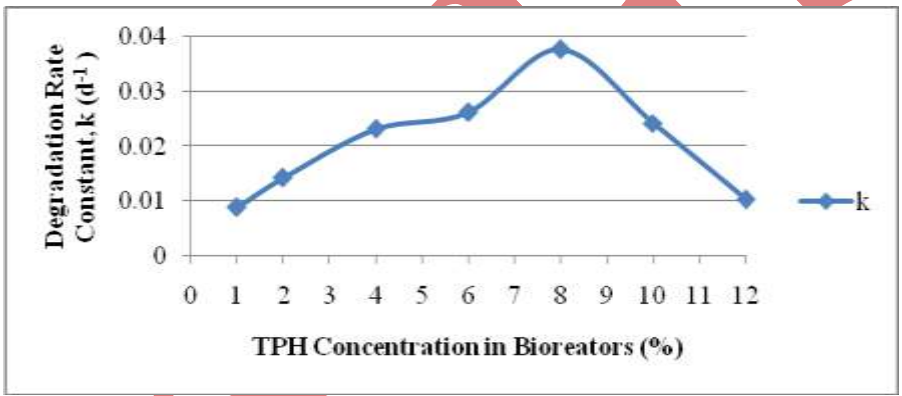

Figure 2: Degradation Rate Constant for all Bioreactors

5. CONCLUSIONS

The study showed that the TPH (substrate) concentration plays a significant role in determining the degradation rate and residual concentrations of the contaminants. The efficiency of TPH reduction in bioreactors having 1%, 2%, 4%, 6% initial TPH concentration was 44.00%, 63.40%, 78.34% and 84.04% respectively and maximum reduction of 90.03% was attained in bioreactor having 8% initialTPH concentration. Bioreactors with 10% and 12% initial TPH concentrations showed reduced degradation efficiencies of 76.14% and 51.26% respectively. Therefore, the highest rate of degradation was observed in bioreactor having initial 8% TPH concentration. Therefore, TPH (substrate) concentration of 8% seems to be advantageous for the growth of indigenous microorganism, which would thereby cause increase in degradation of TPH.

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