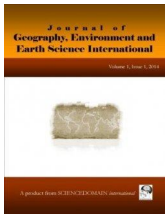

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Biodegradation of Hydrocarbons Present in Soils Contaminated with Different Petroleum Products

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Authors' contributions

This work was carried out in collaboration between both authors. Author DP managed the literature searches, designed the study, performed the analysis, wrote the protocol and wrote the first draft of the manuscript. Author BSG managed the analysed results of the study and reviewed the draft. Both the authors read and approved the final manuscript.

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ABSTRACT

The contamination of soil by petroleum hydrocarbons has resulted in an increased attention towards the development of sound and innovative technologies for its remediation. Biodegradation of hydrocarbons by natural populations of microorganisms is the most eco-friendly and economically viable method for the management of petroleum contaminated sites.

The present study uses the potential of indigenous microorganisms to remediate soil contaminated with different petroleum products. The significance of molecular composition of the hydrocarbons present in the contaminated matrix in deciding the biodegradation rate is illustrated in this work. Two sets of bioreactors were set up for the study, wherein each set had 16 bioreactors. Each bioreactor was filled with fresh soil and contaminated with four different substrates or petroleum products (i.e. kerosene, diesel, lubricating oil and waste oil). One set was maintained at optimum environmental conditions and the other set with no maintenance throughout the study period of 12 weeks and served as the control.

Maximum percentage of total petroleum hydrocarbon (TPH) removal of 85.76% with a degradation rate of 0.0232 d^{-1} was observed in bioreactor contaminated with diesel and maintained at optimum environmental conditions. Minimum percentage of TPH removal of 40.84% with a degradation rate

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of 0.0062 d^{-1} was observed in bioreactor contaminated with waste oil with no maintenance of environmental conditions. The degradation rate was low in control setup. Hence, it could be inferred that environmental conditions have influence on the degradation rate and residual concentrations of the contaminants. Higher degradation was observed in lighter fractions of petroleum when compared to heavier fractions in both the setups. This strengthens the fact that lighter hydrocarbons evaporate in normal conditions and are degraded rapidly, while very long chain alkanes are increasingly resistant to microbial degradation.

Keywords: Petroleum hydrocarbons; biodegradation; petroleum products; lighter fractions; heavier fractions.

1. INTRODUCTION

Petroleum is the principle source of energy and it is used widely in our daily life. Due to this massive use, petroleum has become the most common contaminant of large soil surfaces and eventually is considered as a major environmental problem [1]. Petroleum hydrocarbon induced contamination in soil is due to leaks from underground storage tanks and spills from either pipeline ruptures or tank rail derailments [2,3]. These pollution problems often result in huge disturbances of both 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 organo-pollutants [5]. Although most of the physicochemical methods can be efficient for treating a wide range of pollutants/contaminants, they are extremely expensive [6]. Bioremediation 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 [7].

2. LITERATURE REVIEW

Biological agents, mainly microorganisms i.e. yeast; fungi or bacteria are used to clean up contaminated soil and water [8]. 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 [9]. Hence, it is recommended to treat hydrocarbon-contaminated sites by bio-remediation.

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 [10]. Optimizing these environmental conditions could enhance contaminants biodegradation in the soil [11]. The extent of hydrocarbon biodegradation in contaminated soils is critically dependent upon three factors: a) the creation of optimal environmental conditions to stimulate biodegradative activity, b) the predominant petroleum hydrocarbon types in the contaminated matrix and c) the bioavailability of the contaminants to microorganisms [12]. Additionally, the petroleum hydrocarbon degradation is also affected by the molecular composition of the hydrocarbons [13].

Over the years, lot of studies has been reported on petroleum hydrocarbon degraders [14]. But, there is no comprehensive and conclusive report on the kinetics of biodegradation of crude oil [15]. Few works have been dedicated to investigate the kinetics of soil bioremediation [16,17,18]. 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 [19]. 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 [20,21].

Although there is extensive work done on the degradation of crude oil, comparative studies on the bioremediation using different sources of substrates for contamination are very scarce. Thus, studies on the degradation of different substrates become necessary. Such studies become important since the degradation pattern and kinetics vary for different petroleum fractions owing to their difference in molecular compositions. Hence, this research is geared towards studying the biodegradation of hydrocarbons using different sources of substrates for contamination of the soil.

3. METHODOLOGY

The experiments were designed to investigate the reduction of Total Petroleum Hydrocarbons (TPH) in the soil contaminated with four different substrates i.e. petroleum fractions viz., kerosene, diesel, lubricating oil and waste oil. The study was conducted by setting up 32 bioreactors. Two sets of bioreactors were set up, wherein each set had 16 bioreactors (along with their replicates). The bioreactors were filled with three kilograms of fresh soil. The soil was tested for its physical characteristics to ascertain its suitability for bioremediation. Various chemical and biological analyses were also carried out for the fresh soil (Table 1). Every four bioreactors of each set were contaminated with different substrates to obtain around 10% initial TPH concentration.

The Set 1 bioreactors were maintained at optimum environmental conditions. According to the data available from previous literatures, oxygen was enhanced by means of turning the soil daily, moisture content was maintained at 60% field capacity by adding water, temperature was monitored regularly which was operated under laboratory conditions to maintain the medium. The pH was checked regularly to maintain an optimum range of 6.5 to 8. C: N: P ratio was monitored regularly to maintain an optimum range of 100:10:1. Set 2 bioreactors had no maintenance of environmental conditions throughout the study period of 12 weeks and served as the control. The capacity of indigenous microbes to degrade the petroleum hydrocarbons under natural conditions was observed in this treatment.

Set 1 bioreactors were denoted as $B_{K(O)}$, $B_{D(O)}$, $B_{L(O)}$ and $B_{W(O)}$ since they were contaminated with kerosene (K), diesel (D), lubricating oil (L) and waste oil (W) and maintained at optimum conditions (O) and Set 2 bioreactors used as control (C) were denoted as $B_{K(C)}$, $B_{D(C)}$, $B_{L(C)}$ and $B_{W(C)}$. The replicates of bioreactors were labeled as a, b, c, and d for both sets. Soil samples collected from bioreactors were analyzed regularly for various physical, chemical and biological parameters on a weekly basis for a study period of 12 weeks. The efficiency of bio-treatment was assessed for all the bioreactors.

4. RESULTS AND DISCUSSION

The physico-chemical and biological characteristics of the soil were analyzed before utilizing it for the study (Table 1). The contaminated soil samples collected from the bioreactors were analyzed regularly for various physical, chemical and biological parameters on a weekly basis for a study period of 12 weeks and the results (average of the replicates) of Set 1 and Set 2 bioreactors are shown in Tables 2 and 3.

The readings for bioreactors $B_{K(O)}$ and $B_{K(C)}$ are not tabulated since the TPH content of these bioreactors could not be assessed properly since most of the TPH content present in kerosene contaminated soils would be lost due to volatilization and evaporative losses and some of the residue would be evaporated during TPH extraction procedure. In many of the on-site and field investigations there are evidences to prove that maximum degradation of TPH is achieved for lighter fractions of petroleum i.e. kerosene and minimum/slower degradation rates were observed in heavier fractions such as waste oil as lighter hydrocarbons evaporate in normal conditions. Hence, values were not recorded for these bioreactors and the study of kerosene bioremediation was ignored considering the fact that kerosene was easily remediated in soil even without the intervention of engineered/monitored bioremediation practices.

4.1 Total Petroleum Hydrocarbons Degradation

The effect of time on petroleum degradation was significant. The petroleum degradation rate decreased with increasing time and this observation corresponded with the bacterial growth results. The TPH reduction in bioreactors $B_{D(O)}$, $B_{L(O)}$ and $B_{W(O)}$ during 12 weeks period was 85.76%, 72.52% and 69.86% respectively. The control setup $B_{D(C)}$, $B_{L(C)}$ and $B_{W(C)}$ which had not received bio-stimulation showed a reduction of 68.05%, 48.11% and 40.84% during the study period. Hence, higher TPH removal was witnessed in Set 1 bioreactors when compared with the control setup (Set 2). The differences in hydrocarbon biodegradation between the treated and control setup were due to the large differences in bacterial numbers. Maximum reduction of TPH was observed during the 4^{th} week of treatment in both the setups.

Susceptibility of a hydrocarbon to microbial degradation varies with type and size of the hydrocarbon molecule. Alkanes of intermediate chain length (C_{10}–C_{24}) are often degraded rapidly, while very long chain alkanes are increasingly resistant to microbial degradation. Respiration of microbes occurred very rapidly

during the initial period of incubation when the lighter and more readily degraded fractions were degraded but slowed down as the residue became more difficult to degrade on account of the increase of the heavier fractions. Table 4 shows the weekly reduction of TPH concentration in different bioreactors. The TPH concentration at the end of each week in all the bioreactors is illustrated in Fig. 1.

The weekly percentage TPH reduction for all the bioreactors is shown in Table 5. The percentage of TPH reduction in different bioreactors is depicted in Fig. 2.

Table 1. Physico-chemical and biological characteristics of fresh soil

Parameters	Unit	Fresh soil concentrations
pH	---	7.204
Temperature	°C	23.2
Co-efficient of Uniformity, Cu	---	4.6
Co-efficient of Curvature, Cc	---	1.2
Porosity	%	36.7
Water Holding Capacity	%	32.4
Moisture Content	%	2.04
Total Organic Carbon (TOC)	mg/gm of soil	31.12
Total Petroleum Hydrocarbon (TPH)	mg/kg of soil	0
Nitrogen	mg/gm of soil	3.18*
Phosphorous	mg/gm of soil	0.29*
Bacterial Count	CFU/gm of soil	$46x10^5$

** These values represent the concentrations of nitrogen and phosphorus after amending them with ammonium nitrate and super phosphate to bring them to optimum C: N: P ratio of 100: 10:1*

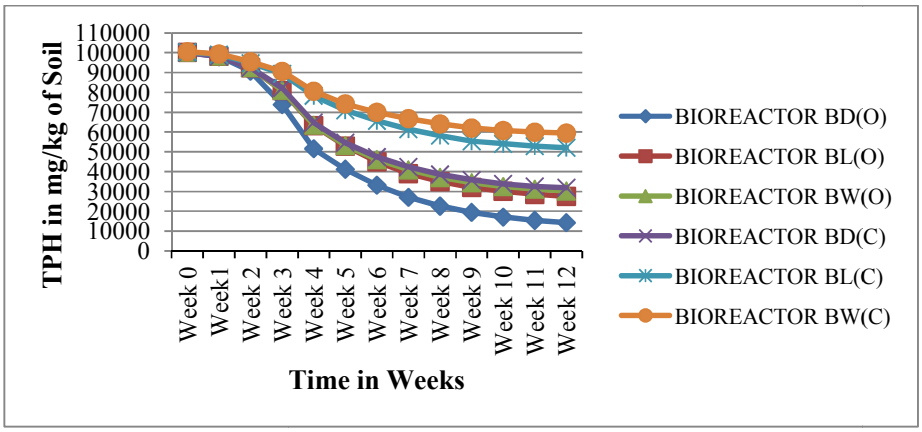

Fig. 1. TPH concentration at the end of different weeks for set 1 and set 2 bioreactors

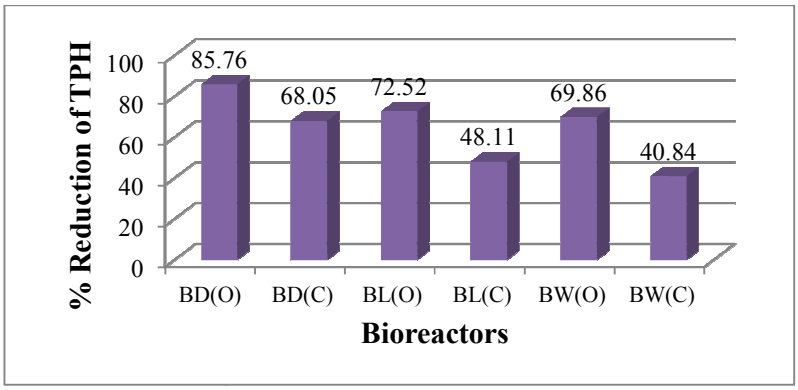

Fig. 2. Percentage of TPH reduction in different bioreactors

Table 2. Initial and final characteristics of the contaminated soil in set 1 bioreactors

Physico-chemical and biological parameters/ characteristics	Set 1 bioreactors					
	$B_{D(O)}$		$B_{L(O)}$		$B_{W(O)}$	
	IR	FR	IR	FR	IR	FR
pH	7.73	6.55	7.70	7.10	7.76	7.08
Temperature (°C)	20.6	22.8	20.7	22.7	20.8	22.6
TOC (mg/gm of soil)	101.45	11.86	91.61	24.82	89.60	29.93
TPH (mg/kg of soil)	100200	14270	100300	27560	100400	30260
Nitrogen (mg/gm of soil)	11.45	0.86	9.20	2.54	9.01	2.86
Phosphorus (mg/gm of soil)	1.25	0.1	0.94	0.22	0.90	0.30
Bacterial Count (CFU/gm×10^6)	32	48	34	35	33	32

Table 3. Initial and final characteristics of the contaminated soil in set 2 bioreactors

Physico-chemical and biological parameters/ characteristics	Set 2 bioreactors					
	$B_{D(C)}$		$B_{L(C)}$		$B_{W(C)}$	
	IR	FR	IR	FR	IR	FR
pH	7.81	6.55	7.58	7.03	7.80	7.11
Temperature (°C)	20.6	22.8	20.8	22.7	20.9	22.8
TOC (mg/gm of soil)	102.00	38.06	92.50	45.92	86.90	52.60
TPH (mg/kg of soil)	100100	31980	100350	52080	100560	59490
Nitrogen (mg/gm of soil)	9.2	0.62	7.80	3.91	7.67	4.51
Phosphorus (mg/gm of soil)	1.05	0.09	0.69	0.36	0.61	0.42
Bacterial Count (CFU/gm×10^6)	24	32	35	23	32	20

** Note: IR indicates initial reading (concentration on the 0th day) and FR indicates final reading (concentration on the 84th day i.e. at the end of 12th week of treatment)*

Table 4. TPH concentration in the bioreactors during the study period

Weeks	Total petroleum hydrocarbons (mg/Kg of soil)					
	$B_{D(O)}$	$B_{L(O)}$	$B_{W(O)}$	$B_{D(C)}$	$B_{L(C)}$	$B_{W(C)}$
Week 0	100200	100300	100400	100100	100350	100560
Week1	98056	98355	98455	98150	99050	99400
Week 2	90707	92480	92600	92457	94706	95460
Week 3	73860	80950	81110	82343	89430	90636
Week 4	51621	63210	63500	64836	78330	80610
Week 5	41258	52980	53260	54642	70962	74240
Week 6	33209	44950	46105	47472	65702	70000
Week 7	27036	39010	40990	42419	61509	66800
Week 8	22625	35050	37140	38625	58250	64100
Week 9	19435	32060	34506	35850	55350	62010
Week 10	17107	30100	32490	33816	54060	60800
Week 11	15382	28585	31180	32502	52900	59950
Week 12	14270	27560	30260	31980	52080	59490

4.2 Biodegradation Rate

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.

$$S = S_0 e^{-kt}$$

Where, S is the concentration of hydrocarbons (mg/kg) at time t, t refers to the study period (day), S_0 is the initial concentration of hydrocarbons (mg/kg) and k is the rate constant of the change in the hydrocarbon content (day^{-1}).

The degradation rate constant "k" of 0.0232 d^{-1}, 0.0154 d^{-1} and 0.0143 d^{-1} was observed in Set 1 bioreactors $B_{D(O)}$, $B_{L(O)}$ and $B_{W(O)}$ respectively. Similarly, the degradation rate observed in Set 2 bioreactors $B_{D(C)}$ was 0.0136 d^{-1}, $B_{L(C)}$ was 0.0078 d^{-1} and $B_{W(C)}$ was 0.0062 d^{-1}. The initial TPH, final TPH, overall TPH reduction and degradation rate constant of the bioreactors is shown in Table 6.

Table 5. Weekly TPH reduction percentage in the bioreactors during the study period

Weeks	Weekly TPH reduction (%)					
	$B_{D(O)}$	$B_{L(O)}$	$B_{W(O)}$	$B_{D(C)}$	$B_{L(C)}$	$B_{W(C)}$
Week1	2.14	1.94	1.94	1.95	1.30	1.15
Week 2	7.33	5.86	5.83	5.69	4.33	3.92
Week 3	16.81	11.50	11.44	10.10	5.26	4.80
Week 4	22.19	17.69	17.54	17.49	11.06	9.97
Week 5	10.34	10.20	10.20	10.18	7.34	6.33
Week 6	8.03	8.01	7.13	7.16	5.24	4.22
Week 7	6.16	5.92	5.09	5.05	4.18	3.18
Week 8	4.40	3.95	3.83	3.79	3.25	2.68
Week 9	3.18	2.98	2.62	2.77	2.89	2.08
Week 10	2.32	1.95	2.01	2.03	1.29	1.20
Week 11	1.72	1.51	1.30	1.31	1.16	0.85
Week 12	1.11	1.02	0.92	0.52	0.82	0.46

Table 6. Overall TPH reduction and degradation rate constants of the bioreactors

Bioreactor		Initial TPH (mg/Kg of soil)	Final TPH (mg/Kg of soil)	TPH reduction (mg/Kg of soil)	TPH reduction (%)	Degradation rate constant (k) d^{-1}
Set 1	$B_{D(O)}$	100200	14270	85930	85.76	0.0232
	$B_{L(O)}$	100300	27560	72740	72.52	0.0154
	$B_{W(O)}$	100400	30260	70140	69.86	0.0143
Set 2	$B_{D(C)}$	100100	31980	68120	68.05	0.0136
	$B_{L(C)}$	100350	52080	48270	48.11	0.0078
	$B_{W(C)}$	100560	59490	41070	40.84	0.0062

5. CONCLUSIONS

The results indicate that rate of degradation is higher in treated setup compared to controlled setup. It can be noted that nutrients and other optimum environmental conditions influence the degradation rate and residual concentrations of the contaminants. Hence, optimal environmental conditions must be created to stimulate the biodegradative activity of the indigenous microorganisms.

Higher degradation was witnessed in lighter fraction than in heavier fraction in both the setups because lighter hydrocarbons evaporate in normal conditions. This may benefit microorganisms to remove more toxic low molecular weight components such as benzene and smaller n-alkanes, while very long chain alkanes become increasingly resistant to microbial degradation. The predominant petroleum hydrocarbon types present in the contaminated soil matrix, molecular composition of the hydrocarbons and the bioavailability of contaminants to the microbes hence play a pivotal role in determining the fate of bioremediation.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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