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**ENVIRONMENTAL SCIENCE**

# Evaluating the Effect of Substrate Type and Concentration in the Biodegradation of Petroleum Hydrocarbons

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**ABSTRACT**

Remediation of contaminated soil has been considered as an important environmental issue. Biological methods are widely acknowledged and used to alleviate the environmental problems associated with contaminated soil. Biodegradation involves a series of metabolic processes that decompose organic compounds into smaller and simpler subunits by the aid of microorganisms. Biodegradation has a great potential to treat soil and groundwater contaminated by a variety of hazardous chemicals such as refractory organics, oils, benzene, styrene, vinyl chloride, pentachlorophenol, polyaromatic hydrocarbons (PAHs), toluene, xylene, phenols, etc. The present study focuses on the biodegradation of total petroleum hydrocarbons (TPH). The study was carried out to understand the effect of substrate concentration and type on the biodegradation rate and efficiency. Three sets of bioreactors were considered for the study, each set had 24 bioreactors. They were contaminated with different substrates i.e. diesel, lubricating oil and waste oil at different concentrations to attain different initial hydrocarbon concentration. These bioreactors had no maintenance of any of the environmental parameters influencing bioremediation throughout the study period of 60 days. The performance and efficiency of the bio-treatment were evaluated for all the bioreactors. Maximum degradation was observed in lighter fraction of petroleum i.e. diesel and minimum degradation was observed in heavier fraction of petroleum i.e. waste oil, as lighter hydrocarbons evaporate in normal conditions.

**Highlights**

- Higher TPH removal was witnessed in bioreactors contaminated with diesel and minimum degradation was observed in bioreactors contaminated with waste oil.
- Hydrocarbons susceptibility to biodegradation depends on the type and concentration of hydrocarbon molecule present in the polluted soil.
- Extremely high or low hydrocarbon concentration may be lethal to microbial activity and limit the biodegradation potential.

**Keywords:** Biodegradation, total petroleum hydrocarbons, diesel, lubricating oil, waste oil

The evolution of human civilization throughout history has led to growing disruption of the natural balance and the occurrence of different types of pollution. Rapid industrialization and urbanization over the past few decades has resulted in the contamination of air, water, soil and even our food. The intensity of hydrocarbon biodegradation in soil is influenced by several environment factors such as quality and quantity of contaminants, indigenous microbial populations, soil properties, pH, temperature, water content and nutrient availability (Riffaldi R. *et al.* 1994). The purpose of this study was to investigate the reduction of Total Petroleum Hydrocarbons (TPH) in the soil contaminated with different petroleum fractions/ products.

## LITERATURE REVIEW

### Petroleum Hydrocarbons

Petroleum primarily consists of a complex mixture of molecules called hydrocarbons. When petroleum comes out of the ground, it is known as crude oil. A variety of consumer products are made from petroleum through refinement process. Gasoline, jet fuel, diesel fuel, kerosene and propane are the common examples of these fuels. It is also used to make asphalt and lubricant grease, and it is used as a raw material for synthetic chemicals. Petroleum products are complex mixtures of hydrocarbon compounds ranging from light and volatile compounds to heavy, long-chained, branched compounds. The composition of petroleum hydrocarbons varies depending upon the source of the crude oil and refining practices used.

### Petroleum Fractions and Composition

When crude oil is pumped out of the ground it will be a complex mixture of a large number of compounds, most of which are hydrocarbons. The hydrocarbons may consist of molecules based on chains of carbon atoms, sometimes linear (straight) and sometimes branched (a side-chain of C atoms) and others are based on rings of carbon atoms (Doc Brown 2000). Petroleum hydrocarbons can be categorized into four simple fractions based on the hydrocarbon molecule present in the crude oil.

- Saturates (or alkanes);
- Aromatics, including compounds such as benzene, toluene, ethyl benzene and xylenes (BTEX) and polyaromatic hydrocarbons (PAHs);
- Resins, consisting of compound containing nitrogen, sulphur, and oxygen that are dissolved in oil;
- Asphaltenes, which are large and complex molecules colloidally dispersed in oil.

The relative proportions of these fractions are dependent on many factors like source, age, migration, etc. Of these fractions, the shorter alkane chain compounds and lighter aromatics tend to be more readily biodegradable (Talaat Balba 2001). The relative percentage of each varies from oil to oil; determining the properties of different oil. The various components of the fractional distillation of crude oil are given below:

- Refinery Gases (LPG) - very short and easy to boil. These are mostly used for cooking and heating.
- Gasoline (Petrol) - these molecules are a little longer but still evaporate easily, flow well and have little colour.
- Naphtha - used to make new substances and is not usually used as a fuel.
- Kerosene - thicker, darker and harder to light but contains more energy. It is a jet-fuel.
- Diesel - thicker, darker and even harder to light but the extra energy makes it a good fuel for trains and trucks.
- Fuel Oil - very thick, only useful for burning in huge engines of ships or for heating purposes since it contains more energy.
- Bitumen - too thick to burn as a fuel, but it is good for tarring roads.

The composition of crude's from different sources varies tremendously, but a typical make-up is as follows: Gases - 5%, Gasoline - 35%, Kerosene - 10%, Diesel - 20%, Lube oil - 2%, Tar and Bitumen - 28%. Boiling ranges are as follows: Gasoline - 40 to 190$^{o}$C, Kerosene - 190 to 260$^{o}$C, Diesel - 260 to 330$^{o}$C, Lube oil - 330 to 400$^{o}$C under vacuum. The very heavy components of the crude (i.e. tars) will not distil even under a vacuum and remain as bottoms or residue (Doc Brown 2000).

### Total Petroleum Hydrocarbons

Total Petroleum Hydrocarbons (TPH) is a term used to describe a broad family of several hundred chemical compounds that originally come from crude oil. They are called hydrocarbons because almost all of them are made entirely from hydrogen and carbon. Crude oils can vary in how much of each chemical they contain and so can the petroleum products that are made from crude oils. The amount of TPH found in a sample is useful as a general indicator of petroleum contamination at that site. However, this TPH measurement or number tells us little about how the particular petroleum hydrocarbons in the sample may affect people, animals and plants (ATSDR 1999).

### Bioremediation

Bioremediation is the process of using micro-organisms (indigenous or introduced) and other manipulations to degrade/detoxify organic substances to harmless compounds, such as carbon dioxide and water, in a confined and controlled environment. Microbes are the key players in bioremediation as they generate the enzymes that catalyze the degradative reactions. The microbes carry out degradative reactions because the microbes use organic substances as a source of carbon and energy. Thus, while transforming the contaminant microbes gain energy and raw material for their multiplication and maintenance. Based on the mechanism by which microbes gain energy, they are broadly categorized into three categories as given in Table 1. However, several xenobiotic contaminants might not be amenable to one of the above described categories and other mechanisms are employed by the microorganisms for degradation of such compounds (Anushree Malik 2006).

**Table 1:** Mechanism of Energy Generation by the Microbes

| Mechanism | Electron Donor | Electron Acceptor | Product |
|---|---|---|---|
| Aerobic respiration | Organic compound | Oxygen | $CO_2$, $H_2O$ |
| Anaerobic respiration | Organic compound | $NO_3$, $SO_4$, $Fe^{3+}$, $Mn^{4+}$, $CO_2$ | $N_2$, $H_2S$, $CH_4$, reduced metals |
| Fermentation | Organic compound | Organic compound | Organic acids, alcohols, $H_2$ and $CO_2$ |

## Methodology

The experimental methodology was formulated to investigate the TPH reduction of different substrates or petroleum fractions in the contaminated soil and to understand the effect of concentration and type of substrate on the biodegradation rate.

## Experimental Setup

A total of 72 bioreactors were used for the study. The study was carried out by setting up three sets of bioreactors (each set had 24 bioreactors) in the Environmental Engineering Laboratory, Department of Civil Engineering, U.V.C.E, JB Campus, Bangalore University, Bangalore. Each set was contaminated with different substrates i.e. diesel, lubricating oil and waste oil at different concentrations to attain different initial TPH concentration. The bioreactors had no maintenance of any of the environmental conditions throughout the study period of 60 days. Natural attenuation of TPH was observed in this treatment. The soil brought from the excavation site was used as it is for the study. The capacity of indigenous microbes to degrade the petroleum hydrocarbons ex-situ under natural conditions was observed in this treatment.

The bioreactors of each set were mentioned as $B_{DC}$, $B_{LC}$ and $B_{WC}$ since they were contaminated with diesel (D), lubricating oil (L) and waste oil (W) and had no maintenance of any of the environmental parameters i.e. they can serve as control (mentioned as "C") to any experimental setup where optimum environmental conditions are provided to study the enhancement of bioremediation rate. Bioreactors having different TPH concentration were labeled as $B_{DC1}$, $B_{DC2}$, $B_{DC3}$, $B_{DC4}$, $B_{DC5}$ and $B_{DC6}$ for diesel contaminated soil, $B_{LC1}$, $B_{LC2}$, $B_{LC3}$, $B_{LC4}$, $B_{LC5}$ and $B_{LC6}$ for lubricating oil contaminated soil and $B_{WC1}$, $B_{WC2}$, $B_{WC3}$, $B_{WC4}$, $B_{WC5}$ and $B_{WC6}$ for waste oil contaminated soil. The replicates of these bioreactors were labeled as a, b, c, and d for all the three sets. The performance and the efficiency of the bio-treatment were evaluated for all the bioreactors.

## RESULTS AND DISCUSSION

The physico-chemical and biological characteristics of fresh soil were analyzed before utilizing the soil for the study. The characteristics of fresh soil are as shown in Table 2.

**Table 2:** Physico-chemical and Biological characteristics of the Fresh Soil

| Parameters | Unit | Fresh Soil Concentrations |
|---|---|---|
| Co-efficient of Uniformity, Cu | — | 4.78 |
| Co-efficient of Curvature, Cc | — | 0.78 |
| Water Holding Capacity | % | 23.90 |
| pH | — | 7.9 |
| Temperature | °C | 27 |
| Moisture Content | % | 2.30 |
| Total Organic Carbon (TOC) | mg/gm of soil | 11.8 |
| Total Petroleum Hydrocarbons (TPH) | mg/kg of soil | 0 |
| Nitrogen | mg/gm of soil | 1.120 |
| Phosphorous | mg/gm of soil | 0.162 |
| Bacterial Count | CFU/gm of soil | $39\times10^6$ |

**Table 3:** Physico-chemical and Biological Characteristics of Diesel Contaminated Soil in all Bioreactors

| Parameter | Unit | Characteristics of Parameters Tested | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | $B_{DC1}$ | | $B_{DC2}$ | | $B_{DC3}$ | | $B_{DC4}$ | | $B_{DC5}$ | | $B_{DC6}$ | |
| | | IR | FR | IR | FR | IR | FR | IR | FR | IR | FR | IR | FR |
| pH | — | 7.11 | 7.08 | 7.56 | 7.18 | 7.66 | 7.10 | 7.65 | 7.18 | 7.69 | 7.00 | 7.69 | 7.16 |
| Temperature | °C | 26.1 | 30.2 | 26.6 | 31.0 | 26.4 | 31.7 | 26.0 | 31.6 | 26.7 | 30.9 | 26.8 | 31.1 |
| TOC | mg/gm of soil | 22.36 | 18.58 | 50.74 | 41.38 | 61.81 | 49.10 | 76.16 | 57.78 | 92.24 | 65.15 | 118.74 | 93.04 |
| TPH | mg/kg of soil | 24600 | 19500 | 51250 | 39400 | 64900 | 48200 | 84000 | 58800 | 102300 | 64900 | 121000 | 88300 |
| Nitrogen | mg/gm of soil | 2.40 | 1.50 | 5.10 | 4.12 | 6.20 | 4.88 | 7.71 | 5.80 | 9.25 | 6.45 | 11.90 | 9.36 |
| Phosphorus | mg/gm of soil | 0.23 | 0.14 | 0.50 | 0.40 | 0.62 | 0.49 | 0.76 | 0.58 | 0.91 | 0.64 | 1.81 | 0.92 |
| Bacterial Count | CFU/gm×$10^6$ | 27 | 18 | 28 | 22 | 31 | 27 | 30 | 31 | 30 | 34 | 31 | 29 |

**Table 4:** Physico-chemical and Biological Characteristics of Lubricating Oil Contaminated Soil in all Bioreactors

| Parameter | Unit | Characteristics of Parameters Tested | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | $B_{LC1}$ | | $B_{LC2}$ | | $B_{LC3}$ | | $B_{LC4}$ | | $B_{LC5}$ | | $B_{LC6}$ | |
| | | IR | FR | IR | FR | IR | FR | IR | FR | IR | FR | IR | FR |
| pH | — | 7.67 | 7.35 | 7.58 | 7.10 | 7.80 | 7.24 | 7.67 | 7.12 | 7.60 | 7.06 | 7.50 | 7.00 |
| Temperature | °C | 26.3 | 30.6 | 26.3 | 31.5 | 26.7 | 31.4 | 26.8 | 31.1 | 26.5 | 31.8 | 26.2 | 30.1 |
| TOC | mg/gm of soil | 25.95 | 21.87 | 50.95 | 41.70 | 62.45 | 50.24 | 78.97 | 61.22 | 99.05 | 74.21 | 109.91 | 87.78 |
| TPH | mg/kg of soil | 26600 | 21400 | 53500 | 41400 | 66200 | 49800 | 84900 | 61000 | 10050 | 68300 | 121900 | 90900 |
| Nitrogen | mg/gm of soil | 2.60 | 2.15 | 5.10 | 4.00 | 6.31 | 4.98 | 7.90 | 6.10 | 9.90 | 7.40 | 11.01 | 8.67 |
| Phosphorus | mg/gm of soil | 0.26 | 0.20 | 0.48 | 0.41 | 0.64 | 0.44 | 0.78 | 0.59 | 0.95 | 0.75 | 1.10 | 0.88 |
| Bacterial Count | CFU/gm×$10^6$ | 26 | 17 | 28 | 20 | 30 | 24 | 31 | 30 | 31 | 33 | 30 | 26 |

**Table 5:** Physico-chemical and Biological Characteristics of Waste Oil Contaminated Soil in all Bioreactors

| Parameter | Unit | Characteristics of Parameters Tested | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | $B_{WC1}$ | | $B_{WC2}$ | | $B_{WC3}$ | | $B_{WC4}$ | | $B_{WC5}$ | | $B_{WC6}$ | |
| | | IR | FR | IR | FR | IR | FR | IR | FR | IR | FR | IR | FR |
| pH | — | 7.90 | 7.02 | 7.64 | 7.10 | 7.78 | 7.1 | 7.50 | 7.07 | 7.70 | 7.18 | 7.50 | 7.13 |
| Temperature | °C | 26.9 | 31.1 | 26.8 | 31.6 | 26.2 | 31.6 | 26.6 | 31.5 | 27.0 | 31.3 | 26.2 | 31.2 |
| TOC | mg/gm of soil | 28.10 | 23.80 | 57.96 | 47.91 | 70.17 | 56.69 | 88.44 | 68.00 | 113.68 | 89.45 | 153.76 | 124.70 |
| TPH | mg/kg of soil | 30900 | 25000 | 62600 | 48800 | 76900 | 58200 | 93400 | 66200 | 126300 | 90800 | 155000 | 117900 |
| Nitrogen | mg/gm of soil | 2.91 | 2.33 | 5.81 | 4.80 | 7.00 | 5.60 | 8.85 | 6.85 | 11.35 | 8.95 | 15.40 | 12.31 |
| Phosphorus | mg/gm of soil | 0.28 | 0.22 | 0.57 | 0.47 | 0.69 | 0.56 | 0.86 | 0.67 | 1.15 | 0.88 | 1.55 | 1.22 |
| Bacterial Count | CFU/gm×$10^6$ | 26 | 15 | 29 | 20 | 29 | 23 | 31 | 30 | 32 | 29 | 29 | 22 |

* ***Note:*** *IR indicates initial reading (concentration on the* $0^{th}$ *day) and FR indicates final reading (concentration on the* $60^{th}$ *day).*

**Table 6:** The Initial and Final TPH Concentrations and Percentage of TPH Reduction in Different Bioreactors

| Bioreactors | Initial TPH (mg/kg) | Initial TPH (%) | Final TPH (mg/kg) | Final TPH (%) | TPH Reduction (mg/kg) | % TPH Reduction |
|---|---|---|---|---|---|---|
| $B_{DC1}$ | 24600 | 2.460 | 19500 | 1.950 | 5100 | 20.73 |
| $B_{DC2}$ | 51250 | 5.125 | 39400 | 3.940 | 11850 | 23.12 |
| $B_{DC3}$ | 64900 | 6.490 | 48200 | 4.820 | 16700 | 25.73 |
| $B_{DC4}$ | 84000 | 8.400 | 58800 | 5.880 | 25200 | 30.00 |
| $B_{DC5}$ | 102300 | 10.230 | 64900 | 6.490 | 37400 | 36.56 |
| $B_{DC6}$ | 121000 | 12.100 | 88300 | 8.830 | 32700 | 27.02 |
| $B_{LC1}$ | 26600 | 2.660 | 21400 | 2.140 | 5200 | 19.55 |
| $B_{LC2}$ | 53500 | 5.350 | 41400 | 4.140 | 12100 | 22.50 |
| $B_{LC3}$ | 66200 | 6.620 | 49800 | 4.980 | 16400 | 24.77 |
| $B_{LC4}$ | 84900 | 8.490 | 61000 | 6.100 | 23900 | 28.15 |
| $B_{LC5}$ | 100050 | 10.005 | 68300 | 6.830 | 31750 | 31.73 |
| $B_{LC6}$ | 121900 | 12.190 | 90900 | 9.090 | 31000 | 25.43 |
| $B_{WC1}$ | 30900 | 3.090 | 25000 | 2.500 | 5900 | 19.09 |
| $B_{WC2}$ | 62600 | 6.260 | 48800 | 4.880 | 13800 | 22.04 |
| $B_{WC3}$ | 76900 | 7.690 | 58200 | 5.820 | 18700 | 24.32 |
| $B_{WC4}$ | 93400 | 9.340 | 66200 | 6.620 | 27200 | 29.12 |
| $B_{WC5}$ | 126300 | 12.630 | 90800 | 9.080 | 32800 | 26.54 |
| $B_{WC6}$ | 155000 | 15.500 | 117900 | 11.790 | 37100 | 23.94 |

The various physico-chemical and biological characteristics of the contaminated soil (average of the replicates) are tabulated in Tables 3 to 5.

The initial and final TPH and percentage TPH reduction in different bioreactors is shown in Table 6. Percentage of TPH reduction in different bioreactors is shown in Fig. 1.

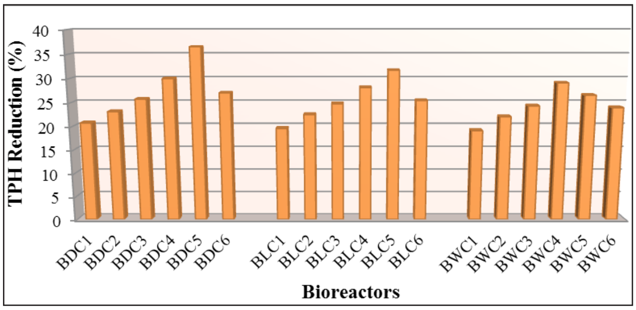


**Fig. 1:** TPH Reduction in all the Bioreactors

Higher TPH removal was observed in soils contaminated with lighter fraction i.e. diesel and less degradation was observed in soils contaminated with heavier fraction of petroleum i.e. waste oil owing to the fact that hydrocarbons susceptibility to microbial degradation depends on the type and size of the hydrocarbon molecule present in the polluted soil. Alkanes of intermediate chain length are degraded rapidly, whereas long chain alkanes are increasingly resistant to microbial degradation. Microbial respiration is very fast during the initial period of incubation when lighter and more readily biodegradable fractions are degraded but gradually slows down as the residue becomes more difficult to degrade due to the increase of the heavier and recalcitrant fractions. Hence, a maximum degradation of 36.56% was observed in $B_{DC5}$ (10.23% initial TPH) followed by $B_{LC5}$ (10.00% initial TPH) showing 31.73% removal and $B_{WC4}$ (9.34% initial TPH) having 29.12% TPH removal.

It was also witnessed that TPH removal rate was very less in bioreactors having extremely high or low initial TPH concentrations since the major factor affecting the rate of biodegradation is the concentration of hydrocarbons in a soil system. Extremely high TPH concentrations have proven to be lethal to microbial activity, thus limiting the biodegradation potential (Admon S. *et al.* 2001). Similarly, extremely low TPH concentrations, while not lethal to organisms, can limit biodegradation because carbon supply may be too low to support microbial growth (Leahy J.H. *et al.* 1990).

The degradation rate constant gives the indication of degradability of an organic compound; high values are preferred for biological treatment to be effective. Table 7 shows the degradation rate constant k for the bioreactors under study.

**Table 7:** Degradation Rate Constants of Different Bioreactors for 60 days

| Bioreactors | Degradation Rate Constant (k) $d^{-1}$ |
|---|---|
| $B_{DC1}$ | 0.0039 |
| $B_{DC2}$ | 0.0044 |
| $B_{DC3}$ | 0.0050 |
| $B_{DC4}$ | 0.0059 |
| $B_{DC5}$ | 0.0076 |
| $B_{DC6}$ | 0.0053 |
| $B_{LC1}$ | 0.0036 |
| $B_{LC2}$ | 0.0043 |
| $B_{LC3}$ | 0.0047 |
| $B_{LC4}$ | 0.0055 |
| $B_{LC5}$ | 0.0064 |
| $B_{LC6}$ | 0.0049 |
| $B_{WC1}$ | 0.0035 |
| $B_{WC2}$ | 0.0042 |
| $B_{WC3}$ | 0.0046 |
| $B_{WC4}$ | 0.0057 |
| $B_{WC5}$ | 0.0055 |
| $B_{WC6}$ | 0.0046 |

The degradation rate constant depends on type and concentration of substrate. Soil contaminated with lighter fraction of petroleum showed highest degradation rate constant "k" of 0.0076 $d^{-1}$ (Bioreactor $B_{DC5}$) when compared to all other bioreactors. Whereas, the least degradation rate constant was observed in waste oil contaminated bioreactor $B_{WC1}$, with a "k" value of 0.0035 $d^{-1}$.

## CONCLUSION

The reduction of TPH in diesel contaminated bioreactors $B_{DC1}$, $B_{DC2}$, $B_{DC3}$, $B_{DC4}$, $B_{DC5}$ and $B_{DC6}$ was 20.73%, 23.12%, 25.73%, 30.00%, 36.56% and 27.02% respectively. Similarly, the TPH reduction in lubricating oil contaminated bioreactors $B_{LC1}$, $B_{LC2}$, $B_{LC3}$, $B_{LC4}$, $B_{LC5}$ and $B_{LC6}$ was 19.55%, 22.50%, 24.77%, 28.15%, 31.73% and 25.43% respectively. Whereas, waste oil contaminated bioreactors $B_{WC1}$, $B_{WC2}$, $B_{WC3}$, $B_{WC4}$, $B_{WC5}$ and $B_{WC6}$ showed a TPH reduction of 19.09%, 22.04%, 24.32%, 29.12%, 26.54% and 23.94% during 60 days of treatment.

Maximum degradation of 36.56% was witnessed in bioreactor $B_{DC5}$ (soils contaminated with lighter fraction of petroleum i.e. diesel) wherein TPH reduced from 102300 mg/kg to 64900 mg/kg of soil and minimum degradation of 19.09% was observed in bioreactor $B_{WC1}$ (soils contaminated with heavier fraction of petroleum i.e. waste oil) wherein TPH degraded from 30900 mg/kg to 25000 mg/kg of soil under natural environmental conditions. This proved the fact that hydrocarbons susceptibility to microbial degradation depends on the type and size of the hydrocarbon molecule present in the polluted soil. It can also be concluded based on the results that extremely high or low TPH concentration may be lethal to microbial activity and limit the biodegradation potential. Thereby, the hydrocarbon degradation rate depends both on the type and concentration of substrate.

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