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LABORATORY STUDIES ON THE BIOREMEDIATION OF SOIL CONTAMINATED BY DIESEL

Dr. B. Santhaveerana Goud
Professor and Former Chairman, Department of Civil Engineering,
University Visvesvaraya College of Engineering,
Bangalore University, Bangalore, Karnataka, India

Fareeda Banu N
Post Graduate Student, Department of Civil Engineering,
University Visvesvaraya College of Engineering,
Bangalore University, Bangalore, Karnataka, India

Prathibha D
Research Scholar, Department of Civil Engineering,
University Visvesvaraya College of Engineering, Bangalore University,
Bangalore, Karnataka, India

ABSTRACT

The most widely used energy and fuel resources are hydrocarbons such as crude oil and petroleum distillates. The accidental discharge of these petroleum products contribute in making hydrocarbons the most common environmental pollutants. Bioremediation helps to destroy or render harmless various contaminants using natural biological activity. The present study utilizes the potential of bioremediation to remediate soil contaminated with diesel. Eight bioreactors were used for the study, out of which four bioreactors were maintained at optimum environmental conditions and the remaining four were kept without any maintenance to serve as control bioreactors. Contaminated soil was prepared by mixing fresh soil and diesel so as to attain 10% TPH concentrations by weight of soil. Each bioreactor was filled with 3 kg of contaminated soil.

About 68.05% of TPH removal was observed in bioreactors used as control and 85.76% of TPH removal was observed in the bioreactors with optimum environmental conditions. Higher TPH degradation was witnessed in the bioreactors maintained at optimum environmental conditions compared to the control setup. The difference in hydrocarbon biodegradation was due to the higher bacterial population observed in bioreactors maintained at *optimum environmental conditions. The microbial count in the bioreactors maintained at optimum environmental conditions varied from 32×10^6 CFU/gm to 87×10^6 CFU/gm in the sixth week and then decreased to 48×10^6 CFU/gm of soil at the end of 12th week. Whereas, in the control bioreactors the microbial count varied from 24×10^6 CFU/gm to 71×10^6 CFU/gm till fifth week and then decreased to 32×10^6 CFU/gm of soil at the end of 12th week. This indicated that biological activity was greatly influenced by the environmental conditions, which in turn affects the degradation of hydrocarbons. The degradation rate observed in bioreactors used as control was 0.0136 d^{-1} and 0.0232 d^{-1} in the bioreactors maintained at optimum environmental conditions.*

Key words: Biodegradation, Bioreactors, Diesel, Environmental Conditions, Hydrocarbons.

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1. INTRODUCTION

Huge quantities of fuel are required for power industries and automobiles. With the number of times each gallon of petroleum is stored, transported, or transferred, accidents and leakages are inevitable, making these hydrocarbons the most common global environmental pollutants (Lily Madhuri Kaushish, 2012). Contaminated soils are a threat to the humans and the ecosystem. To control the environmental risks caused by petroleum products, various new regulations have been introduced and at the same time research focusing on remediation of contaminated soils has increased (Patcharaporn Wongsa, 2004). The microbiological decontamination of oil polluted soils is claimed to be an affordable, eco-friendly, efficient and versatile alternative to physical/chemical treatments. Thus, bioremediation technology has to be developed for treating sites contaminated with variety of pollutants and develop efficient and economical treatment methods.

2. LITERATURE REVIEW

2.1. Petroleum Hydrocarbons

Petroleum is an oily, flammable liquid that occurs naturally in deposits, usually beneath the surface of the earth. It consists principally of a mixture of hydrocarbons with traces of nonmetallic elements such as sulphur, oxygen and nitrogen (Anjana Desai et al., 2006). Compounds such as petrol, diesel, kerosene, naphthalene, bitumen, natural gas and lubricants are derived from fractional distillation of crude oil. The hydrocarbon in crude oil is made up of alkanes, cycloalkanes, phenolics, aromatics, aliphatic and polycyclic aromatic compounds (Adewale Sogo Olalemi et al., 2012).

2.2. Total Petroleum Hydrocarbons

Total Petroleum Hydrocarbons (TPH) is a term used to describe a large family of several hundred chemical compounds that originally come from crude oil. TPH is a mixture of chemicals, but they are all made mainly from hydrogen and carbon, called hydrocarbons. Scientists divide TPH into groups of petroleum hydrocarbons that act alike in soil or water. These groups are called petroleum hydrocarbon fractions. Each fraction contains many individual chemicals. Some chemicals that may be found in TPH are hexane, jet fuels, mineral oils, benzene, toluene, xylenes, naphthalene, and fluorene, as well as other petroleum products like gasoline, diesel and kerosene components. However, it is likely that samples of TPH will contain only some, or a mixture, of these chemicals (ATSDR, 1999).

2.3. Bioremediation

Bioremediation is defined as the process whereby organic wastes are biologically degraded under controlled conditions to an innocuous state, or to levels below concentration limits established by regulatory authorities. By definition, bioremediation is the use of living organisms, primarily microorganisms, to degrade the environmental contaminants into less toxic forms. It uses naturally occurring bacteria and fungi or plants to degrade or detoxify substances hazardous to human health and/or the environment (Kumar A et al., 2011).

2.4. Environmental Factors Influencing Bioremediation

There are numerous factors that influence on the ability of the soil microbes to breakdown pollutants. These factors include nutrients, pH, temperature, moisture, oxygen, soil characteristics and contaminant bioavailability (Bundy G.J. et al., 1989). Optimizing these environmental conditions could enhance contaminants biodegradation in the soil (Margesin R A et al., 2000).

3. MATERIALS AND METHODS

The soil excavated from a land behind Civil Engineering Department, Jnana Bharathi campus at a depth of 50cm from the ground surface was used for the study. 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 considered for research work. The soil was tested for its physical characteristics (soil type, porosity, etc.) and analyzed to ascertain its suitability for bioremediation. Various chemical and biological analyses were also carried out for the fresh soil and the details are as shown in Table 1. The study was carried out by setting up two sets of bioreactors, wherein one set was maintained at optimum environmental conditions (Set 1) and the other set had no maintenance of environmental conditions and served as control (Set 2). Each bioreactor was filled with 3 Kg of diesel contaminated soil. Set 1 had four bioreactors with contaminated soil having 10% TPH by weight maintained at optimum environmental conditions. Wherein, Set 2 had four bioreactors with contaminated soil having 10% TPH by weight with no maintenance of environmental conditions.

Table 1 Physico-chemical and Biological Characteristics of Fresh Soil

Parameters	**Unit**	**Fresh Soil Concentrations**
Type of Soil	---	Sandy (well graded)
Porosity	%	36.7
Co-efficient of Uniformity, Cu	---	4.6
Co-efficient of Curvature, Cc	---	1.2
Water Holding Capacity	%	32.4
pH	---	7.204
Temperature	^0C	23.2
Moisture Content	%	2.04
Total Organic Carbon	mg/gm of soil	31.12
Total Petroleum Hydrocarbon	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	$46x10^5$
Chromium	mg/kg of soil	0.64
Zinc	mg/kg of soil	0.62
Manganese	mg/kg of soil	0.29
Iron	mg/kg of soil	0.23

* 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.

The bioreactors of Set 1 were denoted as $B_{D(O)}$ since they were contaminated with diesel maintained at optimum conditions and Set 2 bioreactors used as control were denoted as $B_{D(C)}$. The replicates of bioreactors were labeled as a, b, c, and d for both the sets. The 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) are shown in Table 2.

Table 2 Initial and Final Characteristics of the Contaminated Soil in the Bioreactors

Physico - chemical and Biological Parameters / Characteristics	**Bioreactors maintained at optimum conditions - $B_{D(O)}$**		**Bioreactors used as control - $B_{D(C)}$**	
	Initial Reading	**Final Reading**	**Initial Reading**	**Final Reading**
pH	7.73	6.55	7.81	6.55
Temperature($^{\circ}C$)	20.6	22.8	20.6	22.8
TOC (mg/gm of soil)	101.45	11.86	102.00	38.06
TPH (mg/kg of soil)	100200	14270	100100	31980
Nitrogen (mg/gm of soil)	11.45	0.86	9.2	0.62
Phosphorus (mg/gm of soil)	1.25	0.1	1.05	0.09
Bacterial Count (CFU/gm×10^6)	32	48	24	32

Note: The initial reading indicates the concentration on the 0^{th} day and the final reading indicates the concentration on the 84^{th} day (i.e. after 12 weeks of treatment).

5. RESULTS AND DISCUSSION

pH: The pH of the soil was decreased with time. The pH of all the bioreactors after 12 weeks was within the pH range of 6.5 to 8.5, which is optimum for bioremediation. Higher or lower values of pH outside the limits would affect the microbial growth and the soil chemistry will be modified.

Temperature: Temperature has a significant influence on petroleum biodegradation by its effect on the composition of the microbial community and its rate of hydrocarbon metabolism, and on the physical nature and chemical composition of the oil. (Ismail Saadoun, 2008). The temperature recorded for the bioreactors ranged from 20.6°C to 23.9°C for the entire study period. This temperature falls within the optimum temperature range that is suggested to be ideal for bioremediation i.e. 15 – 45°C.

Moisture Content: Maintaining adequate moisture content is also important for better bioremediation. The soil moisture content was maintained at 60 (+/-) 10% of field capacity since soil moisture levels in the range of 20% - 80% of saturation generally allow suitable biodegradation to take place, while 100% saturation inhibits aerobic biodegradation because of lack of oxygen.

Total Organic Carbon: Organic compounds serve as sources of carbon and can be estimated based on concentrations of Total Organic Carbon (TOC). TOC reduction, in the bioreactors $B_{D(O)}$ was from 101.45 mg/g to 11.86 mg/g respectively. Where as in controlled bioreactors $B_{D(C)}$ the TOC reduction was less compared to treated bioreactors, i.e. from 102 mg/g to 38.06 mg/g which is due to the lack of nutrition elements might have caused the decrease of microbial population.

Nutrient Concentration: For effective degradation of contaminants microbes must be supplemented with sufficient nutrients. C:N:P in 100:10:1 ratio is optimum of degradation; hence one set of bioreactors (Set 1) were supplemented with Ammonium nitrate as nitrogen source and Super phosphate as phosphorous source. Calculated amount of nitrogen and phosphorus were added to obtain the C:N:P ratio of 100:10:1 and the other set of bioreactors (Set 2) were kept as control without supplying nutrients during the study period.

Microbial Activity: During the study period, all the bioreactors population increased from fourth week and significantly decreased in the later weeks. The microbial count in the bioreactors $B_{D(O)}$ maintained at optimum environmental conditions varied from 32×10^6 CFU/gm to 87×10^6 CFU/gm in the sixth week and then decreased to 48×10^6 CFU/gm of soil at the end of 12th week. Whereas, in the control bioreactors $B_{D(C)}$ the microbial count varied from 24×10^6 CFU/gm to 71×10^6 CFU/gm till fifth week and then decreased to 32×10^6 CFU/gm of soil at the end of 12th week. There was a subsequent increase in bacterial count which was much more remarkable in treatments maintained at optimum environmental conditions, while the control system showed a slow increase in microbial numbers because of the lack of nutrition and environmental conditions. Thus, increase in bacterial counts had an influence on the rate of TPH reduction.

Total Petroleum Hydrocarbons: The TPH reduction in bioreactors $B_{D(O)}$ was 85.76% during 12 weeks period. The control setup $B_{D(C)}$ which had not received nutrition treatment showed a reduction of 68.05% during the study period. Hence, the higher degradation was observed in the setup with optimum conditions compared with the control setup. The differences in hydrocarbon biodegradation between the treated and control setup were due to the large differences between bacterial numbers. The TPH concentrations in the different bioreactors during the study period are as shown in Table 3. Maximum reduction of TPH was observed during the 4th week of treatment in both the setups. Table 4 shows weekly percentage reduction of TPH in different bioreactors. The TPH concentrations at the end of different weeks and weekly TPH reductions of the bioreactors are shown in Figure 1 and Figure 2 respectively. Figure 3 shows weekly TPH degradation rate constants of bioreactors. Table 5 shows the overall TPH reduction and degradation rate constants of the bioreactors.

Table 3 TPH Concentration in the Bioreactors during the Study Period

Weeks	TPH (mg/Kg of soil)	
	Bioreactors – $B_{D(O)}$	**Bioreactors – $B_{D(C)}$**
Week 0	100200	100100
Week1	98056	98150
Week 2	90707	92457
Week 3	73860	82343
Week 4	51621	64836
Week 5	41258	54642
Week 6	33209	47472
Week 7	27036	42419
Week 8	22625	38625
Week 9	19435	35850
Week 10	17107	33816
Week 11	15382	32502
Week 12	14270	31980

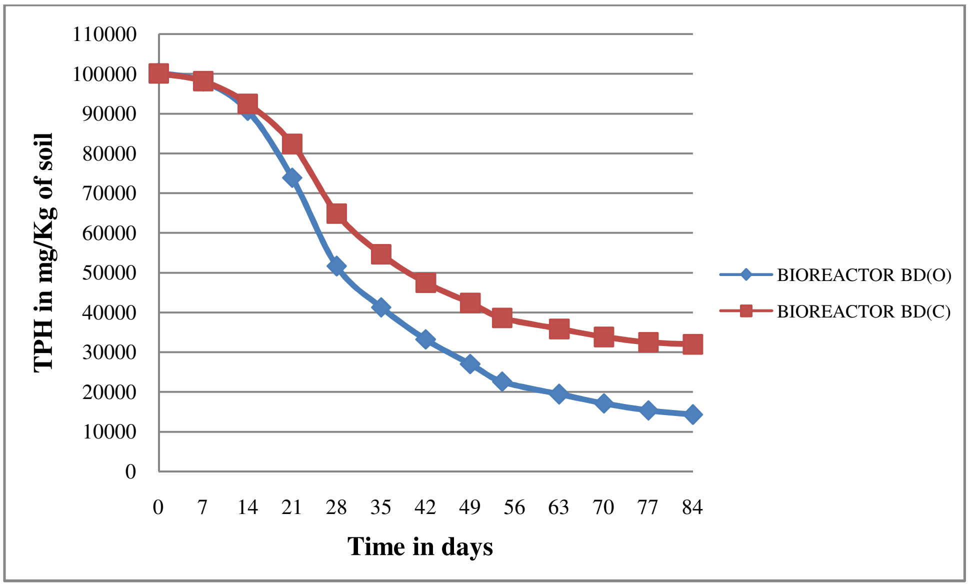

Figure 1 TPH Concentration at the end of different weeks for Bioreactors **$B_{D(O)}$ and $B_{D(C)}$**

Table 4 Weekly TPH Reduction in the Bioreactors during the Study Period

Weeks	Weekly TPH Reduction (%)	
	Bioreactors - $B_{D(O)}$	Bioreactors - $B_{D(C)}$
Week1	2.14	2.14
Week 2	7.33	7.33
Week 3	16.81	16.81
Week 4	22.19	22.19
Week 5	10.34	10.34
Week 6	8.03	8.03
Week 7	6.16	6.16
Week 8	4.40	4.40
Week 9	3.18	3.18
Week 10	2.32	2.32
Week 11	1.72	1.72
Week 12	1.11	1.11

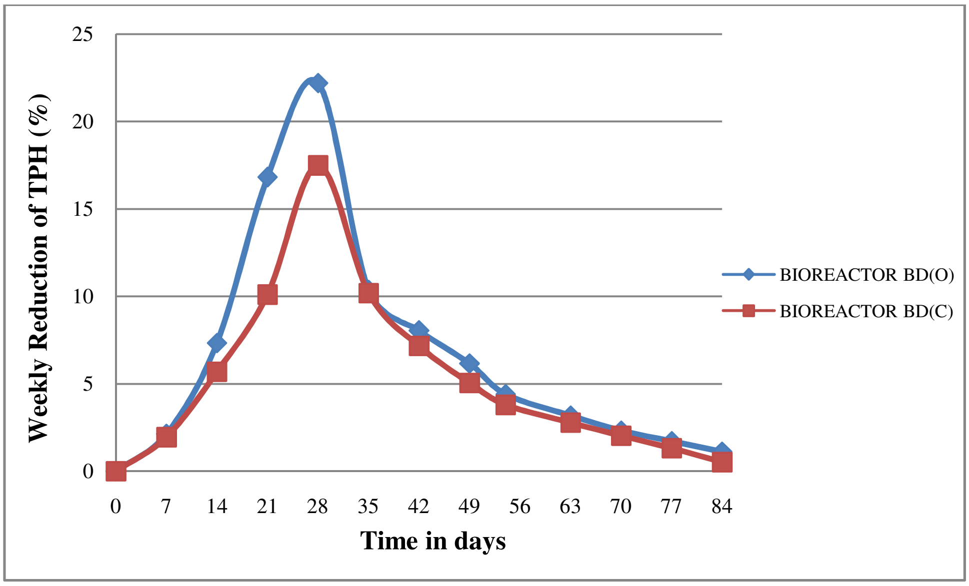

Figure 2 Weekly TPH Reduction for the Bioreactors during the Study Period

Biodegradation Rate

The degradation rate constant gives the indication of degradability of an organic compound; for biological treatment to be effective. The degradation rate constant "k" of 0.0232 d^{-1} was observed in Set 1 bioreactors $B_{D(O)}$. Similarly, the degradation rate observed in Set 2 bioreactors $B_{D(C)}$ was 0.0136 d^{-1} (Table 5).

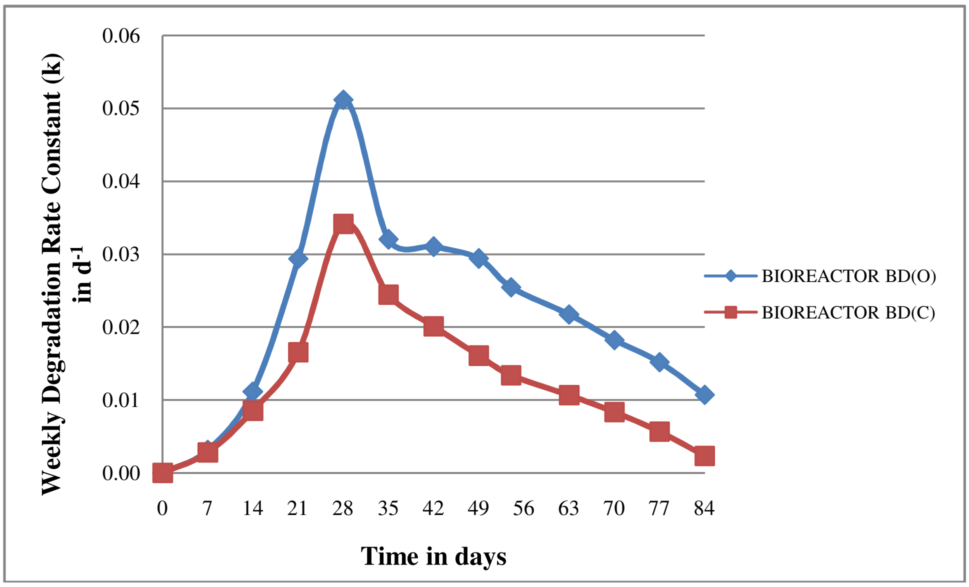

Figure 3 Weekly TPH Degradation rate constants of the Bioreactors

Table 5 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
Set 2 - $B_{D(C)}$	100100	31980	68120	68.05	0.0136

6. CONCLUSIONS

The study showed that the nutrients and other optimum environmental conditions played a significant role in determining the degradation rate and residual concentrations of the contaminants. Susceptibility of a hydrocarbon to microbial degradation varies with type and size of the hydrocarbon molecule. Higher TPH degradation i.e. from 100200 mg/kg to 14270 mg/kg of soil (85.76% TPH reduction) was observed in Set 1 bioreactors $B_{D(O)}$ compared to Set 2 bioreactors $B_{D(C)}$, wherein TPH degraded from 100100 mg/kg to 31980 mg/kg of soil (68.05% TPH reduction). Hence the higher oil degradation was observed in the treated setup compared with the control setup. The differences in hydrocarbon biodegradation between the treated and control setup were due to the large differences between bacterial numbers.

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