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BIOREMEDIATION OF PETROLEUM CONTAMINATED SOIL USING COW DUNG

K. Madhumitha[1]

[1]M.E. Environmental Engineering Department Of Civil Engineering, Annamalai University Chidambaram, India.

kaviyamadhume@gmail.com

ABSTRACT

This research evaluated bioremediation of soil contaminated with petroleum hydrocarbons. The objective of this study was to investigate whether organic nutrient enhances the process of microbial degradation. The samples were collected from the workshops where soil samples contain waste oil spills Cow Dung was applied at 2different proportions namely 30% and 60% respectively. A laboratory scale batch reactor was developed and maintained upto 90 days at room temperature. Bacterial count was done to determine the development of petroleum degrading heterotrophic bacteria. Spectrophotometer was used to determine the petroleum hydrocarbons present. The results indicate that the application of organic nutrients CD at 30% and 60% concentration were able to remediate the soil for efficiency 97.13%.

Keywords: Petroleum Hydrocarbon, Bioremediation, Cow Dung, Microbial Degradation.

1. INTRODUCTION

Environmental pollution is one of the greatest threats to society in the future. One of the primary causes of environmental degradation in a country could be attributed to rapid growth of population, which adversely affects the natural resources and environment. Increasing economic development and a rapidly growing population in India has taken the country from 300 million people in 1947 to over one billion people today was putting a strain on the environment, infrastructure, and the country's natural resources. Rapid industrialization further worsens the situation.

It was the industrial revolution that gave birth to environmental pollution as we know it today. The emergence of large factories and consumption of immense quantities of coal and other fossil fuels gave rise to unprecedented air pollution and the large volume of industrial chemical discharges added to the growing load of untreated human waste. It was estimated that 10,000 more chemicals are introduced worldwide annually and industrialized countries generate more than 90% of the world's annual total of 325-375 million tons of toxic and hazardous waste originating from chemical and petrochemical industries.

Every year spillage of tones of fuel and lubricating oil has become a major environmental hazard. In October 2014, a single oil spillage of 546 tones was reported at Mid-Valley Pipeline of Louisiana, United States. A recent incident highlighted how time consuming a cleanup operation of oil spillage can be. According to CNN International, in Nov 2013, oil-spillage occurred in Philippines due to a typhoon and till date the cleaning up process is going on. There are also long-term effects on ecosystems related to the release of toxic components over a prolonged period as the oil breaks up and the concentration of toxicants in organisms towards the top of the food chain increases.

2. METHODOLOGY

Sample collection

Soil samples were collected from a nearby automobile shop in Chidambaram town. Soil samples were collected in sterile polythene bags. Samples were collected for a maximum depth of 10cm from the surface with a scoop. It is preserved in a room temperature before starting the experiment. Cow dung was used as inoculums. Cow dung was collected from our university cattle farm present at agricultural faculty.

Sample preparation

A measured quantity of 50 gm soil sample was transferred into a conical flask to which 200ml of distilled water was added. The conical flask was tightly closed and placed in rotary shaker for a period of 48hours at 250rpm. The samples were filtered using a standard filter paper. and the filtered samples were analyzed.

Physicochemical analysis of soil

The samples were collected and characterized using a standard method (Laboratory testing procedure for soil & water sample analysis, 2009)

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3. MODELING AND ANALYSIS

Preparation of Growth Medium

5gram of soil samples inoculated with cow dung and at different proportions were added to a mineral salt broth medium (MSM) of 200 ml, which composed of 1L of $(NH_4)_2SO_4$, 1g; KH_2PO_4, 0.2g; K_2HPO_4, 1.6g; $MgSO_4 .7H_2O$, 0.2g; NaCl, 0.1g; $FeSO_4$, 0.1g and $CaCl_2.2H_2O$, 0.02g and 10g/L of glucose were added and pH of the medium was adjusted to 7. The broth medium containing hydrocarbon polluted soil was incubated at room temperature with orbital shaker at 120rpm.

Figure 1. Experimental Set Up On Batch Reactor

Enumeration of Total Heterotrophic Bacteria

Samples were prepared by adding 1ml biomass to 9ml sterile distilled water. Serial dilution of the sample was carried out up to 10^{-4} dilutions. An aliquot (0.1ml) of the dilution was inoculated into a Petri dish containing Plate Count Agar (PCA) in duplicate for total cultivable bacteria. The inoculums were spread on the plate using sterile bent glass wood. The plates were incubated at 28^{o} C for 48 hours. Colonies that were formed during this incubation period were counted.

Determination of Hydrocarbon

Hydrocarbon content of the biomass samples was determined by 10ml of sample in 20ml toluene in a 200ml flask. After shaking for 30 minutes on orbital shaker 120rpm, the liquid of the extract was measured at 420nm using spectrophotometer. The TPH in sample was estimated using the standard curve derived from fresh engine oil diluted with toluene.

4. RESULTS AND DISCUSSION

Physicochemical Analysis

The physicochemical properties of the soil and organic nutrients used for the bioremediation trials are detailed in table1 and 2.

Table 1: Characteritics of Hydrocarbon Polluted Soil

S. No.	Sample Parameter	Sample A	Sample B	Sample c	Cow Dung	Remark
1	**Moisture Content%**	0.908	1.88	9.8	246.26	**mg/l**
2	**pH**	7.16	8.28	7.83	7.7	-
3	**EC**	1.411	1.412	0.335	.304	**Milli mho**
4	**NO_2**	3	1	1.5	30	**mg/l**
5	**PO_4**	3.5	0.5	3	3.1	**mg/l**
6	**SO_4**	45	48	21	310	**mg/l**
7	**Ca**	48.77	61.11	19.90	88	**mg/l**
8	**Na**	51.62	31.45	16.71	412	**mg/l**
9	**K**	76.48	37.75	62.16	17	**mg/l**
10	**Li**	1.42	4.58	0.46	303	**mg/l**
11	**TOC**	185.0	64.46	67.01	122.8	**mg/l**

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Bioremediation of Petroleum Hydrocarbon Contaminated Soil Samples using CD different ratio

Inoculums carry CD containing directly enriched petroleum hydrocarbon degrading microorganisms at 30% and 60% application rates. At the end of the 90th day, bioremediation experiment results revealed that higher amount of petroleum hydrocarbons loss up to 56.78%, 68.17% and 94.12 (for 30% CD), 70.04%, 76.27% and 97.13% (for 60% CD) for sample A, B and C were found respectively. Agamuthu et al (2013) reported that about 94% of PHC can be removed by CD respectively

The petroleum hydrocarbons contaminated soil amended with 30% of CD recorded the highest reduction of petroleum hydrocarbon of nearly 56.78% compared with the 60% of CD which revealed an efficiency of 70.04% for sample-A.

The petroleum hydrocarbons contaminated soil amended with 30% of CD recorded the highest reduction of petroleum hydrocarbon of nearly 68.17% compared with the 60% of CD which revealed an efficiency of 76.27% for sample-B.

The petroleum hydrocarbons contaminated soil amended with 30% of CD recorded the highest reduction of petroleum hydrocarbon of nearly 92.13% compared with the 60% of CD which revealed an efficiency of 94.12% for sample-c.

The petroleum hydrocarbons contaminated soil amended with CD recorded the highest reduction of petroleum hydrocarbon of nearly 97.57% compared with the 30% which was an efficiency of 94.12%. The reasons for results obtained might be due to differences in the nutrient content, particularly N and P in these two organic nutrients in stimulating indigenous microorganisms.

Addition of N and P to a petroleum hydrocarbons polluted soil has been shown to accelerate the biodegradation of the petroleum hydrocarbons in soil. CD with the highest concentration of N and P proves to be effective inoculums in bioremediation of petroleum hydrocarbon contaminated soil. N and P are found to be an important nutrients required by a hydrocarbon utilizing bacteria to carry out effective and efficient bioremediation activities of xenobiotics in the soil environmental.

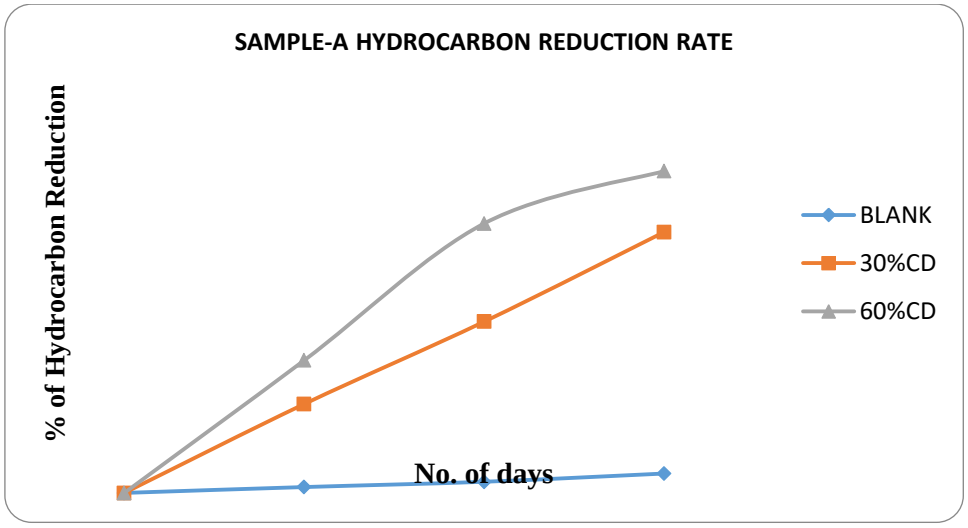

Figure 2: Percentage Of Hydrocarbon Reduction Rate Sample-A

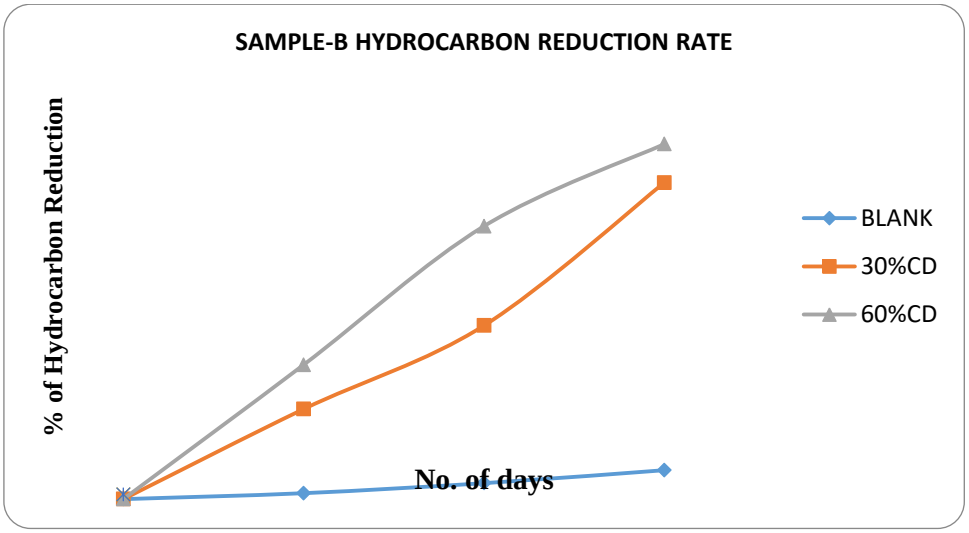

Figure 3 Percentage Of Hydrocarbon Reduction Rate Sample-B

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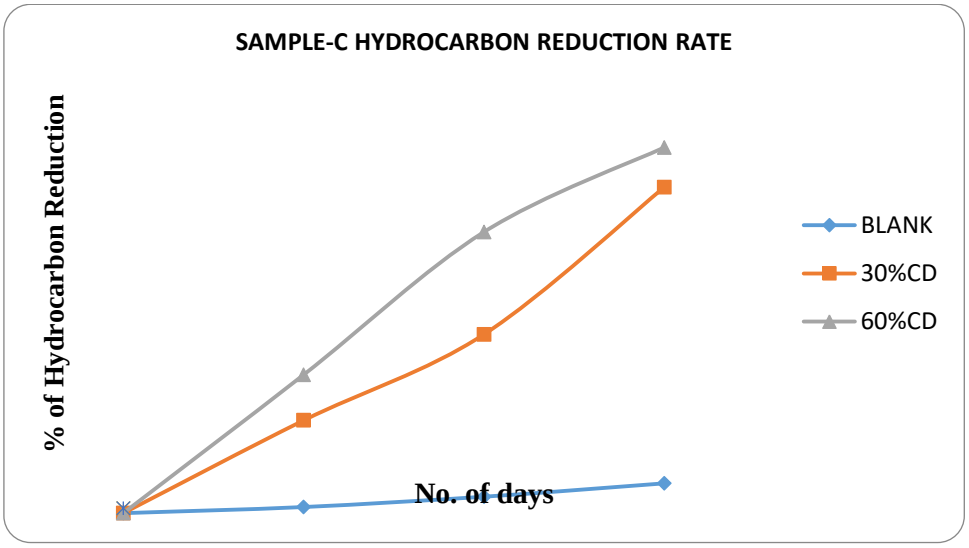

Figure 4: Percentage Of Hydrocarbon Reduction Rate Sample-C

Microbial counts

Counts of hydrocarbon degrading bacteria in soil contaminated with petroleum hydrocarbon organic nutrients is shown figure (5,6 and 7). The count of total heterotrophic bacteria (THB) in soil contaminated with petroleum hydrocarbon and amended with CD ranged between$1.9X10^5$cfu/g to $2.58X10^7$cfu/g. The unamended soil contains THB ranging between $3.2X10^5$cfu/g to $3.44X10^7$cfu/g for sample-A. It was relatively lower compared to organic nutrients amended soil. Microbial count was done at the end of the 90^{th} day.

The THB count was found higher in nutrients amended soil compared to the un-amended polluted soil, it might be due to the presence of appreciable quantities of nitrate and phosphate in the organic nutrients. The higher microbial population counts in petroleum hydrocarbon contaminated soil amended with organic nutrient is accompanied by significant hydrocarbon bioremediation, indicating that the indigenous soil microbes utilized a portion of the C supplied by the diesel fuel as potential nutrients source.

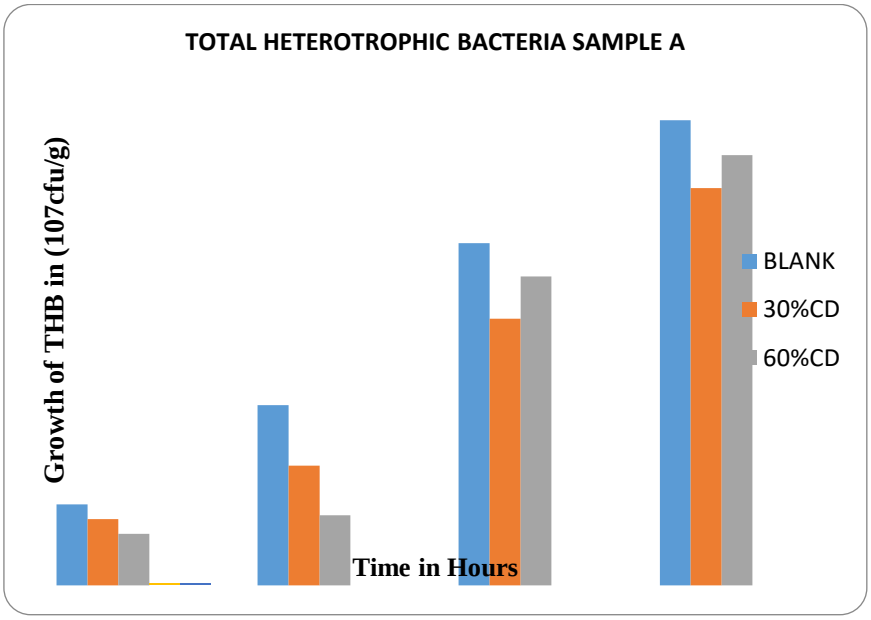

Figure 5: Enumeration Of Total Heterotrophic Bacteria Sample-A

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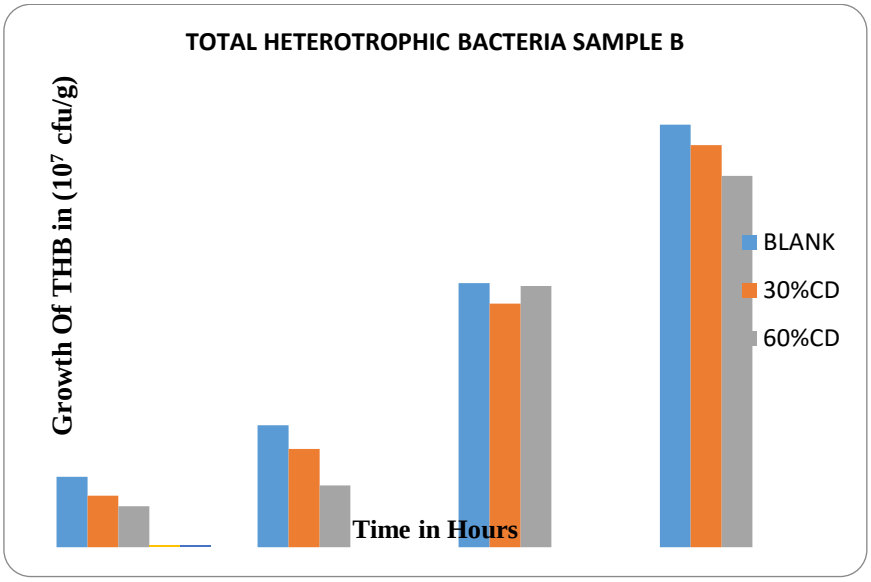

Figure 6: Enumeration Of Total Heterotrophic Bacteria Sample-B

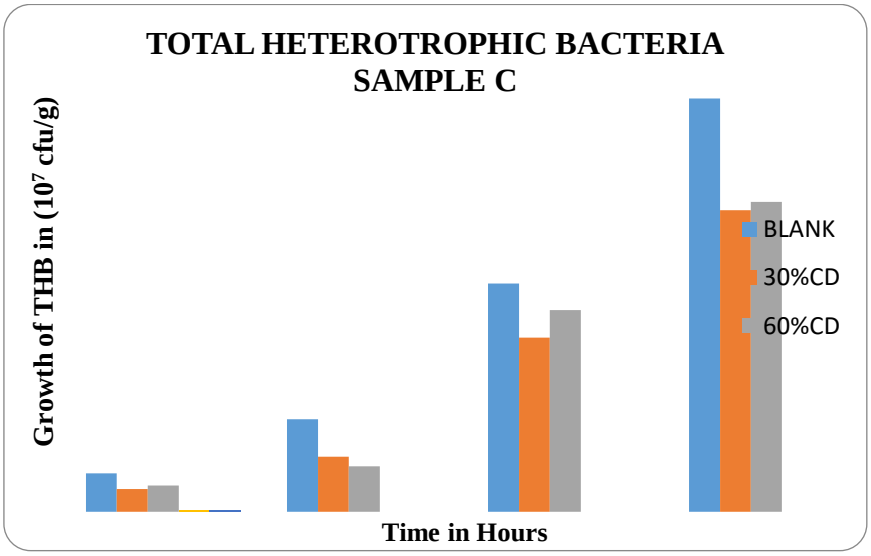

Figure 7: Enumeration Of Total Heterotrophic Bacteria Sample-C

5. CONCLUSION

The results of this study showed that soil contaminated with petroleum hydrocarbon can be treated with CD, Whose reduction potential is 94.12% and 97.13% respectively. Reduction in total petroleum hydrocarbon in contaminated soil by 60% CD proves to be more effective.

The study demonstrated the potential of 30% CD in enhancing the growth of indigenous microorganisms in the soil which in turn increases the bioremediation rate of petroleum hydrocarbon. Both organic matters proved to enhance the multiplication of indigenous microbes thus enabling rapid biodegradation of the contaminated soil.

Taking in to account that the amount of limiting nutrients such as nitrate and phosphate presented in the polluted soil is low. The use of organic nutrients as nitrate and phosphate source can enhance bioremediation process as well as solve the problem of waste management.

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