Enhanced Degradation of Diesel-Contaminated Soil using Organic Wastes

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ABSTRACT This study was carried out to enhance the biodegradation of diesel fuel in soil contaminated with 10 %(w/w) diesel fuel amended with 10% tea leaf (TL), soy cake (SC), potato skin (PS) for a period of 3 months under laboratory condition. At the end of 84 days, the highest percentage of oil biodegradation (76%) was recorded in soil amended with SC; 64% and 53% were recorded with soil amended with PS and TL respectively, while only 27% of oil degraded in control treatment. Hydrocarbon utilizing bacteria (HUB) counts were high in all organic wastes amended treatments, ranging from 45×10^6 CFU/g to 90×10^6 CFU/g of soil compared to unamended control soil (4×10^6 CFU/g to 8×10^6 CFU/g of soil). The count in amended soil was significantly different at ($P< 0.05$) compared to unamended soil. The results obtained showed 90%, 80% and 60% seed germination in remediated soil contaminated with 10% diesel fuel and amended with SC, PS and TL respectively, over the period of 84 days. The results show the high potential of SC for enhanced biodegradation of hydrocarbon in oil contaminated soil.

Key words: degradation, diesel, hydrocarbon, organic waste

INTRODUCTION

Soil contamination by hydrophobic components is one of the main types of pollution [1]. Aliphatic hydrocarbons cause a substantial proportion of organic contamination in the terrestrial environment [2]. Among the hydrocarbon pollutants, diesel fuel is a complex mixture of hydrocarbons with an average carbon number of C_8–C_{26} [3]. The technology of remediation of the petroleum contaminated soil has been a research focus of the academia. They seek an effective, economical and also simple methods to solve this important environmental problem.

Petroleum –contaminated soil is currently treated using three methods: physical, chemical, and biological. Physical or chemical, methods include incineration, chlorination and combustion [4]. Many of these technologies are either costly or do not completely remove contaminants. On the other hand, Biological treatment (bioremediation) appears to be among the most promising methods for dealing with a wide range of organic contaminants.

Bioremediation is an attractive approach for cleaning up contaminated soil because it is simple to maintain, applicable over large areas, cost-effective and leads to the complete destruction of the contaminants [5, 6]. Using fertilizer provides nutrients (N and P) in order to increase the capability of the organisms to degrade hydrocarbon in the soil. However, the use of fertilizer to remove oil spill may be expensive and leads to contamination of underground water.

Effective bioremediation of petroleum-contaminated soil by organic wastes and composting has been proved by many researchers [7, 8, 9]. Microbial activity is often stimulated by the addition of organic material to soil. Organic matter also improves many of the physical and chemical properties of soil such as the water holding capacity, aeration, pH, and other criteria which are necessary for growing bacteria. In this study we investigated the potential of tea leaf, soy cake and potato skin for enhanced biodegradation of diesel fuel in soil and, also to determine the best organic waste that is cheap and available in our environment for stimulating oil degradation.

MATERIALS AND METHOD

COLLECTION OF SAMPLES AND DIESEL OIL

Organic wastes used in this study were collected from different locations; tea leaf (TL) and potato skin (PS) were collected from IPS canteen, University of Malaya and soy cake (SC) was prepared in the laboratory, while the soil used in this study was silty loam and was obtained from the garden section of Asia-European Institute, University of Malaya, Kuala Lumpur. It was transported to the laboratory and air dried, finely ground with a mortar and pestle, and sieved through a 2-mm mesh sieve. Diesel fuel was purchased from petrol station in Petaling Jaya, Malaysia.

MICROCOSM DESCRIPTION

One and half kilogram (1500 gr) of soil (sieved with 2 mm mesh size) was placed in plastic vessels labeled A to E and polluted with 10% (w/w) diesel fuel. After two days, 10% of each dry organic waste (TL, SC and PS) were added into each of the oil-polluted soil labeled A, B and C, respectively. Also, the control (vessel D) was with only soil and diesel fuel. Additional control treatment comprising of autoclaved soil containing 0.5% (w/w) NaN_3 was also set up, to determine non-biological loss of diesel oil from the soil. The soils were mixed daily to provide sufficient air and oxygen. The soil was moistened by the addition of water every two days to adjust water holding capacity to 60% throughout the experimental period. The plastic vessels were incubated at room temperature (30 ± 2° C). All the treatments were set up in triplicates.

PHYSICOCHEMICAL PROPERTIES OF SOIL AND ORGANIC WASTES

Physicochemical property of soil and organic wastes were determined using standard methods. Nitrogen content of soil used for bioremediation and organic wastes were determined by Kjeldahl method, organic carbon was determined using furnace method, pH and phosphorus were determined with pH meter (HANNA HI 8424) on 1:2.5(w/w) soil/distill water after 30 minutes equilibration and ASTM D 5198-92 method, respectively. Triplicate determinations were made.

SAMPLING

The contaminated soils were sampled every two weeks for 84 days from different areas of the container in order to determine pH, total petroleum hydrocarbon and isolation and enumeration of bacteria.

ENUMERATION AND IDENTIFICATION OF HYDROCARBON UTILIZING BACTERIA

0.1 ml of serially diluted samples were plated on nutrient agar medium (Oxoid) for isolation of aerobic heterotrophic bacteria with 50 µg/ml fungazol to suppress the growth of fungi. Plates were incubated at 30°C for 24 h after which the colonies were counted.

Hydrocarbon utilizing bacteria (HUB) in the soil samples were enumerated using oil agar (OA) of Zajic and Supplission [10]; (1.8 g K_2HPO_4, 1.2 g KH_2PO_4, 4.0 g NH_4Cl, 0.2 g $MgSo_4.7H2O$, 0.1 g NaCl, 0.01 g $FeSO_4.7H_2O$, 20 g agar, 2 ml diesel fuel, 1000 ml distilled water). The oil agar plates were incubated for 5 days at 30 °C before counting the colonies. Bacteria colonies were randomly picked and pure culture obtained by repeated sub-culturing on nutrient agar. The bacterial isolates were characterized based on their color and biochemical properties [11].

TOTAL PETROLEUM HYDROCARBON DETERMINATION

The total extent of diesel fuel biodegradation in soil was determined by suspending 10 g of soil in 20 ml of n-hexane in a 250 ml capacity flask. After shaking for 1 h on an orbital shaker (Model N-Biotek), the solvent- oil mixture was filtered using Whatman number 4 filter paper into a round bottom flask of known weight and the solvent was completely evaporated by using rotary evaporation. The new weight of the flasks consisting of residual oil was recorded. Percentage degradation of diesel fuel was calculated using the formula of Ijah and Ukpe [12].

SEED GERMINATION TOXICITY TEST OF REMEDIATED SOIL

Toxicity of the remediated soil was assessed using germination test. Lettuce was used in this study owing to its sensitivity to hydrocarbon in soil [13]. The germination test was conducted over a five days test period. For each soil sample 150g of thoroughly mixed remediated soil was placed in Petri dish bottoms. Ten viable seeds of lettuce (*Lactuca sativa* L.) were placed evenly throughout each Petri dish and covered with 10g of dry sand. The petri dishes were placed in a room with 16 hours light and 8 hours darkness for 5 days. At the end of 5 days, the number of seedlings that emerged from the surface of the sand was counted and percentage of seed germination calculated.

STATISTICAL ANALYSIS

Statistical analysis of data was carried out using Analysis of Variance (ANOVA).

RESULTS AND DISCUSSION

Table 1 showed the results of physicochemical properties of soil and organic wastes. The soil had low N and P content compared to organic wastes. The level of oil biodegradation throughout this study is shown in Figure.1. The rate of biodegradation of diesel fuel in soil increased during 84 days and reached 76% in soil amended with SC. the end of 14 days. There was 20%, 28% and 31% TPH reduction in soil amended with TL, PS and SC, respectively compared to control soil with 9% oil degradation. However, the total biodegradation in soil amended with TL and PS were 53% and 64%, respectively. During this study there was a rapid decrease in total petroleum hydrocarbon (TPH) in all the treatments amended

Table 1: Physicochemical Properties of Soil and Organic Wastes Used for Bioremediation

Parameters	Soil	Organic Wastes		
		TL	SC	PS
Nitrogen (%)	0.8 ± 0.1	1.02± 0.08	1.3± 0.1	1.10±0.04
Phosphorus (%)	0.6± 0.5	0.7± 0.6	0.9±0.9	0.7±0.1
Moisture content (%)	10.2±0.8	34.3±0.5	75.9±1.6	62.1 ±2.0
Organic C (%)	13.1± 1.3	55.6±1.2	72.2± 0.9	66.3±1.1
pH	7.0 ± 1.5	6.5±1.2	6.8±1.2	6.9±0.5

TL: Tea Leaf, SC: Soy Cake, PS: Potato Skin

with organic wastes, compared to unamended soil. Effective bioremediation of soil by organic wastes and compost has been reported [7, 8, 9].

9% of the degradation in autoclaved soil might be due to non biological factors such as evaporation or photodegradation. This was recorded in poisoned control soil i.e. autoclaved contaminated soil treated with 0.5% sodium azide. However, the total extent of diesel fuel biodegradation was about 12% higher in soil amended with SC than that of PS, and about 23% higher than that of soil amended with TL. This might be due to high N and P content in SC (Table1), because these two elements are known as most important nutrients needed by hydrocarbon utilizing bacteria to carry out effective and efficient biodegradative activities of xenobiotics in the soil environment [14,15].

HUB was more abundant in oil polluted soil amended with different organic wastes than that of unamended polluted soil (Figure 2). Statistical analysis revealed that there is significant difference in the counts of HUB between the amended soil and unamended soil ($P<0.05$). HUB recorded in SC treated soil ranged from 10×10^6 to 90×10^6 CFU/g, while HUB counts in PS and TL ranged between 8×10^6 to 55×10^6 CFU/g and 8×10^6 to 45×10^6 CFU/g, respectively (Figure 2). Unamended soil (control) had a range of 1×10^6 to 8×10^6 CFU/g. Generally, the main reason for higher counts of bacteria in SC amended soil can be due to the presence of considerable quantities of N and P in SC, which are necessary nutrients for bacterial biodegradative activities [9, 16].

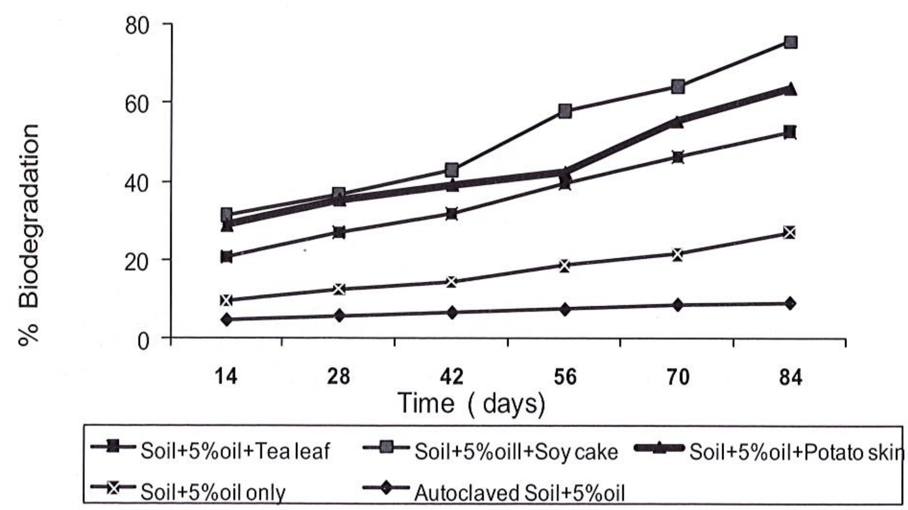

Figure.1. Percentage biodegradation of diesel fuel in soil contaminated with 10% oil

This finding agrees with the report of Odu [17] who reported that the highest application of oil (39%) to Nigerian soil possessed the highest number of bacteria. HUB isolated from the oil contaminated soil were identified as *Bacillus* sp. and *Staphylococcus sp.*.These bacterial species had been implicated in hydrocarbon degradation by different authors [18, 19, 20].

The pH of the soil used for the experiment was 7.03 (Table 1), while the pH for the various treatment of the soil contaminated with 10% diesel fuel and 10% organic wastes varied greatly from slightly acidic to neutral pH throughout the 84 days of this study (Figure3). Results indicated that generally, addition of diesel fuel and organic wastes to soil especially PS and SC, raised the pH of the treatments. This may account for the reason why soil amended with PS and SC recorded highest count of HUB compared to other treatments, while the soil amended with TL lowered the pH. This might be the reason for low biodegradation of oil in soil amended with TL because biodegradation of oil is always favored by neutral or slight alkaline pH [14, 16]. However, pH of soil amended with different organic wastes ranged from 6.7 to 8.42 (Figure 3).

Table 2 shows the result of seed germination index in remediated of soil amended with different organic wastes. Soil treated with SC recorded the highest germination index. The result shows 90 %, 80 % and 60% of seed germination in soil treatment with TL, SC and PS, respectively. These results further proved the effectiveness of SC in enhancing biodegradation of hydrocarbon in oil contaminated soil. These results are similar to the finding of Oleszczuk [21], who reported that compost reduced phytoxicity of diesel and wastewater sludge after 76 days.

Table 2 : Seed germination toxicity test (%)

Treatments					
A	B	C	D	E	F
60±3.0	90±5.0	80±6	40±6	20±0	100

A = Soil + Oil+ TL, **B** = Soil + Oil + SC, **C** = Soil + Oil + PS, D= Soil + Oil, E= Autoclaved soil +Oil +NaN_3, F= Uncontaminated soil

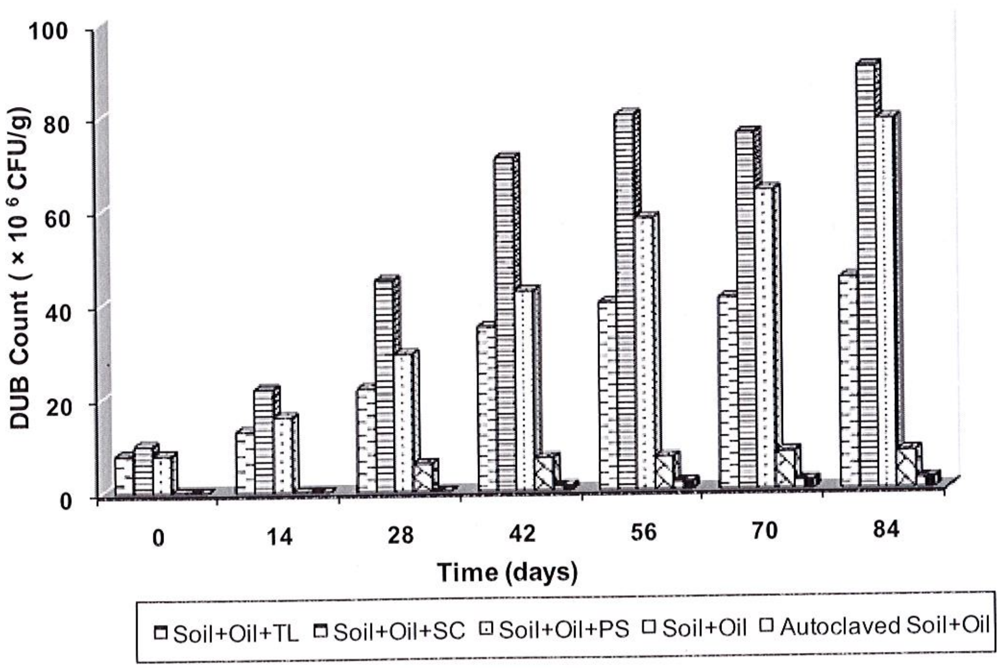

Figure 2: Diesel fuel utilizing bacterial population in contaminated soil

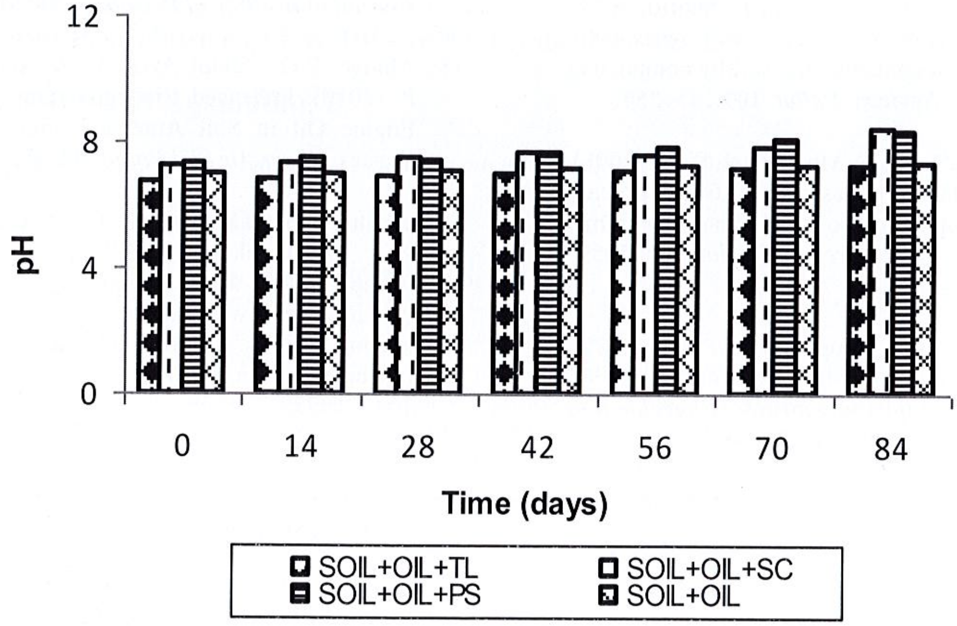

Figure 3: pH of diesel fuel polluted soil

CONCLUSION

Bioremediation by addition of amendments is a viable choice for the remediation of contaminated soil. A significant reduction in the diesel fuel was achieved by adding soy cake, which is waste from soy bean factory, possibly because it was more effective than other amendments in providing an alternative source of N and P.

The counts of AUB in all the soil amended with various organic wastes were higher compared to that of unamended control soil. This may be due to differences in microbial ecology of the soil or characteristics of the experimental soils.

In conclusion, the remediation method adopted in this study is simple and inexpensive.Therefore, the results obtained demonstrated the potential of organic wastes for oil bioremediation in the order SC > PS > TL.

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