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Stimulated Biodegradation of Used Lubricating Oil in Soil Using Organic Wastes

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Abstract Biostimulation studies of soil contaminated with used lubricating oil was undertaken with three organic wastes (Banana skin BS, Brewery spent grain BSG and Spent mushroom compost SMC) used as sources of nutrient to enhance biodegradation of used lubricating oil in soil for a period of 84 days under laboratory conditions. The hydrocarbon loss in the soil shows positive linear correlation with evolution of CO_2 in the soil. 42.05% oil loss was recorded in the unamended polluted soil while 68.73%, 62.03% and 57.01% oil loss were recorded in soil amended with BSG, BS and SMC respectively in 84 days. Hydrocarbon utilizing bacterial (HUB) counts were high in all the organic wastes amended soil ranging between 10.2×10^6 CFU/g of soil to 80.5×10^6 CFU/g of soil compared to unamended control soil (1.0×10^6 CFU/g to 3.5×10^6 CFU/g of soil) throughout the 84 days of study. The counts in amended soil was significantly different at ($P<0.05$) from the unamended polluted soil. The HUB was identified as species of *Acinetobacter, Nocardia, Pseudomonas, Micrococcus and Bacillus*. The results obtained demonstrated the potential of BSG, BS and SMC for enhanced bioremediation of hydrocarbon contaminated soil.

(**Keywords*:*** Lubricating oil, biodegradation, organic wastes, bacteria, and hydrocarbon.)

INTRODUCTION

Waste lubricating oils consist of complex mixture of hydrocarbons that are 80 to 90% by volume and performance enhancing additives that is about 10 to 20% by volume [1]. Since the advent of oil exploration, the environment has been heavily contaminated with hydrocarbon pollutants, which enters the environment through several route. The presence of these pollutants in the terrestrial and aquatic environments constitutes public health and socio-economic hazards [2, 3, 4]. In order to resolve this problem; several techniques have been developed, including bioremediation, which is of great interest because of the possibility of soil reuse. Bioremediation is based on the capacity of microorganisms to degrade organic pollutant compounds, such as hydrocarbons. These compounds are important soil pollutants because of the high toxicity of the polycyclic aromatic hydrocarbon (PAH) fraction. According to the Environmental Protection Agency (EPA), 16 PAHs have been reported as carcinogenic and mutagenic compounds [5], so it is necessary to remove them from contaminated sites.

Biodegradation of hydrocarbon in the natural environment is slow. The major factor responsible for the slow process is the nutritional imbalance created by oil spills. Therefore addition of nutrients (in the form of inorganic fertilizer) coupled with physicochemical processes and microbial seeding has been found to be effective in dealing with oil spills in soil [6, 7, 8]. The fertilizer provides nitrogen and phosphorus to the hydrocarbon degrading microorganisms in the soil. In this way, the growth and hydrocarbon degrading capability of the organisms are promoted. However, the use of fertilizer in treating oil spills may be expensive. Therefore, a cheap alternative like banana skin, brewery spent grain and spent mushroom compost was used in this study to stimulate the activities of the indigenous microorganisms for biodegradation of hydrocarbons in used lubricating oil. The objective of this study was to assess the effectiveness of banana skin; brewery spent grain

and spent mushroom compost in enhancing biodegradation of used lubricating oil in soil.

MATERIALS AND METHOD

Collection of samples

The soil sample used was collected from the Nursery section of Asia-European Institute, University of Malaya, Kuala Lumpur in a sack and transported to the laboratory for analysis. Used engine oil was collected from Perodua car service center Petaling Jaya, while the organic wastes were collected from different locations; banana skin (BS) was collected from IPS canteen, University of Malaya, brewery spent grains (BSG) were collected from Carlsberg brewery, Shah Alam, Selangor and spent mushroom compost (SMC) was collected from Gano mushroom farm, Tanjung Sepat, Selangor.

Measurement of used lubricating oil in soil amended with organic wastes

One thousand and five hundred grams (1500g) of soil (sieved with 2mm mesh size) was placed into each plastic container (PC) and the following treatment were carried out: PC1 had 10% (w/w) used lubricating oil plus 150g ground BS; PC2 had 10% (w/w) used lubricating oil plus 150g ground BSG; PC3 had 10% (w/w) used lubricating oil plus 150g ground SMC; PC4 had only 10% (w/w), it serves as control. The moisture content was adjusted to 60% water holding capacity (WHC) and incubated at room temperature (28 ± 2^0 C). The content of each vessel was tilled twice a week for aeration, and the moisture content maintained at 60% WHC by addition of sterile distilled water. The experiment was set up in triplicate.

Sampling

Periodic sampling from each vessel was carried out at 14 days intervals for 84 days. Composite samples were obtained by mixing 5g of soil collected from four different areas of the PC. For analysis of oil loss from soil, pH and counts of hydrocarbon utilizing bacteria.

Used lubricating oil loss in soil (Biodegradation)

The total extents of used lubricating oil biodegradation in soil were determined by suspending 10g of soil in 20ml of diethyl ether in a 250ml capacity Erlenmeyer flask. After shaking for 30minutes on an orbital shaker (model N-Biotek-101), the solvent – oil mixture was filtered using Whatman No. 1 filter paper into a beaker of known weight and the solvent was allowed to evaporate completely. The new weight of the beaker (now containing residual oil) was recorded. Percentage biodegradation of used lubricating oil was calculated [9].

Physicochemical properties determination of soil and organic wastes

Nitrogen content of soil used for bioremediation and organic wastes was determined using the Kjeldahl method, phosphorus and carbon contents were determined using ICP-QES and furnace method respectively. The pH was determined with pH meter (HANNA HI 8424) on 1:2.5 (w/v) soil/distilled water after 30 minutes equilibration. Triplicate determinations were made.

Enumeration of hydrocarbon utilizing bacteria

Zero point one milliliter (0.1ml) of serially diluted soil samples were plated on oil agar (OA) of Zajic and Supplisson [10]. 1.8g K_2HPO_4, 1.2g KH_2PO_4, 4.0g NH_4Cl, 0.2g $MgSO_4.7H_2O$, 0.01g $FeSO_4.7H_2O$, 0.1g NaCl, 20g agar, 1% used lubricating oil in 1000ml distilled water, pH 7.4. The OA plates were incubated at 30^0C for 5 days and the colonies were counted and randomly picked, pure isolates were obtained by repeated sub-culturing on nutrient agar (Oxoid).

Characterization and identification of bacterial isolates

The bacterial isolates were characterized based on their cultural, microscopic and biochemical properties. The identities of the bacterial isolates were confirmed by comparing their characteristics with those of known taxa as outlined in Bergey's Manual of Determinative Bacteriology [11].

Measurement of CO_2 evolution contaminated soil amended with organic wastes

The rate of microbial breakdown of oil was assessed by the carbon dioxide (CO_2) evolution method of Cornfield [12]. One hundred gram (100g) of soil contained in screw cap bottles in triplicates was treated with 10% (w/w) used lubricating oil. Ten grams (10g) of organic wastes (BS, BSG and SMC) was added individually to each bottle and the soil moisture was adjusted to

60% water holding capacity. To trap the CO_2 liberated during oil biodegradation, glass vials containing 0.5g of barium peroxide and 4.5ml of distilled water were placed in the screw cap bottles containing oil treated samples. Two control experiments were set up as follows: one control without added organic wastes, while the second had neither used lubricating oil or organic wastes. All treatments were incubated at room temperature (28 ± 2^0C) for 28 days. At 7 days intervals, a set of three vials per treatment were withdrawn and titrated with 1M HCl. The amount of CO_2 evolved during oil degradation was calculated by the method of Cornfield [12] and Stotzky [13].

RESULTS AND DISCUSSION

Table 1 showed the results of physicochemical properties of soil and organic wastes used for bioremediation. The results (Figure1) revealed that the rates of biodegradation of used lubricating oil in soil increased with time and reached 68.73% in soil amended with BSG in 84 days. In soil amended with BS and SMC, the total extents of biodegradation were 62.03% and 57.01% respectively in 84days, whereas the extent of biodegradation was 42.05% in unamended polluted soil in 84 days. The results revealed that the total extent of oil biodegradation in amended soil was about 26% higher than that of unamended oil polluted soil, indicating that the organic wastes used enhanced biodegradation of used lubricating oil in soil. Enhanced used lubricating oil biodegradation using organic wastes has been reported [14, 15, 16]. The enhancement may be due to nutrients (N and P) present in the organic wastes (Table 1). However, the total extent of used lubricating oil biodegradation was about 6% higher in soil amended with BSG than that of BS and about 12% higher than that of soil amended with SMC. This may be due to higher nitrogen content in BSG (Table 1). It is also possible that nutrient in BSG were less tied up and available to bacteria than those of BS and SMC.

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

		Organic wastes		
Parameter	**Soil**	**BSG**	**BS**	**SMC**
pH	6.12	6.66	7.04	5.64
Nitrogen (%)	0.4	1.02	0.4	0.5
Phosphorus (mg/kg)	21.8	20.6	21.2	22.5
Organic C (%)	10.3	10.9	10.5	10.2
Moisture (%)	7.0	71.84	38.5	62.3

BSG: Brewery spent grain, BS: Banana skin, SMC: Spent mushroom compost

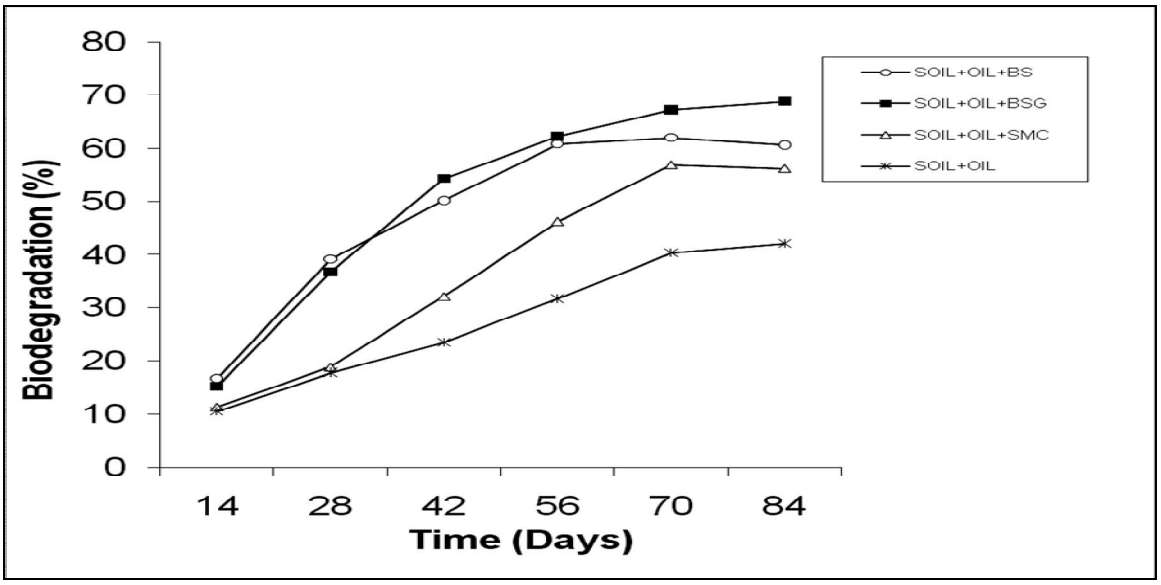

Figure 1. Extent of biodegradation of used lubricating oil in soil

Table 2 shows the results of the amount of CO_2 liberated from soil treated with used lubricating oil and amended with different organic wastes. The cumulative CO_2 production in all samples increased gradually to the last day of sampling. A relatively lower amount of CO_2 (28mg) was liberated in unamended oil polluted soil compared to those of oil polluted amended soil where 52.73mg, 53.17mg and 49.13mg CO_2 were liberated in soil amended with BS, BSG and SMC respectively. The high amount of CO_2 liberated in soil amended with organic wastes is an indication of high utilization of hydrocarbon fractions as a source of carbon and energy by microbial community than that of unamended polluted soil. This is also supported by the results of extent of biodegradation of used lubricating oil (Figure 2), where it shows a positive linear correlation between extent of biodegradation and CO_2 evolution. These agrees with the findings of Ijah and Antai, [17], who reported high evolution of CO_2 in crude oil contaminated soil amended with Chicken droppings as compared to the unamended contaminated soil.

Table 2. Concentration of CO_2 in soil treated with used lubricating oil and amended with organic wastes

Incubation	**CO_2 Concentration (mg)**			
Period (days)	**A**	**B**	**C**	**D**
7	12.83 ± 0.92	12.1 ± 2.1	13.13 ± 1.99	8.69 ± 0.78
14	36.08 ± 2.68	34.83 ± 3	33.22 ± 0.66	18.26 ± 0.93
21	48.99 ± 0.67	48.77 ± 0.34	43.78 ± 0.22	25.0 ± 0.47
28	52.73 ± 0.7	53.17 ± 0.83	49.13 ± 0.46	28.0 ± 0.47

A = Soil + Oil+ BS, B = Soil + Oil + BSG, C = Soil + Oil + SMC, D = Soil + Oil

Hydrocarbon utilizing bacteria (HUB) were more abundant in oil polluted soil amended with different organic wastes than that of unamended polluted soil (Figure 2). The counts ranged from 10.2 x 10^6 CFU/g of soil to 80.5 x 10^6 CFU/g of soil in amended soil compared to that of unamended soil that ranged from 1.0 x 10^6 CFU/g of soil to 3.5 x 10^6 CFU/g of soil. Statistical analysis revealed that there is significant difference in the counts of HUB between the amended soil and unamended soil at ($P<0.05$). Amendment of the oil polluted soil with organic wastes enhanced the proliferation of more bacteria in the soil. This may be due to nutrients contained in these organic wastes which are suitable for microbial growth. This finding agrees with the report of Atlas and Bartha [18] that the amendment of oil polluted soil with fertilizer stimulated the proliferation of oil utilizing bacteria. The HUB was identified as species of *Acinetobacter, Micrococcus, Pseudomonas, Nocardia* and *Bacillus*. These bacterial species had been implicated in hydrocarbon degradation by different investigators [19, 20, 21, 22].

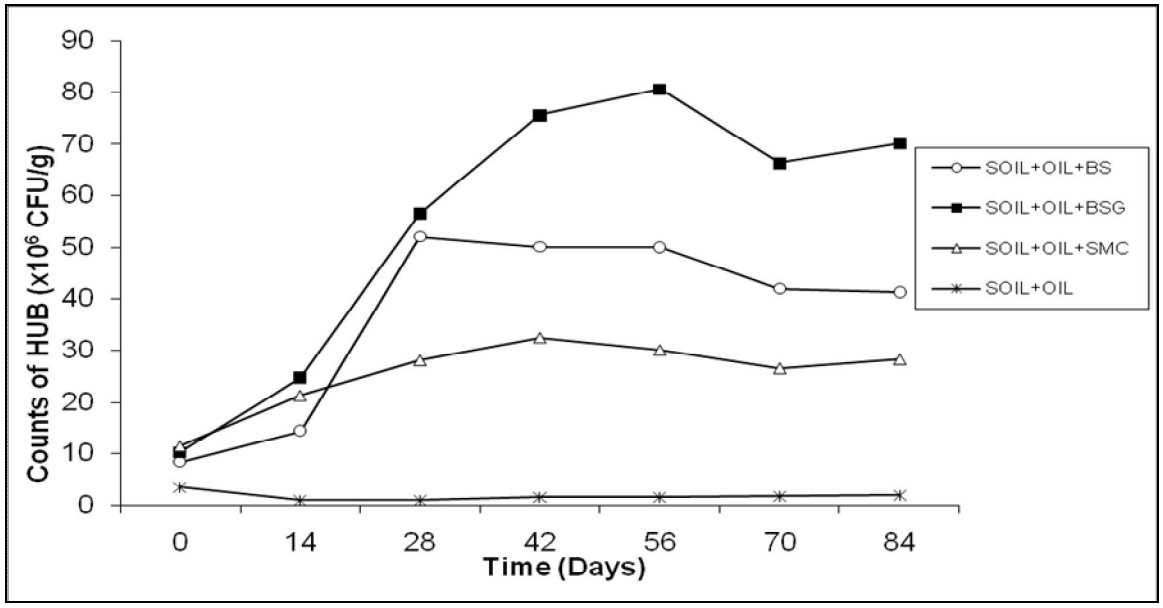

Figure 2. Counts of hydrocarbon utilizing bacteria in oil polluted soil

The pH of the soil used for bioremediation studies was 6.12 (Table 1) while that of unamended oil polluted soil ranged between 6.25 and 6.87 (Figure 3). This indicated that addition of used lubricating oil to soil slightly raised the pH of the soil. The pH of soil amended with different organic wastes ranged from 6.35 to 8.27 (Figure3). Soil amended with BS had the highest pH of 8.27, this may account for the reason why soil amended with BS recorded highest count of HUB compared to other treatments, since the slightly alkaline nature of the soil will encourage the growth of hydrocarbon utilizing bacteria.

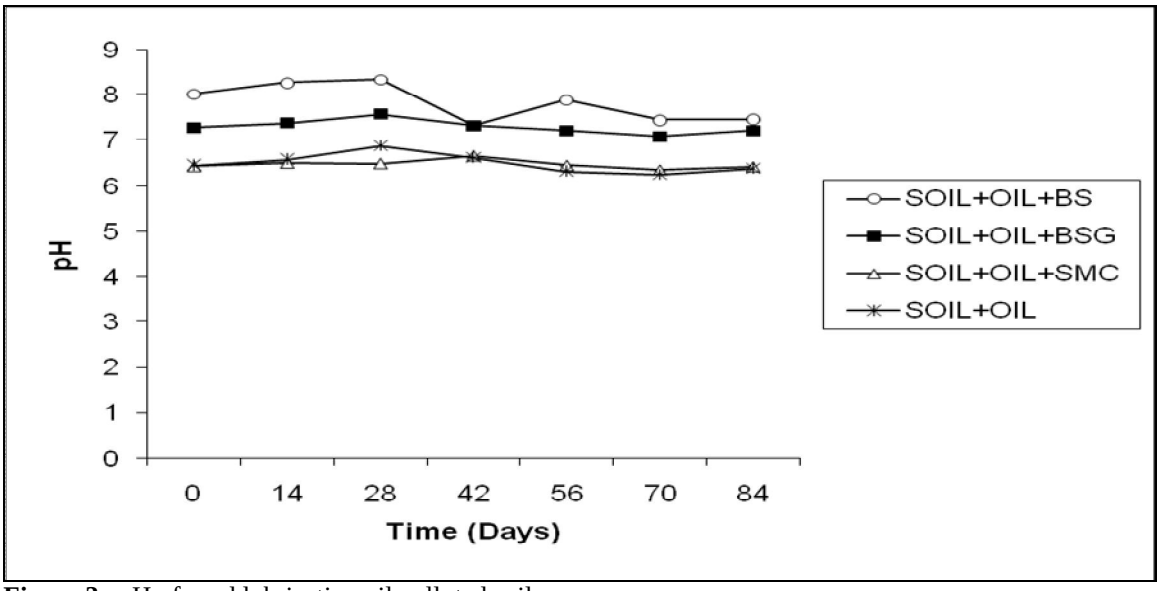

Figure 3. pH of used lubricating oil polluted soil

CONCLUSION

Amendment of used lubricating oil contaminated soil with organic wastes caused more proliferation of hydrocarbon utilizing bacteria and enhanced biodegradation of used lubricating oil in the soil. BSG caused more enhancement of the used oil biodegradation than BS and SMC. Therefore these organic wastes can play an important role in remediation of soil contaminated by used lubricating oil.

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