Water Air Soil Pollut (2010) 209:173–179
DOI 10.1007/s11270-009-0189-3

Enhanced Biodegradation of Used Engine Oil in Soil Amended with Organic Wastes

Peter O. Abioye · A. Abdul Aziz · P. Agamuthu

Received: 22 April 2009 / Accepted: 31 August 2009 / Published online: 16 September 2009

Abstract Three organic wastes (banana skin (BS), brewery spent grain (BSG), and spent mushroom compost (SMC)) were used for bioremediation of soil spiked with used engine oil to determine the potential of these organic wastes in enhancing biodegradation of used oil in soil. The rates of biodegradation of the oil were studied for a period of 84 days under laboratory conditions. Hydrocarbon-utilizing bacterial counts were high in all the organic waste-amended soil ranging between 10.2×10^6 and 80.5×10^6 CFU/g compared to unamended control soil throughout the 84 days of study. Oil-contaminated soil amended with BSG showed the highest reduction in total petroleum hydrocarbon with net loss of 26.76% in 84 days compared to other treatments. First-order kinetic model revealed that BSG was the best of the three organic wastes used with biodegradation rate constant of 0.3163 day^{-1} and half-life of 2.19 days. The results obtained demonstrated the potential of organic wastes for oil bioremediation in the order BSG>BS>SMC.

P. O. Abioye (✉) · P. Agamuthu
Institute of Biological Sciences, University of Malaya,
50603 Kuala Lumpur, Malaysia
e-mail: bisyem2603@yahoo.com

A. Abdul Aziz
Department of Chemical Engineering,
University of Malaya,
50603 Kuala Lumpur, Malaysia

Keywords Bioremediation · Used engine oil · Organic waste · Hydrocarbon · Bacteria

1 Introduction

Engine oil is a complex mixture of hydrocarbons and other organic compounds, including some organometallic constituents (Butler and Mason 1997) that is used to lubricate parts of an automobile engine, in order to smoothened engine operation (Hagwell et al. 1992). Used motor oil contains metals and heavy polycyclic aromatic hydrocarbons that could contribute to chronic hazards including mutagenicity and carcinogenicity (Keith and Telliard 1979; Hagwell et al. 1992; Boonchan et al. 2000). Prolonged exposure to high oil concentration may cause the development of liver or kidney disease, possible damage to the bone marrow, and an increased risk of cancer (Propst et al. 1999; Mishra et al. 2001; Lloyd and Cackette 2001). In Nigeria and some developing countries, about 20 million gallons of waste engine oil are generated annually from mechanic workshops and discharged carelessly into the environment (Faboya 1997; Adegoroye 1997). According to USEPA (1996), only 1 l of used engine oil is enough to contaminate one million gallons of freshwater. Used engine oil also renders the environment unsightly and constitutes a potential threat to humans, animals, and vegetation (ATSDR 1997; Edewor et al. 2004; Adelowo et al. 2006). Environmental pollution with

petroleum and petrochemical products has attracted much attention in recent times. The presence of various kinds of automobiles and machinery vehicles has caused an increase in the use of motor oil. Spillages of used motor oils such as diesels or jet fuels contaminate our natural environment with hydrocarbon (Husaini et al. 2008).

As the usage of petroleum hydrocarbon products increase, soil contamination with diesel and engine oils is becoming one of the major environmental problems. To investigate the countermeasure to remediate soils contaminated with oils, bioremediation provides an effective and efficient strategy to speed up the clean-up processes (Mandri and Lin 2007). Various factors may limit the rate of petroleum hydrocarbon degradation including lack of essential nutrients such as nitrogen. Therefore, the addition of inorganic or organic nitrogen-rich nutrients (biostimulation) is an effective approach to enhance the bioremediation process (Hollender et al. 2003; Semple et al. 2006; Walworth et al. 2007). Positive effects of nitrogen amendment on microbial activity and/or petroleum hydrocarbon degradation have been widely demonstrated (Jørgensen et al. 2000; Margesin et al. 2000, 2007; Brook et al. 2001; Margesin and Schinner 2001; Riffaldi et al. 2006).

The objectives of this work were to determine the potential of banana skin, brewery spent grain, and spent mushroom compost in enhancing biodegradation of used engine oil in soil as an alternative to the use of inorganic fertilizers, which are very expensive, and these organic wastes are widely available as wastes in our environment. Also, we aimed to test a kinetic model to determine the rate of biodegradation of the hydrocarbon in the soil and subsequently determine the half-life of the oil degradation.

2 Methods

2.1 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) was collected from Carlsberg brewery, Shah Alam, Selangor, and spent mushroom compost (SMC) was collected from Gano mushroom farm, Tanjung Sepat, Selangor.

2.2 Microcosm Set-up Description

Soil (1.5 kg; sieved with 2-mm mesh size) was placed in plastic vessels labeled A to D with a volume of about 3,000 cm^3 and polluted with 10% (*w*/*w*; Ijah and Antai 2003a) used engine oil (100,000 mg kg^{-1} soil) and left undisturbed for 2 days. After 2 days, 10% (Ijah and Antai 2003a) of each organic waste (ground dry BS, BSG, and SMC) were individually introduced into each oil-polluted soil labeled A, B, and C, respectively, and thoroughly mixed. The moisture content was adjusted to 60% water holding capacity and incubated at room temperature (28±2°C). Vessel D with only soil and used engine oil served as control. The content of each vessel was tilled twice a week for aeration, and the moisture content was maintained at 60% water holding capacity by the addition of sterile distilled water. The experiment was set up in triplicate.

2.3 Sampling

Periodic sampling from each vessel was carried out at 14-day intervals for 84 days. Composite samples were obtained by mixing 5 g of soil collected from four different areas of the microcosm for isolation and enumeration of bacteria and determination of total petroleum hydrocarbon.

2.4 Physicochemical Property Determination of Soil and Organic Wastes

Nitrogen content of soil used for bioremediation and organic wastes was determined using the Kjeldahl method, and phosphorus and carbon contents were determined using ICP-OES 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-min equilibration. Triplicate determinations were made.

2.5 Total Petroleum Hydrocarbon Determination

Hydrocarbon content of the soil samples was determined gravimetrically by toluene cold extraction

Table 1 Physicochemical properties of soil and organic wastes used for bioremediation

Parameter	Soil	Organic wastes		
		BSG	BS	SMC
pH	6.12±0.23	6.66±0.49	7.04±0.29	5.64±0.25
Nitrogen (%)	0.4±0.02	1.02±0.1	0.4±0.01	0.5±0.03
Phosphorus (mg kg^{-1})	21.8±1.5	20.6±2.0	21.2±1.4	22.5±1.8
Organic C (%)	10.3±1.1	10.9±0.91	10.5±1.3	10.2±1.1
Moisture (%)	7.0±0.3	71.84±3.5	38.5±2.86	62.3±4.12
Sand (%)	37.5±2.6	–	–	–
Silt (%)	18.75±1.95	–	–	–
Clay (%)	43.75±2.75	–	–	–
Texture	Clayey	–	–	–

BSG brewery spent grain, *BS* banana skin, *SMC* spent mushroom compost

method of Adesodun and Mbagwu (2008). Soil sample (10 g) was weighed into 50-ml flask and 20 ml of toluene (AnaLar grade) was added. After shaking for 30 min on an orbital shaker (model N-Biotek-101M), the liquid phase of the extract was measured at 420 nm using DR/4000 spectrophotometer. The total petroleum hydrocarbon (TPH) in soil was estimated with reference to standard curve derived from fresh used engine oil diluted with toluene. TPH data was fitted to first-order kinetics model of Yeung et al. (1997).

$$y = ae^{-kt}$$

Where y is the residual hydrocarbon content in soil (g kg^{-1}), a is the initial hydrocarbon content in soil (g kg^{-1}), k is the biodegradation rate constant (day^{-1}), and t is time (day). The model estimated the biodegradation rate and half-life of hydrocarbons in soil relative to treatments applied. Half-life was then calculated from the model of Yeung et al. (1997) as

$$\text{Half life} = \ln(2)/k$$

This model was based on the assumption that the degradation rate of hydrocarbons positively correlated with the hydrocarbon pool size in soil (Yeung et al. 1997).

2.6 Enumeration and Identification of Bacteria in Soil

Three replicate samples from each oil-polluted soil were withdrawn every 14 days for the enumeration of total aerobic heterotrophic bacteria (AHB). Serially diluted samples (0.1 ml) were plated on nutrient agar medium (Oxoid) supplemented with 50 µg/ml nystatin to suppress the growth of fungi. Triplicate

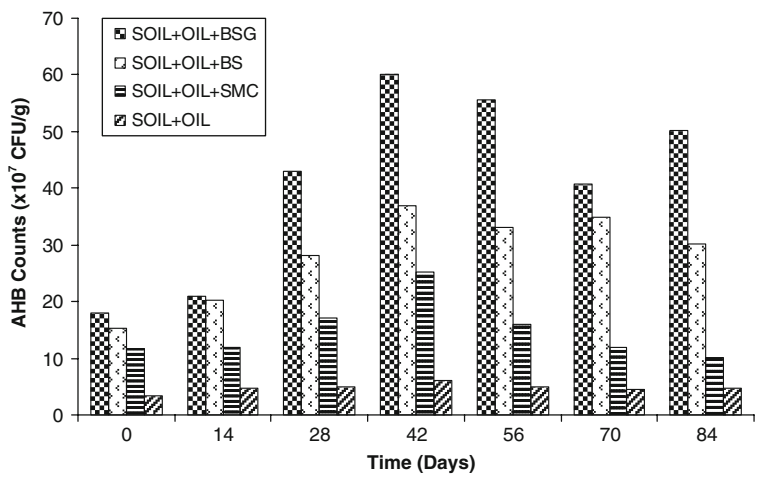

Fig. 1 Counts of aerobic heterotrophic bacterial (*AHB*) population in `oil-polluted soil

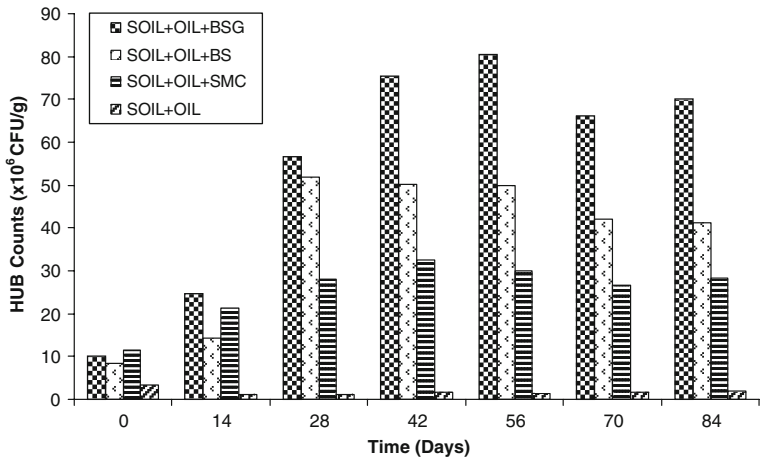

Fig. 2 Counts of hydrocarbon-utilizing bacterial (*HUB*) population in oil-polluted soil

plates were incubated at 30°C for 24 h before the colonies were counted. Hydrocarbon-utilizing bacteria (HUB) in the soil samples were enumerated using mineral salt medium of Zajic and Supplission (1972; 1.8 g K_2HPO_4, 4.0 g NH_4Cl, 0.2 g $MgSO_4.7H_2O$, 1.2 g KH_2PO_4, 0.01 g $FeSO_4.7H_2O$, 0.1 g NaCl, 20 g agar, 1% used engine oil in 1,000 ml distilled water, pH7.4). The oil agar plates were incubated at 30°C for 5 days, and the colonies were counted and randomly picked, and pure isolates were obtained by repeated sub-culturing on nutrient agar (Oxoid). The bacterial isolates were characterized using microscopic techniques and biochemical tests. The identities of the isolates were determined by comparing their characteristics with those of known taxa as described in Bergey's manual of determinative bacteriology.

2.7 Statistical Analysis

Statistical analysis of data was carried out using analysis of variance.

3 Results

Table 1 shows the physicochemical properties of soil and the organic wastes used for bioremediation studies.

3.1 Microbial Counts

The counts of AHB in soil amended with BSG ranged between 18.1×10^7 and 60.0×10^7 CFU/g, while that of soil amended with BS and SMC ranged from 15.3×

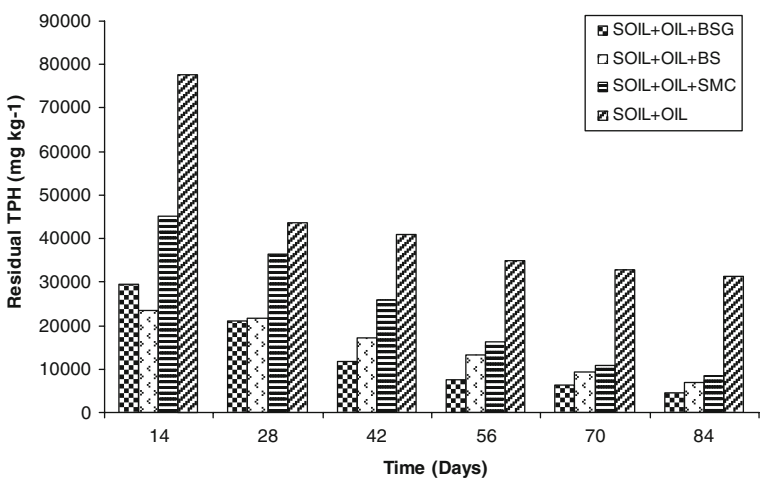

Fig. 3 Residual total petroleum hydrocarbon in soil during bioremediation

Table 2 Net percentage loss of total petroleum hydrocarbon in soil during bioremediation

Treatment	Time (days)					
	14	28	42	56	70	84
A	54.03±3.1	21.79±1.8	23.91±2.1	23.89±0.2	22.07±1.3	24.39±0.6
B	48.26±0.8	22.53±2.5	29.07±3.8	29.46±2.2	25.11±2.9	26.76±1.8
C	32.43±1.7	7.11±0.2	15.16±1.5	20.92±0.7	20.62±1.3	22.80±2.1

A=soil+oil+BS, B=soil+oil+BSG, C=soil+oil+SMC. Net% loss=% loss in TPH of oil-polluted soil amended with organic wastes−% loss in TPH of unamended polluted soil

10^7 to 37.0×10^7 and 10.1×10^7 to 25.3×10^7CFU/g, respectively (Fig. 1). The unamended control soil had the count of AHB ranging between 3.4×10^7 and 5.0×10^7CFU/g. The count of HUB was also higher in oil-contaminated soil amended with different organic wastes (Fig. 2). The count of HUB in soil amended with BSG was about 5% higher than those amended with BS and SMC. HUB count in BSG amended soil ranged from 10.2×10^6 to 80.5×10^6CFU/g, while those amended with BS and SMC ranged from 8.4×10^6 to 52.0×10^6 and 11.5×10^6 and 32.4×10^6CFU/g, respectively. However, the HUB count in unamended control soil was (1.0×10^6 to 3.5×10^6CFU/g) lower than those amended with organic wastes.

3.2 Biodegradation of Used Engine Oil

The level of biodegradation of used engine oil throughout the study period is shown in Fig. 3. There was a rapid reduction in the total petroleum hydrocarbon within the first 14 days of the study in all the soil amended with organic wastes compared to that of unamended soil. At the end of the 14 days, there was 55%, 71%, and 76% TPH reduction in soil amended with SMC, BSG, and BS, respectively, i.e., 54,872, 70,648, and 76,417 mg kg^{-1} TPH reduction, respectively, was observed in amended soil compared to 22% (22,387 mg kg^{-1}) TPH reduction in unamended control soil (Fig. 3).

At the end of 84 days, oil-contaminated soil amended with BSG showed the highest reduction in concentration of used engine oil (95%), followed closely by soil amended with BS (93%), while reduction in TPH in soil amended with SMC showed 92% in the concentration of used oil compared to the unamended control soil that showed 68% reduction at the end of 84 days. The effectiveness of each amendment was determined by calculating the net percentage loss of used oil in the contaminated soil.

The highest net percentage loss was observed at 14 days in soil amended with BS (54.03%) followed by that of BSG (48.26%) and SMC (32.48%), respectively (Table 2). However, the net percentage loss of oil became higher in soil amended with BSG from 28 days to the end of the experimental study (84 days) compared with those of BS and SMC, respectively.

3.3 Biodegradation Rate Constant and Half-Life

First-order kinetics model of Yeung et al. (1997) was used to determine the rate of biodegradation of used oil in the various treatments. Table 3 shows the biodegradation rate constant (k) and half-life ($t_{1/2}$) for the different treatments within the 84 days of study. Data for the sampling periods were combined before this model could be used. Soil amended with BSG shows the highest biodegradation rate of 0.3163 day^{-1} and half-life 2.19 days; the biodegradation rates and half-life of soil amended with BS

Table 3 Biodegradation rate and half-life of hydrocarbon in oil-polluted soil

Treatment	Biodegradation constant (k) day^{-1}	Half-life ($t_{1/2}$) days
A	0.3016 a	2.30
B	0.3163 b	2.19
C	0.2189 a	3.17
D	0.1604 a	4.32

A=soil+oil+BS, B=soil+oil+BSG, C=soil+oil+SMC, D=soil+oil. Values followed by letter b indicate significant difference at the $P<0.05$ level, while values followed by letter a are not different significantly at the $P<0.05$ level

and SMC are 0.3016 day^{-1}, half-life of 2.30 days and 0.2189 day^{-1}, half-life of 3.17 days, respectively. The biodegradation rate of unamended control soil was the least, 0.1604 day^{-1} and half-life of 4.32 days.

4 Discussion

The counts of HUB in all the soil amended with various organic wastes were higher compared to that of unamended control soil; these counts are comparable to those of Ijah and Antai (2003b), who observed counts of hydrocarbon degraders in oil-polluted soil to be $\times 10^6$CFU/g but lower than those obtained by Antai and Mgbomo (1989) whose counts of HUB in hydrocarbon-contaminated soil was $\times 10^8$ CFU/g; this may be due to differences in microbial ecology of the soil or characteristics of the experimental soils. The reason for higher counts of bacteria in amended soil may be the result of the presence of appreciable quantities of nitrogen and phosphorus in the organic wastes, especially high nitrogen content in BSG (Table 1), which is a necessary nutrient for bacterial biodegradative activities (Nakasaki et al. 1992; Ijah and Antai 2003a; Joo et al. 2001, 2007; Adesodun and Mbagwu 2008).

HUB isolated from the soil samples were identified as species of *Acinetobacter*, *Micrococcus*, *Pseudomonas*, *Nocardia*, and *Bacillus*. These bacterial species had been implicated in hydrocarbon degradation by different authors (Ijah 1998; Ahn et al. 1999; Van Hamme et al. 2003; Bento et al. 2005; Das and Mukherjee 2007). Of all the bacterial isolates, *Acinetobacter* and *Nocardia* species demonstrated higher ability in utilizing hydrocarbon when inoculated directly into minimal salt broth containing used oil as the sole source of carbon and energy (results not shown); this may be due to the presence of efficient hydrocarbon degradative enzyme systems and the presence of catabolic genes involved in hydrocarbon degradation in the bacterial species (Kyung-Hwa et al. 2006; Majid et al. 2008).

Total petroleum hydrocarbon was determined in this study rather than individual petroleum components because used oil is highly variable and have altered structure due to combustion process (Tauscher 1988; Adesodun and Mbagwu 2008). Biodegradation of used oil was high (92–95%) in all the soil amended with different organic wastes compared to the unamended soil (68%). The kinetic parameters observed in this study show that the rate of degradation of used oil in soil amended with BSG was higher than other treatments; this may be due to high percentage of nitrogen present in BSG compared to other organic wastes (Table 1) and bioavailabilty of the nutrients in BSG to bacterial species in the oil-polluted soil. However, the rate of oil breakdown in soil amended with SMC was lower than those of BS and BSG; this might be due to its low pH throughout the 84 days of study (result not shown) because low pH condition do affect the growth and biodegradative activities of HUB in soil.

Acknowledgments The authors would like to acknowledge the support of the University of Malaya IPPP Grant PS 244/2008C. Also, we would like to thank the managements of Carlsberg Brewery for providing brewery spent grain and Gano farm for the provision of spent mushroom compost.

References

Adegoroye, G. (1997). Environmental considerations in property design, urban development and renewal. In O. Akinjide (Ed.), *Dimensions of environmental problems in Nigeria* (pp. 12–25). Washington: Friedrich Ebert Foundation.

Adelowo, O. O., Alagbe, S. O., & Ayandele, A. A. (2006). Time-dependent stability of used engine oil degradation by cultures of *Pseudomonas fragi* and *Achromobacter aerogens*. *African Journal of Biotechnology, 5*(24), 2476–2479.

Adesodun, J. K., & Mbagwu, J. S. C. (2008). Biodegradation of waste lubricating petroleum oil in a tropical alfisol as mediated by animal droppings. *Bioresource Technology, 99*, 5659–5665.

Ahn, Y. H., Sanseverino, J., & Sayler, G. S. (1999). Analyses of polycyclic aromatic hydrocarbon-degrading bacteria isolated from contaminated soils. *Biodegradation, 10*, 149–157.

Antai, S. P., & Mgbomo, E. (1989). Distribution of hydrocarbon utilizing bacteria in oil-spill areas. *Microbiology Letters, 40*, 137–143.

ATSDR. (1997). *Public health statements: Used mineral based crankcase oil*. Atlanta: ATSDR.

Bento, F. M., Camargo, F. O. A., Okeke, B. C., & Frankenberger, W. T. (2005). Comparative bioremediation of soils contaminated with diesel oil by natural attenuation, biostimulation and bioaugmentation. *Bioresource Technology, 96*, 1049–1055.

Boonchan, S., Britz, M. L., & Stanley, G. A. (2000). Degradation and mineralization of high-molecular weight polycyclic aromatic hydrocarbons by defined fungal–bacterial co-cultures. *Applied Environmental Microbiology, 66*(3), 1007–1019.

Brook, T. R., Stiver, W. H., & Zytner, R. G. (2001). Biodegradation of diesel fuel in soil under various nitrogen addition regimes. *Soil Sediment Contamination, 10*, 539–553.

Butler, C. S., & Mason, J. R. (1997). Structure–function analysis of the bacterial aromatic ring–hydroxylating dioxygenases. *Advanced Microbial Physiology, 38*, 47–84.

Das, K., & Mukherjee, A. K. (2007). Crude petroleum-oil biodegradation efficiency of *Bacillus subtilis* and *Pseudomonas aeruginosa* strains isolated from petroleum oil contaminated soil from North–East India. *Bioresource Technology, 98*, 1339–1345.

Edewor, T. I., Adelowo, O. O., & Afolabi, T. J. (2004). Preliminary studies into the biological activities of a broad spectrum disinfectant formulated from used engine oil. *Pollution Research, 23*(4), 581–586.

Faboya, O. O. P. (1997). Industrial pollution and waste management. In O. Akinjide (Ed.), *Dimensions of environmental problems in Nigeria* (pp. 12–25). Washington: Friedrich Ebert Foundation.

Hagwell, I. S., Delfino, L. M., & Rao, J. J. (1992). Partitioning of polycyclic aromatic hydrocarbons from oil into water. *Environmental Science and Technology, 26*, 2104–2110.

Hollender, J., Althoff, K., Mundt, M., & Dott, W. (2003). Assessing the microbial activity of soil samples, its nutrient limitation and toxic effects on contaminants using a simple respiration test. *Chemosphere, 53*, 269–275.

Husaini, A., Roslan, H. A., Hii, K. S. Y., & Ang, C. H. (2008). Biodegradation of aliphatic hydrocarbon by indigenous fungi isolated from used motor oil contaminated sites. *World Journal of Microbiology and Biotechnology, 24*, 2789–2797.

Ijah, U. J. J. (1998). Studies on relative capabilities of bacterial and yeast isolates from tropical soil in degrading crude oil. *Waste Management, 18*, 293–299.

Ijah, U. J. J., & Antai, S. P. (2003a). The potential use of chicken-drop microorganisms for oil spill remediation. *The Environmentalist, 23*, 89–95.

Ijah, U. J., & Antai, S. P. (2003b). Removal of Nigerian light crude oil in soil over a 12 month period. *International Biodeterioration and Biodegradation, 51*, 93–99.

Joo, H. S., Phae, C. G., & Ryu, J. Y. (2001). Comparison and analysis on characteristics for recycling of multifarious food waste. *J KOWREC, 9*, 117–124.

Joo, H. S., Shoda, M., & Phae, C. G. (2007). Degradation of diesel oil in soil using a food waste composting process. *Biodegradation, 18*, 597–605.

Jørgensen, K. S., Puustinen, J., & Suortti, A. M. (2000). Bioremediation of petroleum hydrocarbon-contaminated soil by composting in biopiles. *Environmental Pollution, 107*, 245–254.

Keith, L. H., & Telliard, W. A. (1979). Priority pollutants 1—A perspective view. *Environmental Science and Technology, 13*, 416–423.

Kyung-Hwa, B., Byung-Dae, Y., Hee-Mock, O., Hee-Sik, K., & In-Sook, L. (2006). Biodegradation of aliphatic and aromatic hydrocarbons by *Nocardia* sp. H17-1. *Geomicrobiology Journal, 23*(5), 253–259.

Lloyd, C. A., & Cackette, T. A. (2001). Diesel engines: Environmental impact and control. *Air and Waste Management Association, 51*, 805–847.

Majid, Z., Mnouchehr, V., & Sussan, K. A. (2008). Naphthalene metabolism in *Nocardia otitidiscaviarum* strain TSH 1, a moderately thermophilic microorganism. *Chemosphere, 72*, 905–909.

Mandri, T., & Lin, J. (2007). Isolation and characterization of engine oil degrading indigenous microorganisms in Kwazulu-Natal, South Africa. *African Journal of Biotechnology, 6*(1), 23–27.

Margesin, R., & Schinner, F. (2001). Bioremediation (natural attenuation and biostimulation) of diesel-oil-contaminated soil in an Alpine glacier skiing area. *Applied and Environmental Microbiology, 67*, 3127–3133.

Margesin, R., Zimmerbauer, A., & Schinner, F. (2000). Monitoring of bioremediation by soil biological activities. *Chemosphere, 40*, 339–346.

Margesin, R., Hammerle, M., & Tscherko, D. (2007). Microbial activity and community composition during bioremediation of diesel-oil-contaminated soil: Effects of hydrocarbon concentration, fertilizers, and incubation time. *Microbiol Ecol, 53*, 259–269.

Mishra, S. J., Jyot, R. C., & Kuhad, B. L. (2001). Evaluation of inoculum addition to stimulate in situ Bioremediation of oily–sludge-contaminated soil. *Applied Environmental Microbiology, 67*(4), 1675–1681.

Nakasaki, K., Yaguchi, H., Sasaki, Y., & Kubota, H. (1992). Effect of C/N ratio on thermophilic composting of garbage. *Journal of Fermentation and Bioengineering, 73*, 43–45.

Propst, T. L., Lochmiller, R. L., Qualis, C. W., & McBee, K., Jr. (1999). In situ (mesocosm) assessment of immune-toxicity risks to small mammals inhabiting petrochemical waste sites. *Chemosphere, 38*, 1049–1067.

Riffaldi, R., Levi-Minzi, R., Cardelli, R., Palumbo, S., & Saviozzi, A. (2006). Soil biological activities in monitoring the bioremediation of diesel oil-contaminated soil. *Water, Air & Soil Pollution, 170*, 3–15.

Semple, K. T., Dew, N. M., Doick, K. J., & Rhodes, A. H. (2006). Can mineralization be used to estimate microbial availability of organic contaminants in soil? *Environmental Pollution, 140*, 164–172.

Tauscher, W. (1988). The option for re-cycling and re-utilizing used petroleum oils. *Natural Resources Development, 28*, 100–107.

USEPA. (1996). Recycling used oil: What can you do? *Cooperation Extension Services ENRI, 317*, 1–2.

Van Hamme, J. D., Singh, A., & Ward, O. P. (2003). Recent advances in petroleum microbiology. *Microbiology and Molecular Biology Review, 67*(4), 503–549.

Walworth, J., Pond, A., Snape, I., Rayner, J., Ferguson, S., & Harvey, P. (2007). Nitrogen requirements for maximizing petroleum bioremediation in a sub-Antarctic soil. *Cold Regions Science and Technology, 48*, 84–91.

Yeung, P. Y., Johnson, R. L., & Xu, J. G. (1997). Biodegradation of petroleum hydrocarbons in soil as affected by heating and forced aeration. *Journal of Environmental Quality, 26*, 1511–1576.

Zajic, E., & Supplission, B. (1972). Emulsification and degradation of "Banker C" fuel oil by microorganisms. *Biotechnology and Bioengineering, 14*, 331–343.