BIODEGRADATION OF POLYCYCLIC AROMATIC HYDROCARBONS (PAH) FROM PAIKO OIL DEPOT USING A MIXED CULTURE OF BACTERIA

Musa Umaru; Munir, S. M.; Mohammed, I. A.; & Aliyu, A. M.
Department of Chemical Engineering,
Federal University of Technology, Minna, Niger State
E-mail: umardikko2001@yahoo.com*
Phone No: +234-803-231-8723

Abstract
Petroleum development and production have resulted in soil degradation, the generation of air and water pollutants, solid and hazardous wastes. In this study, the degradation potential of polycyclic aromatic hydrocarbons (PAHs) was investigated using a consortia of mixed bacterial culture. Phenanthrene, a three-ringed angular PAH, known to be a human skin photosensitizer and mild allergen was used as a representative of PAH to study the degradation efficiency of a mixed culture of bacteria and fungi. Microorganisms were isolated from hydrocarbon contaminated soils of Pipeline Product Marketing Company Paiko depot, Niger State. The degradation experiments were conducted in liquid cultures. The initial phenanthrene concentration was 60 mg/l at the beginning of degradation experiments. After fifteen days incubation, the mixed culture was capable of degrading about 17% of the phenanthrene with a bacterial population of about 1×10^8 CFU/g. The mixed culture achieved a 100% degradation of the phenanthrene after 34 days. The Lineweaver-Burk plot was used to observe the correlation between the rate of degradation (V_o) and the concentration of the substrate ([S]). The value of Michealis constant (K_M) was determined to be0.00 mg/l.

Keywords: PAH, Phenanthrene, Bacteria, Contaminated soil

Introduction

The contamination of soils with xenobiotic compounds due to industrial activities is widespread. The degradation of soils with polycyclic aromatic hydrocarbons (PAHs) has been implicated into environmental and human health problems since these compounds are toxic, mutagenic and has a carcinogenic potential (Acevedo, 2010). Polycyclic aromatic hydrocarbons (PAHs) comprise of a large and heterogeneous group of organic contaminants which are formed and emitted as a result of the incomplete combustion of organic material (Kumara *et al*., 2006, Bamforth and Singleton, 2005). PAHs are ubiquitous in the natural environment, and originate from two main sources: natural (biogenic and geochemical) and anthropogenic (Bamforth and Singleton, 2005). These compounds are produced by industrial activities such as oil processing and storage, and are often found in contaminated soil (Kim *et al.,* 2001). PAHs are fused-ring compounds that enter soil systems and natural waters via wastewater effluents from coke and petroleum refining industries, accidental spills and leakages, rainwater runoff from highways and roadways, or from intentional disposal in the past (Karthikeyan and Bhandari, 2001). The inertness of these compounds, their low water solubility and strong lipophilic character lead to very high accumulation levels in the environment (Parviz *et al.,* 2006). The persistence of PAHs in the environment is dependent on a variety of factors, such as the chemical structure of the PAH, the concentration and dispersion of the PAH and the bioavailability of the contaminant (Bamforth and Singleton, 2005). Besides this, they possess physical properties, such as low aqueous solubility and high solid water distribution species ratios, which stand against their ready microbial utilization and promote their accumulation in the solid phases of the terrestrial environment (Muller *et al*., 2004).

Polycyclic aromatic hydrocarbons (PAHs) also comprise of a large and heterogeneous group of organic contaminants but the most toxic members of this family known to date are PAH molecules that have four to seven rings (Fatai, 2010). The United States Environmental Protection Agency

currently regulates sixteen (16) PAH compounds as priority pollutants in water and generally considers them as "total PAH" (tPAH) in contaminated soils. The 16 regulated PAHs comprise both low and high molecular weight, and seven of them are designated as known human carcinogens (Arbabi *et al*., 2009). All of the carcinogenic PAH (cPAH) are high molecular weight compounds, hence the improper management and disposal of oily sludge wastes may cause environmental pollution, particularly to the soil and groundwater systems, due to their low volatility and aqueous solubility (Fatai, 2010). PAHs are also recalcitrant in nature and they have high affinity for soil material and particulate matter (Kumara *et al.,* 2006, Bamforth and Singleton, 2005). Overtime, they will accumulate to the extent that they become harder to eliminate (Fatai, 2010). It is also important to note that many of the constituents of PAHs are not only carcinogenic and mutagenic, but they are also potent immune toxicants (Acevedo, 2010). Bamforth and Singleton (2005) reported the impacts of PAHs on critical habitats such as the benthic ecosystems, which may ultimately get into the marine food chain. The recent research and development in the area of PAHs disposal management is focussed on the biodegradation method, which is considered as the effective and sustainable means of controlling the effects of PAHs on the environment.

A good number of researchers have shown interest in the biodegradation mechanism and environmental fate of PAHs as a result of their ubiquitous distribution and their potentially deleterious effects on human health. (Obayori and Salam, 2010). Microbial research over the last two decades have shown that more than 160 genre of bacteria including *Psuedomonas, Alcaligenes, Vibrio, Mycobacterium, Comamonas, Rhodococcus, Neptunomonas, Naphthovorans, Cyanobacterium* and *Cycloclasticus* are capable of degrading PAHs to derive energy and metabolic building blocks (Berardesco et al., 1998). Bacteria generally require the mono- or di-oxygenase enzyme system to break PAH rings (Kulisch and Vilker, 1991). Compared to physicochemical methods, biodegradation offers an effective method for the treatment of crude oil pollution because the majority of molecules in the crude oil and refined products are biodegradable and oil degrading microorganisms are ubiquitous (Chaîneau *et al*., 2003). Several microorganism with an innate ability to degrade phenanthrene - based contaminants have been identified and extensively studied (Karthikeyan and Bhandari, 2001, Abd-Elsalam et al., 2009, Parviz *et al.,* 2006). In this work therefore, the degradation of phenanthrene, a representative PAH was studied to determine the effectiveness of a mixed culture of bacteria in the degradation process. The scope of this work is limited to the biodegradation of phenanthrene in a petroleum depot contaminated soil.

Methodology

Collection of Soil Sample

Soil sample was collected in the range of 3-4 kg from surface and 10 cm deep layer of petroleum contaminated soil at the Pipeline Product Marketing Company, Paiko depot, Niger State. Prior to conducting any analysis on the collected soil sample, the coarse pieces e.g., stones and debris were separated using a sieve and the remaining were mixed well. The sub samples were kept cold in a refrigerator (3-5) °C to be used for isolation of microorganisms.

Extraction of PAHs from Soil Sample

The solid sample (contaminated soil) was placed into an extraction thimble (Pyrex England) where available PAHs were extracted using n-hexane as the solvent. After the solvent had been boiled, the vapour passed through a bypass arm into the condenser where it condensed and dripped back onto the solvent in the thimble. As the solvent got to the top of the siphon arm, the solvent and extract were siphoned back onto the lower flask whereby the solvent reboiled, and the cycle was repeated until the sample was completely extracted into the lower flask (Arbabi *et al*., 2009).

High Performance Liquid Chromatography of Extract

Extracts from the soxhlet extractor were analysed by high performance liquid chromatography (HPLC) (water 600) equipped with ultraviolet (UV) detector (Shimadzu, Japan). Analytical column (250 mm long, 4.6 mm diameter) was packed with totally porous spherical C-18 material (packed size, 5 µm). Acetonitrile-water mixture (75:25) was used as mobile phase at a flow rate of 1.0 ml/ min. Sample (20 µl) was injected into column through sample loop. UV- detector was set at 254nm for compound detection. Stock solutions of PAHs were prepared by dissolving PAHs analytical standard (200-1000 ppm) in acetonitrile. Working standard (10 ppm) PAHs mixture was prepared by suitable dilution of stock solution with acetonitrile. Calibration graph at several dilution of standard mixture of individual compounds of PAHs were used for determining retention time and studying linearity of detector. Concentration of PAHs was calculated by comparing peak areas of sample chromatogram with that of peak area of standard chromatogram (Arbabi *et al.*, 2009)

Soil Microbial Analyses

Microorganisms were extracted from the contaminated soil by mixing of 1g soil with 10 ml of sterile $Na_2P_2O_7$ solution (2.8 g/liter) in 50 ml erlenmayer for 2hrs on a shaker (250 rpm). The soil particles were allowed to sediment for 30 mins. the supernatant was diluted and plated on solid media. Initial microbial analysis was conducted both for fungi and bacteria. Fungi determination test was carried out on solid media of heterotrophic plate count (HPC) containing chloroamphenicole and cyclohexamide compounds for inhibition of bacterial growth. The total Colony Forming Unit (CFU) was determined using plate count agar using Heterophilic Plate Count Agar (HPC) and Brain Heart Infusion (BHI) agar media. Also MPN (most probable number) analyses were conducted using lactose broth media with 15 tubes method (Arbabi *et al.*, 2009).

Microorganisms were extracted from the contaminated soil and the supernatant after dilution by 10^{-3} -10^{-5} times with sterile distilled water and then plated on solid media (BHI). After separation of bacteria, the stock samples of separated microbes were prepared by the addition of 500 µl of glycerine (Arbabi *et al.*, 2009).

Operation Techniques, Using Pilot Plant

A biodegradation concept which uses artificial air flow (Technique known as Bio venting) was selected for decontamination of PAH-contaminated soil. After the addition of microbial mixed culture, MSM containing P&N (phosphorus and nitrogen) and moisture (about 20 %), phenanthrene were filled into the bioreactor in the pilot plant. The supplied air was passed through distilled water in order to prevent reduction of water content. Also to prevent the distribution of off-gas resulting from phenanthrene decomposition, a 4 M NaOH solution was used as an absorbent (Arbabi *et al.,* 2009)

Table 1: Specification of prepared mineral salt medium

Compounds	MSM
K_2HPO_4(g/l)	0.55
NH_4Cl(g/l)	0.90
KNO_3(g/l)	1.75
Na_2SO4(g/l)	2.00
$MgSO_4.7H_2O$(g/l)	0.30
TES(ml)	1.00
pH	6.5-7.2

Results and Discussions

The initial analysis of soil for the determination of fungi yielded a negligible growth on the solid media. Bacteria population analysis as CFU/g for the soil sample is depicted in Table 2. Likewise, other necessary properties of collected contaminated soil (such as phenanthrene concentration, moisture content, pH etc) were analyzed and the result obtained is presented in Table 2.

Table 2: Microbial and Physico-chemical specifications of PAH contaminated soil samples

Properties	Quantity
Initial phenanthrene conc.(mg/l)	60
Microbial population (CFU)	1.0×10^{9}
Moisture content (%)	3.2
pH	7.1
Bulk density (g/ml)	0.7

Table 2 shows the results of microbial and physico-chemical analysis of the soil obtained from the PAH contaminated site in Paiko. The initial phenanthrene concentration was found to be 60 mg/l, moisture content was found to be 3.2 % with a pH of 7.1 and bulk density of 0.7 mg/l.

Table 3: Bacterial population changes over time in the contaminated soil.

Time (days)	Bacteria population(CFU/g)
0	1×10^{9}
2	5×10^{7}
4	7×10^{6}
6	9×10^{5}
8	8×10^{5}
10	3×10^{6}
12	4×10^{7}
14	9×10^{7}
16	1×10^{8}
18	3×10^{8}
20	4×10^{8}
22	6×10^{8}
24	7×10^{8}
26	8×10^{8}
28	9×10^{8}
30	9×10^{8}
32	9×10^{8}
34	8×10^{8}
36	6×10^{8}
38	8×10^{7}
40	4×10^{5}

Table 3 illustrates the bacteria population growth over time. The first four days of contacting the PAH contaminated soil with the cultured bacteria showed that the bacteria population reduced rapidly. This might be due to the fact that the bacteria were not well adapted to the environment (lag phase) and the effect of toxicity of phenanthrene. But after the lag phase, bacterial growth increased gradually between the tenth and the fifteenth day and then rapidly between day sixteen and day twenty-eight (exponential phase). After the twenty-eighth day the bacteria growth entered the stationary phase and the population changes was constant for a period of four days before finally reducing immediately after the stationary phase. This reduction in population was as a result of lack of substrate for the bacteria to feed on. This observation shows close proximity to the report of Arbabi *et al.,* 2009.

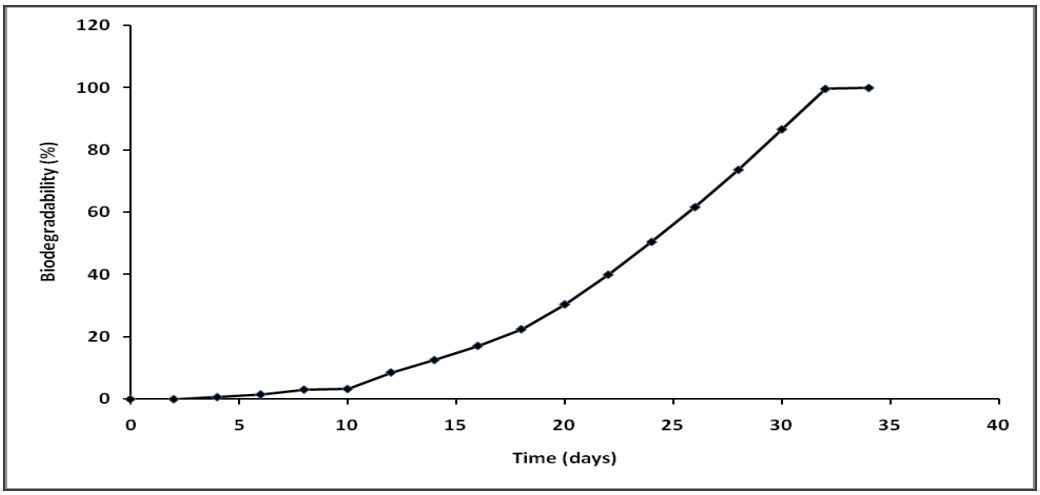

Fig. 1: Biodegradability efficiency of the bacteria

Figure 1 shows the biodegradability efficiency of phenanthrene as a function of time of biodegradation. It was observed that 100 % degradation was achieved after thirty-four days of bio-treatment. This implies that the biodegradation of phenanthrene was effectively completed within this period. This result shows appreciable consistency with the result of Bishnoi *et al.,* 2008 in which 98.6 % degradation was achieved after 42 days of bio-treatment.

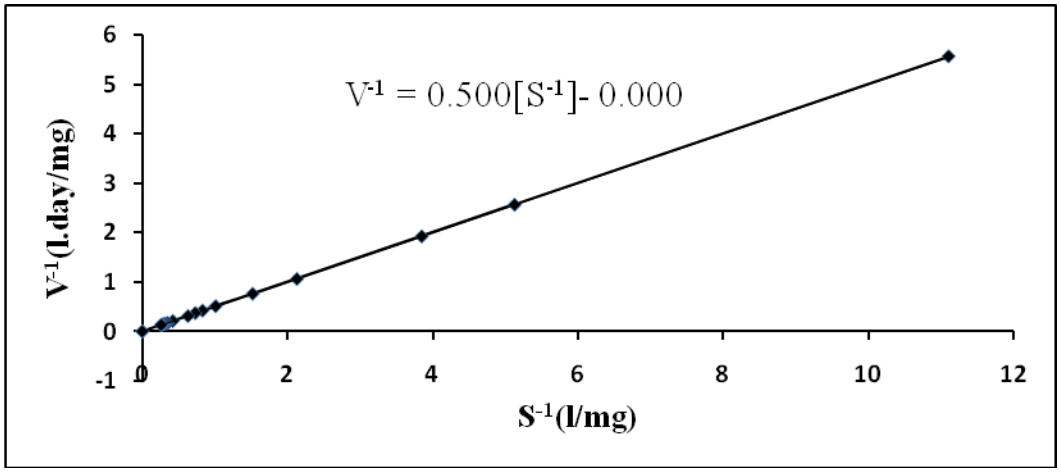

Fig. 2: Lineweaver – Burk Plot of rate against concentration

Figure 2 is a Lineweaver-Burk plot showing the correlation between the rate of degradation (V_o) and the concentration of the substrate [S]. From the graph, the slope and the intercept obtained were 0.50 days and 0.00 (l.day)/mg respectively from which the value of Michealis constant (K_M) which is the constant of degradation was determined to be 0 mg/l.

Conclusion

The biodegradation of polycyclic aromatic hydrocarbon (phenanthrene) by bacteria culture was successfully carried out. Results of the microbial analysis, shows that the prepared media contain only bacteria are available for the biodegradation process as there was no growth for fungi on the medium. It can be concluded that the mixed culture of bacteria used in this study are capable of removing the PAH from the soil. The result of this study clearly attest that mixed culture of indigenous bacterial can be effectively used to degrade PAH specifically phenanthrene as 100 % degradation efficiency was achieved after 40 days of bio-treatment. Generally, it can be concluded that contaminated soil can best be treated or decontaminated by microbial actions basically because of its simplicity, efficiency and cost effectiveness.

References

Abd-Elsalam, H. E., Hafez, E. E., Hussain, A. A., Ali, A. G. & El-Hanafy, A. A. (2009). Isolation and identification of three-rings polyaromatic hydrocarbons (anthracene and phenanthrene) degrading bacteria. *American-Eurasian Journal of Agriculture & Environmental Science,* 5. 57-62.

Acevedo, E. C. F. (2010). *Degradation of polycyclic aromatic hydrocarbons by the whiterot fungus anthracophyllum discolor sp4 and nanoclayimmobilized manganese peroxidase.* PhD, Universidad de La Frontera.

Arbabi, M., Nasseri, S. & Chimezie, A. (2009). Biodegradation of polycyclic aromatic hydrocarbons (pahs) in petroleum contaminated soils. *Iran Journal of Chemical Engineering,* 28**,** 53-59.

Bamforth, S. M. & Singleton, I. (2005). Bioremediation of polycyclic aromatic hydrocarbons: Current knowledge and future directions. *Journal of Chemical Technology and Biotechnology,* 80**,** 723–736.

Berardesco, G., Dyhrman, S., Gallagher, E. & Shiaris, M. P. (1998). Spatial and temporal variation of phenanthrene-degrading bacteria in intertidal sediments. *Applied and Environmental Microbiology, 64, 2560-2565.*

Bishnoi, K., Rajender, K. & Nasri, R. B. (2008) Biodegradation of PAH by white rot fungi phanerochaete chrysosporium in sterile and unsterile soil. *Journal of Scientific and Industrial Research 67, 538-542.*

Chaîneau, C. H., Yepremian, C., Vidalie, J. F., Ducreux, J. & Ballerini, D. (2003). Bioremediation of a crude oil-polluted soil: Biodegradation, leaching and toxicity assessments. *Water, Air, & Soil Pollution, 144, 419-440.*

Fatai, I. O. (2010). *Biodegradation of polycyclic aromatic hydrocarbons.* B.Eng, Federal University of Technology Minna.

Karthikeyan, R. & Bhandari, A. (2001). Anaerobic biotransformation of aromatic and polycyclic aromatic hydrocarbons in soil microcosms: A review. *Journal of Hazardous Substance Research, 3, 1-19.*

Kim, I. S., Park, J. S. & Kim, K. W. (2001). Enhanced biodegradation of polycyclic aromatic hydrocarbons using nonionic surfactants in soil slurry. *Applied Geochemistry, 16, 1419–1428.*

Kulisch, G. P. & Vilker, V. L. (1991). Application of pseduomonas-putida pgg 786 cpmtaomomg p-450 cytochrome monooxygenase for removal of trace naphathalene oncentrations. *Biotechnology Progress, 7, 93-98.*

Kumara, M., Leona, V., Materanoa, A. D. S., Ilzinsa, O. A., Galindo-Castroa, I. & Fuenmayora, S. L. (2006). Polycyclic aromatic hydrocarbon degradation by biosurfactant-producing pseudomonas sp. Ir1. *Z. Naturforsch., 61c, 203-212.*

Muller, H., Breure, A. M. & Rulkens, W. H. (2004). Prediction of complete bioremediation periods for pahs soil pollutants in different physical states by mechanistic models. *Chemosphere, 43, 1085.*

Obayori, O. S. & Salam, L. B. (2010). Degradation of polycyclic aromatic hydrocarbons: Role of plasmids. *Scientific Research and Essays,* 5**,** 4093-4106.

Parviz, S., Abbas, S. S. & Hossein, C. A. (2006). Biodegradation of polycyclic aromatic hydrocarbons by aerobic mixed bacterial culture isolated from hydrocarbon polluted soils. *Iran Journal of Chemical Engineering,* 25**,** 73-78.