Bacterial Degradation of Crude oil Components Using Brewery Effluents as Biostimulation Agents

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Abstract

Wide scale pollution problems associated with crude oil production and consumption have become worrisome to oil producing as well as consuming nations. Also the high cost of environmental clean-up necessitates the adoption of a cost effective alternatives that are more environmentally benign in addition to being cheaper. Some crude oil components particularly Poly Aromatic compounds are ubiquitous environmental contaminants. They are responsible for causing cancer and reproductive abnormalities in living organisms. In this paper part of the data generated in assessing the effect of formulations made purely from brewery effluents on the reduction of Polycyclic Aromatic Hydrocarbons is presented. The formulations were used as sources of nutrients and crude oil was used as the sole source of carbon for the test bacteria used. Quantitation data generated from the residual oil after biodegradation showed that a significant ($P<0.05$) reduction of Polyaromatic Hydrocarbons was achieved. The economic feasibility and multiple benefits of this novel technique used in Bioremediation will also be discussed.

Keywords - Bioenhancement, Bioremediation, Brewery effluents, Crude oil.

Introduction

Bioremediation technology is based on the premise that a large percentage of oil components are readily biodegradable in nature (Atlas, 1992). Bioremediation is more environmentally benign in terms of its end products. The success of oil spill bioremediation depends on one's ability to establish and maintain conditions that favour enhanced oil biodegradation rates in the contaminated environment (Venosa *et al.*, 1997). There are three main approaches to oil spill bioremediation (Prince, 1993).

Bioaugmentation

In this approach, known oil degrading bacteria are added to supplement the existing microbial population. Microbial cultures with metabolic capability to degrade crude oil are now commercially available as "bioenhancement powders". One of the shortcomings of this approach is that when the organisms are applied in an environment where contamination has resulted, their activity is limited to prevailing environmental factors which may be different from the natural environment from which they are isolated. It is, therefore, important for an oil producing country like Nigeria to promote research

leading to Isolation, propagation and processing of indigenous microorganisms with metabolic ability to degrade petroleum based pollutants.

Biostimulation

In this approach, the growth of indigenous oil degraders is stimulated by the addition of nutrient or other growth limiting co-substrates. The advantage of this approach is that the microorganisms being used have already adapted to prevailing environmental factors and the added nutrients could be efficiently utilized leading to maximum growth and activity (Wilfred *et al*., 2002; Ubochi *et al*., 2006; Ibekwe *et al*., 2006). Biostimulation involves the addition of appropriate microbial nutrient to a waste stream. The objective of this process is to stimulate the indigenous microorganisms of the waste to bring about its degradation (Odokuma and Dickson, 2003). Biostimulation can be achieved by the addition of various forms of limiting nutrients such as Phosphorus, Nitrogen and other trace elements. The nutrients are usually added to the subsurface through injection wells, although injection well technology is still emerging. The primary advantage of biostimulation is that bioremediation is undertaken by already present microorganisms that are well distributed spatially within the subsurface. The delivery of nutrients should be done in a manner that allows the additives to be readily available to the microorganisms. Some of the operational bottlenecks faced may be caused by the local geology of the affected area. Areas that are tight and impermeable (tight clays and other fine grained materials) make it difficult to spread the nutrients throughout the affected area. Fractures on the affected area may also create preferential pathways for additives (Lee *et al*., 1993). Biostimulation usually leads to the increase in heterotrophic and hydrocarbon utilizing bacterial populations in response to nutrient supplementation. Using nutrients in soil contaminated with oil can increase biodegradation processes. This could be comparatively cheaper than always trying to use and depend on pure cultures (Yongabi *et al*., 2006).

Materials and Methods

Samples of brewery effluents were obtained from Jos International Breweries, Nigeria. The samples were dried and ground into fine powder. Each powder was used in place of mineral salts medium and employed in the degradation experiment. Parts of the powdery spent grains were also used in proximate analyses. Thus, ash, nitrogen, phosphorus and trace metals contents of the wastes were determined using standard procedures (A.O.A.C., 1999).

Crude oil Biodegradation

The cultures used in the degradation experiment were maintained in crude oil – overlaid – minimal medium. Nutrient broth (10ml) was dispensed into universal bottles. Then the test bacteria were inoculated into these media and the bottles were incubated at 24^{o}C for 48 hours. Eight milliliters of this broth culture containing the actively growing bacteria were inoculated into other sets of universal bottles containing minimal medium overlaid with Bonny light crude oil. Varying concentrations of the spent grains were used, the degradation bottles were labeled with respective concentrations used. They were then left on the bench for two weeks. Both the undegraded and degraded residual crude from the degradation bottles were subjected to gas chromatographic analysis in order to determine the Polyaromatic Hydrocarbons of the two crude samples.

Determination of Polycyclic Aromatic Hydrocarbons (PAHs)

All chemicals and reagents used were of analytical grade. The dichloromethane used as an extractant was obtained from Fischer Scientific (UK). A Poly Aromatic Hydrocarbon mixture (NIST, Baltimore, MD) containing naphthalene, acenaphthylene, acenaphthene, fluorine, phenanthrene, anthracene,

fluoranthene, pyrene, benz (a) anthracene, chrysene, benzo (b) fluoranthene, benzo (k) fluoranthene, benzo (a) pyrene, benzo (ghi) perylene, dibenzo (a, h) anthracene and indeno (1, 2, 2 d) pyrene was prepared and used as standard. Also four isotopically labeled Poly Aromatic Hydrocarbons; acenaphthelene-$_{d10}$, chrysene-$_{d12}$, penanthrene-$_{d10}$ and perylene-$_{d12}$ (ChemService, Westchester, PA) were used as internal standards.

Preparation of Standard Solutions

Five standard solutions each containing 16 target compounds were prepared by diluting the standard mix (i.e. mixture from NIST) to desired concentrations with HPLC grade dichloromethane. Then, 0.5ug of internal standard was added into these solutions. The final mixture was then transferred to a capped and sealed vial until ready for analysis.

Extraction of Residual Oil from Degradation Bottles

The extraction of residual oil from the degradation bottles was carried out in accordance with standard procedures described in USEPA (1994). In this method, a 100ml capacity separating funnel was mounted on a retort stand. The separating funnel was thoroughly washed and dried overnight in a murfle furnace operated at 150^{o}C. Prior to use, the funnel was rinsed vigorously with dichloromethane for several minutes. The funnel was then removed and allowed to drain completely in fumed cupboard. Then 20ml of water-oil mixture was transferred from the degradation bottles to the funnel and to this was added 20ml of dichloromethane. The mixture was shaken vigorously for four minutes and allowed to separate and settle. After ten minutes, the organic layer that formed was removed and the process repeated with the aqueous layer twice. The three portions of the organic phase were combined and evaporated to 1ml volume using a rotary evaporator. The percentage recovery was calculated thus:

$\%R = Q_d/Q_a \times 100$

Where:

Qd= Quantity determined by analysis and

Qa= Quantity added

Calibration

Several dilutions of the standard PAH mixtures made were analyzed to determine detection limits and limit of quantification. For such determinations triplicate analyses were used.

Analysis by GCMS

The GCMS analyses was carried out on a Finnigan Magnum instrument equipped with a CTC A200S auto sampler and a 30um, 0.25 ID DB-5 MS fused silica capillary column (J and W Scientific, Folson CA). Helium was used as a carrier gas and a column head pressure was maintained at 10 psi to give an approximate flow rate of 1ml/minute. The injector and transfer line were maintained at 290 and 250^{o}C respectively. All injection volumes were held at 1ul in the split less mode. The column temperature was initially held at 70^{o}C for four minutes and ramped to 300^{o}C at the rate of 10^{o}C/minute. This temperature was maintained for ten minutes. The mass spectrophotometer was used in electron ionization mode and all spectra were acquired using a mass range of m/z 50-400 and automatic gain control.

Identification and Quantization of the compounds

The identification and quantization of the compounds was based on the retention time match and mass spectra match against the calibration standard. Quantitation was performed using internal standardization method. Acenaphthene-$_{d10}$ was used as the internal standard for naphthalene, acenaphthylene, acenaphthene and fluorine. Phenanthrene was used as the internal standard for phenanthrene, anthracene, fluoranthene and pyrene. Chrysene-$_{d12}$ was used for Benz(a)anthracene and chrysene. Perylene-$_{d12}$ was used for Benzo(b)fluoranthene, Benzo(k)fluoranthene, Benzo(a)pyrene, Benzo(g,h,i)perylene, Dibenz(a,h)anthracene and Indeno(1,2,3-cd)pyrene. The quantitation was based on the ratio of the peak height of the substance being quantified to that of the corresponding internal standard.

Table 1: Polycyclic Aromatic Hydrocarbons contents of Bonny Light Crude before degradation.

Hydrocarbon Component	**Concentration (mg/L)**
Naphthalene	8.77
2-Methyl naphthalene	46.4
Acenaphthylene	67.4
Acenaphthene	91.1
Fluorine	280
Phananthrene	310
Anthracene	354
Fluoranthene	354
Pyrene	410
Benzo (a) anthracene	342
Chrysene	302
Benzo (b) fluoranthene	416
Benzo (k) fluoranthene	322
Benzo (a) pyrene	389
Dibenzo (a, h) anthracene	419
Benzo (g, h, I) Perylene	392
Indeno (1, 2, 3-d) Pyrene	330
Total	**4,833.67**

Table 2: Mean Values of Residual Polycyclic Aromatic Hydrocarbons (mg/L) in bottles seeded with different concentrations of Brewery Effluents and Bacteria.

Hydrocarbon Components (mg/kg)	**Varying Concentration of Brewery effluent (g/L)**		
	3.22	**6.44**	**12.88**
Naphthalene	06.95	02.24	01.53
2-Methyl naphthalene	11.83	05.55	12.29
Acenaphthylene	92.00	52.10	57.95
Acenaphthene	84.75	43.09	66.85
Fluorine	81.05	27.40	25.50
Phananthrene	81.15	26.90	68.21
Anthracene	66.90	32.35	107.46
Fluoranthene	81.45	67.95	32.45
Pyrene	64.20	35.10	14.70
Benzo (a) anthracene	22.90	17.65	27.26
Chrysene	24.30	41.10	32.24
Benzo (b) fluoranthene	51.80	22.75	75.73
Benzo (k) fluoranthene	19.50	12.94	34.25
Benzo (a) pyrene	15.30	20.75	37.25
Dibenzo (a, h) anthracene	07.05	03.87	12.90
Benzo (g, h, I) Perylene	05.38	10.37	09.65
Indeno (1, 2, 3-d) Pyrene	05.40	04.86	08.24

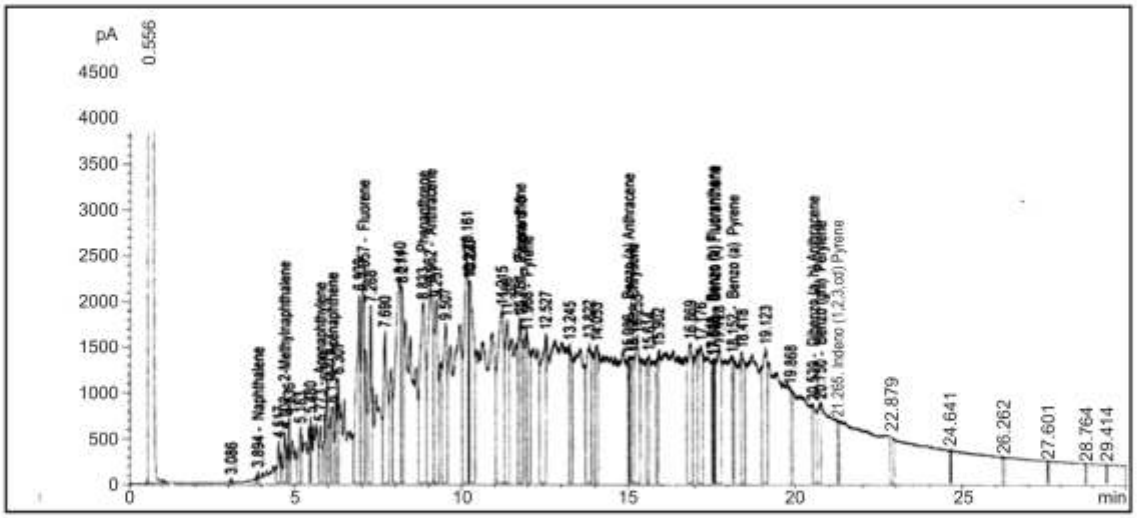

Figure 1: Chromatogram showing the different fractions of undegraded Bonny light crude oil

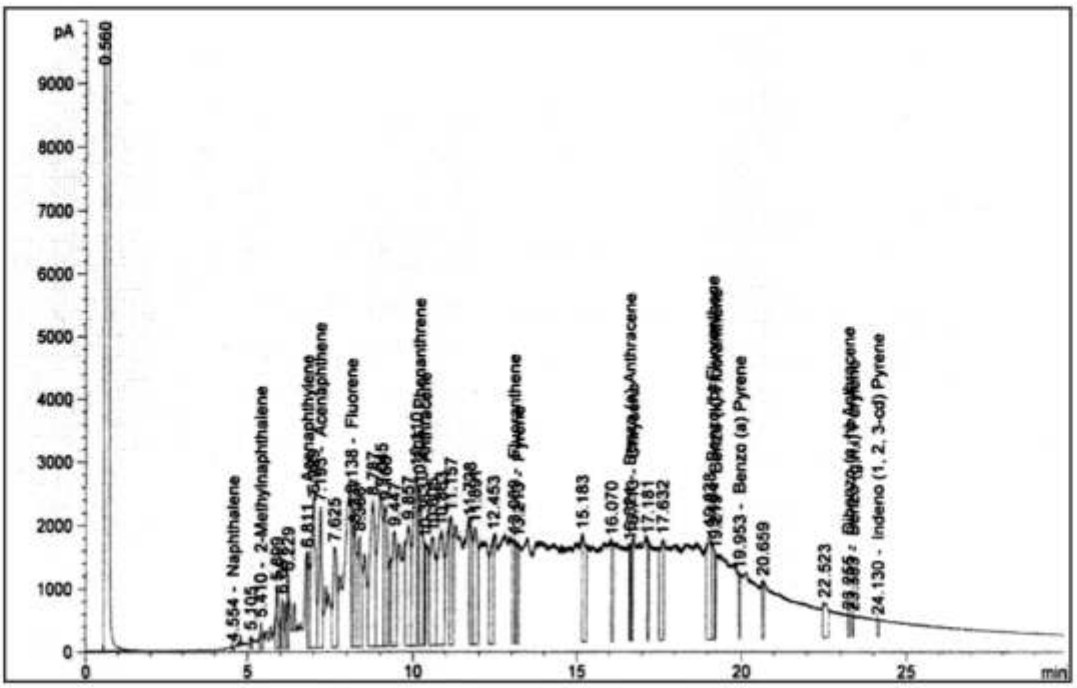

Figure 2: Chromatogram showing different fractions of Bonny light oil seeded with 3.22% of Brewery effuent and bacteria.

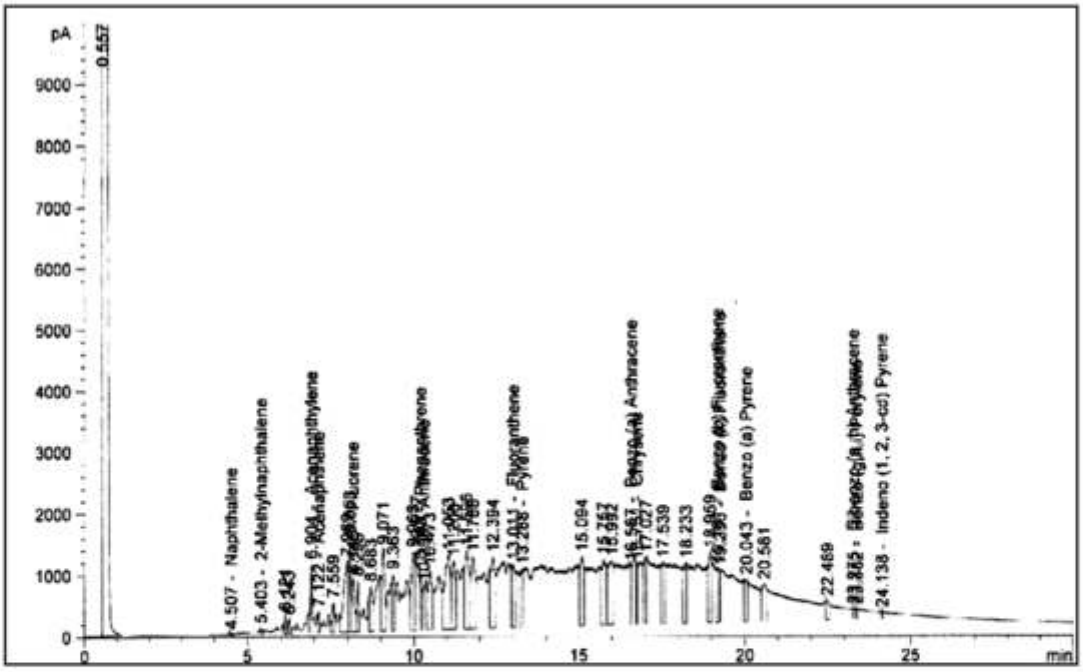

Figure 3: Chromatogram showing different fractions of Bonny light oil seeded with 6.44% of Brewery effuent and bacteria.

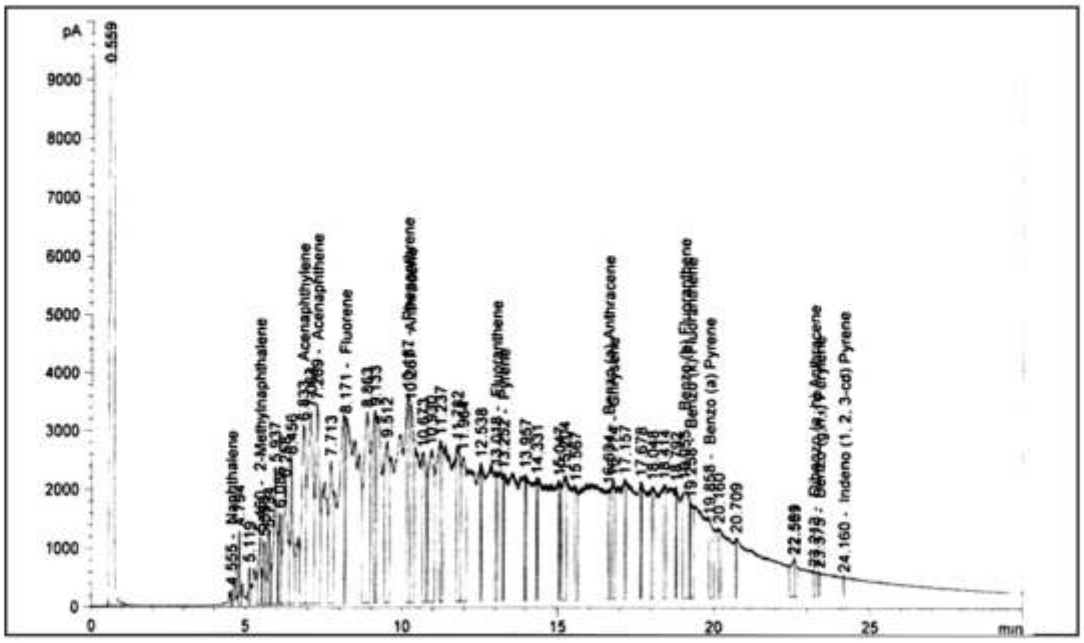

Figure 4: Chromatogram showing different fractions of Bonny light oil seeded with 12.88% of Brewery effuent and bacteria.

Discussion

Comparison between Tables 1 and 2 shows a significant reduction ($P<0.05$) of Polycyclic Aromatic Hydrocarbons. The highest reduction is recorded in the 6.44g/L of the spent grains. This, is therefore, recommended as the optimum concentration for field application. The reduction of Polycyclic Aromatic Hydrocarbons (PAHs) achieved in this research work is of significant importance in view of the numerous toxicity and health problems they cause. The PAHs are ubiquitous environmental contaminants. The health implications of PAHs had been reported by Thomas (1990); Hall and Oris (1991); Chaloupka (1993) and Potter (1994).

A major observation can be made from the reduction of those PAHs that are of serious health hazards. WHO (1998) has provided data on these PAHs. They include Benzo (a) pyrene, Benzo (a) anthracene, Chrysene, Dibenz (a,h) anthracene, Benzo (b) fluoranthene, Indeno (1,2,3 –d) pyrene and Benzo (g,h,i) perylene. It is interesting to note that in this study, all these compounds were significantly ($P<0.05$) degraded using the combination of bacteria and spent grains used as seed.

The outcomes of this research provide a good option for handling industrial wastes in the most economically feasible manner. This is particularly so, because more often than not, the brewery effluents are left clogging drainages in Brewing Industries where their decay results in offensive odour and unpleasant conditions. These brewery effluents can now be processed into bioenhancement powders or other appropriate formulations for application in bioremediation of hydrocarbon contaminated environments.

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