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HYDROCARBON DEGRADING POTENTIALS OF BACTERIAL ISOLATES FROM PETROLEUM REFINERY SLUDGE

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ABSTRACT
Petroleum contain fractions that are potentially toxic, carcinogenic and or mutagenic. Thus the intrinsic biodegradability of petroleum hydrocarbon and distribution in the environment of competent catabolic microorganisms are critical to the implementation of bioremediation strategies for the clean-up of petroleum polluted environments towards ecosystem and public health preservation. In the present study, the petroleum degrading potentials of bacteria species isolated from petroleum refinery sludge was evaluated. The bacterial species were isolated and identified using standard microbiological techniques. The petroleum degrading potentials of isolates was determined by a 14-day enrichment assay using basal salt medium (BSM) supplemented with 2% Escravos light crude oil (ELCO) and assessed by gas chromatography (GC). The petroleum degrading isolates were identified as *Micrococcus* sp., *Bacillus* sp., *Arthrobacter* sp., *Bacillus* sp., *Staphylococcus* sp. and *Streptomyces* sp. The mean bacterial counts during enrichment assay ranged between 0.95×10^1 cfu/ml and 9.7×10^1 cfu/ml. *Staphylococcus* sp. exhibited the highest (96.20%) total petroleum hydrocarbon (TPH) degrading potential; while *Micrococcus* sp. showed the least (35.42%) TPH degrading capacity. The isolates were also remarkable in the degradation of polycyclic aromatic hydrocarbon (PAH), removing between 4 and 10 PAH compounds after treatment in the following order (highest to lowest): *Staphylococcus* sp. >*Streptomyces* sp. >*Arthrobacter* sp. >*Bacillus* sp. >*Micrococcus* sp. >*Bacillus* sp. The study demonstrated that the bacterial isolates were remarkable petroleum degraders with great potentials for deployment as candidates in bioremediation of petroleum polluted sites in the Niger Delta region of Nigeria.

KEYWORDS: Petroleum sludge; Biodegradation; TPH; PAH; Bacteria

INTRODUCTION

Petroleum hydrocarbon remains the principal source of energy globally. Its mining, transport, storage and use for industrial and allied purposes has resulted in numerous occasions of environmental pollution with severe consequences on the ecosystem and public health (Das and Chadran, 2011). Due to the environmental and public health impact of petroleum pollution, various treatment options have been proffered to deal with the challenge over the years. Treatment options include physical, chemical and biological methods/approaches. The physiochemical treatments options include incineration, thermal desorption, chemical oxidation, immobilization and solvent extraction (Liu *et al*., 2010). In general, such treatments are more expensive, energy intensive and unsustainable with respect to their environmental impact (Battikhi, 2014). The biological alternative, otherwise called bioremediation is more reliable, simple and cheap. Bioremediation is a technology that harnesses the metabolic capacity of microorganisms to enzymatically convert toxic chemical contaminants in the environment into harmless or less harmful products. The technology can be applied as green technologies, as it offers an environmentally friendly and cost effective response to environmental oil pollution (Chikere *et al*., 2012). Bioremediation makes use of the catabolic activities of indigenous hydrocarbon utilizing microorganisms (especially bacteria) to decontaminate oil-polluted environments (Mahmoud *et al*., 2009). Hence, knowledge of indigenous oil-degrading bacteria is critical to the search for agents that can enhance the self-purification/cleaning capacity of polluted environments.

Oil sludge is a thick, viscous mixture of sediments, water, oil and high hydrocarbon concentration often encountered during crude oil refining (Prakash *et al*., 2005). A major constituent of oil sludge is polycyclic aromatic hydrocarbons (PAH). PAHs are a group of hydrophobic organic compounds composed of two or more fused aromatic rings in their chemical structure (Hesham *et al*., 2014). An investigation into PAH concentrations in some Niger Delta ecosystems revealed elevated values of these priority pollutants in the studied environments (Ezemonye and Ezemonye, 2005). The petroleum fractions (PAHs) are reported to possess toxic, carcinogenic and mutagenic properties, thus making them be of serious environmental and public health concerns. (Hesham *et al*., 2014). The genotoxicity of PAHs to many microbes and other organisms, implies that microorganisms that are able to survive in petroleum sludge (containing high concentrations of PAH) have evolved capacity not only tolerate toxicity of the compound, but also for effective hydrocarbon degradation. This assertion was corroborated by the observation of Hesham *et al*. (2014) who reported that microbial degradation of petroleum pollutant was the most dominant and significant process for removing PAHs from the environment.

Although a number of studies (Prakash *et al*., 2005; Battikhi, 2014; Musa *et al*., 2015; Obi *et al*., 2016) have reported PAH degrading bacteria isolates from oil sludge around the world, there is dearth of information on the PAH degrading potentials of bacterial strains isolated from oil sludge of the Warri Refinery and Petrochemical Company (WRPC), Ekpan, Delta State, Nigeria. It is therefore imperative to screen WRPC refinery sludge for bacterial strains that can degrade PAH fractions of petroleum as potential candidates for bioremediation of petroleum polluted environments especially in the Niger

Delta region of Nigeria. Thus, the aim of this study was to determine the catabolic potentials of bacterial isolates from sludge of the WRPC refinery wastewater effluent in the biodegradation of target hydrocarbon.

MATERIALS AND METHODS

Sample Collection

Crude oil sludge samples were collected weekly in sterile one litre bottles from the wastewater effluent of the Warri Refenary and Pectrochehemical Company (WRPC), Ekpan, Nigeria between December, 2015 and January, 2016. The refinery is located within latitude 5°31.0'0.12''and longittude 5°45.0'0.00'' in the Niger Delta region of Nigeria. Samples were transported in cooler boxes containing ice to the Benson Idahosa University Microbiology laboratory for analysis. All samples were analysed within 24 hrs of sample collection.

Isolation and Identification of Petroleum-degrading Bacteria

The enrichment culture technique described by Odjadjare *et al*. (2008) was used (with slight modification) for the isolation of petroleum degrading bacteria, using Escravos Light Crude Oil (ELCO) as the sole source of carbon and energy. Basal Salt Medium (BSM) composition was as follows (g/L): K_2HPO_4 (1.5), NH_4NO_3 (1.0), $MgSO_4 \cdot 7H_2O$ (0.5), $CaCl_2$ (0.2), NaCl (30), KCl (0.3) and $FeCl_3$ (0.02). Five (5) grams of each sludge sample was transferred into a 250 ml flask containing 50 ml of sterile BSM broth supplemented with 2% sterile ELCO, sterilized separately in a universal bottle at 121°C for 15 mins before being aseptically added to the BSM. Control was set up comprising BSM broth supplemented with 2% ELCO without the sludge sample. The cultures were incubated on a rotary shaker at 150 rpm and 29±2 °C for 3–7 days. Aliquots of the enriched cultures were inoculated onto BSM agar supplemented with 2% ELCO and incubated at 29±2 °C for 3 to 7 days. Selected pure isolates were identified by their cultural, morphological, and biochemical characteristics with the aid of Bergey's manual determinative bacteriology (Holt *et al*., 1994).

Determination of Petroleum Degradation Potentials of Isolates

The biodegradation potentials and growth profile of the bacterial isolates were determined by inoculating 1.0 ml of standardized (OD 0.1 at wavelength of 540 nm) 24 hrs culture of each selected isolate into a 250 ml Erlenmeyer flask containing 50 ml of BSM broth (pH 7.2) supplemented with 2% ELCO as earlier described. A control flask containing only ELCO without inoculum, was also set up. The culture was incubated on a rotary shaker at 150 rpm and room temperature (29±2°C) for 14 days. At each sampling interval (48 hrs), viable bacterial count analysis was done by standard pour plate techniques (Seeley and Van Denmark, 1981). The residual total petroleum hydrocarbon (TPH) and polycyclic aromatic hydrocarbon (PAH) fractions were determined by gas chromatography (GC).

Gas Chromatography Analysis

Extraction of Residual Hydrocarbon

Thirty millilitre (30 ml) of each bacterial treated sample and control was measured into a separating funnels. Acetone (15ml) and dichloromethane (DCM) (15ml) were added in ratio 1:1 to content of the funnel. The separating funnel was corked and agitated vigorously for 1 to 2 mins with periodic venting to release excess pressure. The organic layer was allowed to separate from the water phase for a minimum of 5 mins by opening the tap of the separating funnel. The solvent extracted was shaken vigorously and the emulsion interface between layers was more than one-third the size of the solvent layer. Mechanical techniques like stirring, filtration etc. were employed to complete the phase separation. The separated solvent was collected in a round-bottom flask and concentrated to about 2 ml. Methylene chloride was exchanged with hexane by adding 10 ml of hexane to the round-bottom flask and re-concentrated to 2 ml final volume. The extract was then transferred to a Teflon-lined screw-cap vial, labelled and refrigerated for further analysis. A blank was extracted with each batch of samples under the same conditions as for the samples, with the use of reagent water.

Determination of Residual Hydrocarbon

The residual petroleum was determined by injecting 2 µL of hydrocarbon extract onto an Agilent model Hewlett Packard 5890 series 2 gas chromatograph (GC) equipped with flame ionization detector (FID) and an HP-5 capillary column (J&W Scientific 25 m × 0.32 mm × 0.52 µm). The carrier gas was helium, set at a flow rate of 6.5 ml/min with an injector temperature of 225ºC. The initial oven temperature was 45 ºC held for 3 mins, followed by a ramp at 12 ºC/min to 225 ºC where it was held for 7 mins. The total run time was 25 mins. Pure alkane standards were used to identify individual petroleum hydrocarbons and to calibrate the response of the GC at each sampling point.

Statistical Analysis

Means and standard deviations of values recorded in this study were derived using Microsoft Excel. One-way analysis of variance (one-way ANOVA) was carried out using SPSS 17.0 statistical software. Values were adjudged to be significant at 95% confidence interval (P< 0.05).

RESULTS

Bacterial Identity and Density during Enrichment Assay

Six strains of bacterial species (*Micrococcus, Bacillus* (two strains), *Arthrobacter, Staphylococcus and Streptomyces* spp.) were isolated and identified using their morphological, cultural and biochemical characteristics (Table 1). Bacterial cell density during enrichment assay on BSM supplemented with 2% ELCO ranged from 0.95×10^1 cfu/ml (*Streptomyces* sp.) to 9.7×10^1cfu/ml (*Bacillus* sp.) (Figure 1). The counts varied significantly (P<0.05) with bacterial strains.

Table 1. Morphological, cultural and biochemical characteristics of selected bacterial isolates

			Biochemical Reactions										Sugar Fermentation Test							
	Morphology	Gram Reaction	Catalase	Coagulase	Oxidase	Starch Hydrolysis	Citrate	Hydrogen Sulphide	Methyl Red	Urease	Vosges Proskauer	Glucose	Lactose	Sucrose	Mannitol	Xylulose	Fructose	Galactose	Probable Identity
	Cocci	+	+	-	+	+	-	-	+	+/-	-	+	-	-	-	-	AG	A	*Micrococcus sp.*
	Rod	+	-	+	+	-	+	-	+	+	+	AG	-	-	A	A	A		*Bacillus sp.*
	Short rods	+	+	-	-	+	+	-	+	+	+	AG	-	-	AG	AG	AG	A	*Arthrobacter sp.*
	Short rods	+	-	-	+	+	-	-	-	+	+	A	-	AG	-	-	-	AG	*Bacillus sp.*
	Cocci	+	+	+	+	-		+	-	+	+/-	A	-	A	AG	AG	AG	A	*Staphylococcus sp.*
	Rods	+	+	-	-	+	-	-	-	+	+	-	-	-	-	A	AG	G	*Streptomyces sp.*

Key: + = positive - = negative A = Acid production AG = Acid and gas production

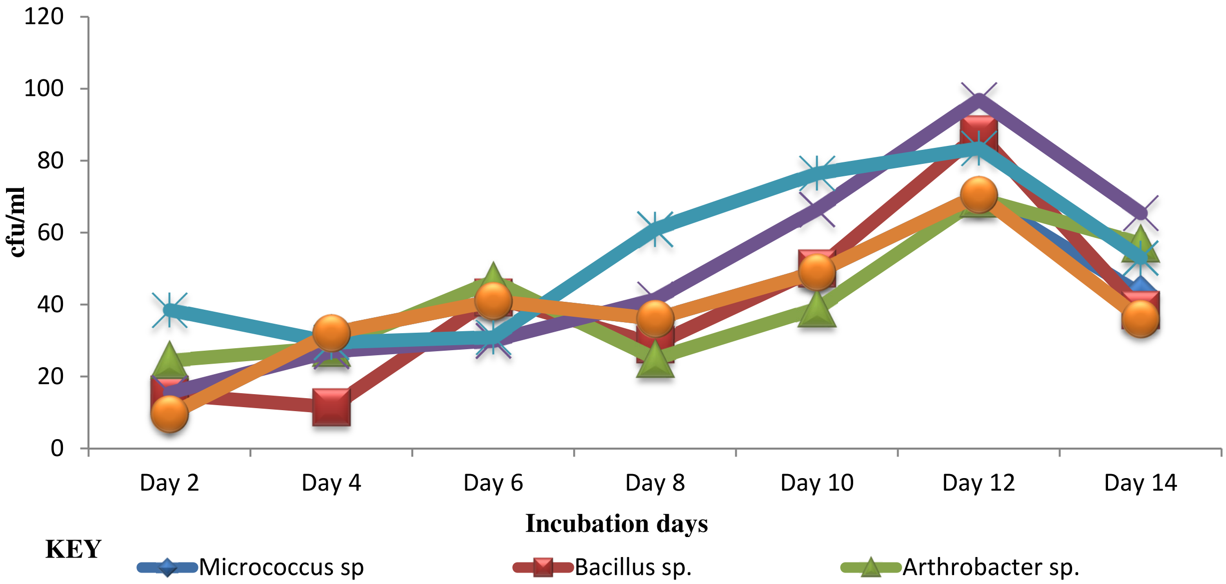


Fig. 1: Bacterial growth patterns in BSM supplemented with 2% Escravos light crude oil during enrichment assay

Determination of Petroleum Degrading Potentials of Isolates

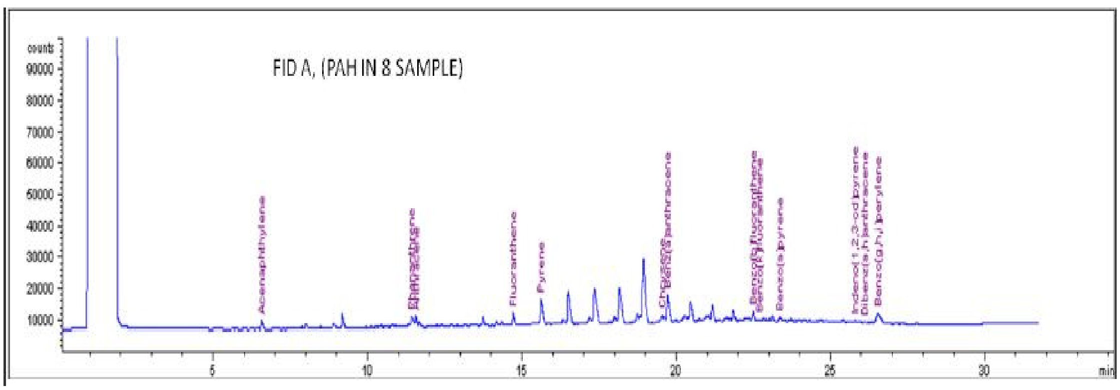
Gas chromatography analysis after the 14-day enrichment assay showed that abiotic factors accounted for loss of 46.87% of the total petroleum hydrocarbon (TPH) fraction of ELCO (Table 2). The percentage biodegradation of TPH of the ELCO ranged from 35.42% (Micrococcus sp.) to 96.20 % (*Staphylococcus* sp.). Others were 51.29%, 67.58%, 72.66% and 83.98% for *Bacillus* sp., *Bacillus* sp., *Arthrobacter* sp. and Streptomyces sp. respectively. A comparison of chromatograms of the two controls (Figure 2a (raw crude oil; Sample 8) and Figure 2b (uninoculated treated crude oil; Sample 7)) indicated that abiotic factors resulted in the removal of phenathrene and dibenz (a,h) anthracene from the raw crude (ELCO) after treatment. However, increased concentrations of indeno (1,2,3 – cd) pyrene was also observed after the treatment.

The PAH degrading potentials of the bacterial isolates were determined by comparing the chromatogram of each isolate with that of control 2. Figure 3 shows that treatment with *Micrococcus* sp. (Sample 1) resulted in the degradation of six (6) PAH compounds: acenaphthylene, phenathrene, indeno (1, 2, 3- cd) pyrene, dibenz (a,h) anthracene and benzo (g, h, i) perylene. Treatment with *Bacillus* sp. (sample 2) resulted in the removal of acenaphthylene, phenathrene, benzo(a) pyrene, indeno(1,2,3 - cd) pyrene, dibenz (a, h) anthracene and benzo (g, h, i) perylene (7 PAH compounds) (Figure 4). While *Arthrobacter* sp. (sample 3) degraded seven (7) PAH compounds including acenaphthylene, phenathrene, anthracene, fluoranthene, indeno (1,2,3 –cd) pyrene, dibenz (a, h) anthracene and benzo (g, h, i) perylene (Figure 5). However, the bacterial treatment also resulted in the introduction of fluorine into the sample (Figure 5).

Table 2. Rate of degradation of TPH components of ELCO after 14-day enrichment

Isolates	Residual TPH (mg/l)	Percentage Undegraded (%)	Percentage Degraded (%)
Micrococcus sp.	9.0195	64.57	35.42
Bacillus sp.	6.8041	48.71	51.29
Arthrobacter sp.	3.8183	27.34	72.66
Bacillus sp.	4.5288	32.42	67.58
Staphylococcus sp.	0.5306	3.80	96.20
Streptomyces sp.	2.2376	16.02	83.98
Control 1 (uninoculated treatment)	13.9674	53.13	46.87*
Control 2 (raw crude)	26.2908	Not applicable	Not applicable

a



Retention time (mins)

b

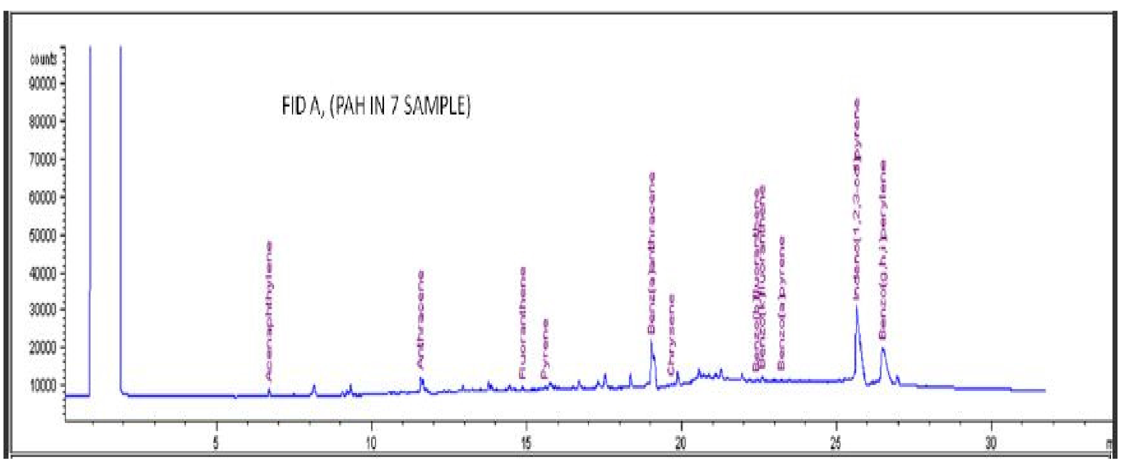


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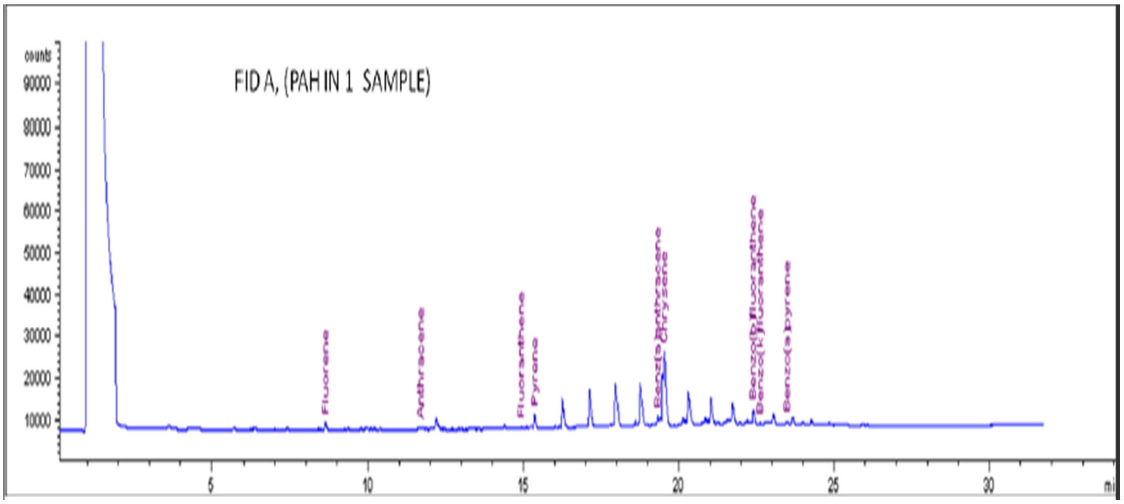
Fig. 2: Chromatogram of PAH fractions of Controls 1 and 2

(a) Chromatogram of PAH component of raw (untreated) ELCO (control 2)
(b) Chromatogram of PAH components of uninoculated ELCO after enrichment assay (Control 1).



Retention Time (mins)

Fig. 3: Chromatogram of the PAH fraction of crude oil substrate after treatment with *Micrococcus* sp.

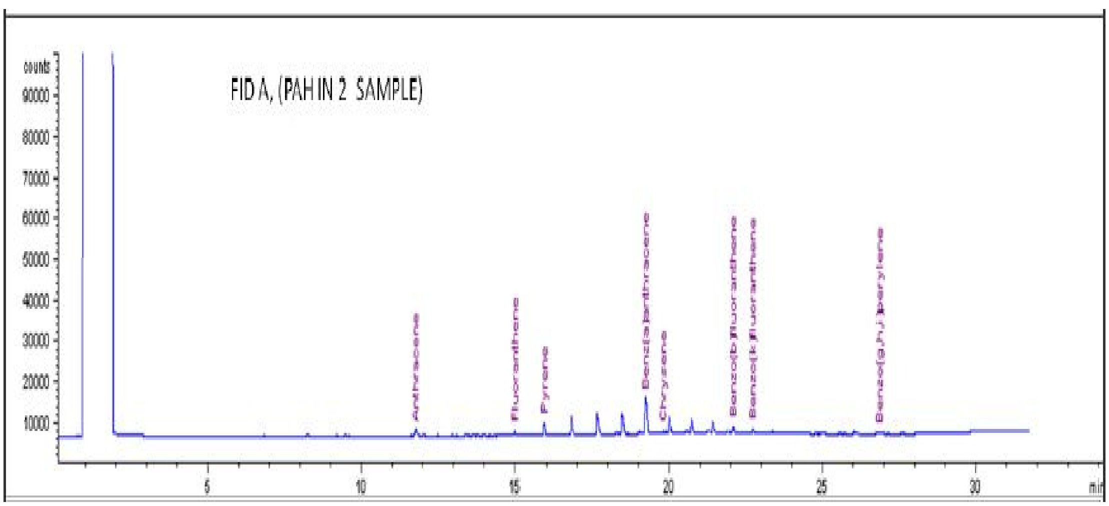


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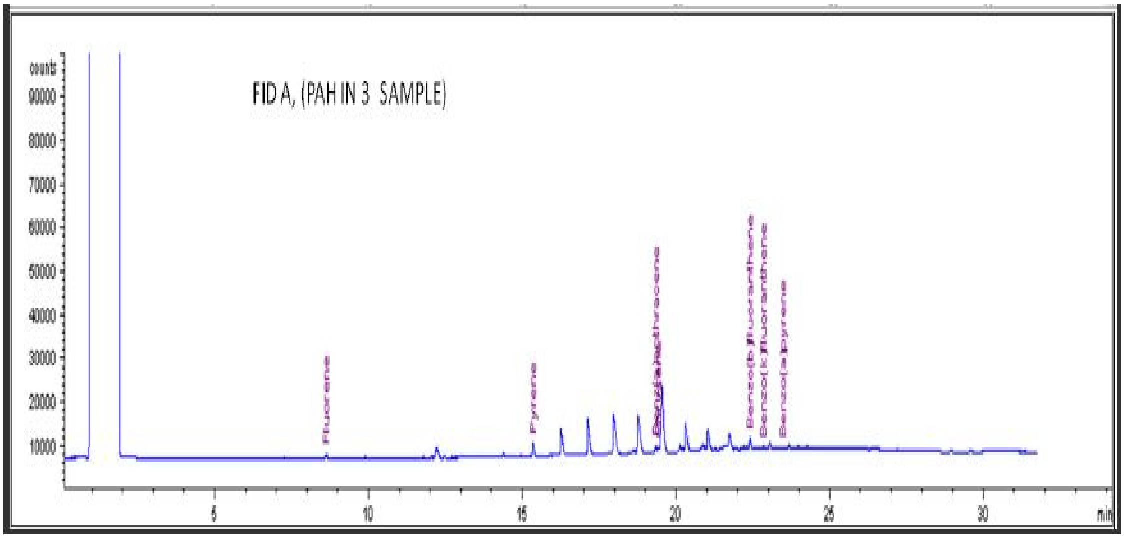
Fig. 4: Chromatogram of the PAH fraction of crude oil substrate after treatment with *Bacillus* sp.



Retention time (mins)

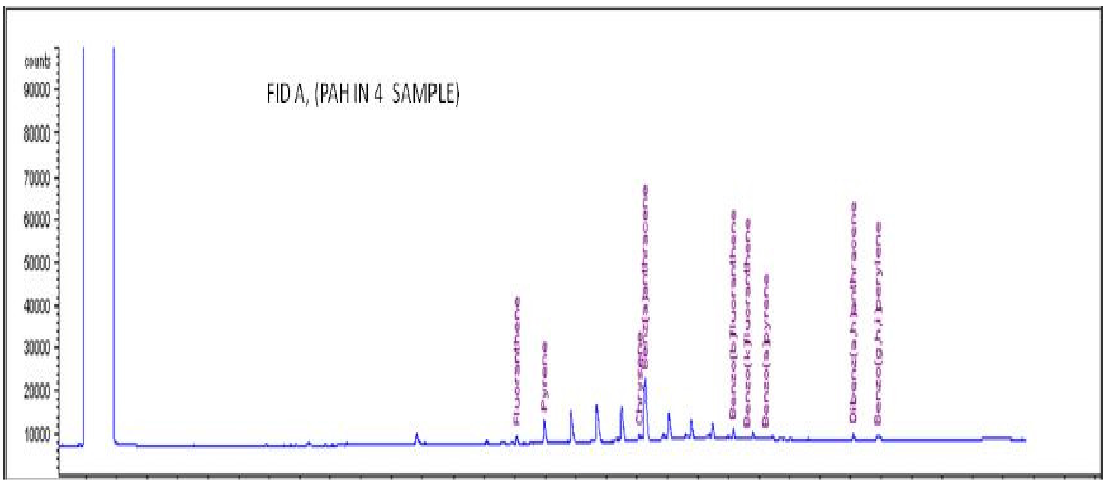
Fig. 5: Chromatogram of the PAH fraction of crude oil substrate after treatment with *Arthrobacter* sp.

ELCO treatment with *Bacillus* sp. (Sample 4) resulted in the degradation of four (4) PAH compounds including acenaphthylene, phenathrene, anthracene and indeno (1,2,3 – cd) pyrene (Figure 6); whereas increased concentrations of fluoranthene and pyrene were observed after treatment with the isolate (Figure 6). *Staphylococcus* sp. (sample 5) degraded ten (10) PAH compounds: acenaphthylene, anthracene, fluoranthene, pyrene, chrysene, benzo(b)fluoranthene, benzo(k)fluoranthene, benzo(a)pyrene, indeno (1,2,3–cd) pyrene and dibenz (a, h) anthracene after treatment (Figure 7). However, the bacterial strain caused the introduction of naphthalene and fluorine after the treatment (Figure 7). Treatment with *Streptomyces* sp. (Sample 6) resulted in the degradation of eight (8) PAH compounds including anthracene, fluoranthene, chrysene, benzo(b)fluoranthene, benzo(k)fluoranthene, benzo(a)pyrene, indeno(1,2,3–cd)-pyrene and dibenz (a, h) anthracene (Figure 8). However, the treatment also resulted in the formation of fluorine in the sample (Figure 8).

Discussion

The current study was initiated to evaluate the catabolic potentials of bacterial isolates from petroleum refinery sludge. Isolates similar to those observed in this study have been previously reported in petroleum refinery sludge (Nkwelang *et al*., 2008; Mansur *et al*., 2014, Musa *et al*., 2015; Ubani *et al*., 2016). The bacterial cell density during enrichment were remarkably lower than that reported by Odjadjare *et al.* (2008) who observed a range of 1.76×10^8 cfu/ml to 4.26×10^{11}. The higher concentration of crude oil supplement (2%) in the current study compared to the 1% used by Odjadjare *et al.* (2008) could be responsible for the observed difference in cell density. High concentrations of petroleum have been reported to be toxic to microbial cells (Das and Chadran, 2011); hence the relatively higher concentration could have affected the growth potentials of the isolates.

The percentage (46.87%) of TPH fraction in ELCO lost to abiotic factors in this study is relatively higher than those reported by Atlas (1999; 30%), Sakalle and Rajkumar (2008; 15%) and Roy *et al.* (2014; 5%); but lower than that (75%) observed by Chikere *et al.* (2012).



Retention time (mins)

Fig. 6: Chromatogram of the PAH fraction of crude oil substrate after treatment with *Bacillus* sp.

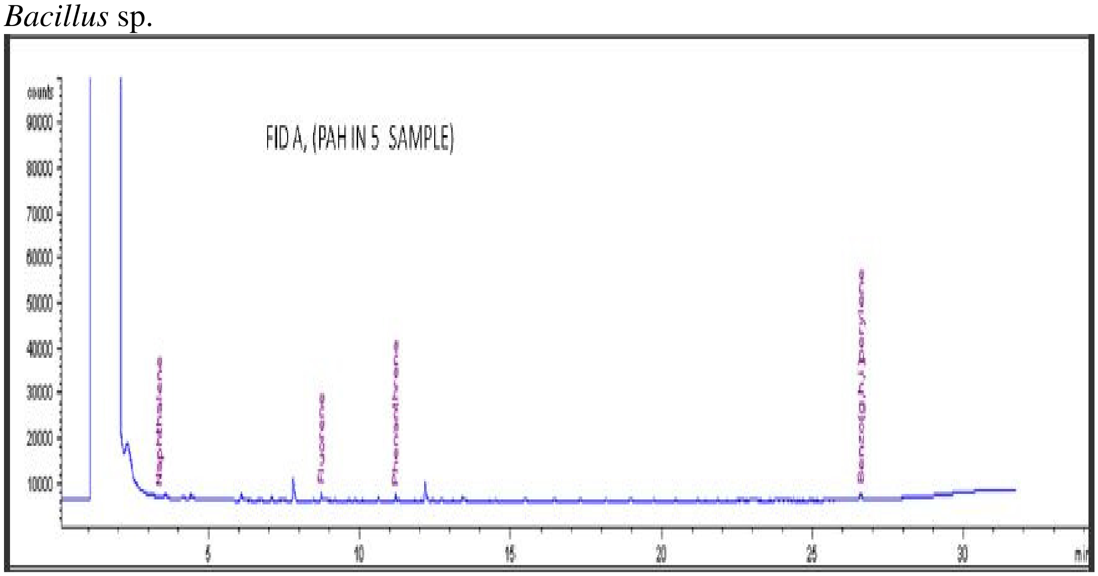


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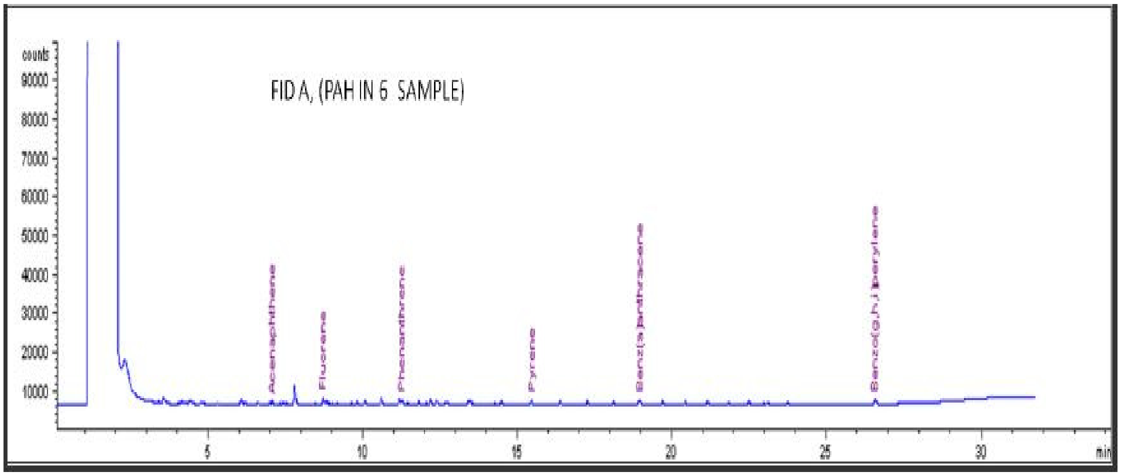
Fig. 7: Chromatogram of the PAH fraction of crude oil substrate after treatments with *Staphylococcus* sp.



Retention time (mins)

Fig. 8: Chromatogram of the PAH fraction of crude oil substrate after treatments with *Streptomyces* sp.

The TPH biodegradation rates in this study were higher than those reported by Yerushalmi *et al.* (2003) (41.3% to 58.9%), but similar to rates (28.71% to 99.01%) observed by Odjadjare *et al.* (2008). The observation suggests that a good number of the test bacterial isolates in this study had remarkable petroleum degrading potentials. The difference in observed rates of biodegradation could be a function of the temperature at which the experiments were conducted. Whereas enrichment assay in the current study and that of Odjadjare *et al.* (2008) were carried out at about 30 ^{o}C, those of Yerushalmi *et al.* (2003) proceeded at 10 ^{o}C. Temperature had previously been reported to be an important factor in determining the rate of biodegradation of petroleum (Trapp *et al*., 2001).

Staphylococcus species had the best (96.20%) TPH degrading potential in this study (Table 2). The observation is consistent with the reports of Matvyeyeva *et al*. (2014) who asserted that *Staphylococcus aureus* was one of the highest biosurfactant producing bacterial strains isolated from petroleum refinery effluent in Nigeria. However, contrary to the observation of this study, Sarma and Sarma (2010) reported *Staphylococcus* strains with relatively poor (17.39%) petroleum degrading potentials.

In agreement with the observation of this study Ferradji *et al.* (2014) reported *Streptomyces* species with high petroleum degrading capacity. A relatively lower (57%) petroleum degrading potential was observed by Oaikhena *et al.* (2016) for *Streptomyces* species. *Arthrobacter sp*. in this study was also observed to be a relatively good TPH degrader (72.66%) contrary to the observation of Odjadjare *et al*. (2008) who reported ELCO degrading potentials of 37.62% by the organism. Strains of *Bacillus* spp. isolated in this study showed petroleum degrading potentials of 51.29% and 67.58%; rates that were lower than those (89.22%)

reported by Omotayo *et al*. (2012) but higher than the observation (28.27%) of Odjadjare *et al.* (2008). Although, Omotayo *et al*. (2012) reported that *Micrococcus varians* was the isolate with highest (93.01%) petroleum degrading capacity amongst bacterial isolates from soil composts in Lagos, Nigeria, observation from the current study suggests that *Micrococcus* sp. was the least TPH degrading isolate with degradation potential as low as 35.42%. However, the observation of this study is consistent with the report of Wolinska *et al*. (2016) who reported degradation rate as low as 27%; while Odjadjare *et al.* (2008) reported a slightly higher (54.95%) degradation potential for *Micrococcus* sp. isolated from the Niger Delta region of Nigeria.

The bacterial isolates in this study demonstrated remarkable potentials for the degradation of PAH fractions of ELCO; removing between 4 to 10 PAH compounds after treatment with the isolates (compare Figures 2, 3 to 8). The PAH degradation potentials of the isolates was in the following order (based on number of PAH compounds removed (highest to lowest)): *Staphylococcus* sp. >*Streptomyces* sp. >*Arthrobacter* sp. >*Bacillus* sp. (sample 2) >*Micrococcus* sp. >*Bacillus* sp. (sample 4). The observation of this study is consistent with the report of Nikitha *et al*. (2017) who observed considerable PAH degrading potentials for *Staphylococcus*, *Arthrobacter* and *Bacillus* spp. Juhasz and Naidu (2000) also observed that *Streptomyces*, *Arthrobacter* and *Bacillus* spp. exhibited good PAH degrading potentials; while PAH degrading capacity of *Micrococcus* species were documented elsewhere (John *et al*., 2012; Kafilzadeh *et al*., 2012).

The observation of this study with regards to bacterial degradation of PAH is quite remarkable considering the fact that many of the PAH compounds degraded by the bacterial treatments are listed as priority environmental pollutants by the United States Environmental Protection Agency (USEPA) (www.epa.gov/sites/production/files/2015-09/documents/priority-pollutant-list-epa.pdf). PAHs are usually considered as high risk environmental pollutants due to their high mutagenic and carcinogenic potentials when exposed to living organisms within the environment (Kafilzadeh *et al*., 2012). Biodegradation using microorganism is usually the preferred and major route of PAH removal from contaminated environments because of its cost effectiveness and capacity to transform the complex pollutants to simpler forms; or completely mineralize the contaminants into harmless substances such as carbon (iv) oxide, water, inorganic compounds, and cell protein (John *et al*., 2012; Nikitha *et al*., 2017). Thus this study demonstrated that the bacterial isolates investigated were good petroleum degraders that could serve as important candidates for bioremediation of hydrocarbon (especially PAH) polluted environments.

REFERENCES

Atlas, R. M. (1999). Microbial hydrocarbon degradation: bioremediation of oil spills. *Journal of Chemical Technology and Biotechnology,* 52: 149-156.

Battikhi, M. N. (2014). Bioremediation of petroleum sludge. *Journal of Microbiology and Experimentation*, 1(2): 00011. DOI: 10.15406/jmen.2014.01.00011.

Chikere, C. B., Chikere, B. O. and Okpokwasili, G. C. (2012). Bioreactor-based bioremediation of hydrocarbon-polluted Niger Delta marine sediment, Nigeria. *3 Biotechnology* 2:53-66. DOI 10.1007/s13205-011-0030-8.

Das, N. and Chandran, P. (2011). Microbial degradation of petroleum hydrocarbon contaminants: an overview. *Biotechnology Research International*, Vol. 2011: 941810, 13 pages http://dx.doi.org/10.4061/2011/941810

Ezemonye, L. I. N. and Ezemonye, N. N. (2005). Polycyclic aromatic hydrocarbons in surface water and sediments of Niger Delta rivers of Nigeria. *Journal Nigerian Environmental Society*, 2: 348–358.

Ferradji, F. G., Mnif, S., Badis, A., Rebecani, S., Fodil, D. Eddouaoda, K. and Sayadi, S. (2014). Naphthalene and crude oil degradation by biosurfactant producing *Streptomyces* spp. isolated from Mitidja plain soil (North of Algeria). *International Biodeterioration and Biodegradation.* 88: 300-308.

Hesham, A. E., Mawad, A. M. M., Mostafa, Y. M. and Shoreit, A. (2014). Biodegradation ability and catabolic genes of petroleum-degrading *Sphingomonas koreensis* strain ASU-06 isolated from Egyptian oily soil. *Biomed Research International,* Vol. 2014: 127674, 10 pages doi: 10.1155/2014/12767

Holt, J. G., Krieg, N. R., Sneath, P. H. A., Staley, J. T. and Williams, S. T. (1994). Bergey's manual of determinative bacteriology, 9th ed. The Williams and Wilkins Co., Baltimore, Md. 754 pp.

John, R. C., Essien, J. P., Akpan, S. B. and Okpokwasili, G.C. (2012). The fate of petroleum pollutants in freshwater ecosystems. *Bulletin of Environmental Contamination and Toxicology*. 88: 1014–1019.

Juhasz, A. L. and Naidu, R. (2000). Coastal diesel fuel pollution effects on the native microbial community. *International Biodeterioration and Biodegradation,* 45: 57-88.

Kafilzadeh, F., Javid, H. and Mohammadi, H. (2012). Isolation and identification of polycyclic aromatic hydrocarbons (PAHs) from Tashk Lake and examination of salt concentration effect on them, *Iranian Journal of Fishery Sciences,* 16(3): 103-111.

Liu, W., Luo, Y., Teng, Y., Li, Z. and Ma, L.Q. (2010). Bioremediation of oily sludge contaminated soil by stimulating indigenous microbes. Environmental Geochemistry and Health, 32(1): 23-29.

Mahmoud, H. M., Al-Hasan, R. H., Sorkhoh, N. A., Eliyas, M. and Radwan, S. S. (2009). Attenuation of oil pollutants in the Arabian Gulf water by bacteria naturally associated with live fish.

International Bio deterioration and Biodegradation, 63:615–620

Matvyeyeva, O. L., Vasylchenko, O. A. and Aliieva, O. R. (2014). Microbial biosurfactantsrole in oil products biodegradation. *InternationalJournal of Environmental Bioremediation and Biodegradation* 2(2): 69-74.

Musa, N. M., Abdulsalam, S., Suleiman, A. D. I. and Abdullahi, S. (2015). Bioremediation of petroleum refinery wastewater effluent via augmented native microbes. *Journal of Emerging Trends in Engineering and Applied Sciences,* 6(1): 1- 6.

Mansur, A. A., Adetutu, E. M., Kadali, K. K., Morrison, P. D., Nurulita, Y. and Ball, A. S. (2014). Assessing the hydrocarbon degrading potential of indigenous bacteria isolated from crude oil tank bottom sludge and hydrocarbon contaminated soil of Azzawiya oil refinery, Libya. *Environmental Science and Pollution Research*, 21: 10725–10735. http://dx.doi.org/10.1007/s11356-014-3018-1.

Nikitta, T., Satyaprakash, M., Satya-Vani, S., Sadhana, B. and Padal, S. B. (2017). Compound differences in aging and sequestration. *International Journal of Current Microbiology and Applied Sciences,* 6(4): 1627-1639.

Nkwelang, G., Kamga, H. F. L., Nkeng, G. E. and Antai, S. P. (2008). Studies on the diversity, abundance and succession of hydrocarbon utilizing microorganisms in tropical soil polluted with oily sludge. *African Journal of Biotechnology*, 7(8): 1075-1080

Obi, L. U., Atagana, H. I. and Adeleke, R. A. (2016). Isolation and characterisation of crude oil sludge degrading bacteria. *SpringerPlus* 5: 1946 DOI 10.1186/s40064-016-3617-z.

Odjadjare, E. E. O., Ajisebutu, S. O., Igbinosa, E. O., Aiyegoro, O. A., Trejo-Hernandez, M. R. and Okoh, A. I. (2008). Escravos light crude oil degradation potentials of axenic and mixed bacterial cultures. *Journal of General Applied Microbiology*, 54: 277-284.

Omotayo, A. E., Ojo, O. Y. and Amund, O. O. (2012). Biodegradation of light crude oil. *Journal of Microbial Research*, **7**(4): 209-218.

Prakash, V., Saxena, S., Sharma, A., Singh, S. and Singh, S. K. (2005). Treatment of oil sludge contamination by composting. *Journal of Bioremediation and Biodegradation*, 6(3): 284. doi:10.4172/2155-6199.1000284

Roy, A. S., Sudarat, B., Margaret, L., Britz, M., Grant, A. and Stanley, O. (2014). Laboratory evaluation of crude oil biodegradation with commercial or natural microbial inocula. *International Biodeterioration and Biodegradation,* 94: 79-89.

Seeley, H. W. and Van Denmark, P. J. (1981). *Microbes in Action: A Laboratory Manual of Microbiology*, 3rd edition, W.H.

Freeman and Company, USA. 240 pp.

Sakalle, K. S. and Rajkumar, R. (2008). Isolation of crude oil degrading marine bacteria and assessment for biosurfactant production. *The Internet Journal of Microbiology,* 7(2): 106-115.

Sarma, A. and Sarma, H. (2010). Enhanced biodegradation by some microbial isolates supplemented with heavy metals. *International Journal of Botany*, 6: 441-448.

Trapp, S., Köhler, A., Larsen, L., Zambrano, K. and Karlson, U. (2001). Phytotoxicity of fresh and weathered diesel and gasoline to willow and poplar trees. *Journal of Soils and Sediments,* 1: 71-76.

Ubani, O., Atagana, H. I., Thantsha, M. P. and Rasheed, A. (2016). Identification and characterisation of oil sludge degrading bacteria isolated from compost. *Archives of Environmental Protection*, 42(2): 67-77.

Wolińska, A., Stępniewska, Z. and Pytlak, A. (2015). The effect of environmental factors on total soil DNA content and dehydrogenase activity. *Archives of Biological Science*, 67: 493–501.

Yerushalmi, L., Rocheleau, S., Cimpoia, R., Sarrazin, M., Sunahara, G., Peisajovich, A., Leclair, G. and Guiot, S.R. (2003). Enhanced biodegradation of petroleum hydrocarbons in contaminated soil. *Bioremediation* 7: 37–51.