Pollution, 4(2): 291-303, Spring 2018
DOI: 10.22059/poll.2017.241366.319
Print ISSN: 2383-451X Online ISSN: 2383-4501
Web Page: https://jpoll.ut.ac.ir, Email: jpoll@ut.ac.ir

Biodegradation of Jet Fuel by Three Gram Negative Bacilli Isolated from Kerosene Contaminated Soil

Adetitun, D. O.[1*], Akinmayowa, O. V. [1], Atolani, O. [2] and Olayemi, A. B. [1]

1. Department of Microbiology, Faculty of Life Sciences, University of Ilorin, Ilorin, Nigeria
2. Department of Chemistry, Faculty of Physical Sciences, University of Ilorin, Ilorin, Nigeria

Received: 2017.09.08 Accepted: 2017.11.03

ABSTRACT: Petroleum hydrocarbon contamination is a major global prevalent issue in the petroleum sector. This research focuses on evaluating biodegradation of three Gram-negative bacilli, isolated from cowpea planted soil, contaminated with kerosene. The Gram negative bacilli strains have been characterized and identified, using Microbact™ ID24E systems for the identification of Enterobacteriaceae and common Miscellaneous Gram-Negative Bacilli (MGNB). The identified organisms include *Aeromonas hydrophila*, *Vibrio parahaemolyticus*, and *Actinobacillus sp*. with the biodegradation indices, monitored for the evaluation of their degrading abilities, being Optical density at 600 nm (OD_{600nm}), pH, and emulsification stability. The chemical profile of single cultures and mixed cultures (consortia) on the jet fuel hydrocarbon has been determined by means of Gas Chromatography Mass Spectrometry (GC-MS), the results of which indicate that all the isolates have undergone above 70% reduction of the hydrocarbon substrates in terms of residual compounds. There has been 48 hydrocarbon compounds in the undegraded jet fuel which, following degradation process, decrease to 5, 13, 7, 10, 6, 9, and 10 compounds for *Aeromonas hydrophila*, *Vibrio parahaemolyticus*, *Actinobacillus* sp., *Aeromonas hydrophila* and *Vibrio parahaemolyticus*, *Aeromonas hydrophila* and *Actinobacillus* sp., *Vibrio parahaemolyticus* and *Actinobacillus* sp., *Aeromonas hydrophila*, *Vibrio parahaemolyticus*, and *Actinobacillus* sp., respectively. The degradation efficiency of the isolates have been relatively high and comparable to the control. Results from this study indicate that all the strains, especially the consortia, are potential candidates for remediating the problem of hydrocarbon contamination in the environment.

Keywords: Bacteria, Bacilli, Biodegradation, Gas chromatography, Jet fuel.

INTRODUCTION

Oil spills are a common event in Nigeria (Baird, 2010). As much as 50% of all spills occur due to pipeline and tanker accidents, while the other causes of oil spill include deliberate destruction (28%) as well as oil production operations (21%), with the remaining 1% being accounted for by inadequate or inoperative production equipment (Nwachukwu et al, 2013). Corrosion of pipelines and tankers is due to cracking or leaking of old production infrastructures that often do not receive inspection and maintenance (Nwilo & Badejo, 2006). In Nigeria, vast portions of land have been contaminated due to spillage, resulting from storage, transportation, or other operations that involve hydrocarbons.

[*] Corresponding author, Email: adetitun.do@unilorin.edu.ng

The dominance of crude oil products in world economy generates the conditions for administration of large amounts of toxins into populated areas and ecosystems around the world (Ojumu, 2004). The latent qualities of microorganisms, indicated as degrading agents of several compounds, pose microbial treatment as the most significant alternative to decrease the ecological impact of oil spills (Facundo et al., 2001, Robert et al., 2003). Pipeline leaks and accidental oil spills happen frequently in nature due to production, distillation, transport, and storage of crude oil and its products. (Kvenvolden & Cooper, 2003).

Biodegradation of petroleum hydrocarbon is a complex process that depends on the nature and the amount of the present hydrocarbons. According to Díaz-Ramírez et al., (2008) biodegradation is the process by which organic substances are broken down into smaller compounds by the enzymes, produced from living microbial organisms. The microbial organism transforms the substance through metabolic or enzymatic processes. The biogeochemical capacities of microorganisms seem almost limitless, and it is often said that microorganisms are "Earth's greatest chemists" (Madigan et al., 2012). Petroleum hydrocarbon degradation is influenced by molecular structure as well as hydrocarbons' weight (Marques-Rocha *et al*, 2000).

The speed of degradation is affected by several physical, chemical, and biological factors such as pH, temperature, nutrient, and quantity of hydrocarbon (Santhini et al., 2009). The wherewithal of microbes as promoters of several compounds' degradation thus indicates biological treatment as the major encouraging substitute to vitiate environmental impact, caused by pollutants (Nweke & Okpokwasili, 2003). According to Jyothi et al. (2012), many microorganisms have the power to use hydrocarbons as sole providers of carbon and energy for metabolic activities.

Jet fuel composition includes, predominantly, n-alkanes and branched alkanes, ranging from 15 to 30 carbon atoms with small amounts of Polycyclic Aromatic Hydrocarbon (PAH) also observed in trace levels (Bernabei et al., 2003). Bacteria that degrade alkane are known to use various metabolic processes, hence they are able to utilize many other compounds as carbon sources, too (Margesin et al., 2003; Harayama et al., 2004).

The present work aims at characterization of some Gram negative bacilli strains as jet-fuel degrading microorganisms.

MATERIALS AND METHODS

The Jet fuel samples (Jet A-1) was fetched from the tank farm of an independent aviation fuel marketing company in Lagos, Nigeria. *Aeromonas hydrophila*, *Vibrio parahaemolyticus*, and *Actinobacillus sp.*, used for the study, were isolated from kerosene-contaminated soil, which was planted with cowpea. They were obtained by utilizing the pour plate technique, characterized and identified via Microbact^{TM} ID 24E systems for identification of Enterobacteriaceae and common Miscellaneous Gram- Negative Bacilli (MGNB). The Microbact^{TM} ID 24E kit was used in accordance with manufacturer's specifications (Oxoid Ltd., Basingstoke, Hants (UK). Gram staining was done before Microbact^{TM} was used.

Mineral Salts Medium (MSM) was prepared as described by Vecchioli et al. (1990), sterilized at 121^{o}C for 15 minutes, and allowed to cool, then to be used for preparation of the biodegradative culture. The starting pH of the reaction mixture was set to 7.0, using a pocket-sized pH meter, manufactured by Hanna Instruments, Italy.

Each flask, containing the 99 mL of MSM, was supplemented with 1 mL of jet fuel as the only carbon and energy source under aseptic conditions, as described by Adetitun et al. (2016); Adetitun et al., (2014); and Oboh et al., (2006). Each of the pure Gram negative bacilli isolates

were aseptically inoculated into the medium. Mixed cultures (consortia) were also introduced in another treatment. Control samples on hydrocarbon free basis were run in parallel. The set-ups were kept at room temperature (25±2 ^{o}C) with continuous agitation on a rotary shaker (Lab Line No 3590) at 10 rpm for 20 days. The continuous agitation is necessary so as to increase the rate of reaction.

The turbidity of the culture fluids, measured by optical density at wavelength of 600 nm, was measured spectrophotometrically in a manner similar to what was described by Rahman et al., (2002). The pH of the various reaction mixtures were monitored as well, using a digital pH meter. The optical density and pH of the culture fluids were monitored at two-day intervals, as indicators of biodegradation. Un-inoculated control was used to monitor abiotic loss of the petroleum product.

The outline of the experimental set up or treatments are as the following:

1. 99ml MSM + 1ml Jet Fuel + Inoculum A,
2. 99ml MSM + 1ml Jet Fuel + Inoculum B,
3. 99ml MSM + 1ml Jet Fuel + Inoculum C,
4. 99ml MSM + 1ml Jet Fuel + Inoculum AB,
5. 99ml MSM + 1ml Jet Fuel + Inoculum AC,
6. 99ml MSM + 1ml Jet Fuel + Inoculum BC,
7. 99mlMSM + 1ml Jet Fuel + Inoculum ABC,
8. Control: 99ml MSM + 1ml Jet Fuel (no bacterial inoculum added).

The hydrocarbon substrates (Jet Fuel) in the MSM were extracted, using the liquid-liquid extraction procedure which employed n-Hexane, also using double extraction. Here, 30 mL of n-hexane was used. Initially, 15 mL of hexane was added to the culture media in the separating funnel, to be capped and shaken thoroughly for about 2 minutes to partition the contaminants into the two phases. Once settled, the mixture in the funnel got separated into two phases, namely the solvent phase and the aqueous one. The latter got drained off into the bottle, from which it was initially poured while the former was kept cold in a refrigerator for 10 days for analysis. These steps were repeated for all samples, including the controls. The aqueous phase was used for the emulsification (E_{24}) index.

The emulsification (E_{24}) indices of the inoculated samples were determined through modification of the procedure, described by Bodour et al., (2004). One mL of the jet fuel mixture was added to the same volume of the culture medium from various treatments in a 15 mL centrifuge tube. The mixture was vortexed for 2 mins and left to stand for 2 hrs. The E_{24} index was expressed as the percentage of the emulsified layer (mm), divided by the total height of the liquid column.

$$E_{24}\text{index} = \frac{\text{Height of emulsified layer (mm)}}{\text{Total height of the liquid column (mm)}}$$

The residual oil was extracted twice from the culture fluid with equal volume of n-hexane, as described by Adebusoye et al. (2006). The hexane extract (1.0 µL) was subsequently analysed, using Hewlett Packward 5890 Series II gas chromatography, equipped with a Flame Ionization Detector (FID) and 30-m long HP-5 column (0.25 mm of I.d. and 0.25 µm of film thickness). The injector and detector temperatures were kept at 300˚C and 350ºC respectively. Nitrogen gas was used as the carrier gas for the analysis. The GC was equipped with a column, packed with polyethylene glycol (EG 200) on chromosorb P (80-100 mesh) solid support, programmed to have a temperature gradient of 70ºC, held for 2 minutes, then ramped at 10ºC / minute to 320ºC and held for 10 minutes isothermally. Exactly 1 µm of the sample was injected into the injection port of the GC, using a micro syringe. The sample was immediately vaporized and swept down the column by the carrier gas. Following this separation in the column, the components were quantitatively analysed by means of the flame ionization detector, the output of which was transmitted to a recorder, producing a chromatogram. The output of the recorder

interfaced with a computer, programmed to indicate data including retention time, peak area, peak height, area percentage, and height percentage. The gas chromatograph was connected to a mass spectrometer, operating at an ionization voltage of 70eV over an acquisition mass range. The hydrocarbon profiling was principally identified through comparison of each GC peak's mass spectral with the data, obtained from National Institute Standard and Technology (NIST 2008) library.

The units of the graphs axis were determined by determining the range of the values and calibrating the axis most appropriately.

RESULTS AND DISCUSSION

Jet A-1 is an aviation fuel, designed to be used by air-craft powered gas-turbines. It appears as a colourless to straw-coloured substance, being the most used fuels for commercial aviation besides Jet A. Kerosene-type jet fuel (jet A-1) has a carbon number distribution between 8 and 16. Preliminary analysis of the Total Petroleum Hydrocarbon (TPH) produced a profile of hydrocarbons, varying from nC_9 to nC_{31}, with the majority of the hydrocarbons ranging between nC_{11} and nC_{16}. Polycyclic aromatic hydrocarbons were also detected in trace levels.

Figure 1 to 7 present the results from the eight treatments of the degradative cultures of the three Gram negative bacilli, namely *Aeromonas hydrophila*, *Vibrio paraheamolyticus*, and *Actinobacillus sp.* along with all their consortia measuring pH and optical density (OD_{600nm}) within a period of 20 days. A general trend was noted in the pH and optical density values across the treatments (Figures 1 to 7), thus a gradual drop in the pH values with proportional rise in the optical density values. An exception to this trend was observed and recorded for treatments in which no microbes were introduced (control). There were marginal increases in the optical density values and very little changes in the pH.

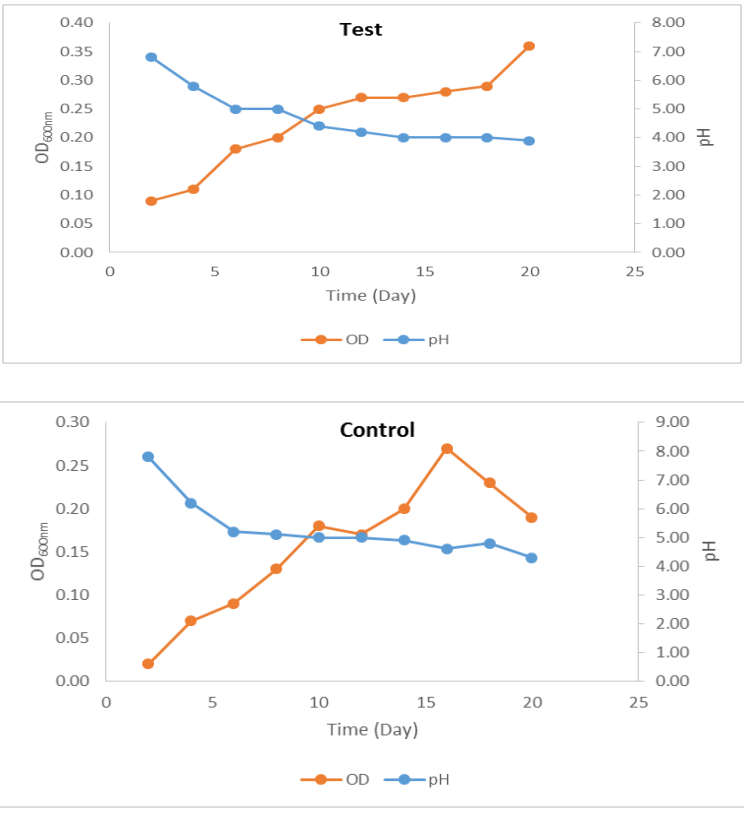

Fig. 1. Growth pattern of *Aeromonas hydrophila* with jet fuel (Test) and without jet fuel (Control)

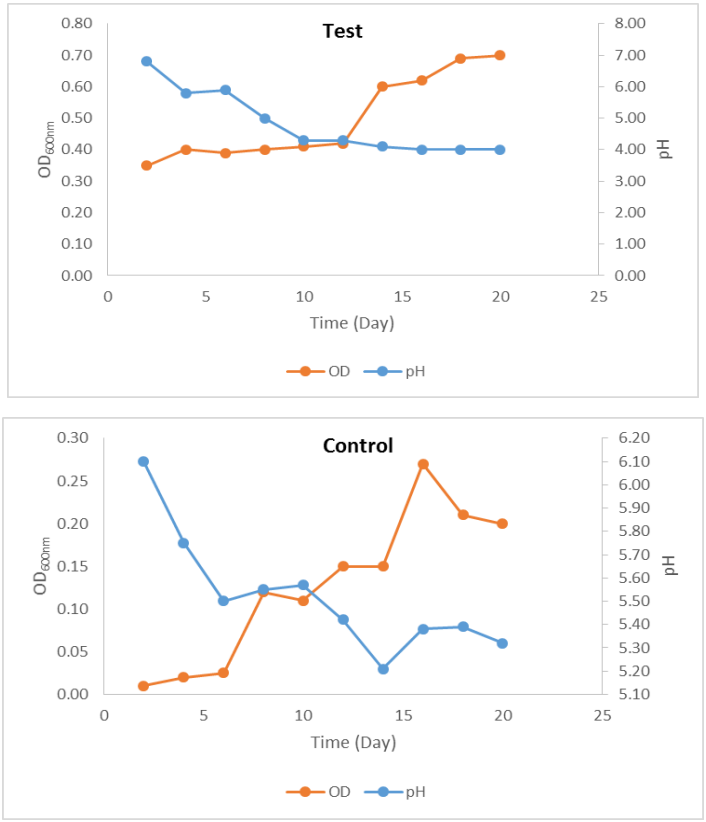

Fig. 2. Growth pattern of *Vibrio parahaemolyticus* with jet fuel (Test) and without jet fuel (Control)

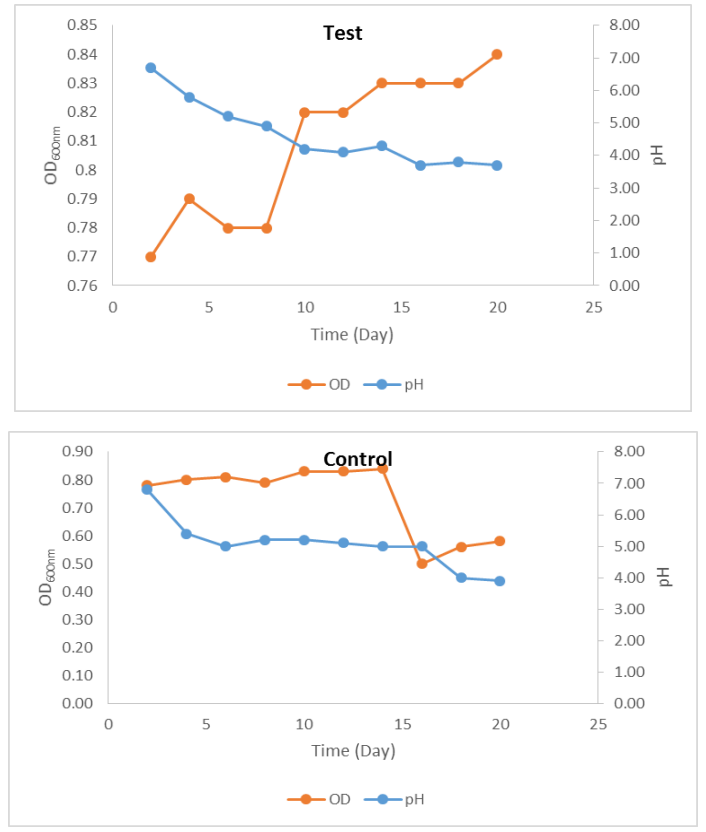

Fig. 3. Growth pattern of *Actinobacillus* sp. with jet fuel (Test) and without jet fuel (Control)

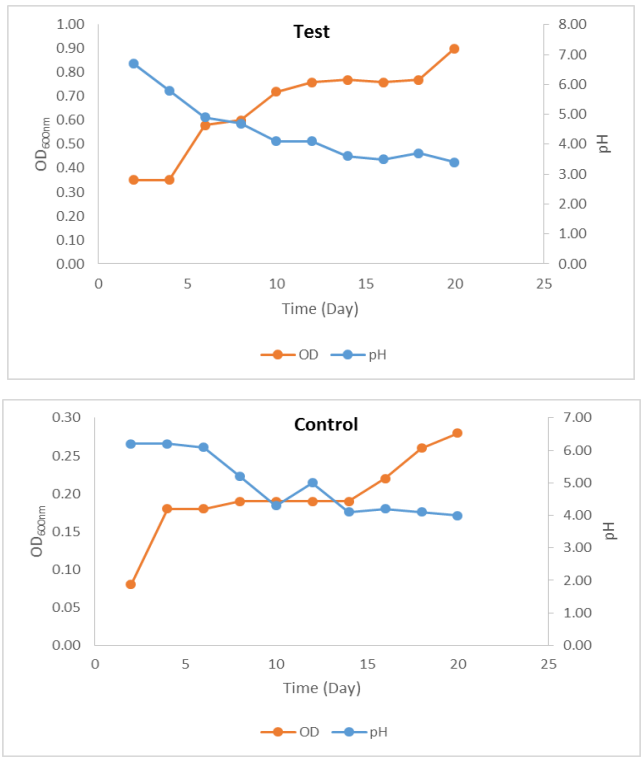

Fig. 4. Growth pattern of *Aeromonas hydrophila* and *Vibrio parahaemolyticus* with jet fuel (Test) and without jet fuel (Control)

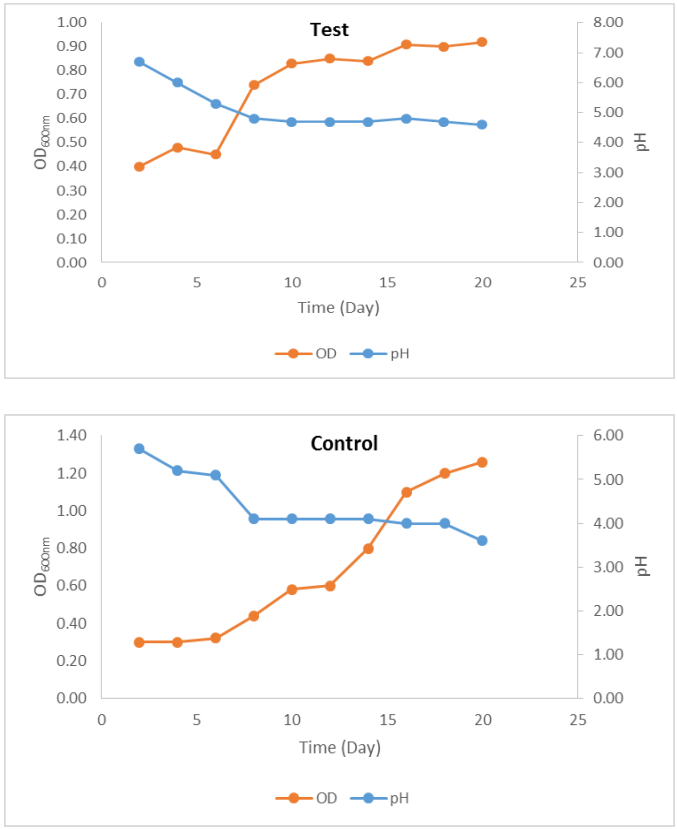

Fig. 5. Growth pattern of *Aeromonas hydrophila* and *Actinobacillus* sp. with jet fuel (Test) and without jet fuel (Control)

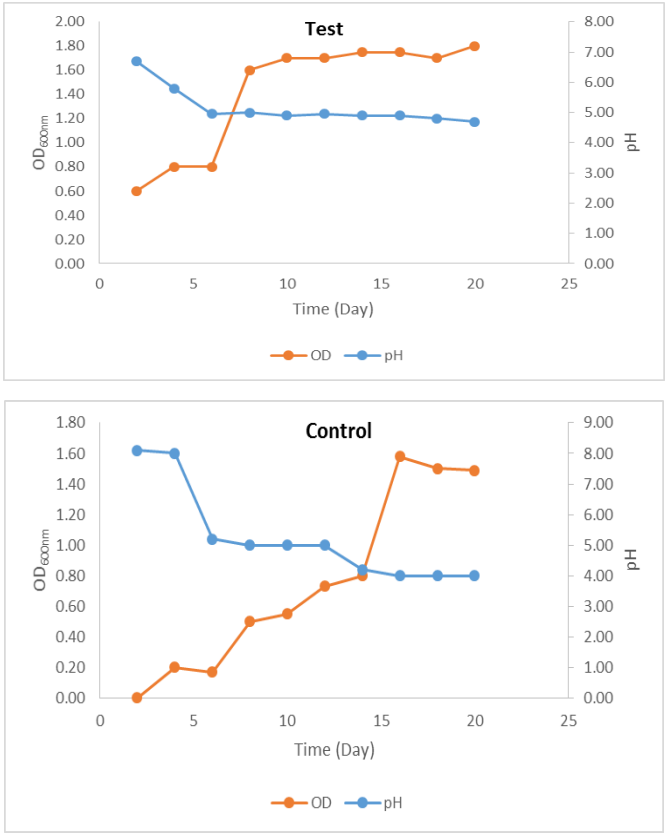

Fig. 6. Growth pattern of *Vibrio parahaemolyticus* and *Actinobacillus* sp. with jet fuel (Test) and without jet fuel (Control)

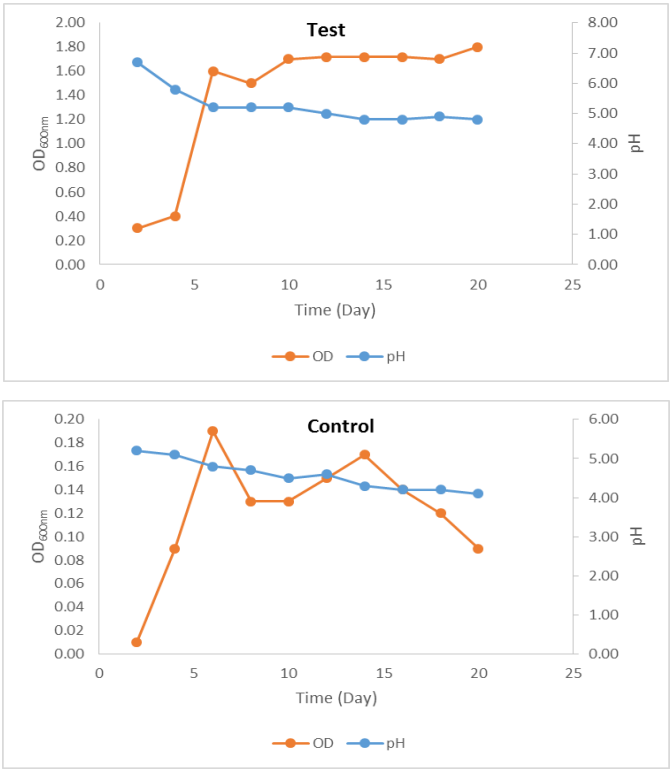

Fig. 7. Growth pattern of *Aeromonas hydrophila, Vibrio parahaemolyticus* and *Actinobacillus* sp. with jet fuel (Test) and without jet fuel (Control)

Adding jet fuel into the culture extracts showed an appreciable emulsion after 24 hours. Jet fuel samples, not inoculated with isolates, did not show any sign of emulsification. Measured emulsification (E_{24}) indices (Table 1) achieved by the single isolates and the consortium ranged from 0.0% to 87.5%, with *Aeromonas hydrophila* giving the highest emulsification (E_{24}) index (87.5%). The E_{24} indices were 87.5%, 62.5%, 75%, 37.5%, 42.5%, 40.0%, 50.0%, and 0.0% for *Aeromonas hydrophila, Vibrio parahaemolyticus, Actinobacillus* sp., *Aeromonas hydrophila* and *Vibrio parahaemolyticus, Aeromonas hydrophila* and *Actinobacillus* sp., *Vibrio parahaemolyticus* and *Actinobacillus* sp., *Aeromonas hydrophila, Vibrio parahaemolyticus, Actinobacillus* sp., and the control respectively. Emulsification stability or indices of emulsions were high, showing no correlation.

Table 2 gives the GC-MS profiles of residual oils, left at the end of the 20-day period (along with the 1-month storage period). The reduction in amount of all treatments show the marked effect of the isolates in utilizing the jet fuel. Also, Table 2 presents the GC profiles of the un-inoculated jet fuel (before degradation) and Table 3 shows chemical composition of the standard jet fuel after degradation. The percentage area of standard jet fuel and the residual hydrocarbons were compared (Table 2).

Results from pH and optical density values of the treatments over a 20-day period of investigation reveal that the top degraders, namely the entire consortia and *Aeromonas hydrophila*, had the optimum pH and optical density values, which were within a range of 6.5 and 1.91, respectively. This combination of pH and optical density is required for effective biodegradation according to Atlas, (1981); Song and Bartha, (1990). In this study all the bacteria exhibited growth ability (measured by increased OD_{600}), lowering the pH of the medium in which they grew. Results show maximal increase in optical density with lowering in pH values for the actives (Figures 1a-7a) while the controls (Figures 1b-7b) experienced minimal decreases.

The high emulsification index of all the organisms in their pure culture and mixed culture, presented in Table 1, implies that the degradative cultures were able to metabolize one or more compounds in the jet fuel, which is similar to a previous report of Monteiro et al., (2007), who recorded an emulsification index of 70% after 30 days of incubation. All microbes produced stable emulsions, capable of being used in the control of environmental contamination. Emulsification index greater or equal to 50% *confer or are associated with biodegradability of organisms. This property may be partly responsible for jet fuel biodegradation, observed here as reported by others (*Chandankere *et al., 2013).*

Table 1. Emulsification index of Individual Bacterium and Consortia

Bacteria and Consortia	Percentage of E_{24} (%)
Actinobacillus sp.	75.0
Aeromonas hydrophila	87.5
Vibrio parahaemolyticus	62.5
Aeromonas hydrophila and *Actinobacillus* sp.	42.5
Aeromonas hydrophila and *Vibrio parahaemolyticus*	37.5
Actinobacillus sp. and *Vibrio parahaemolyticus*	40.0
Actinobacillus sp., *Aeromonas hydrophila* and *Vibrio parahaemolyticus*	50.0
Control	0.0

Table 2. Chemical Composition of Jet Fuel before Biodegradation

S/N	Retention time	Chemical compound	Area %
1	3.588	Cyclohexane, methyl-	1.00
2	4.832	Toluene	0.88
3	5.170	Cyclohexane, 1,3-dimethyl	1.86
4	5.871	Cyclohexane,1,2-dimethyl	0.38
5	6.083	Octane	0.47
6	7.328	Cyclohexane, ethyl	0.84
7	7.472	Cyclohexane,1,3-trimethyl	0.77
8	8.104	Cyclohexane,1,2,4-trimethyl	0.73
9	8.611	Ethylbenzene	0.63
10	8.836	Oxalic acid	0.71
11	8.967	Benzene,1,3- dimethyl	2.28
12	9.142	Heptane,2,5-dimethyl	0.73
13	9.568	Cyclohexane	0.34
14	9.699	Cis-1-ethyl-3-methyl-cyclohexane	1.81
15	9.780	Cyclohexane,1-ethyl-4methyl	1.68
16	9.974	O-xylene	1.46
17	10.287	3-Decy-2-ol	0.78
18	10.443	Nonane	2.66
19	10.554	1-Ethyl-3-methylcyclohexane	1.21
20	11.019	1H-Indene,octahydro	1.54
21	11.194	1-Octadecyne	0.89
22	11.513	1-Dodecanol,3,7,11-trimethyl	3.57
23	11.713	1-Octanol,2-butyl	0.81
24	11.907	Octane,2,6-dimethyl	1.57
25	12.039	Cyclohexane,1-ethyl-2,3-dimethyl	1.23
26	12.182	2-Nonen-1-ol	1.78
27	12.439	2,3,4-Trimethyl-hex-3-enal	1.35
28	12.633	Cyclohexane,1,1,2,3-tetramethyl	3.40
29	13.071	Benzene,1-ethyl-3-methyl	2.80
30	13.351	Dodecanal	0.72
31	13.809	Benzene,1-ethyl-2-methyl-	1.06
32	13.940	m-menthane	1.12
33	14.028	1-Nonylcycloheptane	1.88
34	14.453	Benzene,1,2,3-trimethyl	6.58
35	14.660	10-Heneicosene	1.62
36	14.897	Decane	4.78
37	15.648	Trans-P-mentha-1(7),8-dien-2-ol	7.28
38	15.998	Decane,5-cyclohexyl	1.78
39	16.111	Cyclohexanecarboxylic acid	0.90
40	16.830	Cyclooctene,1,2-dimethyl	9.13
41	17.193	Cis-P-Mentha-2,8,dien-1-ol	6.85
42	17.474	2-Piperidinone	2.38
43	18.592	Methanol	3.62
44	18.988	Undecane	3.46
45	19.451	Spiro[3.5]nona-5,7-dien-1-one,trimrthyl	1.78
46	20.258	Hydrocinnamic acid	2.41
47	36.941	Octadecene	1.02
48	37.785	Tetradecene,2,6,10-trimethyl	2.48

Table 3. Chemical Composition of Degraded Jet Fuel, Facilitated by Gram Negative Bacilli and their Consortiums.

S/N	Chemical Compounds	A	B	C	AB	AC	BC	ABC
1	4H-ThioPyrano[4,3:4,5]Furo[2,3-d]pyridine-3(6H)-amine,5,8-dimethyl-	40.24	29.46	27.44	59.90	31.48	27.90	47.34
2	2-Thiazolamine,4-(3,4-dimethoxy-phenyl)-5-methyl-	25.62	11.65	25.76	24.81	10.68	14.08	24.22
3	Benzo(b)naptho(2,3-d)thiophene,10-dihydro-7-methyl-	0.00	12.08	4.80	0.00	16.22	17.36	1.00
4	Methaqualone	0.00	3.68	0.00	0.00	2.36	0.00	0.00
5	Officinalic acid, methyl ester	0.00	0.00	0.00	0.00	0.00	0.00	0.29
6	1-Ethanone	0.00	0.00	0.00	0.00	0.00	0.00	0.34
7	Anodendrosite E2,monoacetate	4.50	0.00	0.00	0.00	0.00	0.00	3.46
8	Benzimidazole-5-carboxylic acid,2-methyl-1-phenyl-	2.88	0.00	3.15	0.00	10.56	0.00	16.37
9	Benz(c) acridine	0.00	0.00	5.60	0.00	0.00	0.00	0.00
10	2,5,Di-t-butyl-4-methoxy-1, 4dihydrobenzaldehyde	1.20	0.00	5.20	0.00	0.00	0.00	0.00
11	3,5,6-Trimethyl-P-quinone,2-(2,5-dioxotetrahydrofuran-3-yl)thio-	0.00	0.00	1.20	0.00	0.00	0.00	0.00
12	1methyl-2,5-dichloro-1,6-diazaphenaline	0.00	0.00	0.00	1.84	0.00	1.88	0.00
13	Benzoic acid	0.00	0.00	0.00	8.16	0.00	0.00	0.00
14	Acetic acid	0.00	3.26	0.00	2.24	0.00	4.90	0.00
15	1,2-Benzenedicarboxylic acid,4-methyl-5(1-methyl)-dimethyl ester	0.00	0.36	0.00	0.00	0.00	0.00	0.00
16	Pregn-4-en-18-oic acid	0.00	1.81	0.00	0.00	0.00	0.00	0.00
17	Benzothiophene-3-carboxamide,4,5,6,7-tetrahydro-2-amino-6-tert-butyl-	0.00	4.10	0.00	0.00	0.00	0.00	0.00
18	5-Beta-hydroxymethyl-3-beta-tosylamino cholestane	0.00	0.00	0.00	0.00	0.00	2.36	0.00
19	Pyrido(1,2,9)benzimidazole-4-carbonitrile,3-methyl-1-dimetylamino-	0.00	0.00	0.00	0.00	0.00	2.62	0.00
20	(1-Cyclopentenyl)ferrocene perylene	0.00	1.51	0.00	0.89	0.00	0.00	1.03
21	1,2,4-Methenocyclopentlene	0.00	1.21	0.00	1.64	0.00	0.00	0.00
22	6,7,8,9-Tetrahydro-1,2,3-trimethoxy-9-methyl-5H-benzocycloheptane	0.00	2.16	0.00	0.00	0.00	0.00	0.00
23	3-(y-methylaminopropyl)-5(4-bromoo-phenyl)-2-methyl-2H-pyrazole	0.00	0.00	0.00	0.00	0.00	0.00	2.11
24	Methyl7-(5-(methoxycarbonyl)methyl1,2-furyl)heptanoate	0.00	0.00	0.00	0.00	0.00	5.65	0.00
25	Cinnamic acid	0.00	2.16	0.00	1.01	0.86	0.00	1.16

Legend: A=*Aeromonas hydrophila*, B= *Vibrio parahaemolyticus*, C=*Actinobacillus* sp. AB= *Aeromonas hydrophila* and *Vibrio parahaemolyticus*, AC= *Aeromonas hydrophila* and *Actinobacillus* sp. BC= *Vibrio parahaemolyticus* and *Actinobacillus*, sp., ABC=*Aeromonas hydrophila*, *Vibrio parahaemolyticus* and *Actinobacillus* sp.

Gas chromatography mass spectrometry analysis of the undegraded (Table 2) and degraded jet fuel (Table 3) at the end of the degradation period revealed a reduction in the number of compounds as well as percentage area of the inoculated hydrocarbons, compared to un-inoculated controls. The incidence of disappearing peaks may be due to carbonisation and gasification of some of the hydrocarbons during the microbial degradation process. It is believed that during the degradation period, the bacterium/bacteria had utilized and broken down the hydrocarbons, present in the jet fuel (Table 2), into fewer and less complex compounds, including carbon dioxide gas (Table 3). This agrees with Alexander, (2001), who saw biodegradation as the biologically catalysed reduction in complexity of

chemical compounds, based on growth and co-metabolism.

The experiments showed hydrocarbon degrading potentials of the isolates, supporting the point, made by Bento et al., (2005), that biodegradation of petroleum hydrocarbon depends on specific microbial population present. The jet fuel was degraded by all three Gram negative bacilli and their consortia at a relatively fast rate, indicating that jet fuel biodegradation can proceed in the presence of these microbes. The fact that the jet fuel, used in this study, was degraded indicated that they were probably a preferred hydrocarbon compound substrate by the microbial consortia and the individual microbe(s) carrying out the metabolic processes (Table 3).

Results in Table 3 reveal that all the consortia, especially consortium of *Aeromonas hydrophila*, *Vibrio parahaemolyticus*, and *Aeromonas* sp., achieved the highest level of bio-degradation for jet fuel substrates, likely due to the synergistic activity of the trio. However, this does not agree with the work of Ausma et al., (2002), who reported that mixed cultures might produce less effective biodegradation since the activities of the different microorganisms could be antagonistic as a result of the competition for growth factors or unfavourable changes in pH. Relatively, the least successful case was *Actinobacillus* sp., where less amount of the jet fuel was utilized (Table 3).

There is no single strain of bacteria with metabolic capacity to degrade all the components, found within crude oil. This agrees with Das and Mukherjee (2007), who reported that a wide variety of metabolic and physiological factors are required for the degradation of different compounds in jet fuel. All of such properties cannot be found in one organism. Similarly, Adebusoye et al., (2006) demonstrated that mixed culture of microbial community is required for complete biodegradation of oil pollutants because the hydrocarbon mixtures differ significantly in terms of volatility, solubility, and susceptibility to degradation and the necessary enzymes needed cannot be found in a single organism. This also agrees with Ekpo & Udofia (2008), who reported further that individual microorganisms metabolize only a limited range of hydrocarbon substrates and jet fuel is made of a mixture of compounds, hence its biodegradation requires mixtures of different bacterial groups or consortia, functioning to degrade a wider range of compounds.

Apparently, the genetic information in more than one organism is required to produce the enzymes, needed for extensive jet fuel biodegradation obtained under aerobic condition in this study. Obviously, the mixed culture of *Aeromonas hydrophila*, *Vibrio parahaemolyticus*, and *Actinobacillus* sp. had the highest hydrocarbon degradation ability than other individual isolates. The use of pure cultures in the study alongside the mixed cultures eliminated the ambiguity associated with the process. According to these studies, it is probable that the pure cultures alone or a mixed culture can utilize jet fuel and most hydrocarbon fuel substances, as reported by Venkateswaran & Harayama (1995). Although mix cultures gave the highest proportion of degradation efficiency in all treatments, evidence of the cooperation of the mixed cultures in dealing with hydrocarbon contaminations is still relevant as reported by Boonchan et al., (2000).

Survival of microorganisms in petroleum hydrocarbon media during degradation period was a key factor for the rate of biodegradation of hydrocarbons in substrates (Ramos et al., 1991). Since the Gram negative bacilli in the present report were isolated from cowpea- planted kerosene contaminated soil, they survived and adapted to the jet fuel substrates rapidly. This was evident from the significant increase in optical density values with a decline in pH values and (E_{24}) indices in all cultures, as compared to control.

Desouky (2003) had reported that Gram negative bacteria were known to be involved in biodegradation, leaching, and removal of several organic and inorganic man-made hazardous wastes, which agrees with the findings of this work.

The results from this empirical work affirms the increasing awareness that bioremediation as a means of dealing with oil spills or contamination is real and practicable. The GC report indicates that all three species showed a remarkable effect on pollutant removal.

CONCLUSION

The studied Gram-negative bacilli had degradation capabilities. The mixed bacterial cultures, i.e., *Aeromonas hydrophila* and *Vibrio parahaemolyticus*; *Aeromonas hydrophila* and *Vibrio parahaemolyticus*; *Vibrio parahaemolyticus* and *Actinobacillus sp*.; *Aeromonas hydrophila*, and *Vibrio parahaemolyticus* and *Actinobacillus sp.* could facilitate a maximum degradation of jet fuel respectively.

Further comprehension of the metabolic process of these organisms on the hydrocarbons will enhance the potentials of developing models and strategies for remediating hydrocarbon pollutants from hydrocarbon contaminated ecosystems.

REFERENCES

Adebusoye, S.A., Ilori, M.O., Amund, O.O., Teniola, O.D. and Olatope, S.O. (2006). Microbial degradation of petroleum hydrocarbons in a polluted tropical stream. World Journal of Microbiology, 23, 1149-1159.

Adetitun, D.O., Olayemi, A.B., Kolawole, O.M. and Fathepure, B. (2016). Molecular Identification of Hydrocarbon Degrading Bacteria Isolated from alfisol-Loam Experimentally Contaminated with Gasoline. Biokemistri, 28(3), 135-143.

Adetitun, D.O., Olayemi, A.B. and Kolawole, O.M. (2014). Hydrocarbon- degrading capability of Bacteria isolated from maize- planted, kerosene contaminated Ilorin alfisol. Biokemistri, 26(1), 13-18.

Alexander, M. (2001). Role of cometalism. Microbial degradation of pollutants in marine environments. US Environment Protection Agency. EPA-600/9-79-012, 67-75.

Atlas, R.M. (1995). Bioremediation of Petroleum Pollutants. International Biodeterioration and Biodegradation, 35(1-3), 317-327.

Atlas, R.M. (1981). Microbial degradation of petroleum hydrocarbons: an environmental perspective. Microbiol. Rev., 45, 180-209.

Ausma, S., Edwards, G.C., Fitzgerald-Hubble, C.R., Halfpenny-Mitchell, L., Gillespie, T. J. and Mortimer, W.P. (2002). Volatile hydrocarbon emissions from a diesel fuel contaminated soil bioremediation facility. Air Waste Management Association, 52, 769-780.

Baird, J. (2010). "Oil's Shame in Africa". Newsweek (Atlantic Edition). 156(4), 16.

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

Bernabei, M., Reda, R., Galiero, R. and Bocchinfuso, G. (2003). Determination of total and polycyclic aromatic hydrocarbons in aviation jet fuels. Journal of Chromatography, 985, 197-203.

Bodour, A.A., Gerrero-Barajas, C. and Maier, M. (2004). Structure and characterization of Flavolipids, a novel class of Biosurfactants produced by Flavolipid sp. Strain MTN1. *Appl Environ Microbiol.*, 70(1), 114-20.

Boonchan, S., Britz, M.L. and Stanley, G.A. (2000). Degradation and mineralization of high-molecular-weight polycyclic aromatic hydrocarbons by defined fungal- bacterial co- culture. Applied Environmental Microbiology, 66, 1007-1091.

Chandankere, R., Yao, J., Choi, M.M.F., Masakorala, K. and Chan, Y. (2013). An efficient biosurfactant-producing and crude-oil emulsifying bacterium Bacillus methylotrophicus USTBa isolated from petroleum reservoir. Biochemical Engineering Journal**,** 74, 46-53.

Das, K. and Mukherjee, A.K. (2007). Crude petroleum-oil biodegradation efficiency of *Bacillus subtilis* and *Pseudomonas aeruginosa* strains isolated from a petroleum-oil contaminated soil from North-East India. Bioresource Technology, 98(7), 1339-1345.

Díaz-Ramírez, I.J., Escalante-Espinosa, E., Favela-Torres, M. and Ramírez-Saad, H., (2008). Design of bacterial defined mixed cultures for biodegradation of specific crude oil fractions, using population dynamics analysis by DGGE. International Biodeterioration and Biodegradation, 62(1), 1-30.

Desouky, A. (2003). Acinetobacter: environmental and biotechnological applications. African Journal of Biotechnology, 2(4), 71-74.

Ekpo, M.A. and Udofia, U.S. (2008). Rate of biodegradation of crude oil by microorganisms isolated from oil sludge environment. African Journal of Biotechnology, 7(24), 4495-4499.

Facundo, J. M., Vanessa, H. and Teresa, M. L. (2001). Biodegradation of Diesel Oil in Soil by a Microbial Consortium. Water, Air and Soil Pollution, 128 (3-4). 313-320.

Harayama, S., Kasai, Y. and Hara, A. (2004). Microbial Communities in Oil- Contaminated Seawater. Current Opinion in Biotechnology, 15: 205–214.

Jyothi, K., Babu, S. K., Nancy Clara, K. and Kashyap, A. (2012). Identification and Isolation of Hydrocarbon Degrading Bacteria by Molecular Characterization. Helix, 2: 105-111.

Kvenvolden, K. A. and Cooper, C. K. (2003). Natural Seepage of Crude Oil into the Marine Environment. Geo-Marine Letters, 23(3-4):140–146.

Madigan, T.M., Martinko, J.M.A., Stahl, D. and Clark, D.P., (2012). Brock Biology of Microorganisms (13th edition). Pearson Education Incorporated, San Francisco, CA.

Margesin, R., Labbé, D., Schinner, F., Greer, C. W. and Whyte, L. G. (2003). Characterization of Hydrocarbon-Degrading Microbial Populations in Contaminated and Pristine Alpine Soils. Applied and Environmental Microbiology, 69:3085–3092.

Marques-Rocha, F. J., Hernandez-Rodrigues, V. and Lamela, M. A. T. (2000). Biodegradation of Diesel Oil by Microbial Consortium. Water, Soil and Air Pollution, 128: 313-20.

Monteiro, S.A., Sassaki, G.L., Souza, L.M., Meira, J.A., Araújo, J.M., Mitchell, D.A., Ramos, L.P. and Krieger, N. (2007). Molecular and structural characterization of the biosurfactant produced by *Pseudomonas aeruginosa* DAUPE614. Chem. Phys. Lipids, 147, 1-13.

Nwachukwu, C., Kalejaiye, K., Ajala, B. and Ejoh, E. (2013). Shell Spills over 26, 000 barrels of oil in 13 months. Vanguard Newspaper, January 29.

Nweke, C. O. and Okpokwasili, G. C. (2003). Drilling Fluid Base Oil Biodegradation Potential of a Soil *Staphylococcus* species. African Journal of Biotechnology, 2: 293 – 295.

Nwilo, P.C. and Badejo, O.T. (2006). Impacts and management of oil spill pollution along the Nigerian coastal areas. In administering marine spaces: international issues. Ed(s) Sutherland *et al*. A publication of FIG commission 4 and 7 group 4.3. International Federation of Surveyors. FIG Publication No 36: 119-133.

Oboh, B.O., Ilori, O.M., Akinyemi, J.O. and Adebusoye, S.A. (2006). Hydrocarbon degrading potentials of bacteria isolated from a Nigeria Bitumen (Tarsand) deposit. National Science, 4, 51-57.

Ojumu, T. V., Bello, O. O., Sonibare, J. A. and Solomon, B. O. (2004). Evaluation of Microbial Systems for Bioremediation of Petroleum Refinery Effluents in Nigeria. African Journal of Biotechnology, 4(1) :31 35.

Oudot, J. (1994). Soil pollution by petroleum products and treatments. Analysis Magazine**,** 22, 16-18.

Ramos, J.L., Duque, E. and Ramos-Gonzalez, M.I. (1991). Survival in soils of an herbicide-resistant *Pseudomonas putida* strain bearing a recombinant TOL plasmid. Appl. Environ. Microbiol., 57, 260-266.

Rahman, K.S.M., Rahman, J.T., Lakshmanaperumalsamy, P. and Banat, I.M. (2002). Towards Efficient Crude Oil Degradation by a Mixed Bacterial Consortium. Bio resource Technology, 85, 257-261.

Robert, M. G., Stephen, J. R. and Roger, C. P. (2003). Biodegradation of Fuel Oil Under Laboratory and Arctic Marine Conditions. Spill Science TechnoIogy BuIIetin, 8 (3): 297-302.

Santhini, K., Myla, J., Sajani, S. and Usharani, G. (2009). Screening of *Micrococcus* sp from Oil Contaminated Soil with Reference to Bioremediation. Botany Research International, 2(4): 248-252.

Song, H. and Bartha, R. (1990). Effects of jet fuel spills on the microbial community of soil. Applied Environmental Microbiology, 56, 646-651.

Vecchioli, G.I., Del Panno, M.T. and Painceira, M.T. (1990). Use of selected autochthnous soil bacteria to enhance degradation of hydrocarbons in soil. Environmental Pollution, 67, 249-258.

Venkateswaran, K. and Harayama S. (1995). Sequential enrichment of microbial populations exhibiting enhanced biodegradation of crude oil. Can. J. Microb., 41, 767-775.