Journal of Emerging Trends in Engineering and Applied Sciences (JETEAS) 3 (3): 483-489

jeteas.scholarlinkresearch.org

Bioremediation of Engine Oil Contaminated Site

A. O. Ogunbayo; R. A. Bello and U. Nwagbara

Department of Chemical Engineering,
University of Lagos, Lagos**,**

Corresponding Author: A. O. Ogunbayo

Abstract

The dominant microorganisms present in soils, contaminated with hydrocarbon fractions in engine oils (used and unused) at various automobile workshops in five locations in the city of Lagos were isolated. The purpose of the work was to evaluate the effectiveness of the several microorganisms indigenous to the soil in remediating the soil. *Bacillus species* and *Pseudomonas species* were found in all the sites, while *flavobacterium* and *micrococcus* species were found in three of the sites and only one the site had the *rhodococcus* species. The effectiveness and efficiencies of degradation of the hydrocarbon components by the isolated organisms were studied in shake flaks containing minimal salt medium with varying concentrations of engine oil (0.5%, 1.0%, and 1.5%). Each isolated organism and mixtures of them were grown in the various media in an incubator shaker at room temperature. The extent of growth of organisms observed was linked to the ability of the organisms to biodegrade the hydrocarbon fractions present in the medium. The results obtained showed that the *pseudomonas* and *rhodococcus* species gave the best growth at all concentrations of engine oil used, degrading 60% and 80% of oil respectively. A co-culture of these two organisms gave a higher growth than each of them when cultured alone, suggesting a positive interaction between the two organisms. This could be attributed to their ability to degrade different types of hydrocarbons thus creating the synergy. It can be concluded that an efficient bioremediation programme can be put in place by the use of an appropriate mixture of organisms as well as other physico-chemical properties that might also influence the growth of these microorganisms.

Keywords: bioremediation; mixed culture; pseudomonas species; rhodococcus species

INTRODUCTION

Accidental spills, illegal dumping and careless handlings of spent lube oil in mechanic workshops have been a significant source of environmental pollution, because of the predominantly unstructured practice of automobile vehicle repair services. Contaminations of soil and groundwater have been imminent from the continuous disposal of used engine oil, which could lead to a great health problem. Engine oil is a complex mixture of hydrocarbons and other organic compounds including some organo-metabolic constituents (Butler and Mason, 1997) that is used to lubricate the parts of an automobile engine in order to avoid excessive wearing out (Hagwell et al, 1992). Used (also called spent or waste) motor engine oil contains metals and heavy polycyclic aromatic hydrocarbons (PAHs) and these could contribute to chronic hazards including mutagenicity and carcinogenicity (Keith and Telliard, 1979, Hagwell et al, 1992; Boonchan et al, 2000). As it is inevitable for the efficient and effective functioning of the automobile engines, soil contamination with used engine oil is becoming one of the major environmental problems (Mandri and Lin, 2006), mainly due to uncontrollable disposal, particularly in developing economies. The widespread ability of microorganisms to assimilate these hydrocarbons is of great significance and when it occurs in the natural environment, the process is known as biodegradation. Hydrocarbons, including PAHs, have been long recognized as substrates supporting microbial growth. A wide range of Hydrocarbon utilizers (HCUs) found to be useful in the soil include the following species, *Pseudomonas*, *Rhodococcus*, *Mycobacterium,* Bacillus, *Acinetobacter*, *Providecin*, *Flavobacter*, *Carynebacterium, Streptococcus* (Bhattacharya et al, 2002) Other organisms such as fungi are also capable of degrading the hydrocarbons in engine oil to a certain extent, but they take longer periods of time to grow when compared to their bacterial counterparts (Prenafeta-Boldu et al, 2001).

Bioremediation uses biodegradation to achieve its goal as it is defined as any process that uses microorganisms or their enzymes to return the environment altered by contaminants to its original condition. Bioremediation technologies can be classified as in situ or ex-situ. In situ bioremediation involves treating the contaminated material at the site, while ex-situ involves the removal of the contaminated materials to be treated elsewhere. This work involves the isolation and identification of HCUs from soil samples collected from mechanic workshops at five different locations in Lagos. The organisms were identified and their growth

behaviours studied in order to pick those exhibiting the best growths. The best two organisms chosen were grown in three different concentrations of engine oil (0.5%, 1.0%, and 1.5%) in a bioreactor as pure cultures and also as a mixed culture, while keeping other nutrients temperature and pH constant.
The objective of this work is to characterize the organisms indigenous to the soil samples contaminated with engine oil and to apply the knowledge of their rates of degradation in establishing a procedure for cleaning up an environment polluted with such engine oils such as mechanic workshops.

MATERIALS AND METHODS

MATERIALS

SAMPLES

Oil contaminated soil samples were collected from three sites in Yaba, one site in Isolo and one site in Bariga local government areas of Lagos state (Fig1). The samples were subjected to microbiological analyses and some physico-chemical parameters such as moisture content and pH were also measured. Used motor engine oil was collected from one of the mechanic workshops and Engine oil of brand (TOTAL QUART 5000) was used as the fresh engine oil sample. The media used were nutrient agar to solidify for the growth in plates and basal medium (minimal salt medium –MSM) for the growth in shake flasks

METHOD

Enumeration of Microorganisms

Total heterotrophic bacterial population in the soil samples was enumerated by adopting the standard plate counts technique using spread plate method. These involved spreading aliquots of a serially diluted 0.1ml of 10^{-5} dilutions of the soil sample suspension on nutrient agar plates and the plates were incubated for 24hr. at 37^0C. The isolated organisms from the nutrient agar plates were again incubated in minimal salt agar plates containing used engine oil as the sole source of carbon and energy. The plates were incubated at 37^0C for 5 days. The percentages of HCUs relative to the total heterotrophic counts were noted. The ability of the HCUs to grow solely on the used engine oil as the only source of carbon was tested further by inoculating a liquid medium with each isolated organism to identify the best oil degraders from the different organisms isolated. The liquid medium containing 1% used engine oil was made up by adding 0.5 ml used into 49.5 ml basal medium. The medium was inoculated by the various identified organisms and incubated in a shaker incubator rotating at 200 rev/min for 5 days at 30^0C. Optical density and visual turbidity was used to identify the best two HCUs degraders.

Identification of hydrocarbon utilizing organisms

The hydrocarbon degraders amongst the isolated organisms were identified and characterized by morphology, gram staining and biochemical characterization. (Spore staining, oxidase test, haemolysis test, nitrate reduction, methyl red test).

Biodegradation Experiments

The liquid basal minimal media containing 0.5%, 1.0% and 1.5% v/v of fresh engine oil were put in 250 ml flasks, and the ability of each of the isolates and their mixtures to degrade the oil was investigated. The flasks were incubated in an incubator at 30^0C and rotating at 200 rev/min for 7 days. A control experiment with no microorganism was included. The extent of utilization of the hydrocarbons by the microorganisms was evaluated by monitoring growth which was measured by total viable counts. Gas Chromatographic analyses of the medium were used to monitor the percentage degradation, while changes in the pH reflected the changes and in the compositions of the medium.

Bacterial Growth

Bacterial growth was monitored by withdrawing a sample from the culture medium every 24hr for 7 days. Each sample was used to inoculate nutrient agar plates by spread plate technique, and all plates were incubated at room temperature for 24hr. and the viable colonies counted. This was done for each the concentrations of engine oil.

pH Monitoring

The initial pH of the culture medium was measured and the pH at the end of 7 days of incubation was also measured.

Analyses

A gas chromatographic analysis was carried out on each culture sample after 7 days of incubation. The samples were first extracted with n-hexane before one microlitre (1 µl) of the sample was introduced into the gas chromatograph which had been initially calibrated with a hydrocarbon standard. The percentage degradation was calculated from the values of the concentration when compared to the control where there was no degradation.

RESULTS AND DISCUSSION

RESULTS

Enumeration of Microbial Population

The bacterial population determined showed that 13.1% of the total heterotrophic populations are hydrocarbon utilizers (HCUs). (Table 1)

Table 1: Bacterial population density

BACTERIAL POPULATION	RESULT (cfu/ml)
Heterotrophic	4.72×10^7
Hydrocarbon utilizers	0.62×10^7
% Hydrocarbon utilizers	13.1%

Isolation and Identification

The bacterial species capable of utilizing engine oil as a sole source of carbon and energy were isolated.

Five species with good degradation potentials were identified and their phenotypic characteristics are shown in Table.2.

Table 2: Phenotypic characterization of Engine oil degraders

Biochemical characteristics	Isolate1	Isolate 2	Isolate 3	Isolate 4	Isolate 5
Cell morphology	Rod	Coccus	Rod	Rod	Coccus
Gram reaction	+	+	-	-	+
Glucose	+	+	-	+	-
Oxidase	-	-	+	+	+
Methyl red	+	+	-	-	-
Lactose	-	-	-	-	-
Haemolysis	+	+	+	*	*
Spore formation	+	-	-	-	-
Pigmentation	Whitish	Orange	Greenish	Orange	Whitish yellow
Closest relative/tentative identity	*Bacillus sp.*	*Rhodococcus sp.*	*Pseudomonas sp.*	*Flavobacterium sp.*	*Micro-ccus sp.*

Key

\- Negative + Positive * Not tested

Degradation of Hydrocarbon Components

The G.C analysis revealed the percentage degradation of the 1% engine oil over a period of 7 days by each of the organisms and the mixture. (Fig.2). The result showed that *Pseudomonas* and *Rhodococcus* species gave 60% and 80% degradation of oil respectively while the mixture of the organism gave 88% degradation. The total viable counts of the cultures also increased as the degradation increased (Fig. 3 – 5). This suggests that the organisms are capable of utilizing the hydrocarbons and the growth curves obtained followed the pattern of a typical microbial growth curve. *Pseudomonas sp.* has the higher specific growth rate of $0.235hr^{-1}$ while *Rhodococcus sp.* had a specific growth rate of $0.187hr^{-1}$. The growth curves of both organisms and the mixture for the three different concentration of engine oil are shown in Figs. 3 to 5.

Single Culture

The incubation with the liquid medium revealed the best two degraders as *Rhodococcus sp.* and *Pseudomonas sp.* Both *Pseudomonas sp*. and *Rhodococcus sp* have often been found in hydrocarbon contaminated sites (Lloyd-Jones and Truddgil, 1989). The two species are widely spread in nature; they have a broad affinity for hydrocarbon and can degrade selected alkanes, alicylics, thiophenes, pyrene, fluoranthene, and anthracene (Allen et al, 1997; Folsom et al, 1996; Koike et al., 1999). Several reports have also attested to their capability of degrading crude oil (Hamme and Ward, 2000; Anal and Mukherji. 2008). In this work *Rhodococcus sp.* degraded the oil better than *Pseudomonas sp.* (Fig. 2). This could be attributed to the claim that *Rhodococcus sp.* are more hydrophobic and have a higher affinity for hydrocarbon – water interfaces than the Pseudomonas sp. (Stringfellow and Alvararez-Cohen, 1999) Furthermore it has been reported that *Pseudomonas sp*. did not adhere to hydrocarbons and was limited by hydrocarbon insolubility (Hamme and Ward, 2000)

Mixed Culture

The degradation obtained with the mixture of the two organisms (88%) was higher than the values obtained for the pure cultures of *Rhodococcus sp.* (80%) and *Pseudomonas sp.* (60%) which suggested that the two organisms could coexist with no adverse effect and possibly have a synergy. (Fig. 2). The advantages of employing mixed cultures have been demonstrated by several researchers (Hamme and Ward, 2000; Juhasz et al., 1997; Akoachere et al., 2008; Mandri and Lin, 2006). Sequential degradation may be responsible for the observed synergy in this case as it has been reported that *Rhodococcus sp.* metabolized hydrocarbons of range $C_6 – C_{32}$ while *Pseudomonas sp.* metabolized $C_{10} – C_{16}$ (Beilen et al., 2002; Mohanty and Mukheriji.,2007). This implies that more substrate was made available for *Pseudomonas sp.* from the degradation of the higher carbon compounds by *Rhodococcus sp.*

pH

It was observed that the pH of all the cultures decreased while the control recorded no change (Fig.6). This may be due to the production of acidic metabolites in the medium (Moro et al., 2001). It was also observed that the higher the drop in pH the greater the degradation thus suggesting the production of more acidic metabolites.

Pattern of Degradation

It was observed from the result of the G.C analyses that the control (Fig.7) had a larger quantity of C_{21} to C_{25} carbon compounds and smaller quantities of C_{16} to C_{18} carbon compounds when compared to the pure cultures and mixed culture (Figs.8 to 10). This suggests that the organisms have been able to degrade some of the higher number carbon compounds to the lower carbon number compounds. Further observation of the G.C results also revealed that the mixed culture (Fig.10) had smaller quantities of higher number carbon compounds C_{25} to C_{28} than either of the pure cultures (Figs 8 and 9) and this indicates that the mixture of the two organisms gave better degradation of the hydrocarbon compounds than either of the pure culture.

CONCLUSION

Engine oil polluted environments such as Mechanic Workshops can be cleaned up effectively and efficiently using indigenous hydrocarbon utilizing microorganisms. The study isolated and characterized gram positive cocci, *Rhodococcus sp.* and gram negative bacilli, *Pseudomonas sp.* as good hydrocarbon degraders. The result showed that the co-culture of these isolates has the ability to degrade engine oil faster than the individual pure cultures,

hence, offering a more effective and efficient way of remediating engine oil polluted sites. The results indicate that by providing favourable environmental factors, a consortium of microorganisms can be used to remediate engine oil polluted environments such as a Mechanic workshop effectively.

The limitation of this work is that bioremediation takes a long time. Consequently bio-stimulation of the soil with additional nutrients or by bio-augmentation which involves adding beneficial microbes with affinity for a specific pollutant may be neccessary to hasten the process

ACKNOWLEDGEMENT

Azeez Adewale and Nwosu Aristole are acknowledged for their assistance in collection of samples and identification of species.

APPENDIX

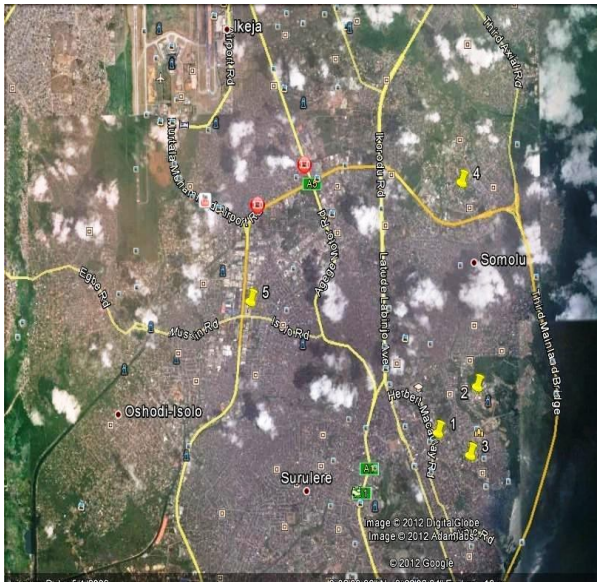

Fig 1 – Map of Lagos showing sample sites

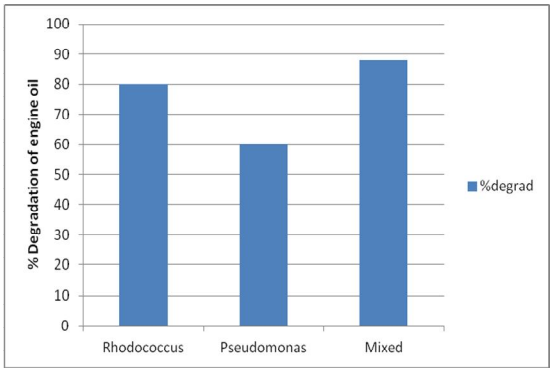

Fig.2: % Degradation of 1% Engine oil by the Organisms

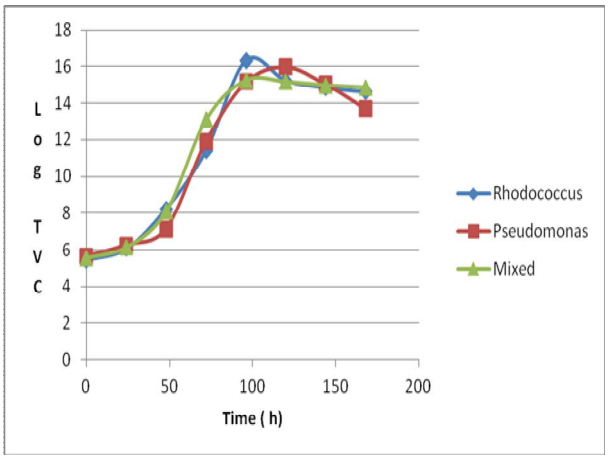

Fig. 3: Growth pattern of the microorganism in medium containing 0.5% oil concentration

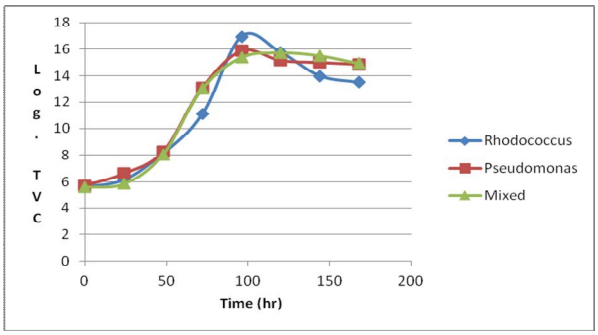

Fig. 4: Growth pattern of the microorganism in medium containing 1.0% oil concentration

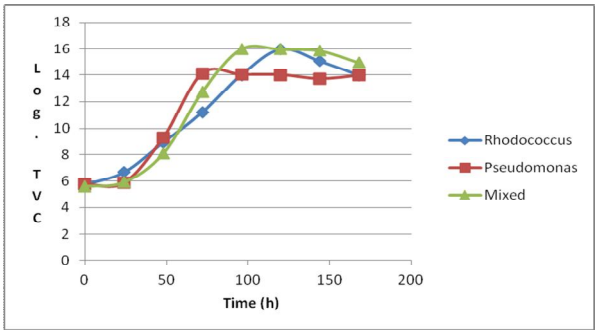

Fig. 5: Growth pattern of the microorganism in medium containing 1.5% oil concentration

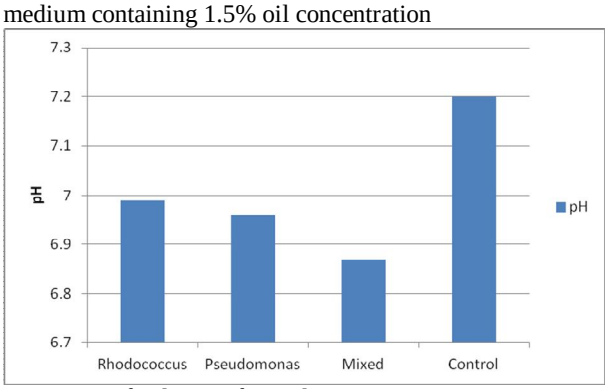

Fig. 6: pH of cultures after 7 days

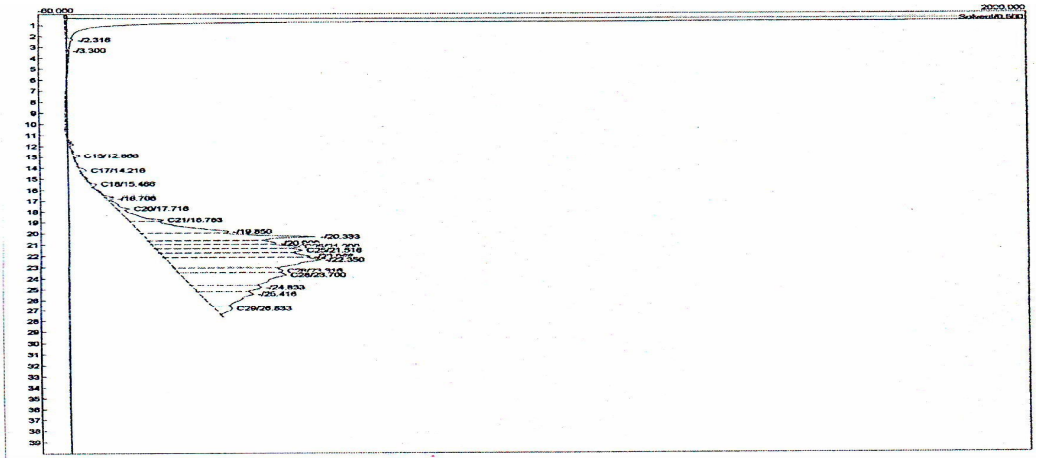

Fig. 7 Chromatogram of Control (day7**)**

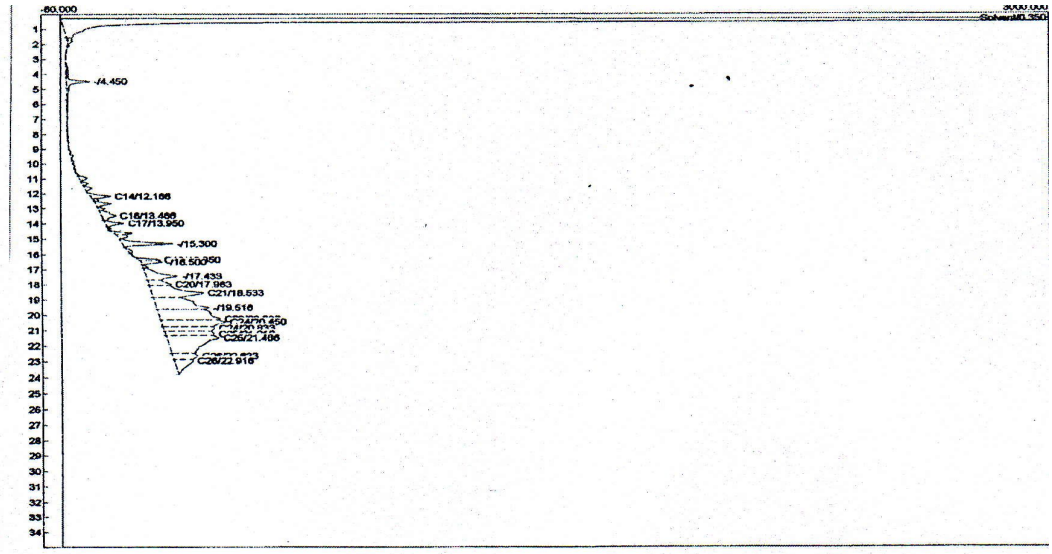

Fig. 8 Chromatogram of products of hydrocarbon degradation using *Pseudomonas sp*. (day7)

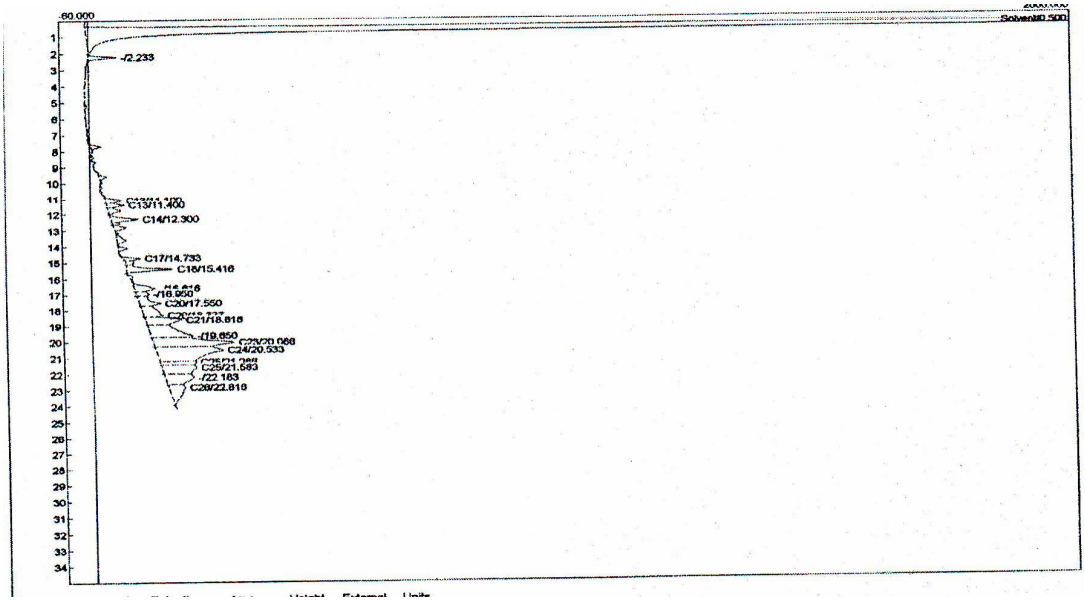

Fig. 9 Chromatogram of products of hydrocarbon degradation using *Rhodococcus sp*. (day7)

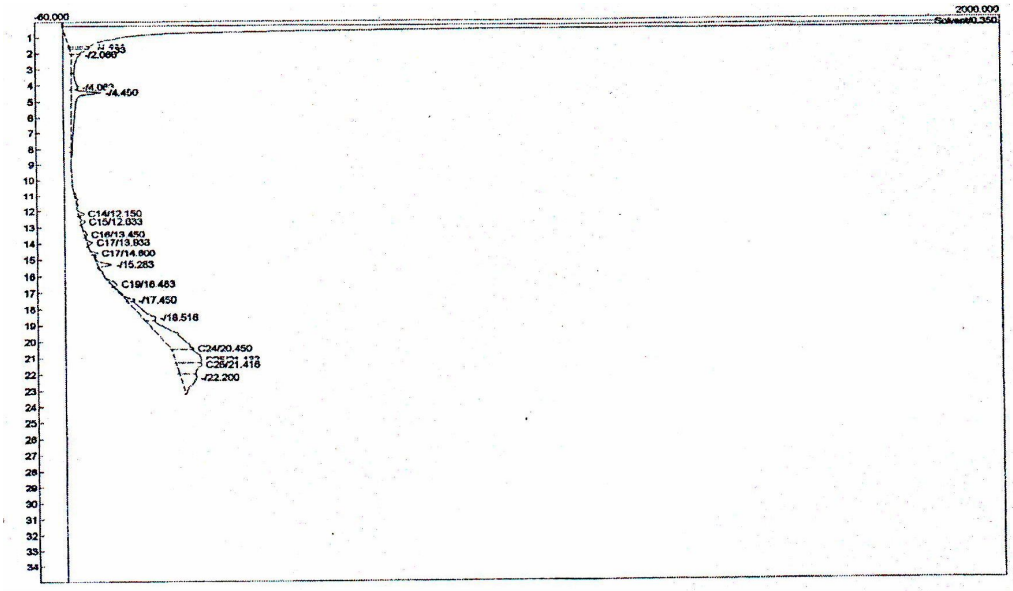

Fig. 10 Chromatogram of products of hydrocarbon degradation using mixed culture (day7)

REFERENCES

Akoachere, Jane-Frances T.K., Akenji, T.N., Yongabi, F.N., Nkwelang, G. and Ndip, R.N. (2008). Lubricating oil-degrading bacteria in soils from filling stations and auto-mechanic workshops in Buea, Cameroon: occurrence and characteristics of isolates. African Journal of Biotechnology. 7 (11): 1700-1706.

Allen, C.R., Boyd, D.R., Larkin, M.J., Reid, K.A., Sharma, N.D., Wilson, K. (1997). Metabolism of naphthalene, 1-naphthol, indene and indole by *Rhodococcus sp*. strain NCIMB 12038. Appl. Environ. Microbiol.63: 151-155.

Anal,C. and Mukherji, A. (2008) Treatment of hydrocarbon rich waste water using oil degrading bacteria and phototrophic microorganism in rotating biological contractor: Effect of N:P ratio. Journal of Hazardous Materials. 154 (1-3): 63-68.

Beilen, J.B., Smits, T.H.M., Whyte, L.G., Schorcht, S., Rothlisberger, M., Plaggemeier, T., Engesser, K.H. and Witholt, B. (2002). Alkane Hydroxylases in Gram-Positive Strains. Environ. Microbiol. 4: 676-682.

Bhattacharya, D., Sarma, P.M., Krishnan, S., Mishra, S., Lal, B. (2002). Evaluation of genetic diversity among *Pseudomonas citronellolis* strains isolated from oily sludge-contaminated sites. Appl. Environ. Microbiol. 69(3): 1435-1441.

Boonchan, S., Britz, M.L., Stanley, G.A. (2000). Degradation and Mineralisation of high-molecular weight polycyclic aromatic hydrocarbons by defined fungal-bacterial cocultures. Appl. Environ. Microbiol. 66(3): 1007-1019.

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

Folson, B.R., Schieche, D.R., DiGrazia, P.M., Werner, J. and Palmer, S. (1999). Microbial desulfurization of alkylated dibenzothiphenes from a hydrodesulfurized middle distillate by *Rhodococcus erythropolis* I-19. Appl. Environ. Microbiol. 65: 4967-4972.

Hagwell, I.S., Delfino, L.M. and Rao, J.J. (1992). Partitioning of Polycyclic Aromatic Hydrocarbons from oil into water. Environ. Sci. Technol. 26: 2104-2110.

Hamme, J.D. and Ward, O.P. (2000). Development of a method for the application of solid-phase microextraction to monitor the biodegradation of volatile hydrocarbons during bacterial growth on crude oil. J. Ind. Microbiol. Biotechnol. 25: 155-162.

Juhasz, A.L., Britz, M.L. and Stanley, G.A. (1997). Degradation of fluoranthene, pyrene, benz[a]anthracene and dibenz[a,h]anthracene by *Burkholderia cepacia*. J. Appl. Microbiol. 83: 189-199.

Keith, L.H. and Telliard, W.A. (1979). Priority pollutants 1 – a perspective view. Environ. Sci. Technol. 13: 416-423.

Koike, K., Ara, K., Adachi, S., Takigawa, H., Mori, H., Inoue, S., Kimura, Y. and Ito, S. (1999). Regiospecific internal desaturation of aliphatic compounds by a mutant Rhodococcus strain. Appl. Environ. Microbiol. 65: 5636-5638.

Lloyd-Jones, G. and Trudgill, P.W. (1989). The degradation of alicyclic hydrocarbons by a microbial consortium. Int. Biodeterioration and Biodegradation. 25: 305-315.

Mandri, T. and Lin, J. (2006). Isolation and characterization of engine oil degrading indigenous microorganism in Kwazulu-Natal, South Africa. African Journal of Biotechnology. 6(1): 023-027.

Mohanty, A. and Mukherji, S. (2007). Biodegradation rate of diesel range n-alkanes by bacterial cultures *Exiguobacterium aurantiacum* and *Burkholderia cepacia*. Int. Biodeterioration and Biodegradation. 61(3): 240-250.

Moro, D.D., Opere, B.O., Latunji, C.M., Nwakaudu, A. and Famurewa, O. (2001). Journal of Research and Review in Science. 2: 82-86.

Prenafeta-Boldu, X.F., Kuhn, A., Dmam, L., Anke, H., Van Groenestijin, J.W. and DeBont, J.A.M. (2001). Isolation and characterization of fungi growing on volatile aromatic hydrocarbon as their sole carbon and energy source. Mycological Res. 4: 477-484.

Springfellow, W.T. and Alvararez-Cohen, L. (1999). Evaluating the relationship between the sorption of Polycyclic Aromatic Hydrocarbons to bacterial biomass and biodegradation. Water Res. 33: 2535-2544.