African Journal of Biotechnology Vol. 5 (24), pp. 2476-2479, 18 December 2006
Available online at http://www.academicjournals.org/AJB
ISSN 1684–5315 © 2006 Academic Journals

Full Length Research Paper

Time-dependent stability of used engine oil degradation by cultures of *Pseudomonas fragi* and *Achromobacter aerogenes*

O. O. Adelowo[1]*, S. O. Alagbe[2] and A. A. Ayandele[1]

[1]Department of Pure and Applied Biology, LAUTECH, P.M.B 4000, Ogbomoso Nigeria.
[2]Department of Chemical Engineering, LAUTECH. P.M.B 4000, Ogbomoso Nigeria.

Accepted 10 November, 2006

***Pseudomonas fragi* and *Achromobacter aerogenes* isolated from used engine oil polluted soils were grown in minimal salts medium (MSM) supplemented with used engine oil as sole carbon and energy source to evaluate their ability to biodegrade used engine oil. The two organisms utilized 73.3 and 80.0% of the oil with a degradation rate of 0.073 and 0.08 ml/day respectively. The utilization rate of the mixed culture did not differ significantly with an 80.0% utilization and 0.08 ml/day degradation rate. However the rate of utilization was reduced significantly after repeated sub culturing of the organisms on nutrient agar for six months with percentage utilization dropping to 33.3, 26.7 and 30.0% respectively for *A. aerogenes, P. fragi* and the mixed culture. This suggests that the presence of hydrocarbons in the growth medium is necessary for the stability of hydrocarbon utilization potentials of the isolates.**

Key words: used engine oil, biodegradation, hydrocarbons, bacteria.

INTRODUCTION

Since the advent of oil exploration, the Nigeria environment has been heavily contaminated with hydrocarbon pollutants, which enters the environment through several route. The presence of these pollutants in the terrestrial and aquatic environments constitutes public health and socio-economic hazards (Edewor et al., 2004; Adelowo and Oloke, 2002; Okerentugba and Ezeronye, 2003). In Nigeria, about 20 million gallons of waste engine oil are generated annually from mechanic workshops and discharged carelessly into the environment (Faboya, 1997; Adegoroye, 1997), out of which only one liter is enough to contaminate one million gallons of freshwater (USEPA, 1996). Apart from this, used engine oil renders the environment unsightly and constitutes a potential threat to humans, animals and vegetation (ATSDR, 1997; Edewor et al., 2004). Several components of the oil, e.g. solvents and detergents added during the blending process, aliph atic hydrocarbon and PAHs distilled from crude oil, and metals from engine wear are either toxic in themselves or can combine with products of combustion to generate carcinogens and endocrine disrupters, (USEPA 1996, ATSDR 1997).

Biological degradation of petroleum hydrocarbon pollutants and petrochemicals by locally isolated bacteria strains have been extensively investigated in Nigeria (Oboirien et al., 2005; Ojumu et al., 2005; Okoh, 2003; Okerentugba and Ezeronye, 2003; Nweke and Okpowasili, 2003; Sanni and Ajisebutu, 2003; Okoh et al., 2003; Ijah and Akpera, 2002; Obire and Nwaubeta, 2001). However, reports on the degradation of used engine oil have not been widespread. Here we report the bio-degradation of used engine oil by locally isolated species of *Pseudomonas fragi* and *Achromobacter aerogenes*.

MATERIALS AND METHOD

Isolation

Contaminated soil samples were collected from mechanical workshops in Ogbomoso, Nigeria. 1 g each of the soil sample were suspended in 9 ml of sterile distilled water and 1 ml each of the suspension were then used to inoculate two sets of duplicate plates of minimal salts oil agar (MSOA) containing (g/l) KH_2PO_4 (4.74), K_2HPO_4 (0.5), $MgS0_4$. $7H_20$ (0.5), $CaCl_2$ (0.1), $FeSO_4$ (trace), Urea (0.5) and agar-agar (2%). Used engine oil sterilized by tyndalliza-

*Corresponding author's E-mail: onomewaleadelowo@yahoo.co.uk. Tel: +2348058044 384.

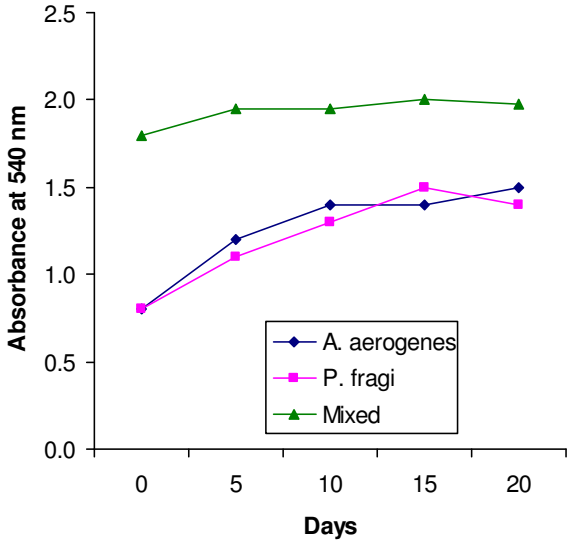

Figure 1. Absorbance measurements during growth of the cultures on used engine oil.

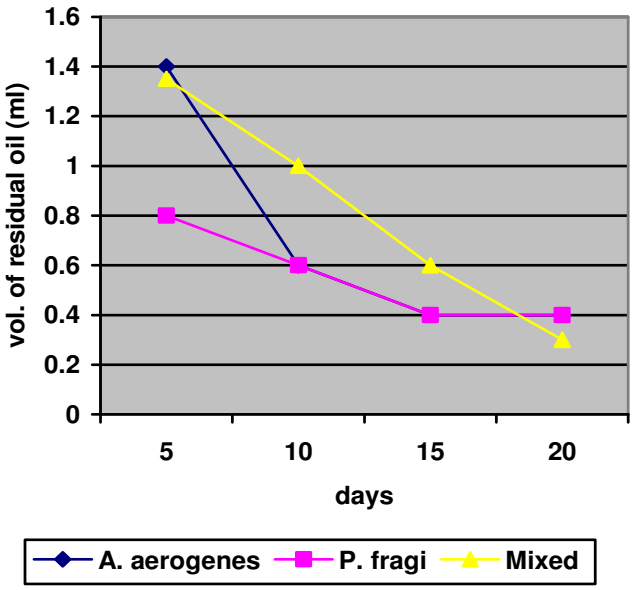

Figure 2 Volume of residual oil of the Fresh Cultures.

tion on surface of the MS0A as a carbon and energy source. One set each of the plates were incubated at 30 and 40°C until colony developed. Distinct colonies were then sub-cultured to obtain pure colonies which are then stored on slants for further studies.

Biodegradation

8.5 ml of MSM was dispensed into McCartney bottles and the volume made up to 10 ml with used engine oil as carbon and energy source. The whole content of the bottles were then sterilized by tyndallization. Eight (8) bottles each of the medium were inoculated with 0.5 ml of a suspension of *P. fragi, A. aerogenes* and a mixture of the two organisms in sterile distilled water. The initial absorbance of the inoculums were measured at 540 nm on a Jenway 6240 visible spectrophotometer and standardized at 0.8, 0.8 and 1.6 A° for *P. fragi, A. aerogenes* and the mixed culture. The inoculated bottles were then incubated at 30°C for 20 days. Controls were similarly set up except that the MSM – oil broth was not inoculated with any organism. The same procedure was repeated for another set of bottles except that the cultures were further supplemented with 1% glucose in addition to used engine oil. Sampling for residual oil was carried out on 0, 5, 10, 15 and 20 days by extracting two bottles each of experimental and control samples with an equal volume of chloroform. The chloroform was allowed to evaporate at room temperature and the volume of residual oil estimated by difference. The aqueous phase of duplicate bottles was similarly aspirated using a sterile syringe for spectrophotometer measurement at 540 nm.

The method of extraction was earlier validated by extracting 5 bottles of uninoculated MSM – oil broth with an equal volume of chloroform. The yield of residual oil was calculated as an average of five extractions to be 96.7%.

Time-dependent stability studies

The organisms were repeatedly sub-cultured on nutrient agar slants at monthly intervals for a period of six-months before being used to inoculate fresh MSM-oil medium as before. The residual oil was extracted and the volume estimated by difference. In addition, samples of residual oil from each treatment were then subjected to IR and UV visible spectroscopy (Figures 4, 5 and 6).

RESULTS AND DISCUSSION

From the soil samples were isolated thirteen different organisms which include three strains of *Pseudomonas fragi*, two strains each of *P. aeruginosa*, *P. nigrificans* and *P. fluorescens* and a strain each of *P. pellucidium, Bacillus licheniformis, P. putrefaciens* and *Achromobacter aerogenes*. The identities of the organisms were confirmed using biochemical tests according to Bergy's manual of determinative bacteriology (Buchannan and Gibbons, 1975). Among these organisms, *A. aerogenes* and one of the *P. fragi* strains showed particularly good growth on used engine oil as the sole carbon and energy source and were hence selected for further studies. Their outstanding utilization of used engine oil as sole carbon and energy source was evidenced by the increase in the absorbance of the culture medium (Figure 1) and the reducetion in the volume of the engine oil from 1.5 ml to 0.4 ml and 0.3 ml, respectively (Figure 2). This corresponds to 80 and 73.3% degradation respectively with a degradation rate of 0.08, 0.073 and 0.08 ml per day for *A. aerogenes, P. fragi* and the mixed culture of the two organisms.

During the period, an emulsification of the engine oil was observed in the culture broth of all the organisms which suggests that the production of extracellular biosurfactant may be one of the mechanisms used by the present isolates in the utilization of the used engine oil. Production of biosurfactant has been reported as one of

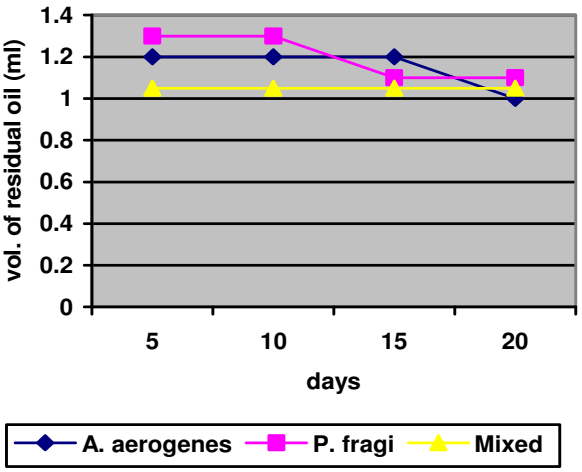

Figure 3 Volume of Residual Oil after Storage of Cultures for 6 months.

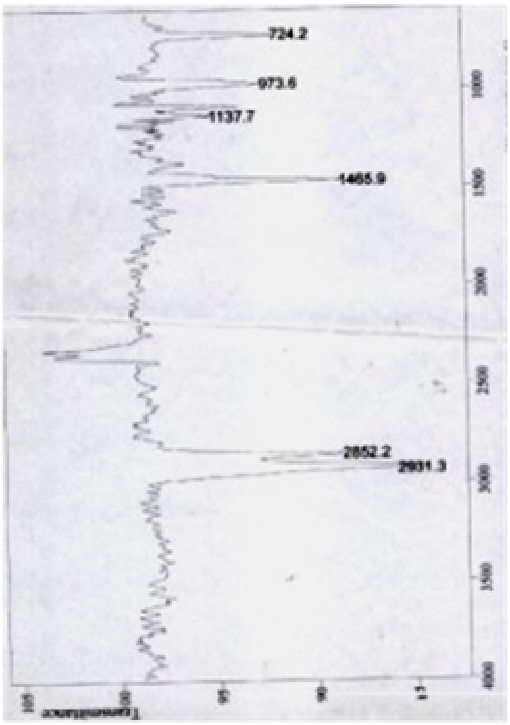

Figure 4. IR spectra of residual Oil from cultures of *A. Aerogenes*.

the mechanisms used by microorganisms in the uptake of hydrophobic substrates (Nweke and Okpowasili, 2003; Adelowo and Oloke, 2002). Addition of glucose (1%, v/v) to the culture medium has no significant effect on the rate of utilization of the used engine oil increasing the rate of degradation to 0.087 and 0.08 ml/day for *A. aerogenes*, and *P. fragi*, respectively while the rate of degradation for the mixed culture of the two organisms remains unchanged (data not shown). This is consistent with the observa-

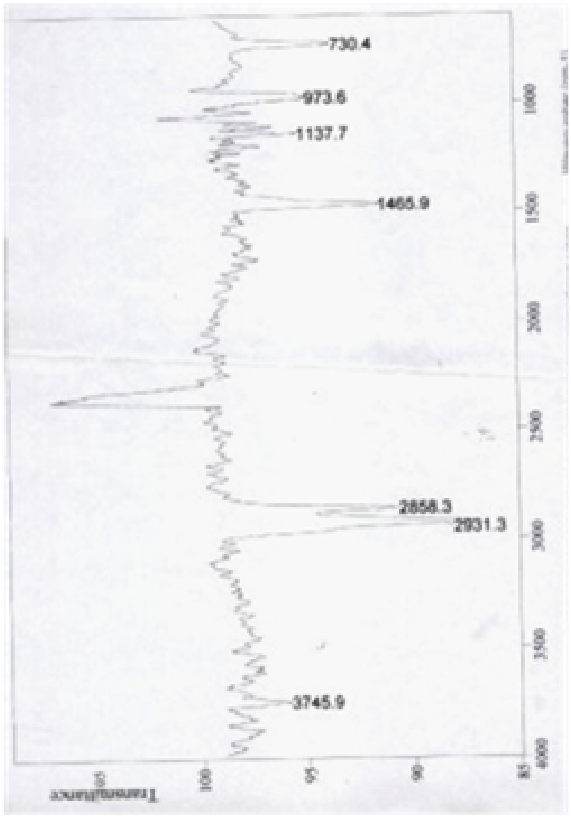

Figure 5. IR spectra of residual oil from the mixed culture.

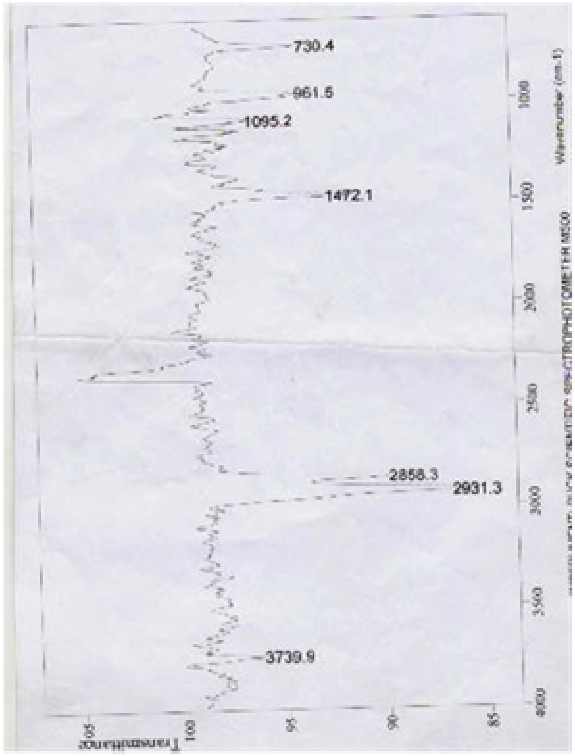

Figure 6. IR spectra of residual oil from *P. fragi*.

tion of Nweke and Okpokwasili (2003) who reported that addition of 1.6% (w/v) glucose to the culture medium have no significant effect on the growth rate of a marine *Staphylococcus* sp. growing on drilling fluid base oil. This observation suggest that the two organisms in this present study are strong primary utilizers of used engine oil and may have very strong potentials in bioremediation of ecosystems contaminated with used engine oil. However, storage of the culture on NA slants for six months reduced the quantity of hydrocarbon utilized significantly (Figure 3). There is a reduction in the quantity of oil utilized by the organisms from 1.2 to 0.5 ml for *A. aerogenes*, 1.1 to 0.4 ml for *P. fragi* and from 1.2 to 0.45 ml for the mixed culture. Several factors have been known to affect the degradation of petroleum hydrocarbon; these includes concentration of the hydrocarbon, addition of other carbon source (Nweke and Okpokwasili, 2003), addition of NaCl (Ijah and Akpera, 2002) viscosity (Amund and Adebiyi, 1991), and physiochemical factors (Atlas, 1981). Result of the stability study suggests that if the degradative capability of the present isolates will be sustained, the organisms must be maintained in a medium containing hydrocarbons.

Infra-red analysis and UV – visible spectroscopy of the control and residual oil reveals several absorption peaks and shifts in the maximum wavelength of absorption which confirms the modification of the original oil by microbial treatment (Figures 4 - 6). In the UV spectra, the maximum absorbance occurred for the control oil at 216.0 cm^{-1}, the maximum absorbance for the residual oil from the cultures of *A. aerogenes, P. fragi* and the mixture of the two organisms occurred at 230, 227 and 233 cm^{-1}, respectively. The spectra of the residual oils reveals absorption peaks at around 730, 970 and 1100 cm^{-1} indicating the presence of C-H bond due to aromatic compound, a C-H bond due to alkanes and at C-O bond due to ethers. There are other peaks at around 1470, 2850, 2931.3 and 3740 cm^{-1} showing the presence of a C-H bond due to saturated alkanes, a C-H stretching vibration due to saturated alkanes and an O-H stretching vibration due to phenols (Figures 4 - 6).

Although the performance of the isolates on the field is yet to be studied, the ability of the isolates in the present study to utilize the used engine oil as a sole carbon and energy source makes them potential organisms for consideration in the bioremediation of sites contaminated by used engine oil.

REFERENCES

Adelowo OO, Oloke JK (2002). Comparison of the Emulsification and Oil Displacement Profile of Some Biosurfactants. NISEB J. Vol. 2(3): 181-186.

Adegoroye G (1997). Environmental Considerations in Property Design, Urban Development and Renewal In. Akinjide O (Ed). Dimensions of Environmental Problems in Nigeria. Friedrich Ebert Foundation. pp: 12-25.

Atlas RM (1981). Biological Degradation of Petroleum Hydrocarbons: An Environmental Perspective. Microbiol. Rev. 45: 180-200.

ATSDR (1997). Public Health Statements : Used Mineral Based Crankcase Oil.

Amund OO, Adebiyi AG (1991). Effect of Viscosity on the Bio-Degradability of Automotive Lubricating Oils. Tribol. Int. 24(4).

Edewor TI, Adelowo OO, Afolabi TJ (2004). Preliminary Studies Into The Biological Activities Of A Broad Spectrum Disinfectant Formulated From Used Engine Oil. Poll. Res. 23(4): 581-586

Faboya OOP (1997). Industrial Pollution and Waste Management in Akinjide O (Ed). Dimensions of Environmental Problems in Nigeria. Friedrich Ebert Foundation. pp: 12-25.

Ijah UJJ, Akpera M (2002). Effect of Salinity on Crude Oil Utilization by Marine Yeast Isolates. J. Pure Appl. Sci. Vol. 3(2): 216-222.

Nweke CO, Okpokwasili GC (2003). Drilling Fluid Base Oil Bio-degradation Potential of a Soil *Staphylococcus* Species. Afr. J. Biotechnol. Vol. 2(9): 293-295.

Oboirien BO, Amigun B, Ojumu TV, Ogunkunle OA, Adetunji OA, Betiku E, Solomon BO (2005). Substrate Inhibition Kinetics of Phenol Degradation by *Pseudomonas aeruginosa* and *Pseudomonas fluorescens*. Biotechnol. 4(1): 56-61.

Obire O, Nwaubeta O (2001). Bio-degradation of Refined Petroleum Hydrocarbon In Soil. JASEM. Vol. 5(1): 43-46.

Ojumu TV, Bello OO, Sonibare JA, Solomon BO (2005). Evaluation Of Microbial Systems for Bioremediation of Petroleum Refinery Effluents in Nigeria. Afr. J. Biotechnol. Vol. 4(1): 31-35.

Okerentugba PO, Ezeronye OU (2003). Petroleum Degrading Potentials of Single and Mixed Microbial Cultures Isolated From Rivers And Refinery Effluents In Nigeria. Afr. J. Biotechnol. Vol. 2(9): 293-295.

Okoh AI (2003). Biodegradation of Bonny Light Crude Oil in Soil Microcosm By Some Bacterial Strains Isolated From Crude Oil Flow Stations Saver Pits In Nigeria. Afr. J. Biotechnol. Vol. 2(5): 104-108.

Okoh IA, Bakare MK, Ajisebutu SO (2002). Bio-Degradation of Bonny Light Crude Oil In Soil Microcosm By A Bacterial Consortium Isolated From Some Wastewater In Nigeria. Sci. Focus. Vol. 1: 32-27.

Sanni GO, Ajisebutu SO (2003). Bio-degradation of Escravos Light Crude Oil by Some Species of Soil Bacteria. Sci. Focus. Vol. 4: 87-95.

USEPA (1996) Recycling Used Oil: What Can You Do? Cooperation Extension Services ENRI-317: 1-2.