BIODEGRADATION POTENTIALS OF BACTERIA ISOLATED FROM ELEME PETROCHEMICAL INDUSTRIAL EFFLUENT

[1] Ajuzie, C.U., Atuanya, E.I. and Amaje, F.U.
Department of Microbiology, Faculty of Life Sciences,
University of Benin, Benin City, Edo State, Nigeria.

[1]Corresponding author: ajuziech@yahoo.co.uk.

ABSTRACT
The biodegradation potentials of microorganisms isolated from different sampling points of Eleme Petrochemical Industrial Effluent was investigated. Physicochemical analyses revealed that process waste water had concentrations of chemical oxygen demand (COD) (156 mg/L), biochemical oxygen demand (BOD) (400 mg/L), total dissolved solids (TDS) (25,000 mg/L), pH (11.11), nitrate (0.952 mg/L), dissolved oxygen (DO) (0 mg/L) and conductivity (7,320 ms/cm). Microbiological enumeration showed that the total heterotrophic bacteria count (THBC) from process waste water (PWW), clarified water (CW), retention pond gate (RPG) and receiving river (RR) ranged from 1.3 x 10^4 to 4.9 x 10^4 CFU/ml. Microbial counts for hydrocarbon utilizing bacteria (HUB) from PWW, CW, RPG and RR ranged from 0 to 2.7 x 10^4 CFU/ml. Characterization of the bacteria revealed ten (10) genera: *Enterobacter*, *Proteus*, *Bacillus*, *Salmonella*, *Citrobacter*, *Flavobacterium*, *Pseudomonas*, *Vibrio*, *Seirratia* and *Escherichia coli*. Efficiency of utilizing petrochemical industrial effluent under aerobic condition was demonstrated by *Pseudomonas* sp, *Serratia* sp. and *Bacillus* sp. Consortium of *Bacillus* sp., *Pseudomonas* sp, and *Serratia* sp, had the greatest efficiency for the utilizing petrochemical industrial effluent. Utilization of the hydrocarbons in petrochemical industrial effluent decreased reduction in percent of COD by *Pseudomonas* sp. (47.32), *Serratia* sp. (92.5) and the Consortium (65.38), with a decrease in the pH values of the cultures.

***Keywords**: petrochemical industrial effluent, consortium, biodegradation potentials, process waste water, physico-chemical, aerobic condition, clarified water.*

INTRODUCTION

Effluents are liquid discharges from homes, industries and establishments which vary in density, treatment stage and composition and bring about considerable effects to the discharge environments (water and soil) and their ecosystem. Petrochemical industries are involved in the conversion of petroleum (crude oil) and natural gas into derived chemical products known as petrochemicals (Matar and Hatch, 2007). Two petrochemical classes are olefins including ethylene and propylene and aromatics including benzene, toluene and xylene isomers (Splitz, 1998).

Oil refineries produce olefins and aromatics by fluid catalytic cracking of petroleum fractions whereas chemical plants produce olefins by steam cracking of natural gas liquids like ethane and propane. Aromatics are produced by catalytic reforming of naphtha. Olefins and aromatics are the building blocks for a wide range of materials such as solvents, detergents and adhesives. Olefins are the basis for polymers and oligomers used in plastics, resins, fibers, elastomers, lubricants and gels (Matar and Hatch, 2007). This of course makes the petrochemical industry a major player in the manufacturing sector. However, the effects of the activities of this unargueably economic boosting sector, which brings about an accompanying production and discharge of effluents into their immediate environments cannot be overemphasized. This is very evident in the Niger Delta region of Nigeria- a mainstay of the Nigerian economy and playing host to quite a number of upstream and downstream oil and gas industries which is otherwise known as a web of environmental hazards. A study of this area reveals to what extent industrial effluents have caused considerable damage to the water and soil environment thus, brining corresponding economic hard times to the people (Chikere and Okpokwasili, 2004). This ranges from toxification of drinking/domestic water sources, destruction of aquatic life (which brings about overall impedance in the people main economic activity-fishing), rendering waters unfit for any other activity as in the case of algal bloom, rendering farmlands unfit for agricultural practices and contamination of ground water (Israel *et al*., 2007)

Exposure to petrochemical industrial effluents discharge could also pose major health hazards. As a matter of fact, petrochemical waste waters consists of both organic (e.g. pesticide residues, solvents and cleaning fluids) and inorganic (brine salts and metals- Pb, Cu, Cr, Ni, As, etc) substances. Toxic metals associated with these effluents tend to occur in high doses in receiving water bodies and aquatic animals (e.g. fishes). Ingestion of Pb, Cu and Cr in high doses (>0.005 mg/L for Cr) results in intoxication and serious medical condition that can be life threatening. These include kidney damage, blood disorders, liver damage and neurological disorders (EPA, 1987).

Eleme Petrochemical Industrial Effluents take various forms which include: Processed waste water (PWW) which refers to water that is intended to come into contact with hydrocarbons or treated chemicals at the petrochemical plant, clarified water (CW) which is a combination of PWW and sewage, and final discharge water (FD) which is the effluent, which has undergone both chemical and biological treatment to eliminate or reduce waste contents to acceptable levels (Isreal *et al.,* 2007).

Studies have revealed that although effluents from Eleme Petrochemical Company Limited generally contain relatively low concentrations of pollutants in the water and sediment, accumulation of these pollutants over time can be fatal to aquatic and human life. Also, continued discharge of improperly treated effluent may further compound the environmental problems of communities living around this company. This therefore makes imperative, the need for an early resolution of the problem of treatment of Eleme Petrochemical effluent (Isreal *et al.,* 2007).

This dire need in recent times have found microorganisms quite instrumental. Microorganisms have been implicated in showing strong ability to biotreat petrochemical effluents. The potentials of microorganisms to catabolize and metabolize xenobiotic compounds has been recognized as a potentially effective means of toxic and hazardous wastes disposal (Kuehn *et al.,* 1995). Phenol and its derivatives have long been recognized as some of the most persistent chemicals in petroleum refinery/petrochemical waste wasters, with high toxicity even at low concentrations. Two species of *Pseudomonas*, *P. aeruginosa* and *P. fluorescence* have been studied for their biodegration potential on phenol present in a refinery wastewater under a batch fermentation process. Phenol was successfully degraded by both species and there was high positive correlation between phenol biodegradation and microbial growth (Ojumu *et al.,* 2004).

Biodegradation of these compounds have been recognized as a potential solution for their disposal owing to its cost effectiveness and simplicity. This study was therefore conducted to isolate and identify indigenous bacteria present in Eleme Petrochemical industrial effluent and ascertain its potentials to degrade process waste water (PWW) of the petrochemical industrial effluent.

MATERIALS AND METHODS

Study Site

The study site was Eleme Petrochemical Plant located in the oil rich Niger Delta in Port Harcourt, Rivers State, Nigeria which generates large quantities of effluent daily.

Sample Collection

Effluent samples were collected from the process waste water (PWW), clarified water (CW), retention pond gate (RPG) and receiving river (RR) of Eleme Petrochemical Company Limited (EPCL), Port Harcourt, Rivers State. Samples were collected using sterile, air tight plastic containers properly labeled. They were then transported in ice packed coolers to the laboratory and those that could not be analyzed immediately were stored in the refrigerator at a temperature of 4^0c.

Enumeration, Isolation, characterization and identification of petrochemical industrial effluent utilizing bacteria

The total heterotrophic bacteria counts of the effluent samples were performed in triplicates by plating about 0.1ml of the effluent samples in nutrient agar plates using the spread plate technique as described by Pelezar *et al*. (2001). Plates were enumerated after 24 hrs of incubation. The isolation of petrochemical industrial effluent utilizing bacteria were performed in triplicates of about 0.1ml of effluent samples on modified mineral salts medium of Marquez-Rocha *et al*. (2001) to which 1% (50 ml) of petrochemical industrial effluent was added using the spread plate techniques.

The mineral salts medium had in litres; Mg, So_4 $7H_20$, 0.12 g/L; KCL, 0.30g/L; kH_2P0_4, 0.8g/Ll; K_2 $HP0_2$, 1.3g/L, $NaNO_3$; 0.42g/L; pH 7.4; Agar, 15g/L. The medium was formulated by dissolving the basal component in 750 ml of distilled water and sterilized. The phosphate components were dissolved in 200 ml of distilled water, sterilized and allowed to cool and added to the basal component. Enumeration was done after incubation of plates at room temperature for 7 days. Pure stock cultures of petrochemical industrial effluent utilizing bacteria were identified and characterized after being subcultured in nutrient agar using the criteria in Krieg and Holt (1994).

Standard cultures of text bacteria isolates

Standard cultures were prepared for the isolates according to the methods of Seely and Van-Denmark (1981). One hundred milliliters of nutrient broth were dispensed into each of the five different conical flasks and inoculated with each isolates from different conical flasks and inoculated with each isolates from pure culture stock using a sterilized loop and then incubated at 28 ± 2^0c for 24 hours. After incubation, 0.1ml of each broth were cultured onto nutrient agar plates and the values obtained were expressed as standard numbers of cells present in 1ml of the broth.

Screening test of abilities of isolates to utilize effluent

The method employed was adopted from Okpokwasili and Okorie (1988). The mineral salts medium was contained in 9.9 ml amounts in two sets of test tubes. To one set of tubes was added 0.1 ml each of petrochemical industrial effluent. The other set of tubes contained no petrochemical industrial effluent. After capping, all the test tubes were sterilized by autoclaving at 121^0c for 15 minutes, after which they were allowed to cool. On cooling, each set of tubes was inoculated with two drops of cells suspension of an isolate in sterile mineral salt broth. The cell suspension was prepared by suspending a loopfull of bacteria isolates from nutrient agar plates into 2 ml of mineral salt solution. A set of control test tubes remained uninoculated. All test tubes were incubated at 28 ± 2^0c for 14 days, after which each tube was scored for turbidity.

Shake Flask Degradation Test

Pure isolates from stock cultures were first revived by inoculating in nutrient broth and then incubated at 37^0c for 24 hours. Pure isolates of *Pseudomonas* sp., *Serratia* sp. and *Bacillius* sp., were selected because they showed high capacity to utilize the effluent. Ten percent (10%) effluent concentration of mineral salts medium was prepared by mixing 100 ml of effluent to 900 ml of mineral salts medium. This was then autoclaved at 121^0c for 15 minutes. Two milliliter (2 ml) each of the 3 pure isolates (in broth culture) were then dispensed into 3 flasks containing the medium. A fourth flask containing the medium was used for the consortium in which 1ml each of the 3 bacterial isolates was dispensed and were all properly mixed. Serial dilution was then performed for each of the inoculated media and 0.1ml of the dilutions were cultured on nutrient agar and incubated at 37^0c for 24 hours for the total viable count.

All the capped flasks were then conveyed into a rotary shaker (Stuart Orbital Incubator/5150). Samples were then analyzed every 4 days for 20 days starting with the 1^{st} day which served as control (i.e. 0 hour). The parameters analyzed include chemical oxygen demand (COD), biochemical oxygen demand (BOD), dissolved oxygen (DO), pH, turbidity, total dissolved solids (TDS), nitrate and conductivity.

Physiochemical analysis of effluent samples

Some physicochemical parameters of the effluent samples were determined before treatment with bacteria and after treatment.

they include pH, biochemical oxygen demand (BOD), chemical oxygen demand (COD), dissolved oxygen (DO), turbidity, nitrate and conductivity. All analyses were in accordance with standard methods for the examination of water and waste water. pH was read using Equiptronics digital pH meter mode EQ-610 at 30^0c. Conductivity was determined using a conductivity meter after meter had been calibrated and stabilized at $0.0mScm^{-1}$. Total dissolved solids (TDS) was determined using a weigh balance Setra BL-4105. Chemical oxygen demand (COD) was determined by the Open reflux method of (APHA, 1989). Nitrate level was determined using phenol-disulphoric acid method and absorbance was read at 4.15nm. Genesys 20 thermospectronic spectrophotometer was used for turbidity reading.

RESULTS AND DISCUSSION

The results of the analyses of the physicochemical parameters of the process waste water (untreated) petrochemical industrial effluent are shown on table 1. Analysis of raw effluent (i.e. process waste water) revealed a very high concentration of common environmental toxicants and toxicity indicating parameters. This is evident in the results of physicochemical parameters: COD (156mg/L), BOD (400mg/L), DO (0mg/L), pH (11.11), Nitrate (0.952mg/L), Total Dissolved Solids (TDS) (25,000mg/L) and low conductivity (7.320) which are all above the guidelines by FEPA (1991). These parameters are high because the effluent had not undergone any form of treatment whether chemical or biological. This implies that discharging these effluents (which physicochemical parameters are quite high compared to FEPA standards (FEPA, 1991)) to recipient environment will over time produce accumulated effects that pose great threats to overall ecological balance hence, the need for treatment.

Table 1: Physicochemical parameters of process waste water (untreated)

Parameter	Value	FEPA Limitation 1991 (mg/l)	Effluent guideline
pH	11.11	6-9 (no unit)	
COD	156 mg/L	40	
DO	Nil	20	
BOD	400 mg/L	10	
Nitrate	0.952 mg/L	1.0	
TDS	25,000 mg/L	2000	
Turbidity	938	-	
Conductivity	7,320μS/cm	-	

Table 2: Mean bacteria counts of effluent from various sampling points and major genera bacterial isolates of eleme petrochemical industry.

Sample	THBC (cfu/ml) x 10^4	HUB (cfu/ml) x 10^4	Bacterial isolates
Clarified water (CW)	9.0	1.05	*Bacillus, salmonella, pseudomonas, citrobacter*
Process waste water (PWW)	1.3	1.5	*Proteus, Bacillus, Flavobacterium, vibrio, Serratia*
Retention pond gate (RPG)	4.9	2.7	*Bacillus, Proteus*
Receiving river (RR)	9.0	-	*Bacillus, Escherichia coli, Enterobacter.*

THBC = Total heterogeneous bacteria
HUB = Hydrocarbon utilizing bacteria

Table 2 revealed the total heterotrophic bacteria and hydrocarbon utilizing mean bacteria counts present in the various effluent samples analyzed as well as the major bacterial utilizing isolates. It was observed that retention pond gate (RPG) contained the highest number of total heterogeneous bacterial counts (4.9 x 10^4 CFU/ml) and hydrocarbon (HC) utilizing bacterial count (2.7 x 10^4CFU/ml).

This implies that the effluent was very prone to microbial degradation as they did harbor organisms that showed great potentials in utilizing hydrocarbon. The high counts recorded in RPG for the presence of indigenous microbes is not a direct indication that the indigenous microbes primarily utilize the HC present in the effluent as the carbon and energy sources. They may rather be utilizers of the HC as their secondary carbon and energy sources. This aligns with the report of Okpokwasili and Okorie (1988).

Isolation, characterization and identification of indigenous bacteria found in petrochemical effluent revealed 10 bacteria genera as shown in (table 2). These bacteria include *Enterobacter* sp., *Proteus* sp., *Bacillus* sp., *Serratia* sp. *Citrobacter* sp., *Flavobacterium* sp., *Pseudomonas* sp., *Vibrio* sp., and *E. coli*. It was observed that 44.4% of isolated bacteria were Gram positive while 55.5% were Gram negative. This observation is very well in consonance with the report of Calomaries *et al.* (1986) and Fought and Westlake (1987), who reported that most of the bacterial flora of hydrocarbon sources was Gram negative.

Table 3: screening test for utilization of petrochemical industrial effluent and Total viable count of isolates from effluent treated with bacteria over a 20-day period (CFU/ml) x 10^4

Organisms/DAY	0	4	8	12	16	20	Turbidity
Pseudomonas sp.	2.92	7.15	8.30	2.14	2.14	6.90	-
Serratia sp.	4.80	2.08	7.60	1.18	1.80	1.14	++
Bacillus sp.	3.40	5.60	1.39	4.80	4.80	4.40	+++
Consortium	1.49	4.85	1.26	5.60	5.60	4.60	-
Control							-

***Key**: +++ = Heavy growth ++ = moderate growth + =little growth - = No growth*

***Note**: Consortium consists a mixture of Pseudomonas, Serratia and Bacillus sp.*

Results of table 3 shows that the highest TVC was recorded in *Serratia* sp (on day 20). The gradual appreciation of the TVC for *Serratia* sp., starting from day 0 to day 20 indicates that not only did *Serratia* species show great potentials for biodegradation, they also primarily utilized the effluent HCs for growth and metabolism. Appreciation in the growth trends was also noticed in *Pseudomonas* sp., *Bacillus* sp. and the Consortium (*Pseudomonas* sp., *Bacillus* sp., and *Serratia* sp.), thus, the degradation process of the effluent by the various bacterial isolates and the Consortium can be said to move concurrently with the growth of the organisms, establishing a direct proportion between the rate of effluent biodegradation and bacterial growth. This is in consonance with reports of Thomas *et al.* (2002) and Collins and Daugulis (1997).

Three relatively abundant bacteria in petrochemical effluent were screened for their ability to utilize the HC present in petrochemical effluents as their carbon and energy sources. Results revealed that *Serratia* sp. showed the highest turbidity followed by *Pseudomonas* sp. and *Bacillus.* Suggesting that *Serratia* sp. was the most efficient utilizers of the effluent to derive carbon and energy (table 3).

The observed COD and DO reduction levels as seen in (table 4) further proved the efficiency of bacteria in biodegradation process. The reduction noticed in the nitrate value (table 5) by *Pseudomonas* sp., *Serratia* sp., and consortium shows the capability of *Pseudomonas* sp. and other bacteria to utilize different nitrogen sources including in an oil degradation assay. This is in agreement with Mandri and Lin (2007). The COD reduction levels were observed for bacterial isolates as shown in (Table 6) Viz: *Pseudomonas* 47.32%, *Serratia* 92.5% and consortium of *Pseudomonas, Serratia* and *Bacillus* recorded 65.38% reduction in BOD. Similar outcome was obtained for the reduction levels in BOD; *Pseudomonas* 70%, *Serratia* 86.7% and consortium 85.7%. COD showed highest reduction on 12th day by both Consortium and *Serratia* sp (table 6). These percentage changes were derived from the initial results of effluent analysis before treatment with bacteria and after treatment over a period of 20 days as shown in (Table 6). *Bacillus* sp. showed less degradative abilities as this could be attributed to formation of spores thereby making it a slow degrader as evidenced in the percentage (%) changes in (Table 6) with changes occurring on the 12th and 16th day of observation.

Table 4: Chemical oxygen demand (COD) and dissolved oxygen values of effluent treated with bacteria over a 20-day period (mg/L)

Organisms/DAY	0	4	8	12	16	20
Pseudomonas sp.	224 (0.80)	272 (0.80)	280 (4.00)	118 (0.20)	- (-)	- (-)
Serratia sp.	240 (-)	108 (3.20)	140 (-)	208 (2.80)	18 (4.40)	- (-)
Bacillus sp.	34 (1.12)	12 (2.40)	- (2.00)	212 (4.80)	156 (5.60)	- (-)
Consortium	208 (1.60)	280 (0.80)	270 (4.80)	72 (0.48)	216 (2.40)	202 (2.40)

***Key:** Dissolved oxygen (DO) = values in parenthesis*

Table 5: Biochemical oxygen demand (BOD_5) and nitrate values of effluent treated with bacteria over a 20-day period (mg/L)

Organisms/DAY	0	4	8	12	16	20
Pseudomonas sp.	- (5.78))	480 (6.40)	320 (5.79)	144 (5.63)	144 (-)	- (-)
Serratia sp.	600 (6.27)	480 (5.79)	240 (5.07)	360 (5.69)	360 (5.87)	- (-)
Bacillus sp.	40 (4.90)	160 (5.50)	160 (5.91)	160 (6.02)	- (5.62)	- (-)
Consortium	- (5.48)	560 (6.31)	160 (5.89)	160 (5.22)	80 (5.63)	160 (5.69)

***Key**: Nitrate (NO_3) = values in parenthesis*

Table 6: Percentage (%) changes in the physicochemical properties of petrochemical effluent treated with bacteria over a 20-day period.

Parameterse /Organism	*Pseudomonas* sp. (%)	*Serratia* sp. (%)	*Bacillus* sp (%)	Consortium (%)
COD	47.32	92.5	26.42*	65.38
BOD	70	86.7	-	85.7
DO	80	12.5	-	70
NO_3	1.37	19.1	6.57*	4.65

** represent % changes noticed in Bacillus sp. between the 12th and 16th day.*

From study, it was observed that best degradation occurred in the consortium of the three bacteria isolates (*Pseudomonas* sp., *Serratia* sp. and *Bacillus* sp.). The advantages of employing mixed cultures than pure cultures in remediation processes have been demonstrated by Benker-Coker and Ekundayo (1997); Alden *et al.* (2001) and Atuanya and Purohit (2001). Enhanced degradation in this study can be attributed to the fact that some microorganisms act as primary utilizers, utilizing organic carbon or substrate molecules while others act as secondary utilizers utilizing the breakdown products of effluent substrates after initial attack by primary utilizers (Okpokwasili and Okorie, 1988).

CONCLUSION

This study has shown that indigenous microorganisms present in petrochemical effluent are able to biodegrade some of the effluent components with an accompanying increase in number/viable counts (growth). It becomes very vital that further studies are initiated in a bid to exploring newer, more effective, less costly and better satisfactory ways of petrochemical effluent management.

REFERENCES

Alden, L., Demoling, F. and Batth, E. (2001). Rapid method of determining factors limiting bacterial growth in soil. *Applied Environmental Microbiology.* **67**:1830-1838.

APHA, (1989). *Standard Methods for the Examination of Water and Wastewater* 17th Ed. America Public Health Association. Washington D.C. 215pp.

Atuanya, E.I. and Purohit, H.J. (2001). Bacteria consortium for polycyclic aromatic hydrocarbon (PAH) mixture remediation. *Tropical Journal of Science and Health.* 4(**1**): 20-27.

Benka-Coker, M.O. and Ekundayo, J.A. (1997). Applicability of evaluating the ability of microbes isolated from an oil spill site to degrade oil. *Environmental Monitoring and Assessment.* **45**: 259-272.

Calomaries, J.J., Austin, B., Walker, J.D. and Colwell, R.R. (1986). Enrichment for estuaries petroleum degrading bacteria using liquid and solid media. *Journal of Applied Bacteriology.* **42:** 135-144.

Chikere, B.O., and Okpokwashi, G.C. (2004). Frequency of occurrence of microorganisms at petrochemical effluent outfall. *Journal of Tropical Biosciences* **4**: 12-18.

Collins, L. D. and Daugulis, A. J. (1997). Biodegradation of Phenol at High Initial Concentration in Two-Phase Partitioning Batch and Fed-batch Bioreactors. *Biotechnology Bioengineering* **55**: 155-162.

Environmental Protection Agency (EPA) (1987). Soil Microbial Community Toxicity Test. EPAOCER Part 797 3700. Toxic Substances Control Act Test Guideline Proposed Rule 50pp.

Federal Environmental Protection Agency (FEPA) (1991). *National Environmental Protection (Effluent Limitation) Regulation. (5.1.8).* FEPA, FGL, Lagos, Nigeria.

Fought, J.M. and Westlake, D.W.S. (1987). Biodegradation of Hydrocarbons in Fresh Waters *In: Oil in Freshwater Chemistry, Biology Counter Measure Technology* (Vandermeulen, J.A and Hradey, S.E. Eds.) Perpamon Press, NewYork. pp. 252-263.

Isreal, A.U., Obot, I.B., Umoren, A.U., Mkpenie, V. and Ebong, G.A. (2007). Effluents and Solid Waste Analysis in a Petrochemical Company- A case Study of Eleme Petrochemical Company Ltd., Port Harcourt, Nigeria. *Electronic Journal of Chemistry*. **5 (1)**: 74-80.

Krieg, W.R. and Holt, J.G. (1994). *Bergey's Manuals of Systematic Bacteriology.* William and Wilkins Baltimore Ltd, New York, U.S.A. 101pp.

Kuehn, R. L., Berlin, K.D., Hawkins, W.E. and Ostrander, G.K. (1995). Relationships among petroleum refining, water and sediment contamination, and fish health. *Journal of Toxicology and Environmental Health.* **46**: 101-116.

Mandri, T. and Lin, Y. (2007). Isolation and characterization of engine oil degrading indigenous microorganisms in Kwazulu-Natal, South Africa. *African Journal of Biotechnology.* **6**: 23-27.

Marquez-Rocha, F.J., Hernandez-Rodriguez, V. and Tamela, T.M.A. (2001). Biodegradation of Diesel Oil in Soil by a Microbial Consortium. *Water, Air and Soil Pollution.* **128:** 313-320.

Matar, S. and Hatch, L.F. (2001). *Chemistry of Petrochemical Processes* (2nd ed.). Gulf Professional Publishing, USA, 375pp.

Ojumu, T.V., Bello, O.O., Sonibare, J.A. and Solomon, B.O. (2005). Evaluation of microbial systems for the bioremediation of petroleum refinery effluents in Nigeria. *African Journal of Biotechnology*. **4 (1)**: 31 – 35.

Okpokwasili, G.C. and Okorie, B.B. (1988). Biodegradation potentials of microorganisms isolated from car-engine lubricating oil. *Tribology International.* **24:** (4) 215-220.

Petezar, M.I., Chan, E.C.S. and Krieg, I. (2001). *Microbiology: Applications and Practice* 6th ed. McGraw-Hill Inc. London. 518pp.

Seely, H.W. and Van-Denmark, P.J. (1981). Determination of microbial numbers in microbes in action. *A Laboratory Manual for Microbiology* 3rd Ed. Freeman W.H. Publishing Company U.S.A 53pp.

Spitz, H. (1998). *Petrochemical: The rise of an industry.* Wiley Interscience, New York. 588pp.