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Full Length Research Paper

Effect Of varying concentration of sodium nitrate on biological oxidation of petroleum hydrocarbon polluted water

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The bioremediation of petroleum hydrocarbon polluted water using *Aspergillus niger* and varying concentrations of sodium nitrate was investigated in this study. Five samples of petroleum hydrocarbon polluted water innoculated with *A. niger* containing 0.05, 0.1, 0.2, 0.3 and 0.4 M $NaNO_3$(aq) and the control was monitored for parameters such as Total Hydrocarbon Content (THC), Biological Oxygen Demand (BOD), pH and Turbidity over a period of forty nine days. The results obtained showed that the sample remediated with 0.4M $NaNO_3$ (aq) showed the greatest THC drop of 97.5% followed by 0.3M $NaNO_3$ (aq) with 97.1% drop, 0.2M $NaNO_3$ (aq) with 96.5% drop 0.1 M $NaNO_3$ (aq) with 95.8% and finally 0.05M $NaNO_3$ (aq) with 95.1% drop. The sample amended with 0.4M $NaNO_3$ (aq) showed the greatest BOD drop of 98.8% followed by 0.3 M $NaNO_3$ (aq) with drop of 98.5% then followed by 0.2 M $NaNO_3$ (aq) with drop of 98.4% then followed by 0.1M $NaNO_3$ (aq) with 98.3% drop. All four samples above fall within stipulated values of 30 mg/l by regulatory agencies like FEPA and DPR during the experimentation period. Bioremediation as a strategy for clean up of petroleum hydrocarbon polluted water has been shown to be efficient considering the level of drop in THC, pH, BOD and Turbidity which fall within the FEPA and DPR limits over the period of study. *A. niger* offer an efficient and interesting possibility of degrading petroleum hydrocarbon polluted water. 0.2, 0.3 and 0.4 M $NaNO_3$ (aq) are efficient in stimulating *A. niger*. Scale up of this bioremediation strategy is very promising and should be encouraged.

Key words: Bioremediation, crude oil polluted water, sodium nitrate, biochemical oxygen demand, total hydrocarbon content.

INTRODUCTION

Petroleum like all fossil fuels primarily consists of a complex mixture of molecules called hydrocarbons. In large concentrations, the hydrocarbon molecules that make up crude oil and petroleum products are highly toxic to many organisms, including humans (Alexander, 1994). The dominance of petroleum products in world economy creates the conditions for distributing large amount of these toxins into populated areas and ecosystems around the globe (Ojumu et al, 2004).

Petroleum is also the raw material for many chemical products such as plastics, paints and cosmetics. The transport of petroleum across the world is frequent and the amounts of petroleum stocks in developed countries are enormous. Consequently, the potential for oil spills is significant. The volume of spills is significant. The volume of spills usually exceeds the inherent remediation capacity for any given environment, resulting in a significant ecological impact (Yehuda, 2002).

The most rational way of decontamination of the environment loaded with petroleum derivatives is an application of methods based mainly on metabolic activity of microorganisms (Leahy and Colwell, 1990). Microbial degradation is the major mechanisms for the elimination of spilled oil from the environment (Colwell and Walker, 1977; Ibe and Ibe, 1984; Atlas, 1995). The ability to actively decompose specified fractions of petroleum oil is expressed by many microorganisms (Bartha and Atlas,

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Nomeclature: THC; Total hydrocarbon content, **BOD;** biological oxygen demand, **FEPA;** federal environmental protection agency, **DPR-** department of petroleum resources.

1977).

Crude oil is a complex but biodegradable mixture of hydrocarbons and the observation that hydrocarbon degrades can be enriched in many, if not most types of environments (Atlas, 1981) have contributed to the development of oil bioremediation techniques (Margesin and Schinner 1997). Although the optimum temperature for biodegradation of petroleum products has generally been found to be in the range of 20 - 30°C (Atlas and Bartha 1992). Local environmental conditions may select for a population with a varying optimum temperature.

Bioremediation strategy can be as simple as applying a garden fertilizer to an oil-contaminated beach or as complex as an Engineered treatment "cell" where soils or other media are manipulated aerated; heated or treated with various chemical compounds to promote degradation (Hildebrandt and Wilson 1991). The bioremediation strategy of choice ultimately will depend on the peculiarity of the contaminated site. Many published articles have documented the potentials of microorganisms to degrade oil both in the laboratory and in field trials. Bioremediation which is defined as any process that uses micro- organism or their enzymes to return the environments to its original condition is an attractive process due to its cost effectiveness and the benefit of pollutant mineralized to CO_2 and H_2O (Obahiagbon and Owabor,2008). It also provides highly efficient and environmentally safe clean up tools (Margesin, 2000).This technology accelerates the naturally occurring biodegradation under optimized conditions such as oxygen supply, temperature, pH, the presence or addition of suitable microbial population (bio argumentation) and nutrients (biostimulation), water content and mixing (Trindade et al., 2005).

The major goal of bioremediation is to biodegrade the organic pollutants to concentration that are either undetected or if detected, to concentration below the limits established as safe or acceptable by regulating agencies

MATERIALS AND METHODS

Sample collection

The crude oil (Escravoes light) used for this study was obtained from an Oil Producing Company located in the Niger Delta region of Nigeria.

Sample preparation

The Crude oil polluted water was made by adding 100 ml of Escravoes light to 1000 ml of water. This crude oil polluted water of ratio 1:10 was then stored in black plastic containers until required. Before the experiment was started the crude oil polluted water was allowed to stand for one week allowing the indigenous microbes to grow and accustom to the medium.

ISOLATION AND ENUMERATION OF FUNGAL CULTURE

Fungal culture

The fungi were isolated from the water samples using Czapek Dox agar unto which sterile streptomycin (50 mg ml^{-1}) had been added to suppress bacteria growth. Pure cultures of the fungal isolates were made and transfered unto Czapek Dox agar slants as stock cultures. The microscopic and macroscopic features of the hyphal mass. morphology of cells and spores and nature of the fruiting bodies were used for identification

TOTAL HYDROCARBON CONTENT (THC)

Procedure

The oil content of the water was determined by shaking 5 g of a representative waster sample with 10 ml of toluene or carbon tetrachloride and the oil extracted was determined by the absorbance of the extract at 450 nm in a spectronic 70 spectrophotometer.

The wastewater extract ratio was varied where necessary depending on the concentration of oil in the water. In some cases where oil content was very high, it was found more accurate to dilute the extract before reading from the spectrophotometer than reducing the weight of water sample extractant.

A standard curve of the absorbance of different known concentrations of oil in extractant was first drawn; after taking readings from the spectrophotometer, oil concentrations, in the water sample were then calculated after reading the concentration of the oil in the extract from the standard curve. With reference to the standard curve and multiplication by the dilution factor, the oil concentration was calculated.

pH Measurement

Apparatus

(a) An electronic pH meter (Fisher Accruement pH meter mode 320) with temperature compensation adjustment, loss electrode and reference electrode.
(b) Buffer Solutions
(c) Sample
(d) Stirrers
(e) Thermometer

Procedure

The temperatures of the buffer solutions and sample were taken using a thermometer and the temperature is manually compensated for in the meter. The electrode system of the pH was then calibrated. After calibration, the sample was thoroughly mixed together using a stirrer and its pH was taken.

The pH value obtained was then recorded. Based on the efficiency of the electrodes, the corrected value was then recorded

Biochemical Oxygen Demand (BOD)

Reagents used: Winkler's solution A, Winkler's solution B, starch solution

Two 250 ml reagent bottles were filled up completely with the sample and stoppered tightly. To one of the bottles, 1.5 ml each of Winkler's Solution A and B were added, and precipitant was formed. The precipitant was dissolved with 2 ml of concentrated hydrochloric or sulphuric acid to form a golden brown solution. 100 ml of the resulting solution was poured into 250 ml flask and 3 drops of starch indicator were added and titrated against 0.1 N Sodium thiosulphate ($Na_2 S_2 O_4$) initial blue black coloration and the volume of 0.1 N ($Na_2 S_2 O_4$) solution used was recorded.

The second bottle was covered with black cellophane bag or aluminum foil to prevent the penetration of light and then incubated at

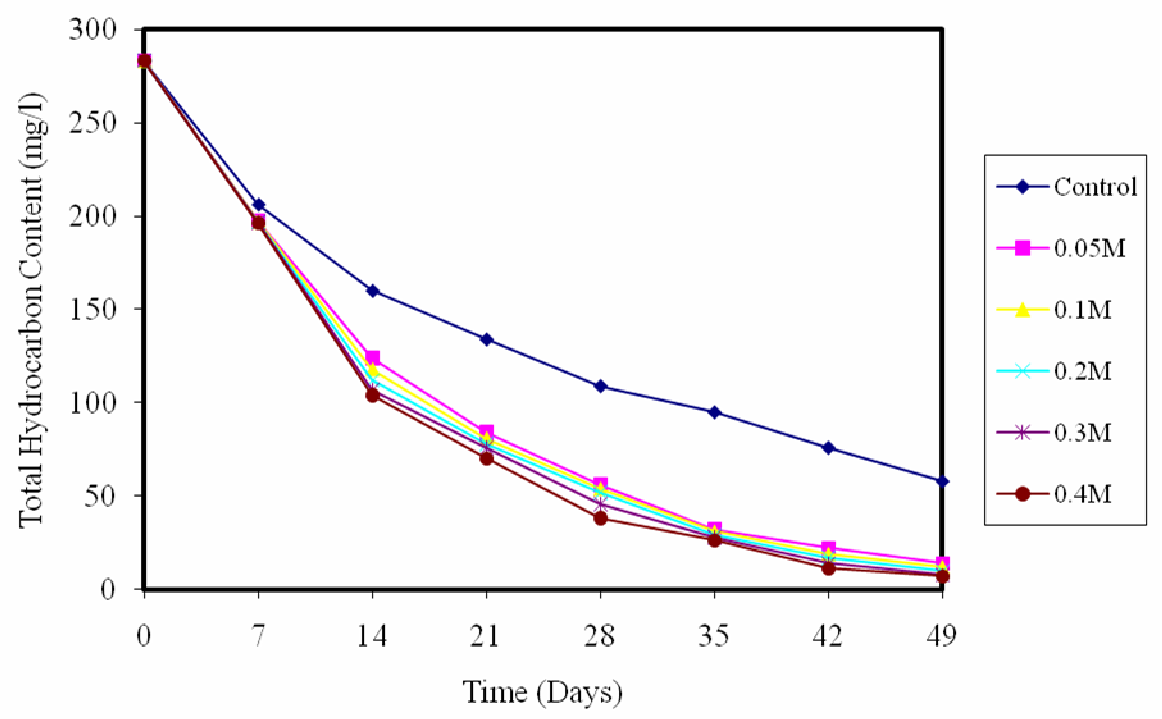

Figure 1. Variation of total hydrocarbon content with time for samples remediated with sodium nitrate.

20℃ for 5 days. At the end of 5 days, step (a) was repeated and the volume of 0.1N $Na_2 S_2 O_4$ used was recorded
The BOD of the sample was calculated as follows:

$$BOD_5 = \frac{DO_0 - DO_5}{P}$$

Where;
DO_O = Dissolved oxygen concentration at zero time
DO_5 = Dissolved oxygen concentration after 5 days incubation period
P = dilution factor

RESULTS AND DISCUSSION

The role of *Aspergillus niger* (fungus) and varying concentration of sodium nitrate on the biological oxidation of petroleum hydrocarbons was investigated in this study. The five samples with 0.05, 0.1, 0.2, 0.3 and 0.4 M sodium nitrate and the control was monitored for physico-chemical para-meters such as Total Hydrocarbon Content (THC), Biolo-gical Oxygen Demand (BOD), pH and Turbidity as indica-tors of the degree of bioremediation.

Different samples of the hydrocarbon polluted water was innoculated with *A. niger* (fungus) and varying concentration of sodium nitrate under appropriate condition of pH, temperature and oxygen supply (Trindede et al., 2005). The samples were monitored for forty nine days.

The total hydrocarbon content for all samples was observed to drop with time. The control samples showed the least drop in THC (283.2-58mg/l) in forty nine days signifying the activity of indigenous micro-organism (Obahiagbon and Ezeokeke, 2000).

All samples remediated with sodium nitrate as shown in Figure 1 showed significant decline in the THC. The sample remediated with 0.4M solution of sodium nitrate showed the greatest drop of 97.5% (283.2 - 7.0mg/l) followed by 0.3M $NaNO_3$ (aq) with 97.1% drop. (283.2 - 8.0 mg/l), then 0.2 M $NaNO_3$ (aq) solution with 96.5% drop (283.2 – 10 mg/l) and 0.1 M $NaNO_3$ (aq) with 95.8% (283.2 - 12.0 mg/l) and finally 0.05 M $NaNO_3$ (aq) with 95.1% drop (283.2 - 14.0 mg/l) all within the forty nine days of experimentation.

The drop in the THC values signifies a drop in hydrocarbon contents as a result of the release of enzymes by the micro-organisms to mineralize the organics to less toxic substances such as CO_2 and H_2O (Da Cunha, 1996, Obahiagbon and Aluyor, 2001). Of all samples, the ones remediated with 0.2, 0.3 and 0.4 M $NaNO_3$ (aq) at the end of the remediation period (49 days) fall within the acceptable limit of 10 mg/l recommended by regulatory agencies such as FEPA, DPR etc To make the process cost effective and reduce likelihood of waste water contamination by nitrates, it is recommended that 0.2 M $NaNO_3$ (aq) should be used in the remediation of petroleum hydrocarbon polluted water.

The pH values was observed to drop sharply within the first week as can be seen from Figure 2 for the samples remediated with $NaNO_3$ (aq) due to the acidic nature of the salt. The control sample showed a gradual decline signifying the activities of indigenous microbes converting the hydrocarbons into acidic products such as alkanoic acids. By and large all samples showed a steady rise in

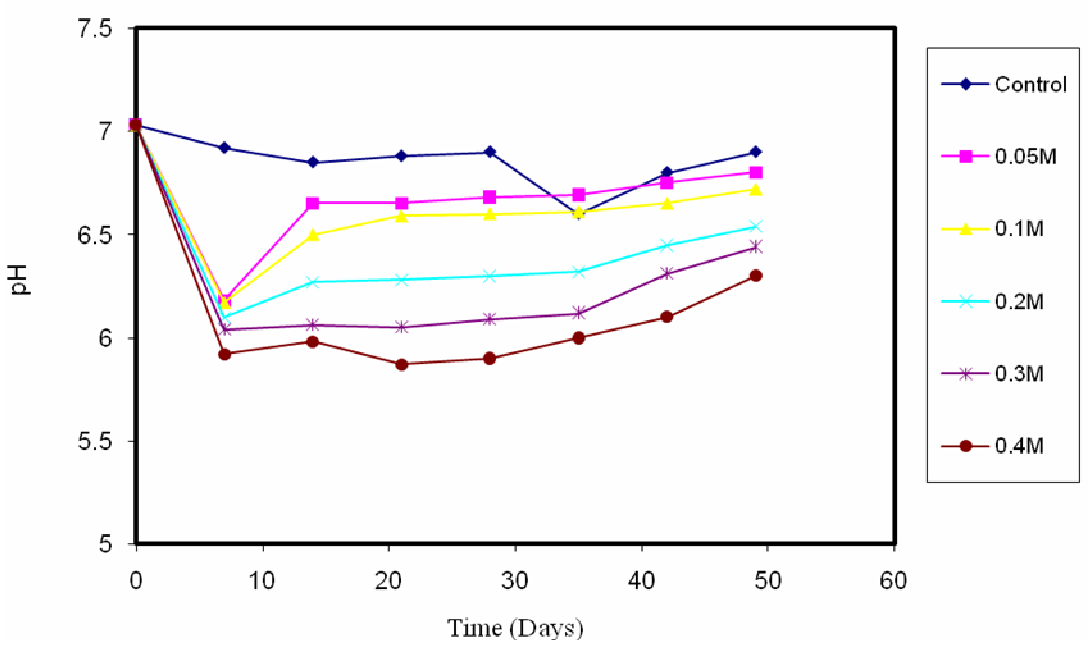

Figure 2. Variation of pH with time for samples remediated with sodium nitrate.

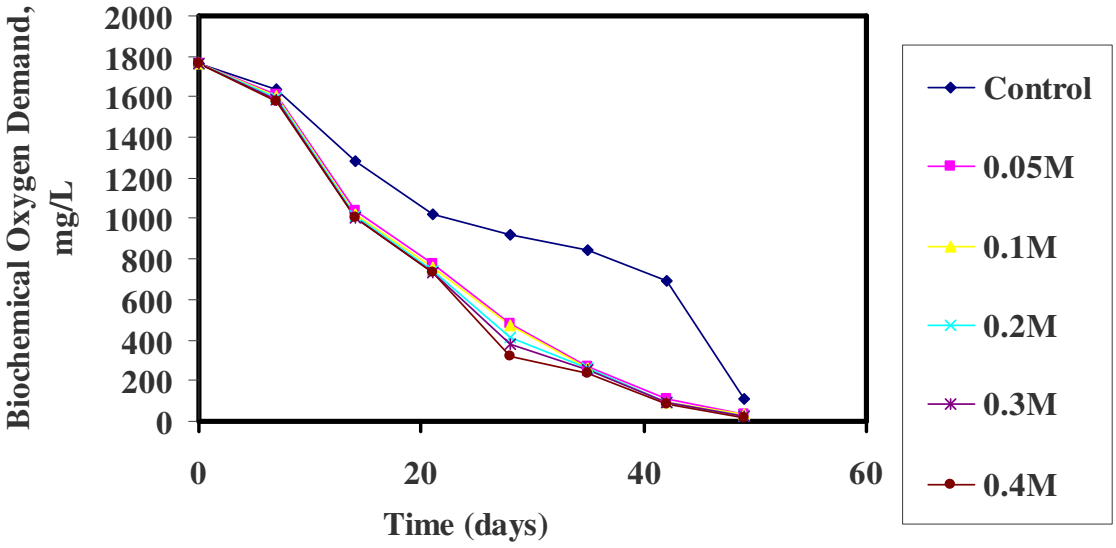

Figure 3. Variation of biochemical oxygen demand with time for samples remediated with sodium nitrate.

pH with time showing the conversion of hydrocarbons into less toxic acidic products. The pH of all samples falls within acceptable limit of 6-9(Obahiagbon and Agbajoh, 2000).

The BOD value of all samples was observed to drop as time progresses. Figure 3 shows the trend of BOD drop for the various samples. The sample amended with 0.4M $NaNO_3$ (aq) showed the greatest BOD drop of 98.8% (1760 – 21 mg/l) followed by 0.3M $NaNO_3$ (aq) amended sample with BOD drop of 98.5% (1760 – 26 mg/l) then followed by 0.2M $NaNO_3$ (aq) amended sample with drop in BOD of 98.4% (1760-28mg/l),then followed by 0.1 M $NaNO_3$ (aq) amended sample with 98.3% drop (1760 – 30 mg/l). All four samples above fall within stipulated values of 30 mg/l by regulatory agencies like FEPA and DPR during the experimentation period of 49 days. The samples remediated with 0.05 M $NaNO_3$ (aq) and the control had BOD drop of 98.1 and 93.8% respectively.

The two values fall short of the stipulated values. The drop in BOD for samples can be attributed to activities of

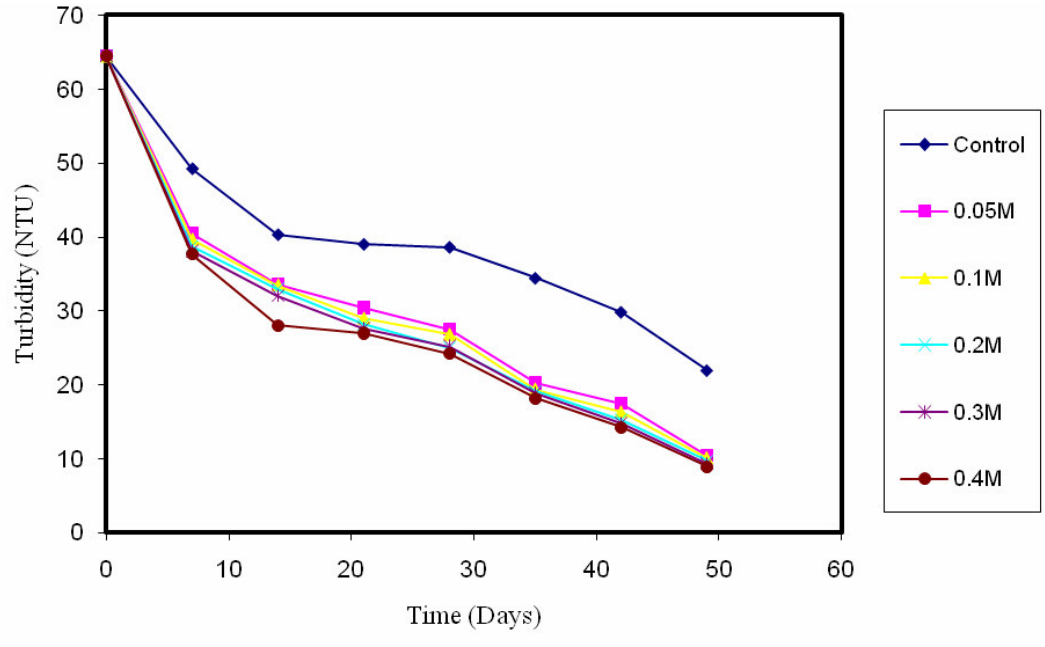

Figure 4. Variation of turgidity with time for samples remediated with sodium nitrate.

the *A. niger* and other indigenous microbes present in the samples which converts the hydrocarbons into less toxic substances such as CO_2, H_2O and many intermediates like organic acids, lipids, esters, complex alcohols and microbial proteins in form of enzymes (Obahiagbon and Owabor, 2008).

The turbidty value from Figure 4 was observed to decrease with time for all samples including the control. The drop in turbidity was observed to be greatest for the sample amended with 0.4M $NaNO_3$ (aq) (64.49 - 8.9 NTU), then followed by 0.3 M $NaNO_3$ (aq) (64.49 - 9.3 NTU). By and large turbidity was found to decrease with increase in the amount of $NaNO_3$ in the sample. The drops in turbidity was a result of the reduction in the hydrocarbon content of the sample which hitherto prevents light from penetrating the media.

At the end of the bioremediation period only three samples amended with 0.4, 0.3 and 0.2 M $NaNO_3$ (aq) respectively fall within the stipulated limits of less than 10 NTU by regulatory agencies such as FEPA and DPR.

Conclusion

Bioremediation as a strategy for clean up of petroleum hydrocarbon polluted water has been shown to be efficient considering the level of drop in THC, pH, BOD. and Turbidity which fall within the FEPA and DPR limits over the period of study *A. niger* offer an efficient and interesting possibility of degrading petroleum hydrocarbon polluted water 0.2, 0.3 and 0.4 M $NaNO_3$ (aq) are efficient in stimulating the microorganisms.

Scale up of this bioremediation strategy is very promising and should be encouraged

REFERENCES

Alexander M (1994). Biodegradation and Bioremediation. Academic Press, Inc. USA. pp. 24-30.

Atlas RM (1981).Microbial degradation of petroleum hydrocarbon. An environmental perspective. Microbiol. Rev. 45:180-20.

Atlas RM, Bartha R (1992). Hydrocarbon biodegradation and oil spill bioremediation. In Advances in Microbial Ecology, Marshall KC. (ed) Plenum, New York, USA. 12: 287-338.

Atlas RM (1995). Bioremediation of petroleum Pollutants. Int. Biodeterior. Biodegradation pp. 317-327.

Bartha R, Atlas RM (1997). Biodegradation of oil in Seawater, Writing Factor and Artificial Stimulation in the Microbial Degradation of oil Pollutants (D.G. Ahern and S.P. Meyers (eds), Centre for Wetland Resources, Louissiana pp.147-152.

Colwell RR, Walker JD (1977). Role of Autochtonous Bacteria in the Removal of Spilled Oil from Sediments. Environ. Pollut. 12: 51-56.

Federal Environmental Protection Agency (FEPA) (1997). Guidelines and `Standards for Environmental Impact Assessment in Nigeria pp. 87-95.

Ibe SN, Ibe EC (1984). Control and dispersion potential of oil spills by bacteria seeking in the petroleum industry and the Nigerian Environment Proceedings of the 1983 International Seminar. Nigeria National Petroleum Corporation (NNPC), Lagos pp. 188-191.

Leaby JG, Colwell RR (1990). : Microbial degradation of hydrocarbons in the environment. Microbiol Rev. 54: 305 – 315.

Margesin R (2000). Bioremediation of diesel oil contaminated alpine soils at low temperature. Appl. Microb. Biotech. 47:462-468.

Margesin R, Schinner F (1997). Efficiency of Indegenous and Innoculated Cold-adapted Soil Micro-organisms for Biodegradation of Diesel Oil in Alpine Soil. Appl. Environ. Microbiol. 63(7): 2660-2664.

Obahiagbon KO, Owabor CN (2008). "Biotreatment of Crude Oil polluted water using mixed microbial populations of *P. aureginosa, Penicillium notatum, E. coli* and *Aspergillus niger"* proceedings of the 2nd International Conference on Engineering Research and

Development: Innovations (15th - 17th April, 2008), Benin City, Nigeria.
Obahiagbon K, Aluyor E(2001): Bioremediation of Refinery wastewater using mixed microbes. Nig. J. Biomed. Eng. 1(1): 4-8.
Obahiagbon K, Agbajoh KG (2000): Bioremediation of crude oil spills in water using fertilizer and cow dung. J. Civil. Environ. Syst. Eng. 1(2): 61-72.
Obahiagbon KO, Ezeokeke I (2000). Bioremediation of crude oil contaminated soil using inorganic fertilizer. J. Nig. Inst. Prod. Eng. (NiProdE). 5(2): 44-50
Ojumu TV, Bello OO, Sonibare JA, Solomo, BO (2004). Evaluation of microbial Systems for Bioremediation of Petroleum Refimery Effluents in Nigeria. Afr. J. Biotech. 4(1): 31-35
Trindade PVO, Sobral LG, Rizzo ACL, Leite SGF, Soriano AU (2005). Bioremediation of a weathered and a recently oil-contaminated soils from Brazil: a comparison study. Chemosphere 58: 515-522
Yehuda C (2002). Bioremediation of oil by marine microbial mats, Int. Microbiol. 5:189-193.