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RESEARCH ARTICLE

COMPARATIVE STUDY ON THE RATE OF BIODEGRADATION OF CRUDE OIL BY BACTERIA AND FUNGI ISOLATES

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ABSTRACT

Comparative study on the rate of biodegradation of crude oil by bacteria and fungi isolates was carried out using growth and biodegradation indices in an oil supplemented mineral salt medium. The bacterial genera that were isolated from oil-polluted sample from location 1 of Shell Petroleum Development Company (SPDC), at Ukwugba Village in Egbema include: *Bacillus, Pseudomonas, Micrococcus and Acinetobacter*, while the genera of fungi include: *Trichosporon, Aspergillus* and *Mucor*. *Bacillus* sp. and *Trichosporon* sp. were used for the comparative study. The microbial growth was monitored by UV spectrophotometer **(**Astell UV- Vis Grating, 752W) at absorbance (OD520nm) while degradation was monitored by gas chromatography (model HP 5890 series II; GC Injection and detector temperature: 65°C and 275°C; Column: Capillary Column (HP5) -30m length, 0.32mm internal diameter, 0.25 µm film diameter; and Detector: Flame Ionization Detector) . An increased growth from 0.17 - 0.53% was recorded for *Bacillus* sp. and 0.11 – 0.45% for *Trichosporon* sp. The degradation analysis showed quantitative reductions in the total petroleum hydrocarbons (TPHs) which ranged from 12, 405 to 122mg/1 and 12, 405 to 712mg/1 for *Bacillus* sp. and *Trichosporon* sp. respectively during the 21 days incubation period. Statistically, significant differences were detected in the growth and degradation parameters measured between *Bacillus* sp. and *Trichosporon* sp. Observations of the study conclude that both *Bacillus* and *Trichosporon* species have potentials for degradation of Bonny light crude oil, however, comparatively *Bacillus* species are better utilizers and degraders of Bonny light crude oil than *Trichosporon* species**.**

Key words: *Bacillus* sp., *Trichosporon* sp., Growth, Bonny-light crude oil, Total Petroleum Hydrocarbons (TPHs), Biodegradation.

INTRODUCTION

Since the discovery of the first commercial oil field in 1956 at Oloibiri in the Niger Delta of Nigeria, the frontiers of oil exploration has been expanding in producing medium and light crude oils. (Amund and Akanghou, 1993). Increasing petroleum exploration refining and other allied industrial activities in the Niger Delta have led to the wide scale contamination of most of its creeks, swamps, rivers and streams (Okpokwasili and Odokuma, 1990) with hydrocarbon and dispersal products. The contamination of these habitats constitutes public health and socio-economic hazards (Smith and Dragun, 1984). The traditional treatment of oily waste such as containment and collection using floating booms, adsorption by natural or synthetic materials e.t.c. cannot degrade crude oil thoroughly (Ollis, 1992). Hence microbial degradation appears to be the most environment friendly method of removal of oil pollutants (Oboh *et al*., 2006). Microbial degradation of oil has been shown to occur by attack on the aliphatic or light aromatic fractions of the oil. High molecular weight aromatics, resins and asphaltenes are generally considered to be recalcitrant or exhibit only low rates of biodegradation or mineralization. In aquatic ecosystems, dispersion and emulsification of oil in slicks appear to be prerequisites for rapid biodegradation (Okerentugba and Ezeronye, 2003). Several authors have made lists containing bacteria and fungi genera that are able to mineralize wide spectrum pollutants proceeding from marine atmosphere as well as the soil (Juhaz *et al.,* 2000). An ability to isolate high numbers of certain oil-degrading microorganisms from an environment is commonly taken as the evidence that those microorganisms are the active degraders in that environment (Okerentugba and Ezeronye, 2003). Walker *et al*. (1975) have observed that bacteria and fungi have similar patterns of hydrocarbon degradation. The most prevalent genera associated with the utilization and degradation of crude oil in aquatic environment include: *Pseudomonas, Achromobacter, Vibrio, Brevibacterium, Nocardia, Arthrobacter, Acinetobacter, Micrococcus, Corynebacterium, Flavobacterium, Candida, Rhodotorula* (Bartha and Atlas, 1977).

In one of his studies, Okoh, (2003), demonstrated degradation or mineralization rates of different strains of *Pseudomonas aeruginosa* on crude oil with evidence of significant reduction of major peak components of the oil. Also, in their preliminary experiment, Lemos *et al*. (2001), pointed out that *Aspergillus versicolor* was bearing the highest potential to degrade petroleum hydrocarbons (10.8%) when compared to *Aspergillus niger* (7.5%) responsible for the second best result, in relation to the other fungi whose biodegradation efficiency were between 7.3% and 6.6%. In this study, the aim was to isolate pure cultures of bacteria and fungi species

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from an oil polluted environment and to compare the bio-degradation potentials of both isolates.

MATERIALS AND METHODS

Description of Sampling Site

The oil-polluted water sample for this study was obtained from location 1 of Shell Petroleum Development Company (SPDC), at Ukwugba Village in Egbema, Ohaji-Egbema Local Government Area of Imo State. The sampling site is about 150km east from Anambra State University (ANSU), Uli Campus main gate. The main source of the water is a stationary body called Utu swamp. Geomorphologically, its location is on level plain land. Anthropologenic survey revealed that there were no human activities such as refuge dumping, washing e.t.c. that took place within the water location area.

Collection of Water Sample

Crude oil-polluted water sample was obtained from location 1 Shell Petroleum Development Company (SPDC) Ukwugba Village Egbema, in Ohaji-Egbema Local Government Area of Imo State. Collection of the sample was done aseptically with the aid of a sterile conical flask (250ml) from a depth of about 0 – 15cm. It was then immediately delivered to the Microbiology lab. of Anambra State University, Uli, Anambra State for isolation of bacteria and fungi native populations by adopting the method described by Ojo, (2005).

Source of Crude Oil Sample

The Bonny-light crude oil sample was obtained from the Bonny Terminal of the Shell Petroleum Development Company of Nigeria at Port Harcourt, Rivers State

Isolation, Identification and Maintenance of Bacteria and Fungi Isolates

Isolation was carried out using Nutrient agar (NA) for bacteria and Sabouraud Dextrose agar (SDA) for fungi (Okerentugba and Ezeronye, 2003, Lemos *et al*., 2001). The bacterial isolates obtained were characterized and identified based on their cultural, morphological and biochemical characteristics using the scheme of Bergey's Manual of Determinative Bacteriology (Chikere and Okpokwasili, 2003; Oboh *et al*., 2006). Cultural and microscopic features of fungi including Gram's stain and sugar fermentation tests were used for the characterization of the fungi isolates (Ekpo and Ekpo, 2006).

Crude Oil Utilization Adaptation Test

By adopting the method of Wang (1984), the bacterial and fungal isolates (identified as *Bacillus* sp. and *Trichosporon* sp.) were adapted for crude oil utilization in 99ml mineral salt medium containing 1ml of Bonny-light crude oil as the carbon source (Mills *et al*., 1978).This mixture was agitated manually and incubated at ambient temperature (30^0C) for 10 days. Loopful of the adapted culture media was then transferred onto Nutrient agar and Sabouraud Dextrose agar and incubated for 2 days. Single discrete colonies of the isolates were later transferred into slants incubated and stored in the refrigerator at 4^0C for further use (Chikere and Okpowasili, 2003).

Laboratory Biodegradation Studies

By adopting the method of Ekpo and Ekpo (2006), the biodegradation studies of the Bonny light crude oil was carried out in liquid medium. Mineral salt medium broth was autoclaved in 2 litres conical flasks. 99ml of the liquid medium was dispensed into nine (9) sterile 250ml dark amber bottles into which 1ml of sterile crude oil was added (Ekpo and Ekpo, 2006). Five millilitres of the adapted cultures of both bacterial and fungal isolates were inoculated into four (4) dark amber bottles in two sets each for the bacterium and fungus. The ninth bottle was uninoculated and served as control. The bottles were incubated at ambient temperature (30^0C) in the dark with manual shaking of 100 strokes per minute for 30 minutes (Wang, 1984) each day for 3weeks. Sampling period was set for every seven days for 21 days (Okoh, 2003). Bacterial and fungal utilization or growth rate were monitored by optical density at 520nm wavelength (Ekpo and Ekpo, 2006). The total petroleum hydrocarbon was measured and changes in the hydrocarbon profile of the crude oil were monitored by gas chromatography (Onyeike and Osuji, 2003).

RESULTS

In these studies, four genera of bacteria and three genera of fungi were isolated from oil-polluted water. The bacterial genera were *Bacillus, Pseudomonas, Micrococcus,* and *Acinetobacter* (Table 1). On the other hand, fungi isolates were *Trichosporon, Aspergillus,* and *Mucor* (Table 2). The genera *Bacillus* was identified based on formation of endospores, Gram positive rod, motile, catalase positive and acid production from glucose. Also, the identification of *Trichosporon* is based on elevated creamy colonies, long oval

Table 1: Colonial, cellular and biochemical characteristics of bacteria

Isolate designation	Colonial and cellular morphology	Cell morphology	Catalase	Oxidase	Indole	M.R.	V.P	Citrate	Starch	Spore staining	H_2S	Motility	Glucose	Monnitol	Sucrose	Lactose	Maltose	Probable Genera
As	Light-yellow, Round, irregular, 1.5 – 2.5mm	Rods	-	-	-	-	-	-	+	+	-	-	-	A	A/G	A	A/G	A/ G	*Acinetobacter*
Mac	Creamy, circular, entire, 2mm	Rods	-	+	+	-	-	-	+	-	-	+	+	A/G	A/G	A	A	A/G	*Pseudomonas*
P	Milky, irregular, Erose, 1-2.5mm	Rods	+	+	-	-	-	-	-	+	+	-	+	A	A/G	A/G	A	A/G	*Bacillus*
Q	Yellow, circular, entire, 1-2mm	Cocci	+	+	-	-	+	-	+	-	-	-	-	A	A	A	A/G	A	*Micrococcus*
X	Milky, irregular, erose,1-2.5mm	Rods	+	+	-	-	+	-	-	+	+	-	+	A	A/G	A/G	A/G	A	*Bacillus*
Y	Yellow, circular, entire,1-2mm	Cocci	+	+	-	-	+	-	-	-	-	-	-	A	A	A	A/G	A/G	*Micrococcus*

Key: + = Positive, A = Acid production, A/G = Acid and gas production, - = Negative. V.P. = Voges Proskauer, M.R. = Methyl Red, H_2S = Hydrogen Sulphide Production

Table 2: Characterization of fungal isolates

Isolates	Colonial morphology	Microscopy	GR	Fermentation test; sugar		Identity
1	Elevated colonies with a mycelial boarder, initially creamy, later dark	Long oval blastospores and zig-zag arrangements of arthrospores	+	Glucose, lactose, sucrose Maltose Mannitol	A A A/G -	*Trichosporon* sp.
2	Velvety surface due to sporulation. Grey-green in hyphae colour	Septate hyphae with conidiophores borne laterally	+	NT		*Aspergillus* sp.
3	Long fibred rough woolly network of hyphae. Initially white, later grey, Petri dish filled within a few days	Thick non – septate hyphae, sporangiophore departing laterally from mycelium and sporangia filled with spores	+	NT		*Mucor* sp.

Key: A = Growth with acid production, A/G = Growth with acid and gas production, - = No growth, NT= not tested, GR= Gram reaction.

blastospores. Bacterial and fungal utilization of crude oil for growth monitored by optical density at 520nm recorded better growth in the *Bacillus* inoculated samples than *Trichosporon* (Table 3). Low values of optical densities for both bacterial and fungal isolates on day 0 can be explained that both organisms had not yet got adapted to the crude oil and thus were adjusting to the new condition of environment and producing new enzymes for the degradation of the Bonny light crude oil. Increase in the absorbance values for both isolates with *Bacillus* sp having 0.53 and *Trichosporon* sp. having 0.45 on days 21 was recorded. Growth and biodegradation parameters of *Bacillus* sp inoculated samples were significant more than *Trichosporon* sp inoculated samples ($p < 0.05$). These differences could be attributed to the enzymatic capabilities of the isolates. Biodegradation studies and results obtained (Figures 1-5) showed reduction in the heights and numbers of the chromatograms after 21 days for both *Bacillus* and *Trichosporon* species. Comparatively, the reduction in the samples that indicates *Bacillus* species was more than the reduction in the *Trichosporon* species inoculated samples.

Table 3: Mean values of absorbance

Days	*Bacillus* sp.	*Trichosporon* sp.	Control
0	0.17	0.11	0.13
7	0.19	0.16	0.12
14	0.22	0.25	0.14
21	0.53	0.45	0.13

Figure 1. Chromatographic profile of TPH for *Bacillus* sp. Day 0

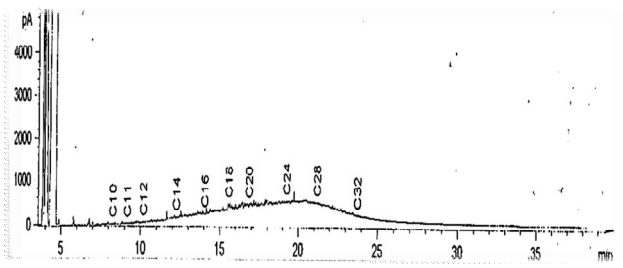

Figure 2: Chromatographic profile of TPH for *Bacillus* sp. Day 21

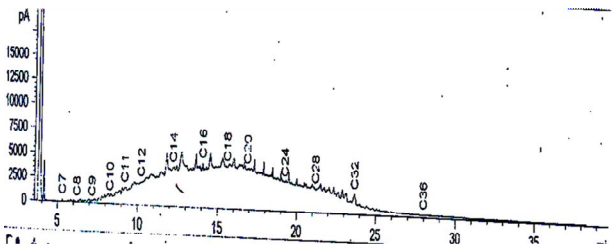

Figure 3. Chromatographic profile of TPH for *Trichosporon* sp. Day 0

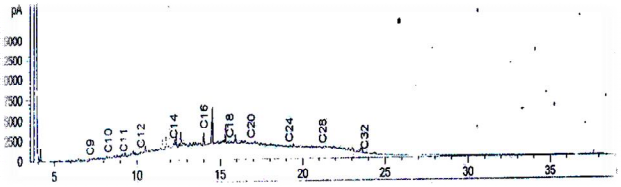

Figure 4: Chromatographic profile of TPH for *Trichosporon* sp. Day 21

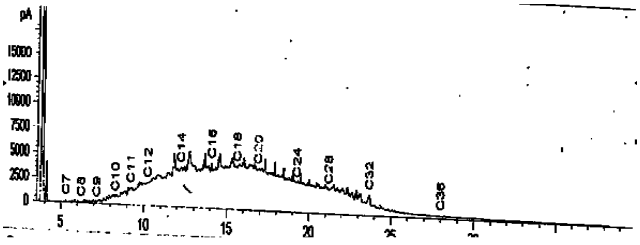

Figure 5: Chromatographic profile of TPH for control on day 21

DISCUSSION

Isolation of bacteria; *Bacillus, Pseudomonas, Micrococcus, Acinetobacter* and fungi; *Trichosporon, Aspergillus,* and *Mucor* from crude oil environments have been documented by Bartha and Atlas (1977); Chikere and Okpokwasili (2003); and Okpokwasili and Okorie (1988). Low values of optical densities for both bacterial and fungal isolates on day 0 were similar to the report made by Okerentugba and Ezeronye, (2003). The differences in the growth recorded using absorbance could be attributed to the enzymatic capabilities of the isolates. The report agrees with the observations of Chikere and Okpokwasili, (2003) that the difference in enzymatic capabilities of degraders is said to be the major limiting factor in the transformation of complex substances through the process of biodegradation. The absorbance value remained unchanged in the control. The reductions in both

the number and sizes of the peaks and height in the chromatogram report of *Bacillus* sp. and *Trichosporon* sp corroborate with the report by Okpokwasili and Okorie, (1988) that the organisms are secondary invaders which utilize the breakdown products of hydrocarbon after an initial attack had occurred. The findings of this study also lend more weight to the report of Ekpo and Ekpo (2006). Comparatively, *Bacillus* sp. had the highest utilization and degradation rate than *Trichosporon* sp as the result of *Bacillus* sp. increase and decrease significantly at ($P<0.05$) than *Trichosporon* sp.. The finding is well supported by Ekpo and Ekpo, (2006) that bacteria are better degraders of Bonny light crude oil compared to fungi.

Conclusion

The study revealed that the location 1 of Shell Petroleum Development Company (SPDC) oil polluted water at Ukwugba Village in Egbema harbour a number of microbial hydrocarbon degraders which are capable of utilizing Bonny light crude oil as source of carbon when monitored using absorbance. The increasing values obtained indicate that both test organisms: *Bacillus* sp. and *Trichosporon* sp. can grow and utilizing Bonny light as carbon source but *Trichosporon* sp. grow at a slower rate as the days of incubation increase further. The results of the chromatographic analyses of the total petroleum hydrocarbons (TPHs) also showed that *Bacillus* sp. can degrade Bonny light crude oil more efficiently that *Trichosporon* sp. and this evidence is shown from the reductions of both the number and sizes of the peaks. Therefore, it appeared that *Bacillus* sp. a bacterium could be more useful in the bioremediation of water bodies contaminated with Bonny light crude oil and is recommended for controlling aquatic aliphatic pollution.

REFERENCES

Amund, O. O. and Akangbou, T. S. (1993). Microbial Degradation of four Nigerian Crude Oils in an Estuarine Microcosm. *Lett. Appl. Microbiol*. 16: 188 – 192. .

Bartha, R. and Atlas, R.M. (1977). The Microbiology of Aquatic Oil Spills. *Appl. Microbiol.* 32: 225 – 266. .

Chikere, B.O. and Okpokwasili, G.C. (2003). Enhancement of Biodegradation of Petrochemicals by Nutrient Supplementation. *Nig. J. Microbiol.* 17(2): 130- 135.

Ekpo, M.A. and Ekpo, E.I. (2006). Utilization of Bonny Light and Bonny Medium Crude Oil by Microorganisms isolated from Qua Iboe River Estuarine. *Nig. J. Microbiol*. 20(1): 832 – 839.

Juhasz, A.L. and Naidu, R. (2000). "Bioremediation of High Molecular Weight Polycyclic Aromatic Hydrocarbons: A Review of the Microbial Degradation of Benzo (a) pyrene". *Int. Biodeterioration and Biodegradation.* 45: 57 – 88.

Lemos, J. S. L., Pizzo, A.C. Millioli; V. S., Seriano, A. U., Surquis, M.I.M. and Santos, R. (2001). Petroleum Degradation by Filamentous Fungi. Institute Oswaldo Cruz – Fiocruz, Rio de Janeiro. Pp. 1 – 7.

Mills, A. L., Breul, C. and Colwell, R. R. (1978). Enumeration of Petroleum Degrading Marine and Estuarine Microorganisms by the Most Probable Number Method. *Can. J. Microbiol.* 24: 552 – 557.

Oboh, B.O., Ilori, M.O.I. Akinyemi, J. O. and Adebusoye, S. A. (2006). Hydrocarbon Degrading Potentials of Bacteria Isolated from a Bitumen (Tarsand) Deposit. *Nature and Science* 4(5):51-57.

Ojo, O.A. (2005). Petroleum Hydrocarbon Utilization by Native Bacteria Population from a Waste Water Canal Southwest, Nigeria. Department of Microbiology, Unilag. Publication, Lagos. Code No. jb06054. Pp. 1 – 4.

Okerentugba, P.O. and Ezeronye, O. U. (2003). Petroleum Degradation of Potentials of Single and Mixed Microbial Cultures Isolated from Rivers and Refinery Effluent in Nigeria. *Afr. J. Biotechnol.* 2(9): 288 – 292.

Okoh, I. A. (2003). Biodegradation of Bonny Light Crude Oil in Soil Pits in Nigeria *Afr. J. Biotechnol.* 2 (5): 104 – 108.

Okpokwasili, G. C. and Okorie, B. B. (1988). Biodegradation Potentials of Microorganisms Isolated from Car Engine Lubricating Oil. *Tribol. Int*. 21:215-220.

Okpokwasili, G. C. and Odokuma, L.O. (1990). Effect of Salinity on Biodegradation of Oil Spill Dispersal. *Wastes Management* 10: 141 – 146.

Ollis, D. (1992). Slick solutions for Oil Spills. *Nature*, 358: 453 – 456.

Onyeike, E. N. and Osuji, J. O. (2003). Research Techniques in Biological and Chemical Services. Spring Field Publishers, Ltd. Imo. Pp. 321 – 327.

Smith, L. R. and Dragun, J. (1984). Degradation of Volatile Chlorinated Aliphatic Priority Pollutants in Ground Water. *Environ. Int.* 10: 291 – 298.

Walker, J. D., Austin, B. F. and Colwell, R. R. (1975). Utilization of Mixed Hydrocarbon Substrate by Petroleum Microorganisms. *J. Gen. Microbiol.* 21: 27 – 39. .

Wang, W. (1984). Acclimation and Response of Algal Communities from Different Sources to Zinc Toxicity. Illinois State Water Survey. Peoria, Illinois. Pp. 205 – 210.

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