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Biodegradation of Two Nigerian Crude Oils by Four-Membered Consortium of Hydrocarbonoclastic Bacteria

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Abstract: Degradation of two Nigerian crude oils by a four-membered consortium of hydrocarbonoclastic bacteria was studied. The consortium consisted of *Alcaligenes paradoxus*, *Aeromonas* sp., *Bacillus licheniformis* and *Pseudomonas fluorescens* isolated from a crude oil-polluted freshwater body. The degradation of the crude oils in aerated batch and static-flask cultures as well as the effect of biostimulation using inorganic nitrogenous fertilizer (NPK-15) on the degradation were studied for a period of 15 days. Biodegradation of the crude oils was monitored as primary degradation. In the aerated batch cultures 21.2 and 26.0% of Bonny Medium and Qua-Iboe crude oils respectively were utilized by the consortium in 15 days. Whereas in the static-flask cultures 10 and 13.6% of Bonny Medium and Qua-Iboe crude oils respectively were utilized in the same period. In the NPK-15 supplemented aerated-batch cultures 24.2% and 29.6% of Bonny Medium and Qua-Iboe respectively were utilized; while 14% and 18.8% of Bonny Medium and Qua-Iboe respectively were utilized in the supplemented static-flask cultures. This study demonstrates the importance of aeration and biostimulation with inorganic nitrogenous fertilizer (NPK-15) in biodegradation of crude oils.

Key words: Crude oil · Bonny Medium · Qua-Iboe · Biostimulation · Aeration

INTRODUCTION

Crude oil and its refined products play important roles in Nigeria's economy as the largest earner of foreign exchange. Along side, increase in degradation of land and pollution of water environments in the Niger-Delta region caused by oil spills, oil installation vandalism and bunkering is an issue even at international discourse [1]. While urgent machineries are being put in place by the Government to resolve the many volatile issues revolving around resource control in the region, measures must also be taken to reclaim the already degraded natural resources.

The issue of environmental pollution has become a key element in assessing impacts of development. Managing this problem is a necessary pre-requisite for making development more sustainable. Pollution management therefore should be in the context of achieving sustainable development i.e. integrating the objectives of environmental protection, social needs and economic development. On these criteria, bio2emediation has scored very high both in terms of 'Best Practicable Environmental Option' and 'Best Available Technology Not Entailing Excessive Cost' [2].

Various biotechnologies employing the principle of microbial infallibility have been developed to treat contaminated ecosystems. The applicability of any of these technologies depends on several factors including the nature and concentration of the contaminants and other interactive: not-well-defined environmental conditions on one hand [3] and the level of decontamination acceptable on the other hand [4]. These topics have been extensively reviewed [5, 6] and environmental factors such as availability of sufficient oxygen and nutrients have been identified as major rate-limiting factors [7]. Commercial nitrogen-and phosphorus-containing fertilizers that have an affinity for hydrocarbons are often used for treating oil pollution, though some of them have not been effective [7].

This study examines the degradation of two Nigerian crude oils using a consortium of hydrocarbonoclastic bacteria isolated from a crude oil polluted water body in the Niger Delta region. Degradation of the crude oils by a concortium of four of the hydrocarbonclastic bacteria was

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examined under aerated batch and static-flask conditions. The effect of biostimulation using inorganic nitrogenous fertilizer (NPK-15) on the degradation of the crude oils was also studied. Degradation of the crude oils was monitored as primary degradation.

MATERIALS AND METHODS

Preparation of Consortium of Hydrocarbonclastic Bacteria: The bacteria used in the study were selected from among hydrocarbonoclastic bacteria previously isolated from a crude oil-polluted fresh water body in Abereke, Ondo State, Nigeria. The isolates were screened for ability to initiate and undertake primary degradation of the oil by growing each as pure culture on mineral agar containing the crude oil. They were then acclimatized in 0.5% (v/v) crude oil mineral medium for 72 hours on an orbital shaker (120 rpm) at room temperature and then standardized [8]. Equivolumes of the standardized cultures were combined as a consortium and used for the degradation studies.

Degradation Study: One milliliter of the standardized four-membered-consortium was used to inoculate flasks containing 200ml each of sterile mineral medium containing 0.05% (w/v) of Bonny Medium and Qua-Iboe crude oils. One set of the flasks were incubated on an orbital shaker (120rpm) as aerated-batch cultures while another set were left stationary as static-flask cultures. Uninoculated flasks containing the same medium were incubated along with each set to serve as control. Degradation was monitored by gravimetrically determining the amount of residual crude oil in 10ml of the aliquots after extraction with petroleum ether [9].

The effect of biostimulation was studied by adding inorganic fertilizer (NPK-15) was added to the medium containing the crude oil at a concentration of 0.75mg/l. The flasks were inoculated with the four-membered consortium; one set of the flasks were incubated on an orbital shaker (120rpm) as aerated-batch cultures while another were left stationary as static-flask cultures. Uninoculated flasks containing the same medium were incubated along with each set to serve as control. Degradation was also monitored by gravimetrically determining the amount of residual crude oil in 10ml of the aliquots after extraction with petroleum ether [9].

Bacterial growth was monitored by enumeration of bacterial population using pour plate technique. The plates were incubated at 37°C for 48 hrs after which the colonies were counted. The changes in pH were monitored using a pH meter (PYE unicorn model 291MK). All parameters were monitored starting from the day of incubation and subsequently at a 5-day interval for 15 days.

RESULTS

There was gradual decline in pH and steady increase in the microbial populations during the period. The consortium degraded the crude oils at different rates under the different culture conditions; aeration favoured the degradation of the crude oil (Figures 1 and 2). The addition of NPK fertilizer enhanced the degradation of the crude oils (Figures 3 and 4). The combination of aeration and biostimulation with NPK-15 resulted in the fastest rate of biodegradation of the crude oils. The Qua-Iboe crude oil was more readily degraded under both culture conditions compared to the heavier Bonny Medium crude oil.

DISCUSSIONS

The decrease in pH of the medium is suggestive of production of acidic products resulting from metabolic activities of members of the consortium. Biological metabolism of various aliphatic and aromatic components of petroleum hydrocarbons is the most important factor influencing their fate and distribution in a water body [4]. Increase in the microbial population is attributable to the fact that microorganisms readily metabolized fractions of the crude oils. The increase in counts corresponded with rapid decrease in the concentrations of the crude oils (Figures 1, 2, 3 and 4).

The difference in rate of degradation of the crude oils under the culture conditions used confirms that growth conditions influence the rates of degradation. About two-fold increases were observed in the aerated-batch cultures as compared with the static-flask cultures. The availability of sufficient oxygen is important in microbial metabolism of aromatic hydrocarbons. Its importance in managing oil pollution in aquatic environments has been discussed by Crawford and Crawford [6]. Rosenberg and Ron [10] suggest that oxygen may not be a limiting factor on or near surface of water; however it becomes a factor with increase in depth because oxygen concentration reduces with increase in water depth. Thus, oxygen concentration is likely to affects the rate of removal of heavy oils that may form tar balls and sink to the sediment as dense-non-aqueous-phase liquid (DNAPL) [4].

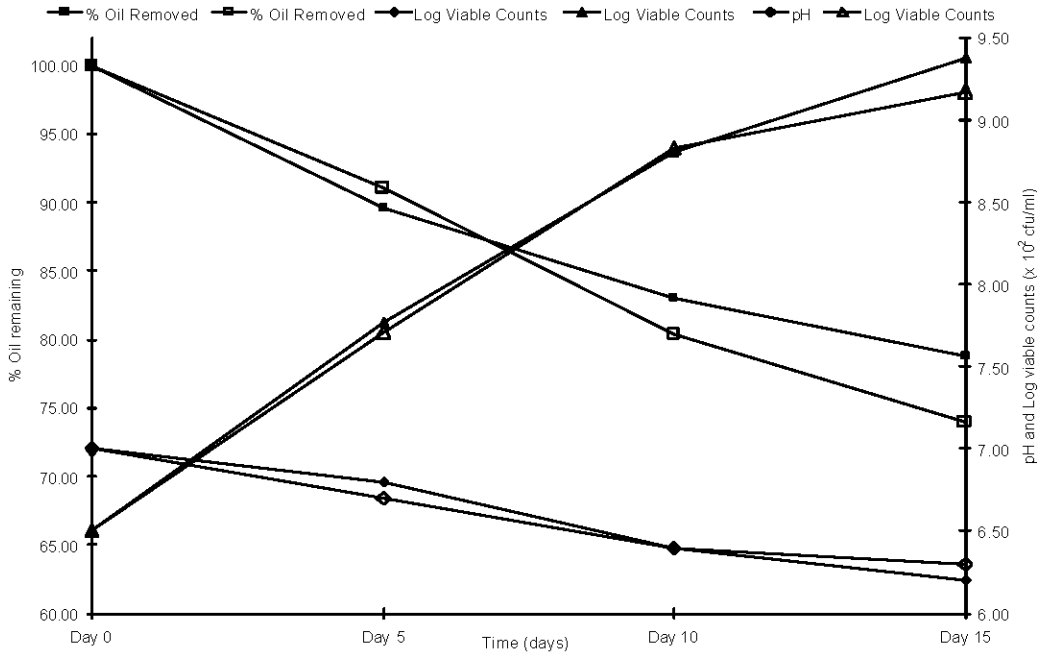

Fig. 1: Biodegradation profiles of Bonny Medium and Qua-Iboe crude oils by consortium of hydrocarbonoclastic bacteria during a 15-day aerated batch culture

Key: Black shapes represent parameters for Bonny Medium crude oil while Unshaded shapes represent parameters for Qua-Iboe crude oil

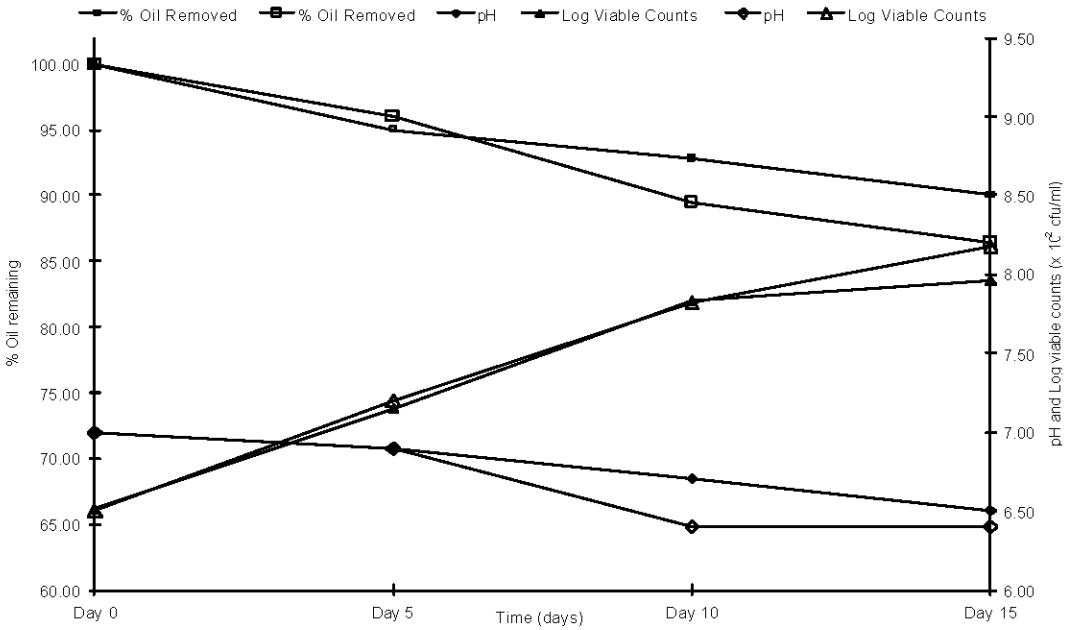

Fig. 2: Biodegradation profiles of Bonny Medium and Qua-Iboe crude oils by consortium of hydrocarbonoclastic bacteria during a 15-day static-flask culture

Key: Black shapes represent parameters for Bonny Medium crude oil while Unshaded shapes represent parameters for Qua-Iboe crude oil

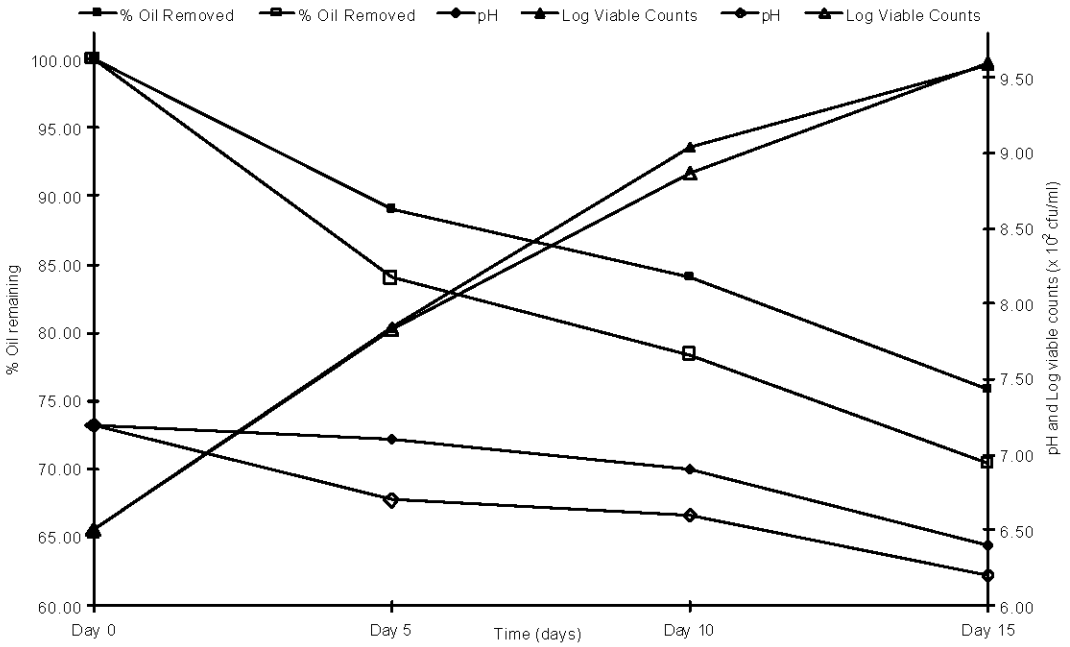

Fig. 3: Biodegradation profiles of Bonny Medium and Qua-Iboe crude oils by consortium of hydrocarbonoclastic bacteria during a 15-day NPK-15 supplemented aerated-batch culture

Key: Black shapes represent parameters for Bonny Medium crude oil while Unshaded shapes represent parameters for Qua-Iboe crude oil

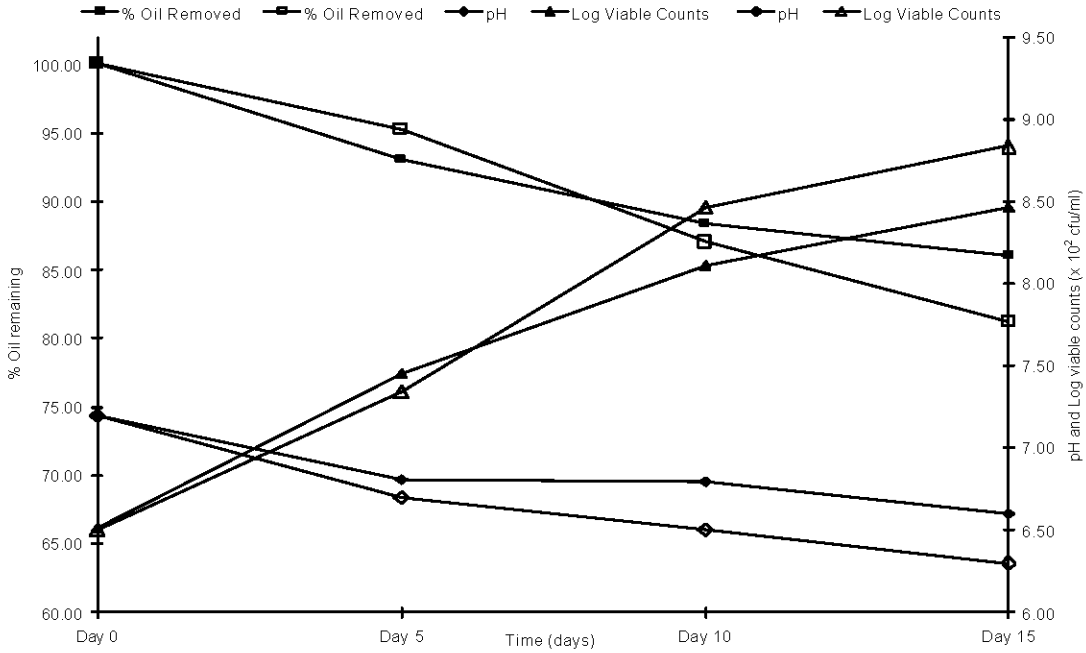

Fig. 4: Biodegradation profiles of Bonny Medium and Qua-Iboe crude oils by consortium of hydrocarbonoclastic bacteria during a 15-day NPK-15 supplemented static-flask culture

Key: Black shapes represent parameters for Bonny Medium crude oil while Unshaded shapes represent parameters for Qua-Iboe crude oil

The results from this study show that the nature of the substrates affected the rate of biodegradation. More of Qua-Iboe crude oil, which is lighter, was removed under both culture conditions compared to the heavier Bonny Medium crude oil (Figures 1 and 2). The degradation of crude oils is associated with their densities, which reflects the relative fractions of saturates, aromatic and asphaltenic components of the crude oils [11]. Crude oils containing large percentages of high molecular weight polyaromatic hydrocarbons and asphaltenes are reported to be more resistant to microbial degradation [4, 8].

The positive effect of biostimulation with NPK-15 (Figures 3 and 4) on degradation of the crude oils is similar to the submission of Odokuma and Dickson [12]. They reported significant bioremediation using fertilizer and tilling. Biodegradation of the crude was most rapid under aeration and biostimulation (with NPK-15) followed by aeration alone. Although biostimulation increased rate of biodegradation under static flask condition, it was less than the rate in aerated batch culture. The trend of rates of biodegradation is: Aeration and Biostimulation> Aeration> Static and Biostimulation> Static; similar trends have been observed by Henry and Grbic-Galic [13] and Hutchins [14].

CONCLUSION

This study demonstrates that the crude oils were readily biodegradable and that the environmental conditions would influence the persistence of crude oils in Nigerian freshwater bodies. In case of oil spill, agitators to introduce oxygen and supply of NPK-15 fertilizer can be readily used to cleanup the environment. In addition, hydrocarbonclastic bacteria that have been isolated from the environment can be used to augument in areas where it may be necessary to achieve bioremediation of crude oil-polluted environments.

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