Effects of Crude Oil on Biomass and Protein Production by Aquatic Bacteria

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Abstract

Some effects of Bonny light crude oil on the biomass and protein production by three aquatic bacteria namely, Pseudomonas aeruginosa, Aeromonas hydrophyla and Vibrio fisheri were investigated. The species showed different responses to the toxic influences of various crude oil concentrations. The growth response was measured spectrophotometrically using optical density (OD) at 600nm. Pseudomonas aeruginosa responded positively to all crude oil levels. A general assessment indicated that 2% (v/v) crude oil concentration stimulated maximum biomass and protein production of this organism. Lower biomass and protein yields were observed at reduced crude oil levels. Biomass production decreased gradually among Aeromonas hydrophyla and Vibrio fisheri in comparison to the control. Biomass of Aeromonas hydrophyla increased from 0.1 (OD_{600nm}) at 0 h to 0.58 after 20 h at 0.5% crude oil concentration. This level gradually declined to 0.03 after 20 h cultivation at 1.5 % crude oil concentration. Maximum decline in optical density of this organism was observed at crude oil concentration of 2.0%. Protein levels for Aeromonas hydrophyla decreased from 0.12 mg/mL after 20 h at crude oil concentration of 0.5% to 0.06 mg/mL after 20 h at maximum crude oil concentration of 2%. The biomass of Vibrio fisheri increased slightly from 0.1(OD_{600nm}) at 0 h to 0.03 after 20 h at 0.5% crude oil level. Further decreases in OD values of this organism occurred progressively as the crude oil concentration was increased. Lowest protein yield for this organism was observed at a crude oil concentration of 2% at which the least protein production of 0.03 mg/mL was produced after 20 h cultivation.

Key words: Crude oil, aquatic bacteria; protein; biomass.

Introduction

Crude oil is a complex mixture that consists of hydrocarbon, (including alkanes, cycloalkanes and anomatic hydrocarbons) and non-hydrocarbon (including resin and asphalt) (Jiang *et al*; 2010). Crude oil is physically, chemically and biologically harmful to the environment because it contains many toxic compounds in relatively high concentrations. Ever since the discovered of oil in Nigeria in the 1950's, the country has been suffering the negative environmental consequences of oil exploration and exploitation. The growth of the country's oil industry, combined with a population explosion and lack of enforcement of environmental regulations has led to significant contamination of the environment. The effects of numerous and varied types of spillages on estuarine wetlands over the years has become a source of concern. A vast amount of petroleum wastes are produced during activities related to exploration, production refinement and transportation of oil and gas products which cause serious damage especially to the marine and estuarine environments. The major contributor to the environmental pollution is oil spillage which refers to the accidental discharge of crude oil or refined petroleum products on land or water during the process of transportation or distribution resulting in environmental pollution. This incidence of oil spillage happen in some parts of the world. Nigeria is a major exporter of crude oil and has experienced several oil spills.

Petroleum hydrocarbons are the most common environmental pollutants (El-Sheshtawy *et al*., 2013). The contamination of habitats constitutes public health hazards. The hydrocarbons so discarded may pose serious aquatic toxicity problems. Xenobiotics may affect the microorganisms, biochemically and genetically and may also lead to inhibition of microbial growth. Pollutants many inhibit some microbial communities that are important in some biogeochemical cycles of the ecosystem as this affects microbial productivity in such ecosystem. With the development of the oil industry the general environment particularly the wetland ecosystem has become vulnerable to the toxic effects of oil pollution. Contamination of aquatic environment by crude oil and petroleum products constitute an additional source of stress to aquatic organisms (Omoregie *et al*., 1997).

Bacteria are ubiquitos and capable of rapid growth when provided with nutrients and conditions favourable for metabolism and cell division. Many marine bacteria have demonstrated the capacity to remove, under optimal conditions, some selected fractions of crude oil in a matter of days or weeks so that a certain percentage of oil by weight will disappear in a proportionate time but the remaining fractions may be more refractory to microbial attack. The abundance of bacterioplankton and phagotrophic protists in the presence of crude oil suggested enhanced carbon transfer from bacterial production to higher order organisms in an ecological loop. This maybe an important ecosystem component based on bacterial production in an oil contaminated habitat. Common crude oil degraders found in the environments are *Pseudomonas, Achromobacter*, *Flavobacterium, Acienetobacter, Bacillus, Arthrobacter, Nocardia* (Ubalua 2011). The aim of this present work was to investigate the effects of various concentrations of crude oil on biomass and protein production by some aquatic bacteria.

Materials and Methods

Bacterial isolation: Water sample was collected from Nembe River in Bayelsa State, Nigeria. This river is periodically polluted with crude oil. The sample was collected into a screw – capped conical flask at a depth of about 10cm from the surface and was taken into the laboratory for microbiological analysis. About 0.1mL of the water sample was plated onto Nutrient agar (Oxoid, Ltd., UK) plates containing 0.1% clotrimazole to suppress fungal contaminants. Plates were incubated at 30±2^{o}C for 24 h. Pure cultures were obtained by streaking on fresh agar plates and the isolated bacteria were identified based on the taxonomic scheme given in Bergey's Manual of Determinative Bacteriology (Holt *et al.*, 1994).

Inoculation and biomass production: Into Nutrient broth (100 mL) contained in conical flasks was each added 0.5, 1.0, 1.5 and 2 % (v/v) Bonny light crude oil. A control experiment devoid of crude oil was separately prepared. The medium was dispensed in 10 mL aliquots in test tubes and autoclaved at 121^{o}C for 15 min. The isolates were diluted to optical density (OD) 0.1 at 600nm measured in a Spectrum lab 23A spectrophotometer and 0.5 mL aliquot was each added into the tubes. The inoculated tubes were incubated for 20 h at 30±2^{o}C on a shaker (Fisher Roto Rack Model 343). Optical density readings were measured at 600nm.

Protein determination: Bacterial protein was precipitated with 10% trichloroacetic acid and determined according to the method of Lowry *et al.* (1951) using bovine serum albumin (Sigma-Aldrich) as a standard.

Statistical Analysis

Analysis of variance (ANOVA) and least significant difference (LSD) were used to determine the significant differences among mean values where by $p \leq 0.05$ was considered significant.

Results and Discussion

A total of three bacteria were isolated from the river sample. The bacteria were identified as *Pseudomonas aeruginosa, Aeromonas hydrophyla* and *Vibrio fisheri*. Figs. 1-3 show the average optical densities of the isolates. Biomass production by the organisms was concentration dependent. There was a correspondingly higher biomass yield of *Pseudomonas aeruginosa* at all concentrations of the crude oil. The best optimum growth of 0.88 (OD_{600nm}) was observed after 20 h when the level of crude oil in Nutrient broth was maximum (2%). The lowest growth of 0.77 (OD_{600nm}) after 20 h was achieved when the level of crude oil in the growth medium was lowest (0.5%). These levels were higher than control samples devoid of crude oil (Fig. 1) but showed no statistical significance. There are reports on the utilization of complex hydrocarbon mixtures like crude oil by isolated *Pseudomonas* species. Shailubhai *et al*. (1985) reported the degradation of oil sludge by pure strains of *Rhodotorula rubra* and *Pseudomonas aeruginosa*. Foght *et al*. (1989) reported the utilization of crude oil by pure bacterial strains of *Acinetobacter calcoaceticus* RAG-1 and *Pseudomonas sp*. HL7b. Margesin and Schinner (1999) investigated biodegradation of diesel oil by two psychotrophic bacteria that were assigned to the genera *Pseudomonas sp*. and *Arthobacter sp*. *Pseudomonas* was able to biodegrade crude oil and its components and used them as its sole carbon source (Leahy and Colwell, 1990).

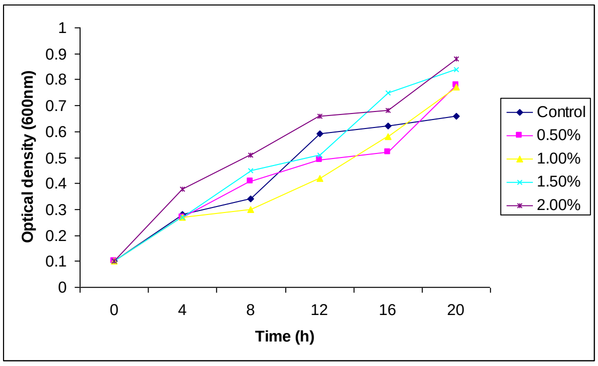

Figure 1: Effects of crude oil on biomass production by *Pseudomonas aerouinosa*

Figure 2 shows the effects of crude oil on biomass production by *Aeromonas hydrophyla*. Growth of this bacterium was low throughout the experiment. At the lowest concentration of crude oil (0.5%), maximum biomass level of 0.58 (OD_{600nm}) was observed after 20 h cultivation. This level decreased with increasing crude oil concentration (Fig. 2). Lowest growth of 0.04 (OD_{600nm}) was observed after 20 h at the highest crude oil level (2%). These levels were statistically ($p \le 0.05$) different from the control cultures.

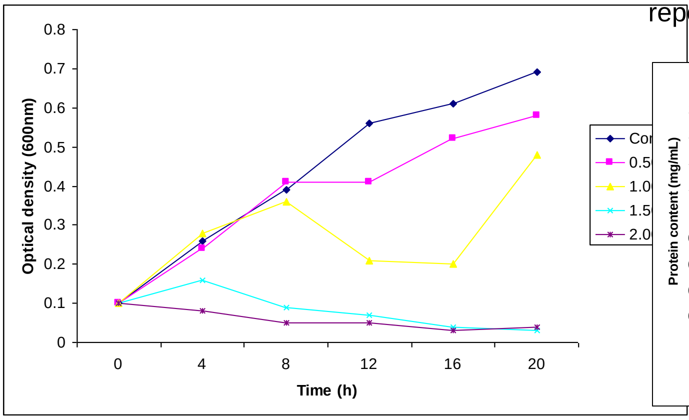

Figure 2: Effects of crude oil on biomass production by *Aeromonas hydrophila*

The effects of crude oil on biomass production in *Vibrio fisheri* culture is shown in Fig 3. Growth suppression ability of crude oil on this bacterium was evident at higher concentrations. At 0.5% crude oil concentration, maximum biomass level was produced. This level decreased progressively with increasing crude oil concentration. Maximum growth suppression occurred at the highest crude oil concentration of 2% at which the bacterial biomass level of 0.01(OD_{600nm}) was produced. These levels showed statistical difference ($p \le 0.05$) from the control samples.

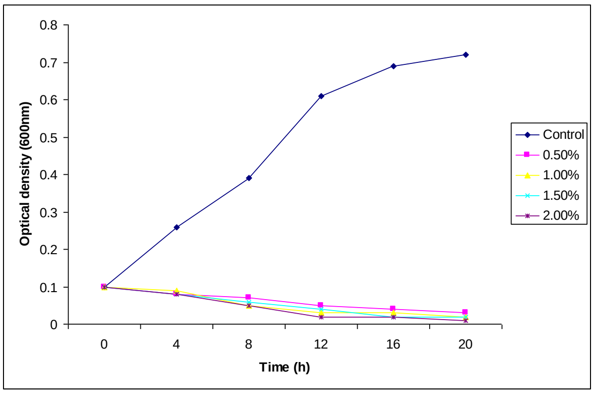

Figure 3: Effects of crude oil on biomass production by *Vibrio fischeri*

The concentrations of protein produced by *Pseudomonas aeruginosa* is shown in Fig. 4. This reveals a trend of protein increases with increases in crude oil concentration. At a crude oil concentration of 2%, maximum protein production (1.76 mg/mL) was produced after 20 h. This level was much lower at 0.5% crude oil level (Fig. 4). Control culture produced only 1.05 mg/mL of bacterial protein after 20 h. This phenomenon was attributed to the fact that the bacterium utilized the oil as a carbon source for growth as previously reported (Leahy and Colwell, 1990).

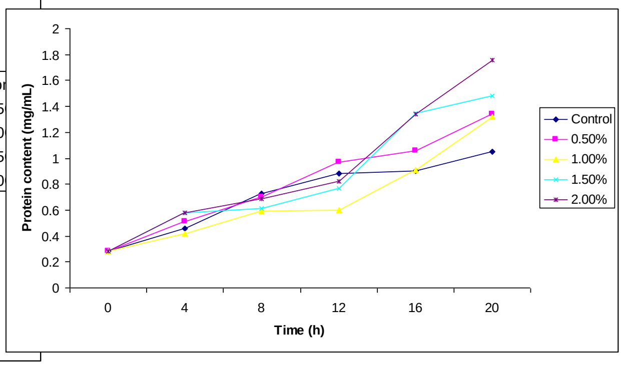

Figure 4: Effects of crude oil on protein production by *Pseudomonas aeruginosa*

Protein production by *Aeromonas hydrophyla* cultures is shown in Fig. 5. Protein content started to decrease from 0.12 mg/mL after 20 h at crude oil concentration of 0.5%, reaching a minimum of 0.06 mg/mL after 20 h at a maximum crude oil concentration of 2%. Protein of control cultures increased from 0.28 mg/mL at 0 h to a maximum level of 1.2 mg/mL after 20 h.

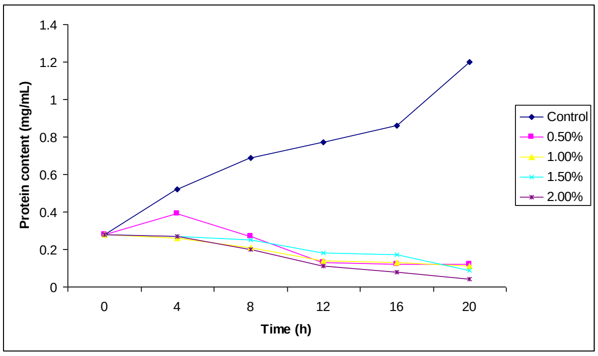

Figure 5: Effects of crude oil on protein production by *Aeromonas hydrophila*

The maximum protein production by culture of *Vibrio fisheri* was observed for control sample while that of oil treated cells gradually fell from 0.28 mg/mL at 0 h to 0.03 mg/mL after 20 h at the highest crude oil concentration of 2% (Fig. 6).

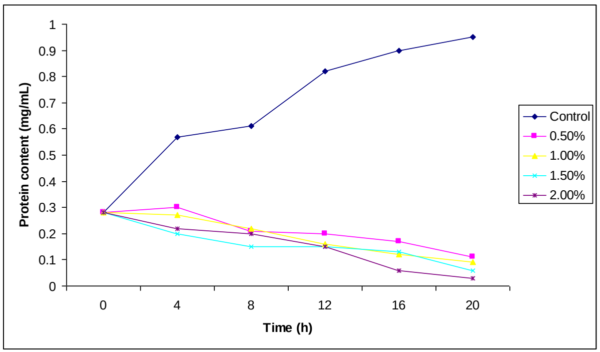

Figure 6: Effects of crude oil on protein production by *Vibrio fischeri*

Quantitative structure – activity relationship studies show that the toxicity of organic compounds depends primarily on the accumulation capacity in organisms and the ability to interact with the receptors (Jiang *et al*., 2010). There are a few reports on the toxicity of crude oil on aquatic bacteria. Most research work centers on the degradation of crude oil by bacteria (Shailubhai *et al*., 1985; Margesin and Schinner 1999; Mukherjee and Bordoloi, 2012). Das and Mukherjee (2007) reported that *Bacillus subtilis* and *Pseudomonas aeruginosa* utilized crude petroleum oil as sole source of carbon and energy which was evident from the increase in bacterial dry biomass, protein contents and cell density after 120 h incubation.

Hydrocarbons exert their toxic effects by entering the lipophilic layer of the cell membrane and preventing the transport of ions in and out of the cell. Toxicity of hydrocarbons on bacteria also occurs through disruption of cellular metabolism. Adapted microbial communities have higher proportion of hydrocarbon degraders that can respond to the presence of hydrocarbon pollutants (Okerentugba and Ezeronye, 2003). The principal forces limiting the biodegradation of polluting petroleum in the aquatic environments are the resistant and toxic components of oil itself, low water temperatures, scarcity of mineral nutrients (especially nitrogen and phosphorus), the exhaustion of dissolved oxygen, and in previously unpolluted areas, the scarcity of hydrocarbon – degrading microorganisms (Atlas, 1981). The water soluble fraction of crude oils has been found to reduce the growth rate and biomass of some bacteria. Aquatic bacterial population was reduced in stimulated oil spill experiment (Lekunberri *et al*., 2010). Higher concentrations of crude oil caused growth inhibition due to the higher amounts of toxic components of crude oil. Growth inhibition of bacteria at higher crude oil concentrations has been ascribed to several effects such as decreased growth rate (Amadi *et al*., 1996); decreased cell number (Adesina and Adelasoye 2004); decreased biomass yield (Yao *et al*., 2003); changes in respiration rate (Minoui *et al.,* 2008); changes in nitrifying activity (Urakawa *et al*., 2012); induction of membrane toxicity (Sikkema *et al*., 1995); changes in bacterial activity and composition (Lekunberri *et al*., 2010). Biomass and protein contents of *Aeromonas hydrophyla* and *Vibrio fisheri* were lower in crude oil polluted media. It is not surprising that *Pseudomonas aeruginosa* produced high biomass and bacterial protein than the control at maximum crude oil level of 2%. From this finding, the ability of *Pseudomonas aeruginosa* to produce the best biomass and protein in maximum crude oil levels was therefore related with its capacity to colonize environments contaminated with petroleum hydrocarbon and to use petroleum hydrocarbon compounds as source of carbon and energy for growth.

Conclusion

This study demonstrated that different indigenious aquatic bacteria namely *Pseudomonas aeruginosa, Aeromonas hydrophyla* and *Vibrio fisheri* responded differently to various concentrations of crude oil. Higher concentrations of crude oil decreased the growth and protein production among *Aeromonas hydrophyla* and *Vibrio fisheri* cultures. *Pseudomonas aeruginosa* produced maximum biomass and protein at highest crude oil levels and the values obtained for this bacterium were not significantly different in comparison to control cultures, devoid of crude oil. The eological implication from this study is that growth stimulation or inhibition of bacterioplankton in aquatic environments by petroleum hydrocarbon pollution could effect an imbalance on the ecological structure and trophic inter-relationship in an aquatic eco-system.

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