Full Length Research Paper

Uptake of crude petroleum hydrocarbons by mudflat bacteria exposed to nitrogenous fertilizer plant effluents

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In the Niger delta, due to the large-scale exploration and exploitation of crude petroleum, hydrocarbon spills frequently occur. The adverse impacts of these occurrences have frequently been causes for concerns in the area. However, in open estuarine environments, minor spills have short-term effects due to washout by stream water and increased microbial action. The microbial degradation of low level spills is enhanced by the 'continuous' input of nitrogenous fertilizer (NPK) components. The 'continuous' input assists in mitigating dilution effects of the stream water thus increasing the availability of nutrients to the petroleum degrading microbes. The net result is the increased recovery potential of this estuarine environment in the event of pollution by crude oil hydrocarbons. Enumeration of viable aerobic heterotrophic bacteria showed that counts were higher in hydrocarbon amended nitrogenous fertilizer plant effluent than in the raw effluent. The higher counts were accompanied by a rapid decline in the level of crude petroleum in the amended effluent. The ability of aerobic heterotrophic mudflat bacteria to grow on three different refined petroleum products namely kerosine, diesel and engine oil was also studied. At the levels tested, there were no appreciable differences in growth patterns of petroleum utilizing bacteria in three refined products after 5 weeks incubation at ambient temperature.

Key words: Crude, petroleum, degradation, heterotrophic, bacteria, fertilizer, effluent.

INTRODUCTION

Aquatic bacteria exhibit a variety of responses to pollutants. Whereas organic wastes frequently increase bacterial abundance, e.g. in river waters (Rheinheimer, 1965; Deufel, 1972; Coleman et al., 1974; Kachan, 1976; Starzecka, 1979) and sediments (Ossowska-Cypryk, 1981), inorganic wastes may provoke a different response. Inorganic wastes such as phosphates and nitrogenous materials that may be derived from fertilizer factory effluent discharges may result in algal blooms in streams and rivers. Pollution may also cause change in the taxonomic composition of aquatic bacterial communities.

For bacteria to grow efficiently they require about ten parts carbon to one part nitrogen (Olivieri et al., 1976). If the ratio is greater such as can occur during oil spills, e.g. 100 : 1 or 1000 : 1, growth of the bacteria and utilization of the carbon(s) will be retarded. Biodegradation by aquatic micro organisms plays an important role in the fate of organic pollutants. Biodegradation of petroleum hydrocarbons in the aquatic and terrestrial environments has been studied extensively (Blumer and Sass, 1972; Farrington, 1980; Lee, 1980; Fusey and Oudot, 1984; Oudot, 1984).

Several approaches at solving the problem of oil pollution have been described (Garret, 1969; Meijs et al., 1969; Wang et al., 1975; Olivieri et al., 1976), but each has its limitations. Numerous investigators have studied the possibility of using micro-organisms to increase the natural biodegradation of petroleum (Le-Petit and Barthelemy, 1968; Byrom and Beastall, 1971; Atlas and Bartha, 1972; Mulkins-Philips and Stewart, 1972; Soli and Bens, 1972; Schwarz et al., 1974), but it is too slow to have any practical importance for oil clean up. Inoculation of oil-polluted areas with oil-decomposing micro-organisms
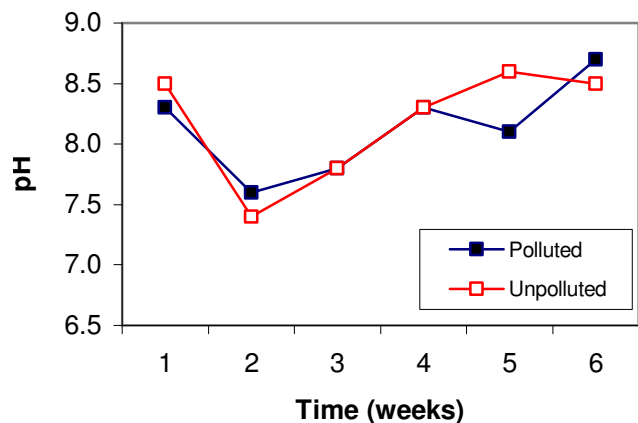
Figure 1. Bacteria induced changes in pH of fertilizer factory effluent amended with crude petroleum hydrocarbons.

Figure 2. Nitrate and phosphate profiles of fertilizers factory effluent amended with crude petroleum hydrocarbons.

seems to be ineffective because of growth-limiting nitrogen and phosphorus concentrations in seawater (Le-Petit and Barthelemy, 1968; Zobell, 1969).

Biodegradation of crude petroleum by heterotrophic bacteria in systems receiving NPK fertilizer factory effluents has been studied (Adoki et al., 1999). Reports show that urea and phosphorus sources were able to enhance biodegradation of crude petroleum. The present study further examines biodegradation of crude and refined petroleum by sediment bacteria in NPK fertilizer factory effluent. The results should provide an insight to the fate of crude petroleum and refined petroleum in an estuarine system receiving these fertilizer effluents.

MATERIALS AND METHODS

Samples

Effluent and sediment samples from the outfall point of the National Fertilizer Factory at Onne in Rivers State, Niger delta were collected using procedures outlined by APHA (1975).

Biodegradation tests

Biodegradation tests were carried out in 1000 ml bottles. Only batch cultivations were monitored. To each of twelve 1000 ml pre-sterilized bottles was added 495 ml of fertilizer factory effluent as nutrient medium, 5 ml of Bonny medium crude oil using a sterile glass pipette and 2 g sediment as inoculant. The bottles were subsequently stoppered and incubated at room temperature (28 ± 2ºC) with aeration/agitation through Teflon tubes by means of Teacax AP-1500 air pump. Seawater with no sediment addition was also included in the tests.

At intervals of seven days, one bottle was withdrawn for microbiological and physicochemical analyses. Viable counts of heterotrophic and petroleum-utilizing bacteria and determination of residual hydrocarbon contents were made using the methods of Institute of Pollution Studies (IPS) (1988), Cruickshank et al. (1975), Cowan and Steele (1974) and Olivieri et al. (1978), respectively. Petroleum utilizing bacteria were enumerated on a medium with the following composition (IPS, 1988): ammonium chloride (NH_4Cl), 0.5
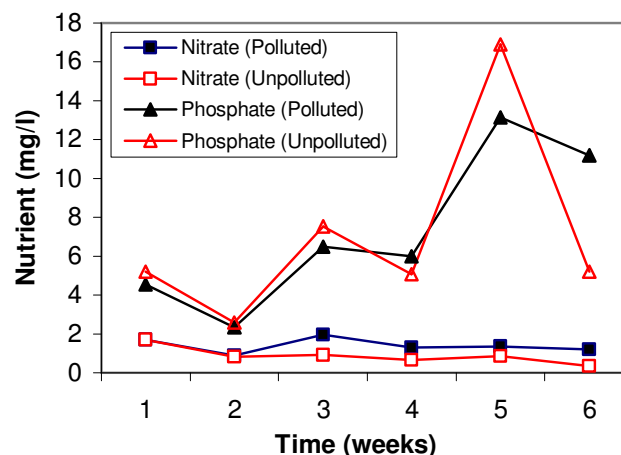
g; disodium hydrogen phosphate ($Na_2HPO_4.12H_2O$), 2.5 g or 1 g of anhydrous salt; dipotassium hydrogen phosphate (K_2HPO_4), 0.5 g; engine oil mixture (Engine oil: diesel; 50:50), 0.5% v/v; agar (Oxoid), 15 g; estuarine salt solution, 750 ml; and distilled water, 250 ml. The estuarine salt solution had the following composition: sodium chloride (NaCl), 10 g; magnesium chloride ($MgCl_2$), 2.5 g; potassium chloride, 0.3 g; and distilled water, 1L.

Water chemistry

The following physico-chemical parameters, which include pH, conductivity, turbidity, salinity, dissolved oxygen (fresh sample only) ammonium nitrogen, phosphate, nitrate and sulphate were determined by methods and procedures outlined by APHA (1975).

RESULTS AND DISCUSSION

Figure 1 shows changes in pH of fertilizer factory effluent by stream sediment bacteria induced by the addition of crude petroleum hydrocarbons. The figure shows that for about 4 weeks no significant trend in pH was observed in both the unpolluted and polluted conditions. However, beyond 4 weeks reversed trends were recorded. By contrast, no such reverse trends were recorded for phosphate and nitrate levels (Figure 2). The levels of these nutrients did not show very significant differences in the polluted and unpolluted conditions. However, a very sharp drop in phosphate level was recorded in the unpolluted condition after 5 weeks incubation at room temperature. Beyond 5 weeks, a very sharp decline was recorded in phosphate level in the unpolluted culture. Uptake of nitrate was generally higher in the unpolluted culture as reflected in the lower levels recorded between 2 and 6 weeks of incubation. Nutrient uptake was adversely affected by amendment with petroleum hydrocarbons.

Figure 3 shows that sulphate levels were generally low in hydrocarbon polluted samples. Uptake patterns were
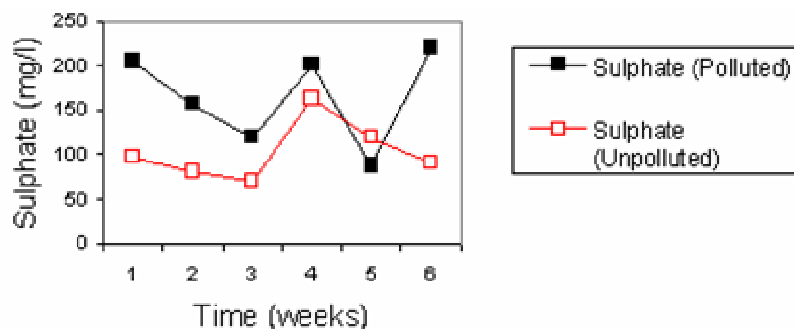
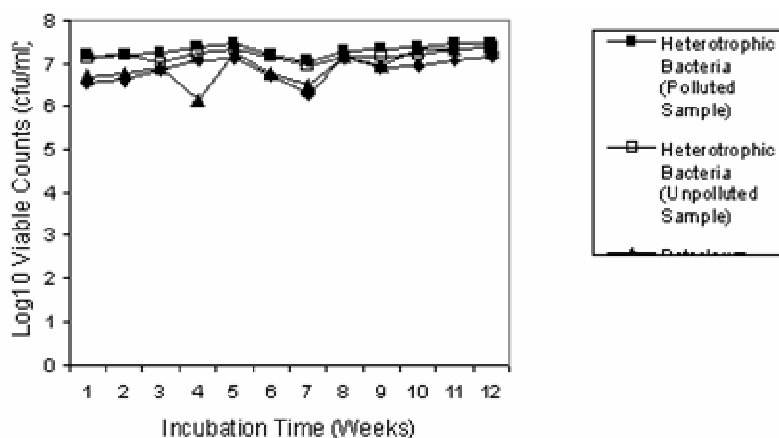**Figure 3.** Sulphate profile of fertilizer factory effluent amended with crude petroleum hydrocarbons.

Figure 4. Viable counts of bacteria in crude petroleum hydrocarbon amended effluent.
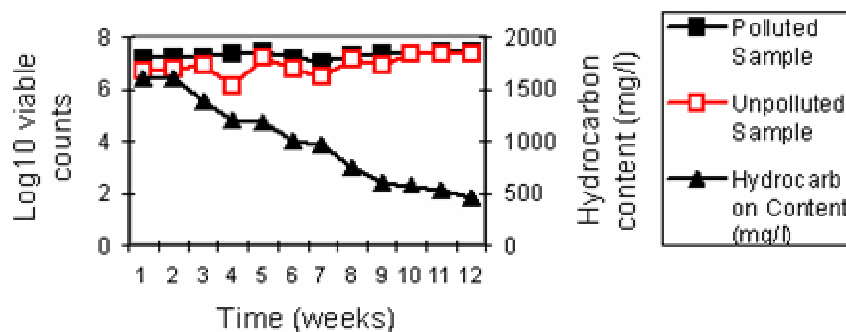

Figure 5. Uptake of crude petroleum hydrocarbons by aerobic heterotrophic bacteria in fertilizer effluent.

however similar in both cultures. These trends were also exhibited in counts of viable aerobic heterotrophic bacteria. Counts were slightly higher in cultures exposed to petroleum hydrocarbons. This could be ascribed to the additional carbon source provided by the hydrocarbons (Figure 4). Similar trends were recorded in counts of petroleum-utilizing bacteria (Figure 4).

Enumeration of viable aerobic heterotrophic bacteria showed that counts were higher in hydrocarbon amended effluent. The higher count was accompanied by a rapid decline in the level of crude petroleum in the amended effluent as shown in Figure 5. The ability of aerobic heterotrophic mudflat bacteria to grow on refined petroleum products was also studied. The results (Figure 6)
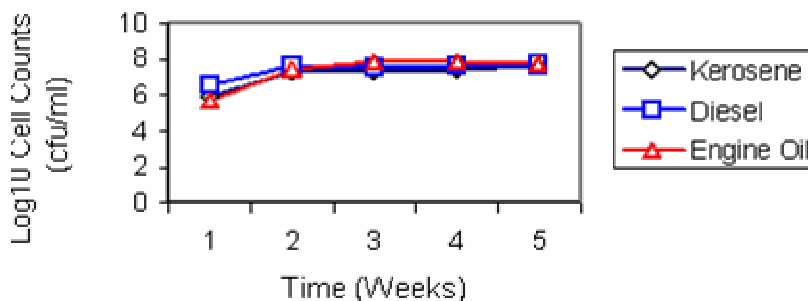
Figure 6. Growth profile of aerobic heterotrophic bacteria in cultures amended with refined petroleum products.

show that there was no significant difference in growth patterns of petroleum utilizing bacteria in the three refined products tested after 5 weeks incubation at room temperature.

Several approaches at solving the problem of oil pollution have been described (Garret, 1969; Meijs et al., 1969; Wang et al., 1975; Olivieri et al., 1976), but each has its limitations. The possibility of using micro-organisms to increase the natural biodegradation of petroleum (Le-Petit and Barthelemy, 1968, Byrom and Beastall, 1971; Atlas and Bartha, 1972; Mulkins-Philips and Stewart, 1972; Soli and Bens, 1972; Schwarz et al., 1974) has been investigated, but this process is too slow to have any practical importance for oil clean-up. Inoculation of oil-polluted areas with oil-decomposing micro-organisms seems to be ineffective because of growth-limiting nitrogen and phosphorus concentrations in seawater (Le-Petit and Barthelemy, 1968; Zobell, 1969).

In the Niger delta, due to the large-scale exploration and exploitation of crude petroleum hydrocarbon spills frequently occur. The adverse impacts of these occur-rences have frequently been causes for concerns in the area. However, in open estuarine environments such as the NAFCON creek, minor spills have short-term effects due to washout by stream water and increased microbial action. The microbial degradation of low level spills is enhanced by the 'continuous' input of nitrogenous fertili-zer (NPK) components. The 'continuous' input assists in mitigating dilution effects of the stream water thus increasing the availability of nutrients to the petroleum degrading microbes. The net result is the increased recovery potential of this estuarine environment in the event of pollution by crude oil hydrocarbons.

REFERENCES

Adoki A, Sokari TG, Ibiebele DD (1999). Effects of amendments on biodegradation of crude petroleum by sediment bacteria from Bonny River Estuary. Afr. J. Ecol. 7: 258-266.

APHA (1975). Standard Methods for the examination of Water and Wastewater. 14th edn. American Public Health Association APHA-AWWA-WPCF. John D. Lucas Co. Baltimore

Atlas RM, Bartha R (1972). Degradation and mineralization of petroleum in seawater: limitation by nitrogen and phosphorus. Biotechnol. Bioeng. 14: 309-318.

Blumer M, Saas J (1972). Oil pollution: persistence and degradation of spilled fuel oil. Science 176: 1120-1122.

Byrom JA, Beastall S (1971). Microbial degradation of crude oil with particular emphasis on pollution. Environ. Pollut. 69: 237-257.

Coleman RN, Campbell JN, Cook FD, Westlake DWS (1974). Urbanization and the microbial content of the North Saskatchewan River. Appl. Microbiol. 27: 93-101.

Cowan ST, Steele KJ (1974). Manual for the Identification of Medical Bacteria. 2nd edn. Cambridge University Press, London.

Cruickshank R, Duguid JP, Marmion BP, Swain RHA (1975). Medical Microbiology, Vol. 2. The Practice of Medical Microbiology 12th Edn. Churchill Livingstone, Edinburgh. pp. 273-283.

Deufel J (1972). Die Bacterian –und Keimzahlem in Oberlauf der Donau bis Ulm. Arch. Hydrobiol. 44(Suppl.): 1-9.

Farrington JW (1980). An overview of the biogeochemistry of fossil fuel hydrocarbon in the marine environment. In: Petroleum in the Marine Environment (Eds L. Petrakis and F.T. Weiss). Adv. Chem. Ser. 185: 1-22.

Fusey P, Oudot J (1984). Relative influence of physical removal and biodegradation in the depuration of petroleum-contaminated seashore sediments. Mar. Pollut. Bull. 15: 136-141.

Garret WD (1969). Confinement and control of oil pollution on water with monomolecular surface films. In: Proceedings of the Joint Conference on Prevention and Control of Oil Spills. American Petroleum Institute, New York.

Institute of Pollution Studies (IPS) (1988). NNPC-EPSD Baseline Studies Project: Biological baseline Data Acquisition at NNPC Installations. A Technical Report. Institute of Pollution Studies (IPS), Rivers State University of Science & Technology, Port Harcourt, Nigeria.

Kachan VI (1976). Microbiology of Ros' River and some of its tributaries. Hydrobiol. J. 12: 76-78.

Lee RF (1980). Processes affecting the fate of oil in the sea. In: Marine Environ. Poll. Vol. 1 (Ed. Geyer RF).

Le-Petit J, Barthelemy MH (1968). The hydrocarbon in water: the problem of microorganisms using the littoral zones. Ann. Inst. Pasteur, Paris 144: 149-158.

Meijs FH, Schmit Jionbloed LJ, Tadema (1969) New methods for combating oil slicks. In: Proceedings of the Joint Conference on Prevention and Control of Oil Spills. American Petroleum Institute, New York.

Mulkins-Philips GJ, Stewart JF (1972). Effect of four dispersants on biodegradation and growth of bacteria on crude oil. J. Appl. Microbiol. 28: 547-552.

Olivieri R, Bacchin P, Robertiello A, Oddo N, Degen L, Tonolo A (1976). Microbial degradation of oil spills enhanced by a slow-release fertilizer. J. Appl. Environ. Microbiol. 33: 629-634.

Olivieri R, Robertiello A, Degen L (1978). Enhancement of microbial degradation of oil pollutant using lipophilic fertilizers. Mar. Pollut. Bull. 9: 217-220.

Ossowska-Cypryk K (1981). Microbiological studies of waters and bottom sediments of Utrata River. Pol. Arch. Hydrobiol. 38: 357-375.

Oudot J (1984). Rates of microbial degradation of petroleum components as determined by computerized capillary gas chromatography and computerized mass spectrometry. Mar. Environ. Res. 13: 277-302.

Rheinheimer G (1965). Mikrobiologische Untersuehungen in der Elbe zwischen Schnackenburg und Cuxhaven. Arch. Hydrobiol. 29: 181-251.

Schwarz JR, Walker JD, Colwell RR (1974). Deep-sea bacterial growth and utilization of hydrocarbon at ambient and *in-situ* pressure. J. Appl. Microbiology, 28: 982-986.

Soli G, Bens EM (1972). Bacteria which attack petroleum hydrocarbons in a saline medium. Biotech. Bioeng. 14: 319-330.

Starzecka A (1979). Bacteriological characteristics of water in the River Nida and its tributaries. *Acta.* Hydrobiol. Krakov, 21: 341-360.

Wang IK, Yank JY, Dahm DB (1975). Separation of emulsified oil from water. Chem. Ind. London, 13: 562-564.

Zobell CE (1969). Microbial modification of crude oil in the sea. In: Proceedings of the Joint Conference on Prevention and Control of Oil Spills. American Petroleum Institute, New York.