# Microbial degradation of four Nigerian crude oils in an estuarine microcosm

**Article** *in* Letters in Applied Microbiology · June 2008

Impact Factor: 1.66 · DOI: 10.1111/j.1472-765X.1993.tb01374.x



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*Letters in Applied Microbiology* 1993, **16**, 118–121

# Microbial degradation of four Nigerian crude oils in an estuarine microcosm

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*MS/277: received 9 September 1992 and accepted 11 September 1992*

Amund, O.O. & Akangbou, T.S. 1993. Microbial degradation of four Nigerian crude oils in an estuarine microcosm. *Letters in Applied Microbiology* **16**, 118–121.

Four Nigerian crude oils (Bonny Light, Bonny Medium, Escravos Light and Forcados Blend) that differ substantially in fractional composition were exposed to the Lagos Lagoon waters in microcosm experiments with oil-impregnated membrane filters. Changes in microbial numbers on the membranes and in the residual oil concentration showed a relationship between the fractional composition and the biodegradation rates of the oils, with the lighter oils disappearing more rapidly. After 10 weeks exposure in the lagoon, only 15% (w/w) of the Bonny Light crude remained on the filters as compared with 20, 32 and 45% (w/w) for Escravos Light, Bonny Medium and Forcados Blend respectively. The hydrocarbon-utilizing microbial colonizers of the oil-impregnated membranes were *Micrococcus, Bacillus, Pseudomonas, Flavobacterium, Alcaligenes* and *Aspergillus niger*.

Microbial degradative mechanisms appear to be the natural processes which eliminate the bulk of oil pollutants after initial physical and chemical breakdown has occurred (Floodgate 1972; Zobell 1964; Atlas 1981). Crude oils vary in their physical properties, chemical composition and between fields of origin; of special importance are the relative amounts of high and low molecular weight compounds present in crude oils. Heavy oils contain a greater percentage of high molecular weight components. The low biodegradation rates of heavy oils probably reflect the resistance of the complex high-molecular weight components to microbial degradation (Atlas 1975).

Two major types of crude oil (light and medium) are produced in various oilfields within the Niger Delta area of Nigeria. Increased production of crude oil makes it necessary to open new fields and involves repeated spillages and pollution incidents in the creeks and salt marshes of the Niger Delta. The work reported here was undertaken to assess the biodegradability and relative persistence of four Nigerian crude oils as a function of their fractional composition under the estuarine conditions typical of the Lagos lagoon.

## Materials and Methods

### SOURCES OF CRUDE OIL SAMPLES

Bonny Light and Bonny Medium crude samples were obtained from the Bonny Terminal of the Shell Petroleum Development Company of Nigeria. Escravos Light crude sample was supplied from the Escravos Terminal of Chevron (Nigeria) Limited while the Forcados Blend crude sample was obtained from the Forcados Terminal of Shell. The specific gravity of the oils was measured gravimetrically with specific gravity bottles.

### FRACTIONATION OF CRUDE OIL SAMPLES

The fresh crude oil samples were separated into class fractions by liquid chromatography on a glass column (2 × 40 cm) packed with silica gel G. One millilitre of each oil was placed on the column and separation of the paraffinic (saturates), aromatic and asphaltenic fractions was accomplished by successive elution with 120 ml aliquots of hexane, benzene and methanol respectively. Each solvent phase was evaporated under reduced pressure and the residue weighed. The relative percentages of the fractions as well as the unresolved residues were determined gravimetrically.

### EXPOSURE OF OILS TO THE ENVIRONMENT

Typical microcosm experiments for the *'in situ'* measurement of oil degradation rates were carried out as previously described (Gilbert and Higgins 1978; Mahadi and Watkinson 1988; Amund and Igiri 1990). Oiled membrane filters (0·25$\mu$ pore size) were inserted into perforated plastic balls which were sealed with a hot knife and placed in perforated aluminum containers before immersion into Lagos lagoon water as previously described (Amund and Igiri 1990). Non-biological leaching controls were set up in the laboratory with sterile lagoon water as the immersion medium. Filters withdrawn from the field and the laboratory at 2 week intervals were extracted with 10 ml of carbon disulphide while the absorbance of the extract was measured with an infra-red spectrophotometer (Phillips) as described by Simard *et al.* (1951). The oil content was extrapolated from a standard calibration curve.

### ASSESSMENT OF MICROBIAL POPULATIONS

On recovery of filters from the environment, each was placed in 10 ml of sterile distilled water in McCartney bottles and shaken vigorously for 5 min with the aid of a vortex mixer to free the micro-organisms from the filter surfaces. Total heterotrophic counts were carried out by plating aliquots (0·1 ml) of appropriate dilutions of the cell suspension on nutrient agar and incubating at room temperature (28 $\pm$ 2·0°C) for 48 h and counting the resulting colonies. Total counts of hydrocarbon-utilizing micro-organisms were similarly determined on minimal agar plates as previously described by Amund *et al.* (1987) and Amund and Igiri (1990). Crude oils used as carbon sources were introduced by vapour-phase transfer by placing filter discs impregnated with the oils into the lids of Petri dishes (Raymond *et al.* 1976). Hydrocarbon-utilizing bacterial strains were isolated and identified by the identification schemes of Cowan (1974). Diagnostic properties used include Gram reaction, motility, colonial morphology, production of cytochrome oxidase, catalase, indole and urease, gelatin liquefaction, starch hydrolysis, oxidation/fermentation of sugars, Methyl red test, Voges Proskaeur test, growth at 42°C and 5°C, and sugar utilization tests. Fungal isolates were identified on the basis of morphological properties.

## Results and Discussion

The four crude oil samples used in this study differed in their specific gravities as well as in their fractional composition (Table 1). The lighter oils (Escravos and Bonny light crudes) were richer in saturate fractions and lower in asphaltenes than the heavier oils (Table 1). This is consistent with the observations of Atlas (1975) who contended that lighter oils contain a greater percentage of low-molecular weight components.

The oil-impregnated membrane filters exposed to the lagoon water were colonized by bacteria and fungi. The counts of heterotrophs and hydrocarbon utilizers increased with exposure time for each crude oil sample (Fig. 1). These increases in counts were accompanied by

**Table 1.** Fractional composition and specific gravity of Nigerian crude oils

| | Crude oil fraction (%) | | | | |
|---|---|---|---|---|---|
| | Saturates | Aromatics | Asphaltenes | Residue | Specific gravity |
| Bonny | | | | | |
| light | 81·11 | 7·20 | 2·48 | 9·21 | 0·84 |
| medium | 64·90 | 13·37 | 13·37 | 8·36 | 0·98 |
| Escravos light | 69·74 | 22·05 | 2·56 | 5·65 | 0·78 |
| Forcados blend | 58·89 | 11·10 | 3·40 | 26·61 | 0·88 |

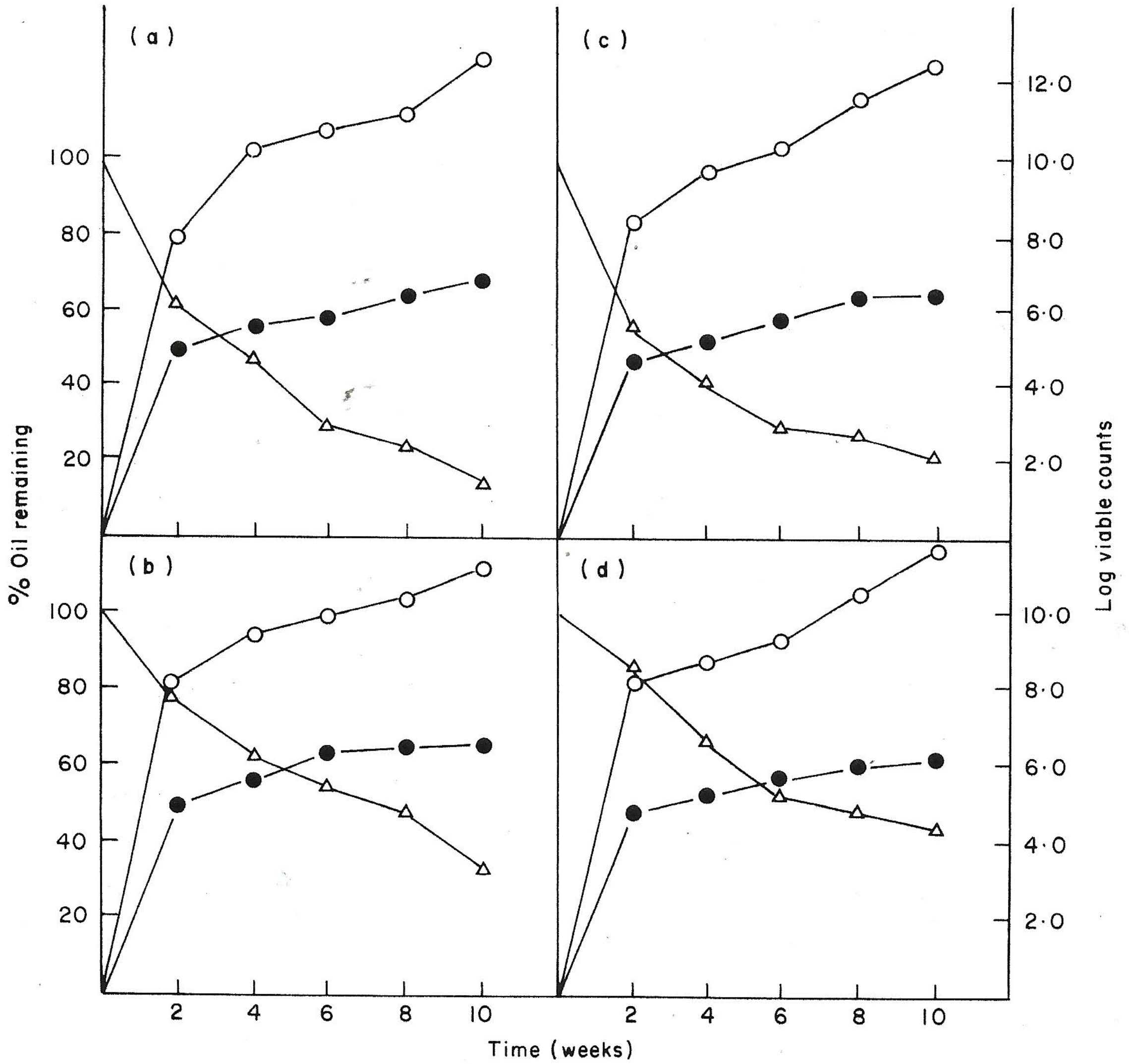


**Fig. 1.** Changes in the bacterial population density and crude oil concentration on oil-impregnated membranes exposed to the Lagos lagoon water. (a) Bonny Light Crude; (b) Bonny Medium Crude; (c) Escravos Light Crude; (d) Forcados Blend Crude. ○, Total heterotrophs; ●, hydrocarbon utilizers; △, crude oil concentration.

corresponding decreases in oil content of the membrane filters. However, the various oils differed in their degradation rates. After the 10-week exposure, 15–25% (w/w) of the lighter oils remained undegraded (Fig. 1a, c) while about 32–45% (w/w) of the heavier oils remained (Fig. 1b, d). Oil losses from membranes in the laboratory controls varied between 3·2 and 6·7% (w/w). The considerably high levels of residual oils extracted from membrane filters in the laboratory controls indicate that physical mechanisms including dissolution, evaporation, dispersion, emulsification, sedimentation and adsorption played a small role in oil losses in the aquatic environment. The lower biodegradation rates exhibited by the heavier oils (Bonny Medium and Forcados Blend) probably reflect their lower content of saturates and their high asphaltene content. Asphaltenes are known to be more resistant to microbial degradation (Atlas 1975). It should, however, be noted that although the total heterotrophic counts on membranes increased exponentially during weeks 2–10 of exposure, the number of hydrocarbon-utilizing bacteria stayed relatively constant after an initial increase during the first 2 weeks. Similar observations had earlier been made under temperate and tropical environmental conditions. (Gilbert and Higgins 1978; Mahadi and Watkinson 1988; Amund and Igiri

1990). These results were consistent with the rapid utilization of the growth-supporting fractions of the crude oils within the first 2 weeks leading to the initial population increases observed while the remaining fractions could only support slower rates of microbial growth. However, the rapid and continued increases in the total heterotrophic bacterial populations would suggest that the metabolic products of oil degradation on the membranes could support the growth of other heterotrophs present. These increases may also be consistent with the ability of some micro-organisms to attach to surfaces resulting in elevated population counts.

The hydrocarbon-utilizing microbial populations supported by the oiled filters included five bacterial species identified with the aid of diagnostic schemes of Cowan (1974) as *Micrococcus*, *Bacillus*, *Pseudomonas*, *Flavobacterium* and *Alcaligenes* and *Aspergillus niger*. The bacterial species were similar to those consistently reported as the dominant hydrocarbon utilizers in the Lagos lagoon (Amund and Igiri 1990; Amund and Adebiyi 1991). However, this is the first report of a hydrocarbon-utilizing fungus in the Lagos lagoon. The hydrocarbon utilizers constituted less than 1·0% of total heterotrophs and this could be attributed to the fact that the Lagos lagoon had not suffered from oil spillage other than minor accidental discharges of waste oils. Elevated counts of these organisms would reflect the degree of environmental exposure to hydrocarbon contaminants (Hampson and Sander 1969).

It is apparent from this investigation that the fractional composition of the Nigerian crude oils under test had a major effect on their biodegradation rates, with the lighter oils disappearing more rapidly from the environment. Oils with higher saturate fractions were also degraded more quickly indicating that the saturate fraction is the most important component with regard to oil biodegradability. It is this fraction that is first metabolized by oil-degrading micro-organisms in the environment.

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