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Effects of Discarded Drill Muds on Microbial Populations [and Discussion]

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Effects of discarded drill muds on microbial populations

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Drilling operations from platforms in the North Sea result in the production of large quantities of drill cuttings. These are a variable mixture of rock chippings, clays and original drilling fluids. Drilling mud is cleaned on the platform to remove rock chips before re-use of the mud. The rejected fraction from the clean-up plant (the cuttings) contains some of the base drilling fluid, and this can lead to an organically rich input to the sea-bed. Cuttings are discarded immediately underneath the platform jacket and thus build-up over the natural seabed sediment. In many cases this cuttings pile may cover considerable areas of seabed, leading to seabed biological effects and potential corrosion problems.

Different types of cuttings have different environmental impacts, this being partly dependent upon their hydrocarbon component. Diesel-oil based cuttings contain significant amounts of toxic aromatic hydrocarbons, whereas low-toxicity, kerosene-based cuttings contain less. Both types of cuttings support an active microbiological flora, initiated by hydrocarbon oxidation. This paper presents a study of microbiological degradation of hydrocarbons in cuttings piles around two North Sea platforms. Results indicate that there is a close correlation between microbiological activity and hydrocarbon breakdown in the surface of cuttings piles and that both of these parameters reach their maximum values closer to the platform when low-toxicity muds are in use.

Introduction

Drilling operations from North Sea platforms have a number of ecological impacts. By far the most important of these is the deposition of drilling-mud cuttings onto the seabed. This deposition has a number of effects on life in the sediment, primarily physical smothering and organic enrichment but also a direct toxic effect from the hydrocarbons, especially the aromatic fractions (Heildelberger 1975).

Drilling muds are used to lubricate the drill bit, to remove rock chips to the surface and to control reservoir pressure (figure 1).

Drilling mud is pumped down the inside of the drill string and forced up the well casing, carrying the drilled rock chips with it. The used mud is cleaned up by means of shale shakers, mud cleaners, to separate the rock chips (cuttings) from the mud. Mud is then re-used for drilling; cuttings are generally deposited down a cuttings caisson onto the seabed immediately beneath the platform structure, in most cases a tubular steel crossed braced jacket. In the northern North Sea, cuttings can build up into a deep pile on the seabed because of low residual seabed currents. The cuttings pile may be more than 3 cm deep at a distance of 250 m from the platform jacket. In spite of the clean-up and separation process, the cuttings contain significant amounts of mud associated with the rock chips. It is this residual mud, and its associated hydrocarbons, that causes some of the observed environmental impacts (Davies *et al.* 1984; Addy *et al.* 1984).

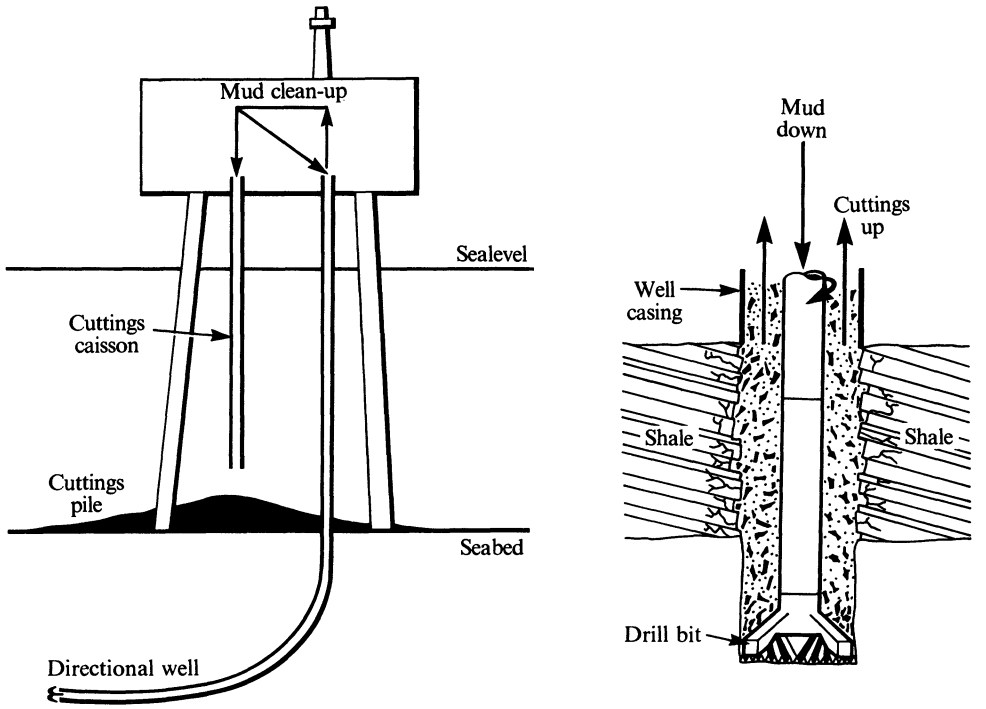

FIGURE 1. Diagrammatic representation of typical cuttings discharges and drilling operations in the North Sea.

There are many types of drilling mud, but the three main types are: water-based, diesel-based and low-toxicity muds. The composition of these three fluids is given in table 1.

Water-based muds are the most simple of the three types and have been used extensively in the North Sea. Their organic content is usually low and their environmental impact from hydrocarbon enrichment is not as significant as the other types. In addition, because the continuous phase is water, rather than hydrocarbons, they are more easily dispersed by seabed currents.

Water-based muds suffer from a number of limitations especially when used in deep, directionally drilled, wells. To reduce these limitations, oil-based muds have been increasingly used in the North Sea since 1978, either for drilling the entire well or for drilling the final sections. Owing to the large amount of diesel-oil in such fluids, there was concern about the

TABLE 1. TYPICAL COMPOSITION OF THREE TYPES OF DRILLING FLUIDS USED IN THE NORTH SEA

water-based muds	diesel-based muds	low-toxicity muds
water	water in oil emulsion	water in oil emulsion
polymers	diesel oil (70–80%)	mineral oil–kerosine (70–80%)
clays	water	water
barite	polymers	polymers
drilled solids (cuttings)	clays	clays
	barite	barite
	drilled solids (cuttings)	drilled solids (cuttings)

immediate and long-term environmental impact of dumping diesel-based cuttings because fresh cuttings may contain up to 17% residual hydrocarbons (Wilkinson 1982; Addy *et al.* 1984). In response to pressure to minimize these effects, low-toxicity drilling muds were developed. These are based upon mineral-oils or kerosenes, are low in aromatic components and have largely replaced diesel-based muds since 1982.

Diesel is the petroleum distillate fraction immediately above (higher boiling point than) kerosene. Gas–liquid chromatography (GLC) analysis of diesel oil generally displays a carbon number range of approximately C_{12} to C_{26} with the greatest concentration at C_{15} to C_{16}. This 'cut' contains a high relative abundance of aromatic hydrocarbons such as naphthalene, fluorene and phenanthrene and their alkylated homologues, which are thought to contribute greatly to the overall toxicity of diesel. Most of the low-toxicity base-oils, however, are based on the kerosene fraction of a crude-oil, and are further refined by solvent washing to remove the majority of the aromatic hydrocarbons. GLC analysis of a low-toxicity base-oil of this type generally shows a carbon number range of approximately C_9 to C_{16}, with the greatest concentration at C_{12}.

Oil discharged with the cuttings undergoes both biological and chemical weathering and physical loss by dispersion. The major factor involved in the degradation of petroleum hydrocarbon in the marine environment is likely to be microbiological, with numbers of hydrocarbon degrading microbes increasing because of the elevated hydrocarbon concentration (Atlas 1981). Active hydrocarbon degradation has been found in seabed sediment even in cold-water systems (Haines & Atlas 1983). Microbiological hydrocarbon oxidation is exclusively an aerobic phenomenon, mainly mediated by aerobic bacteria (eg. *Pseudomonas* spp., *Micrococcus* spp. and *Acinetobacter* spp.) although other aerobic organisms, such as yeasts and fungi, may also be involved. These microbes are able to utilize a very wide range of hydrocarbon fractions as sources of both carbon and energy. Oxidation liberates breakdown products such as fatty acids, aldehydes and alcohols (McKenna & Kallio 1965). There is usually a gradual succession in the fractions of oil which are degraded; *n*-alkanes followed by branched alkanes, cycloalkanes and then aromatic compounds. Although branched alkanes, such as the isoprenoids pristane (Pr) and phytane (Ph) are biodegraded, the process is slower than for the *n*-alkanes because branching, in general, increases the resistance of hydrocarbons to microbial attack (Pirnik *et al.* 1974). On analysis by GLC this can be observed as a decrease in the value of n-C_{17}/Pr and n-C_{18}/Ph.

Because of the similar physical properties of n-C_{17} and pristane, and n-C_{18} and phytane, these ratios are not significantly altered by other physical weathering processes. GLC analysis of an oil generally produces a complex series of resolved components (peaks) superimposed on a 'hump', which is known as the unresolved complex mixture (UCM). The UCM comprises thousands of structurally complex (multi-branched and cyclic) compounds, which, as stated above, are resistant to biodegradation (Farrington & Tripp 1977). Biodegradation therefore results in an increase in the relative concentration of UCM (Davies & Tibbetts 1987). This effect can be quantified by calculating the value of *n*-alkanes/UCM%.

Co-oxidation is also known to occur (Raymond *et al.* 1967; Perry 1979). This enables a compound, which cannot be degraded in isolation, to be enzymatically attacked by a consortium of micro-organisms growing primarily on other hydrocarbons within the oil. Aromatic compounds, the most toxic fraction to macrobenthic species, may thus be utilized by a mixed microbiological population. Such microbiological biodegradation increases the

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organic status of the cuttings and, although this may reduce the direct toxic effect of the aromatic fractions, partial biodegradation may also produce further toxic or carcinogenic compounds (Cerniglia *et al.* 1982). Microbiological hydrocarbon degradation and the effects of hydrocarbon pollution on microbiological processes are well reviewed by Atlas (1984).

Microbiological processes in sediment are complex, with different microbiological populations being spatially separated due to physical and chemical stratification (Jørgensen 1977*a*, *b*, 1982). The surface of the sediment is aerobic; the depth of this aerobic layer will be dependent upon the organic status and physical properties of the sediment. An organically enriched sediment, such as a drill-cuttings pile, will have a very active aerobic microflora in the surface layer. The consequences of this growth are twofold: low molecular mass substrates are produced from incomplete hydrocarbon oxidation and oxygen is rapidly consumed from the pore water. This sets up an anaerobic zone in the subsurface sediment and also provides a wide range of carbon sources, stimulating further microbial populations.

In oil-based cuttings piles, the aerobic layer may be less than 2 cm thick, the remainder of the sediment being anaerobic and highly reduced. Fermentation reactions by anaerobic bacteria liberate yet more carbon compounds and produce reduced conditions suitable for the growth of sulphate-reducing bacteria (SRB). These bacteria are unable to utilize hydrocarbons directly (Postgate 1984) but are able to grow on the products of aerobic hydrocarbon degradation (Laanbroek & Pfennig 1981). SRB produce sulphide as a by-product of their metabolism and, under favourable conditions, maintain high concentrations of toxic sulphide (both soluble and insoluble) and very low redox potentials. These effects add to the direct toxic effects of the discarded cuttings. In addition, such sulphide generation and low redox potential produces a corrosive environment which poses a risk to structures, pipelines and associated steel covered by the mud (Fischer 1983; Sanders 1984; Sanders & Hamilton 1986). This simplified microbiological consortium is shown in figure 2.

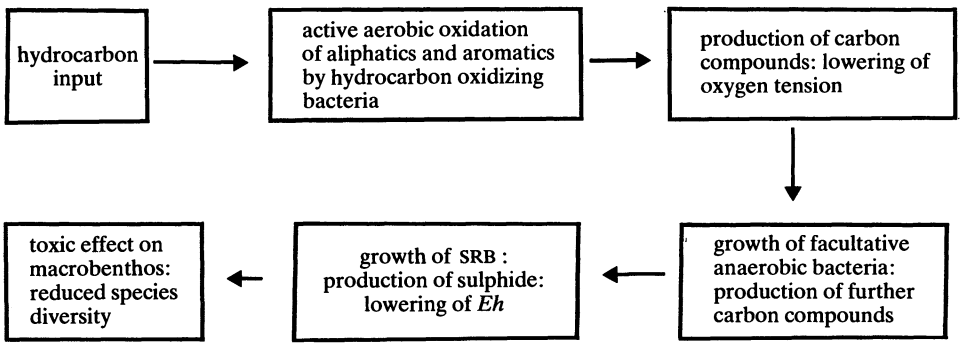

FIGURE 2. Microbiological processes involved in the degradation of hydrocarbons in the seabed sediment.

In sediment with a low organic content (e.g. unpolluted sandy beaches), the aerobic layer is thicker; nutrients for SRB are in low concentration, sulphide production is low and therefore toxic effects are minimal. A high species diversity of the sediment macrobenthos is commonly found in such environments, although biomass and productivity may be low.

The aim of this paper is to highlight the effects of drill-cuttings piles on seabed microbiological populations by means of hydrocarbon, chemical and microbiological analysis around two comparable North Sea installations. A brief drilling history of the two locations is shown in table 2.

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Table 2. Simplified drilling histories of the two fields studied

Field A	Field B
16 water-based mud wells to 1979	16 wells to Jan. 1986
28 diesel-based mud wells to 1983	depth: 3400–4300 m
3 low-toxicity mud wells to 1985	All low-toxicity mud, little diesel
depth: 3700–4300 m	
drilling ceased end 1985	

Field A used predominantly diesel-based muds (28 wells), with 3 final low-toxic mud wells before cessation of drilling in 1985. The field is situated in 160 m of water in the northern North Sea, with a small residual seabed current in a south easterly direction. The natural seabed sediment is a fine sand (mean particle size *ca.* 0.2 mm diameter) with a diverse macrobenthic community.

Field B, south of Field A, by comparison, used low-toxicity muds exclusively, 16 wells being drilled up to January 1986, with the drilling programme still in progress at the time of the survey. This field is situated in 110 m of water, with a residual seabed current of 2 cm s^{-1} in a southerly direction. The natural seabed sediment is of a fine clay consistency (6–15% silt), again with a diverse macrobenthic community.

Methods

Samples were mostly collected from the seabed by using Perspex hand-held coring devices, shown in figure 3. Some samples were collected by taking Day grab samples, with cores being taken immediately after recovery to surface. The cores of sediment were immediately subsampled for microbiological and hydrocarbon analysis. The samples for hydrocarbon analysis were kept frozen (−20 °C) in pre-cleaned and de-greased aluminium cans.

Hydrocarbon analysis

Cores for hydrocarbon analysis were transported to the laboratory in a frozen condition to minimize hydrocarbon breakdown by the microbiota. Frozen cores were allowed to thaw for 2 h before being extruded onto pre-cleaned aluminium foil using a wooden ram covered with pre-cleaned aluminium foil. The sediment core was marked with a clean stainless steel spatula, at intervals of 2 cm from the top down. The core in its semi-frozen state was then easily sectioned with the spatula, which was wiped clean and rinsed with isopropanol between each sectioning. Each section was placed in a Kilner jar and mixed thoroughly with isopropanol (50 cm^3), which acts as a biocide.

Hexane (20 cm^3) and isopropanol (80 cm^3) were added to the sediment and the sample extracted by using ultrasonication (2 × 5 min with stirring in-between). The solvent was then decanted and partitioned between water and hexane (2:3 by volume; 120 cm^3). The organic layer was then collected in a pre-cleaned round-bottomed flask. A further 100 cm^3 of isopropanol and hexane (4:1) was added to the sediment, the extraction procedure repeated, and the two organic extracts combined and evaporated to dryness at ambient temperature under vacuum (Buchi Rotavap R).

The resulting total organic extract (TOE) was weighed to estimate total hydrocarbon

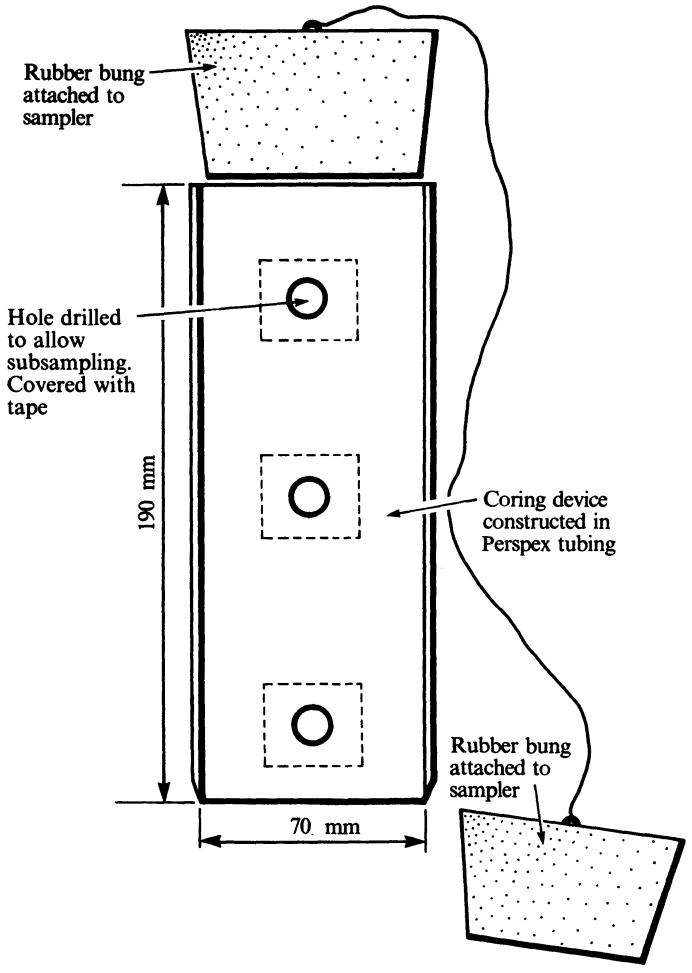

FIGURE 3. Diagram of Perspex hand-held corer used for collecting undisturbed seabed sediment.

content of the sample. A known aliquot of the TOE was taken and 2,21-dimethyldocosane (25 μg) was added as internal standard. The aliquot was dissolved in hexane (2 cm^3) and 0.5 μl was injected for analysis by GLC under the following conditions: instrument, Carlo Erba 4160; detector, flame ionization at 320 °C; column (aliphatic), 25 m × 0.32 mm internal diameter MEGA OV-1 WCOT fused silica; temperature programme, 40 °C–320 °C at 8 °C min^{-1}; carrier gas, helium.

To estimate the concentrations of *n*-alkanes actually removed from the oil in the sediment by biodegradation it was assumed that the UCM in the oil remains unbiodegraded (Van Der Linden 1978). Thus, by calculating the concentration of *n*-alkanes remaining in the sediment and comparing this with the concentration of *n*-alkanes relative to UCM in the oil used in the drilling-mud formulation on the platform (reference oil), it is possible to estimate the amount of *n*-alkanes lost by biodegradation as follows:

$$n\text{-alkanes biodegraded} = \frac{(n\text{-alkanes (reference oil)} \times \text{UCM (sample)})}{(\text{UCM (reference oil)})} - n\text{-alkanes (sample)}.$$

Microbiological analysis

All the samples for microbiological analysis were stored in an insulated coolbox, with frozen ice-packs to keep them cool. They were analysed immediately after collection on board the diving vessel. This minimized any changes in the microbial population which begin immediately after sample collection (the so-called 'bottle effect'). Samples were processed and analysed within a maximum of 12 h of collection. Samples were shipped to Aberdeen in a cold, insulated icebox for final analysis.

(*a*) *Microbial numbers*

Sediment samples were sub-sampled by placing a known mass of the material (approximately 1 g) into a tube of sterile, anaerobic, reduced diluent (10 cm^3) (medium 1, table 3). In some cases, sub-samples were taken at various depths in the core to obtain a sediment profile. An even suspension was made by shaking, stirring and mixing, followed by ultra-sonic dispersion to break up clumps of cells.

The first dilution tube, set up offshore, was used to prepare a 1:9 dilution series in Aberdeen. A fresh, sterile syringe was used to add 1 cm^3 of this suspension to a fresh tube containing 9 cm^3 of anaerobic dilution fluid and mixed. Similar 1:9 dilutions were made to achieve a final dilution of $1:10^{-8}$ (eight tubes).

The dilution series were tested for a number of bacterial groups by inoculation onto selective nutrient media. Results for SRB and hydrocarbon oxidizing bacteria are presented here.

SRB were enumerated by injecting 1 cm^3 amounts of each dilution tube into 9 cm^3 of Postgate broth medium (medium 2, table 3) in triplicate by using standard procedures modified from

TABLE 3. MICROBIOLOGICAL MEDIA USED

(All amounts are percentages, mass/volume or volume/volume.)

medium 1: dilution fluid (anaerobic) (pH 7.6)	
peptone	0.5
yeast extract	0.3
ascorbic acid	0.01
sodium thioglycollate	0.01
seawater	75
medium 2: seawater Postgate medium (pH 7.6) for SRB	
Na_2SO_4	0.1
$CaCl_2 . 6H_2O$	0.01
$MgSO_4 . 7H_2O$	0.2
yeast extract	0.2
$FeSO_4 . 7H_2O$	0.05
sodium thioglycollate	0.01
ascorbic acid	0.01
sodium lactate (70% solution)	0.35
NaCl	2.5
medium 3: Bushnell–Haas broth (pH 7.0) for hydrocarbon oxidizers	
$MgSO_4 . 7H_2O$	0.02
$CaCl . 6H_2O$	0.002
KH_2PO_4	0.1
K_2HPO_4	0.1
$FeCl_3 . 6H_2O$	0.005
seawater	75
carbon source (hydrocarbon)	1

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the American Petroleum Institute (API) recommended practices (1975). Growth of SRB was indicated by blackening of the tube; SRB numbers were estimated by most probable number (MPN) tables from the highest dilution giving blackening after 28 days growth at 30 °C.

Aerobic diesel-oil oxidizing bacteria were enumerated in Bushnell–Haas broth, by using diesel-oil or mineral-oil as the carbon source (medium 3, table 3). Samples (100 µl) of each dilution were added to 10 cm^3 of the broth in triplicate, with 100 µl of sterile hydrocarbon added afterwards. Growth is indicated by an increase in turbidity and emulsification of the oil after 10 d growth with gentle shaking at 30 °C. Numbers were estimated from MPN tables as above.

(*b*) *SRB activity measurement*

SRB activity was measured by using methods similar to those previously described for coastal sediments (Jørgensen 1978; Rosser & Hamilton 1983). A known mass of the sediment was placed in the tube, as shown in figure 4. The tube contained 4 cm^3 of vacuum de-gassed sulphate-free artificial seawater, to which 1 µCi† of [^{35}S] sodium sulphate was added. The tube

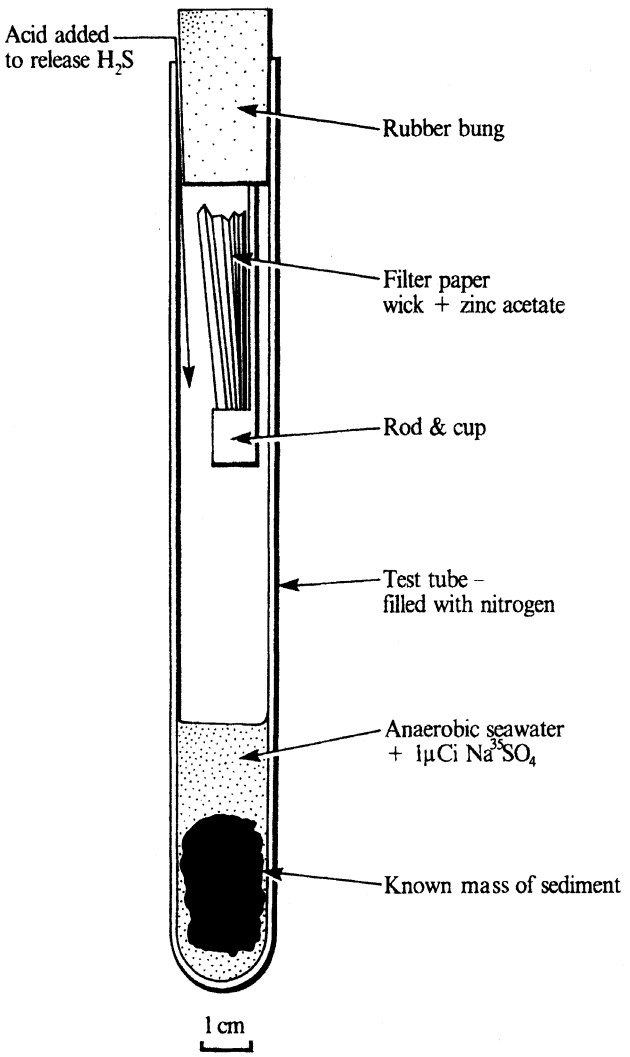

FIGURE 4. Diagram of tube used to assess SRB activity in sediment.

† 1 Ci = 3.7×10^{10} Bq.

was gassed with oxygen-free nitrogen before sealing with an H_2S impermeable butyl rubber bung, from which a rod, cup and wick assembly was suspended. Zinc acetate was added to the filter paper wick at the start of the 24 h incubation period (20 °C) to trap any H_2S evolved. Five tubes were set up from each sample. Four of these were used as 'live' tubes, incubated for 24 h to allow the $^{35}SO_4^{2-}$ to be reduced by SRB to $^{35}S^{2-}$. The fifth tube was killed with acid to liberate H_2S and kill bacteria at the start of the incubation, thus acting as a background, control, tube. The four live tubes were killed with hydrochloric acid after incubation, the H_2S being released by gentle shaking in a shaking water-bath.

The rods, cups and wicks were removed after shaking, placed in scintillation vials and the amount of ^{35}S quantified by liquid scintillation counting. The sulphate reduction activity was calculated from the amount of sulphate in the tube, the amount of $^{35}SO_4^{2-}$ added, the amount of $^{35}S^{2-}$ produced, the mass of deposit and the incubation time, by subtracting the background from the live values.

Results and discussion

Field A, 1982

Microbiology

The depth profile for SRB activity shown in figure 5 is similar to that found in natural sediments, with sulphate reduction being localized within depths of 2–5 cm below the sediment surface. There were also more aerobic bacteria in the surface layers, but they were distributed throughout the sediment profile. Aerobic bacterial activity (including microbial hydrocarbon

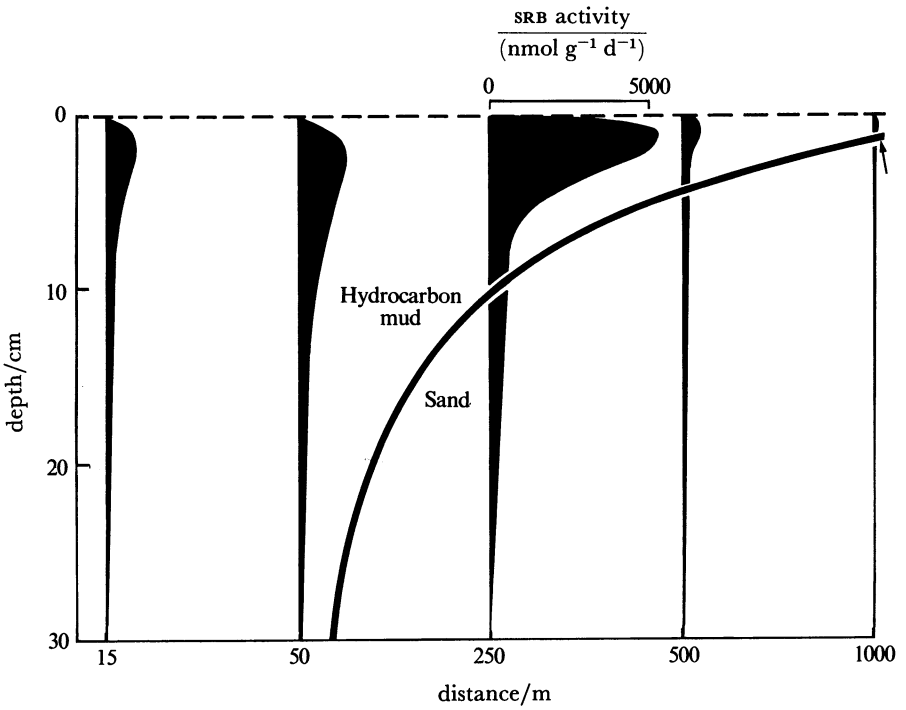

FIGURE 5. Depth profile of SRB activity along a transect at Field A in 1982. Maximum SRB activity was found in the surface or subsurface core at a distance of 250 m from the jacket. The arrow denotes the limit of hydrocarbon-affected sediment.

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oxidation) would be localized in the more oxidized surface layers, because high sulphide concentrations and low redox potentials were found at all depths below 2 cm. The stations closest to the jacket were composed entirely of drill cuttings, whereas the top 10 cm at 250 m was affected in this way. Even at 500 m, there was a thin layer of the fine fractions of the mud, overlying the natural seabed sand, with elevated hydrocarbon concentrations in the top 4 cm. The highest sulphate reduction rates were found at the 250 m station (5300 nmol g^{-1} d^{-1}), with lower rates being found close to the jacket (200–1300 nmol g^{-1} d^{-1}). At stations unaffected by the hydrocarbon input, sulphate reduction rates were very low (less than 50 nmol g^{-1} d^{-1}). It thus appears that the microbiological profiles in these mud deposits are similar to those in natural, undisturbed sediment, but that the activity and population size of SRB is enhanced in the subsurface layers of the sediment close to the platform jacket. This is likely to be because of the organic loading from the cuttings.

Hydrocarbons

Examples of the GLC traces obtained from sediment extracts from Field A in 1982 are shown in figure 6. The uppermost trace (50 m from the platform) shows a range of alkanes from C_{12}

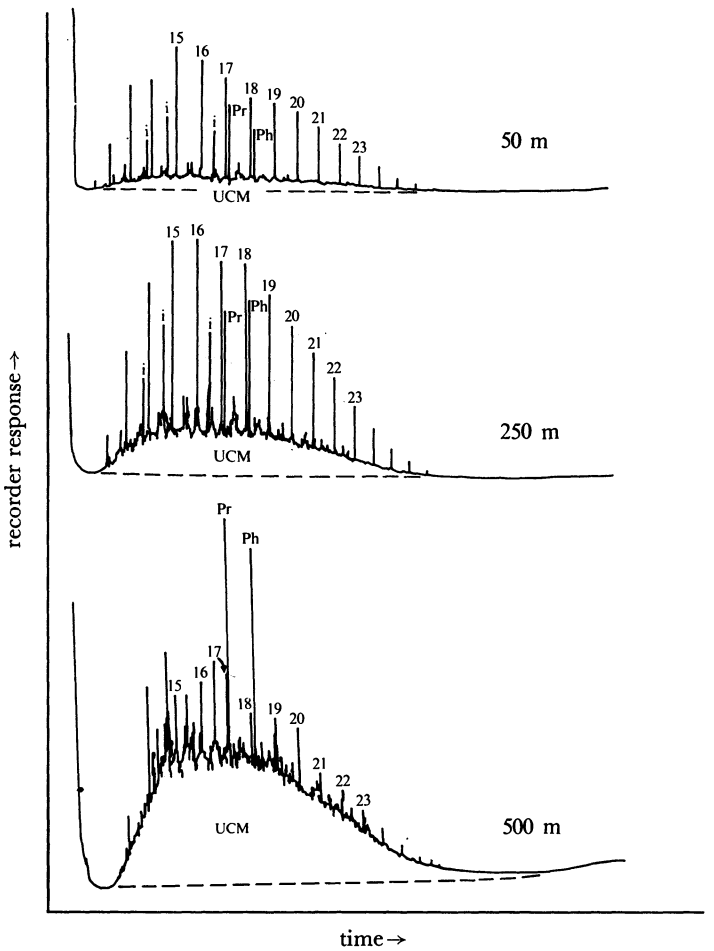

FIGURE 6. GLC traces of TOE from samples along a transect at Field A in 1982. The traces are typical of a biodegraded diesel-oil, especially at the outer stations. Key: i, isoprenoids; Pr, pristane; Ph, phytane; numbers refer to *n*-alkane carbon number; UCM, unresolved complex mixture.

to C_{26}, which is typical of a diesel-oil. As expected, all of the sediments in this survey contained a major contribution from diesel, which had been weathered to a greater or lesser extent.

The degree of biodegradation, estimated from the value *n*-alkanes/UCM (table 4), indicates that the degree of biodegradation of the oil in the sediment increases with increasing distance from the platform. This effect can be seen in figure 6 where the relative concentration of the

TABLE 4. RESULTS OF GLC ANALYSIS (BY MASS) OF DRY SEDIMENT, FIELD A, 1982 AND 1986

	distance /m	$\frac{n\text{-alkanes}}{\text{UCM}}$ (%)	*n*-alkanes biodegraded (p.p.m.)	(%)
1982	15	18	0	0
	50	11	280	61
	250	3	520	79
	500	2	180	89
	1000	1	30	91
1986	50	40	12100	33
	250	0	4900	100
	500	0	340	100
	750	0	100	100
	1000	0	70	100

UCM in the oil appears to increase away from the platform and the $n\text{-}C_{17}$/Pr value falls from approximately 1.4 to 0.3. However, if the sedimentary concentration of the diesel is taken into consideration, the actual concentration of *n*-alkanes removed by biodegradation is at a maximum 250 m from the platform (table 4).

This effect has been observed previously around other North Sea platforms (Tibbetts & Large 1986). At the innermost stations it is thought that the constant rain of drill cuttings causes rapid burial under oily sediment, through which there can be little water (and therefore oxygen) percolation. Aerobic biodegradation therefore has a very limited time in which to take place. In the outermost stations, the amount of *n*-alkanes removed by biodegradation is limited by the lower total concentration of *n*-alkanes deposited in the sediment.

General

The above results show a close correlation between the profiles of SRB activity and the concentration of *n*-alkanes biodegraded (figure 7), with maximum values of both parameters being observed at a distance of 250 m from the platform. SRB activity is the final microbiological stage but is dependent upon initial hydrocarbon oxidation by aerobic bacteria. These results therefore suggest that SRB activity can be used as an indirect indicator of the extent of hydrocarbon biodegradation.

Field A, 1986

Sediment profiles were examined for microbiology in 1986 and SRB were again found to be most active in the subsurface zone. Results from this stratum are presented in figures 8 and 9. Although depth profiles for hydrocarbon were not carried out in 1986 a physical examination of the cores suggested a similar depth profile to that seen in 1982.

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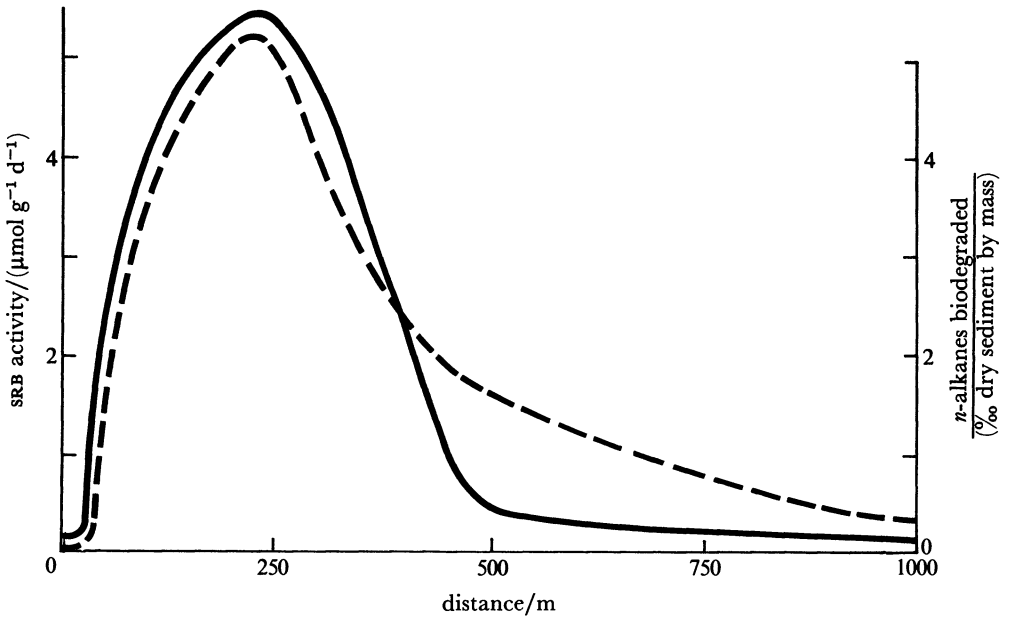

FIGURE 7. Comparison of SRB activity and *n*-alkane biodegradation around Field A in 1982, showing a close correlation.

Hydrocarbons

The GLC traces of the TOE from the sediments around Field A in 1986 are shown in figure 8. Because of the utilization of low-toxicity base-oil on the platform since 1983, the 50 m station shows a kerosene-type input (alkanes from *n*-C_{12} to *n*-C_{16}), which represents the major source of contamination in the sediment. The other stations, however, all show weathered diesel as the major contaminant.

Although biodegradation of the *n*-alkanes appears complete (table 4) from 250–1000 m, when the hydrocarbon loading of the sediment is taken into consideration these are not the stations from which the most *n*-alkanes have been removed. On this survey the maximum concentration of *n*-alkanes biodegraded was only 50 m from the platform (table 4 and figure 9), at which point over 1200 p.p.m. dry sediment by mass of *n*-alkanes have been removed. This represents a ten- to twentyfold increase in the concentration of *n*-alkanes removed compared with that found in 1982.

Microbiology

Figure 9 summarizes the microbiological results from the survey in 1986. SRB activity was found to be maximal at the 50 m station, compared with the 250 m station in 1982. Maximum sulphate reduction rates were lower than in 1982 (maximum 2.2 μmol g^{-1} d^{-1}); this apparent decrease in overall SRB activity may be due to a number of factors, one of which is the change in the nature and level of the platform discharges. SRB numbers were also highest at the 50 m and 125 m stations (10^5 g^{-1}). Immediately beneath the jacket (0 m), numbers were lower by an order of magnitude, and low numbers (10^1–10^2 g^{-1}) of SRB were found between 500 m and 1000 m. Hydrocarbon oxidizing bacteria were also found in highest numbers close to the platform (10^3 g^{-1} at 0–125 m), reaching low background values (0–10) between 500 m

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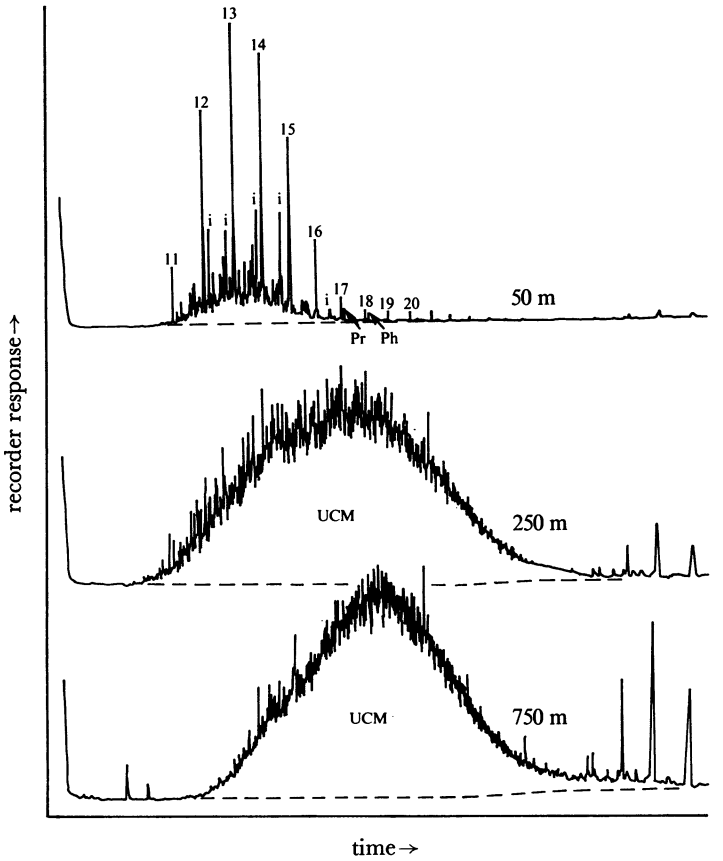

FIGURE 8. GLC traces of TOE from samples around Field A in 1986. Diesel-oil is completely biodegraded at the outer stations whereas the trace at 50 m shows partly biodegraded low-toxicity mud. Key: i, isoprenoids; Pr, pristane; Ph, phytane; numbers refer to *n*-alkane carbon number; UCM, unresolved complex mixture.

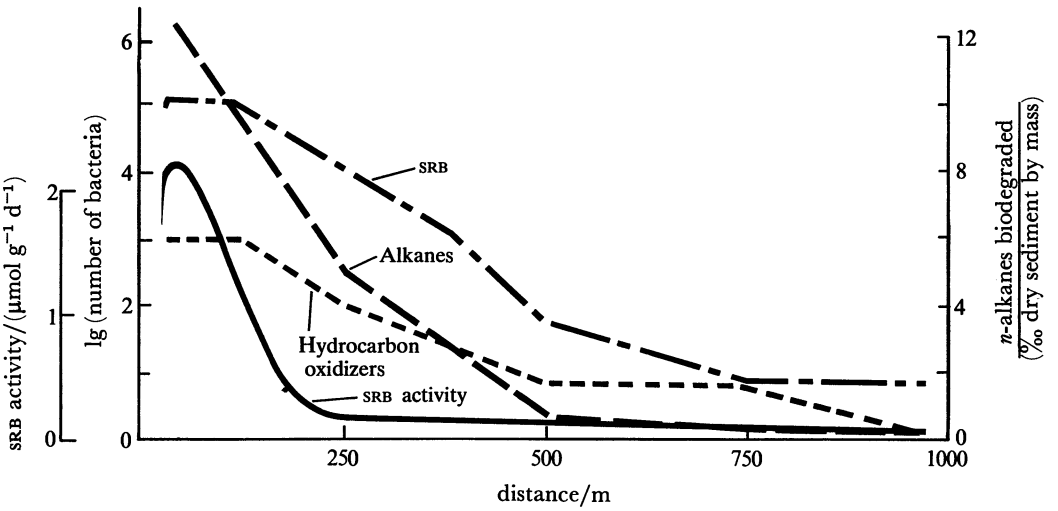

FIGURE 9. Comparison of hydrocarbon and microbiological analyses of Field A, 1986, showing a slightly different zonation when compared with 1982 (figure 7).

and 1000 m. Aerobic and facultatively anaerobic bacteria (not shown) followed similar trends, being present in high numbers (10^6 g^{-1}) close to the platform, but with a less pronounced decrease in numbers with distance.

All these results indicate a significant change to the pattern in 1982, with microbiological processes taking place much closer to the platform in 1986 than in 1982.

General

By comparison with the 1982 data, the results of this survey, summarized in figure 9, show the greatest values of both SRB activity and *n*-alkanes removed by biodegradation to occur at a distance of only 50 m from the platform. Unfortunately, no hydrocarbon data were obtained immediately beneath the platform, but a study of the hydrocarbon oxidizing bacteria (figure 9) suggests that the greatest biodegradation of *n*-alkanes would be occurring there. Since 1982, major parameters that have been altered are:

(1) the replacement of diesel with low-toxicity base-oil;

(2) a decrease in drilling activity with only three wells being drilled in the two years when low-toxicity muds were used;

(3) cessation of cuttings discharges approximately 2 months before the 1986 survey.

It is not clear from these results alone which of these three factors has had most influence on the change in microbial distribution since 1982. This aspect is discussed further, below, in the light of results from Field B.

Field B, 1986

Hydrocarbons

As with Field A in 1986, kerosene-type low-toxicity base-oil is the major contaminant in the sediment within 40 m of the platform (figure 10). At 250 m 96% of *n*-alkanes have been removed (table 5) but isoprenoid hydrocarbons (i) are still present. At 500 m and beyond, however, all of the *n*-alkanes and isoprenoids have been removed by biodegradation.

In this case, the greatest concentration of *n*-alkanes removed by biodegradation (1900 p.p.m. dry sediment by mass) was immediately under the platform (table 5). Even though this only represents 12% of the *n*-alkanes available, the absolute concentration of *n*-alkanes removed is similar to that observed around Field A in 1982.

Microbiology

Figure 11 summarizes the microbiological results from the survey of Field B in 1986. SRB activity was found to be maximal within 20–30 m of the jacket, with a sharply defined peak of sulphate reduction. This is a similar pattern to that found in Field A in 1986. Results of bacterial numbers are also similar, with numbers of SRB and hydrocarbon-oxidizing bacteria being highest close to, or under, the platform. Numbers decline between 125 m and 250 m from the platform and reach background values by 750 m. As with Field A, numbers of aerobic and facultatively anaerobic bacteria follow similar trends but the decline with distance is less pronounced.

General

These results again show close correlation between the profiles of SRB activity and the concentration of *n*-alkanes removed by biodegradation (figure 11), except immediately under

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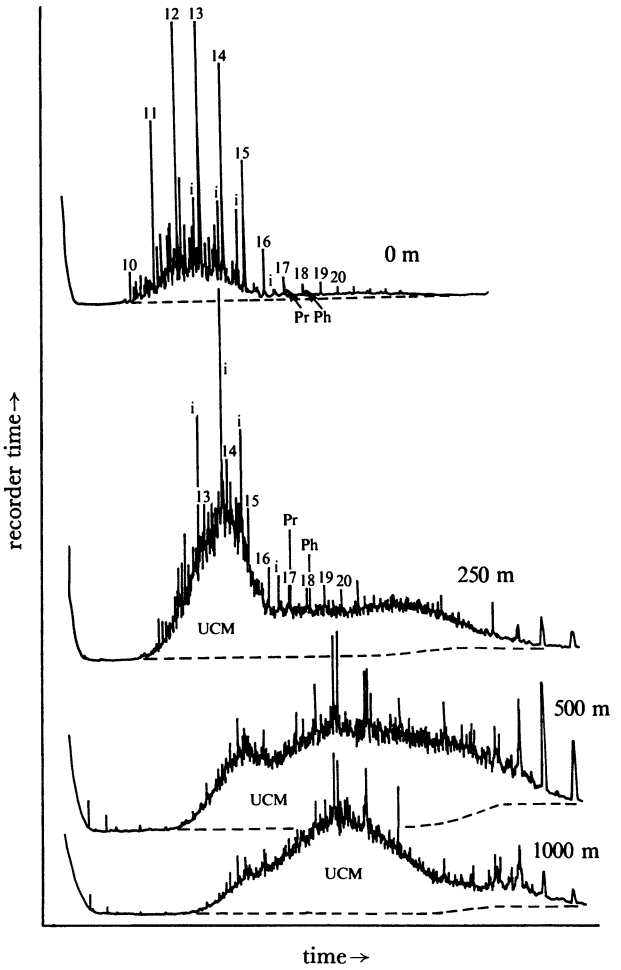

FIGURE 10. GLC traces of TOE from samples around Field B in 1986. Traces indicate biodegradation of the low-toxicity oil, together with some diesel-oil at the outer stations. Key: i, isoprenoids; Pr, pristane; Ph, phytane; numbers refer to *n*-alkane carbon number; UCM, unresolved complex mixture.

the platform, where little SRB activity was observed but where the greatest concentration of *n*-alkanes is estimated to have been biodegraded.

Inter-field comparison

From a comparison of the data obtained for Field A in 1982 and 1986 (table 4), it appears that there has been a general increase in the quantity of *n*-alkanes removed from the sediment by biodegradation since 1982. This could be accounted for by any of the three changes in drilling activity between 1982 and 1986 outlined earlier. However, when the results from Field B are considered (table 5) it can be seen that the absolute concentrations of *n*-alkanes removed by biodegradation are similar to those in Field A in 1982. In both of these cases drilling activity was in progress. This suggests that the increase in the concentrations of *n*-alkanes removed by biodegradation around Field A in 1986 is mainly due to the reduction and subsequent cessation of drilling activity at Field A rather than the change to low-toxicity drilling mud. It is unknown at this stage whether this is because of an increase in the rate of biodegradation or merely reflects

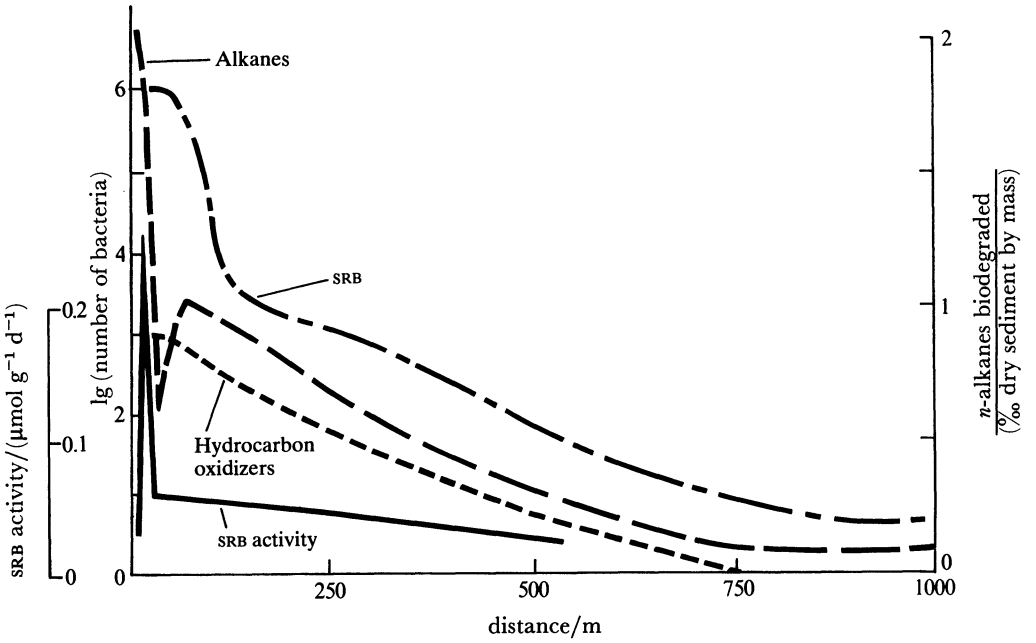

FIGURE 11. Comparison of hydrocarbon and microbiological analyses of Field B, 1986, showing a pattern similar to that of Field A in 1986 (figure 9).

TABLE 5. RESULTS OF GLC ANALYSIS (BY MASS) OF DRY SEDIMENT, FIELD B, 1986

distance/m	$\frac{n\text{-alkanes}}{\text{UCM}}$ (%)	*n*-alkanes biodegraded (p.p.m.)	*n*-alkanes biodegraded (%)
0	31	1900	12
40	28	550	23
100	22	1070	40
250	2	520	96
500	0	120	100
1000	0	64	100

the greater length of time for biodegradation to occur as a result of the reduction in drilling activity.

It is evident from a comparison of the three surveys (figures 7, 9 and 11) that both SRB activity and *n*-alkane biodegradation peak closer to the platforms when low-toxicity drilling muds were in use (Field A, 1986 and Field B, 1985) than at Field A in 1982 when diesel-based mud was in use. The similarity of the profiles in the two low-toxicity mud studies and their marked difference to the profiles seen when diesel-based mud was used, suggests that the spatial distribution of *n*-alkane biodegradation and SRB activity is governed by the base-oil type. This effect may occur because at the same concentration of total oil in the sediment, diesel contains higher relative concentrations of aromatic hydrocarbons than low-toxicity oil. Because it is generally accepted that the aromatic hydrocarbons are the toxic fraction of an oil it is likely that diesel could suppress microbial activity to a greater distance from a platform.

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Discussion

R. E. Jones (*School of Ocean Sciences, University College of North Wales, U.K.*). Does the nature of drilling cuttings change from field to field depending on the strata being drilled and the techniques used? Would such changes account for some of the variability by altering the permeability of the sediments and the transport of oxygen into and hydrocarbons out of the sediments?

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P. F. Sanders. Drilling cuttings will vary from field to field, since the rock cuttings derive from the strata drilled. The residual mud in which the rock chips are embedded, however, will be dependent on the type of drilling mud in use. It is quite likely that such localized changes in mud chemical and physical characteristics could account for differences in biodegradation between the sites. We would, however, expect such differences to be slight because the predominant effect is from the mud itself.

R. J. Watkinson (*Shell Research Ltd., Sittingbourne, Kent, U.K.*). The analytical data on hydrocarbon distribution between the two sites are used to make statements on relative degradability. Oxygen will have a large influence on rates of degradation of the two mud-type hydrocarbons. Could the differences between the two sites be explained in terms of rates of oxygen diffusion rates between the two sites?

P. F. Sanders. This study was purely concerned with the relative degree of biodegradation of *n*-alkanes in the base-oil and no attempt was made to establish the rate of biodegradation. The same degree of biodegradation is possible with either a high rate for a short time or a low rate for a long time. Similar considerations apply when trying to extrapolate these data to assess rates of seabed recovery.

R. A. A. Blackman (*MAFF, Burnham-on-Crouch, Essex, U.K.*). I would like to ask Dr Sanders for clarification on the relative degradability of diesel and low-toxicity alternative base-oils. He has suggested that the zone of maximum degradation of the latter lies much closer to the discharge point than for diesel-oil but Dr Kingston suggested (this symposium) that at sediment concentrations of 3% (still very high concentrations only to be expected very close to the platform) diesel-oil was more readily degradable than low-toxicity oils.

P. F. Sanders. There are many examples in the literature where studies conducted under controlled conditions in the laboratory with single pure cultures provide conflicting data to those obtained from field measurements.

P. F. Kingston (*Institute of Offshore Engineering, Heriot-Watt University, Edinburgh, U.K.*). In answer to Dr. Blackman's question I should like to make it clear that the experimental results that I quoted from Gillam *et al.* (1986) referred to one species of filamentous bacterium isolated from diesel-contaminated cuttings (*Streptomyces* sp.). The fact that such species exist and that at diesel concentrations below 3% may utilize diesel as a substrate twice as fast as low-toxicity base-oil suggests that diesel may be biodegraded at a much greater rate than was previously thought when compared with 'low-toxicity' base-oils.

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P. F. Sanders. Undoubtedly, some organisms use diesel-oil equally rapidly, if not more rapidly, than other oils, including low-toxicity base-oils. The reverse is also true, however, and the microbial population in nature will be adapted to the oil types in the environment. The rate

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of breakdown of the oil will ultimately depend upon the nature of the consortium of microbes so that results from single pure cultures may not reflect the situation in the natural environment. It is also possible that concentrations of diesel greater than 3% occur close to the discharge. This would explain our findings of depressed bacteria activity close to the platforms using diesel oil-based muds.

W. A. Hamilton (*Department of Microbiology, University of Aberdeen, U.K.*). The last few questions have discussed various factors concerned with breakdown of hydrocarbon fractions. I should like to shift our attention back to what is the main point being made by Dr Sanders; that is to say, the direct relation between hydrocarbon breakdown and the sulphate-reducing bacteria.

Earlier in this meeting C. G. Moore discussed the effects of meiofauna in both a mudflat environment and also under production platforms. In our own early work we were looking at a polluted estuary where the organic input was in the form of cellulose. Each of these three environments is therefore quite different in terms of physical and chemical components, and of the microorganism responsible for the primary biodegradation. What they all have in common however is the consequences of organic overloading: reduced conditions, growth and activity of sulphate-reducing bacteria, production of sulphide. What Dr Sanders has shown is this direct relation between these indices of sulphate-reducing bacteria and of hydrocarbon loading and its biodegradation in discarded drill cuttings.

The point we would make is that low redox conditions and sulphide production are major direct causes of the effects that can be observed in the meiobenthos and macarobenthos, and on corrosion.

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