FOREWORD

Marine contamination by petroleum, whether by natural seepage or by spills from ships at sea, by accidents in harbour or at offshore installations or by atmospheric or terrigenous input is by no means a new or rare phenomenon. In recent years however, the problems have been highlighted not only by the increased utilisation and marine transport of oil but also by a number of spectacular accidents which have raised questions about possible effects on the ecosystem. A number of detailed studies have been carried out in an attempt to answer these questions. The demands for such knowledge have been further increased by the various questions raised as a result of expansion of offshore exploration and exploitation for oil, particularly in environments hostile to these operations, in regions as far apart as the northern North Sea and the coast of Alaska.

Consequently, diverse aspects of the problem are being studied in several parts of the world by chemists and biologists who are often asking the same questions but using different approaches and sometimes producing conflicting views. Against this background, it seemed timely therefore to bring together a group of scientists from university, industry and government, actively engaged in such work, to examine and discuss common problems relevant to petroleum hydrocarbon contamination of the marine ecosystem and so a Workshop was sponsored by the International Council for the Exploration of the Sea, and held in Scotland at Aberdeen in September 1975.

The Workshop considered methodology, occurrence and fate in the environment, and effects on the ecosystem of petroleum hydrocarbons in the sea. Most of the papers presented and updated where necessary, are brought together in the present volume together with an edited version of the recorded discussion that followed each session. Of necessity, the reportage of the discussion is very brief although the proportion of time available for discussion compared favourably with that set aside for formal presentation of the papers. In preparing the discussion reports, the editors were assisted in particular by Dr R. Hardy, Dr R. Johnston, Mr P. R. Mackie and Dr I. C. White, and by comments from several contributors.

No attempt was made to produce specific recommendations but a study of the papers in this volume does give a clear indication of several lines of research which must be followed up before an adequate understanding can be reached of the effects of petroleum in the sea and it is evident that widespread monitoring operations will be fully effective only when the basis of our knowledge has been thus extended.

A list of participants to the workshop may be found in Appendix I.

A. D. McINTYRE
K. J. WHITTLE

Rapp. P.-v. Réun. Cons. int. Explor. Mer, 171: 129–138. 1977.

RATE MEASUREMENTS AND RATE-LIMITING FACTORS IN OIL BIODEGRADATION IN THE MARINE ENVIRONMENT

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The interpretation and relevance of various measurements of oil biodegradation are discussed, e. g. oxygen uptake, recovery of oil, etc. The concepts of degradative capacity of a given body of water viz. the degradation rate of a small amount of oil in unlimited water are considered. Two new respirometric methods are described in which relationships between oxidation rate and nutrient supply were obtained. Extent of degradation and changes in properties were also noted. A "chocolate mousse" exposed on a raft at sea showed changes in physical and chemical properties, apparently without net loss of material.

INTRODUCTION

The subject of oil biodegradation as a whole has been reviewed too frequently to bear repetition here, and the reader is referred to the reviews of Freide et al (1972), Floodgate (1972a, 1972b and 1973), Ahearn and Meyers (1973) and ZoBell (1973). Some of the problems may be summed up by the following extracts: "Conspicuous by its absence from the discussions in this Workshop as well as in published papers is meaningful information on the absolute rates of biodegradation of oil pollutants... With few exceptions, only relative rates are reported." (ZoBell, 1973) and "... The term "microbial degradation of oil in the marine environment" means therefore the degradation of a complex and variable mixture of hundreds of substrates by unknown mixed populations of microorganisms, in an erratically changing medium... The confusion is compounded by the fact that biodegradation is not the only means whereby the oil is changed chemically... evaporation, solubilization, photo-oxidation and possibly other abiological mechanisms, operate at the same time." (Floodgate, 1973).

It is very easy to obtain "results" showing oil degradation in artificial culture conditions, with high temperatures, and nutrient concentrations orders of magnitude higher than in nature, but very difficult to determine the rate and extent of the process under environmental conditions. It is intended to concentrate attention here on approaches to the latter problem.

Even the limited amount of work in moderately "natural" conditions gives widely differing results, and this is not surprising in view of the number of factors which may drastically affect the rate of degradation (Table 41). Some of these differences, probably water quality and microbial acclimatization in particular, must account for contrasting results in Britain and America.

INTERPRETATION AND DIMENSIONS

The two-phase structure of any oil-seawater system, as well as presenting practical difficulties in experimental design, complicates the interpretation of any results. It poses the question "what are the dimensions of the numerical result obtained?" which is closely linked to the rate-limiting factors in the particular experiment. For example, is the measured rate of oil destruction expressed as a rate per quantity of oil present (as in a first order reaction), or per surface area of the oil, or per volume of water present, or in relation to the

Table 41. Factors affecting rate and extent of oil biodegradation

Substrate	Environment
Type of oil:	Chemistry and microbiology:
Crude;	Quantity of nutrients
composition dependent	Number and type of organisms
upon source	Turnover rates of nutrients
Distillate products	Nutrient input through "run-off"
Lubricating oil	Presence of other organic
Residual fuel oils;	matter
variable according	Bacterial predators
to viscosity etc.	"Acclimatization" to oil
Waxy tank washings	
Physical form of oil:	Temperature
Thin film	Turbulence
Oil-in-water emulsion	
Water-in-oil emulsion (mousse)	
Tar balls	

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total quantity of some essential nutrient, the number of bacteria present, or a combination of these factors? This consideration is critical in drawing general conclusions and extrapolating to environmental conditions.

The knowledge required in the environmental context can be simplified to the following questions:

A) How much oil can a given amount of sea cope with per year?
B) How long will any individual spill last before destruction?
C) What proportion of any given oil will degrade?
D) What happens to the remainder?

The following sections review various attempts in this laboratory to measure oil biodegradation rates with some understanding of the rate-limiting factors, under conditions as near natural as possible while still allowing measurements.

PRACTICAL METHODS OF RATE MEASUREMENT

There appears to be an environmental equivalent of Heisenburg's uncertainty principle, whereby the closer one's experimental model is to natural conditions, the more nearly impossible it becomes to measure the processes occurring. Oil biodegradation poses particular problems, for example:

a) Oil is a complex mixture of many substrates so one rate measurement cannot represent the process as a whole, and long term measurements are necessary. If possible these should continue until degradation ceases.
b) The two-phase nature of the system renders "aliquot" sampling extremely difficult, as well as complicating interpretation.
c) The rate of "degradation" depends on the characteristic chosen for measurement. A number of possible approaches, with their environmental relevance and limitations are given in Table 42.

Table 42. Practical methods of measuring oil biodegradation

Experimental characteristic	Relevance	Limitations
Disappearance of visible film	Short term relevance to effects on birds and surface film chemistry	No knowledge of actual fate (emulsification, chemical modification, mineralization)
Loss of oleophilic (solvent extractable) material	More relevant in long term as "oily" characteristic destroyed	Sampling destructive (aliquot sampling usually impossible). Is oil mineralized or solubilized?
Loss of total organic carbon	Measures extent of complete mineralization	Conversion of oil to biomass and modification not measured. Sampling destructive
CO_2 production	ditto	ditto, but non-destructive
Oxygen uptake	Includes both mineralization and partial oxidation	Quick and sensitive, non-destructive. Does not differentiate between partial and complete oxidation, or conversion to biomass.
Bacterial numbers	Qualitative indication of organisms concerned	No quantitative indication of rate or extent of degradation
Use of radioactive substrates		Limited to single substrates.
Properties of oil	Properties affect pollution hazard	Quantitative estimates of loss less reliable

Of the possible experimental approaches, measurement of oil loss by extraction and weighing appears the best, at first sight. However, owing to the two-phase nature of the oil-seawater system and the impossibility of representative "aliquot" sampling, the whole container must be extracted. Thus only one measurement per vessel can be made, limiting the information per experiment. This approach was used by S. O'Hara and M. F. Spooner (1973, personal communication), using moderately enriched seawater, wherein Kuwait crude residues lost about half their weight in one year. Using unsupplemented seawater, Kinney et al (1970) claimed that 20 mg per litre of Cook Inlet crude oil was degraded virtually completely in a year. Gunkel (1967) found that 50 % of heavy gas oil in unsupplemented seawater was destroyed in 8 weeks.

Measurement of carbon dioxide evolution allows continuous long term measurements, and this method has been used by Atlas and Bartha (1972). However the disadvantages include the loss of the carbonate-bicarbonate buffering characteristic of seawater, owing to aeration by CO_2-free air, and as with other closed systems, metabolic products can build up and affect biodegradation. The latter effect was thought to explain cessation of biodegradation while degradable hydrocarbons and essential nutrients were still present.

Bacterial numbers provide useful supplementary information, but do not necessarily bear any quantitative relationship to the rate of oil destruction. Where the main rate measurement is indirect (CO_2 production, O_2 uptake), it is desirable to know the total biomass produced, but it is not always possible to estimate this reliably. Fungi may be important as well as bacteria.

Changes in oil properties are often quoted as indicators of biodegradation, particularly loss of *n*-alkanes as measured by gas chromatography. These are however usually of qualitative significance only, since *n*-

alkanes only represent a small proportion of the involatile fraction of crude oil. The most quantitatively significant measurement is probably metal concentration (vanadium and nickel). It is believed (Duckworth 1971; Brunnock et al, 1968) that these metal complexes are not lost by weathering, so any net destruction of oil should be reflected in an increase in their concentrations in the residue. As far as is known, this has not been proven positively. Chemical modifications (as opposed to mineralisation) are reflected in property changes such as viscosity and specific gravity.

Oxygen uptake is a very attractive method for measuring biodegradation, in that it is sensitive and non-destructive. Oxidative modifications such as fatty acid production are to some extent reflected in the measurements, but to discriminate between these and complete mineralisation to carbon dioxide and water requires some supplementary method. Manometric methods have been applied to oil biodegradation (e.g. Bridie and Bos, 1971), but suffer considerable limitations. Unless a "carbon dioxide buffer" (Pardee, 1949; Krebs, 1951; Warburg and Krippahl, 1960) is used instead of alkali, the carbonate-bicarbonate buffering character of sea water is destroyed, and this may affect metabolism (Dixon, 1951, p. 75; Laser and Rothschild, 1939). Long-term experiments would render such buffering difficult, and accumulation of metabolic products becomes a problem in any closed system. A further problem is nutrient supply. During oil biodegradation, available nitrogen particularly is utilized rapidly, so in a closed system, biodegradation soon slows down owing to nutrient depletion unless these nutrients are initially present in unnaturally high concentrations.

Thus no single experimental method fully defines the changes occurring, and combinations of methods are desirable. The author has favoured oxygen uptake, and much of the work described here was designed to overcome the above problems. Other methods (supplementary to oxygen uptake or alone) are also mentioned.

"SEMI-CLOSED" RESPIROMETRY (CRUDE OIL/SEA-WATER)

A first approach to the problem (Gibbs, 1972) provided, in a flask, a layer of oil over seawater which was aerated by an external reservoir / aerator / air lift pump. At intervals the flask was isolated by stopcocks, and the drop in oxygen concentration was measured by Winkler titration of abstracted samples. This allowed measurements at natural carbon dioxide levels, but available nitrogen soon dropped to very low concentrations, with oxidation rate slowing as a result. Other problems included the oil water ratio being too high, and too low a surface area between oil and water.

Modifications gave the apparatus shown in Figure 86, six sets being made and used in a constant temperature room. This work has already been described in detail (Gibbs, 1975; Gibbs et al, 1975). Nitrogen and phosphorus nutrients were supplied continuously via the capillary tubing, at a variety of rates and in various ratios. A current of aerated water was maintained through the apparatus, except when oxidation rates were being measured. Then, the stopcocks (T_2 and T_3) were closed to prevent ingress of oxygen, and duplicate samples for Winkler titration were taken via T_3, immediately and after one and two days. Samples for nutrient analysis were also taken. The drop in oxygen concentration combined with vessel volume gave the rate of oxidation, and the known addition of nutrients, coupled with measured concentrations (of nitrate, nitrite, ammonium, phosphate and total phosphorus) allowed calculation of nutrient uptake. After

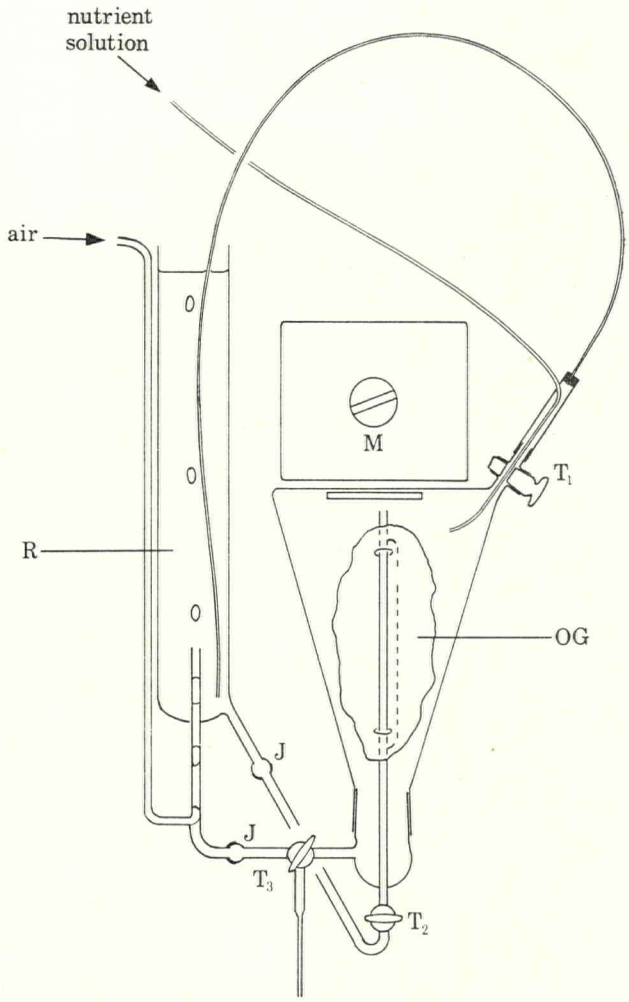

Figure 86. "Semi-closed" respirometer vessel. M, magnetic stirrer; OG, oil on glass-fibre filter paper; T_1, T_2, stopcocks; T_3, "T bore" stopcock; J, ball in socket joints; R, reservoir with air-lift pump.

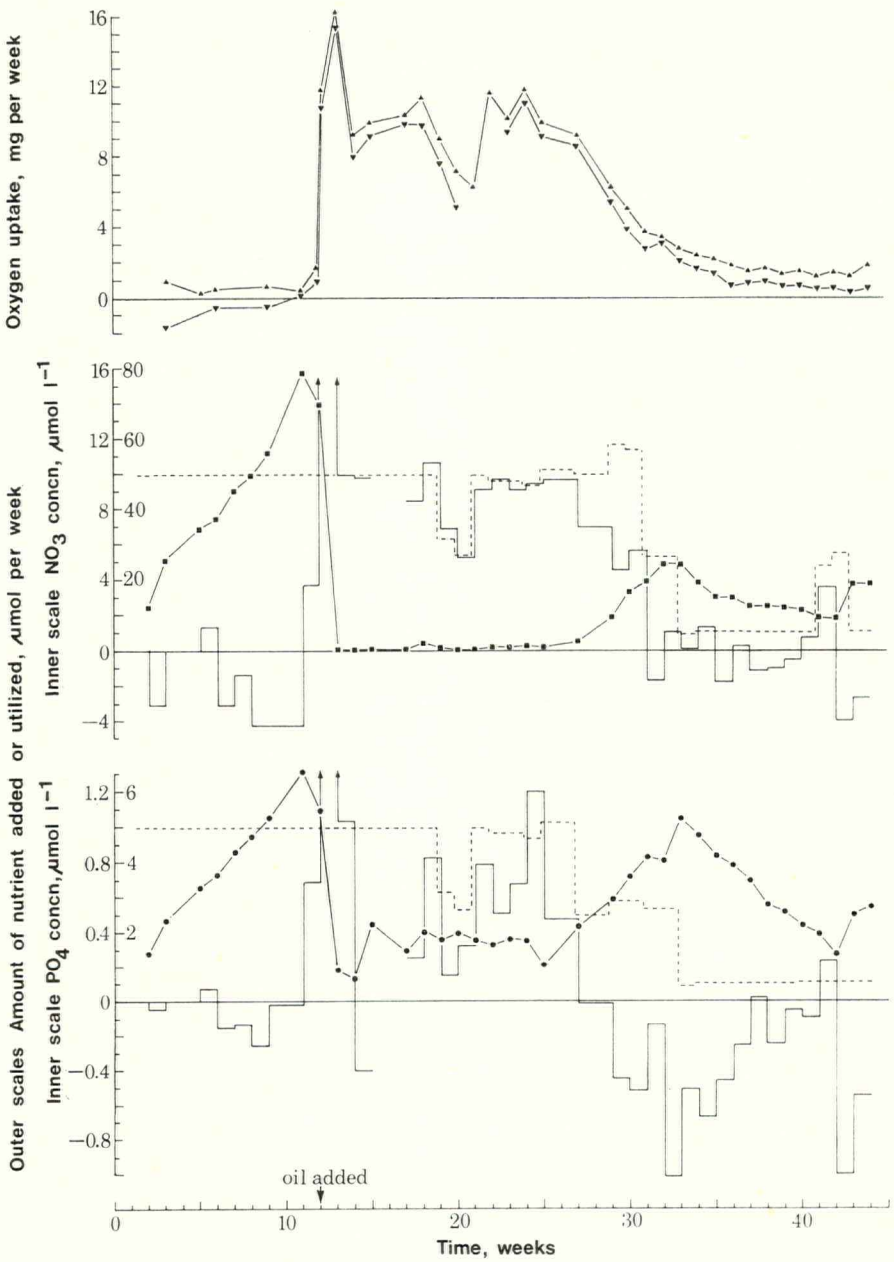

Figure 87. Activity against time in respirometer with most rapid nutrient addition. ▲, rate of oxygen uptake (gross); ▼, rate of oxygen uptake less control; – – –, nutrient added; —— nutrient utilized; ■, nitrate concentration; ●, phosphate concentration.

twelve weeks baseline measurement, without oil, 200 mg of Kuwait residue absorbed on glass fibre filter paper was added to five of the six vessels, as shown. This provided a large surface area with a small quantity of oil. The accumulation of soluble products was minimized in this system since the weekly sampling and replenishment by fresh seawater provided a slow flushing.

The history of the respirometer system receiving most nutrients is summarized in Figure 87. It is seen that in the presence of oil, virtually all the nitrate added was taken up, concentration remaining low, until degradable components in the oil became limiting. Nitrate absorption then ceased, and (though not shown in Fig. 87) nitrogen was released as ammonia. Phosphate was also taken up to a less dramatic extent, and was

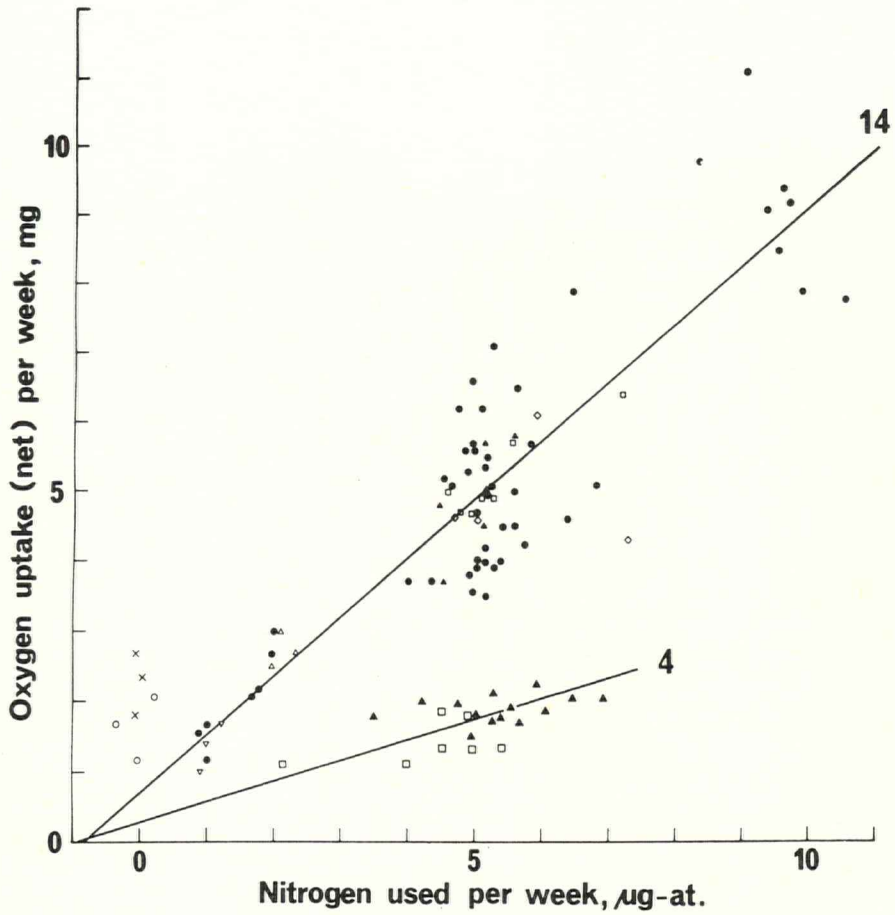

Figure 88. Rate of oxygen uptake (net) against nitrogen utilized per week at 14° and 4° C. P/N ratios: □, 0·02; ◇, 0·06; ●, 0·10; △, 0·25; ▽, 0·50; x, 0·5/0; ○, 0/0; ▲, N as ammonium.

released again as oil biodegradation ceased. The latter part of this history thus probably represents the death and decay of the oil oxidizing organisms.

The rate-limiting factor was found to be the rate of supply of available nitrogen, as evidenced by the upper plot in Figure 88. Phosphate supply did not become limiting, even when the P/N supply ratio was much less than the natural concentration ratio. The effect of temperature is shown by comparison of the two plots of Figure 88. (The figures plotted are oxygen against nitrogen utilized, but the latter is almost the same as nitrogen added.) The Q_{10} value between 14°C and 4°C was 2·7, in agreement with the range quoted by ZoBell (1964) for enriched culture conditions. Assuming a theoretical oxygen demand of 3·5 g O_2 per g oil, the figures suggest a nitrogen requirement of 4 µg-at N per mg oil at 14°C and 11 µg-at N per mg oil at 4°C, at least with the water and associated biota sampled locally.

Extrapolation of these conclusions to the natural environment suggests that the long term degradative capacity of local sea water depends on the rate of recycling of available nitrogen, which is not accurately known. Assuming a one month recycling time (Harvey, 1966, pp. 75—76) and a total N concentration of 10 µg-at l^{-1}, figures of about 30 g m^{-3} a^{-1} at summer temperatures and 11 g m^{-3} a^{-1} at winter temperatures are obtained.

These experiments were repeated (with slight modification) using different crude oils — Brega (Libyan) and Forties (North Sea) Davis et al, (in manuscript). Rather different behaviour was noted, in particular a less complete utilization of nitrate, and lower oxidation rates in relation to nitrate uptake at high temperatures. However, checking with Kuwait crude showed that this was not primarily due to the oil type. It was probably a characteristic of the sample of seawater with its associated biota, which was collected during the winter (when nitrate concentration is high) in contrast to the previous work with summer collected water. This illu-

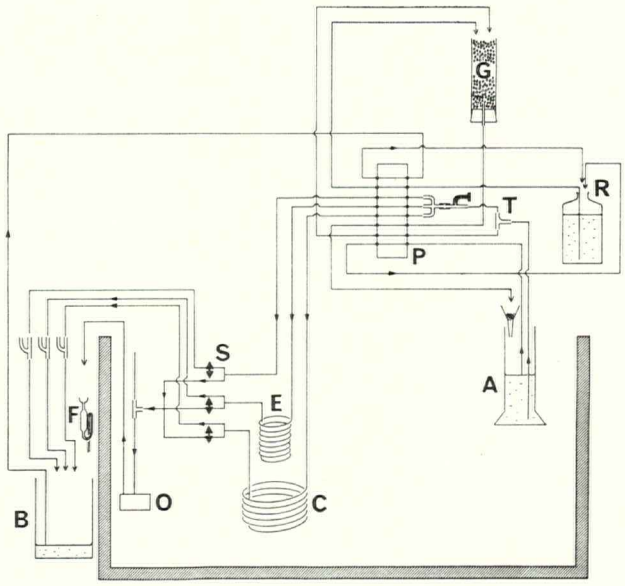

Figure 89. Continuous flow respirometer. A, aeration cylinder; B, beaker for collection of waste; C, control coil; E, experimental coil; F, flow meter; G, gravel column; O, oxygen electrode; P, peristaltic pump; R, reservoir; S, flow switching system; T, "T" piece.

strates some of the complexities and dangers inherent in the subject.

In all these experiments, integrated oxygen uptakes suggested approximately 25 % of the oil was oxidized, and recovery of oil indicated a 50 % loss, after which the process was very slow. This difference is thought to arise mainly from soluble intermediates washed out, although the biomass remaining is unknown. The undegraded remainder from Kuwait 350°C residue and Brega 350°C residue were dense enough to sink in seawater, but that from Forties (estimated 250°C residue) was not.

CONTINUOUS FLOW RESPIROMETRY

(a) Oil / water. The above work clearly relates oxidation rate of oil (in excess) to nutrient supply (limiting), which is interpretable tentatively in terms of degradative capacity of seawater (i.e. question A, p. 130). It is possible to model an unlimited supply of sea water acting on a limited amount of oil? This was attempted by coating the internal surface of a coiled glass tube with oil, and arranging a flow of seawater through it. The oxygen tension of the water entering and leaving the coil (and an untreated control coil) was measured by an automatic continuously recording polarographic respirometer (Gibbs, 1976), the system being thermostatted at 14°C. This apparatus recorded flow rates, as well as the oxygen tensions of input water and both effluents, the rate of oil oxidation being calculated from the difference in oxygen concentration (control — experimental) multiplied by flow rate.

Figure 89 shows this apparatus in schematic form, the components within the cross hatching being thermostatted in a water bath. Three identical, aerated seawater supplies were pumped from the aeration cylinder (A) to the control (C) and experimental (E) coils, and direct to the switching gear (S). This switching gear sampled consecutively through an oxygen electrode (O), the direct "input" water supply, and the effluent from the control and experimental coils. Oxygen tensions of the three seawaters were recorded as a series of steps on a chart recorder, and the automatic syphon (F) connected to an event marker recorded the flow rates. In the system illustrated, the combined effluent water was returned to the reservoir (R) and recycled via a biological filter (G) to the aeration cylinder (A). A continuous supply of new seawater could be used instead of this recycling facility. When required, nutrients could be injected through the capillary arm "h" piece (shown blanked off in Fig. 89).

At first, oxygen uptake occurred in the oiled coil during the day, but not at night. This was found to be a purely photochemical process probably involving the conversion, discovered by Burwood and Speers (1974), of thiacyclanes to thiacyclane-1-oxides, which are water-soluble. (This illustrates another of the aforementioned problems in oil biodegradation studies.) This photochemical reaction was of limited duration and after it had almost ceased, biodegradation eventually began. This system probably did not achieve the aim of an "unlimited" supply of seawater to the oil, since oxidation rate was again strongly correlated with rate of nitrate supply:

Oxygen uptake mg h^{-1} = 0·005 + 0·33 (N supply μg-at h^{-1})
Correlation coefficient r = 0·91

However, with unsupplemented sea water, at the highest experimental flow rate, the bio-oxidation rate was such as would degrade the labile half of the oil present in just over a year. This was calculated from the following information:

Amount of oil applied	= 770 mg
Amount of oil recovered at end of experiment	= 619 mg
Amount of oil lost (by mineralization, solubilization, etc.)	= 151 mg
Integrated oxygen consumption (biodegradation only)	= 158 mg
Amount of oil lost / oxygen used	= 0·95

Rate of oxygen uptake (maximum flow unsupplemented water) = 0·96 mg day^{-1}
Time to convert $^1/_2 \times 770$ mg oil = 405 days = 1·1 year

This should only be considered an estimate of the order of magnitude of the rate, for an oil film about 7 μm thick at 14°C. Within these limitations the figure represents an upper limit for the time involved.

(b) Oil / sand / water. The pioneering work on this subject was done by Johnston (1970) using unenriched seawater percolating through columns of medium sand, contaminated lightly or heavily with oil. Heavy oiling produced anaerobic conditions.

It was felt that in natural sandy beaches oxygen is more likely to limit oil degradation rate than is nutrient supply, but with increasing particle size, porosity and water percolation rates, available nitrogen could again become the most important factor. The continuous flow respirometer was set up with two columns of shingle (median grain diameter 1·6 mm) replacing the two glass coils shown in Figure 89. One column was loaded with 2·7 g of Kuwait crude residue as in Figure 90. Biodegradation was slow to start even with nutrient enrichment, but once underway the baseline rate (with unenriched seawater) increased during the first two months. Injection of mineral nutrients (sodium nitrate and sodium dihydrogen phosphate) to give concentrations in the order of 10 μg-at N and 1 μg-at P per litre markedly increased oxidation rate. The effluent from the oiled shingle was low in nitrate in comparison with the control. The steady increase in oil oxidation rate with unenriched water made interpretation of nutrient uptake in relation to oxidation rate difficult, but the proportionality found was in the same order of magnitude as before. It was determined in two ways — from calculated nitrate input, and from the measured depletion in the oiled viz. control column. Results were fairly similar, and combining both sets of figures gave an equation:

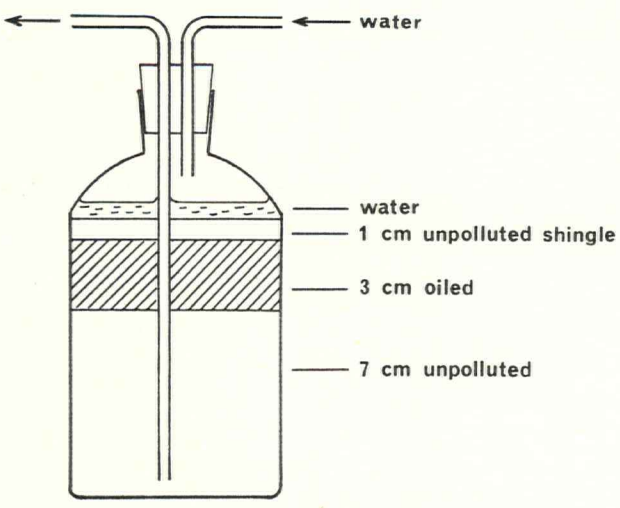

Figure 90. Column of shingle in 500 ml bottle.

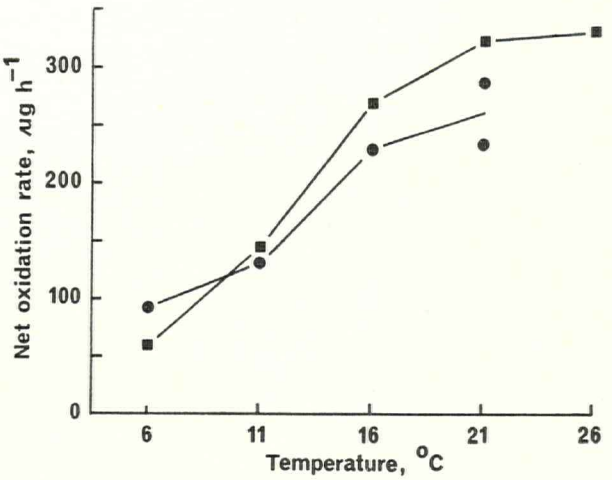

Figure 91. Effect of temperature on oil oxidation rate in shingle column.

Oxygen uptake mg h^{-1} = 0·12 + 0·23 (nitrate uptake, μg-at h^{-1})
Correlation coefficient r = 0·82 (with n = 18).

The "constant", i.e. the rate of oxidation without nitrate supplementation was thus much higher than in oil/water experiments, consistent with rapid nutrient recycling in the beach environment.

Temperature had a marked effect, two series of measurements being shown in Figure 91. At the highest temperatures the flow rate had to be increased to avoid anaerobic conditions.

This work (Gibbs and Davis, 1976) was complementary to studies using model beaches which mimic more closely the natural environment (Pugh, 1975), mentioned below.

INFERENCES FROM OIL ANALYSIS

In some simulated environmental situations, rate measurements such as oxygen uptake and carbon dioxide evolution are not possible, and the heterogeneous condition renders estimation of oil content during experiments impossible. Extent of degradation can then only be followed by sampling and analysing the oil. As mentioned before, the almost universally measured disappearance of n-alkanes is a useful qualitative indicator of biodegradation. While no oil property has yet been proven a quantitative measure of oil destruction, vanadium and nickel concentrations may serve this purpose.

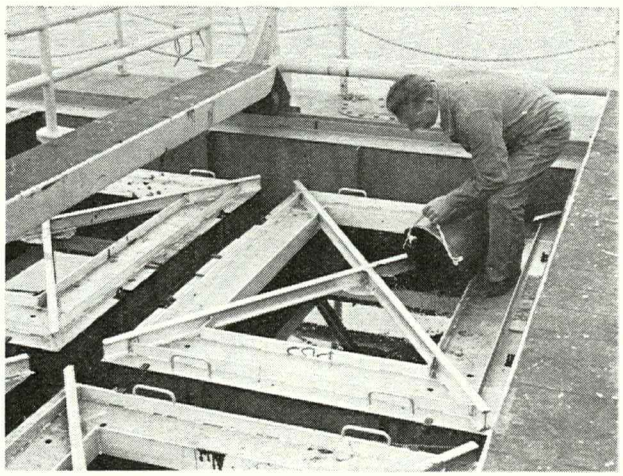

Figure 92. Oil exposure tanks. Reproduced by permission of the Ministry of Defence.

(a) Oil degradation on model beaches. Of three model beaches as described by Pugh (1975), involving tide and wave action on a wedge of fine sand, two have been polluted with Kuwait and Forties crude oil residues respectively. Changes in oil properties are being followed, also the effects of oil on beach chemistry (nitrate, nitrite, ammonia, phosphate, and oxygen) and microbiology, at two temperatures, 5 and 15°C. Hopefully it will be possible to estimate net loss of oil by comparing properties with oils from other quantitative experiments. Indications from *n*-alkane contents are that at 5°C at least, the process is slow. (Pugh et al, in preparation).

(b) Weathering of "chocolate mousse" at sea. Physical conditions during an oil spill often convert crude oil into a stable water-in-oil emulsion or "chocolate" mousse", the degradation rate of which will be very different from that of oil-in-water emulsions or thin films. Thick layers (0·7 cm) of Kuwait crude residue were exposed in two tanks on a raft in Langstone Harbour (Portsmouth) (Fig. 92), with the co-operation of the Exposure Trials Station (Central Dockyard Laboratory) of the Ministry of Defence. One tank was sealed off below the waterline and the other was open to allow flushing by seawater. Samples of oil (and water) were analysed frequently during two years (Davis and Gibbs, 1975). The oil soon formed a water-in-oil emulsion of about 50 % water. Vanadium and nickel concentrations did not change during two years, suggesting that no net loss of oil occurred, but physical and chemical properties changed significantly. The *n*-alkane content dropped by about 50 % in the "open" tank, but scarcely changed in the "closed" tank. The weight loss from *n*-alkane destruction was probably balanced by incorporation of oxygen, which is consistent with the elemental analyses. Asphaltenes, specific gravity and viscosity all increased as shown in Figure 93. Partial oxidation and polymerization were probably the main processes, but it is not known whether biological or abiological agencies predominated. It is suspected that abiological processes were the more important in this case.

Although there is a large oil/water interface in "chocolate mousse", the water is not continuous with the external sea water, so biodegradation is probably limited by the slow access of oxygen and/or mineral nutrients into the interior of the mass. It is therefore impossible to give any quantitative description of the destruction rate for "chocolate mousse", except that it is extremely slow unless physical factors fragment the mass into small particles.

CONCLUSIONS AND SUMMARY

It is possible to use respirometric methods to follow the rate of oil degradation under fairly (but not completely) natural conditions, but these measurements should be supplemented by other methods when possible. Referring back to the four questions on page 130:

Question A. Quantitative relationships have been obtained between oil oxidation and mineral nutrient supply and uptake (Table 43). These figures allow better estimates of the degradative capacity of sea water than before.

Question B. It is more difficult to quantify the lifetime of a limited oil spill, allowed (or caused) to disperse freely. However an upper limit of about one year is suggested for 50 % degradation of one oil, existing as a film or droplets of about 7 μm thickness. Thick water-in-oil emulsion weathers with little if any net destruction unless physically dispersed.

Question C. With three crude oil residues, approximately 50 % appears very resistant to degradation, about one quarter being mineralized and one quarter being lost by modification, probably as dissolved organic matter.

Question D. The undegraded material from Kuwait and Brega residues will sink in seawater. Less severely topped residues from Forties crude slowed drastically in biodegradation rate without exceeding the density of seawater. Extended weathering including photo-oxidation etc. may of course cause it to sink eventually.

These conclusions from work in local seawater are not necessarily applicable world-wide.

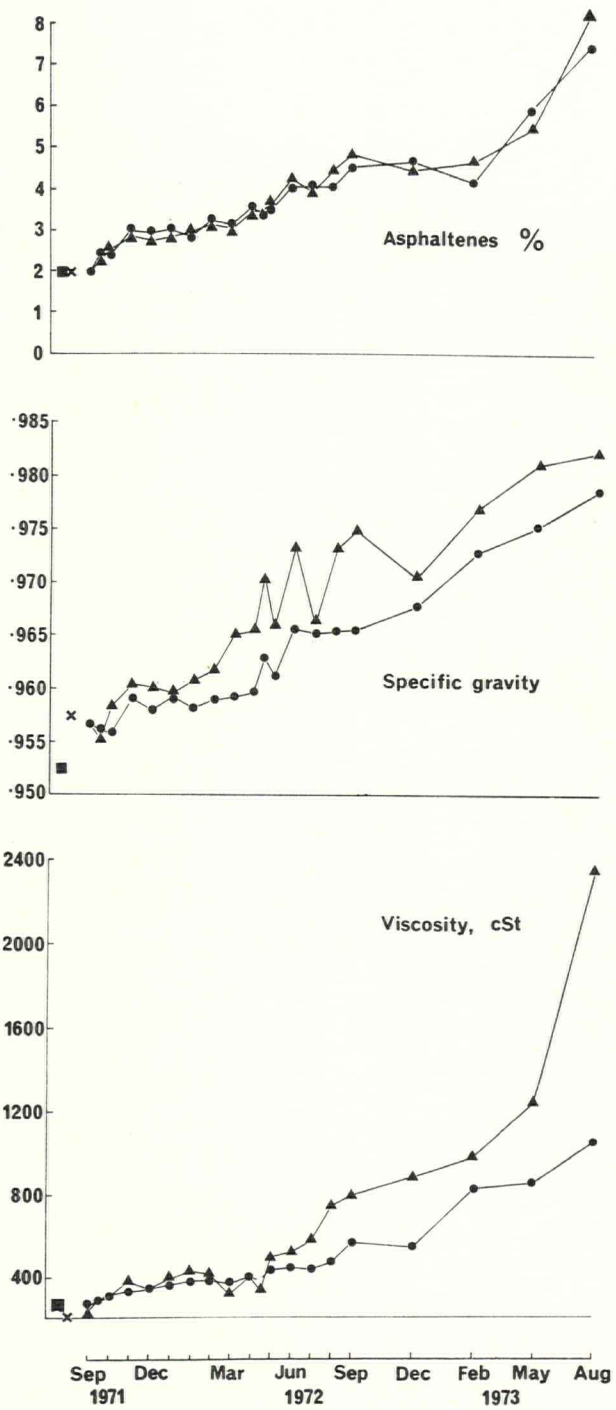

Figure 93. Some properties of Kuwait crude residues exposed at sea. ▲, open to tidal flushing; •, tank closed below water-line; ■, unexposed residue; x, unexposed residue subjected to recovery procedure.

Table 43. Relationship between oil oxidation rates and avaiable nitrogen uptake

Experimental conditions	mg O_2 per µg-at N	Correlation coefficient *r*	No. of observations
"Semi-closed" respirometry:			
Water collected in summer,			
Experiment at 14° C.	0·83	0·89	74
Experiment at 4° C.	0·3		21
Water collected in winter,			
Experiment at 10° C.	0·37	0·91	35
Continuous flow respirometry:			
Oil coated glass tube.	0·33	0·91	22
Oil on shingle column.	0·23	0·82	18

ACKNOWLEDGEMENTS

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