HELGOLÄNDER MEERESUNTERSUCHUNGEN
Helgoländer Meeresunters. 49, 143–158 (1995)

Oil pollution in the North Sea – a microbiological point of view

W. Minas[1] & W. Gunkel[2]

[1]*California Institute of Technology, Department of Chemical Engineering; Pasadena, CA 91125, USA* *;

[2]*Biologische Anstalt Helgoland, Zentrale; Notkestraße 31, D-22607 Hamburg, Germany*

ABSTRACT: In this study we determined oil degradation rates in the North Sea under most natural conditions. We used the heavy fuel oil, Bunker C, the major oil pollutant of the North Sea, as the model oil. Experiments were conducted in closed systems with water sampled during winter and repeated under identical conditions with water collected during summer. No nitrogen or phosphorous was added and conditions were chosen such that neither oxygen nor nutrients, present in the water, would become limiting during the experiments. We detected a fourfold increased degradation rate for water samples taken in summer (18 °C water temperature) as compared to water sampled in winter (4 °C water temperature). Under the assumption that biodegradation of oil can be regarded as a Michaelis-Menten type kinetic reaction, the kinectic constants V_{max} and K_M were determined for oil biodegradation at 4 °C and 18 °C. At both temperatures K_M was about 40 ppm, whereas V_{max} was 3–4 times higher at 18 °C. From both V_{max} and the results of fermentation studies, we determined the maximum rates of Bunker C oil degradation in the North Sea as ~20 g $m^{-3}a^{-1}$ at 4 °C in winter and 60–80 g $m^{-3}a^{-1}$ at 18 °C in summer. Furthermore, while over 25 % of the oil was degraded within 6 weeks in summer, only 6.6 % of the oil was degraded in winter. A higher incubation temperature in winter (18 °C) increased both the rate and the percentage of oil degraded, but degradation did not reach the level obtained during the summer. While these data reflect the oxidation only of the hydrocarbons, we conducted experiments directly in the open sea to determine the contribution of abiotic factors to oil removal. Approximately 42 % of the oil was lost within 6 weeks under these conditions in summer and 65 % in winter. However, GC-MS analysis of the recovered oil showed no significant change in the alkane pattern that would indicate enhanced degradation. Thus, mainly abiotic factors such as erosion and dispersion rather than degradation were responsible for enhanced oil removal. Especially the high loss during winter can be attributed to frequent storms resulting in greater dispersion. In conclusion, the higher oil degrading potential of the microbial population in the North Sea was represented by a four times faster oil degradation during the summer. In-situ experiments showed that abiotic factors can have an equal (summer) or even higher (winter) impact on oil removal.

INTRODUCTION

Oil pollution of the oceans and coastal environments has been a problem ever since man began to use fossil fuels. Large-capacity supertankers and increased traffic on the oceans, especially on the shipping routes near the coasts, have heightened the risk of

* Current address: Institut für Biotechnologie, ETH-Hönggerberg; CH-8093 Zürich

large and catastrophic oil spills (National Research Council, 1985). The "EXXON Valdez" spill in 1989 and the Kuwait spill in 1991 were only two recent and spectacular examples of a long list of tanker or oil rig accidents that resulted in the release of thousands of tons of oil into the marine environment (Galt et al., 1991; Rao et al., 1991). Every time a large spill occurs, a variety of techniques are employed to (a) contain the spill, (b) to recover as much as possible of the spilled oil, and (c) to remove the remaining oil from sight by either dispersion, chemically or mechanically, or by enhancing biological degradation of the oil (Atlas, 1991).

Several estimates on the oil input into the oceans have been released during the last decade (National Research Council, 1985). An example of a best estimate is given in Table 1. According to this estimate, about 40 % of the anthropogenic oil input is due to tanker operation, accidents and oil drilling activities. Roughly the same amount of oil enters the sea as urban and industrial wastewater. Harbour activities and shipping account for the rest. In addition to anthropogenic sources, natural oil seeps, as found at the bottom of the Gulf of Mexico and the Caribbean Sea, sediment erosion, and most

Table 1. Annual input of hydrocarbons into the oceans. Data from National Research Council (1985, modified)

Source	Best estimate (in million tons per year)
Anthropogenic sources	**3.0**
Offshore drilling	0.05
Transport	
Tanker	0.7
Dry docking	0.03
Harbours	0.02
Fuel and Bilge oils	0.3
Tanker accidents	0.4
Non-tanker accidents	0.02
(Total)	(1.47)
Urban and industrial wastewater	
Urban sewage	0.7
Refineries	0.1
Industrial sewage	0.2
Urban runoff	0.12
Rivers	0.04
Dumping	0.02
(Total)	(1.18)
Atmosphere (unburned fuels)	0.3
Natural sources	**10.25**
Oils seeps	0.2
Sediment erosion	0.05
Photosynthetic hydrocarbons	10*
TOTAL	**13.2**

* Global primary production $\sim 5 * 10^{10}$ t/a, hydrocarbon content 0.03 % (Floodgate, 1984)

important, hydrocarbons (mostly aliphatics, saturated and olefinic) produced photosynthetically by land- and sea plants account for most of the hydrocarbons released into the sea (Floodgate, 1984).

Looking at hydrocarbons as natural products, it is not surprising to find organisms that are able to degrade these energy-rich substrates. Bacteria, several fungi, yeasts, cyanobacteria, and some algae are able to oxidize hydrocarbons. Environmentally significant biodegradation of hydrocarbons is accomplished by bacteria only in marine environments and by mainly bacteria and fungi in freshwater environments. However, no single species is able to completely oxidize an oil (Leahy & Colwell, 1990). The complexity and varying composition of crude oil or refined products require a complex population of different microorganisms adapted to oxidize the various compounds of an oil. Generally, a wide variety of bacteria will quickly degrade n-alkanes and light-weight aromatics. Iso-alkanes, high molecular weight aromatics, and heterocyclic compounds are oxidized at a slower rate by fewer bacteria. The higher the number of rings, the more persistent the component (Leahy & Colwell, 1990). So called "Tar Balls" are examples of a very persistent form of oil mainly comprised of waxes, polycyclic asphaltenes, and resins. Introduction of oil into a previously oil-free environment results in an enrichment for oil-degrading microorganisms by several orders of magnitude within a few days (Atlas, 1991; Gunkel, 1967). The presence of an initially high number of oil-degrading bacteria indicates a previous history of oil contaminations or a chronic contamination by oil (Atlas, 1991).

Other factors affecting biodegradation of oil are environmental conditions, such as the availability of oxygen, nutrients, and the water temperature. Environmentally significant amounts of oil are only degraded under aerobic conditions (Atlas, 1991; Ward et al., 1980). As a rule of thumb, about 3.5 g of oxygen are required for the complete oxidation of one gram of oil (Gibbs, 1975; Gibbs & Davis, 1976). Low concentrations of fixed forms of nitrogen and phosphorous are the limiting factors for bacterial growth at sea and will inhibit oil biodegradation. Increased temperature speeds up growth and metabolic activity, and will enhance oil oxidation. However, higher temperature also increases the solubility and toxicity of hydrocarbons (Bossert & Bartha, 1984), and reduces the amount of dissolved oxyen in the water which in turn could inhibit oil degradation.

Several factors have prompted us to study the biodegradation of the heavy fuel oil Bunker C in the North Sea and the German Bight, in particular. The German Bight and the Wadden Sea are of both ecological and economic importance. They are the nursery for the North Sea fish stocks as well as of great recreational value (Tromp & Coenen, 1991). In addition, the North Sea is the sea with the highest traffic worldwide. This heavy traffic has led to an increased number of oil spills, over 80 % of which involved Bunker C fuel oil (Dahlmann, 1985). Interestingly, oil contaminations were observed more frequently during the winter months (Reineking, 1984; Vauk, 1984).

In this study, we addressed the question of seasonal variations in the oil degrading potential in the North Sea. Experimental conditions for laboratory experiments were chosen, to closely resemble conditions in the open North Sea. Biodegradation rates and oil degradability were determined in experiments conducted during winter and summer. The effect of the water temperature on oil oxidation rates and its degradability is discussed. In addition, results are presented from in-situ experiments conducted in the North Sea near Helgoland, and the role of physical factors for oil removal are evaluated.

MATERIAL AND METHODS

Bunker C-oil was obtained from the ESSO refinery in Hamburg. It consisted of a 350 °C residue of crude oil, containing long chain alkanes (mostly > C_{20}), and "up to some mass percentages" polycyclic aromatics and heterocyclic hydrocarbons (ESSO AG Safety Data Sheet DIN 52900, Waso Nr 4400, 8. 4. 1984). Prior to use, the oil was autoclaved at 120 °C for 20 min. A stock solution of this oil was prepared in diethyl-ether/chloroform (1:1[v:v]) to permit precise handling of small quantities.

Seawater samples were collected at the station "Kabeltonne" (cable buoy) on the Helgoländer Roads (Gunkel, 1964) from 1 m below the surface. Water samples were processed immediately.

Microbiological Methods

Viable counts were determined using either marine agar 2216 E solidified with 2 % Bacto agar (Difco), or a liquid minimal salt medium (MS) containing 7 mM Na_2HPO_4, 2.9 mM K_2HPO_4, 9.35 mM NH_4Cl in 75 % aged seawater (Gunkel & Trekel, 1967) to which, after autoclaving and pH adjustment to 7.6 with NaOH, a 1:1 (v:v) mixture of sterile Diesel oil and Bunker C was added to a final concentration of about 5 % (v:v). Numbers of oil-degrading bacteria were determined by the most probable number (MPN) method, as outlined by Gunkel & Trekel (1967). Ten ml of MS medium were inoculated with 1 ml of serial dilutions of water samples and incubated for 12 weeks in the dark at 18 °C on a reciprocal shaker at 100 strokes/min. The number of other heterotrophic bacteria was determined by plating appropriate dilutions of water samples onto 2216 E seawater agar and incubating for 3 weeks at 18 °C in the dark (Gunkel, 1964).

Analytical Methods

The oxygen content of water samples was determined by a modified Winkler method (Kalle, 1939). Oil oxidation was determined by measuring oxygen consumption in water samples (in triplicates), incubated in the presence of oil. The oxygen consumption in the oil-free water sample was subtracted from that of the oil-containing sample in order to correct for the degradation of other organic material in the water. Knowing the biological oxygen demand (BOD), which is about 3.5 g O_2 per gram of oil (Gibbs, 1975; Gibbs & Davis, 1976), the net oxygen consumption in the oil-containing water was used to calculate the amount of oil oxidized. Dividing the amount of oil degraded by the incubation time leads to the oil degradation rate, which was expressed as mg oil degraded per litre of water per day (mg l^{-1} d^{-1}) or, if multiplied by 365, as g oil degraded annually per cubic metre (g m^{-3} a^{-1}). We attempted to keep the oxygen concentrations above 0.5 mg/l to eliminate inhibitions caused by oxygen limitation (ZoBell, 1940). This method does not distinguish between complete oxidation of the hydrocarbons, partial oxidation, or conversion into biomass. However, this method is fast and accounts quantitatively for oxidation of all the components of the oil (National Research Council, 1985).

The formation of oxidation products during hydrocarbon degradation was followed by measuring the formation of carbonyl compounds in water samples in a semi-quantitative mode, as described by Peach & Tracey (1955). Water samples were passed through a

0.45 µm filter to remove cells, debris, and particles. To a 50-ml sample, 2 ml of 2,-4,-dinitrophenylhydrazin (DNPH) solution was added (DNPH was dissolved in 2-n hydrochloric acid at a final concentration of 0.02 %). The sample was mixed and incubated for 30 min at 22 °C. Then 2 ml carbontetrachloride (CTC) were added and the sample was shaken vigorously for one minute. After phase separation was completed, the organic phase was recovered and washed with 2 ml of 0.5-n NaOH before measuring its absorption at 335 nm against CTC. Analysis was performed in duplicates. Unoiled seawater samples, treated the same way, served as controls.

Data for concentrations of fixed nitrogen (NH_4^+, NO_2^-, NO_3^-) and phosphorous were obtained from the hydrographic group at the Biologische Anstalt Helgoland. Seawater samples, taken at the same location and time as the water samples for our experiments, were analysed using standard techniques (Gillbricht, 1985).

Determination of oil degradation rates

Oil degradation rates were determined by measuring the oxygen consumption over a period of 16 days. Bunker C oil from the Bunker C-diethyl-ether/chloroform stock was dispensed into clean, sterile, dry 500-ml glass bottles containing 5 glass beads (0.5 mm). Constant rotation of the bottles ensured a thin oil cover coating the glass walls. The final concentration of oil was 20 ppm. After complete evaporation of the diethylether/chloroform, the bottles were filled bubble-free with freshly collected seawater. The bottles were closed air-tight and incubated for up to 16 days in the dark at either 18 °C or 4 °C, slowly rotating head-down in a carousel. The rolling glass beads facilitated the mixing of the water. Oil-free water samples were incubated under identical conditions and served as controls. For the determination of the oxygen consumption, a bottle each of oil-free water and oil-containing water was taken for immediate measurement of the amount of dissolved oxygen in the water. Subsamples were used to determine the number of oil-degrading bacteria and other heterotrophic bacteria, and to monitor the levels of carbonyl components in the water.

Determination of Bunker C oil degradability

Long-term incubation of water samples was necessary to determine the extent to which the Bunker C oil could be degraded by the indigenous microbial population in the North Sea. The experimental design was the same as for the determination of the rates, except that the incubation time was extended to up to 6 weeks and the oil content was reduced to 4 ppm. Under these conditions, the amount of oxygen and nutrients present in the water was sufficient for the degradation of 50 % of the oil.

Fermentation studies

A different approach for measuring oil degradation was to run a batch-fermentation with seawater and Bunker C oil as the sole carbon source. We incubated 4 l of freshly collected seawater at 4 °C in winter and at 18 °C in summer in a fermentor (Eschweiler & Co, Kiel, Germany), aerated with sterile air at a rate of 650 l/h. A glass fritted disk (4.5 cm in diameter), previously coated with 100–150 mg of oil, was lowered into the

water. The disk was retrieved after 21 days of incubation, and the elimination of oil was measured gravimetrically by comparing the weight of the oiled glass disk before and after incubation. In addition, the remaining oil was extracted and its weight was determined.

In-situ experiments for the determination of oil removal from sediment in an open system

We designed a flow-through chamber system which was placed in the North Sea near Helgoland at a depth of 6 m and 50 cm above ground. This design permitted us to evaluate the contribution of physical factors to the removal of oil from sediment. The system is described in detail in a previous paper (Minas et al., 1986). Briefly, it consists of a series of identical plastic tubes, 9 cm in diameter and 17 cm in length. Placed inside these tubes were aluminium trays that contained about 70 g of coarse sediment that was dried at 150 °C overnight before it was coated with a defined amount, about 500 mg, of Bunker C oil from the Bunker C-diethylether/chloroform stock solution. After complete evaporation of the solvents, the tubes containing the trays with the oiled sediment were capped on either side with nylon gauze (mesh size 1000 µm). Several of these assemblies were attached to concrete foundations on the bottom of the North Sea and incubated for up to 15 weeks. An Anderaa RCM 4 recording unit (Aanderaa Instruments, Bergen, Norway) was used to measure the water temperature. After retrieval of a tube by divers, the sediment was dried at 130 °C overnight. Dry-weight measurements showed that no sediment was lost during incubation at sea. The oil content of the sediment was determined gravimetrically after extraction of the oil with ether:chloroform (1:1[v:v]) in a Soxhlet extractor. The solvent was heated at 80 °C and condensed over the sample at 4 °C. Extraction was stopped when the solvent remained colourless after passing through the sediment. The amount of oil initially added to the sediment was determined by extracting the oil from three sediment samples. These samples were not incubated in the water but otherwise treated the same way as those that were exposed in the North Sea. The mean value of these extractions was used as reference for all other samples.

RESULTS

Oil biodegradation in closed laboratory systems

Biodegradation of oil by indigenous microorganisms of the North Sea was determined by measuring the oxygen consumption in water samples that were incubated at 4 °C or 18 °C. These temperatures were a close approximation to the surface water temperatures of the North Sea reached in winter and summer, respectively. Oil concentrations used in the experiments were adjusted to the duration of the experiment, the initial amount of dissolved oxygen, and the concentrations of nitrogen and phosphorous in the water. Under these conditions, neither oxygen nor nutrients were limiting, and we were able to determine the initial degradation rates for Bunker C oil. In addition, we were able to determine the degradability of this oil after incubation over a 6-week period. For the determination of biodegradation rates, we used an oil concentration of 20 ppm in the water sample, while only 4 ppm were used for degradability studies. At this low

concentration, 50 % of the oil could be degraded using the nutrients and dissolved oxygen present in the water sample. Degradation of less than 50 % would indicate that the oil contains components that are not easily degraded, or the bacterial population present in the water sample does not have the enzymatic capability to degrade this oil any further. Incubation time was restricted to a maximum of 6 weeks, since the possibility of adaptation or succession in the bacterial population degrading the oil was very limited in these experiments.

Oil degradation rates from experiments conducted once in winter and repeated during summer are summarized in Table 2. Water collected in winter showed a slower overall degradation as compared to water collected during summer. During the winter experiment, a 3.4-times faster biodegradation was measured when samples were incubated at 18 °C as compared to 4 °C. A 2.9-times difference was determined between incubation at 4 °C and 18 °C in the summer experiment. Interestingly, when comparing the rate measured at 4 °C in winter and the rate for 18 °C incubation in summer, a more than 4-fold faster degradation was obtained in summer.

A similar result was obtained for the biodegradability of the Bunker C oil measured as the amount degraded in 6 weeks (Table 3). Comparing the degradability at 4 °C and 18 °C, measured during the winter and summer experiments respectively, 1.9- and 2.7-fold increases in the amount of oil degraded were determined at the higher temperature. Again, an almost 4-fold difference in the amount of oil degraded was measured when comparing results from incubations at 4 °C during the winter experiment with results obtained during the summer experiment incubated at 18 °C. This difference was likely to be even greater, since no dissolved oxygen was left in the 6th-week sample of the summer experiment which inhibited further degradation of the oil.

As an alternative approach to the experiments in which oxygen consumption was used as a measure for oil degradation, we conducted batch-fermentations in which we measured oil degradation gravimetrically. Four litres of sea water were sparged with sterile, moist air to avoid oxygen depletion. A defined amount of oil applied on a fritted glass disk, hanging in the fermentor vessel, served as the carbon source in these fermentations. The fermentations were stopped after 21 days. The amount of oil degraded was determined by comparing the weight of the oiled glass disk prior to and after the fermentation. Degradation rates in these experiments were 22.2 g $m^{-3}a^{-1}$ during the winter fermentation at 4 °C, and 80.4 g $m^{-3}a^{-1}$ during the summer fermentation at

Table 2. Summary of oil-degradation rates as determined by oxygen consumption (or gravimetrically*) in seawater samples to which 20 ppm (or 35 ppm) fresh Bunker C oil was added

Incubation temperature	Oil degradation rates			Difference Summer–Winter
	Winter (g m^{-3} a^{-1})		Summer (g m^{-3} a^{-1})	
4 °C	5.58		6.66	19.4 %
Factor 4 °C–18 °C	3.42	4.06	2.93	
18 °C	19.06		22.64	18.8 %
	(22.16 at 4 °C	Factor 3.63	80.39 at 18 °C)	

* In parentheses, the results from 21-day batch fermentations with seawater sparged with air

Table 3. Summary of oil-degradability as determined by oxygen consumption in sea-water samples to which 4 ppm fresh Bunker C oil was added

Incubation temperature	Oil removal Winter (%)		Oil removal		Oil removal Summer (%)	Difference Summer–Winter
4 °C	6.6	↔			9.6	45.5 %
Factor 4 °C–18 °C	1.9		3.9	↔	2.7	
18 °C	12.6				25.6*	103.2 %

* Oxygen limited due to complete oxygen consumption

18 °C. Again almost a 4-fold difference in the degradation rate was observed between summer and winter. Measurements of carbonyl components in the water at the beginning of the fermentation and after 21 days, showed a 63-fold increase after the 4 °C fermentation, while only a 2.6-fold higher concentration was measured at the end of the summer fermentation. This indicated that a significant amount of partially oxidized hydrocarbons accumulated in the water during the winter experiment, whereas hydrocarbons are either oxidized completely or transformed into biomass during the summer experiment. Alternatively, sparging with air could have volatilized some of the compounds.

Determination of the kinetic constants V_{max} and K_M

We studied degradation at various concentrations of oil ranging from 2 ppm to 20 ppm. Within this range, we found a positive and linear correlation between degradation rate and oil concentration. Figure 1 shows degradation rates obtained with 2, 4, 8, and 20 ppm oil during a 16-day incubation. The linear correlation between degradation rates and oil concentrations indicates that all concentrations were below saturation of the microbial and enzymatic activities. One exception was the measurement for the 20 ppm winter water sample incubated at 18 °C and marked as 'winter' in Figure 1. We substituted this value by the rate obtained under the same condition in summer (marked summer). Assuming that oil biodegradation follows Michaelis-Menten kinetics, we used these rates to determine V_{max} and K_M for Bunker C oil degradation. From a Lineweaver-Burk plot, we determined V_{max} (Fig. 2) which was 0.0528 mg l^{-1} d^{-1} or 19.3 g m^{-3} a^{-1} for samples incubated at 4 °C. V_{max} at 18 °C was 0.1676 mg l^{-1} d^{-1} or 61.2 g m^{-3} a^{-1}. The K_M for the 4 °C and 18 °C incubation was 43.9 ppm and 40.7 ppm, respectively. As expected, a change in temperature had a great influence on V_{max}, a determinant for the speed with which a reaction takes place. The change in temperature had no effect on the Michaelis constant K_M, which is a measure for substrate affinity.

Viable cell counts

We determined the number of heterotrophic bacteria and the percentage of oil-degrading bacteria of all water samples processed. Medium, incubation temperature and

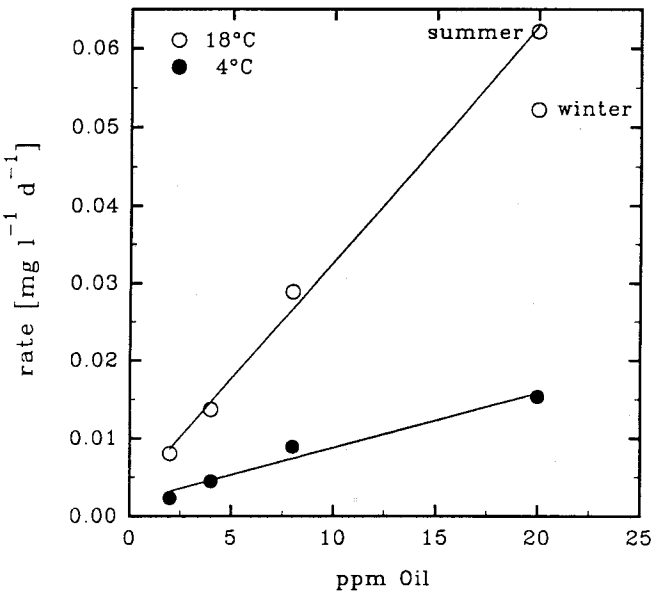

Fig. 1. Oil degradation rates determined in water samples that contained oil concentrations between 2 ppm and 20 ppm. Rates were measured in water samples incubated at 4 °C (filled circles) and 18 °C (open circles). See text for details

duration will all influence the number of viable cells counted for a water sample. For the purpose of monitoring biodegradation of oil, however, the standard method employed proved sufficient to follow the increase of total heterotrophic bacteria and oil degrading bacteria (ODB) and to demonstrate an increase in ODB over time in water samples that contained oil. The initial number of bacteria in the water samples used in the experiments was between 10^3 and 10^4 cells per ml (data not shown). Typically, the number of heterotrophic bacteria remained within one order of magnitude of the initial value, while the number of ODB among these increased from between a 0.01 to 5 % base level to up to 100 % and higher (data not shown). More than 100 % ODB are possible, since these bacteria do not necessarily grow under the conditions used to determine the number of heterotrophic bacteria.

In-situ experiments in the North Sea

Oiled sediment samples were exposed in the North Sea for up to 15 weeks. After recovering the sediment, the remaining oil was extracted and its weight was determined on a microbalance. A temperature probe was used to monitor the water temperature during the experiment. The results of two experiments, one conducted during winter and the other during summer, are summarized in Figure 3. The highest oil removal was obtained during the first 6–8 weeks of both experiments. Over 70 % and 40 % of the oil was removed during the winter and summer experiment, respectively. Only minor changes in the oil content of the sediment were measured after these first weeks. Measuring the dry weight of the sediment after exposure in the North Sea revealed that

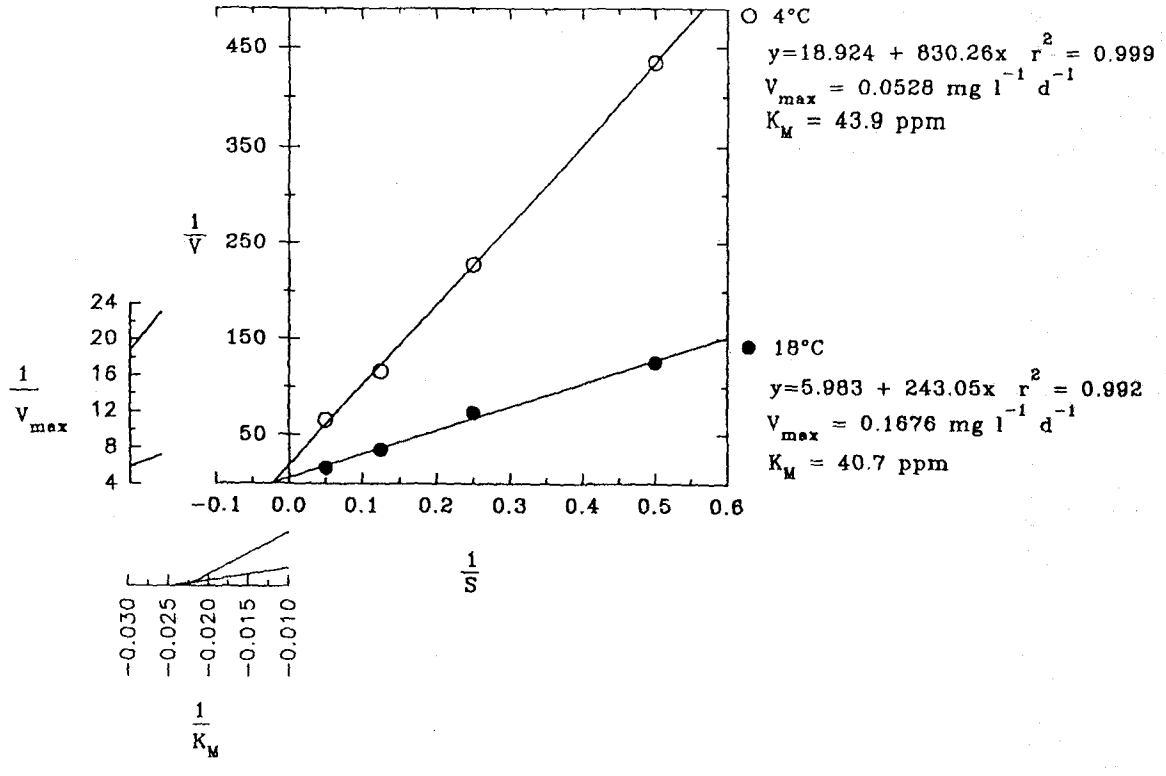

Fig. 2. Lineweaver-Burk plot of oil-degradation rates determined in water samples that contained oil concentrations between 2 ppm and 20 ppm. Water samples were incubated at 4 °C (filled circles) and 18 °C (open circles). V_{max} is calculated from the intercept of the Y-axis which represents $1/V_{max}$. The Michaelis constant K_M is calculated from the intercept of the X-axis which is $-1/K_M$. The regions where the graphs intercept the axes are enlarged on the left and lower left of the plot. The function for each graph is given on the right, together with the calculated values for V_{max} and K_M. Substrate concentrations are given in ppm, and rates are expressed as mg oil degraded per liter of water per day (mg l^{-1} d^{-1})

no sediment was lost during incubation. On the contrary, often 2–3 times the initial amount of sediment was found inside the holding device. This sediment consisted of fine sand overlying the original oil coated sediment, causing it to become anaerobic. Samples with a very high influx of sediment are marked in Figure 3. The major differences between oil removal in winter and summer were: (a) an immediate and more rapid onset of oil removal during the summer as compared to winter, and (b) a maximal removal that amounted to some 40 % in summer and to over 70 % in winter. Samples from the recovered oil were sent to Dr. Dahlmann at the German Hydrographic Institute (DHI, Hamburg) for analysis by gas chromatography, coupled with mass spectroscopy (GC-MS). The GC-MS analysis of the samples was performed to examine possible changes in the alkane pattern of the oil which would indicate biological oil degradation. We found that the alkane pattern of the samples incubated in the sea was not significantly different from that of the fresh oil (data not shown), indicating that the oil removal from the sediment was mainly due to abiotic factors. Under this assumption, the stormy winter weather and strong currents would explain the high loss of oil. During the summer experiment, algal growth on the gauze membranes, used to contain the sample, restricted

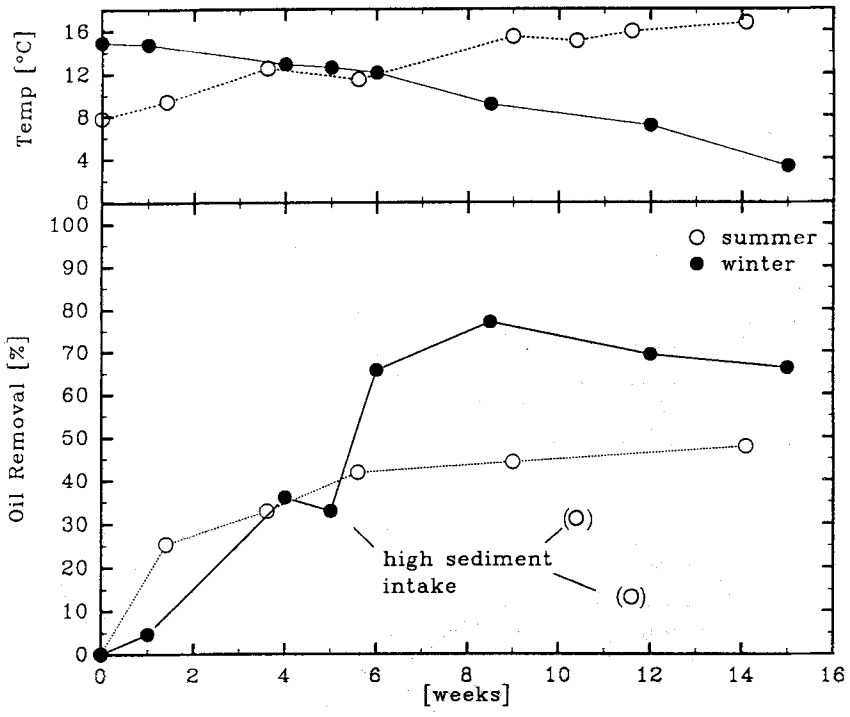

Fig. 3. Oil removal from sediment samples that had been exposed in the North Sea for up to 15 weeks during winter (filled symbols and solid lines) and summer (open symbols and dotted lines). Changes in North Sea water temperature are shown in the upper portion. Points marked 'high sediment intake' represent samples in which more than twice the initial amount of sediment was found after incubation at sea

the flow of water over the sample. Despite constant removal of the algae, the reduced water exchange over the sample could be the main reason for the relatively small loss of oil.

DISCUSSION

In this study, we examined the microbial degradation of Bunker C oil in the North Sea. Different sets of experiments were designed in which freshly sampled seawater was exposed to oil and incubated at different temperatures. These experiments were conducted once during winter and repeated during summer. This allowed us to study seasonal variations int he oil-degrading potential of the microbial community present in the water. In addition to closed-system laboratory experiments, a set of experiments was conducted in the open North Sea in order to evaluate the significance of biodegradation versus abiotic removal of oil from a model environment.

For the two sets of laboratory experiments described here, we chose oxygen consumption as the sumparameter for the determination of oil degradation. Using this method, one does not distinguish between mineralization of the oil to CO_2 and water, partial oxidation, or transformation into biomass. Other analytical methods, such as gas chromatography and spectroscopy (mass, UV, or IR), are frequently used for fingerprinting of oil samples (Dahlmann, 1985). However, these methods are very specific for the various classes of hydrocarbons. This makes quantification of the overall degradation of oil difficult. Thus, measurement of oxygen consumption was the most suitable method for the purpose of this study. An alternative way of measuring the degradation of oil was a gravimetrical determination of oil removal from a water sample. This approach was utilized when higher quantities of oil were used, as in batch-fermentations and in in-situ experiments.

Enhancement of oil biodegradation by the addition of nitrogen and phosphorus to the water has been well documented (Gunkel, 1967). For the purpose of this study, we have investigated the oil-degrading potential of the indigenous microbial population in the North Sea under natural conditions with no nitrogen or phosphorus added. At the very low oil concentrations used, the amounts of nitrogen and phosphorus present in the seawater were high enough not to limit oil degradation. This was confirmed by experiments in which nutrients were added, but in which no enhancement of oil degradation was observed (data not shown).

From oxygen consumption measurements, we obtained rates for the Bunker C degradation during winter and summer. A 3.4- to 2.9-fold difference in degradation was measured between water samples incubated at 4 °C and 18 °C in winter and summer, respectively. From an environmental point of view, the more interesting comparison was between the rates obtained at 4 °C in winter and at 18 °C in summer. This showed that oil degradation was over 4 times faster during the summer. Gravimetrical determination of oil degradation by measuring the oil removal from a fritted glass filter disk after 21-day batch-fermentations confirmed a 3- to 4-times higher degradation rate during summer. Oil degradation rates in the fermentations were higher than those obtained in experiments using oxygen consumption as a measure for oil degradation. Oil dissolution or emulsion from the disk into the water, or the sparging with air, could account for the higher rates. Sparging the water with air could have volatilized some components of the

oil similar to the situation found at the water surface in the open sea. However, Bunker C is a heavy 350 °C residue of crude oil, comprised mostly of long-chain ($>C_{20}$) aliphatics, 4- to 6-ring aromatics and heterocyclic compounds that are not very volatile at 4 °C and 18 °C. Since the rates were close to the V_{max} determined for oil degradation (see below), we consider the higher rates mainly to be the result of higher oil concentrations used in the fermentations. Measurement of carbonyl compounds in the water showed an over 60-fold increase after 21 days during the winter experiment at 4 °C, compared to only 2.5-times more carbonyl compounds after 21 days at 18 °C in water collected in summer. The accumulation of carbonyl compounds, as a result of a partial oxidation of hydrocarbons mainly observed in winter, indicated that the microbial population in winter had a different oil-degrading potential than the population present at a higher water temperature in summer. In addition, higher volatility at 18 °C could further reduce the concentration of carbonyl compounds in the water.

Kinetic constants V_{max} and K_M for oil degradation could be determined from rates determined by measurements of oxygen consumption for oil concentration between 2 ppm and 20 ppm. A change in temperature from 4 °C to 18 °C had a greater effect on V_{max}. The K_M values for both temperatures were about the same. Thus, changes in oil degradation at different temperatures are due to variations in rates rather than changes in substrate affinity. Interestingly, V_{max} determined at 4 °C under these conditions was about the same as the rate measured in the 4 °C winter fermentation, despite the experimental differences. At 18 °C, however, V_{max}, as determined by oxygen consumption, was 61.2 g m^{-3} a^{-1} which was 25 % lower than the rate of 80.4 g m^{-3} a^{-1} obtained in the batch-fermentation at 18 °C in summer. As discussed, increased solubility and volatility at 18 °C might account for the difference between the two experimental systems.

Results similar to those for degradation rates were obtained for the degradability of the Bunker C oil. The conditions in these experiments were such that degradation of less than 50 % of the oil would indicate that the oil contains components that are not easily degraded, or that the bacterial population present in the water sample does not have the enzymatic capability to degrade this oil any further. With a maximum amount of 25.6 % oil degraded during summer at 18 °C and a minimum of 6.6 % in winter at 4 °C, we determined a ca 4-fold difference in degradability. This low degradability indicated that this oil contained a high portion of compounds that are not readily degraded. Walker et al. (1975) found under similar conditions that only 11 % of Bunker C oil was degraded at 15 °C. Johnston (1970), using gravel sand columns, determined that the first 10 % of oil was rapidly degraded, while the remaining 90 % was very persistent. Gibbs & Davis (1976) showed that 12 % of the 350 °C residue of Kuwait crude oil was degraded within 12 weeks at temperatures ranging from 6–21 °C. An increase in temperature by 10 °C resulted in a 2.7-fold increased degradation (Q_{10} = 2.7). In our study, a 14 °C increase in temperature alone (4 °C −> 18 °C) resulted in a 3.4-fold and 2.9-fold enhancement in winter and summer, respectively, which is equivalent to a Q_{10} of 2.4 and 2.1. The environmentally more relevant comparison of degradation between winter at 4 °C and summer at 18 °C resulted in a Q_{10} of 2.9, which was higher than that reported in the literature (Gibbs & Davis, 1976).

All our findings combined: (a) different degradability in winter and summer, (b) 4-times-higher degradation rates for Bunker C oil in summer, and (c) the accumulation of

oxidation products, suggested differences in the oil-degrading potential of the microbial populations present in the water. Since the number of cells in the North Sea water was similar throughout the experiments, we assume that the difference in the potential to degrade the oil is due to seasonal changes in the microbial population as a function of water temperature and nutrient concentrations. Such changes in populations are well known and documented for the North Sea (Sieburth, 1967), and it should not be surprising that this will influence oil biodegradation.

In addition to laboratory experiments in closed systems under controlled conditions, we conducted a series of experiments in the open North Sea in which we had little control over the experiment. However, we found them useful to determine the fate of an oil contamination in the environment itself, and to answer the questions: (a) how long it would take for this oil to be removed or degraded, and (b) what are the major factors contributing to the removal: biotic or abiotic? Compared to lab experiments in which 6.6 to 25.6 % of oil was degraded within 6 weeks, in the in-situ experiments we determined an oil removal of over 70 % and over 40 % during the same time-period in winter and summer, respectively. From the largely unaltered alkane pattern of the recovered oil, however, we concluded that this high removal rate was due to physical factors such as washout and solution, rather than biological degradation. Thus, the oil was not removed from the environment but rather redistributed, diluted, and dispersed. Since these processes usually result in increased surface to volume ratios, subsequent biodegradation would be faster. It also showed that next to biodegradation of oil, physical factors can have an equal (summer) or even greater (winter) influence on the removal of a contaminant from the environment.

In conclusion, we found that Bunker C oil is degraded in summer at an approximately 4-times faster rate than in winter. In-situ experiments in the North Sea showed that even though biodegradation is slowed down during winter, physical removal of oil from surfaces could be enhanced due to higher wave activities and stormy weather conditions. This could prove advantageous for the subsequent biodegradation of the oil. However, many other factors effect the degradation of large quantities of oil under spill conditions, the most important being the type of oil. Spreading on the water surface and distribution in the water column will increase the surface area on which microbial degradation takes place. Microbial activity can be enhanced by dispersing floating oil slicks, either chemically or mechanically, further increasing the oil-water interface and improving degradation (Tramier & Sirvins 1983; Halmo 1985), or by adding oleophilic fertilizers. The "EXXON Valdes" spill and the successful application of oleophilic fertilizers to clean some of the polluted beaches in Prince William Sound is one of the more recent examples for large-scale bioremediation (Lindstrom et al., 1991).

Finally, it is important to note that oil degradation rates are always specific for the type of oil and the environmental conditions studied. In this work, we have presented data on the degradation of one of the most persistent oils, the heavy Bunker C fuel oil, in the North Sea. This information might be useful for clean-up operations, now and in the future.

Acknowledgements. We thank Professor Dr. H. Kaltwasser and the late Professor Dr. R. Schweisfurth for helpful discussions, also the crew of the MB Aade and the BAH underwater diving team for their help during sampling. This study (Project No. 335-01) was funded by the German Society for Petroleum Science and Coal Chemistry.

LITERATURE CITED

Atlas, R. M. (Ed.), 1984. Petroleum microbiology. Macmillan, New York, 692 pp.

Atlas, R. M., 1991. Microbial hydrocarbon degradation – bioremediation of oil spills. – J. chem. Technol. Biotechnol. *52*, 149–156.

Bossert, I. & Bartha, R., 1984. The fate of petroleum in soil ecosystems. In: Petroleum microbiology. Ed. by R. M. Atlas. Macmillan, New York, 434–476.

Dahlmann, G., 1985. Herkunft der Ölverschmutzungen an der deutschen Nordseeküste. – Seevögel *6* (Sonderbd.), 73–80.

Floodgate, G. D., 1984. The fate of petroleum in marine ecosystems. In: Petroleum microbiology. Ed. by R. M. Atlas, Macmillan, New York, 355–397.

Galt, J. A., Hehr, W. J. & Payton, D. L., 1991. Fate and transport of the "Exxon Valdez" oil spill. Environ. Sci. Technol. *25*, 202–209.

Gibbs, C. F., 1975. Quantitative studies on marine biodegradation of oil. I: Nutrient limitation at 14 °C. – Proc. R. Soc. (Ser. B) *188*, 61–82.

Gibbs, C. F. & Davis, S. J., 1976. The rate of microbial degradation of oil in beach gravel column. – Microb. Ecol. *3*, 55–64.

Gillbricht, M., 1985. Hydrographie, Nährstoffe und Phytoplankton bei Helgoland. –Jber. Biol. Anst. Helgoland *1984*, 27–30.

Gunkel, W., 1964. Einwirkungen des kalten Winters 1962/63 auf die Bakterienpopulation vor Helgoland. –Helgoländer wiss. Meeresunters. *10*, 246–256.

Gunkel, W., 1967. Experimentell-ökologische Untersuchungen über die limitierenden Faktoren des mikrobiellen Ölabbaus im marinen Milieu. – Helgoländer wiss. Meeresunters. *16*, 210–225.

Gunkel, W. & Trekel, H. H., 1967. Zur Methodik der quantitativen Erfassung ölabbauender Bakterien in verölten Sedimenten und Böden, Öl-Wassergemischen und teerartigen Substanzen. – Helgoländer wiss. Meeresunters. *16*, 336–348.

Halmo, G., 1985. Enhanced biodegradation of oil. In: Proceedings of the 1985 Oil Spill Conference, Los Angeles, California. American Petroleum Institute, Washington, 531–537.

Johnston, R., 1970. The decomposition of crude-oil residues in sand columns. – J. mar. biol. Ass. U. K. *50*, 925–937.

Kalle, K., 1939. Einige Verbesserungen zur Bestimmung des gelösten Sauerstoffs im Meerwasser. – Annln Hydrogr., Berlin *67*, 267–269.

Leahy, J. G. & Colwell, R. R., 1990. Microbial degradation of hydrocarbons in the environment. – Microbiol. Rev. *54*, 305–315.

Lindstrom, J. E., Prince, R. C., Clark, J. C., Grossman, M. J., Yeager, T. R., Braddock, J. F. & Brown, E. J., 1991. Microbial populations and hydrocarbon biodegradation potentials in fertilized shoreline sediments affected by the T/V "Exxon Valdez" oil spill. – Appl. environ. Microbiol. *57*, 2514–2522.

Minas, W., Gunkel, W. & Tadday, G., 1986. An open flow-through chamber system – a new tool for experimental ecological investigations in the marine sublittoral. – Mar. environ. Res. *20*, 299–305.

National Research Council (Ed.), 1985. Oil in the sea. National Academy Press, Washington, 601 pp.

Peach, K. & Tracey, M. V., 1955. Moderne Methoden der Pflanzenanalyse. Springer, Heidelberg *2*, 421–431.

Rao, U. R., Chamdrasekhar, M. G., Radhakrishnan, K., Jayaraman, V., Desai, P. S., Pal, P. K. & Joshi, P. C., 1991. Environmental impacts of the Persian Gulf oil spill and oil-fire smoke. – Curr. Sci. *60*, 486–492.

Reineking, B., 1984. Zum Seevogelsterben durch Ölpest an der deutschen Nordseeküste im Winter 1982/83. – Seevögel *5*, 43–49.

Sieburth, J. McN., 1967. Seasonal selection of estuarine bacteria by water temperature. – J. Exp. mar. Biol. Ecol. *1*, 98–121.

Tramier, B. & Sirvins, A., 1983. Enhanced oil biodegradation: a new operational tool to oil spills. In: Proceedings of the 1983 Oil Spill Conference, San Antonio, Texas. American Petroleum Institute, Washington, 115–119.

Tromp, D. & Coenen, R. C. A., 1991. The third international conference on the protection of the North Sea – review and challenges. – Wat. Sci. Technol. *24*. XV–XX.

Vauk, G., 1984. Oil pollution dangers on the German coast. – Mar. Pollut. Bull. *15*, 89–93.
Walker, J. D., Petrakis, L. & Colwell, R. R., 1975. Comparison of the biodegradability of crude and fuel oils. – Can. J. Microbiol. *22*, 598–602.
Ward, D., Atlar, R. M., Boehm, D. K. & Calder, J. A., 1980. Microbial degradation and the chemical evolution of "Amoco Cadiz" oil pollutants. – Ambio *9*, 277–283.
ZoBell, C. E., 1940. The effects of oxygen tension on the rate of oxidation of organic matter in seawater by bacteria. – J. mar. Res. *3*, 211–223.